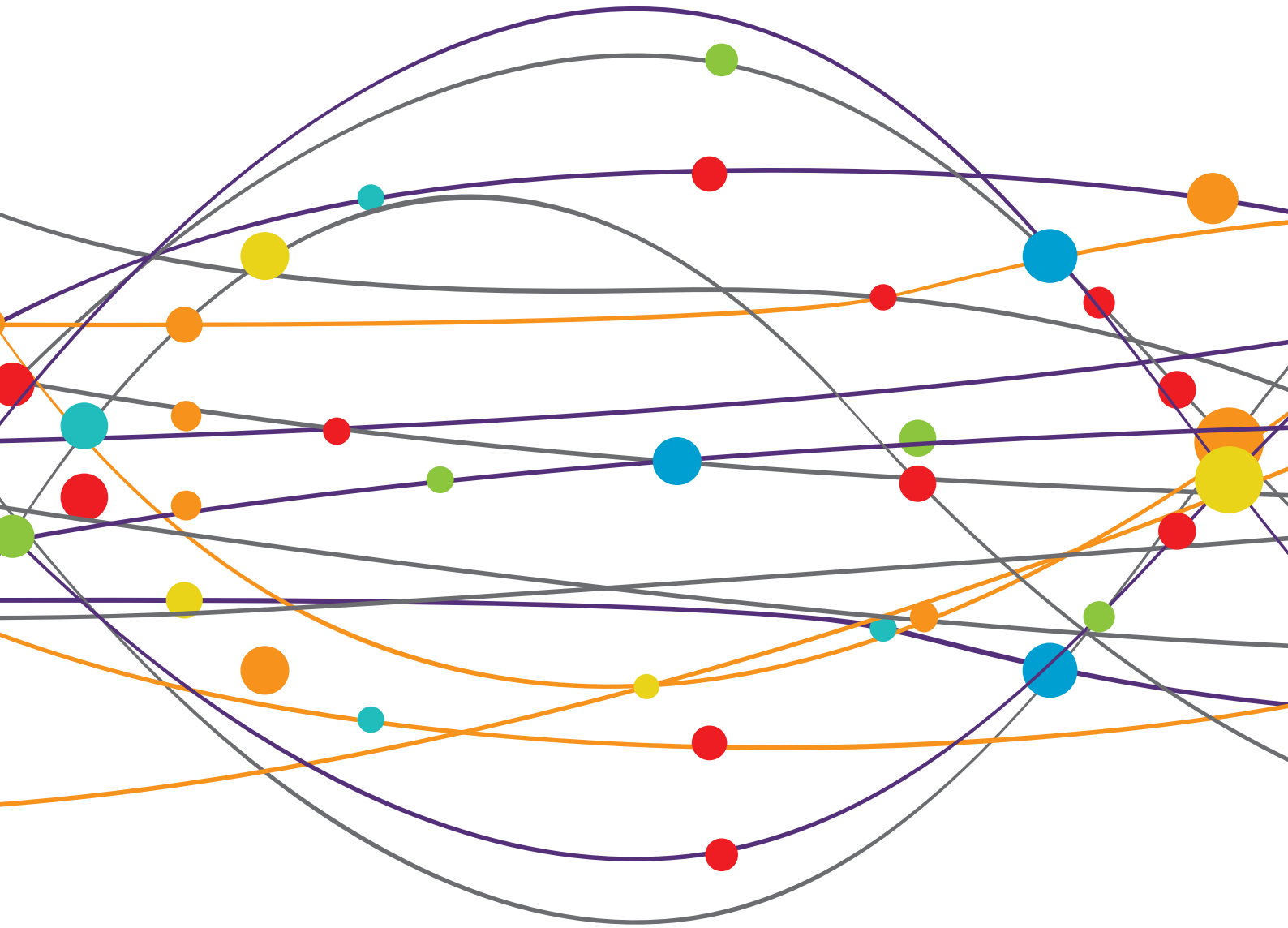
INTRACRANIAL BLEEDING AFTER REPERFUSION THERAPY IN ACUTE ISCHEMIC STROKE

EDITED BY: Nishant K. Mishra, Richard Leigh and Bruce Campbell
PUBLISHED IN: Frontiers in Neurology



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88971-508-4
DOI 10.3389/978-2-88971-508-4

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

INTRACRANIAL BLEEDING AFTER REPERFUSION THERAPY IN ACUTE ISCHEMIC STROKE

Topic Editors:
Nishant K. Mishra, Yale University, United States
Richard Leigh, Johns Hopkins University, United States
Bruce Campbell, The University of Melbourne, Australia

Citation: Mishra, N. K., Leigh, R., Campbell, B., eds. (2021). Intracranial Bleeding After Reperfusion Therapy in Acute Ischemic Stroke. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88971-508-4

Table of Contents

frontiers in Neurology

EDITORIAL
published: 31 August 2021
doi: 10.3389/fneur.2021.745993

Editorial: Intracranial Bleeding After Reperfusion Therapy in Acute Ischemic Stroke

Nishant K. Mishra[1]*, *Richard Leigh*[2] and *Bruce C. V. Campbell*[3]

[1] Department of Neurology, Division of Stroke and Vascular Neurology, Yale University, New Haven, CT, United States, [2] Department of Neurology, Johns Hopkins University, Baltimore, MD, United States, [3] Department of Medicine and Neurology, Melbourne Brain Centre at the Royal Melbourne Hospital, University of Melbourne, Parkville, VIC, Australia

Keywords: hemorrhagic transformation, stroke, blood brain barrier, permeability imaging, endovascular treatment, hypothermia (induced)

Editorial on the Research Topic

Intracranial Bleeding After Reperfusion Therapy in Acute Ischemic Stroke

The use of reperfusion therapy [IV thrombolysis with tissue plasminogen activator [tPA] or tenecteplase [TNK], and/or mechanical thrombectomy (MT)] in ischemic stroke patients is now an established standard of care (1, 2). The risk of intracranial bleeding after reperfusion therapy continues to be the most feared complication of reperfusion therapy [(1); also see review article by Charbonnier et al.]. In routine clinical practice, we aim to identify the occurrence of hemorrhagic transformation (HT) soon after the reperfusion therapy is offered. This is because early identification of HT may lead to precautionary steps such as stopping ongoing antithrombotic therapies, use of bleeding reversal agents and surgical interventions (1). HT appears as a hyperdensity on a non-contrast CT head and is not difficult to detect if the patient did not receive iodinated contrast previously (3). If, however, the patient did receive iodinated contrast prior to the CT head, as part of pre-treatment imaging with CT angiogram (CTA) and CT perfusion (CTP) or catheter angiography during MT, it is challenging to distinguish contrast staining from HT because both look similarly hyperdense. If an MRI cannot be obtained because of contraindications or unavailability, a typical course is to follow the evolution of the hyperdensity on the subsequent CT scans. This approach delays critical therapies such as antiplatelet agents in patients who have contrast staining but no HT. Dual Energy CT (DECT) offers a solution to this problem by exploiting the fact that the use of two X-ray radiation energies can distinguish tissue composition based on the differential Xray attenuation, including distinguishing iodine from blood (4). Three original papers examine the role of DECT in distinguishing HT from iodine contrast staining in stroke patients. Almqvist et al. found that, compared to a non-contrast CT head, DECT changed the diagnosis from intracranial hemorrhage to iodine contrast staining in about 10 percent of the stroke patients (n/N 3/31). Similarly, Liu et al. found that the conventional CT images obtained at the conclusion of MT led to the diagnosis of HT in 74.5% of the patients; this proportion, however, reduced to 10.4% when images were reviewed from the DECT. Liu et al. and Lun et al. showed that the interrater reliability for the detection of blood and/or contrast using DECT in stroke patients was fair ($k = 0.3$) with zero agreement between 18 readers. Interestingly, the intra rater agreement was higher at $k = 0.7$ in this study by Lun et al. Larger studies are needed to confirm these findings and standardize the image interpretation.

OPEN ACCESS

Edited and reviewed by:
Jean-Claude Baron,
University of Cambridge,
United Kingdom

***Correspondence:**
Nishant K. Mishra
Nishant.Mishra@yale.edu

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *23 July 2021*
Accepted: *23 July 2021*
Published: *31 August 2021*

Citation:
Mishra NK, Leigh R and Campbell BCV (2021) Editorial: Intracranial Bleeding After Reperfusion Therapy in Acute Ischemic Stroke. Front. Neurol. 12:745993. doi: 10.3389/fneur.2021.745993

HT is classified into hemorrhagic infarction (HI) and parenchymal hematoma (PH) (3). Petechial hemorrhage without mass effect is referred as HI1 unless it is confluent which is classified as HI2 (3, 5). PH refers to HT associated with space-occupying effect (3, 5). Not every HT is of similar clinical significance, however (3, 6). It is typically the PH type HT that is clinically meaningful as it is commonly associated with clinical worsening (3). Patients with PH type HT and >30 percent involvement of the infarcted tissue are referred as PH2 type HT; these patients are typically the ones with the worst clinical outcomes (3). The classification of HT into HIs and PHs is typically done by visual inspection of the patient images (3). van Kranendonk et al. performed volumetric assessment of the HT and found that only the PH2 hemorrhage volume was associated with worse outcomes (OR 0.37; 95%CI 0.14–0.98).

Reperfusion therapy is associated with a greater risk of HT. However, this HT did not translate into increased mortality in clinical trials, an argument used to assuage the concerns of those worried about the excess bleeding risk [(1, 7–9), Charbonnier et al.]. In real world clinical practice, the goal is to prevent HT that causes clinical deterioration, mostly related to parenchymal hematoma (PH, blood clot with mass effect). If PH does occur, the goal is to rapidly diagnose it and emergently initiate therapies to prevent further clinical and/or radiological worsening (1). The two comprehensive review articles contributed by Charbonnier et al. and Maier et al. provide an excellent overview of the state of knowledge relevant to the identification and management of HT. The treating physicians are also advised to review the contemporary guidelines from relevant societies e.g., American Heart Association when needing to manage patients with post MT intracranial hemorrhage (1). The review article by Spronk et al. provides a detailed description of the post stroke inflammatory cascades which have been linked to HT. Spronk et al. discuss the therapeutic approaches to prevent HT and edema that have been previously tested in animals (e.g., Otaplimastat) and humans (e.g., NXY-059), and describe the biological pathways that can be targeted to inhibit specific inflammatory target to prevent HT. Similarly the review article by Bernardo-Castro et al. provides a detailed overview of the pathomechanism linked to the BBB permeability and its association with HT.

A survey of expert vascular neurologists found that the factors associated with the risk of HT include the volume of ischemia, previous use of antithrombotic medication, neurological severity, age, hyperglycemia at presentation, hypertension on admission, and cardio embolism (10). A secondary analysis of the WAKE-UP trial by Jensen et al. found that treatment with IV tPA, baseline NIHSS, ischemic stroke volume, baseline glucose levels, and atrial fibrillation are associated with the increased risk of HT or HI-type HT. The study also found that the patient's baseline NIHSS predicted PH-type HT in this population (Jensen et al.). It is not surprising that the survey of the expert vascular neurologists included the prior use of antithrombotic agents as one of the variables that would increase the risk of HT after MT (10). In an analysis of the NORDICTUS registry, Ramos-Araque et al. found that it was only the vitamin K inhibitors (OR 1.9, 95% CI 1.0–3.5, $P = 0.04$; $N = 1{,}455$) and not the newer oral anticoagulants (OR 0.3, 95%CI 0.0–2.4, $p = 0.3$) that were associated with an increased risk of symptomatic intracerebral hemorrhage in MT patients. These registry data add to the growing body of evidence in support of the safety of NOACs for secondary stroke prevention (11). Various other predictors of HT have been reported e.g., very low cerebral blood volume or significantly prolonged Tmax delay (12), Elands et al. reported that angiographic finding known as early venous filling is associated with increased risk of HT and could be used to identify high risk patients (OR 6.7, $p = 0.005$) [(13), Elands et al.].

From a biological stand point, cerebral ischemia activates the cascade of destructive processes like the progressive failure of the Na/K pumps, activation of NMDA receptors, alteration in the ionic homeostasis, buildup of acidotic environment, and the activation of inflammatory cascades [(14), Choi et al.]. The inflammatory response is characterized by the release of activated neutrophils, production of reactive oxygen species, and the expression of metalloproteinases (like MMP 9 and 2) (Spronk et al.). Post stroke inflammatory response, the ischemic cascade, and the patient specific clinical variables (e.g., the extent and degree of ischemic injury or the prior use of oral anticoagulant) interact with each other to result in the increased permeability of BBB Spronk et al., Bernardo-Castro et al. CT and MR based imaging algorithms can provide information on the degree and the extent of BBB permeability (15, 16). Arba et al. conducted a meta-analysis to study the association of BBB permeability with HT and found a significant association of BBB permeability with HT. The strength of association with HT was greater for the MRI based BBB permeability measures with the OR of 9.3 (95% CI, 3.2–27.6) which in case of CT based BBB permeability was 3.42 (95% CI, 1.62–7.23) (Arba et al.).

Despite their utility in predicting BBB disruption, these approaches have not been applied for use in routine clinical practice; the permeability imaging algorithms need to be standardized in order to permit comparability across all platforms and adoption in the routine clinical practice (Arba et al.). Additionally, analysis of BBB disruption will need to be performed in real time if it is to be used as part of clinical decision making. Heidari et al. investigated how BBB analysis may complement penumbral imaging for patients presenting in an extended time window. They found that patients with larger penumbral volumes had less disruption of the BBB, possibly explaining the low hemorrhage rates in studies that used penumbral imaging to guide thrombolysis in the extended time window (17). Their study highlights the potential value of incorporating a standardized BBB imaging protocol in routine clinical practice; however, this will require detailed validation in larger studies, standardization of the protocol, and clearance by the regulatory bodies.

Endovascular technologies have made huge strides in the last decade and have resulted in the higher recanalization rates over 80 percent (18). Even though successful recanalization can be achieved in large proportion of patients, the success with recanalization procedures has not translated into comparable improvement in the patient's outcomes (19–21). Only 25 percent of the stroke patients treated with MT are left with no disability (Rankin 0–1) (18). One of the reasons why the successful recanalization does not translate into comparably improved

outcomes is that the ischemia activates a cascade of destructive processes like progressive failure of the Na/K pumps, activation of NMDA receptors, altered ionic homeostasis, local acidosis, and the activation of proinflammatory processes like the release of cytokines; [(14), Choi et al.] the restoration of blood flow into this ischemic, acidotic, brain tissue can at times be more destructive causing reperfusion injury and HT (22). Neuroprotective strategies are therefore urgently needed to immediately arrest the ischemic cascades at the stroke onset and prevent the buildup of proinflammatory and acidotic environment [(22, 23), Choi et al.]. Therapeutic hypothermia is one such strategy; however, its application in the clinical trials has been limited by the difficulty in quickly achieving hypothermia. Choi et al. report a novel approach that uses endovascular delivery of hypothermia to offer focused cooling of the ischemic brain tissue [selective endovascular brain cooling (24)] and avoids the systematic complication of systemic hypothermia like pneumonia and altered coagulability. This approach, however, needs testing in randomized controlled trials to demonstrate safety and efficacy (Choi et al.). Another potential neuroprotective agent is Magnesium (25). In an analysis of retrospective dataset of 242 patients, Cheng et al. found lower magnesium level at the stroke onset is associated with an increased risk of HT at 24–36 h after thrombolytic therapy. This relationship, however, does not hold true if the baseline magnesium levels are above a threshold of 0.88 mmol/L (Cheng et al.). They used this argument to indicate why the previous trials like FAST MAG did not show treatment response from the use of Magnesium. The magnesium levels were indeed higher than the physiological concentrations in the FAST MAG study (25, 26).

A common challenge encountered in contemporary stroke management is the risk of distal embolization of the clot that reduces the proceduralist's ability to achieve full reperfusion, perhaps more likely after IV tPA. This is particularly the case when the thrombectomy device is unable to reach the smaller distal branches of the vessel because of smaller vessel diameter (27, 28). A meta-analysis of the trial data by the HERMES collaborators reported no hemorrhagic complication and showed favorable outcomes from the MT of the M2 branches of the MCA (29). The analysis of MR CLEAN data ($N = 1{,}349$) showed that the thrombus location commonly changes between the CTA and cerebral angiography ($N = 302$; 22%) and the use of iv tPA is associated with an increased odds of thrombus migration (OR 2.0 95% CI 1.3–3.1) (30). In the subgroup of patients with MCA M1 occlusion, distal migration of the clot was associated with better odds of functional recovery (OR 1.5, $p < 0.05$) (30). Chang et al. report outcome data from the ischemic stroke patients ($N = 170$) with large vessel occlusion who received IV tPA plus mechanical thrombectomy (MT) or only (MT). In contrast to the recent DIRECT-MT trial (30), significantly greater proportions of the patients achieved recanalization (TICI 2b-3) from the combination therapy (tPA & MT) compared to MT alone (83 vs. 67%; $p = 0.03$) (Chang et al.). Importantly, the study found that the rate of clot migration was lower at 11% and the rate was only slightly increased in the IV tPA group (Chang et al.). Also, there was no difference in the rate of symptomatic hemorrhage between the two groups (Chang et al.). IV tPA remains standard in eligible patients in the setting of the drip-and-ship model of stroke care. Its role in patients for whom MT is immediately available may become clearer with the release of further results from randomized trials of the direct to MT strategy (SWIFT DIRECT trial, NCT03192332; DIRECT SAFE trial, NCT03494920) (30). An analysis of the MT data lodged within the STRATIS registry showed that the risk of subarachnoid hemorrhage was greater in patients with distal vessel occlusion and needing >3 passes (Lee et al.). Despite these findings, these is a degree of clinical equipoise regarding MT of the M2 occlusions, and it will be interesting to see the results of the MT trials that would target MCA M2 occlusions.

The American Heart Association recommends targeting blood pressure (BP) below 180/105 mmHg after reperfusion therapy. The ENCHANTED trial showed a significant reduction in the rate of intracranial hemorrhage when BP in the range of 130–140 mmHg was targeted within 1 h of tPA administration (OR 0.75, 95% CI 0.60–0.94, $p = 0.01$); this trial however showed no improvement in functional outcomes in the groups in which BP was targeted at <180 mmHg vs. BP 130-140 (31), Silverman et al. have contributed a comprehensive review article about the impact of cerebral hemodynamics after MT on patient outcomes. They report that ischemic stroke patients show a significant variability in the trajectory of their post MT systolic blood pressures (32). The review article indicates that the patients in each trajectory would require different BP goals (32). The presence of acutely elevated blood pressure or persistently high blood pressure in the post-MT phase is associated with significantly worse outcomes, including a higher rate of symptomatic HT (32). Acutely elevated blood pressure or persistently high blood pressure amongst successfully recanalized ischemic stroke patients reflects the presence of underlying untreated hypertension (32). A chronically hypertensive patient develops a pressure passive cerebral hemodynamic system; and restoration of blood flow in this system from successful recanalization increases the risk of HT (32). Silverman et al.'s review article highlights that there is a need to conduct trials that will test a differential response to BP management based on the observation that post MT BP oscillates within the autoregulatory limits or deviates from it (32). Cheng et al. reported that the mean, maximum, range, and the standard deviation of systolic BP measured at the conclusion of the MT were associated with PH2 type HT in ischemic stroke patients. This study suggested that in patients with successful recanalization the systolic BP should be kept to low levels, below 120 mmHg, following the conclusion of MT and fluctuations in blood pressure should be avoided (Cheng et al.; Appleton et al.) conducted a pooled analysis of the three glyceryl trinitrate (GTN) trials, ENOS, RIGHT, and RIGHT 2 ($N = 715$) (Appleton et al.). These trials mainly included patients treated with IV tPA (>99%) (Appleton et al.). The study failed to detect an association of the use of GTN with the risk of intracranial hemorrhage. However, among the patients randomized within 6 h, there was a trend toward reduced risk of HT and also improved functional outcomes(Appleton et al.).

We are delighted to present this research topic. It provides review articles that give a detailed overview of the epidemiology, pathophysiology, diagnosis, and management

of hemorrhagic transformation after reperfusion therapy. It also identified important hypotheses for testing in future trials.

AUTHOR CONTRIBUTIONS

NM conceived of the presented research topic and invited co-editors RL and BC. NM drafted the editorial. All authors reviewed and edited the editorial and contributed to its final version.

FUNDING

NM was supported by NIH R25 award (Award No: 5R25NS079102-08) and Leon Levy Foundation during his affiliation with the Icahn School of Medicine at Mt. Sinai, New York; by NIH StrokeNET fellowship during his affiliation with UCLA, Los Angeles. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, Leon Levy Foundation, or NM's past or present employers.

REFERENCES

1. Yaghi S, Willey JZ, Cucchiara B, Goldstein JN, Gonzales NR, Khatri P, et al. Treatment and outcome of hemorrhagic transformation after intravenous alteplase in acute ischemic stroke: a scientific statement for healthcare professionals from the american heart association/american stroke association. *Stroke.* (2017) 48:e343–61. doi: 10.1161/STR.0000000000000152
2. Powers WJ, Derdeyn CP, Biller J, Coffey CS, Hoh BL, Jauch EC, et al. 2015 american heart association/american stroke association focused update of the 2013 guidelines for the early management of patients with acute ischemic stroke regarding endovascular treatment: a guideline for healthcare professionals from the american heart association/american stroke association. *Stroke.* (2015) 46:3020–35. doi: 10.1161/STR.0000000000000074
3. Fiorelli M, Bastianello S, von Kummer R, del Zoppo GJ, Larrue V, Lesaffre E, et al. Hemorrhagic transformation within 36 hours of a cerebral infarct: relationships with early clinical deterioration and 3-month outcome in the european cooperative acute stroke study i (ecass i) cohort. *Stroke.* (1999) 30:2280–4. doi: 10.1161/01.STR.30.11.2280
4. McCollough CH, Leng S, Yu L, Fletcher JG. Dual- and multi-energy ct: Principles, technical approaches, and clinical applications. *Radiology.* (2015) 276:637–53. doi: 10.1148/radiol.2015142631
5. Hacke W, Kaste M, Fieschi C, von Kummer R, Davalos A, Meier D, et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ecass ii). second european-australasian acute stroke study investigators. *Lancet.* (1998) 352:1245–51. doi: 10.1016/S0140-6736(98)08020-9
6. Thomalla G, Sobesky J, Kohrmann M, Fiebach JB, Fiehler J, Zaro Weber O, et al. Two tales: hemorrhagic transformation but not parenchymal hemorrhage after thrombolysis is related to severity and duration of ischemia: Mri study of acute stroke patients treated with intravenous tissue plasminogen activator within 6 hours. *Stroke.* (2007) 38:313–8. doi: 10.1161/01.STR.0000254565.51807.22
7. Lees KR, Bluhmki E, von Kummer R, Brott TG, Toni D, Grotta JC, et al. Time to treatment with intravenous alteplase and outcome in stroke: an updated pooled analysis of ecass, atlantis, ninds, and epithet trials. *Lancet.* (2010) 375:1695–703. doi: 10.1016/S0140-6736(10)60491-6
8. Campbell BCV, Mitchell PJ, Churilov L, Yassi N, Kleinig TJ, Dowling RJ, et al. Tenecteplase versus alteplase before thrombectomy for ischemic stroke. *N Engl J Med.* (2018) 378:1573–82. doi: 10.1056/NEJMoa1716405
9. Campbell BCV, Majoie C, Albers GW, Menon BK, Yassi N, Sharma G, et al. Penumbral imaging and functional outcome in patients with anterior circulation ischaemic stroke treated with endovascular thrombectomy versus medical therapy: a meta-analysis of individual patient-level data. *Lancet Neurol.* (2019) 18:46–55. doi: 10.1016/S1474-4422(18)30314-4
10. Andrade JBC, Mohr JP, Lima FO, Barros LCM, Nepomuceno CR, Portela LB, et al. Predictors of hemorrhagic transformation after acute ischemic stroke based on the experts' opinion. *Arq Neuropsiquiatr.* (2020) 78:390–6. doi: 10.1590/0004-282x20200008
11. Meinel TR, Kniepert JU, Seiffge DJ, Gralla J, Jung S, Auer E, et al. Endovascular stroke treatment and risk of intracranial hemorrhage in anticoagulated patients. *Stroke.* (2020) 51:892–8. doi: 10.1161/STROKEAHA.119.026606
12. Mishra NK, Christensen S, Wouters A, Campbell BC, Straka M, Mlynash M, et al. Reperfusion of very low cerebral blood volume lesion predicts parenchymal hematoma after endovascular therapy. *Stroke.* (2015) 46:1245–9. doi: 10.1161/STROKEAHA.114.008171
13. Shimonaga K, Matsushige T, Takahashi H, Hashimoto Y, Mizoue T, Ono C, et al. Early venous filling after reperfusion therapy in acute ischemic stroke. *J Stroke Cerebrovasc Dis.* (2020) 29:104926. doi: 10.1016/j.jstrokecerebrovasdis.2020.104926
14. Zheng Z, Yenari MA. Post-ischemic inflammation: molecular mechanisms and therapeutic implications. *Neurol Res.* (2004) 26:884–92. doi: 10.1179/016164104X2357
15. Bang OY, Saver JL, Alger JR, Shah SH, Buck BH, Starkman S, et al. Patterns and predictors of blood-brain barrier permeability derangements in acute ischemic stroke. *Stroke.* (2009) 40:454–61. doi: 10.1161/STROKEAHA.108.522847
16. Puig J, Blasco G, Daunis IEP, van Eendendburg C, Carrillo-Garcia M, Aboud C, et al. High-permeability region size on perfusion ct predicts hemorrhagic transformation after intravenous thrombolysis in stroke. *PLoS ONE.* (2017) 12:e0188238. doi: 10.1371/journal.pone.0188238
17. Campbell BCV, Ma H, Ringleb PA, Parsons MW, Churilov L, Bendszus M, et al. Extending thrombolysis to 4.5-9 h and wake-up stroke using perfusion imaging: a systematic review and meta-analysis of individual patient data. *Lancet.* (2019) 394:139–47. doi: 10.1016/S0140-6736(19)31053-0
18. Badhiwala JH, Nassiri F, Alhazzani W, Selim MH, Farrokhyar F, Spears J, et al. Endovascular thrombectomy for acute ischemic stroke: a meta-analysis. *JAMA.* (2015) 314:1832–43. doi: 10.1001/jama.2015.13767
19. Albers GW, Marks MP, Kemp S, Christensen S, Tsai JP, Ortega-Gutierrez S, et al. Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. *N Engl J Med.* (2018) 378:708–18. doi: 10.1056/NEJMoa1713973
20. Saver JL, Goyal M, van der Lugt A, Menon BK, Majoie CB, Dippel DW, et al. Time to treatment with endovascular thrombectomy and outcomes from ischemic stroke: a meta-analysis. *JAMA.* (2016) 316:1279–88. doi: 10.1001/jama.2016.13647
21. Nogueira RG, Jadhav AP, Haussen DC, Bonafe A, Budzik RF, Bhuva P, et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. *N Engl J Med.* (2018) 378:11–21. doi: 10.1056/NEJMoa1706442
22. Al-Mufti F, Amuluru K, Roth W, Nuoman R, El-Ghanem M, Meyers PM. Cerebral ischemic reperfusion injury following recanalization of large vessel occlusions. *Neurosurgery.* (2018) 82:781–9. doi: 10.1093/neuros/nyx341
23. Yenari MA, Han HS. Influence of hypothermia on post-ischemic inflammation: role of nuclear factor kappa b (nfkappab). *Neurochem Int.* (2006) 49:164–9. doi: 10.1016/j.neuint.2006.03.016
24. Choi JH, Marshall RS, Neimark MA, Konstas AA, Lin E, Chiang YT, et al. Selective brain cooling with endovascular intracarotid infusion of cold saline: a pilot feasibility study. *AJNR Am J Neuroradiol.* (2010) 31:928–34. doi: 10.3174/ajnr.A1961
25. Saver JL, Starkman S, Eckstein M, Stratton SJ, Pratt FD, Hamilton S, et al. Prehospital use of magnesium sulfate as neuroprotection in acute stroke. *N Engl J Med.* (2015) 372:528–36. doi: 10.1056/NEJMoa1408827
26. Shkirkova K, Starkman S, Sanossian N, Eckstein M, Stratton S, Pratt F, et al. Paramedic initiation of neuroprotective agent infusions: successful achievement of target blood levels and attained level effect on clinical outcomes in the fast-mag pivotal trial (field

administration of stroke therapy - magnesium). *Stroke.* (2017) 48:1901–7. doi: 10.1161/STROKEAHA.116.015664
27. Guedin P, Larcher A, Decroix JP, Labreuche J, Dreyfus JF, Evrard S, et al. Prior iv thrombolysis facilitates mechanical thrombectomy in acute ischemic stroke. *J Stroke Cerebrovasc Dis.* (2015) 24:952–7. doi: 10.1016/j.jstrokecerebrovasdis.2014.12.015
28. Behme D, Kabbasch C, Kowoll A, Dorn F, Liebig T, Weber W, et al. Intravenous thrombolysis facilitates successful recanalization with stent-retriever mechanical thrombectomy in middle cerebral artery occlusions. *J Stroke Cerebrovasc Dis.* (2016) 25:954–9. doi: 10.1016/j.jstrokecerebrovasdis.2016.01.007
29. Menon BK, Hill MD, Davalos A, Roos Y, Campbell BCV, Dippel DWJ, et al. Efficacy of endovascular thrombectomy in patients with m2 segment middle cerebral artery occlusions: meta-analysis of data from the hermes collaboration. *J Neurointerv Surg.* (2019) 11:1065–9. doi: 10.1136/heartjnl-2014-307109.258
30. Alves HC, Treurniet KM, Jansen IGH, Yoo AJ, Dutra BG, Zhang G, et al. Thrombus migration paradox in patients with acute ischemic stroke. *Stroke.* (2019) 50:3156–63. doi: 10.1161/STROKEAHA.119.026107
31. Yang P, Treurniet KM, Zhang L, Zhang Y, Li Z, Xing P, et al. Direct intra-arterial thrombectomy in order to revascularize ais patients with large vessel occlusion efficiently in chinese tertiary hospitals: a multicenter randomized clinical trial (direct-mt)-protocol. *Int J Stroke.* (2020) 15:689–98. doi: 10.1177/1747493019882837
32. Anderson CS, Huang Y, Lindley RI, Chen X, Arima H, Chen G, et al. Intensive blood pressure reduction with intravenous thrombolysis therapy for acute ischaemic stroke (enchanted): an international, randomised, open-label, blinded-endpoint, phase 3 trial. *Lancet.* (2019) 393:877–88. doi: 10.1016/S0140-6736(19)30038-8

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Neurology

ORIGINAL RESEARCH
published: 19 May 2020
doi: 10.3389/fneur.2020.00357

Dual-Energy CT Follow-Up After Stroke Thrombolysis Alters Assessment of Hemorrhagic Complications

Håkan Almqvist[1,2†], Niklas S. Almqvist[3†], Staffan Holmin[1,2†] and Michael V. Mazya[1,4†]*

[1] Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden, [2] Department of Neuroradiology, Karolinska University Hospital, Stockholm, Sweden, [3] Medical Programme, Karolinska Institutet, Stockholm, Sweden, [4] Department of Neurovascular Diseases, Karolinska University Hospital, Stockholm, Sweden

OPEN ACCESS

Edited by:
Nishant K. Mishra,
Icahn School of Medicine at Mount Sinai, United States

Reviewed by:
Ken Uchino,
Cleveland Clinic, United States
Kumar Rajamani,
Wayne State University, United States

***Correspondence:**
Michael V. Mazya
michael.mazya@sll.se

†ORCID:
Håkan Almqvist
orcid.org/0000-0002-0363-1284
Niklas S. Almqvist
orcid.org/0000-0003-1030-0878
Staffan Holmin
orcid.org/0000-0002-1628-1615
Michael V. Mazya
orcid.org/0000-0002-0377-3506

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: 08 March 2020
Accepted: 14 April 2020
Published: 19 May 2020

Citation:
Almqvist H, Almqvist NS, Holmin S and Mazya MV (2020) Dual-Energy CT Follow-Up After Stroke Thrombolysis Alters Assessment of Hemorrhagic Complications. Front. Neurol. 11:357. doi: 10.3389/fneur.2020.00357

Background and Purpose: We aimed to determine whether dual-energy CT (DECT) follow-up can differentiate contrast staining (CS) from intracranial hemorrhage (ICH) in stroke patients treated with intravenous thrombolysis (IVT), who had undergone acute stroke imaging using CT angiography (CTA), and CT perfusion (CTP).

Materials and Methods : Between November 2012 and January 2018, 168 patients at our comprehensive stroke center underwent DECT follow-up within 36 h after IVT and acute CTA with or without CTP but did not receive intra-arterial imaging or treatment. Two independent readers evaluated plain monochromatic CT (pCT) alone and compared this with a second reading of a combined DECT approach using pCT and water- and iodine-weighted images, establishing and grading the ICH diagnosis, per Heidelberg and Safe Implementation of Treatments in Stroke Monitoring Study (SITS-MOST) classifications.

Results: On pCT alone within 36 h, 31/168 (18.5%) patients had findings diagnosed as ICH. Using combined DECT (cDECT) changed ICH diagnosis to "CS only" in 3/168 (1.8%) patients, constituting 3/31 (9.7%) of cases with initially pCT-diagnosed ICH. These three cases had pCT diagnoses of one SAH, one minor, and one more extensive petechial hemorrhage (hemorrhagic infarction types 1 and 2), respectively. pCT alone had a 100% sensitivity, 98% specificity, 90% positive predictive value (PPV), 100% negative predictive value (NPV), and 98% accuracy for any ICH, compared to the cDECT. Inter-reader agreement for ICH classification using pCT compared to DECT was weighted kappa 0.92 (95% CI 0.87–0.98) vs. 0.91 (0.85–0.95).

Conclusion: Compared to pCT, DECT within 36 h after IV thrombolysis for acute ischemic stroke, changes the radiological diagnosis of post-treatment ICH to "CS only" in a small proportion of patients. Studies are warranted of whether the altered radiological reports have an impact on patient management, for example initiation timing of antithrombotic secondary prevention.

Keywords: spectral computed tomography, acute ischemic stroke, intravenous thrombolysis, computed tomography, intracerebral hemorrhage (ICH)

INTRODUCTION

The 2018 American Heart Association (AHA)/American Stroke Association (ASA) acute ischemic stroke (AIS) guidelines recommend a follow-up CT or MRI scan at 24 h after intravenous thrombolysis (IVT), prior to initiation of secondary preventive treatment with antithrombotic agents (1).

Previous dual-energy CT (DECT) studies have focused on imaging after intra-arterial endovascular therapy (EVT), contrast staining (CS) concealing infarcts, the mimicking of hemorrhagic events in 10–85% depending on the time window, prognostication of infarct size, or a later hemorrhage depending on the amount of immediate iodine leakage (2–7). In the neuroradiological field, there are other DECT applications and improvement of CT angiography (CTA) including bone removal, calcification scoring, contrast-to-noise ratio (CNR) and beam hardening artifacts, the value of virtual non-contrast (VNC) images, blooming artifact of calcium and iodine in carotid stenosis with different keV levels [which correspond to different kVp levels with single-energy (SECT) technology], and CS during the investigation after a spontaneous hemorrhagic stroke (8–12).

The added value and aim are to understand whether there is CS or not in the brain that may mimic hemorrhagic events on a routine 24-h follow-up after IVT in patients examined with CTA and/or CT perfusion (CTP), without additional EVT. The secondary aim was to assess inter-reader agreement between two experts when using DECT images.

MATERIALS AND METHODS

The included patients were treated with IVT as the only acute recanalization therapy for AIS between November 2012 and January 2018, with a workup protocol including a non-enhanced CT brain, IV iodine contrast injection for CTA and/or CTP, with non-enhanced follow-up DECT within 36 h after the start of IVT. The time cutoff chosen was motivated by its basis in literature and clinical practice, with nearly all symptomatic intracerebral hemorrhages known to occur within 36 h and with a 22- to 36-h standard follow-up time used in large-scale IVT registry studies (13, 14).

Patients receiving intra-arterial iodine injections or treatment were excluded. Hospital administrative systems and the radiological information system (RIS) were queried for patients with IVT-treated AIS, including information on the radiological modality chosen for follow-up.

Following the acquisition of a dual-energy-capable CT scanner in 2012, DECT was selected for routine follow-up imaging 22–36 h after IVT in AIS, and earlier or later follow-up in the acute phase, if clinically indicated. For this study, patients were grouped by timing of DECT follow-up in relation to start of IVT: those with scans performed within 22–36 h (routine interval) and <22 h (early follow-up due to clinical indication). If patients had DECT scans in both time intervals, they were included in the 22–36 h group only, and only the images obtained in this time interval was assessed for the purposes of the study.

TABLE 1 | Classification of intracranial hemorrhage.

Class	Definition
HI1	Scattered small petechiae along the margins of the infarct, no space-occupying effect
HI2	Confluent petechiae within the infarcted area, no space-occupying effect
PH1	Local, intra-ischemic, confluent hematoma in ≤30% of the infarcted area, with at the most mild space-occupying effect
PHr1	Small- to medium-sized hematoma located remotely from the infarct(s), with mild space-occupying effect
PH2	Local or intra-ischemic confluent hematoma >30% of the infarcted area, with substantial space-occupying effect
PHr2	Large confluent hematoma in an area remote from the actual infarct(s), with substantial space-occupying effect
IVH	Intraventricular hemorrhage
SAH	Subarachnoid hemorrhage
SDH	Subdural hemorrhage

Image Acquisition and Analysis

The follow-up scan was performed with CT750HD (General Electric Health Company, Milwaukee, USA) using a fast kVp-switching technique, with a routine protocol with $CTDIvol_{16cm}$ of 57 mGy. The radiation dose is lower than the CT accreditation program requirements from the American College of Radiology (ACR)[1]. From this single DECT scan, routine 5-mm MPRs in three planes for plain monochromatic non-contrast CT (pCT) images at 67 keV were transferred to the picture archive communication system (PACS) together with a triad of series used for a combined DECT approach (cDECT), containing a 5-mm axial series of the pCT, water-weighted DECT (wDECT), and iodine-weighted DECT (iDECT) series. The triad of series containing pCT, wDECT, and iDECT was automatically postprocessed in the CT scanner and transferred to the PACS. pCT images obtained with DECT technology are considered to have an equal or improved image quality compared to SECT and the advantage of the ability to separate iodine from ordinary brain tissue (3–5, 7, 10).

DECT scans were evaluated for diagnosis and grading of ICH using the Heidelberg classification (HI1, HI2, PH1, PH2, SAH, IVH, and SDH) (15). Since the Heidelberg system did not provide a subclassification of hemorrhages located remotely from

Abbreviations: AIS, acute ischemic stroke; cDECT, combined approach of dual-energy CT images; CNR, contrast-to-noise ratio; CS, contrast staining; CTA, CT angiography; CTP, CT perfusion; DECT, dual-energy CT; EVT, endovascular therapy; ICH, intracranial hemorrhage; iDECT, iodine-weighted dual-energy CT images; IVT, intravenous thrombolysis; keV, kiloelectron volt; kVp, kilovolt peak; PACS, picture archive communication system; pCT, plain monochromatic CT; RIS, radiological information system; SECT, single-energy CT; SICH, symptomatic intracranial hemorrhage; VNC, virtual non-contrast; wDECT, water-weighted dual-energy CT images.

[1] Available online at: https://www.acraccreditation.org/-/media/ACRAccreditation/Documents/CT/Requirements.pdf?la=en (accessed September 25, 2019).

infarcted tissue, the Safe Implementation of Treatments in Stroke Monitoring Study (SITS-MOST) classification was used for these: PHr1 and PHr2 (16, 17). The ICH grades were pooled into five categories: no ICH, HI1 and/or HI2, SAH and/or IVH, PH1 and/or PHr1, and PH2 and/or PHr2. For definitions, see **Table 1**.

Additionally, two definitions of symptomatic ICH (SICH) were used. SICH is defined by the European Cooperative Acute Stroke Study (ECASS) II as a ≥4-point NIHSS deterioration at 24 h or, if unavailable, the first available NIHSS score within 7 days or death within 7 days, combined with any ICH on 22- to 36-h DECT follow-up or, in cases where the latter was unavailable, on <22-h DECT follow-up (18). SICH is define by SITS-MOST as a ≥4-point NIHSS deterioration at 24 h or if unavailable, for example, due to general anesthesia, the first available NIHSS score within 7 days or death within 7 days, combined with PH2 or PHr2 or, in cases where the latter was unavailable, on <22-h DECT follow-up (16).

A neuroradiologist with 20 years of experience of acute stroke imaging (HA) and a neurologist with specific expertise in hemorrhagic complications of stroke therapies (MM) separately reviewed first the pCT images alone and in a second reading the pCT combined with wDECT and iDECT images.

Disagreement was resolved by consensus in a subsequent joint reading session involving both readers. Hyperattenuating findings of hemorrhagic appearance (e.g., in parenchyma, intraventricular, or elsewhere in the subarachnoid space) on pCT images were judged to be an ICH. Hyperattenuating findings with a suspicion of hemorrhage on wDECT images were judged to be hemorrhagic (3, 8).

Statistical Analysis

The consensus judgment of cDECT images was used as reference. Continuous values were reported as medians with interquartile ranges and categorical values as frequencies, excluding cases with missing data from the denominator. The significance of difference between proportions was calculated using exact binomial or McNemar's test as appropriate. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated with 95% confidence intervals (CI). Unweighted and categorized quadratic weighted Cohen's kappa with 95% CI was used to assess the level of inter-reader agreement. A difference between kappa values was considered significant if the 95% CIs did not overlap. SPSS version 25.0 (IBM, Armonk, NY) was used.

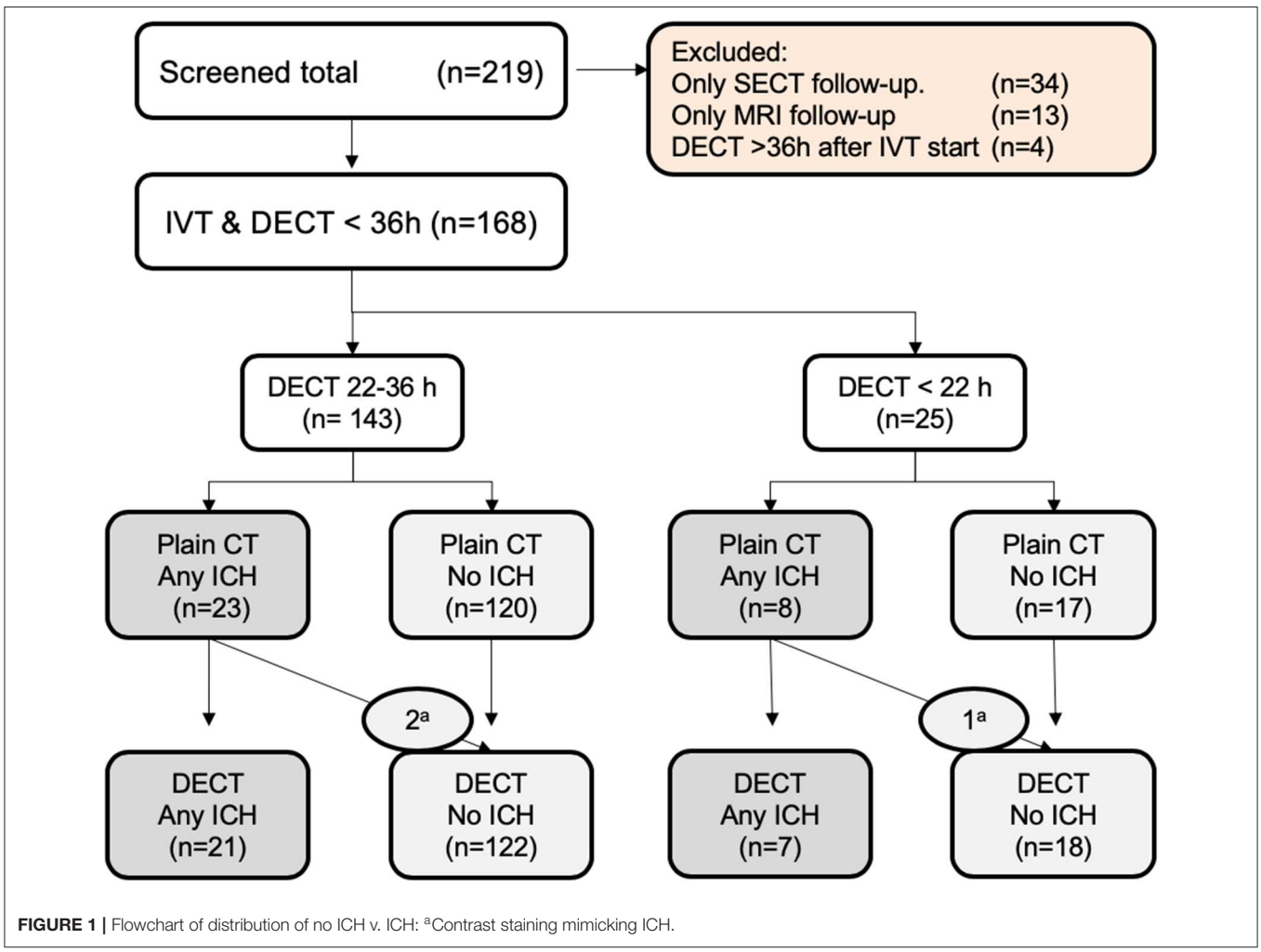


FIGURE 1 | Flowchart of distribution of no ICH v. ICH: [a]Contrast staining mimicking ICH.

TABLE 2 | Clinical characteristics.

Characteristic	Median (IQR) or %, $N = 168$
Age	71 (59–80)
NIHSS baseline[a]	7 (4-13)[a]
NIHSS 24 h after IVT[a]	3 (1-8)[b]
NIHSS 24 h worsened with 4p or more	11/166 (6.6%)
Gender (f)	78/168 (46%)
Atrial fibrillation	35/168 (21%)
Hypertension	94/168 (56%)
Diabetes mellitus	21/168 (13%)
Previous stroke	19/168 (11%)
Smoking	23/168 (14%)
Antiplatelet treatment	47/168 (28%)
Oral anticoagulants	6/168 (3.6%)

[a]*n = 167,* [b]*n = 167, one missing case each but not the same patient.*

Ethics

Ethics approval including waiver of patient consent was obtained from the Stockholm Regional Research Ethics Committee (approval 2018/1602-31/2) for retrospective review of imaging, electronic health records, and clinical patient data registered in the local Safe Implementation of Treatments in Stroke (SITS) Registry.

RESULTS

A total of 219 screened patients with AIS were treated with IVT, had IV contrast CT examination at baseline (CTA and/or CTP), and had no endovascular imaging or treatment. Of these, 34/219 (16%) cases were excluded due to having SECT-only follow-up, and 13/219 (6%) had only an MRI follow-up. Of the remaining 172 with DECT exams, 4/172 (2.3%) cases were excluded because >36 h has passed between IVT initiation and DECT. A combined recruitment and result flowchart is shown in **Figure 1**. Demographic and clinical data for the 168 included cases are shown in **Table 2**.

A total of 143 cases were scanned 22–36 h after IVT start and 25 within 22 h. The findings on pCT alone of the 168 cases were 14 HI1/HI2, 3 SAH, 10 PH1/PHr1, and 4 PH2/Phr2. With cDECT, the proportion of all patients diagnosed with ICH on pCT was reduced from 31/168 (18.5%) to 28/168 (16.7%), $p = 0.25$ (exact binomial test): one case each of HI1, HI2, and SAH was judged to be CS only (no ICH). No cases had an altered ICH classification. The frequency of SICH per ECASS II was 5/166 (3.0%), and that of SICH per SITS-MOST was 2/166 (1.2%), with no changes between pCT and cDECT. The patients with SICH had the following ICH findings (on both pCT and DECT): one HI2, two PH1/PHr1, and two PH2. The distribution of ICH and SICH findings is shown in **Figure 2**.

The ICH classification using pCT alone compared to cDECT had a 100% (88–100) sensitivity, 98% (94–100) specificity, 90% (75–97) PPV, 100% NPV, and 98% (95–100) accuracy for the dichotomous classification of any ICH vs. no ICH.

Inter-reader agreement of any ICH vs. no ICH using pCT had Cohen's kappa value of 0.86 (95% CI 0.76–0.96), and that using the combined DECT approach was 0.85 (0.75–0.95). Categorized ICH had unweighted Cohen's kappa values of 0.77 (0.66–0.88) and 0.73 (0.61–0.85) for pCT and DECT, respectively, and weighted kappa values of 0.92 (0.87–0.98) and 0.91 (0.85–0.97), respectively.

DISCUSSION

The main finding of our study is that pCT follow-up within 36 h may result in a false-positive diagnosis of ICH due to contrast medium extravasation in patients initially given IV contrast for CTA or CTP and treated with IVT as the sole recanalization therapy. Using DECT for radiological follow-up, we found that the proportion of patients diagnosed with any ICH was reduced by 3/168 (1.8%) patients, constituting 3/31 (9.7%) of cases with findings assessed as ICH on pCT. This phenomenon has been previously described in studies of patients undergoing endovascular thrombectomy (2–7). Meanwhile, we have been unable to find published literature on the same occurring in patients who have not undergone any endovascular procedure but have only been treated with IVT.

Our study showed that 2/23 (8.7%) of ICH findings in the routine 22- to 36-h follow-up period are caused by CS mimicking blood. In the early group scanned within 22 h, the proportion was 1/8 (13%). Although our number of cases is low, early follow-up may show CS more frequently, as reported in previous studies of DECT, which specifically covered early follow-up after EVT (3–6). For an illustrative case, please see **Figure 3**. The choice and timing of a follow-up exam with either SECT/DECT or MRI (with or without heme-sensitive sequences such as T2* and susceptibility-weighted imaging) may affect the results and potentially subsequent clinical decision making (19).

Our findings can be related to what is previously known on the topic from a routine 24-h DECT follow-up after start of EVT in the 18- to 36-h window, which showed that CS could be misinterpreted as ICH, shifting the rate of any ICH from a total of 37% on pCT to 27% with the use of DECT technology (7). In comparison, in our study, in the 22- to 36-h window, the shift went from any ICH in 16.1% (23/143) on pCT to 14.7% (21/143) using the cDECT concept. The lower stroke severity with our median NIHSS score 7 vs. 15 in the mentioned EVT study, as well as intravenous injection vs. intra-arterial injection, can be explanations for these differences.

The 2018 AHA/ASA guidelines for management of patients with AIS recommend obtaining a follow-up CT or MRI scan at 24 h after IVT before starting anticoagulants or antiplatelets (1). Meanwhile, the AHA/ASA gives no guidance on whether, and by how much, antithrombotic medication should be delayed, if follow-up imaging reveals any ICH. A recent multicenter observational study of patients with AIS and atrial fibrillation showed that patients with hemorrhagic transformation had a

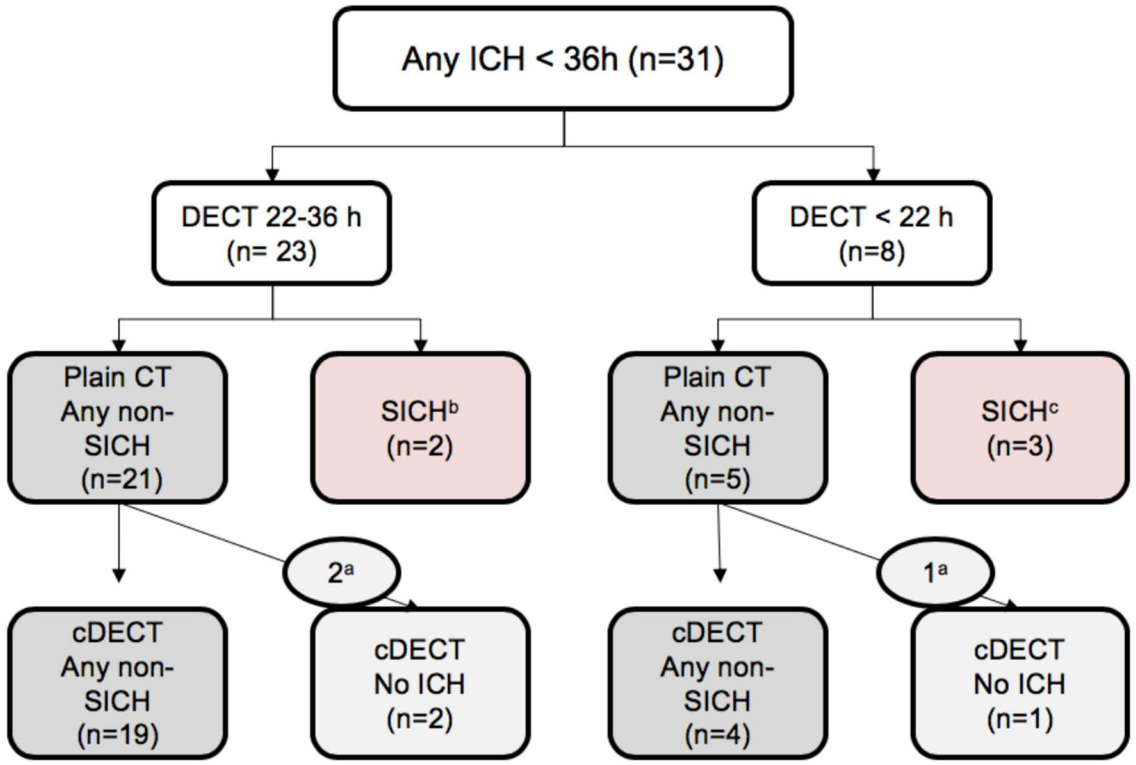


FIGURE 2 | Flowchart of the ICH findings. SICH, symptomatic intracranial hemorrhage. [a]Contrast staining mimicking ICH. [b]SICH fulfilling both ECASS II and SITS-MOST criteria, both cases with PH2. [c]SICH per the ECASS II definition, with one case each of HI2, PH1, and PHr1. All SICH showed the same image on pCT or cDECT. The differences between different subcategories are non-significant with $p > 0.25$.

mean time from stroke onset to oral anticoagulation initiation of 23 days, compared to 12 days in cases without hemorrhage (20). We have been unable to find any published studies of initiation timing of antiplatelets or anticoagulants specifically in patients with ICH following IVT or EVT.

Our findings suggest that DECT may be somewhat better than SECT for follow-up after IVT, in particular for differentiation between hemorrhage and CS. There are several techniques to increase diagnostic certainty. Previous studies of CT follow-up after EVT reported that diagnostic uncertainty regarding hyperattenuating findings could be resolved using a repeated exam within 1–3 days, when contrast medium, but not extravasated blood, could be expected to have cleared (19, 21). This is likely generalizable also to CT follow-up after IVT. However, apart from the increased use of resources and additional radiation dose of one more exam, this could imply postponement of antithrombotic secondary prevention until the second scan. DECT is a simple and rapid solution for differentiating blood from CS, avoiding the wait for a repeat exam and avoiding the limitations of MRI (safety, contraindications, and limited resource issues).

DECT does have some technical limitations. Motion artifacts pose difficulties in all follow-up imaging and in DECT specifically. The DECT technique requires that the image elements (voxels) are in the same exact position during the readout with two different energy spectra for the analysis of iodine removal. Motion artifacts may compromise the process of iodine removal (22). When relating DECT to MRI for the currently discussed purpose, it is important to note that in MRI, iodine can affect several sequences, for example, T1, T2, and diffusion-weighted images (DWI) (19, 23–25). Meanwhile, on hemosiderin-sensitive sequences, it is unlikely that iodine could mimic hemorrhage (26, 27).

There are other limitations to this study which warrant mention. It is a retrospective observational study of patients at a single comprehensive academic stroke center. The relatively low number of IVT cases without endovascular treatment at our center is explained by the fact that until October 2017, our hospital was the primary IVT service for only a small geographic area with a population of ∼120,000, while being the only neuroendovascular center for a region of over three million inhabitants. A further aspect requiring mention is that without verification with heme-sensitive MR sequences or a later follow-up and/or neuropathology, we cannot completely rule out any blood extravasation on wDECT images classified as no ICH. It is likely that MRI follow-up would increase the number of cases with hemorrhagic findings, especially types HI1 and HI2 (28–30). Meanwhile this issue pertains to both routine SECT and the more novel DECT technology. Furthermore, 34/219 (16% of all screened)

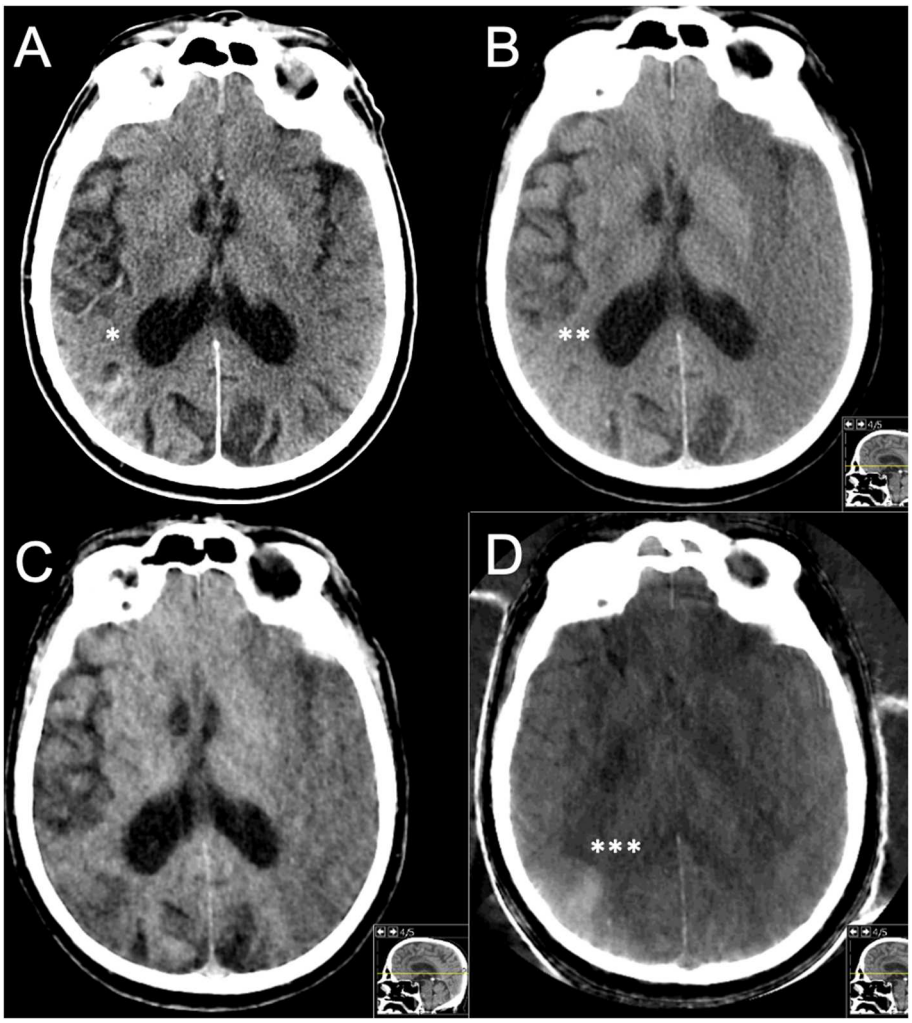


FIGURE 3 | Case example. Male, 72 years old, with hypertension. Right-sided hemiparesis with NIHSS 24 points at stroke onset and a left-sided M1 occlusion and a (missed) very subtle infarction with ASPECT 3. Routine drip-and-ship from a primary stroke center following IVT initiation to a thrombectomy center. IVT was stopped due to clinical worsening before arrival. The repeated stroke imaging there showed extensive manifest infarction (ASPECT 3) on the left side but also a hemorrhage within an infarct on the right side. Rejected for thrombectomy. **(A)** SECT 1 h 15 min after IVT initiation with signs of hemorrhage*. **(B)** pCT, **(C)** wDECT, and **(D)** iDECT, all at 10 h, on pCT alone judged to be a HI2 bleeding**; however, wDECT and iDECT showed iodine*** within an infarct, originating from an IV contrast injection for a CTA done immediately prior to initial IVT administration. Clinical workup showed a previously undiagnosed atrial fibrillation, with cardioembolism deemed to be the likely cause of stroke.

patients were excluded as they underwent only SECT follow-up. The decision to use SECT in these cases was not systematic nor patient related but was explained in the early days after DECT acquisition by an incomplete awareness of the availability of the technique among staff and, in singular cases, non-availability of the DECT machine due to caseload or technical maintenance.

CONCLUSIONS

Compared to pCT, DECT within 36 h after IVT for AIS, changes the radiological diagnosis of posttreatment ICH to "CS only" in a small proportion of patients. Studies on whether the altered radiological reports have an impact on patient management, for example, initiation timing of antithrombotic secondary prevention, are warranted.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Stockholm Regional Research Ethics Committee.

Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HA: idea conception, study planning, data acquisition and management, statistical analysis, manuscript drafting, and manuscript revision for intellectual content. NA: data acquisition, data management, statistical analysis, and manuscript revision for intellectual content. SH: idea conception, study planning, supervision, funding, and manuscript revision for intellectual content. MM: study planning, data acquisition, supervision, and manuscript revision for intellectual content.

FUNDING

HA was supported by Health, Medicine and Technology (HMT), and VINNOVA foundations, and the Stockholm County Council. SH was supported by MedTechLabs, VINNOVA, HMT, and the Söderberg Foundations. MM was supported by the Stockholm County Council (clinical postdoctoral appointment and ALF grant).

ACKNOWLEDGMENTS

We acknowledge Tiago Moreira, MD, Ph.D. at the Dept. of Neurology, Karolinska University Hospital, Stockholm, Sweden, the local coordinator of the SITS Registry, and Mats Danielsson, professor at the Royal Institute of Technology, Stockholm, Sweden, who contributed to funding.

REFERENCES

1. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. 2018 guidelines for the early management of patients with acute ischemic *stroke*: a guideline for healthcare professionals from the american heart association/american stroke association. *Stroke.* (2018) 49:e46–e110. doi: 10.1161/STR.0000000000000158
2. Phan CM, Yoo AJ, Hirsch JA, Nogueira RG, Gupta R. Differentiation of hemorrhage from iodinated contrast in different intracranial compartments using dual-energy head CT. *AJNR Am J Neuroradiol.* (2012) 33:1088–94. doi: 10.3174/ajnr.A2909
3. Morhard D, Ertl L, Gerdsmeier-Petz W, Ertl-Wagner B, Schulte-Altedorneburg G. Dual-energy CT immediately after endovascular stroke intervention: prognostic implications. *Cardiovasc Intervent Radiol.* (2014) 37:1171–8. doi: 10.1007/s00270-013-0804-y
4. Tijssen MP, Hofman PA, Stadler AA, van Zwam W, de Graaf R, van Oostenbrugge RJ, et al. The role of dual energy CT in differentiating between brain haemorrhage and contrast medium after mechanical revascularisation in acute ischaemic stroke. *Eur Radiol.* (2014) 24:834–40. doi: 10.1007/s00330-013-3073-x
5. Renu A, Amaro S, Laredo C, Roman LS, Llull L, Lopez A, et al. Relevance of blood-brain barrier disruption after endovascular treatment of ischemic stroke: dual-energy computed tomographic study. *Stroke.* (2015) 46:673–9. doi: 10.1161/STROKEAHA.114.008147
6. Bonatti M, Lombardo F, Zamboni GA, Vittadello F, Curro Dossi R, Bonetti B, et al. iodine extravasation quantification on dual-energy ct of the brain performed after mechanical thrombectomy for acute ischemic stroke can predict hemorrhagic complications. *AJNR Am J Neuroradiol.* (2018) 39:441–7. doi: 10.3174/ajnr.A5513
7. Almqvist H, Holmin S, Mazya MV. Dual energy CT after stroke thrombectomy alters assessment of hemorrhagic complications. *Neurology.* 93:e1068–75. (2019) doi: 10.1212/WNL.0000000000008093
8. Johnson TRC, Fink C, Schönberg SO, Reiser MF. (eds.). *Dual Energy CT in Clinical Practice.* S New York, NY: Springer Heidelberg Dordrecht London (2011). doi: 10.1007/978-3-642-01740-7
9. Watanabe Y, Nakazawa T, Higashi M, Itoh T, Naito H. Assessment of calcified carotid plaque volume: comparison of contrast-enhanced dual-energy CT angiography and native single-energy CT. *AJR Am J Roentgenol.* (2011) 196:W796–9. doi: 10.2214/AJR.10.4938
10. Pomerantz SR, Kamalian S, Zhang D, Gupta R, Rapalino O, Sahani DV, et al. Virtual monochromatic reconstruction of dual-energy unenhanced head CT at 65-75 keV maximizes image quality compared with conventional polychromatic CT. *Radiology.* (2013) 266:318–25. doi: 10.1148/radiol.12111604
11. Postma AA, Das M, Stadler AA, Wildberger JE. Dual-energy CT: what the neuroradiologist should know. *Curr Radiol Rep.* (2015) 3:16. doi: 10.1007/s40134-015-0097-9
12. Saba L, Argioas GM, Lucatelli P, Lavra F, Suri JS, Wintermark M. Variation of degree of stenosis quantification using different energy level with dual energy CT scanner. *Neuroradiology.* (2019) 61:285–91. doi: 10.1007/s00234-018-2142-x
13. Strbian D, Sairanen T, Meretoja A, Pitkaniemi J, Putaala J, Salonen O, et al. Patient outcomes from symptomatic intracerebral hemorrhage after stroke thrombolysis. *Neurology.* (2011) 77:341–8. doi: 10.1212/WNL.0b013e3182267b8c
14. Keselman B, Cooray C, Vanhooren G, Bassi P, Consoli D, Nichelli P, et al. Intravenous thrombolysis in stroke mimics: results from the SITS International Stroke Thrombolysis Register. *Eur J Neurol.* (2019) 26:1091–7. doi: 10.1111/ene.13944
15. von Kummer R, Broderick JP, Campbell BC, Demchuk A, Goyal M, Hill MD, et al. The heidelberg bleeding classification: classification of bleeding events after ischemic *stroke* and reperfusion therapy. *Stroke.* (2015) 46:2981–6. doi: 10.1161/STROKEAHA.115.010049
16. Wahlgren N, Ahmed N, Davalos A, Ford GA, Grond M, Hacke W, et al. Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST): an observational study. *Lancet.* (2007) 369:275–82. doi: 10.1016/S0140-6736(07)60149-4
17. Mazya MV, Ahmed N, Ford GA, Hobohm C, Mikulik R, Nunes AP, et al. Remote or extraischemic intracerebral hemorrhage–an uncommon complication of stroke thrombolysis: results from the safe implementation of treatments in stroke-international stroke thrombolysis register. *Stroke.* (2014) 45:1657–63. doi: 10.1161/STROKEAHA.114.004923
18. Hacke W, Kaste M, Fieschi C, von Kummer R, Davalos A, Meier D, et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). Second European-Australasian Acute Stroke Study Investigators. *Lancet.* (1998) 352:1245–51. doi: 10.1016/S0140-6736(98)08020-9
19. Yedavalli V, Sammet S. Contrast extravasation vs. hemorrhage after thrombectomy in patients with acute stroke. *J Neuroimaging.* (2017) 27:570–6. doi: 10.1111/jon.12446
20. Paciaroni M, Bandini F, Agnelli G, Tsivgoulis G, Yaghi S, Furie KL, et al. Hemorrhagic transformation in patients with acute ischemic stroke and atrial fibrillation: time to initiation of oral anticoagulant therapy and outcomes. *J Am Heart Assoc.* (2018) 7:e010133. doi: 10.1161/JAHA.118.010133
21. Payabvash S, Qureshi MH, Khan SM, Khan M, Majidi S, Pawar S, et al. Differentiating intraparenchymal hemorrhage from contrast

extravasation on post-procedural noncontrast CT scan in acute ischemic stroke patients undergoing endovascular treatment. *Neuroradiology.* (2014) 56:737–44. doi: 10.1007/s00234-014-1381-8
22. Dinkel J, Khalilzadeh O, Phan CM, Goenka AH, Yoo AJ, Hirsch JA, et al. Technical limitations of dual-energy CT in neuroradiology: 30-month institutional experience and review of literature. *J Neurointerv Surg.* (2015) 7:596–602. doi: 10.1136/neurintsurg-2014-011241
23. Hergan K, Doringer W, Langle M, Oser W. Effects of iodinated contrast agents in MR imaging. *Eur J Radiol.* (1995) 21:11–7. doi: 10.1016/0720-048X(95)00677-I
24. Ganguly A, Gold GE, Butts Pauly K, Mayer D, Moseley MM, Pelc NJ, et al. Quantitative evaluation of the relaxivity effects of iodine on GD-DTPA enhanced MR arthrography. *J Magn Reson Imaging.* (2007) 25:1219–25. doi: 10.1002/jmri.20934
25. Ogura A, Hayakawa K, Miyati T, Maeda F. Effects of iodinated contrast agent on diffusion weighted magnetic resonance imaging. *Acad Radiol.* (2009) 16:1196–200. doi: 10.1016/j.acra.2009.03.021
26. Nikoubashman O, Jablawi F, Dekeyzer S, Oros-Peusquens AM, Abbas Z, Lindemeyer J, et al. MRI appearance of intracerebral iodinated contrast agents: is it possible to distinguish extravasated contrast agent from hemorrhage? *AJNR Am J Neuroradiol.* (2016) 37:1418–21. doi: 10.3174/ajnr.A4755
27. You SH, Kim B, Kim BK, Suh SI. MR imaging for differentiating contrast staining from hemorrhagic transformation after endovascular thrombectomy in acute ischemic stroke: phantom and patient study. *AJNR Am J Neuroradiol.* (2018) 39:2313–9. doi: 10.3174/ajnr.A5848
28. Arnould MC, Grandin CB, Peeters A, Cosnard G, Duprez TP. Comparison of CT and three MR sequences for detecting and categorizing early (48 hours) hemorrhagic transformation in hyperacute ischemic stroke. *AJNR Am J Neuroradiol.* (2004) 25:939–44.
29. Fiebach JB, Bohner G. T2*-weighted imaging enables excellent interobserver concordance but should not be considered as sole gold standard imaging for hemorrhagic transformation classification after thrombolysis. *Cerebrovasc Dis.* (2010) 29:605–6. doi: 10.1159/000312868
30. Alvarez-Sabin J, Maisterra O, Santamarina E, Kase CS. Factors influencing haemorrhagic transformation in ischaemic stroke. *Lancet Neurol.* (2013) 12:689–705. doi: 10.1016/S1474-4422(13)70055-3

Conflict of Interest: SH was a medical advisor for Prismatic Sensors AB.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

MINI REVIEW
published: 17 July 2020
doi: 10.3389/fneur.2020.00728

Hemodynamics and Hemorrhagic Transformation After Endovascular Therapy for Ischemic Stroke

Andrew Silverman†, *Sreeja Kodali*†, *Kevin N. Sheth and Nils H. Petersen**

Department of Neurology, Yale School of Medicine, New Haven, CT, United States

Hemorrhagic transformation remains a potentially catastrophic complication of reperfusion therapies for the treatment of large-vessel occlusion ischemic stroke. Observational studies have found an increased risk of hemorrhagic transformation in patients with elevated blood pressure as well as a high degree of blood pressure variability, suggesting a link between hemodynamics and hemorrhagic transformation. Current society-endorsed guidelines recommend maintaining blood pressure below a fixed threshold of 180/105 mmHg regardless of thrombolytic or endovascular intervention. However, given the high recanalization rates with mechanical thrombectomy, it is unclear if the same hemodynamic goals from the pre-thrombectomy era apply. Also, individual patient factors such as the degree of reperfusion, infarct size, and collateral status likely need to be considered. In this review, we will discuss current evidence linking hemodynamics to hemorrhagic transformation after mechanical thrombectomy. In addition, we will review the clinical relevance of cerebral autoregulation in stroke, highlighting recent studies that have harnessed autoregulatory physiology to define and trend individualized limits of autoregulation. This review will go on to emphasize the translatability of this approach to stroke management. Finally, we will discuss novel statistical approaches like trajectory analysis to post-thrombectomy hemodynamics.

Keywords: thrombectomy, blood pressure, stroke, autoregulation dysfunction, neurocritical care management

OPEN ACCESS

Edited by:
Nishant K. Mishra,
Icahn School of Medicine at Mount Sinai, United States

Reviewed by:
Georgios Tsivgoulis,
National and Kapodistrian University of Athens, Greece
Ken Uchino,
Cleveland Clinic, United States

***Correspondence:**
Nils H. Petersen
nils.petersen@yale.edu

†*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *25 April 2020*
Accepted: *15 June 2020*
Published: *17 July 2020*

Citation:
Silverman A, Kodali S, Sheth KN and Petersen NH (2020) Hemodynamics and Hemorrhagic Transformation After Endovascular Therapy for Ischemic Stroke. Front. Neurol. 11:728. doi: 10.3389/fneur.2020.00728

INTRODUCTION

Hemorrhagic transformation (HT) is a feared complication of acute ischemic stroke and is independently associated with neurological deterioration and worse functional outcomes (1–4). Accurate prediction and triage of patients at risk for HT would be of tremendous value, and yet the underlying mechanisms and potential biomarkers of HT remain elusive. While animal and human studies have invoked pathomechanisms involving neuroinflammation, neurovascular unit impairment, blood brain barrier disruption, and vascular remodeling, this clinically oriented review will focus on cerebral autoregulation and optimal blood pressure (BP) management following endovascular thrombectomy (EVT) for large-vessel occlusion (LVO) acute ischemic stroke (5, 6).

Mechanical thrombectomy preceded by intravenous thrombolytics has become standard of care treatment in stroke patients with acute ischemia secondary to LVO (7). This shift occurred after 2015, a year that witnessed five randomized trials (MR CLEAN, ESCAPE, SWIFT PRIME, REVASCAT, and EXTEND IA), showing the efficacy of EVT over standard medical care (8–12). A subsequent meta-analysis (HERMES) included a total of 1,287 patients and demonstrated a significant reduction in 90-days disability compared to controls, though 90-days mortality did not

differ between the two study populations (7). Two additional trials (DAWN, DEFUSE-3) were published in 2018. They provided evidence that thrombectomy can be offered up to 24 h after symptom onset in selected patients with a mismatch between infarct size and clinical deficit (13, 14).

In all seven of these major trials, the rates of symptomatic HT were key safety outcomes, reported as serious adverse events following treatment. In the first five studies that looked at EVT in the early window (up to 12 h), symptomatic HT in the treatment group ranged from 0 to 7.7%. Of note, in these five studies, most patients (>80%) in both intervention and control groups received intravenous thrombolysis in addition to EVT. In both extended time window trials, symptomatic hemorrhagic complications occurred in 6–7% of patients in the treatment group. The DEFUSE 3 trials' rates of symptomatic intracranial bleeding did not differ between the EVT and control group (7 vs. 4%, respectively; $P = 0.75$) (13). Five patients with symptomatic HT in the EVT group died, compared with two in the control group. In the DAWN trial, the rates of symptomatic intracranial bleeding did not significantly differ between the EVT and control groups (6 vs. 3%, respectively; $P = 0.50$) (14). The HERMES pooled analysis of patient-level data concluded that the rates of symptomatic intracranial hemorrhage are not higher in patients receiving EVT than in patients receiving medical therapy alone (4.4 vs. 4.3%, respectively; risk difference 0.1%), suggesting that reperfusion alone may not be the primary driver of symptomatic HT (7). Observational studies have shown an increased risk of HT with sustained post-procedural hypertension and higher BP variability (15). Interestingly, mean systolic BP (SBP) was lower among patients with successful reperfusion, indicating a possible difference in the threshold for reperfusion injury depending on recanalization status. Furthermore, radiographic hemorrhagic infarction (HI) is common following EVT and has been associated with poor outcome, thereby questioning the purported benign nature of HI (4). While these studies suggest a possible role of hemodynamics in the development of HT, they do not prove a causal relationship. Identification of patients at risk for HT (both radiographic and symptomatic) may allow for early preventative strategies like BP control post-EVT.

BLOOD PRESSURE MANAGEMENT FOLLOWING THROMBECTOMY

Current American Heart Association guidelines recommend maintaining BP < 180/105 mmHg for all patients treated with intravenous thrombolysis or EVT to promote perfusion to ischemic territories while mitigating potential risks of intracranial hemorrhage. Still, guidelines acknowledge a lack of prospective trials to substantiate this position, and the language of these consensus statements reflects this uncertain area of care: "In patients who undergo mechanical thrombectomy, it is reasonable to maintain the BP ≤ 180/105 mmHg during the first 24 h after the procedure. In patients who undergo mechanical thrombectomy with successful reperfusion, it might be reasonable to maintain BP at a level <180/105 mmHg." (16). Randomized controlled trials are unavailable, and the evidence in support of these recommendations is moderate to weak (class of recommendation IIa&IIb, level of evidence B-NR). Furthermore, trial protocols regarding post-procedural BP control in the studies that contributed to guideline development were vague, and BP management likely varied across sites. The vast majority of patients enrolled in under 6-h randomized trials received intravenous thrombolytic therapy, and the trial protocols stipulated management according to local guidelines with pressures generally under 180/105 mmHg for the first 24 h after the procedure. Only two trial protocols provided additional recommendations. The ESCAPE protocol states that systolic BP ≥ 150 mmHg is probably useful in promoting and sustaining adequate collateral flow while the artery remains occluded (9). The protocol further states that controlling pressure once reperfusion has been achieved, aiming for normal pressures, is a reasonable route for individual patients. Second, the DAWN protocol endorses systolic pressures under 140 mmHg in the first 24 h for subjects who achieve successful reperfusion (17). As a result of the limited data, current management strategies are based on guidelines that favor a one-size-fits-all approach that neglects the heterogeneity of stroke and differences in individual patient characteristics. The care of patients with stroke is, therefore, poorly individualized.

Despite the efficacy of EVT, many patients with LVO stroke still suffer morbidity, mortality, and functional dependence in longitudinal studies (7, 18). Observational studies, including a recent meta-analysis, have shown higher rates of HT, worse outcomes, and increased mortality in patients with higher peak SBP values or hemodynamic variability in the first 24 hours after EVT (15, 19–21). However, it remains unclear if post-procedural hypertension is simply an epiphenomenon, or if it reflects a valid therapeutic target. In a recent multicenter study of 1,245 patients who achieved successful reperfusion after EVT, Anadani et al. divided patients into three groups based on SBP goal in the first 24 h post-EVT. The investigators found that higher SBP targets were associated with higher odds of symptomatic intracranial hemorrhage, mortality, and hemicraniectomy (22). The results agree with earlier findings by Goyal et al., who published a single-center experience after the implementation of more aggressive BP control following successful EVT. Compared to patients treated with permissive hypertension (<180 mmHg), those treated with moderate (<160 mmHg) and intensive (<140 mmHg) BP control showed improved functional outcome and lower mortality at three months (19). Although we currently lack rigorous clinical evidence, these studies, as well as compelling conceptual reasons, suggest that BP optimization may represents a post-EVT neuroprotective strategy.

Indeed, while a higher BP may be beneficial in patients with incomplete reperfusion by promoting perfusion to ischemic territories and the penumbra, it could lead to relative hyperperfusion. Such hyperperfusion could cause cerebral edema and hemorrhage in those patients with complete reperfusion. This phenomenon is well-described in chronic ischemia after carotid revascularization (via endarterectomy or stenting) but may also occur in acute stroke (23–25). For example, Hashimoto *et al.* reported cerebral hyperperfusion syndrome in a 77-year-old patient with acute internal carotid and middle cerebral

artery occlusions. Due to the patient's neurologic deterioration, the authors suggest that it is essential to routinely monitor regional oxygen saturation with near-infrared spectroscopy, evaluate cerebral blood flow, and maintain antihypertensive therapy to prevent hyperperfusion after revascularization (25). It is also possible that this complication is more prevalent than the handful of published case reports might suggest. Following recanalization, lower BP targets may be warranted to decrease reperfusion injury and promote penumbral recovery. Nevertheless, optimal, personalized BP targets remain undefined. To complicate the matter, individual patient factors such as degree of reperfusion, infarct size, concomitant carotid revascularization, antithrombotic therapy, and hemodynamic status likely need to be considered. Because of these factors, there is a high degree of practice variation in BP management following EVT (26).

Recent studies have shown that real-time autoregulation monitoring can be used to identify a dynamic BP range in individual patients at which autoregulation is optimally functioning (27–31). Such an autoregulation-derived, personalized BP range may provide a favorable physiologic landscape for the acutely injured brain. Accordingly, the following section will review the use of cerebral autoregulation monitoring in patients with acute ischemic stroke, highlighting the hypothesis that exceeding a personalized upper limit of autoregulation predisposes patients to reperfusion injury and HT (27, 29)

CEREBRAL AUTOREGULATION AND BLOOD PRESSURE PERSONALIZATION

Cerebral autoregulation describes the intrinsic capacity of the cerebral vasculature to preserve stable blood flow in the face of systemic BP changes (or, more precisely, cerebral perfusion pressure changes) (32). Autoregulatory capacity in acute stroke is critical for the maintenance of stable blood flow to the ischemic penumbra and avoidance of excessive hyperperfusion (33, 34). There is fairly widespread agreement that stroke is associated with impaired autoregulation, even in cases of minor stroke (33–35). This impairment may exist ipsilateral to the stroke site in a focal fashion, or globally throughout both hemispheres (34). Interestingly, Immink et al. reported dynamic autoregulatory disturbance ipsilateral to middle cerebral artery (MCA) territory strokes but bilaterally in lacunar ischemic strokes (36). These results were bolstered in more recent analyses by Guo et al., showing that dynamic autoregulatory markers were impaired ipsilaterally in a stroke of large artery atherosclerosis but bilaterally in stroke of small artery occlusion (37). Petersen et al. then examined autoregulation on a more longitudinal basis, reporting dynamic autoregulatory failure up to 1 week following acute LVO strokes in the MCA. More specifically, this investigation showed that the autoregulatory parameter phase was lower in the affected cerebral hemisphere compared to the contralateral hemisphere, indicating an impaired ability to buffer against BP fluctuations (38).

Furthermore, in stroke patients with impaired autoregulation, recovery tends to be delayed for up to 3 months, underlining the clinical relevance of autoregulation in stroke research (35, 39). That said, only a handful of studies have looked at functional outcome prognostication with respect to autoregulation physiology in stroke. For example, Reinhard et al. enrolled 45 patients within 48 h of LVO MCA strokes and showed that ipsilateral lower phase shifts were related to worse functional outcomes (40). In light of the prolonged enrollment timeframe, the authors conceded that autoregulatory impairment might reflect initial stroke severity, rather than functioning as an independent contributing factor to outcome. To help resolve this question, Castro et al. measured autoregulation in 30 patients with LVO MCA ischemic stroke within 6 h of symptom onset (39). This report demonstrated that autoregulatory impairment operated as a statistically independent predictor of functional autonomy at the 90-days endpoint (odds ratio 14.0, 95% confidence interval 1.7–74.0; $P = 0.013$). In yet another study, these authors reported that final infarct volume is significantly lower in patients with preserved autoregulation in a similar acute window post-stroke (41). In a review summarizing these findings, Castro *et al.* conclude that early autoregulatory measures wield considerable import in the guidance of acute stroke management, secondary injury prevention, and outcome improvement (35).

Autoregulatory physiology has thus been invoked as a biological avenue with possible deterrent and restorative benefits concerning HT and associated neurologic worsening. In an invasive neuromonitoring study, Dohmen et al. enrolled 15 patients with MCA ischemic strokes and calculated the cerebral perfusion pressure-oxygen reactivity index (COR) (42). They found COR indices were higher (worse) in the eight patients with malignant courses (i.e., massive brain edema) compared to the seven patients with relatively benign courses. The study concludes that dysautoregulation appears to play an essential role in the development of cerebral edema. In a study mentioned above, Castro et al. calculated cerebrovascular resistance, coherence, gain, and phase in 46 patients within 24 h of MCA ischemic stroke (41). At admission, phase was lower (indicative of worse autoregulation) in patients with HT. Also, progression to edema was related to lower cerebrovascular resistance values and increased blood flow velocities at the initial presentation. These lower resistances, the authors submit, reflect paradoxical cerebral vasodilation, as cerebrovascular resistance is equal to the quotient between mean arterial pressure and mean flow velocity (CVR = MAP/MFV). Thus, they argue that breakthrough hyperperfusion and microvascular injury may underlie the development of malignant edema and HT.

Cumulatively, there is substantial evidence for impaired autoregulation after stroke. It follows that an autoregulation-guided approach can be applied to the cerebrovascular hemodynamics of stroke pathophysiology. The Cambridge group has been refining this work over several decades, particularly in patients with traumatic brain injury (43). With this hypothesis in mind, a recent study harnessed autoregulation monitoring to identify and track personalized BP limits in 90 patients undergoing EVT for LVO ischemic stroke (27, 29).

This cohort revealed that continuous estimations of optimal BP and autoregulatory limits are feasible in post-EVT care. The study further demonstrated that exceeding individualized autoregulatory thresholds was associated with HT and worse outcome (**Figure 1**). In more detail, every 10% increase in time spent above the upper limit of autoregulation was associated with a doubling in the odds of shifting toward a more unfavorable 3-months outcome. The study also observed a progressive increase in percent time above this upper limit with worsening grades of HT (11.4% of the time for no HT, 13.5% for hemorrhagic infarctions 1 and 2, and 20.9% for parenchymal hematoma 1 and 2; $P = 0.03$). Also, patients who developed symptomatic intracranial hemorrhage spent more time above the upper autoregulatory limit when compared to patients without this complication (11.9 vs. 24.6%; $P = 0.1$) (29).

This relationship between deviation from the upper autoregulatory limit and outcome is supported by the construct that above the upper autoregulatory limit, the cerebral vasculature functions as a pressure-passive system, in which increases in cerebral blood flow are not counteracted by vasoconstriction (44). This system permits periods of hyperperfusion in the setting of an elevated systemic BP (33). Furthermore, higher cerebral blood flow after reperfusion therapy (measured via arterial spin labeling magnetic resonance imaging) has been shown to increase the risk of HT (45). Several retrospective studies reported an association between sustained hypertension after EVT and HT (15, 46), although others did not unearth this relationship (19, 47). Divergence of autoregulatory capacity among different patients may be at least one explanation for these discordant results.

An additional aim of this post-EVT monitoring study was to compare personalized, autoregulation-guided BP targets with two commonly used clinical approaches: 1) maintaining BP below a fixed, pre-determined value as recommended by current guidelines and 2) stratifying BP thresholds based on reperfusion status (29). Ultimately, there was no association between time spent above any of the fixed SBP thresholds and HT or functional outcome, even after stratifying by reperfusion status. This supplementary analysis was particularly important because optimal BP ranges after EVT are likely influenced by numerous factors; stratifying by reperfusion status alone might not be sufficient. For instance, chronic hypertension and flow-limiting extracranial carotid disease may shift a person's autoregulatory curve toward higher pressures. Aggressively lowering BP after successful EVT in this scenario may result in cerebral hypoperfusion and infarct expansion (48, 49). In comparison, optimal BP ranges could shift toward lower pressures in patients without hypertension or pre-existing large-vessel disease. Overall, then, these results argue for future research in prospective, multicenter, and randomized

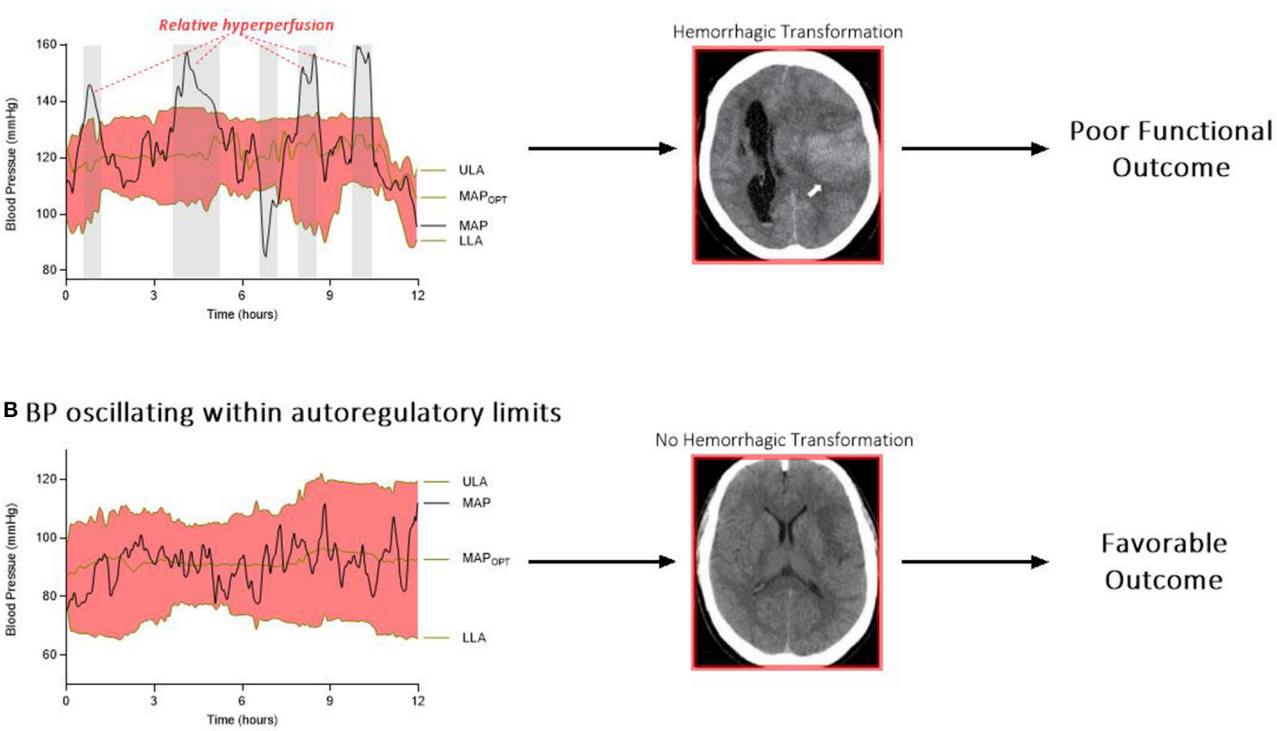


FIGURE 1 | (A) Relative hyperperfusion above the upper limit of autoregulation may predispose patients to hemorrhagic transformation and worse outcomes. **(B)** In contrast, patients who oscillate within their personalized limits of autoregulation may be protected from secondary brain injury after stroke. ULA, upper limit of autoregulation; MAP_{OPT}, optimum mean arterial pressure; MAP, mean arterial pressure; LLA, lower limit of autoregulation.

trials. Finally, another interesting avenue of investigation revolves around the question of restoring dysautoregulation by dynamically adjusting BP. In other words, by targeting an optimum BP within autoregulatory limits, intensivists may be able to shift patients to a more favorable position on the autoregulatory curve, but this hypothesis remains untested.

BLOOD PRESSURE TRAJECTORY ANALYSIS AFTER STROKE

In addition to autoregulation monitoring, researchers in recent years have applied innovative statistical tools to study BP data in the acute window post-stroke. For instance, in 2018, Kim et al. used trajectory modeling to examine longitudinal BP data from a prospective multicenter registry of 8,376 stroke patients (50). Their characterization of post-stroke BP courses has been hitherto a missing element in the field. In their work, the authors applied the TRAJ procedure from SAS software to separate heterogeneous, longitudinal BP data into trajectory groups with similar patterns. This analysis identified the optimal number and shape of trajectories; it then assigned patients to estimated trajectory groups. Five distinct BP trajectories were generated over the acute period following stroke. The risk of recurrent stroke, myocardial infarction, or death was greater in patients who fell into the acutely elevated or persistently high BP trajectory groups.

In 2019, Li et al. published a *post-hoc* BP trajectory analysis of a large BP lowering trial in 4,036 patients with stroke (51). Using similar statistical methods, the authors generated five BP trajectories over seven days following stroke. Patients who sustained high BP over time had significantly higher mortality rates at 3-months and 2-years follow-up. Patients in the experimental arm of the original trial who received BP lowering interventions were more likely found in lower BP trajectories than patients in the control arm, demonstrating that pharmacological intervention can affect a patient's BP trajectory and potentially their outcome. These two studies, then, reaffirm the association between elevated post-stroke BP and poor outcome.

In recent work by Petersen et al., trajectory analysis was conducted on a prospective, multicenter, international cohort of 1,060 patients who underwent EVT for LVO ischemic stroke (52). Five unique post-EVT systolic BP trajectories were generated over 72 h (**Figure 2**). Compared to patients in the moderate trajectory (2), patients in the acutely elevated (4) and persistently high (5) trajectories had a significantly increased risk of unfavorable functional outcome after adjustment for several covariates (odds ratio 1.6 and 2.5, respectively). While the elevated BP in high trajectory groups may reflect an acute, post-stroke hypertensive response, it may also reflect underlying, untreated hypertension. Patients in higher trajectories had higher rates of hypertension and received more antihypertensive medication pre-admission. Additionally, elevated BP may reflect reperfusion status, as non-recanalized patients were more likely to be in higher trajectory groups. Overall, patients who maintained lower BP

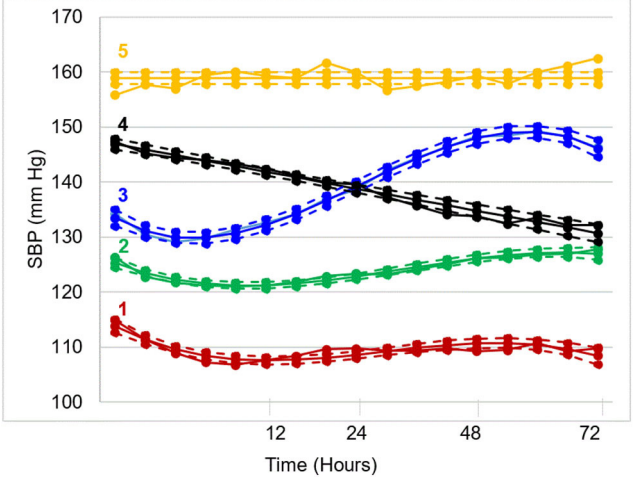


FIGURE 2 | Systolic blood pressure trajectories over 72 h post-EVT. Five distinct trajectories emerged: (1) low (17%), (2) moderate (38%), (3) moderate-to-high (21%), (4) high-to-moderate (17%), and (5) high (7%).

trajectories had better 90-days functional outcomes, but this trend was not observed for symptomatic HT. Patients in the acutely elevated (4) trajectory had the highest rate of symptomatic HT, even more than patients in the persistently high trajectory (5). In contrast, patients in the moderate-to-high (3) trajectory (who had the highest rates of in-hospital antihypertensive treatment) had markedly lower rates of symptomatic HT than any other trajectory group. These findings raise questions about alternative mechanisms, such as cerebral edema, through which elevated BP may impact functional outcome.

It is unknown whether lowering a patient's trajectory from persistently high (5) to acutely elevated (4) will improve outcomes, as this retrospective analysis of a prospective cohort was purely observational. However, these findings may help identify ideal candidates for future trials. This work, along with the previously described studies on autoregulation-based BP goals, are hypothesis-generating and aim to identify a subset of patients who may benefit most from post-stroke BP intervention. Additionally, this body of work demonstrates the impact of emerging analytical techniques on understanding post-stroke hemodynamics, prevention of secondary injuries like HT, and more personalized BP management.

CONCLUSION

In the era of endovascular thrombectomy, hemorrhagic transformation remains a potentially devastating complication of acute ischemic stroke. Intracranial bleeds after thrombectomy likely occur as a result of a multifactorial process. Still, this clinical review of BP optimization shows that hemodynamic management represents a titratable, neuroprotective avenue in the care of critically ill patients. Exceeding the upper limit of autoregulation may predispose patients to reperfusion injury;

maintaining BP within autoregulatory limits may achieve favorable outcomes while avoiding hemorrhagic complications. Additionally, trajectory analysis has the potential to provide more tailored hemodynamic management in the post-thrombectomy intensive care setting.

AUTHOR CONTRIBUTIONS

AS and SK contributed in equal parts to the manuscript's concept and design. KS and NP provided critical feedback and revisions for intellectual content.

REFERENCES

1. Khatri P, Wechsler LR, Broderick JP. Intracranial hemorrhage associated with revascularization therapies. *Stroke.* (2007) 38:431–40. doi: 10.1161/01.STR.0000254524.23708.c9
2. Fiorelli M, Bastianello S, von Kummer R, del Zoppo GJ, Larrue V, Lesaffre E, et al. Hemorrhagic transformation within 36 hours of a cerebral infarct: relationships with early clinical deterioration and 3-month outcome in the European Cooperative Acute Stroke Study I (ECASS I) cohort. *Stroke.* (1999) 30:2280–4. doi: 10.1161/01.STR.30.11.2280
3. van Kranendonk KR, Treurniet KM, Boers AMM, Berkhemer OA, van den Berg LA, Chalos V, et al. Hemorrhagic transformation is associated with poor functional outcome in patients with acute ischemic stroke due to a large vessel occlusion. *J Neurointerv Surg.* (2019) 11:464–8. doi: 10.1136/neurintsurg-2018-014141
4. Nogueira RG, Gupta R, Jovin TG, Levy EI, Liebeskind DS, Zaidat OO, et al. Predictors and clinical relevance of hemorrhagic transformation after endovascular therapy for anterior circulation large vessel occlusion strokes: a multicenter retrospective analysis of 1122 patients. *J Neurointerv Surg.* (2015) 7:16–21. doi: 10.1136/neurintsurg-2013-010743
5. Jickling GC, Liu D, Stamova B, Ander BP, Zhan X, Lu A, et al. Hemorrhagic transformation after ischemic stroke in animals and humans. *J Cereb Blood Flow Metab.* (2014) 34:185–99. doi: 10.1038/jcbfm.2013.203
6. Salas-Perdomo A, Miro-Mur F, Gallizioli M, Brait VH, Justicia C, Meissner A, et al. Role of the S1P pathway and inhibition by fingolimod in preventing hemorrhagic transformation after stroke. *Sci Rep.* (2019) 9:8309. doi: 10.1038/s41598-019-44845-5
7. Goyal M, Menon BK, van Zwam WH, Dippel DW, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet.* (2016) 387:1723–31. doi: 10.1016/S0140-6736(16)00163-X
8. Berkhemer OA, Fransen PS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med.* (2015) 372:11–20. doi: 10.1056/NEJMoa1411587
9. Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. *N Engl J Med.* (2015) 372:1019–30. doi: 10.1056/NEJMoa1414905
10. Saver JL, Goyal M, Bonafe A, Diener HC, Levy EI, Pereira VM, et al. Stent-retriever thrombectomy after intravenous t-PA vs. *t-PA alone in stroke*. *N Engl J Med.* (2015) 372:2285–95. doi: 10.1056/NEJMoa1415061
11. Campbell BC, Mitchell PJ, Kleinig TJ, Dewey HM, Churilov L, Yassi N, et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N Engl J Med.* (2015) 372:1009–18. doi: 10.1056/NEJMoa1414792
12. Jovin TG, Chamorro A, Cobo E, de Miquel MA, Molina CA, Rovira A, et al. Thrombectomy within 8 hours after symptom onset in ischemic stroke. *N Engl J Med.* (2015) 372:2296–306. doi: 10.1056/NEJMoa1503780
13. Albers GW, Marks MP, Kemp S, Christensen S, Tsai JP, Ortega-Gutierrez S, et al. Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. *N Engl J Med.* (2018) 378:708–18. doi: 10.1056/NEJMoa1713973
14. Nogueira RG, Jadhav AP, Haussen DC, Bonafe A, Budzik RF, Bhuva P, et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. *N Engl J Med.* (2018) 378:11–21. doi: 10.1056/NEJMoa1706442
15. Mistry EA, Mistry AM, Nakawah MO, Khattar NK, Fortuny EM, Cruz AS, et al. Systolic blood pressure within 24 hours after thrombectomy for acute ischemic stroke correlates with outcome. *J Am Heart Assoc.* (2017) 6:e006167. doi: 10.1161/JAHA.117.006167
16. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the american heart association/american stroke association. *Stroke.* (2018). 49:e46–e110. doi: 10.1016/j.jvs.2018.04.007
17. Jovin TG, Saver JL, Ribo M, Pereira V, Furlan A, Bonafe A, et al. Diffusion-weighted imaging or computerized tomography perfusion assessment with clinical mismatch in the triage of wake up and late presenting strokes undergoing neurointervention with Trevo (DAWN) trial methods. *Int J Stroke.* (2017) 12:641–52. doi: 10.1177/1747493017710341
18. Tsivgoulis G, Safouris A, Krogias C, Arthur AS, Alexandrov AV. Endovascular reperfusion therapies for acute ischemic stroke: dissecting the evidence. *Expert Rev Neurother.* (2016) 16:527–34. doi: 10.1586/14737175.2016.1168297
19. Goyal N, Tsivgoulis G, Pandhi A, Chang JJ, Dillard K, Ishfaq MF, et al. Blood pressure levels post mechanical thrombectomy and outcomes in large vessel occlusion strokes. *Neurology.* (2017) 89:540–7. doi: 10.1212/WNL.0000000000004184
20. Kim TJ, Park HK, Kim JM, Lee JS, Park SH, Jeong HB, et al. Blood pressure variability and hemorrhagic transformation in patients with successful recanalization after endovascular recanalization therapy: a retrospective observational study. *Ann Neurol.* (2019) 85:574–81. doi: 10.1002/ana.25434
21. Malhotra K, Goyal N, Katsanos AH, Filippatou A, Mistry EA, Khatri P, et al. Association of blood pressure with outcomes in acute stroke thrombectomy. *Hypertension.* (2020) 75:730–9. doi: 10.1161/HYPERTENSIONAHA.119.14230
22. Anadani M, Orabi MY, Alawieh A, Goyal N, Alexandrov AV, Petersen N, et al. Blood pressure and outcome after mechanical thrombectomy with successful revascularization. *Stroke.* (2019) 50:2448–54. doi: 10.1161/str.50.suppl_1.153
23. van Mook WN, Rennenberg RJ, Schurink GW, van Oostenbrugge RJ, Mess WH, Hofman PA, et al. Cerebral hyperperfusion syndrome. *Lancet Neurol.* (2005) 4:877–88. doi: 10.1016/S1474-4422(05)70251-9
24. Galyfos G, Sianou A, Filis K. Cerebral hyperperfusion syndrome and intracranial hemorrhage after carotid endarterectomy or carotid stenting: a meta-analysis. *J Neurol Sci.* (2017) 381:74–82. doi: 10.1016/j.jns.2017.08.020
25. Hashimoto T, Matsumoto S, Ando M, Chihara H, Tsujimoto A, Hatano T. Cerebral hyperperfusion syndrome after endovascular reperfusion therapy in a patient with acute internal carotid artery and middle cerebral artery occlusions. *World Neurosurg.* (2018) 110:145–51. doi: 10.1016/j.wneu.2017.11.023
26. Mistry EA, Mayer SA, Khatri P. Blood pressure management after mechanical thrombectomy for acute ischemic stroke: a survey of the strokenet sites. *J Stroke Cerebrovasc Dis.* (2018) 27:2474–8. doi: 10.1016/j.jstrokecerebrovasdis.2018.05.003
27. Petersen NH, Silverman A, Wang A, Strander S, Kodali S, Matouk C, et al. Association of personalized blood pressure targets with hemorrhagic transformation and functional outcome after endovascular stroke therapy. *JAMA Neurol.* (2019) 76:1256–8. doi: 10.1001/jamaneurol.2019.2120
28. Silverman A, Kodali S, Strander S, Gilmore EJ, Kimmel A, Wang A, et al. Deviation from personalized blood pressure targets is associated with worse outcome after subarachnoid hemorrhage. *Stroke.* (2019) 50:2729–37. doi: 10.1161/STROKEAHA.119.026282
29. Petersen NH, Silverman A, Strander SM, Kodali S, Wang A, Sansing LH, et al. Fixed compared with autoregulation-oriented blood pressure thresholds after mechanical thrombectomy for ischemic stroke. *Stroke.* (2020) 51:914–21. doi: 10.1161/STROKEAHA.119.026596
30. Beqiri E, Smielewski P, Robba C, Czosnyka M, Cabeleira MT, Tas J, et al. Feasibility of individualised severe traumatic brain injury management using an automated assessment of optimal cerebral perfusion pressure: the COGiTATE phase II study protocol. *BMJ Open.* (2019) 9:e030727. doi: 10.1136/bmjopen-2019-030727
31. Donnelly J, Czosnyka M, Adams H, Robba C, Steiner LA, Cardim D, et al. Individualizing thresholds of cerebral perfusion pressure using

estimated limits of autoregulation. *Crit Care Med.* (2017) 45:1464–71. doi: 10.1097/CCM.0000000000002575
32. Paulson OB, Strandgaard S, Edvinsson L. Cerebral autoregulation. *Cerebrovasc Brain Metab Rev.* (1990) 2:161–92.
33. Wang A, Ortega-Gutierrez S, Petersen NH. Autoregulation in the Neuro ICU. *Curr Treat Options Neurol.* (2018) 20:20. doi: 10.1007/s11940-018-0501-x
34. Xiong L, Liu X, Shang T, Smielewski P, Donnelly J, Guo ZN, et al. Impaired cerebral autoregulation: measurement and application to stroke. *J Neurol Neurosurg Psychiatry.* (2017) 88:520–31. doi: 10.1136/jnnp-2016-314385
35. Castro P, Azevedo E, Sorond F. Cerebral autoregulation in stroke. *Curr Atheroscler Rep.* (2018) 20:37. doi: 10.1007/s11883-018-0739-5
36. Immink RV, van Montfrans GA, Stam J, Karemaker JM, Diamant M, van Lieshout JJ. Dynamic cerebral autoregulation in acute lacunar and middle cerebral artery territory ischemic stroke. *Stroke.* (2005) 36:2595–600. doi: 10.1161/01.STR.0000189624.06836.03
37. Guo ZN, Liu J, Xing Y, Yan S, Lv C, Jin H, et al. Dynamic cerebral autoregulation is heterogeneous in different subtypes of acute ischemic stroke. *PLoS ONE.* (2014) 9:e93213. doi: 10.1371/journal.pone.0093213
38. Petersen NH, Ortega-Gutierrez S, Reccius A, Masurkar A, Huang A, Marshall RS. Dynamic cerebral autoregulation is transiently impaired for one week after large-vessel acute ischemic stroke. *Cerebrovasc Dis.* (2015) 39:144–50. doi: 10.1159/000368595
39. Castro P, Serrador JM, Rocha I, Sorond F, Azevedo E. Efficacy of cerebral autoregulation in early ischemic stroke predicts smaller infarcts and better outcome. *Front Neurol.* (2017) 8:113. doi: 10.3389/fneur.2017.00113
40. Reinhard M, Rutsch S, Lambeck J, Wihler C, Czosnyka M, Weiller C, et al. Dynamic cerebral autoregulation associates with infarct size and outcome after ischemic stroke. *Acta Neurol Scand.* (2012) 125:156–62. doi: 10.1111/j.1600-0404.2011.01515.x
41. Castro P, Azevedo E, Serrador J, Rocha I, Sorond F. Hemorrhagic transformation and cerebral edema in acute ischemic stroke: link to cerebral autoregulation. *J Neurol Sci.* (2017) 372:256–61. doi: 10.1016/j.jns.2016.11.065
42. Dohmen C, Bosche B, Graf R, Reithmeier T, Ernestus RI, Brinker G, et al. Identification and clinical impact of impaired cerebrovascular autoregulation in patients with malignant middle cerebral artery infarction. *Stroke.* (2007) 38:56–61. doi: 10.1161/01.STR.0000251642.18522.b6
43. Steiner LA, Czosnyka M, Piechnik SK, Smielewski P, Chatfield D, Menon DK, et al. Continuous monitoring of cerebrovascular pressure reactivity allows determination of optimal cerebral perfusion pressure in patients with traumatic brain injury. *Crit Care Med.* (2002) 30:733–8. doi: 10.1097/00003246-200204000-00002
44. Budohoski KP, Czosnyka M, Kirkpatrick PJ, Smielewski P, Steiner LA, Pickard JD. Clinical relevance of cerebral autoregulation following subarachnoid haemorrhage. *Nat Rev Neurol.* (2013) 9:152–63. doi: 10.1038/nrneurol.2013.11
45. Okazaki S, Yamagami H, Yoshimoto T, Morita Y, Yamamoto H, Toyoda K, et al. Cerebral hyperperfusion on arterial spin labeling MRI after reperfusion therapy is related to hemorrhagic transformation. *J Cereb Blood Flow Metab.* (2017) 37:3087–90. doi: 10.1177/0271678X17718099
46. Butcher K, Christensen S, Parsons M, De Silva DA, Ebinger M, Levi C, et al. Postthrombolysis blood pressure elevation is associated with hemorrhagic transformation. *Stroke.* (2010) 41:72–7. doi: 10.1161/STROKEAHA.109.563767
47. Anadani M, Orabi Y, Alawieh A, Chatterjee A, Lena J, Al Kasab S, et al. Blood pressure and outcome post mechanical thrombectomy. *J Clin Neurosci.* (2019) 62:94–9. doi: 10.1016/j.jocn.2018.12.011
48. Marshall RS, Pavol MA, Cheung YK, Strom I, Slane K, Asllani I, et al. Dissociation among hemodynamic measures in asymptomatic high grade carotid artery stenosis. *J Neurol Sci.* (2016) 367:143–7. doi: 10.1016/j.jns.2016.05.011
49. Strandgaard S. Autoregulation of cerebral blood flow in hypertensive patients. The modifying influence of prolonged antihypertensive treatment on the tolerance to acute, drug-induced hypotension. *Circulation.* (1976) 53:720–7. doi: 10.1161/01.CIR.53.4.720
50. Kim BJ, Cho YJ, Hong KS, Lee J, Kim JT, Choi KH, et al. Trajectory groups of 24-hour systolic blood pressure after acute ischemic stroke and recurrent vascular events. *Stroke.* (2018) 49:1836–42. doi: 10.1161/STROKEAHA.118.021117
51. Li C, Zhang Y, Xu T, Peng H, Wang D, Xu T, et al. Systolic blood pressure trajectories in the acute phase and clinical outcomes in 2-year follow-up among patients with ischemic stroke. *Am J Hypertens.* (2019) 32:317–25. doi: 10.1093/ajh/hpy174
52. Kodali S, Meng C, Strander S, Nguyen CK, Silverman A, Kimmel A, et al. Systolic blood pressure trajectories 72 hours after mechanical thrombectomy are associated with poor functional outcome: a multicenter analysis of individual patient blood pressure data. *Stroke.* (2020) 51:A120. doi: 10.1161/str.50.suppl_1.TP411

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 28 August 2020
doi: 10.3389/fneur.2020.00957

Clinical Characteristics and Outcome of Patients With Hemorrhagic Transformation After Intravenous Thrombolysis in the WAKE-UP Trial

Märit Jensen[1], Eckhard Schlemm[1], Bastian Cheng[1], Iris Lettow[1], Fanny Quandt[1], Florent Boutitie[2,3,4], Martin Ebinger[5,6], Matthias Endres[5,7,8,9], Jochen B. Fiebach[5], Jens Fiehler[10], Ivana Galinovic[5], Vincent Thijs[11,12], Robin Lemmens[13,14,15], Keith W. Muir[16], Norbert Nighoghossian[17], Salvador Pedraza[18], Claus Z. Simonsen[19], Christian Gerloff[1] and Götz Thomalla[1]*

[1] Klinik und Poliklinik für Neurologie, Kopf- und Neurozentrum, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2] Hospices Civils de Lyon, Service de Biostatistique, Lyon, France, [3] Université Lyon 1, Villeurbanne, France, [4] CNRS, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive, Equipe Biostatistique-Santé, Villeurbanne, France, [5] Centrum für Schlaganfallforschung Berlin (CSB), Charité—Universitätsmedizin Berlin, Berlin, Germany, [6] Neurologie, Medical Park Berlin Humboldtmühle, Berlin, Germany, [7] Klinik und Hochschulambulanz für Neurologie, Charité-Universitätsmedizin Berlin, Berlin, Germany, [8] German Center for Neurodegenerative Disease (DZNE), Partner Site Berlin, Berlin, Germany, [9] German Center for Cardiovascular Research (DZHK), Partner Site Berlin, Berlin, Germany, [10] Department of Diagnostic and Interventional Neuroradiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [11] Florey Institute of Neuroscience and Mental Health, University of Melbourne, Heidelberg, VIC, Australia, [12] Austin Health, Department of Neurology, Heidelberg, VIC, Australia, [13] Department of Neurology, University Hospitals Leuven, Leuven, Belgium, [14] Department of Neurosciences, Experimental Neurology, KU Leuven—University of Leuven, Leuven, Belgium, [15] VIB, Laboratory of Neurobiology, Center for Brain & Disease Research, Leuven, Belgium, [16] Institute of Neuroscience and Psychology, University of Glasgow, Glasgow, United Kingdom, [17] Department of Stroke Medicine, Université Claude Bernard Lyon 1, Hospices Civils de Lyon, Lyon, France, [18] Department of Radiology, Institut de Diagnostic per la Image (IDI), Hospital Dr. Josep Trueta, Institut d'Investigació Biomèdica de Girona (IDIBGI), Girona, Spain, [19] Department of Neurology, Aarhus University Hospital, Aarhus, Denmark

OPEN ACCESS

Edited by:
Bruce Campbell,
The University of Melbourne, Australia

Reviewed by:
Carlo W. Cereda,
Ente Ospedaliero Cantonale (EOC), Switzerland
Richard Leigh,
National Institutes of Health (NIH), United States

***Correspondence:**
Märit Jensen
m.jensen@uke.de

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *12 June 2020*
Accepted: *23 July 2020*
Published: *28 August 2020*

Citation:
Jensen M, Schlemm E, Cheng B, Lettow I, Quandt F, Boutitie F, Ebinger M, Endres M, Fiebach JB, Fiehler J, Galinovic I, Thijs V, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Simonsen CZ, Gerloff C and Thomalla G (2020) Clinical Characteristics and Outcome of Patients With Hemorrhagic Transformation After Intravenous Thrombolysis in the WAKE-UP Trial. Front. Neurol. 11:957. doi: 10.3389/fneur.2020.00957

Background: Hemorrhagic transformation (HT) is an important complication of intravenous thrombolysis with alteplase. HT can show a wide range from petechiae to parenchymal hematoma with mass effect with varying clinical impact. We studied clinical and imaging characteristics of patients with HT and evaluated whether different types of HT are associated with functional outcome.

Methods: We performed a *post-hoc* analysis of WAKE-UP, a multicenter, randomized, placebo-controlled trial of MRI-guided intravenous alteplase in unknown onset stroke. HT was assessed on follow-up MRI or CT and diagnosed as hemorrhagic infarction type 1 and type 2 (HI1 and HI2, combined as HI), and parenchymal hemorrhage type 1 and type 2 (PH1 and PH2, combined as PH). Severity of stroke symptoms was assessed using the National Institutes of Health Stroke Scale (NIHSS) at baseline. Stroke lesion volume was measured on baseline diffusion weighted imaging (DWI). Primary endpoint was a favorable outcome defined as a modified Rankin Scale score 0–1 at 90 days.

Results: Of 483 patients included in the analysis, 95 (19.7%) showed HI and 21 (4.4%) had PH. Multiple logistic regression analysis identified treatment with alteplase (OR, 2.08 [95% CI, 1.28–3.40]), baseline NIHSS score (OR, 1.11 [95% CI, 1.05–1.17]), DWI lesion volume (OR, 1.03 [95% CI, 1.01–1.05]), baseline glucose levels (OR, 1.01 [95% CI,

1.00–1.01]) and atrial fibrillation (OR, 3.02 [95% CI, 1.57–5.80]) as predictors of any HT. The same parameters predicted HI. Predictors of PH were baseline NIHSS score (OR, 1.11 [95% CI, 1.01–1.22]) and as a trend treatment with alteplase (OR, 2.40 [95% CI, 0.93–6.96]). PH was associated with lower odds of favorable outcome (OR 0.25, 95% [CI 0.05–0.86]), while HI was not.

Conclusion: Our results indicate that HI is associated with stroke severity, cardiovascular risk factors and thrombolysis. PH is a rare complication, more frequent in severe stroke and with thrombolysis. In contrast to HI, PH is associated with worse functional outcome. The impact of HT after MRI-guided intravenous alteplase for unknown onset stroke on clinical outcome is similar as in the trials of stroke thrombolysis within a known early time-window.

Keywords: ischemic stroke, WAKE-UP, thrombolysis, intracerebral hemorrhage, hemorrhagic transformation

INTRODUCTION

Hemorrhagic transformation (HT) represents an important complication of intravenous thrombolysis with alteplase for acute ischemic stroke. However, HT of ischemic stroke can show a wide range from small petechiae with no clinical impact to massive parenchymal hematoma with space-occupying effect associated with neurological deterioration. The following four subtypes of HT have been distinguished radiologically: hemorrhagic infarction type 1 (HI1; scattered small petechiae, no mass effect), hemorrhagic infarction type 2 (HI2; confluent petechiae, no mass effect), parenchymal hemorrhage type 1 (PH1; hematoma within infarcted tissue, occupying $< 30\%$, no substantive mass effect) and parenchymal hemorrhage type 2 (PH2; hematoma occupying 30% or more of the infarcted tissue, with obvious mass effect) (1). In addition, intracerebral hemorrhage outside infarcted brain tissue or intracranial-extracerebral hemorrhage is considered a separate category, including subarachnoidal hemorrhage and subdural hematoma. The clinical significance of different types of HT after thrombolysis is a matter of debate (2). While there is no doubt that massive HT, meeting criteria of PH2, is likely to be associated with clinical worsening, mere hemorrhagic infarction (HI) may also be understood as a marker of successful recanalization into partially ischemic damage with no adverse clinical effect (3, 4). Previous work has suggested a different pathogenesis for HI and PH. While HI might be a clinically irrelevant epiphenomenon of ischemic damage and reperfusion, PH appears to be related to biological effects of alteplase and other pre-existing pathologic conditions and also carries the potential of clinical deterioration (4). At the same time, it is still uncertain how—if at all—patients at high risk of severe intracerebral hemorrhage after thrombolysis can be identified beforehand based on clinical or imaging characteristics. In the present study, our first objective was to identify possible clinical and imaging parameters that predict HT after acute ischemic stroke. Second, we aimed to study the functional outcome of patients with different types of HT to get further insights into the clinical significance of HT after intravenous thrombolysis.

METHODS

Study Design

In this exploratory *post-hoc* analysis of the WAKE-UP trial, we reviewed patients for intracerebral hemorrhage on follow-up imaging 22–36 h after stroke. WAKE-UP was a multicenter, randomized, double-blind, placebo-controlled clinical trial to study the efficacy and safety of intravenous thrombolysis with alteplase in patients with an acute stroke of unknown onset time, guided by MRI. Inclusion criteria comprised the mismatch between an acute ischemic lesion visible on diffusion-weighted imaging (DWI) but with no corresponding marked parenchymal hyperintensity on fluid-attenuated inversion recovery (FLAIR) as a surrogate marker of lesion age, indicating that the stroke had most likely occurred within 4.5 h (5). Patients or their legal representatives provided written informed consent according to national and local regulations. There was an exception from explicit informed consent in emergency circumstances in some countries. For each study site, the competent authorities and the corresponding ethics committee approved the trial. The detailed trial protocol has been published together with its main results (5). The trial was registered at ClinicalTrials.gov number (NCT01525290) and EudraCT (2011-005906-32).

In this analysis, we examined demographic characteristics, medical history, clinical and imaging data at baseline and follow-up, including final follow-up at 90 days after stroke. HT was assessed on MRI or, if MRI was not feasible, on CT 22–36 h after randomization and categorized into established radiologic subtypes HI1, HI2, PH1, PH2, and other hemorrhages (1). Image reading was performed by the central image reading board consisting of experienced neuroradiologists. For statistical analysis, we collapsed the subtypes of HT into two categories HI (HI1 and HI2), and PH (PH1 and PH2). Other subtypes of hemorrhages outside the infarcted brain tissue or intracranial-extracerebral hemorrhage were disregarded in the current analysis. DWI lesions were segmented and quantified on apparent diffusion coefficient (ADC) maps calculated from DWI using a semi-automated procedure based on an upper ADC threshold of 620 mm^2/s.

Outcome Measures and Endpoints

Clinical outcome was assessed at 90 days after stroke. The primary endpoint was favorable outcome defined as a score of 0–1 on the modified Rankin Scale (mRS). Secondary endpoint in this analysis comprised the ordinal analysis of the mRS ("shift analysis").

Statistical Analysis

Baseline characteristics were compared between patients with and without hemorrhage using Chi-square test, *t*-tests, ANOVA or non-parametric Kruskall-Wallis test as appropriate. In addition, multiple logistic regression analysis was used to assess the association between HT and baseline clinical and imaging characteristics in a joint model. To investigate the association between HT and functional outcome, we fitted three separate unconditional logistic regression models to associate the log odds of achieving a mRS score of 0–1 with the occurrence of any HT, HI, or PH. All models were adjusted for the stratification parameters age and NIHSS score, treatment group, and parameters that were predictive of any HT, HI, or PH in multivariate analysis (i.e., baseline NIHSS score, baseline glucose levels, atrial fibrillation (AF), and DWI lesion volume). Odds ratios were tested against the null hypothesis of no association using *t*-tests and presented with 95% confidence intervals obtained from profiling the likelihood function. Associations between HT and a shift in the distribution of mRS scores were assessed by fitting ordinal logistic regression models under the proportional odds assumptions with the same covariates as above. All tests were carried out with a two-sided significance level of 5% without correction for multiple comparisons.

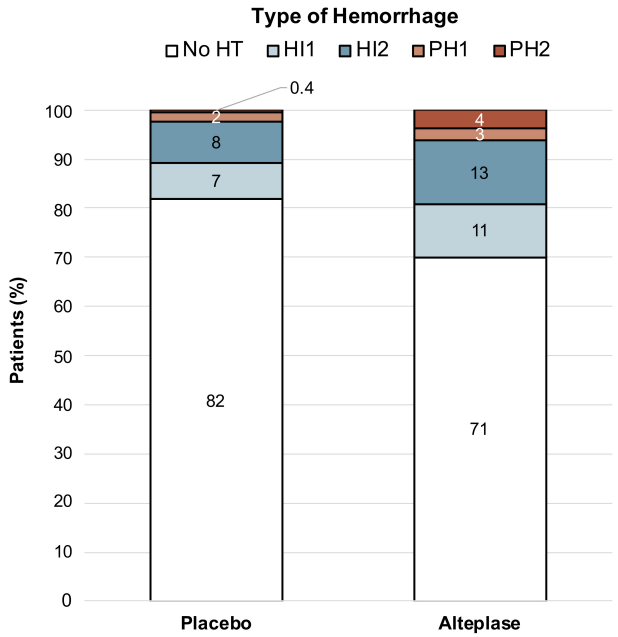


FIGURE 1 | Distribution of Hemorrhages in relation to the treatment group. Distribution of Hemorrhage in relation to the treatment groups shows that any hemorrhage was more frequent in patients treated with alteplase. While HI occurred in both treatment groups, PH was mainly seen in patients treated with alteplase. ICH, intracerebral hemorrhage; HI1, hemorrhagic infarction type 1; HI2, hemorrhagic infarction type 2; PH1, parenchymal hemorrhage type 1; PH2, parenchymal hemorrhage type 2.

RESULTS

Patient Characteristics

Of 503 patients randomized in WAKE-UP, follow-up imaging and data on the primary endpoint was available for 486 patients. Follow-up imaging was performed by MRI in 457 (94%) patients and by CT in 29 patients (6%). Three patients with hemorrhage outside the infarcted brain tissue were excluded from further analysis. HT, either HI or PH, was present in 116 (24%) patients. We observed HI1 in 44 (9.1%), HI2 in 51 (10.6%), PH1 in 11 (2.3%), and PH2 in 10 (2.1%) patients. **Figure 1** illustrates the distribution of HT type in relation to the treatment group. Subgroup analysis revealed that PH2 was more frequent with alteplase as compared to placebo (3.7 vs. 0.41%, $p = 0.01$), while HI1 (10.8 vs. 7.45%, $p = 0.26$), HI2 (12.9 vs. 8.3%, $p = 0.14$), and PH1 (2.5 vs. 2.1%, $p = 0.99$) did not show significant differences between treatment groups.

Clinical and imaging characteristics of patients with and without HT are shown in **Table 1**. Treatment with alteplase was more prevalent in patients with HT (62 vs. 46%, $p = 0.004$). Those with hemorrhage were more severely affected, with a higher median NIHSS score on admission (9 vs. 5, $p < 0.001$), and more often had cardiovascular comorbidities including AF (25 vs. 9%, $p < 0.001$) and diabetes mellitus (26 vs. 13%, $p = 0.002$). Accordingly, medication with antidiabetics was more frequent (23 vs. 11%, $p = 0.001$), and glucose levels on admission were higher in patients with HT (median 124 vs. 113 mg/dL, $p < 0.001$). DWI lesion volume at baseline was significantly higher (median 7.33 vs. 1.53 mL, $p < 0.001$) in patients with hemorrhage.

Clinical and Imaging Predictors of Hemorrhagic Transformation

For analysis, data from 29 patients were excluded due to missing data (i.e., 1 patient without information on glucose level and AF, 11 patients without information on glucose levels, 7 patients without information on AF, and 10 patients in whom quantification of DWI lesion volume was not possible). Among all randomized patients, the following parameters were associated with any HT on follow-up in multiple regression analysis (see **Table 2**): treatment with alteplase (OR, 2.08 [95% CI, 1.28–3.40]), baseline NIHSS score (OR, 1.11 [95% CI, 1.05–1.17]), DWI lesion volume (OR, 1.03 [95% CI, 1.01–1.05]), serum glucose levels (OR, 1.01 [95% CI, 1.00–1.01]), and AF (OR, 3.02 [95% CI, 1.57–5.80]). HI was predicted by treatment with alteplase (OR, 1.73 [95% CI, 1.05–2.89]), baseline NIHSS score (OR, 1.08 [95% CI, 1.02–1.14]), DWI lesion volume (OR, 1.03 [95% CI, 1.01–1.05]), serum glucose (OR, 1.01 [95% CI, 1.00–1.01]), and AF (OR, 2.58 [95% CI, 1.32–4.97]). NIHSS score on admission predicted the occurrence of PH (OR, 1.11 [95% CI, 1.01–1.22]), and there was a trend for an association of alteplase treatment with PH (OR, 2.40 [95% CI, 0.93–6.96]).

TABLE 1 | Baseline characteristics of patients with and without HT.

Variable	Patients, No. (%)					
	No HT ($n = 367$)	Any HT ($n = 116$)	Group comparison No HT vs. any HT *p*-value	HI ($n = 95$)	PH ($n = 21$)	Group comparison No HT vs. HI vs. PH *p*-value
Age, mean (SD), y	64.8 (11.7)	66.4 (10.9)	0.19	66.4 (11.3)	66.4 (9.38)	0.45
Female	128 (34.9)	44 (37.9)	0.63	36 (37.9)	8 (38.1)	0.84
Medical history or risk factors						
Arterial hypertension	187 (51.2)	67 (58.3)	0.23	58 (61.7)	9 (42.9)	0.12
Diabetes mellitus	48 (13.2)	30 (26.3)	0.002	28 (30.1)	2 (9.5)	0.001
Hypercholesterolemia	128 (36.4)	46 (42.2)	0.32	43 (48.9)	3 (14.3)	0.007
Atrial fibrillation	31 (8.6)	28 (24.6)	<0.001	23 (24.5)	5 (25)	<0.001
History of ischemic stroke	49 (13.4)	17 (14.7)	0.85	15 (15.8)	2 (9.5)	0.79
Medication classes						
Antiplatelets	118 (32.2)	40 (34.5)	0.72	34 (35.8)	6 (28.6)	0.73
Statins	106 (28.9)	43 (37.1)	0.12	39 (41.1)	4 (19)	0.04
Antihypertensives	173 (47.1)	62 (53.4)	0.28	52 (54.7)	10 (47.6)	0.42
Antidiabetics	40 (10.9)	27 (23.3)	0.001	26 (27.4)	1 (4.8)	<0.001
Laboratory parameters						
Platelet count, median (IQR), $10^3/\mu L$	230 (191–273)	222 (189–270)	0.67	226 (188-281)	200 (195–232)	0.34
Serum glucose, median (IQR), mg/dL	113 (101–133)	124 (107–164)	<0.001	124 (107–167)	124 (110–146)	0.001
National Institute of Health Stroke Scale score, median (IQR)	5 (3–8)	9 (6–15)	<0.001	9 (5–15)	11 (6–12)	<0.001
Diffusion-weighted imaging lesion volume at baseline, median (IQR), mL	1.53 (0.61–5.68)	7.33 (3.16–23)	<0.001	7.02 (3.12–22.7)	10.3 (5.1–23.3)	<0.001
Time from last-seen-well to treatment initiation, median (IQR), min	605 (485–714)	660 (520–751)	0.044	658 (537–739)	690 (455–815)	0.12
Time from symptom recognition to treatment initiation, median (IQR), min	186 (155–230)	192 (144–236)	0.72	192 (146–234)	175 (133–240)	0.72
Treatment with Alteplase	169 (46)	72 (62.1)	0.004	57 (60)	15 (71.4)	0.007

HT, hemorrhagic transformation; HI, hemorrhagic infarction; PH, parenchymal hemorrhage; SD, standard deviation; IQR, interquartile range.

Influence of Intracerebral Hemorrhage on Stroke Outcome

Figure 2 shows the distribution of modified Rankin Scale scores at 90 days after stroke by hemorrhage group. Multivariate analysis adjusted for age, baseline NIHSS, treatment group, AF, serum glucose and DWI lesion volume revealed an independent association of PH with lower odds of favorable outcome. Favorable outcome was observed in 3 of 21 patients (14.3%) in the PH group and in 227 of 462 patients (49.1%) in the group without PH [OR, 0.25 [95% CI, 0.05–0.86]; see **Table 3**]. In ordinal analysis of the mRS at 90 days, PH was associated with a shift toward worse functional outcomes (OR, 0.39 [95% CI, 0.17–0.89]). Neither any HT nor HI showed a significant association with outcome.

DISCUSSION

In this *post-hoc* analysis of HT after intravenous thrombolysis for acute ischemic stroke in the WAKE-UP trial we demonstrate that: (1) HI, although slightly more prevalent in alteplase-treated patients, may occur as part of natural history of ischemic stroke with or without thrombolysis, while severe HT was more frequently seen in the context of alteplase treatment; (2) treatment with alteplase, baseline NIHSS Score, DWI lesion volume, serum glucose levels, and AF are predictors of HT and HI, and (3) PH but not HI was associated with worse functional outcome after correction for baseline predictors.

In our cohort, HT occurred in 18.2% of patients with placebo and in 29.9% with alteplase. The observed rate of hemorrhage was slightly lower as compared to a pooled analysis of previous clinical trials of stroke thrombolysis, in which radiographic evidence of HT occurred in 24.2% of placebo-treated patients and 32.5% of alteplase-treated patients (6, 7). However, the distribution between both groups was similar. The lower rate of hemorrhage is also remarkable, as we used MRI for follow-up imaging in the vast majority (>90%) of patients, and MRI is known to be more sensitive to HT than CT (8), which was the main follow-up imaging modality in the pooled stroke thrombolysis trials. Amongst other factors, the overall lower rate of HT in our population may be attributable to the trial design,

which led to the exclusion of very elderly patients (>80 years of age) as age was associated with HT in other studies (9–11). The fact that our population was less severely affected with a median NIHSS of 6 as compared to 11 in previous pooled stroke thrombolysis trials may also have contributed to overall lower rates of HT.

It has been suggested that the underlying mechanisms of HI and PH differ from each other (12). Our results showed that HI occurred in 15.7% of placebo-treated patients and 23.7% of alteplase-treated patients. In contrast, PH is uncommon and was found in only 2.5% of placebo-treated patients, but in 6.2% of alteplase-treated patients. Especially PH2 (0.4 vs. 3.7%, $p = 0.01$) was associated with intravenous thrombolysis. These findings are in line with previous studies, that showed that HI is also encountered without the use of thrombolytic agents and thus occurs as a part of natural history of ischemic stroke, while severe PH appears mainly attributable to biological effects of treatment with alteplase (12).

However, the coagulopathy induced by alteplase is not the only determinant of HT occurring after ischemic stroke. We additionally identified NIHSS score and DWI lesion volume on admission, blood glucose levels and AF as independent predictors of HT. The same risk factors were observed for HI, which accounts for the majority of HTs (i.e., 82%). These results are in line with previous findings. DWI lesion volume was associated with the occurrence of HT/HI (13). Higher NIHSS score values, which were also associated with HT, also reflect more severe strokes and larger infarcts (4). The association of AF with HT that we observed in our analysis has also been reported in previous studies (14–16). AF is associated with higher volumes of more severe baseline hypoperfusion leading to greater infarct growth (17). The more severe ischemia with this type of stroke has been postulated to damage blood vessel integrity, resulting in increased HT, especially with reperfusion (18). Elevated glucose levels are usually considered to be a risk factor for HT, especially in the setting of thrombolysis (19). Accordingly, in our study patients with HT had higher baseline glucose levels. Experimental studies have suggested that several pathological mechanisms, e.g., increased activity of matrix metalloproteinases and enhanced apoptosis of smooth muscle cells, might result in diffuse damage of the microvasculature and thus increase infarct size and risk of HT (20).

Although the absolute difference between the percentage of patients treated with alteplase in the PH and no HT group was high (71 vs. 46%), multiple regression analysis failed to demonstrate a significant association of alteplase treatment with PH ($p = 0.08$). In our study, solely NIHSS score on admission was significantly associated with PH ($p = 0.027$). The reviewed literature additionally identifies large lesions attributable to cardioembolism, hyperglycemia, extent of parenchymal hypoattenuation on baseline CT scan, a history of congestive heart failure, increasing age, and baseline systolic blood pressure as predictors of PH (9, 21, 22). We assume that the low number of patients with PH in our study limits the statistical power to detect possible associations of risk factors with PH.

Previous studies have suggested that most types of HT in acute stroke do not have a relevant effect on the clinical outcome in

TABLE 2 | Clinical and imaging predictors of any HT, HI, and PH.

	p-value	OR* (95% CI)
Predictors of any HT		
National Institute of Health Stroke Scale score	< 0.001	1.11 (1.05–1.17)
Baseline blood glucose	0.004	1.01 (1.00–1.01)
Atrial fibrillation	< 0.001	3.02 (1.57–5.80)
Diffusion-weighted imaging lesion volume at baseline, mL	0.005	1.03 (1.01–1.05)
Age, y	0.32	1.01 (0.99–1.04)
Treatment with Alteplase	0.003	2.08 (1.28–3.40)
Predictors of HI		
National Institute of Health Stroke Scale score	0.005	1.08 (1.02–1.14)
Baseline blood glucose	0.003	1.01 (1.00–1.01)
Atrial fibrillation	0.005	2.58 (1.32–4.97)
Diffusion-weighted imaging lesion volume at baseline, mL	0.006	1.03 (1.01–1.05)
Age, y	0.42	1.01 (0.99–1.04)
Treatment with Alteplase	0.033	1.73 (1.05–2.89)
Predictors of PH		
National Institute of Health Stroke Scale score	0.027	1.11 (1.01–1.22)
Baseline blood glucose	0.90	1.00 (0.99–1.01)
Atrial fibrillation	0.26	1.90 (0.57–5.43)
Diffusion-weighted imaging lesion volume at baseline, mL	0.80	1.00 (0.97–1.04)
Age, y	0.58	1.01 (0.97–1.07)
Treatment with Alteplase	0.08	2.40 (0.93–6.96)

*HT, hemorrhagic transformation; HI, hemorrhagic infarction; PH, parenchymal hemorrhage; OR, odds ratio; CI, confidence interval. *multiple logistic regression analysis.*

TABLE 3 | Association of any HT, HI, and PH with favorable functional outcome.

	Type of hemorrhage					
	Any HT (HI and PH)		HI		PH	
Assessment Variable at 90 days	**OR* (95% CI)**	***p*-value**	**OR* (95% CI)**	***p*-value**	**OR* (95% CI)**	***p*-value**
Primary end point (Modified Rankin Scale score 0–1)	0.64 (0.37–1.09)	0.10	0.83 (0.47–1.46)	0.52	0.25 (0.05–0.86)	0.043
Secondary end point ("Shift analysis")	0.69 (0.46–1.06)	0.09	0.88 (0.57–1.36)	0.56	0.39 (0.17–0.89)	0.026

**Multiple logistic regression analysis including age > 60, baseline NIHSS score > 10, DWI lesion volume, treatment with alteplase, baseline glucose, and atrial fibrillation. HT, hemorrhagic transformation; HI, hemorrhagic infarction; PH, parenchymal hemorrhage; OR, odds ratio; CI, confidence interval.*

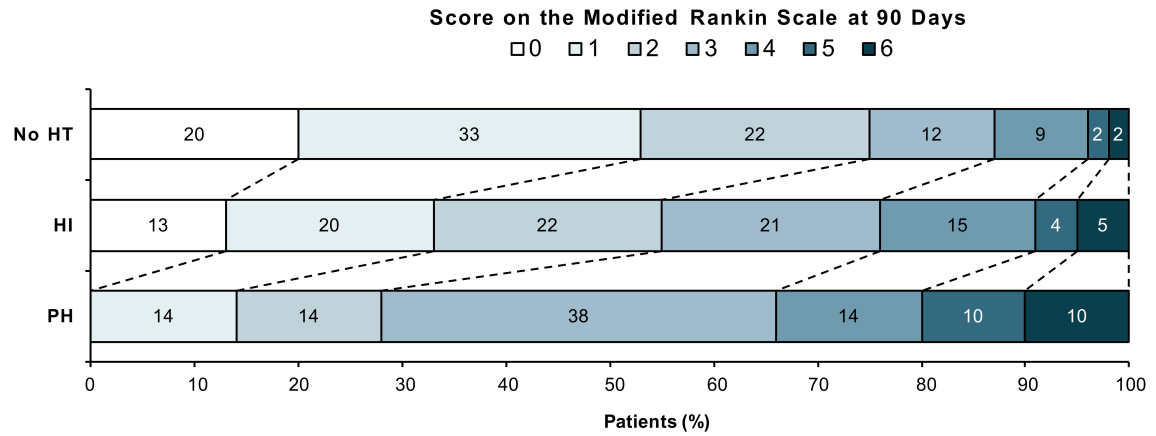


FIGURE 2 | Distribution of Modified Rankin Scale scores at 90 days after stroke by hemorrhage groups. Modified Rankin Scale scores range from 0 to 6 (0, no symptoms; 1, no clinically significant disability; 2, slight disability; 3, moderate disability; 4, moderately severe disability; 5, severe disability; and 6, death). HT, hemorrhagic transformation; HI, hemorrhagic infarction; PH, parenchymal hemorrhage.

the majority of cases. On the contrary it has been suggested that mild to moderate HT represents a marker of successful treatment and vascular recanalization (4, 23, 24). In support of this notion, in our analysis HI was not associated with poor outcome at 3 months. In contrast and in agreement with previous studies, PH was associated with worse functional outcome (2, 9).

There are limitations to our study. Due to the observational design of the analysis of hemorrhage, we cannot claim a causal relationship between hemorrhage and functional outcome. In addition, as study inclusion and exclusion criteria entailed a selected and relatively young sample of stroke patients, generalizability of our results is limited. Finally, the small number of patients with severe intracerebral hemorrhagic complications limits the statistical power of our analysis.

CONCLUSION

HT is a frequent observation in acute ischemic stroke and more frequent with intravenous thrombolysis as compared to placebo. Our results support the hypothesis that HI pathogenesis may be related to stroke severity, cardiovascular risk factors and thrombolysis but does not negatively influence stroke outcome. PH is a rare complication, more frequent in severe stroke and after treatment with alteplase, and is associated with worse functional outcome. The impact of HT in the WAKE-UP trial of MRI-guided intravenous alteplase for unknown onset stroke on clinical outcome is similar as in the trials of stroke thrombolysis within a known early time-window.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of the Hamburg chamber of physicians, Weidestr. 122b, 22083 Hamburg, Germany was the primary ethics committee that approved the trial (PVN3857). For each study site, the competent authorities and the corresponding ethics committee approved the trial. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MJ and GT developed the study protocol, interpreted the data, and drafted the manuscript. ES and FB provided support with statistical analysis. BC, MEb, MEn, JBF, JF, IG, VT, RL, KM, NN, SP, CS, CG, and GT collected data. ES, BC, IL, FQ, FB, MEb, MEn, JBF, JF, IG, VT, RL, KM, NN, SP, CS, and CG interpreted the data and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

WAKE-UP received funding from the European Union Seventh Framework Program [FP7/2007-2013] under grant agreement no. 278276 (WAKE-UP). The European Union Seventh Framework Program [FP7/2007-2013] had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

REFERENCES

1. von Kummer R, Broderick JP, Campbell BC, Demchuk A, Goyal M, Hill MD, et al. The Heidelberg bleeding classification: classification of bleeding events after ischemic stroke and reperfusion therapy. *Stroke*. (2015) 46:2981–6. doi: 10.1161/STROKEAHA.115.010049
2. Berger C, Fiorelli M, Steiner T, Schabitz WR, Bozzao L, Bluhmki E, et al. Hemorrhagic transformation of ischemic brain tissue: asymptomatic or symptomatic? *Stroke*. (2001) 32:1330–5. doi: 10.1161/01.STR.32.6.1330
3. Fiehler J, Remmele C, Kucinski T, Rosenkranz M, Thomalla G, Weiller C, et al. Reperfusion after severe local perfusion deficit precedes hemorrhagic transformation: an MRI study in acute stroke patients. *Cerebrovasc Dis*. (2005) 19:117–24. doi: 10.1159/000083180
4. Thomalla G, Sobesky J, Kohrmann M, Fiebach JB, Fiehler J, Zaro Weber O, et al. Two tales: hemorrhagic transformation but not parenchymal hemorrhage after thrombolysis is related to severity and duration of ischemia: MRI study of acute stroke patients treated with intravenous tissue plasminogen activator within 6 hours. *Stroke*. (2007) 38:313–8. doi: 10.1161/01.STR.0000254565.51807.22
5. Thomalla G, Simonsen CZ, Boutitie F, Andersen G, Berthezene Y, Cheng B, et al. MRI-guided thrombolysis for stroke with unknown time of onset. *N Engl J Med*. (2018) 379:611–22. doi: 10.1056/NEJMoa1804355
6. Yaghi S, Willey JZ, Cucchiara B, Goldstein JN, Gonzales NR, Khatri P, et al. Treatment and outcome of hemorrhagic transformation after intravenous alteplase in acute ischemic stroke: a scientific statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. (2017) 48:e343–61. doi: 10.1161/STR.0000000000000152
7. Lees KR, Bluhmki E, von Kummer R, Brott TG, Toni D, Grotta JC, et al. Time to treatment with intravenous alteplase and outcome in stroke: an updated pooled analysis of ECASS, ATLANTIS, NINDS, and EPITHET trials. *Lancet*. (2010) 375:1695–703. doi: 10.1016/S0140-6736(10)60491-6
8. Arnould MC, Grandin CB, Peeters A, Cosnard G, Duprez TP. Comparison of CT and three MR sequences for detecting and categorizing early (48 hours) hemorrhagic transformation in hyperacute ischemic stroke. *AJNR Am J Neuroradiol*. (2004) 25:939–44.
9. Larrue V, von Kummer RR, Muller A, Bluhmki E. Risk factors for severe hemorrhagic transformation in ischemic stroke patients treated with recombinant tissue plasminogen activator: a secondary analysis of the European-Australasian Acute Stroke Study (ECASS II). *Stroke*. (2001) 32:438–41. doi: 10.1161/01.STR.32.2.438
10. Hacke W, Donnan G, Fieschi C, Kaste M, von Kummer R, Broderick JP, et al. Association of outcome with early stroke treatment: pooled analysis of ATLANTIS, ECASS, and NINDS rt-PA stroke trials. *Lancet*. (2004) 363:768–74. doi: 10.1016/S0140-6736(04)15692-4
11. Tanne D, Kasner SE, Demchuk AM, Koren-Morag N, Hanson S, Grond M, et al. Markers of increased risk of intracerebral hemorrhage after intravenous recombinant tissue plasminogen activator therapy for acute ischemic stroke in clinical practice: the Multicenter rt-PA Stroke Survey. *Circulation*. (2002) 105:1679–85. doi: 10.1161/01.CIR.0000012747.53592.6A
12. Cerebral Embolism Study Group. Immediate anticoagulation of embolic stroke: brain hemorrhage and management options. *Stroke*. (1984) 15:779–89. doi: 10.1161/01.STR.15.5.779
13. Selim M, Fink JN, Kumar S, Caplan LR, Horkan C, Chen Y, et al. Predictors of hemorrhagic transformation after intravenous recombinant tissue plasminogen activator: prognostic value of the initial apparent diffusion coefficient and diffusion-weighted lesion volume. *Stroke*. (2002) 33:2047–52. doi: 10.1161/01.STR.0000023577.65990.4E
14. He W, Ruan Y, Yuan C, Cheng Q, Cheng H, Zeng Y, et al. High neutrophil-to-platelet ratio is associated with hemorrhagic transformation in patients with acute ischemic stroke. *Front Neurol*. (2019) 10:1310. doi: 10.3389/fneur.2019.01310
15. Nogueira RG, Gupta R, Jovin TG, Levy EI, Liebeskind DS, Zaidat OO, et al. Predictors and clinical relevance of hemorrhagic transformation after endovascular therapy for anterior circulation large vessel occlusion strokes: a multicenter retrospective analysis of 1122 patients. *J Neurointerv Surg*. (2015) 7:16–21. doi: 10.1136/neurintsurg-2013-010743
16. Tan S, Wang D, Liu M, Zhang S, Wu B, Liu B. Frequency and predictors of spontaneous hemorrhagic transformation in ischemic stroke and its association with prognosis. *J Neurol*. (2014) 261:905–12. doi: 10.1007/s00415-014-7297-8
17. Tu HT, Campbell BC, Christensen S, Desmond PM, De Silva DA, Parsons MW, et al. Worse stroke outcome in atrial fibrillation is explained by more severe hypoperfusion, infarct growth, and hemorrhagic transformation. *Int J Stroke*. (2015) 10:534–40. doi: 10.1111/ijs.12007
18. Campbell BC, Christensen S, Butcher KS, Gordon I, Parsons MW, Desmond PM, et al. Regional very low cerebral blood volume predicts hemorrhagic transformation better than diffusion-weighted imaging volume and thresholded apparent diffusion coefficient in acute ischemic stroke. *Stroke*. (2010) 41:82–8. doi: 10.1161/STROKEAHA.109.562116
19. Demchuk AM, Morgenstern LB, Krieger DW, Linda Chi T, Hu W, Wein TH, et al. Serum glucose level and diabetes predict tissue plasminogen activator-related intracerebral hemorrhage in acute ischemic stroke. *Stroke*. (1999) 30:34–9. doi: 10.1161/01.STR.30.1.34
20. Kerenyi L, Kardos L, Szasz J, Szatmari S, Bereczki D, Hegedus K, et al. Factors influencing hemorrhagic transformation in ischemic stroke: a clinicopathological comparison. *Eur J Neurol*. (2006) 13:1251–5. doi: 10.1111/j.1468-1331.2006.01489.x
21. Paciaroni M, Agnelli G, Corea F, Ageno W, Alberti A, Lanari A, et al. Early hemorrhagic transformation of brain infarction: rate, predictive factors, and influence on clinical outcome: results of a prospective multicenter study. *Stroke*. (2008) 39:2249–56. doi: 10.1161/STROKEAHA.107.510321
22. Bruno A, Levine SR, Frankel MR, Brott TG, Lin Y, Tilley BC, et al. Admission glucose level and clinical outcomes in the NINDS rt-PA stroke trial. *Neurology*. (2002) 59:669–74. doi: 10.1212/WNL.59.5.669
23. Molina CA, Alvarez-Sabin J, Montaner J, Abilleira S, Arenillas JF, Coscojuela P, et al. Thrombolysis-related hemorrhagic infarction: a marker of early reperfusion, reduced infarct size, and improved outcome in patients with proximal middle cerebral artery occlusion. *Stroke*. (2002) 33:1551–6. doi: 10.1161/01.STR.0000016323.13456.E5
24. von Kummer R. Brain hemorrhage after thrombolysis: good or bad? *Stroke*. (2002) 33:1446–7. doi: 10.1161/01.STR.0000016923.99605.75

Conflict of Interest: FB reports grants from University Medical Center Hamburg-Eppendorf during the conduct of the study. BC reports grants from European Union 7th Framework Program during the conduct of the study. MEb reports grants from European Union 7th Framework Program during the conduct of the study. MEn reports grant support from Bayer, the German Research Foundation (DFG), the German Federal Ministry of Education and Research (BMBF), the German Center for Neurodegenerative Diseases (DZNE), the German Center for Cardiovascular Research (DZHK), the European Union, Corona Foundation, and Fondation Leducq; and fees paid to the Charité from Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Pfizer, Daiichi Sankyo, Amgen, GlaxoSmithKline, Sanofi, Covidien, Novartis, all outside the submitted work. JBF reports grants from European Union 7th Framework Program during the conduct of the study and personal fees from Bioclinica, Artemida, Cerevast, Brainomix, BMS, Merck, Eisai, Biogen, Guerbet, and Nicolab outside the submitted work. JF reports grants and personal fees from Acandis, personal fees from Cerenovus, grants and personal fees from Medtronic, grants and personal fees from Microvention, personal fees from Penumbra, personal fees from Route92, outside the submitted work. IG reports grants from European Union 7th Framework Program during the conduct of the study. VT reports grants from European Union 7th Framework Program during the conduct of the study, and personal fees and non-financial support from Boehringer Ingelheim, Pfizer/BMS, Bayer, Sygnis, Amgen and Allergan, outside the submitted work. RL reports institutional fees from Bayer, Boehringer Ingelheim, Genentech, Ischemiaview, Medtronic and Occlutech outside the submitted work. KM reports grants from European Union 7th Framework Program during the conduct of the study, and personal fees and non-financial support from Boehringer Ingelheim, outside the submitted work. SP reports grants from European Union 7th Framework Program during the conduct of the study. CS reports grants from Novo Nordisk Foundation and personal fees from Bayer, outside the submitted work. CG reports, outside the submitted work, funding from German Research Council (DFG), European Union, Federal Ministry of Education and Research (BMBF), German Statutory Pension Insurance Scheme (RV Nord), National Innovation Fond, Wegener Foundation, Hertie Foundation, and Schilling Foundation, and received, outside the submitted work, personal fees from Abbott, Amgen, Bayer Vital, Bristol-Myers-Squibb, Boehringer Ingelheim, Daiichi-Sankyo, Sanofi Aventis, and Prediction Biosciences. GT reports grants from European Union 7th Framework Program during the conduct of the study

and personal fees from Acandis, Boehringer Ingelheim, BMS/Pfizer, Stryker, Daiichi Sankyo, grants and personal fees from Bayer, grants from Corona Foundation, German Innovation Fonds and Else Kroener Fresenius Foundation, outside the submitted work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 02 September 2020
doi: 10.3389/fneur.2020.00962

Low Serum Magnesium Levels Are Associated With Hemorrhagic Transformation After Thrombolysis in Acute Ischemic Stroke

Zicheng Cheng[1], *Xiaoyan Huang*[1], *Farah Mohamed Muse*[1], *Lingfan Xia*[1], *Zhenxiang Zhan*[1], *Xianda Lin*[2], *Yungang Cao*[1] *and Zhao Han*[1]*

[1] *Department of Neurology, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China,* [2] *Department of Neurology, The Wenzhou Third Clinical Institute Affiliated to Wenzhou Medical University, Wenzhou, China*

Background: In patients with acute ischemic stroke, hemorrhagic transformation is a major complication after intravenous thrombolysis. This study aimed to investigate the relationship between serum magnesium levels and hemorrhagic transformation (HT) after thrombolytic therapy.

Methods: We retrospectively analyzed data from 242 patients who received thrombolytic therapy at the Second Affiliated Hospital of the Wenzhou Medical University in China. Baseline serum magnesium levels were measured before intravenous thrombolysis, and the occurrence of HT was evaluated using computed tomography images reviewed within 24–36 h after therapy. The relationship between serum magnesium levels and HT was examined using multivariate logistic regression, subgroup analysis, and restricted cubic spline models.

Results: Of the 242 included patients, 43 (17.8%) developed HT. Patients with HT had significant lower serum magnesium levels than those without HT (0.81 ± 0.08 vs. 0.85 ± 0.08 mmol/L, $p = 0.007$). Multivariable logistic regression analysis indicated that patients with higher serum magnesium levels had lower risk of HT (OR per 0.1-mmol/L increase 0.43, 95% CI 0.27–0.73, $p = 0.002$). However, this association did not persist when baseline levels of serum magnesium were higher than the median value (0.85 mmol/L) in subgroup analysis (OR per 0.1-mmol/L increase 0.58, 95% CI 0.14–2.51, $p = 0.47$). This threshold effect was also observed in the restricted cubic spline model when serum magnesium levels were above 0.88 mmol/L. No association between symptomatic HT and serum magnesium levels was observed in our study (OR per 0.1-mmol/L increase 0.52, 95% CI 0.25–1.11, $p = 0.092$).

Conclusions: Lower serum magnesium levels in patients with ischemic stroke are associated with an increased risk of HT after intravenous thrombolysis, but perhaps only when serum magnesium is below a certain minimal concentration.

Keywords: magnesium, hemorrhagic transformation, thrombolysis, recombinant tissue plasminogen activator, acute ischemic stroke

OPEN ACCESS

Edited by:
Nishant K. Mishra, University of California, United States

Reviewed by:
Georgios Tsivgoulis, National and Kapodistrian University of Athens, Greece
Lina Palaiodimou, University General Hospital Attikon, Greece

***Correspondence:**
Zhao Han
wzhanzhao@aliyun.com

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *30 May 2020*
Accepted: *24 July 2020*
Published: *02 September 2020*

Citation:
Cheng Z, Huang X, Muse FM, Xia L, Zhan Z, Lin X, Cao Y and Han Z (2020) Low Serum Magnesium Levels Are Associated With Hemorrhagic Transformation After Thrombolysis in Acute Ischemic Stroke. Front. Neurol. 11:962. doi: 10.3389/fneur.2020.00962

INTRODUCTION

Intravenous thrombolysis (IVT) with recombinant tissue plasminogen activator (rt-PA) is the preferred treatment for acute ischemic stroke patients in super earlier period (≤4.5 h) (1). However, hemorrhagic transformation (HT) is common in patients with IVT, occurring as a consequence of coagulation dysfunction and blood-brain barrier (BBB) disruption induced by rt-PA (2, 3). Limited administration of thrombolytic drugs in stroke patients is largely due to the fear of HT. Since patients with HT are susceptible to early death or long-term disability (4, 5), it is imperative to identify the modifiable risk factors of HT after IVT.

Magnesium is an abundant endogenous neuroprotective agent that has close association with ischemic stroke (6). It also maintains the integrity of the vascular endothelial barrier through anti-inflammatory and anti-oxidation effects (7). Brain microvascular endothelium is the fundamental component of BBB and the initiation of ischemia-related BBB disruption is predominantly triggered by endothelial damage (8). Recent evidence indicates the protective effect of magnesium on BBB in rats with transient focal cerebral ischemia (9). In addition, magnesium is involved in the coagulation cascade (10, 11) and platelet activation (12); magnesium deficiency would lead to dysfunction of coagulation system. The relationship between serum magnesium levels and functional outcomes in patients with acute ischemic stroke has been widely studied. But two large-sample randomized controlled trials [the IMAGES (Intravenous Magnesium Efficacy in Stroke) trial and the FAST-MAG (Field Administration of Stroke Therapy–Magnesium) trial] showed regrettable results, which early intravenous magnesium sulfate therapy did not improve the outcomes for patients with acute stroke (13, 14). By contrast, there are very few studies on the association between serum magnesium levels and the occurrence of HT. These studies had inconsistent conclusions and did not specifically address patients with IVT (15, 16). Thus, we investigated the association of serum magnesium levels with development of HT in patients with acute ischemic stroke after IVT.

METHODS

Patients

This retrospective, observational, single-center study was conducted at the Second Affiliated Hospital of the Wenzhou Medical University, China. All ischemic stroke patients who received IVT at the Department of Neurology between January 2015 and January 2020 were evaluated for eligibility. All the included patients received a confirmed diagnosis of acute ischemic stroke via magnetic resonance imaging (MRI) or computed tomography (CT), and received rt-PA infusion of 0.9 mg/kg (a maximum of 90 mg) on arrival in the emergency room. We excluded patients with no available data on baseline serum magnesium levels or no follow-up CT images within 24–36 h after IVT. This study was approved by the Ethics Committee of the Second Affiliated Hospital and Yuying Children's Hospital of the Wenzhou Medical University. Written informed consent was exempted for the retrospective nature of the study.

Data Collection

From the medical records, we obtained demographic data (age and sex), baseline clinical parameters [systolic and diastolic blood pressure, blood glucose level, international normalized ratio (INR), activated partial thromboplastin time (APTT), platelet count, serum magnesium, and serum calcium levels, National Institutes of Health Stroke Scale (NIHSS) score, onset-to-treatment time, and current antithrombotic therapy], data on vascular risk factors (hypertension, diabetes, hyperlipidemia, atrial fibrillation, coronary heart disease, history of stroke, current smoking, and current drinking), and acceptance of bridging therapy, as well as additional laboratory data [total cholesterol, low-density lipoprotein cholesterol (LDL-C), and HbA1c]. All patients underwent CT scanning during admission, as well as within 24–36 h after IVT. Antithrombotic agents were administered to all patients only after a follow-up CT was conducted.

Assessment of Hemorrhagic Transformation

All CT images were retrospectively evaluated by two experienced neurologists who were blinded to patients' clinical data, and disagreements were settled by further discussion. Based on the European Cooperative Acute Stroke Study (ECASS) criteria, we classified HT into hemorrhagic infarctions (HI 1 or 2) and parenchymal hemorrhage (PH 1 or 2) (17). Symptomatic hemorrhagic transformation (sHT) was defined as HT accompanied by neurological deterioration (18).

Statistical Analysis

Continuous variables are presented as mean and standard deviations in the case of normally distributed data, or medians (interquartile range) in the case of skewed data. Differences in continuous variables were analyzed using unpaired *t*-tests or Mann–Whitney *U*-tests. Categorical variables are presented as percentages. Inter-group differences were analyzed using Pearson's chi-square test or Fisher's exact probabilities test. Measured serum magnesium levels were collapsed into quartiles, and the first quartile values were used as the reference category for the logistic regression analysis. To examine a potential independent association between serum magnesium levels and development of HT, two multivariate logistic regression models were evaluated: model 1 adjusted for all variables with $p < 0.1$ in the univariate analysis, while model 2 tested the variables from model 1 along with other potential risk factors of HT (age, hypertension, and baseline blood glucose levels), identified based on the literature (19). Serum magnesium concentrations were entered into the models in two formats, as a continuous variable (in which case OR was calculated per 0.1 mmol/L increase) or as a four-categorized variable (in which case OR was calculated compared to the first quartiles).

A subgroup analysis was conducted after stratifying the patients using the median value of baseline serum magnesium levels as a cut-off. In addition, we used the R package "rms" to

construct restricted cubic splines with three knots to understand patterns in the association between serum magnesium levels and HT, using the median value of baseline serum magnesium as a reference point (20). All statistical tests were two-tailed, and $p < 0.05$ was considered statistically significant. All analyses were performed using SPSS 25.0 (IBM, Armonk, NY, USA) and R 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

A total of 268 acute ischemic stroke patients who received IVT treatment were screened for eligibility. After excluding patients with no available data on baseline serum magnesium levels ($n = 21$) or follow-up CTs ($n = 5$), we analyzed clinic data from 242 patients to determine the effect of serum magnesium levels on the development of HT. Mean patient age was 68.6 ± 14.1 years, and 154 (63.6%) were men. Among the included patients, 43 (17.8%) presented with HT, comprising 15 (6.2%) with HI 1, 13 (5.4%) with HI 2, 10 (4.1%) with PH 1, and 5 (2.1%) with PH 2. Of these 43 patients, 13 (5.4%) developed sHT.

The average concentration of serum magnesium across all patients was 0.84 ± 0.08 mmol/L. Serum magnesium levels in patients who developed HT were significantly lower than levels in those who did not develop HT (0.81 ± 0.08 vs. 0.85 ± 0.08 mmol/L, $p = 0.007$, **Table 1**). Patients with HT also had a significantly higher incidence of atrial fibrillation and bridging therapy, higher INRs and NIHSS scores, and lower LDL-C levels than those without HT (**Table 1**). Multivariate logistic regression showed that, after adjusting for potential confounding factors, serum magnesium levels had an independent negative relationship with HT risk (OR per 0.1-mmol/L increase 0.43, 95% CI 0.27–0.73, $p = 0.002$; **Table 2**). Patients in the third and fourth serum magnesium quartiles showed a significant decline in HT risk (OR 0.18, 95% CI 0.05–0.60, $p = 0.005$; OR 0.33, 95% CI 0.12–0.92, $p = 0.035$; respectively) compared to those in the first quartile.

Patients were then stratified based on the median serum magnesium level of 0.85 mmol/L. Subgroup analysis showed a negative association between serum magnesium levels and HT risk when the levels were below the median value (OR per 0.1-mmol/L increase 0.33, 95% CI 0.12–0.93, $p = 0.037$), but no association when serum magnesium levels were above the median value (OR per 0.1-mmol/L increase 0.58, 95% CI 0.14–2.51, $p = 0.47$; **Table 3**). The restricted cubic spline model corroborated the significant negative linear relationship between serum magnesium and HT, but also showed that ample serum magnesium levels did not further lead to a reduction in HT risk [the reference line (OR = 1) cross the corresponding 95% CI of OR when serum magnesium levels were higher than 0.88 mmol/L, **Figure 1**].

Based on the univariate analysis, we found that patients with sHT had insignificantly lower serum magnesium levels compared to those who did not develop sHT (0.81 ± 0.10 vs. 0.84 ± 0.08 mmol/L, $p = 0.21$; **Table S1**). After adjusting for confounding factors, our analysis showed that high serum magnesium levels did not lead to a significant decrease in sHT risk (OR per 0.1-mmol/L increase 0.52, 95% CI 0.25–1.11, $p = 0.092$; **Table S2**).

TABLE 1 | Clinical characteristics of thrombolytic patients, stratified by the development of HT.

	No HT ($n = 199$)	HT ($n = 43$)	*P*
Age in years, mean ± SD	68.1 ± 14.2	70.8 ± 13.6	0.25
Female, *n* (%)	71 (35.7%)	17 (39.5%)	0.63
Hypertension, *n* (%)	151 (75.9%)	34 (79.1%)	0.66
Diabetes mellitus, *n* (%)	59 (29.6%)	13 (30.2%)	0.94
Hyperlipidemia, *n* (%)	81 (40.7%)	14 (32.6%)	0.32
Atrial fibrillation, *n* (%)	53 (26.6%)	27 (62.8%)	**<0.001**
Coronary artery disease, *n* (%)	18 (9.0%)	6 (14.0%)	0.33
History of stroke, *n* (%)	24 (12.1%)	7 (16.3%)	0.45
Current Smoking, *n* (%)	47 (23.6%)	11 (25.6%)	0.78
Current Drinking, *n* (%)	36 (18.1%)	9 (20.9%)	0.66
Current antithrombotic therapy, *n* (%)	28 (14.1%)	11 (25.6%)	0.063
OTT in min, median (IQR)	169 (125–210)	180 (125–205)	0.84
Baseline NIHSS score, median (IQR)	8 (4–13)	13 (8–19)	**<0.001**
Baseline SBP in mm Hg, mean ± SD	156.6 ± 21.2	156.9 ± 23.1	0.93
Baseline DBP in mm Hg, mean ± SD	87.2 ± 15.3	89.5 ± 16.9	0.37
Baseline blood glucose in mmol/L, median (IQR)	6.91 (5.95–8.98)	7.27 (6.47–9.69)	0.10
Platelet count in 10^9/L, median (IQR)	191 (167–238)	190 (154–219)	0.24
INR, median (IQR)	1.03 (0.99–1.09)	1.07 (1.04–1.14)	**<0.001**
APTT in sec, median (IQR)	33.8 (30.6–36.6)	33.3 (31.7–36.0)	0.94
Serum magnesium in mmol/L, mean ± SD	0.85±0.08	0.81±0.08	**0.007**
Serum calcium in mmol/L, mean ± SD	2.25±0.11	2.22±0.11	0.081
HbA1c in %, median (IQR)	5.90 (5.50–6.50)	6.07 (5.70–6.70)	0.40
TC in mmol/L, median (IQR)	4.40 (3.79–5.10)	4.11 (3.53–4.76)	0.12
LDL_C in mmol/L, median (IQR)	2.60 (2.07–3.32)	2.35 (1.84–2.75)	**0.033**
Bridge therapy, *n* (%)	17 (8.5%)	8 (18.6%)	**0.049**

APTT, activated partial thromboplastin time; DBP, diastolic blood pressure; HT, hemorrhagic transformation; INR, international normalized ratio; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; NIHSS, National Institutes of Health Stroke Scale; OTT, onset-to-treatment time; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol. The bold values represent significant results in univariate analysis (P < 0.05).

Serum magnesium levels were similar between HI and PH (0.81 ± 0.08 vs. 0.81 ± 0.09 mmol/L, $p = 0.87$).

DISCUSSION

This retrospective study evaluated the association between serum magnesium levels and occurrence of HT in 242 acute ischemic stroke patients after IVT treatment. Our findings indicate that there is a significant association between low serum magnesium levels at admission and an increased risk of developing HT within 24–36 h after IVT. However, this negative relationship does not appear to hold when serum magnesium levels are above a certain minimum.

How serum magnesium concentrations influence the risk of HT in stroke patients after IVT remains unclear. The reason may relate to the role of magnesium in neuroprotection and hemostasis. Rt-PA can cause post-stroke HT by inducing oxidative stress that further compromise the BBB (3), and magnesium-deficiency would lead to enhanced permeability of the cerebrovascular endothelial barrier as proved in mouse models (7). Intraperitoneal injections with magnesium sulfate in normal mice reduced histamine-induced endothelial hyperpermeability mainly by suppressing oxidative stress and inflammatory responses, while up-regulating endothelial barrier-stabilizing mediators such as cyclic adenosine monophosphate (7). In rats subjected to transient focal cerebral ischemia, magnesium showed the ability to protect BBB integrity by increasing the activity of anti-oxidant enzymes and decreasing lipid peroxide levels (9). Another pivotal pathogenesis of HT after IVT is rt-PA induced coagulopathy (2, 21). Magnesium can shorten prothrombin time by accelerating the activation of factor X through the factor IXa- and factor VIIa-tissue factor-mediated pathways (10, 22). Magnesium also promotes the adherence of platelets to collagen, independent of platelet activation, and secretion (12). In patients with intracerebral or subarachnoid hemorrhage, magnesium shows hemostatic properties: serum magnesium levels are negatively associated with hematoma volume (23–25).

Similarly to our study, an increase in risk of HT in patients with low serum magnesium levels was observed in another Chinese patient sample (16). Differently, thrombolytic patients were not included, besides association between adequate serum magnesium levels and HT was not analyzed in their study (16). Our finding that the association between serum magnesium levels and the occurrence of HT after thrombolysis disappeared at serum magnesium concentrations ≥0.88 mmol/L may reflect a threshold effect for the capacity of magnesium to prevent HT. In fact, Dong et al. (26) found that magnesium, at physiological levels, could maintain integrity for human endothelial cells *in vitro*, while extra magnesium did not bring about an increase in integrity. This suggests that the maximal protective effect of

TABLE 2 | Multiple logistic regression analysis to identify relationships between serum magnesium levels and risk of hemorrhagic transformation after thrombolysis.

	Model 1		Model 2	
	OR (95% CI)	***P***	**OR (95% CI)**	***P***
Serum magnesium, per 0.1-mmol/L increase	0.44 (0.27–0.73)	0.001	0.43 (0.27–0.73)	0.002
Serum magnesium, quartiles				
Q1 (<0.80 mmol/L)	Reference		Reference	
Q2 (0.80–0.84 mmol/L)	0.40 (0.15–1.04)	0.060	0.45 (0.17–1.23)	0.12
Q3 (0.85–0.90 mmol/L)	0.32 (0.12–0.88)	0.029	0.33 (0.12–0.92)	0.035
Q4 (> 0.90 mmol/L)	0.18 (0.06–0.58)	0.004	0.18 (0.05–0.60)	0.005
P for trend	0.002		0.003	

Model 1: adjusted for atrial fibrillation, ongoing antithrombotic therapy, baseline NIHSS score, INR, serum calcium (per 0.1-mmol/L increase), LDL-C, and bridging therapy.
Model 2: adjusted for the variables in model 1, as well as age, hypertension, and baseline blood glucose levels.
CI, Confidence interval; INR, international normalized ratio; LDL-C, low-density lipoprotein cholesterol; NIHSS, National Institutes of Health Stroke Scale; OR, Odds ratio.

TABLE 3 | Stratified logistic regression analysis to identify relationships between serum magnesium levels and risk of hemorrhagic transformation after thrombolysis.

	OR (95% CI)	***P***
Serum magnesium, per 0.1-mmol/L increase		
<median (< 0.85 mmol/L)	0.33 (0.12–0.93)	0.037
≥median (≥ 0.85 mmol/L)	0.58 (0.14–2.51)	0.47

*The model was adjusted for the same confounding factors as in model 1 in **Table 2**.*
CI, Confidence interval; OR, Odds ratio.

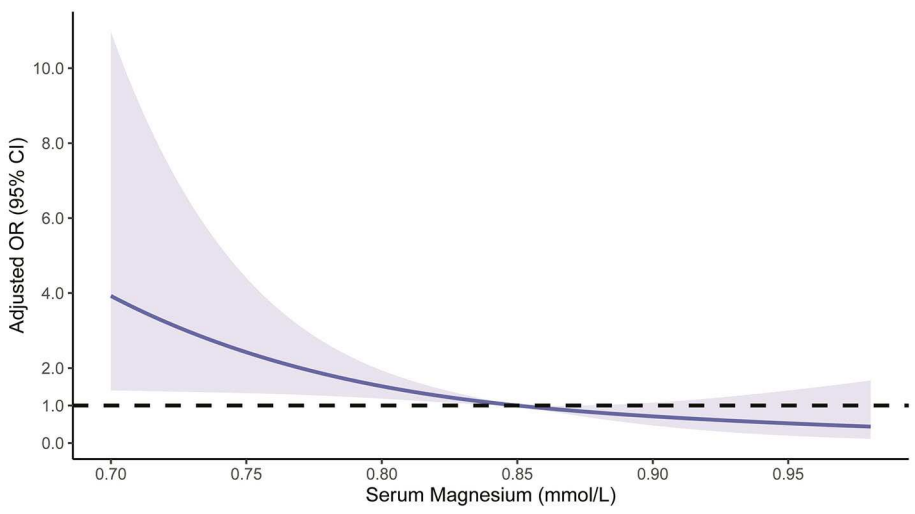


FIGURE 1 | Restricted cubic spline regression model of the relationship between serum magnesium levels and risk of hemorrhagic transformation. The solid blue line represents the odds ratio (OR), and the blue shading depicts the corresponding 95% confidence interval (CI). The dotted black line is the reference line for which OR = 1. The model was adjusted for the same confounding variables as in model 1 in **Table 2**.

magnesium on the BBB may appear within the physiological concentration range. Moreover, although magnesium is an essential component to the coagulation system, *in vitro* studies suggest that excessive magnesium levels may inversely prolong prothrombin time (10) and inhibit human platelet function (26, 27). Decreased platelet activity and increased bleeding time have also been observed in healthy volunteers given intravenous magnesium sulfate, accompanied by an elevation in mean serum magnesium concentrations from 0.85 to 1.50 mmol/L (28). These findings are consistent with our results, suggesting that the risk of HT does not decline further after serum magnesium levels exceed a certain value.

Our findings imply that magnesium supplementation may reduce risk of HT in patients undergoing IVT, at least up to a certain point. By contrast, the IMAGES and FAST-MAG clinical trials showed that magnesium sulfate treatment did not improve outcomes for stroke patients (13, 14). Researchers attributed the failure to the delay of magnesium in crossing the BBB, so that the concentration did not accumulate immediately in brain tissues (14). It is noteworthy that the patients included in both their studies were not only ischemic stroke, but hemorrhagic stroke and stroke mimics, besides only part of ischemic stroke patients received thrombolytic therapy. Also, Patients were randomized to receive intravenous magnesium sulfate or placebo regardless of the baseline serum magnesium levels. Therefore, it is still necessary to explore whether magnesium supplementation therapy can improve the prognosis of thrombolytic patients with low serum magnesium levels, which is mediated by the protective effect on preventing HT.

In our study, no statistically significant reduction of serum magnesium levels was observed in patients with sHT. This is different from the positive findings that serum magnesium levels affect the risk of HT after thrombolysis. A reasonable explanation is that low serum magnesium levels are associated with larger infarct volume (9, 29). Thus, subsequent hemorrhage in ischemic lesions are not sufficient to cause further deterioration of neurological function. Nonetheless, the negative result in our study may also be a type II error caused by our small sample (30). Among FAST-MAG patients, only a portion of whom was given thrombolysis, sHT was less frequent in those treated with magnesium sulfate than those who receive placebo (2.1 vs. 3.3%, $p = 0.12$) (14). Although *post-hoc* analysis of the FAST-MAG trial concluded that serum magnesium levels measured within 24 h after IVT were not associated with sHT (15), it should be noted that the sHT and non-sHT patients in their study showed higher average serum magnesium levels than physiological concentrations, and the neutral findings may result from that the additional magnesium did not further affect risk of sHT because of the threshold effect mentioned above. Given adverse outcomes of sHT are quite serious, further studies are needed to verify whether a relationship exists between serum magnesium and risk of sHT.

There are some limitations to this study. Our results should be interpreted with caution since we measured total magnesium rather than ion magnesium, which plays direct physiological roles. The single-center, retrospective design, and limited sample size restricted our ability to identify the effects of serum magnesium levels on patients affected by HT, especially sHT. The relationship between serum magnesium and sHT remains unclear. Additionally, we could not evaluate and correct for potentially confounding neuroimaging metrics such as ischemic core volume before thrombolysis and cerebral microbleeds. Regrettably, due to the observational nature of this study, serum magnesium concentrations beyond the upper normal limit were not obtained and the efficacy and safety of excess magnesium supplementation for thrombolytic patients could not be fully evaluated.

Despite these limitations, our results suggest that lower baseline serum magnesium levels are associated with increased risk of HT in acute ischemic stroke patients after IVT, but this association does not appear to hold when serum magnesium reaches a certain physiological concentration. Further randomized controlled trials are needed to determine whether early initiation of magnesium supplementation therapy in thrombolytic patients with low serum magnesium levels can reduce their risk of HT and, subsequently, improve their prognosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee of the Second Affiliated Hospital and Yuying Children's Hospital of the Wenzhou Medical University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ZH and ZC conceived and designed the study. All authors acquired the data, which ZC and FM analyzed. ZC, ZH, and XH assisted in data interpretation and wrote the manuscript. All authors participated in revising the article and approved the final version.

FUNDING

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81771267 and 81571114) and Clinical Scientific Research Foundation of the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University (SAHoWMU-CR 2017-01-212).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00962/full#supplementary-material

REFERENCES

1. Emberson J, Lees KR, Lyden P, Blackwell L, Albers G, Bluhmki E, et al. Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. *Lancet*. (2014) 384:1929–35. doi: 10.1016/S0140-6736(14)60584-5
2. Yaghi S, Eisenberger A, Willey JZ. Symptomatic intracerebral hemorrhage in acute ischemic stroke after thrombolysis with intravenous recombinant tissue plasminogen activator: a review of natural history and treatment. *JAMA Neurol*. (2014) 71:1181–5. doi: 10.1001/jamaneurol.2014.1210
3. Wang W, Li M, Chen Q, Wang J. Hemorrhagic transformation after tissue plasminogen activator reperfusion therapy for ischemic stroke: mechanisms, models, and biomarkers. *Mol Neurobiol*. (2015) 52:1572–9. doi: 10.1007/s12035-014-8952-x
4. Dzialowski I, Pexman JH, Barber PA, Demchuk AM, Buchan AM, Hill MD, et al. Asymptomatic hemorrhage after thrombolysis may not be benign: prognosis by hemorrhage type in the Canadian alteplase for stroke effectiveness study registry. *Stroke*. (2007) 38:75–9. doi: 10.1161/01.STR.0000251644.76546.62
5. Park JH, Ko Y, Kim WJ, Jang MS, Yang MH, Han MK, et al. Is asymptomatic hemorrhagic transformation really innocuous? *Neurology*. (2012) 78:421–6. doi: 10.1212/WNL.0b013e318245d22c
6. Kirkland AE, Sarlo GL, Holton KF. The role of magnesium in neurological disorders. *Nutrients*. (2018) 10:730. doi: 10.3390/nu10060730
7. Zhu D, You J, Zhao N, Xu H. Magnesium regulates endothelial barrier functions through TRPM7, MagT1, and S1P1. *Adv Sci.* (2019) 6:1901166. doi: 10.1002/advs.201901166
8. Krueger M, Bechmann I, Immig K, Reichenbach A, Hartig W, Michalski D. Blood-brain barrier breakdown involves four distinct stages of vascular damage in various models of experimental focal cerebral ischemia. *J Cereb Blood Flow Metab*. (2015) 35:292–303. doi: 10.1038/jcbfm.2014.199
9. Shadman J, Sadeghian N, Moradi A, Bohlooli S, Panahpour H. Magnesium sulfate protects blood-brain barrier integrity and reduces brain edema after acute ischemic stroke in rats. *Metab Brain Dis*. (2019) 34:1221–9. doi: 10.1007/s11011-019-00419-y
10. van den Besselaar AM. Magnesium and manganese ions accelerate tissue factor-induced coagulation independently of factor IX. *Blood Coagul Fibrinolysis*. (2002) 13:19–23. doi: 10.1097/00001721-200201000-00003
11. Messer AS, Velander WH, Bajaj SP. Contribution of magnesium in binding of factor IXa to the phospholipid surface: implications for vitamin K-dependent coagulation proteins. *J Thromb Haemost*. (2009) 7:2151–3. doi: 10.1111/j.1538-7836.2009.03634.x
12. Santoro SA. Identification of a 160,000 dalton platelet membrane protein that mediates the initial divalent cation-dependent adhesion of platelets to collagen. *Cell*. (1986) 46:913–20. doi: 10.1016/0092-8674(86)90073-5
13. Muir KW, Lees KR, Ford I, Davis S, Intravenous Magnesium Efficacy in Stroke Study I. Magnesium for acute stroke (intravenous magnesium efficacy in stroke trial): randomised controlled trial. *Lancet*. (2004) 363:439–45. doi: 10.1016/S0140-6736(04)15490-1
14. Saver JL, Starkman S, Eckstein M, Stratton SJ, Pratt FD, Hamilton S, et al. Prehospital use of magnesium sulfate as neuroprotection in acute stroke. *N Engl J Med*. (2015) 372:528–36. doi: 10.1056/NEJMoa1408827
15. Shkirkova K, Starkman S, Sanossian N, Eckstein M, Stratton S, Pratt F, et al. Paramedic initiation of neuroprotective agent infusions: successful achievement of target blood levels and attained level effect on clinical outcomes in the FAST-MAG pivotal trial (field administration of stroke therapy - magnesium). *Stroke*. (2017) 48:1901–7. doi: 10.1161/STROKEAHA.116.015664
16. Tan G, Yuan R, Wei C, Xu M, Liu M. Serum magnesium but not calcium was associated with hemorrhagic transformation in stroke overall and stroke subtypes: a case-control study in China. *Neurol Sci*. (2018) 39:1437–43. doi: 10.1007/s10072-018-3445-8
17. Hacke W, Kaste M, Fieschi C, Toni D, Lesaffre E, von Kummer R, et al. Intravenous thrombolysis with recombinant tissue plasminogen activator for acute hemispheric stroke. The european cooperative acute stroke study (ECASS). *JAMA*. (1995) 274:1017–25. doi: 10.1001/jama.274.13.1017
18. National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med*. (1995) 333:1581–8. doi: 10.1056/NEJM199512143332401
19. Alvarez-Sabin J, Maisterra O, Santamarina E, Kase CS. Factors influencing haemorrhagic transformation in ischaemic stroke. *Lancet Neurol*. (2013) 12:689–705. doi: 10.1016/S1474-4422(13)70055-3
20. Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med*. (1989) 8:551–61. doi: 10.1002/sim.4780080504
21. Lee VH, Conners JJ, Cutting S, Song SY, Bernstein RA, Prabhakaran S. Elevated international normalized ratio as a manifestation of post-thrombolytic coagulopathy in acute ischemic stroke. *J Stroke Cerebrovasc Dis*. (2014) 23:2139–44. doi: 10.1016/j.jstrokecerebrovasdis.2014.03.021
22. van den Besselaar AMHP, van Dam W, Sturk A, Bertina RM. Prothrombin time ratio is reduced by magnesium contamination in evacuated blood collection tubes. *Thromb Haemostasis*. (2001) 85:647–50. doi: 10.1055/s-0037-1615647
23. Liotta EM, Prabhakaran S, Sangha RS, Bush RA, Long AE, Trevick SA, et al. Magnesium, hemostasis, and outcomes in patients with intracerebral hemorrhage. *Neurology*. (2017) 89:813–9. doi: 10.1212/WNL.0000000000004249
24. Goyal N, Tsivgoulis G, Malhotra K, Houck AL, Khorchid YM, Pandhi A, et al. Serum magnesium levels and outcomes in patients with acute spontaneous intracerebral hemorrhage. *J Am Heart Assoc*. (2018) 7:e008698. doi: 10.1161/JAHA.118.008698
25. Liotta EM, Karmarkar A, Batra A, Kim M, Prabhakaran S, Naidech AM, et al. Magnesium and hemorrhage volume in patients with aneurysmal subarachnoid hemorrhage. *Crit Care Med*. (2020) 48:104–10. doi: 10.1097/CCM.0000000000004079
26. Dong JF, Cruz MA, Aboulfatova K, Martin C, Choi H, Bergeron AL, et al. Magnesium maintains endothelial integrity, up-regulates proteolysis of ultra-large von Willebrand factor, and reduces platelet aggregation under flow conditions. *Thromb Haemost*. (2008) 99:586–93. doi: 10.1160/TH07-11-0694
27. Sheu JR, Hsiao G, Shen MY, Fong TH, Chen YW, Lin CH, et al. Mechanisms involved in the antiplatelet activity of magnesium in human platelets. *Br J Haematol*. (2002) 119:1033–41. doi: 10.1046/j.1365-2141.2002.03967.x
28. Ravn HB, Vissinger H, Kristensen SD, Wennmalm A, Thygesen K, Husted SE. Magnesium inhibits platelet activity—an infusion study in healthy volunteers. *Thromb Haemost*. (1996) 75:939–44. doi: 10.1055/s-0038-1650398
29. Demougeot C, Bobillier-Chaumont S, Mossiat C, Marie C, Berthelot A. Effect of diets with different magnesium content in ischemic stroke rats. *Neuroscie Lett*. (2004) 362:17–20. doi: 10.1016/j.neulet.2004.01.034
30. Akobeng AK. Understanding type I and type II errors, statistical power and sample size. *Acta Paediatr*. (2016) 105:605–9. doi: 10.1111/apa.13384

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 07 October 2020
doi: 10.3389/fneur.2020.583411

The Role of Dual Energy CT in Evaluating Hemorrhagic Complications at Different Stages After Thrombectomy

Keqin Liu[1†], *Lin Jiang*[1†], *Jie Ruan*[1], *Wenqing Xia*[1], *Huan Huang*[1], *Guozhong Niu*[1], *Shenqiang Yan*[2*] *and Congguo Yin*[1*]

[1] *Department of Neurology, Hangzhou First Hospital, Zhejiang University School of Medicine, Hangzhou, China,* [2] *Department of Neurology, School of Medicine, The 2nd Affiliated Hospital of Zhejiang University, Hangzhou, China*

Background: Contrast media extravasation can mimic hemorrhage after endovascular thrombectomy (EVT). Dual energy CT (DECT) has the potential to distinguish hemorrhage from iodine contrast.

Methods: We retrospectively examined clinical and radiological data from 106 consecutive acute ischemic stroke patients who received EVT and underwent DECT immediately and 24 h after EVT. Iodine overlay map, virtual non-contrast, and mixed images are reconstructed.

Results: With the use of DECT, the proportion of all patients diagnosed with hemorrhagic transformation on mixed images immediately after EVT was reduced from 74.5% (79 of 106) to 10.4% (11 of 106), with a very poor consistency ($\kappa = 0.076$, $p = 0.041$). Correspondingly, hemorrhagic transformation on mixed images 24 h after EVT was reduced from 41.5% (44 of 106) to 30.2% (32 of 106), with a moderate consistency ($\kappa = 0.757$, $p < 0.001$).

Conclusions: The use of DECT both immediately and 24 h after EVT changes the diagnosis of hemorrhagic transformation in a considerable proportion of acute ischemic stroke patients with EVT. This could affect decision making with respect to antithrombotic strategy.

Keywords: dual energy CT (DECT), ischemic stroke, thrombectomy, hemorrhagic transformation (HT), contrast staining

OPEN ACCESS

Edited by:
Bruce Campbell,
The University of Melbourne, Australia

Reviewed by:
Julian Maingard,
Monash Health, Australia
Hamed Asadi,
University of Melbourne, Australia

****Correspondence:***
Congguo Yin
yincg716@aliyun.com
Shenqiang Yan
shenqiangyan@zju.edu.cn

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *14 July 2020*
Accepted: *03 September 2020*
Published: *07 October 2020*

Citation:
Liu K, Jiang L, Ruan J, Xia W, Huang H, Niu G, Yan S and Yin C (2020) The Role of Dual Energy CT in Evaluating Hemorrhagic Complications at Different Stages After Thrombectomy. Front. Neurol. 11:583411. doi: 10.3389/fneur.2020.583411

INTRODUCTION

Intravenous thrombolysis (IVT) is an effective and broadly applicable treatment for acute ischemic stroke (1). One of the most important complications of IVT is intracranial hemorrhagic transformation, which is associated with poor outcome and even death (2). Recently, the benefit of endovascular thrombectomy (EVT) has also been established in acute ischemic stroke patients with large artery occlusion (3). Both IV iodine contrast during advanced imaging and intra-arterial injection of iodine contrast during the EVT procedure can lead to contrast staining due to blood-brain barrier breakdown; as a consequence, hyperdense areas were frequently detected on non-contrast CT immediately after EVT (4). Early differentiation between hemorrhage and

contrast staining is important for clinical decision making, such as the use of glycoprotein IIb/IIIa inhibitor (tirofiban) in some patients with high risk of early reocclusion after EVT (5), or initiation of secondary preventive treatment with antiplatelet or anticoagulant agents after 24 h (6).

The use of dual energy CT (DECT) might change the radiologic report regarding post-treatment hemorrhagic transformation in a considerable proportion of patients with EVT compared to conventional non-contrast CT (7). In DECT, iodine overlay map (IOM) and virtual non-contrast (VNC) images are reconstructed from two different X-ray spectra at different kilovoltage (kV) either from one X-ray source using kV switching or from two X-ray sources (8, 9). The differentiation between hemorrhage and contrast medium became feasible since the attenuation characteristics of iodine and blood are different at two energy levels.

However, there are few studies trying to verify the diagnostic confidence in differentiation between hemorrhage and contrast medium extravasation after EVT, and to evaluate the clinical value of DECT at different stages. Therefore, in the current study, we aimed to (1) investigate how DECT immediately after EVT changes the diagnosis of hemorrhagic transformation and compare its radiologic report with follow-up CT of 24 h and (2) investigate how DECT 24 h after EVT changes the diagnosis of hemorrhagic transformation and compare its radiologic report with a follow-up CT of 3 days.

METHODS AND MATERIALS

Study Subjects

We retrospectively reviewed our prospectively collected database for consecutive patients with acute ischemic stroke received EVT between January 2016 and October 2018. We then enrolled patients who (i) underwent DECT immediately after EVT; (ii) underwent DECT 24 h after EVT; (iii) underwent conventional non-contrast CT 3 days after EVT. We excluded patients who had recent previous ischemic stroke within 3 months to avoid any potential intracranial hemorrhage and contrast staining findings related to subacute blood-brain barrier breakdown.

Study Protocols

DECT was performed immediately and 24 h after EVT, and conventional non-contrast CT was performed 3 days after EVT. DECT images were acquired with a dual source 128 slice CT scanner (SOMATOM Force, Siemens Healthcare, Forchheim, Germany). Acquisition and reconstruction of CT parameters were as follows: a dedicated dual-source protocol with simultaneous imaging at 80 kV/392 mAs eff. and 140 kV(Sn)/196 mAs eff., collimation of 0.6 mm and pitch of 0.7 was employed. The raw spiral projection data were rebuilt in three different series, with two sets corresponding to 80 and 140 kV (0.6 mm slice thickness) and a third set corresponding to a mixed map of both energies (80/140 kV), simulating a conventional 120 kV CT. VNC images and IOM were calculated using a dedicated brain hemorrhage algorithm (Syngo; CT Dual-Energy Brain Hemorrhage; Siemens) (**Figure 1**).

Image Analysis

Hemorrhagic transformation was classified by using the following radiological criteria: hemorrhagic infarction (HI, including HI-1 and HI-2), parenchymal hemorrhage (PH, including PH-1 and PH-2) (10). DECT images were evaluated for the diagnosis and grading of hemorrhagic transformation. Definitions of contrast material extravasation and intracranial hemorrhage on DECT were previously described in detail elsewhere (11). Mixed images of DECT and conventional non-contrast CT images were evaluated for the presence of hyperdense areas. Hyperdensities were defined as areas with objective higher density than the surrounding brain parenchyma. Hyperdensities visible on mixed images of DECT were interpreted as hemorrhage, contrast extravasation, or both. The interpretations of DECT were compared with conventional non-contrast CT 3 days after EVT. Washout or near-complete clearing of the hyperdensities on follow-up CT was classified as contrast material extravasation, while persisting hyperdensities on follow-up CT were classified as hemorrhage.

Two neurologists with 10 years of experience with acute stroke imaging (KL & LJ) separately reviewed first the mixed images alone and, in a second reading, the IOM and VNC images. Disagreement was resolved by consensus, and in cases with remaining disagreement, the final decision was made by an interventional neurologist (CY).

Statistical Analysis

The consensus judgment of DECT and conventional non-contrast CT was used as reference. Categorical variables were presented as number and percentage. Continuous variables were summarized as mean ± SD or median with interquartile range (IQR). Significance of difference between proportions was calculated with the Pearson χ^2 or Fisher's exact test. All analyses were performed blind to participant identifying information.

RESULTS

Inter-reader agreement of any hemorrhagic transformation immediately after EVT had a κ value of 0.79 on mixed images, and a κ value of 0.76 on VNC images, respectively. Inter-reader agreement of any hemorrhagic transformation 24 h after EVT had a κ value of 0.81 on mixed images, and a κ value of 0.73 on VNC images, respectively. Inter-reader agreement of any hemorrhagic transformation 3 days after EVT had a κ value of 0.71 on conventional non-contrast CT.

A total of 106 remaining patients were included for the final analysis. Of the patients included, 39 (36.8%) were women, with a median age of 74 years (mean 71.6 ± 10.3 years, range 27–86 years). Mean time from onset to puncture was 291.5 (237.3–367.0) min, and mean time from puncture to reperfusion was 50.5 (40.0–82.3) min. The detailed clinical data and demographics are shown in **Table 1**.

Based on the IOM and VNC images of DECT immediately after EVT (**Table 2**), 11 patients (10.4%) were classified as hemorrhagic transformation, and all of them were mixed with iodine contrast. In all, 68 patients (64.2%) were classified as pure iodine contrast, while the remaining 27 patients (25.5%) showed

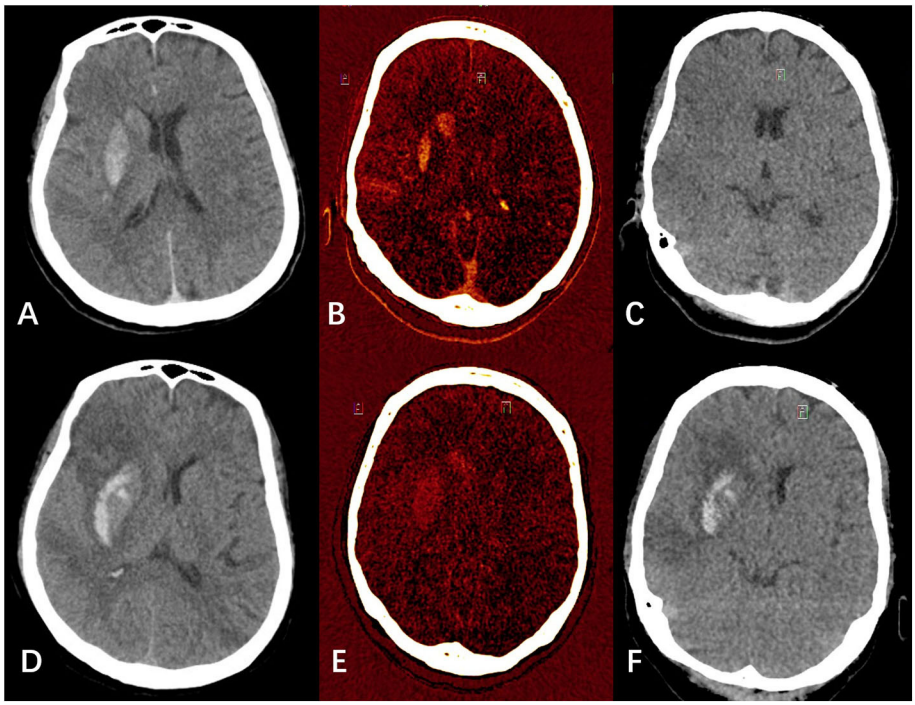


FIGURE 1 | Examples of hemorrhagic transformation and contrast extravasation with iodine overlay map (IOM), virtual non-contrast (VNC), and mixed images. **(A–C)** were mixed image, IOM, and VNC, respectively from a patient's dual energy CT (DECT) immediately after endovascular thrombectomy (EVT). **(A)** showed hyperdensities in the right lentiform nucleus and caudate nucleus. In **(B,C)** combined, the hyperdensities were classified as pure iodine contrast. **(D–F)** were mixed image, IOM, and VNC, respectively from the same patient's DECT 24 h after EVT. **(D)** also showed hyperdensities in the right lentiform nucleus and caudate nucleus. In **(E,F)** combined, the hyperdensities were classified as hemorrhage with iodine contrast.

TABLE 1 | Clinical characteristics ($n = 106$).

Variable	Mean ± SD or median (IQR) or *n* (%)
Age (year)	71.6 ± 10.3
Female, *n* (%)	39 (36.8%)
Co-morbid conditions	
Hypertension, *n* (%)	61 (57.5%)
Diabetes mellitus, *n* (%)	13 (12.3%)
Hyperlipidemia, *n* (%)	14 (13.2%)
Atrial fibrillation, *n* (%)	48 (45.3%)
Smoking, *n* (%)	20 (18.9%)
Prior stroke or TIA, *n* (%)	11 (10.4%)
Clinical variables	
NIHSS score	17 (14–20)
Intravenous thrombolysis, *n* (%)	29 (27.4%)
Anterior circulation stroke, *n* (%)	90 (84.9%)
Onset to puncture (min)	291.5 (237.3–367.0)
Puncture to reperfusion (min)	50.5 (40.0–82.3)
Poor clinical outcome, mRS ≥3	51 (48.1%)

TIA = transient ischemic attack; NIHSS = National Institutes of Health Stroke Scale; mRS = modified Rankin Scale.

TABLE 2 | Interpretations of hyperdensities on DECT immediately and 24 h after EVT.

	Immediate DECT	24 h DECT
Any hemorrhagic transformation	11 (10.4%)	32 (30.2%)
Hemorrhage with iodine contrast	11 (100.0%)	17 (53.1%)
Hemorrhage without iodine contrast	0 (0.0%)	15 (46.9%)
Pure iodine contrast	68 (64.2%)	12 (11.3%)
No hyperdensities	27 (25.5%)	62 (58.5%)

DECT = dual energy CT; EVT = endovascular thrombectomy.

no hyperdensities. Based on the IOM and VNC images of DECT 24 h after EVT (**Table 2**), 32 patients (30.2%) were classified as hemorrhagic transformation, and 17 of them (53.1%) were mixed with iodine contrast. Twelve patients (11.3%) were classified as pure iodine contrast, while the remaining 62 patients (58.5%) showed no hyperdensities.

The 11 patients diagnosed with hemorrhage immediately after EVT (all mixed with iodine) were still shown as hemorrhage on both mixed images 24 h after EVT and non-contrast CT 3 days after EVT, while 5 of them (45.5%) had clearance of iodine contrast based on the DECT 24 h after EVT (**Figure 2**). The 27 patients with no hyperdensities immediately after EVT remained clean on both mixed images 24 h after EVT and non-contrast CT 3 days after EVT. In 68 patients classified as pure iodine contrast immediately after EVT, 35 of them (51.5%) showed no hyperdensities on mixed images 24 h after EVT, while 12 of them (17.6%) had clearance of iodine contrast on non-contrast CT 3

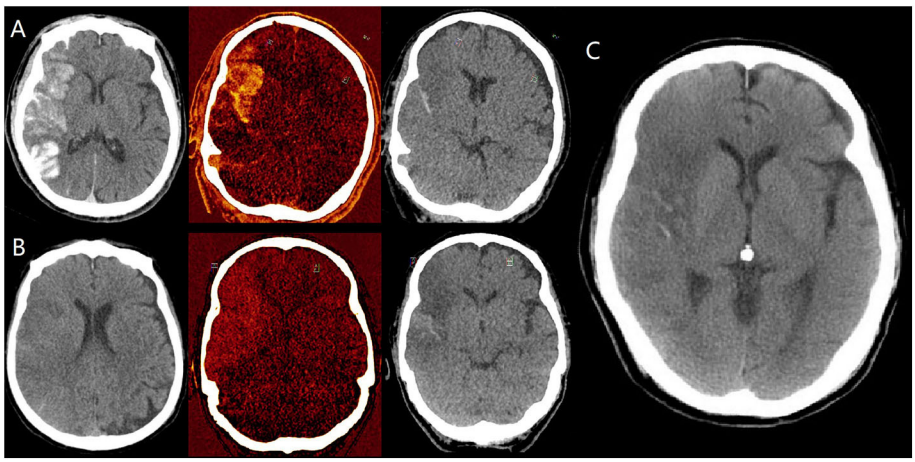


FIGURE 2 | Dynamic changes of hyperdensities in a patient diagnosed with hemorrhagic transformation (mixed with iodine) on dual energy CT (DECT) immediately after EVT. **(A)** are mixed images, iodine overlay map (IOM), virtual non-contrast (VNC) images immediately after EVT, respectively, indicating the presence of both hemorrhage and iodine contrast. **(B)** are also mixed images, iodine overlay map (IOM), virtual non-contrast (VNC) images 24 h after EVT, respectively, indicating the persistent hemorrhage and clearance of iodine contrast. **(C)** is conventional non-contrast CT 3 days after EVT, indicating the partial absorption of hemorrhage.

TABLE 3 | Comparison of hemorrhage judgment on mixed and VNC images.

	Mixed images	VNC images	κ	*p*-value
DECT immediately after EVT			0.076	0.041
Any hemorrhagic transformation	79 (74.5%)	11 (10.4%)		
No hemorrhage	27 (25.5%)	95 (89.6%)		
DECT 24 h after EVT			0.757	<0.001
Any hemorrhagic transformation	44 (41.5%)	32 (30.2%)		
No hemorrhage	62 (58.5%)	74 (69.8%)		

VNC = virtual non-contrast; DECT = dual energy CT; EVT = endovascular thrombectomy.

TABLE 4 | Grades of hemorrhagic transformation at different stages after thrombectomy.

	Immediate VNC	24 h VNC	3 day NCCT
Hemorrhagic transformation grades			
No hemorrhage	95 (89.6%)	74 (69.8%)	71 (67.0%)
Hemorrhagic infarction	5 (4.7%)	21 (19.8%)	21 (19.8%)
Parenchymal hemorrhage	6 (5.7%)	11 (10.4%)	14 (13.2%)

VNC = virtual non-contrast; NCCT = non-contrast CT.

days after EVT. And the remaining 21 patients (30.9%) developed hemorrhagic transformation on both VNC images 24 h after EVT and non-contrast CT 3 days after EVT.

With the use of DECT, the proportion of all patients diagnosed with hemorrhagic transformation on mixed images immediately after EVT was reduced from 74.5% (79 of 106) to 10.4% (11 of 106), with very poor consistency ($\kappa = 0.076$, $p = 0.041$) (**Table 3**). Correspondingly, the proportion of all patients diagnosed with hemorrhagic transformation on mixed images 24 h after EVT was reduced from 41.5% (44 of 106) to 30.2% (32 of 106), with moderate consistency ($\kappa = 0.757$, $p < 0.001$) (**Table 3**).

The classification of hemorrhagic transformation at different stages after thrombectomy was shown in **Table 4**. New hemorrhage mostly occurred within 24 h after EVT (from 10.4 to 30.2%). Three patients with no hyperdensities on 24 h DECT developed delayed hemorrhagic transformation (1 HI and 2 PH), while one patient with HI on 24 h DECT became PH on 3 day conventional non-contrast CT.

DISCUSSION

Hyperdense areas were frequently (74.5%) detected on CT immediately after EVT. With the use of DECT, the proportion of patients diagnosed with hemorrhagic transformation immediately after EVT was reduced to 10.4%. Although the phenomenon of contrast medium extravasation became less common at 24 h, the use of DECT still changed the diagnosis of hemorrhagic transformation in a considerable number of patients (11.3%). New hemorrhages mostly occurred within 24 h after EVT (from 10.4 to 30.2%). After excluding some delayed new and progressive hemorrhages, the hemorrhagic transformation classified on 24 h VNC was quite consistent with the 3 day conventional non-contrast CT.

Lummel et al. (12) reported the frequency of hyperdense lesions was 84.2% in patients after EVT, even higher than our current study. Our study showed that 27.2% (12 in 44) of hemorrhage findings in the routine 24 h follow-up group are caused by contrast staining mimicking blood. This proportion increases to 86.1% (68 in 79) in the group scanned immediately after EVT. The former finding is similar to the report from Almqvist et al.'s (7) study of DECT. The latter finding of pure contrast staining proportion is higher than in three previously published studies of a post-interventional DECT strategy within 30, 60, or 120 min (68, 47, and 32%, respectively) (8, 13, 14).

There are several techniques to distinguish contrast staining from hemorrhage. Commonly, the issue can be resolved with a repeated CT examination within 1–3 days (12, 15), which may

postpone antithrombotic therapy or anticoagulation. Although iodine can affect several magnetic resonance sequences (16), hemosiderin-sensitive sequences can be used since it is unlikely that iodine could mimic hemorrhage on magnetic resonance imaging (17). By contrast, DECT is a simple and fast solution differentiating hemorrhage and contrast staining, avoiding the delayed time for a repeat examination and the limitations of magnetic resonance, such as contraindications and limited resource issues (7).

Early differentiation between hemorrhage and contrast medium extravasation immediately after EVT is important for clinical decision making, such as whether to start treatment with glycoprotein IIb/IIIa inhibitor (tirofiban) after EVT to prevent early reocclusion due to endothelial damage (5), or might be useful where repeat intervention is necessary. On the other hand, the AHA/ASA guidelines for the management of acute ischemic stroke patients recommended obtaining follow-up imaging 24 h after IVT before starting antiplatelets or anticoagulants (6). However, the high occurrence of hyperdensities on CT after EVT brought concerns to clinicians about the use of antithrombotic agents for secondary preventive treatment, since contrast staining could mimic hemorrhage. Based on the current study, the use of DECT might in our opinion provide this differentiation.

Limitations include a retrospective design in a single stroke center, though we prospectively collected data using a stroke registry, which might present a potential risk of selection bias. Some severe stroke patients might be transferred to an intensive care unit or receive surgical treatment the next day making them unable to undergo follow-up DECT within 24 h. The sample size is moderate; future large multicenter studies and individual patient data meta-analysis are needed to further investigate the importance of DECT in the patients with EVT. On the other hand, the best verification of our confidence in DECT's diagnostic differentiation between hemorrhage and contrast medium extravasation is by performing hemosiderin-sensitive magnetic resonance sequences at the same time, which is clinically difficult. Considering the clearance of iodine contrast, we used a conventional non-contrast CT 3 days after EVT for the verification, although a few patients developed new or progressive hemorrhagic transformation.

Standard non-contrast CT alone should be used with caution for the diagnosis and grading of hemorrhagic transformation after EVT, because contrast staining can mimic hemorrhage. We concluded that DECT with IOM and VNC has potential and is essential for early differentiation of hemorrhage and contrast material extravasation after EVT, offering additional information for clinical decision making regarding antithrombotic and anticoagulant therapy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by human ethics committee of Hangzhou First Hospital, Zhejiang University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KL: drafted/revised the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, statistical analysis, and study supervision. LJ: drafted/revised the manuscript, study concept or design, analysis or interpretation of data, and acquisition of data. JR and WX: drafted/revised the manuscript, study concept or design, and analysis or interpretation of data. HH: acquisition of data and analysis or interpretation of data. GN: drafted/revised the manuscript. SY and CY: drafted/revised the manuscript, study concept or design, analysis or interpretation of data, contribution of vital reagents, tools, patients, study supervision, and obtained funding. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by grant from the Zhejiang provincial public welfare research project (LGF18H090017 and LGF20H090008) and the National Natural Science Foundation of China (81701150).

REFERENCES

1. Berkowitz AL, Mittal MK, McLane HC, Shen GC, Muralidharan R, Lyons JL, et al. Worldwide reported use of IV tissue plasminogen activator for acute ischemic stroke. *Int J Stroke*. (2014) 9:349–55. doi: 10.1111/ijs.12205
2. Wahlgren N, Ahmed N, Davalos A, Ford GA, Grond M, Hacke W, et al. Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST): an observational study. *Lancet*. (2007) 369:275–82. doi: 10.1016/S0140-6736(07)60149-4
3. Goyal M, Menon BK, van Zwam WH, Dippel DW, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet*. (2016) 387:1723–31. doi: 10.1016/S0140-6736(16)00163-X
4. Yoon W, Seo JJ, Kim JK, Cho KH, Park JG, Kang HK. Contrast enhancement and contrast extravasation on computed tomography after intra-arterial thrombolysis in patients with acute ischemic stroke. *Stroke*. (2004) 35:876–81. doi: 10.1161/01.STR.0000120726.69501.74
5. Kellert L, Hametner C, Rohde S, Bendszus M, Hacke W, Ringleb P, et al. Endovascular stroke therapy: tirofiban is associated with risk of fatal intracerebral hemorrhage and poor outcome. *Stroke*. (2013) 44:1453–5. doi: 10.1161/STROKEAHA.111.000502
6. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. 2018 Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. (2018) 49:46–110. doi: 10.1161/STR.0000000000000163

7. Almqvist H, Holmin S, Mazya MV. Dual energy CT after stroke thrombectomy alters assessment of hemorrhagic complications. *Neurology*. (2019) 93:1068–75. doi: 10.1212/WNL.0000000000008093
8. Tijssen MP, Hofman PA, Stadler AA, van Zwam W, de Graaf R, van Oostenbrugge RJ, et al. The role of dual energy CT in differentiating between brain haemorrhage and contrast medium after mechanical revascularisation in acute ischaemic stroke. *Eur Radiol*. (2014) 24:834–40. doi: 10.1007/s00330-013-3073-x
9. Ferda J, Novak M, Mirka H, Baxa J, Ferdova E, Bednarova A, et al. The assessment of intracranial bleeding with virtual unenhanced imaging by means of dual-energy CT angiography. *Eur Radiol*. (2009) 19:2518–22. doi: 10.1007/s00330-009-1495-2
10. Larrue V, von Kummer RR, Muller A, Bluhmki E. Risk factors for severe hemorrhagic transformation in ischemic stroke patients treated with recombinant tissue plasminogen activator: a secondary analysis of the European-Australasian Acute Stroke Study (ECASS II). *Stroke*. (2001) 32:438–41. doi: 10.1161/01.STR.32.2.438
11. Gupta R, Phan CM, Leidecker C, Brady TJ, Hirsch JA, Nogueira RG, et al. Evaluation of dual-energy CT for differentiating intracerebral hemorrhage from iodinated contrast material staining. *Radiology*. (2010) 257:205–11. doi: 10.1148/radiol.10091806
12. Lummel N, Schulte-Altedorneburg G, Bernau C, Pfefferkorn T, Patzig M, Janssen H, et al. Hyperattenuated intracerebral lesions after mechanical recanalization in acute stroke. *Am J Neuroradiol*. (2014) 35:345–51. doi: 10.3174/ajnr.A3656
13. Morhard D, Ertl L, Gerdsmeier-Petz W, Ertl-Wagner B, Schulte-Altedorneburg G. Dual-energy CT immediately after endovascular stroke intervention: prognostic implications. *Cardiovasc Intervent Radiol*. (2014) 37:1171–8. doi: 10.1007/s00270-013-0804-y
14. Bonatti M, Lombardo F, Zamboni GA, Vittadello F, Curro Dossi R, Bonetti B, et al. Iodine extravasation quantification on dual-energy CT of the brain performed after mechanical thrombectomy for acute ischemic stroke can predict hemorrhagic complications. *Am J Neuroradiol*. (2018) 39:441–7. doi: 10.3174/ajnr.A5513
15. Payabvash S, Qureshi MH, Khan SM, Khan M, Majidi S, Pawar S, et al. Differentiating intraparenchymal hemorrhage from contrast extravasation on post-procedural non-contrast CT scan in acute ischemic stroke patients undergoing endovascular treatment. *Neuroradiology*. (2014) 56:737–44. doi: 10.1007/s00234-014-1381-8
16. Ganguly A, Gold GE, Butts Pauly K, Mayer D, Moseley MM, Pelc NJ, et al. Quantitative evaluation of the relaxivity effects of iodine on GD-DTPA enhanced MR arthrography. *J Magn Reson Imaging*. (2007) 25:1219–25. doi: 10.1002/jmri.20934
17. Ogura A, Hayakawa K, Miyati T, Maeda F. Effects of iodinated contrast agent on diffusion weighted magnetic resonance imaging. *Acad Radiol*. (2009) 16:1196–200. doi: 10.1016/j.acra.2009.03.021

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

SYSTEMATIC REVIEW
published: 20 October 2020
doi: 10.3389/fneur.2020.584038

Intracranial Bleeding After Reperfusion Therapy in Acute Ischaemic Stroke Patients Randomized to Glyceryl Trinitrate vs. Control: An Individual Patient Data Meta-Analysis

Jason P. Appleton[1,2], Lisa J. Woodhouse[1], Nikola Sprigg[1,3], Joanna M. Wardlaw[4] and Philip M. Bath[1,3] on behalf of the ENOS, RIGHT, and RIGHT-2 Investigators*

[1] Stroke Trials Unit, Division of Clinical Neuroscience, University of Nottingham, Nottingham, United Kingdom, [2] Stroke, University Hospitals Birmingham National Health Service Foundation Trust, Birmingham, United Kingdom, [3] Stroke, Nottingham University Hospitals National Health Service Trust, Nottingham, United Kingdom, [4] Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, United Kingdom

OPEN ACCESS

Edited by:
Nishant K. Mishra,
University of California, Los Angeles, United States

Reviewed by:
Jean-Marc Olivot,
Center Hospitalier Universitaire de Toulouse, France
Kumar Rajamani,
Wayne State University, United States

***Correspondence:**
Philip M. Bath
philip.bath@nottingham.ac.uk

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: 16 July 2020
Accepted: 24 September 2020
Published: 20 October 2020

Citation:
Appleton JP, Woodhouse LJ, Sprigg N, Wardlaw JM and Bath PM (2020) Intracranial Bleeding After Reperfusion Therapy in Acute Ischaemic Stroke Patients Randomized to Glyceryl Trinitrate vs. Control: An Individual Patient Data Meta-Analysis. Front. Neurol. 11:584038. doi: 10.3389/fneur.2020.584038

Background: Thrombolysis, with or without thrombectomy, for acute ischaemic stroke is associated with an increased risk of intracranial bleeding. We assessed whether treatment with glyceryl trinitrate (GTN), a nitric oxide donor, may influence the associated bleeding risk.

Methods: We searched for completed randomized controlled trials of GTN vs. no GTN in acute ischaemic stroke with data on reperfusion treatments (thrombolysis and/or thrombectomy). The primary efficacy outcome was functional status as assessed by the modified Rankin Scale (mRS) at day 90; the primary safety outcome was intracranial bleeding. Secondary safety outcomes included symptomatic intracranial hemorrhage and haemorrhagic transformation of infarction. Individual patient data were pooled and meta-analysis performed using ordinal or binary logistic regression with adjustment for trial and prognostic variables both overall and in those randomized within 6 h of symptom onset.

Results: Three trials met the eligibility criteria. Of 715 patients with ischaemic stroke who underwent thrombolysis (709, >99%) or thrombectomy (24, 3.4%), 357 (49.9%) received GTN and 358 (50.1%) received no GTN. Overall, there was no difference in the distribution of the mRS at day 90 between GTN vs. no GTN (OR 0.94, 95% CI 0.72–1.23; $p = 0.65$); similarly, there was no difference in intracranial hemorrhage rates between treatment groups (OR 0.90, 95% CI 0.43–1.89; $p = 0.77$). In those randomized to GTN vs. no GTN within 6 h of symptom onset, there were numerically fewer bleeding events, but these analyses did not reach statistical significance.

Conclusions: In ischaemic stroke patients treated predominantly with thrombolysis, transdermal GTN was safe, but did not influence functional outcome at 90 days.

Keywords: bleeding, glyceryl trinitrate, ischaemic stroke, reperfusion, thrombolysis, thrombectomy, meta-analysis

INTRODUCTION

Thrombolysis with alteplase for acute ischaemic stroke is an efficacious treatment if given within 4.5 h of onset, but is associated with an increased risk of symptomatic intracranial hemorrhage (sICH: 6.8% thrombolysis vs. 1.3% placebo) (1). In the context of large vessel occlusion of the anterior cerebral circulation, thrombectomy is highly effective at improving clinical outcomes when performed within 6 h of onset (2), and upto 24 h in those with perfusion mismatch (3). In trials including patients who received alteplase, there was no difference in sICH rates between those randomized to thrombectomy and no thrombectomy (2). Further, a recent trial assessing tPA + thrombectomy vs. thrombectomy alone, found no difference in sICH rates (4).

Raised blood pressure (BP) in those undergoing thrombolysis has been associated with increased risk of haemorrhagic transformation of infarction (HTI) and sICH. The ENCHANTED-BP trial found that intensive lowering of BP in those undergoing thrombolysis did not improve functional outcome but did reduce the rate of sICH as compared with guideline BP management (5). It is unclear whether specific BP agents exert effects that may augment reperfusion strategies. The nitric oxide donor, glyceryl trinitrate (GTN), has been associated with improved clinical outcomes when administered as a patch within 6 h of stroke onset in a subgroup of the large ENOS trial (6). However, when administered in the pre-hospital setting within 4 h of onset, GTN had a neutral effect on functional outcome (7). Data from a small pilot study in the ambulance found that treatment with GTN was associated with a tendency toward increased rates of thrombolysis compared with no GTN, suggesting that GTN may prime patients for thrombolysis by lowering their BP into the treatment range (8). Little is known about whether GTN influences the bleeding risk associated with reperfusion treatments. Here, we assessed the effect of GTN in those undergoing reperfusion therapies and the associated bleeding risk.

METHODS

The study was registered with PROSPERO (CRD42020193427) and followed PRISMA guidance (**Supplementary Table 1**).

Search Strategy and Selection Criteria

We searched EMBASE, PubMed, and Cochrane Library for randomized controlled trials of GTN in adults with acute stroke and data on reperfusion treatments, including thrombolysis or thrombectomy, using the search terms: "stroke" OR "cerebral ischaemia," AND "glyceryl trinitrate" OR "nitroglycerin," AND "randomized," OR "randomized." Non-randomized studies were excluded. We aimed to perform an individual patient data meta-analysis of the trials found, focusing on acute ischaemic stroke patients treated with reperfusion strategies. Participants with available data on reperfusion therapy and modified Rankin Scale (mRS) score at 90 days were eligible for inclusion.

Search results were screened, abstracts reviewed and full-text manuscripts assessed for inclusion criteria by one author (JPA). We assessed risk of bias of the included studies using Cochrane's "risk of bias" tool as high, low or unclear risk across the following elements: random sequence generation; allocation concealment; blinding of participants and personnel; blinding of outcome assessment; incomplete outcome data; selective reporting; and other sources of bias (9).

The chief investigator of the included studies was approached to share individual patient data for use in the meta-analysis. Ethical approval was obtained for each of the included trials and informed consent was received from participants or their legal representative.

Clinical Outcomes

The primary efficacy outcome was functional outcome measured using the mRS at day 90; a seven level ordinal scale ranging from 0 = no symptoms, through increasing levels of dependency, to 6 = death.

The primary safety outcome was intracranial hemorrhage; secondary safety outcomes focused on bleeding using accepted definitions of sICH, HTI, extracranial bleeding (ECB), and major extracranial hemorrhage (MEH). In the included trials, imaging data were collected at sites and adjudicated centrally by trained neuroradiologists using a set proforma (10). Safety data on death were also collected at day 7 and day 90.

Outcomes were assessed overall across the total population of the included trials, and in those participants randomized to GTN vs. no GTN within 6 h of stroke onset in order to assess the effect of GTN in close proximity to reperfusion therapies.

Data Analysis

The individual patient databases of the included trials were merged by LJW. Participants with a non-ischaemic stroke diagnosis, or those who did not receive reperfusion therapies, were removed from the dataset. Data on recruitment, age, sex, baseline mRS, prior medical problems, baseline National Institutes for Health Stroke Scale (NIHSS), stroke etiology, stroke syndrome, stroke type, time to randomization, baseline hemodynamics, mRS at day 90, death at day 7, and 90, intracranial bleeding, HTI, sICH, ECB, and MEH were extracted.

Data are number (%), mean (standard deviation, SD), median [interquartile range]. Baseline characteristics were compared across trials by Chi-square test, Kruskal–Wallis test, or one-way ANOVA as appropriate. Data between treatment groups were assessed by intention-to-treat. Ordinal or binary logistic regression was used to assess differences in clinical outcomes between treatment groups with resultant odds ratio (OR) with 95% confidence intervals (CI) provided. Analyses were adjusted for prognostic variables including age, sex, baseline mRS, baseline NIHSS, baseline systolic BP, time to randomization,

Abbreviations: ECB, Extracranial bleeding; ENOS, Efficacy of nitric oxide in stroke; HTI, transformation of infarction; MEH, Major extracranial hemorrhage; mRS, modified Rankin Scale; RIGHT, Rapid intervention with glyceryl trinitrate in hypertensive stroke trial; RIGHT-2, Rapid intervention with glyceryl trinitrate in hypertensive stroke trial-2; sICH, symptomatic intracranial hemorrhage.

and trial. Subgroup analyses were performed by adding an interaction term to an unadjusted ordinal logistic regression model. Significance was set at $p < 0.05$. Analyses were performed using Statistical Package for the Social Sciences (SPSS) version 23.

RESULTS

Included Studies

Three trials met the inclusion criteria: RIGHT (8), ENOS (11), and RIGHT-2 (7) trials (**Supplementary Figure 1**). All three trials assessed transdermal GTN 5 mg patches vs. no patch, but varied in design and delivery. RIGHT ($n = 41$) and RIGHT-2 ($n = 1149$) were both pre-hospital paramedic-delivered trials that randomized patients with presumed stroke to transdermal GTN or no GTN within 4 h of onset. There were 6 further days of trial treatment in hospital in RIGHT and 3 further days in RIGHT-2. In contrast, ENOS ($n = 4{,}011$) was a large in-hospital trial that randomized participants with stroke within 48 h of onset to GTN or no GTN given for 7 days. Therefore, patients in RIGHT and RIGHT-2 received GTN or no GTN in the ambulance prior to reperfusion therapies in hospital, whilst most ENOS participants with ischaemic stroke were randomized after the thrombolysis window. All participants underwent baseline neuroimaging. Follow-up imaging was stipulated as part of the RIGHT-2 trial at day 2 and as clinically indicated in all three trials e.g., 24 h post-thrombolysis/thrombectomy. All neuroimaging scans were adjudicated centrally by trained neuroradiologists. All participants had data extracted for the clinical outcomes specified, where available. Further details pertaining to the conduct and results of the individual trials are published (7, 8, 11).

Risk of Bias of Included Studies

Full details are provided in a "risk of bias" table (**Supplementary Table 2**) and associated figures (**Supplementary Figures 2, 3**). In summary, all three trials had low risk of bias in relation to the elements assessed except for blinding of participants and personnel (performance bias), which was an unclear risk of bias in all three included studies. There was no placebo patch available in any of the included studies and therefore a gauze dressing was applied over the GTN patch or equivalent area of skin in ENOS (11) and RIGHT (8), whilst in RIGHT-2 (7) a Duoderm dressing was placed on the skin of those randomized to no GTN with a gauze dressing on top. As such, participants were blinded but investigators applying the patch/dressing were not.

Baseline Characteristics

Data were available for 715 participants (GTN 357, no GTN 358) with acute ischaemic stroke who underwent reperfusion therapies from the three included studies. Data on reperfusion treatment were available in 414 patients recruited into ENOS (from 2003 to 2013), 10 patients in RIGHT (2010–2011) and 291 patients in RIGHT-2 (2015–2018) (**Table 1**). Overall, the average age was 72 years old, 45% were female, 62% had hypertension, and 21% atrial fibrillation (AF). The mean NIHSS score was 12.5 and median GCS 15. A substantial proportion of participants (36%) were deemed to have a cardioembolic etiology for their stroke. Baseline BP was 162 / 87 mmHg, with a median time to randomization of 4.9 h. The vast majority underwent thrombolysis (709, 99%) with 24 (3.4%) patients being treated with thrombectomy. Of the 24 participants who underwent thrombectomy, six did not receive thrombolysis (**Table 1**).

Across the three trials the participants were older and baseline mRS was higher in RIGHT and RIGHT-2 than in ENOS (**Table 1**). Similarly, AF rates differed across the trials with a higher proportion in RIGHT-2. Median GCS was lower in RIGHT-2 than ENOS and RIGHT. Large vessel and small vessel stroke etiologies were more common in ENOS than RIGHT or RIGHT-2. Baseline BP and heart rate also varied across the trials. Due to the different randomization periods across the trials, participants were randomized at around 1 h after symptom onset in RIGHT and RIGHT-2, and at 22 h in ENOS. The 24 patients who received thrombectomy were all from the RIGHT-2 trial (**Table 1**).

Clinical Outcomes

Overall, the distribution of the mRS at day 90 did not differ by randomization to GTN or no GTN in unadjusted and adjusted analyses: adjusted OR 0.94, 95% CI 0.72–1.23, $p = 0.65$ (**Table 2** and **Figure 1**). There were 32 (4.5%) intracranial bleeds, of which 12 (1.7%) were sICH. There were no differences in bleeding rates between the GTN and no GTN groups. There were numerically fewer HTIs in the GTN than no GTN groups (8 vs. 11) although this was not statistically significant. ECB and MEH were uncommon and did not differ by randomized treatment. No differences between GTN groups were noted for death at day 7 or day 90 (**Table 2**).

In those randomized to GTN or no GTN within 6 h of onset, there was a non-significant tendency toward improved 90 day functional outcome in both unadjusted and adjusted analyses: OR 0.81, 95% CI 0.56 to 1.18, $p = 0.27$ (**Table 2**, **Figure 2**). Similarly, there were numerically fewer intracranial bleeds, sICH, and HTI in those randomized to GTN than no GTN, but none of these analyses reached statistical significance. Rates of ECB and MEH, and death at day 7 and 90 did not differ between treatment groups (**Table 2**).

Subgroup Analyses

In an unadjusted ordinal logistic regression model there were no significant interactions noted between subgroups and randomization to GTN vs. no GTN (**Figure 3**). The baseline systolic BP subgroup interaction narrowly missed statistical significance ($p = 0.053$), with a suggestion that treatment with GTN in participants with increasing systolic BP may be associated with tendencies toward improved functional outcome at 90 days. Further, treatment with GTN in those with baseline systolic BP <140 mmHg may be associated with worse functional outcome (**Figure 3**).

TABLE 1 | Baseline characteristics of ischaemic stroke participants who underwent reperfusion strategies by trial.

	All	ENOS	RIGHT	RIGHT-2	*p*
Number of patients	715	414	10	291	
Years of recruitment, range	2003–2018	2003–2013	2010–2011	2015–2018	–
Median, [IQR]	2013 [2011, 2017]	2012 [2010, 2012]	2010 [2010–2011]	2017 [2016–2017]	<0.001
Age (years)	72.4 (11.6)	71.0 (11.4)	78.2 (5.9)	74.2 (11.8)	<0.001
Sex, male (%)	393 (55.0)	239 (57.7)	7 (70.0)	147 (50.5)	0.10
mRS [/6]	0 [0, 1]	0 [0, 0]	0 [0, 1]	0 [0, 1]	<0.001
Medical history (%)					
Hypertension	444 (62.1)	265 (64.0)	7 (70.0)	172 (59.1)	0.37
Diabetes mellitus	118 (16.5)	57 (13.8)	2 (20.0)	59 (20.3)	0.07
Atrial fibrillation	136 (20.6)	73 (17.6)	2 (20.0)	61 (25.7)	0.048
Stroke	88 (12.3)	43 (10.4)	1 (10.0)	44 (15.1)	0.17
TIA	92 (13.1)	53 (13.2)	3 (30.0)	36 (12.4)	0.27
IHD	103 (14.5)	59 (14.5)	0	44 (15.1)	0.41
PAD	22 (3.1)	18 (4.5)	0	4 (1.4)	0.058
Smoking, current	139 (19.4)	85 (20.5)	4 (40.0)	50 (17.2)	0.14
Alcohol >21 units per week	51 (7.1)	33 (8.0)	0	18 (6.2)	0.45
Qualifying event (%)					
Ischaemic stroke	714 (99.9)	414 (100.0)	10 (100.0)	290 (99.7)	0.48
TIA	0	0	0	1 (0.3)	0.48
NIHSS (/42)	12.5 (6.0)	12.2 (5.3)	11.7 (5.3)	13.0 (7.0)	0.17
GCS [/15]	15 [13,15]	15 [14,15]	15 [13,15]	14 [12,15]	<0.001
TOAST classification (%)					
Cardioembolic	252 (35.7)	136 (32.9)	5 (50.0)	111 (39.4)	0.14
Large vessel	157 (22.2)	106 (25.6)	2 (20.0)	49 (17.4)	0.037
Small vessel	121 (17.1)	85 (20.5)	1 (10.0)	35 (12.4)	0.017
Other	179 (25.4)	88 (21.3)	2 (20.0)	89 (31.6)	0.008
Hemodynamics					
BP, Systolic (mmHg)	161.6 (19.2)	163.1 (16.0)	176.5 (27.7)	158.9 (22.4)	0.001
BP, Diastolic (mmHg)	87.2 (14.1)	85.9 (12.4)	92.5 (22.8)	88.7 (15.9)	0.019
Heart rate	78.8 (18.5)	76.6 (15.7)	84.7 (22.6)	81.8 (21.3)	0.001
Time to randomization [hours]	4.9 [1.1, 23.5]	21.7 [7.6, 28.7]	1.1 [0.7, 2.1]	1.0 [0.7, 1.4]	<0.001
Thrombolysis (%)	709 (99.2)	414 (100.0)	10 (100.0)	285 (97.9)	0.012
Thrombectomy (%)	24 (3.4)	-	-	24 (8.5)	-

Data are number (%), mean (standard deviation, SD), median [interquartile range, IQR]; comparison across trials by Chi-square test, Kruskal–Wallis test, or one-way ANOVA.
BP, blood pressure; ENOS, Efficacy of nitric oxide in stroke trial; GCS, Glasgow coma score; IHD, ischaemic heart disease; mRS, modified Rankin Scale; NIHSS, National Institutes for Health Stroke Scale; PAD, peripheral arterial disease; RIGHT, Rapid intervention with glyceryl trinitrate in hypertensive stroke trial; TIA, transient ischaemic attack.

DISCUSSION

In this individual patient data meta-analysis of trials assessing transdermal GTN vs. no GTN in acute ischaemic stroke patients who underwent reperfusion therapies (>99% thrombolysis), treatment with GTN was safe but did not influence functional outcome at 90 days. Bleeding complications after reperfusion therapies were infrequent and not influenced by GTN treatment overall. However, in those randomized to GTN vs. no GTN within 6 h of onset there was a tendency toward improved functional outcome at 90 days and reduced rates of intracranial haemorrhagic complications following reperfusion therapies, but these findings did not reach statistical significance. Randomization to GTN in those with higher systolic BP may be associated with a tendency toward improved functional outcome.

BP lowering in patients with acute ischaemic stroke is primarily reserved for patients with BP >185/110 mmHg who are otherwise eligible for thrombolysis (12). There is some evidence to suggest that in the context of large vessel occlusion, intensive lowering of elevated BP may be harmful (13). However, a *U*-shaped association with outcome was noted in the MR CLEAN registry with both high and low levels of baseline systolic BP being associated with worse clinical outcomes, whilst increasing systolic BP was associated with increased rates of sICH (14). Although there were too few thrombectomy patients in the current study to add to this evidence base, transdermal GTN was safe and did not worsen clinical outcomes nor affect the occurrence of haemorrhagic complications in a population predominantly treated with thrombolysis. This is in keeping with the overall results of prior GTN in acute stroke trials to date; GTN

TABLE 2 | Clinical outcomes of acute ischaemic stroke patients who underwent reperfusion strategies by GTN vs. no GTN.

Overall	GTN	No GTN	OR (95% CI)	*p*
N (%)	357 (49.9)	358 (50.1)		
Primary efficacy outcome				
mRS at day 90 [/6]–adjusted	3 [2,5]	3 [2,5]	0.94 (0.72, 1.23)	0.65
Unadjusted			0.96 (0.74, 1.24)	0.74
Primary safety outcome				
Intracranial hemorrhage (%)	15 (4.2)	17 (4.7)	0.90 (0.43, 1.89)	0.77
Secondary safety outcomes				
sICH (%)	6 (1.7)	6 (1.7)	1.01 (0.31, 3.28)	0.99
HTI (%)	8 (2.2)	11 (3.1)	0.74 (0.29, 1.91)	0.53
ECB (%)	2 (0.6)	3 (0.8)	0.62 (0.10, 3.86)	0.61
MEH (%)	1 (0.3)	1 (0.3)	1.55 (0.08, 31.60)	0.78
Death				
By day 7 (%)	14 (3.9)	13 (3.7)	1.21 (0.52, 2.80)	0.66
By day 90 (%)	57 (16.0)	57 (15.9)	1.03 (0.64, 1.64)	0.91
Randomized <6 h				
N (%)	206/357 (57.7)	183/358 (51.1)		
Primary outcome				
mRS at day 90 [/6]–adjusted	3 [1,5]	3 [2,5]	0.81 (0.56, 1.18)	0.27
Unadjusted			0.84 (0.59, 1.19)	0.33
Primary safety outcome				
Intracranial bleeding (%)	9 (4.4)	14 (7.7)	0.60 (0.24, 1.48)	0.27
Secondary safety outcomes				
sICH (%)	3 (1.5)	4 (2.2)	0.74 (0.16, 3.52)	0.70
HTI (%)	5 (2.4)	10 (5.5)	0.46 (0.15, 1.41)	0.17
ECB (%)	1 (0.5)	2 (1.1)	0.35 (0.02, 5.79)	0.47
MEH (%)	0	0	–	–
Death				
By day 7 (%)	4 (2.0)	3 (1.7)	1.05 (0.21, 5.26)	0.95
By day 90 (%)	32 (15.5)	32 (17.5)	0.86 (0.44, 1.69)	0.67

Data are number (%), odds ratio (OR), hazard ratio (HR) with 95% confidence intervals (CI) using ordinal or binary logistic regression.

CI, confidence interval; ECB, extracranial bleeding; GTN, glyceryl trinitrate; HR, Hazard ratio; HTI, haemorrhagic transformation of infarction; mRS, modified Rankin Scale; OR, odds ratio; sICH, symptomatic intracranial hemorrhage.

Adjusted for age, sex, baseline mRS, baseline NIHSS, baseline systolic BP, time to randomization, and trial.

lowers BP, is safe but does not influence functional outcome at 90 days (7, 15). NO has significant antiplatelet properties, but NO donors may differ in their clinical antiplatelet effects: for example, GTN, an organic NO donor, may have antiplatelet properties *in vitro* (16) but did not *in vivo* in stroke patients (17); in contrast, sodium nitroprusside, an inorganic NO donor, has both *in vitro* and *in vivo* antiplatelet effects (18). Further, GTN may interrupt the early vasoconstrictory phase in hemorrhage (19), thus leading to more severe bleeding. In a subgroup analysis involving the intracerebral hemorrhage patients recruited in the RIGHT-2 trial, there was a tendency toward worse clinical outcomes in those randomized to GTN vs. no GTN (20). However, this was a small subgroup of the RIGHT-2 trial and the findings may represent undetected baseline imbalances. Reassuringly, in the present meta-analysis there were few bleeding events and we did not see any signal of increased haemorrhagic complications. Instead, there was a suggestion that GTN administered within 6 h of symptom onset in those undergoing reperfusion treatments may be associated with fewer bleeding complications, but this did not reach statistical significance.

The interaction between baseline systolic BP and treatment with GTN vs. no GTN narrowly missed statistical significance ($p = 0.053$), with increasing baseline BP associated with a tendency toward improved functional outcome in the presence of GTN. This may suggest that modest lowering of elevated BP in the context of thrombolysis may be beneficial, perhaps priming patients for earlier thrombolysis without the need for intravenous BP lowering, whilst also reducing the risk of potential haemorrhagic complications. This is line with the ENCHANTED-BP trial demonstrating less intracranial bleeding in those treated with intensive BP lowering vs. standard BP lowering, although there was no effect on functional outcome at 90 days (5). The present study also demonstrated that treatment with GTN in those with a baseline systolic BP <140 mmHg was associated with a tendency toward worse functional outcome at 90 days. BP lowering in this group may have compromised cerebral blood flow, extending the ischaemic core resulting in worse clinical outcomes. Therefore, this approach should be avoided in those with systolic BP <140 mmHg in the context of reperfusion therapies.

The strengths of this analysis include the ability to assess treatment effects at an individual patient level across the included trials and a high proportion of available outcome data. However, there are limitations. First, the timing of thrombolysis and thrombectomy was not recorded in the trials. In RIGHT and RIGHT-2, randomization to GTN vs. no GTN occurred in the ambulance prior to hospital and therefore reperfusion therapies. In ENOS, the relationship between time of randomized treatment administration and thrombolysis is less clear. We attempted to address this by assessing those participants randomized within 6 h of symptom onset separately, as in this group the randomized treatment is likely to have been received in close proximity to thrombolysis. Second, we do not have angiographic data on the proportion of patients with a confirmed large vessel occlusion at baseline, nor the recanalisation rate on any subsequent imaging. We were therefore unable to ascertain whether GTN was safe and efficacious in this population, or whether GTN had an effect based upon recanalisation status. Third, data regarding other BP lowering medications administered in relation to reperfusion therapies were not available. Such additional medication may have attenuated any treatment effect of GTN. Last, all included trials were

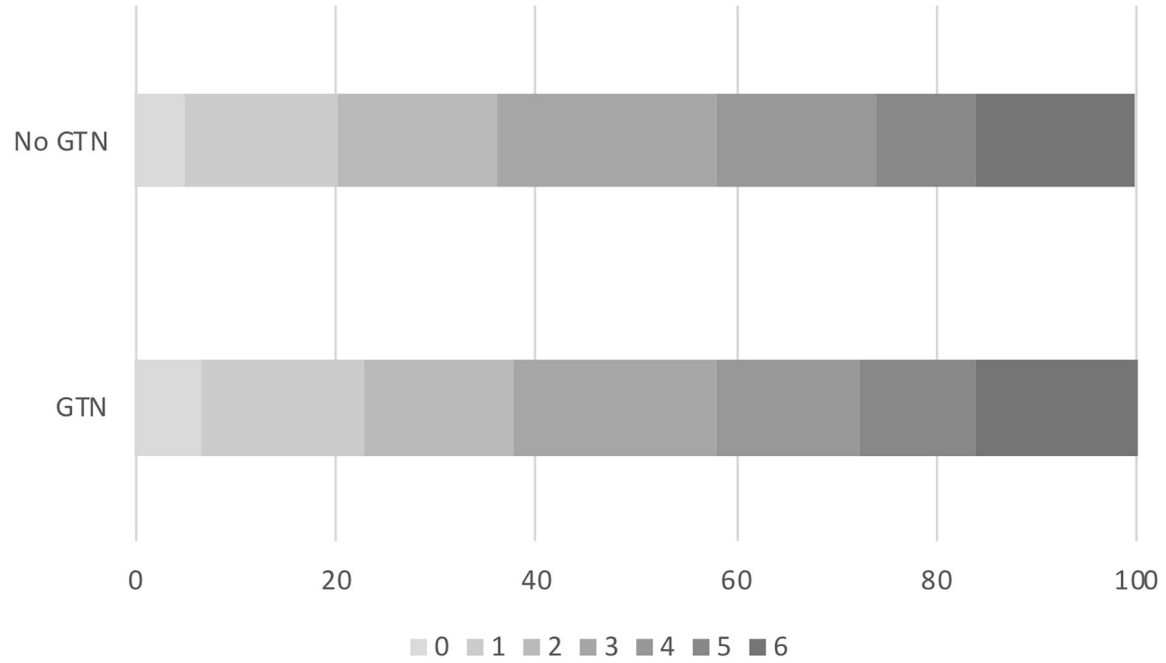


FIGURE 1 | Acute ischaemic stroke patients who underwent reperfusion strategies. Distribution of mRS at day 90 by GTN vs. no GTN: adjusted OR 0.94, 95% CI 0.72–1.23, $p = 0.65$. CI, confidence interval; GTN, glyceryl trinitrate; mRS, modified Rankin Scale; OR, odds ratio.

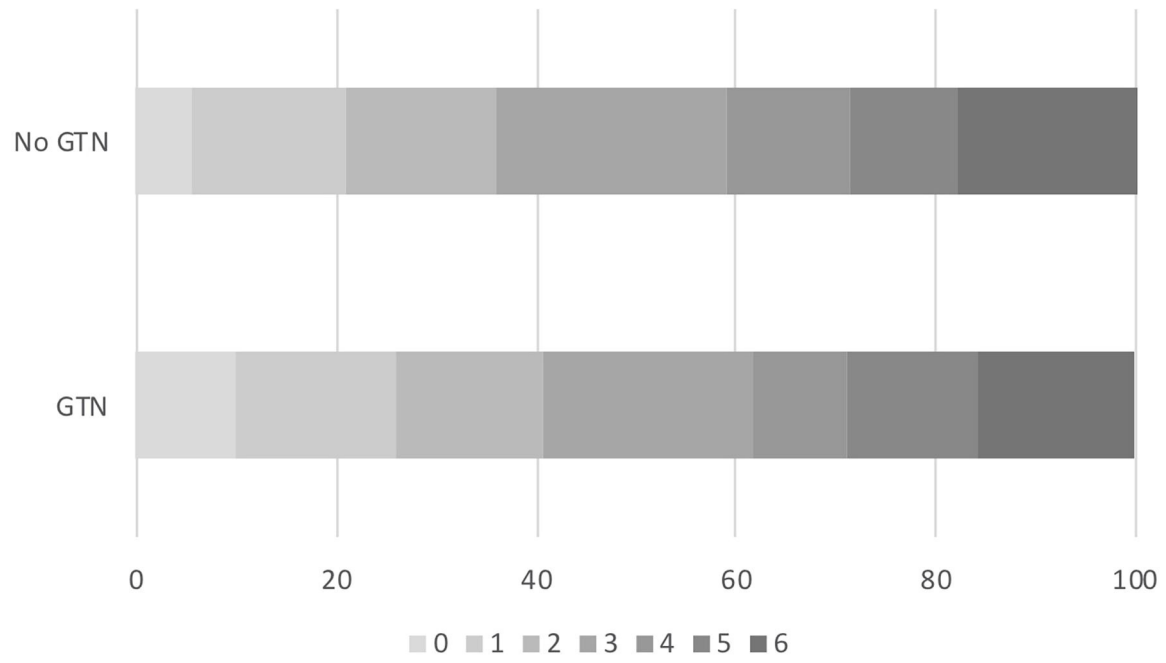


FIGURE 2 | Acute ischaemic stroke patients who underwent reperfusion strategies and randomized to GTN vs. no GTN within 6 h of onset. Distribution of mRS at day 90 by GTN vs. no GTN: adjusted OR 0.81, 95% CI 0.56–1.18, $p = 0.27$. CI, confidence interval; GTN, glyceryl trinitrate; mRS, modified Rankin Scale; OR, odds ratio.

performed by the same group and unfortunately included very few participants treated with thrombectomy. The ongoing MR ASAP trial in the Netherlands is assessing the use of a single GTN patch in the ambulance with a particular focus on patients with large vessel occlusions, adding vital data on this population (ISCRTN:99503308) (21).

In summary, this individual patient data meta-analysis has demonstrated that transdermal GTN is safe in patients with

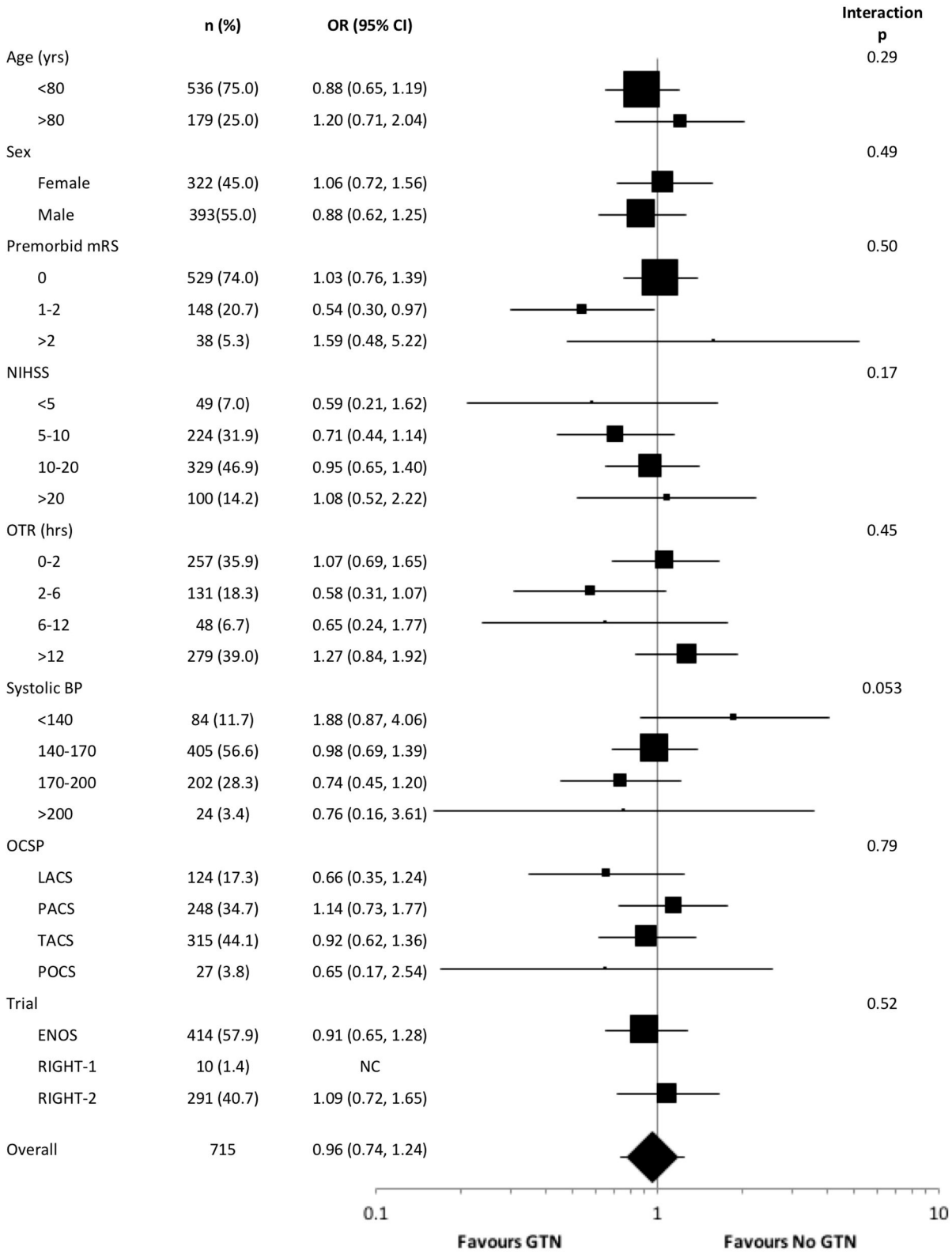


FIGURE 3 | Forest plot of effect on mRS at day 90 in pre-defined subgroups for acute ischaemic stroke patients who underwent reperfusion strategies by GTN vs. no GTN. Unadjusted ordinal logistic regression for mRS at day 90 with interaction between subgroup and GTN vs. no GTN. BP, blood pressure; CI, confidence interval; ENOS, Efficacy of nitric oxide in stroke trial; GTN, glyceryl trinitrate; LACS, lacunar syndrome; mRS, modified Rankin scale; NIHSS, National Institutes for Health Stroke Scale; OCSP, Oxfordshire community stroke project; OTR, onset to randomization; PACS, partial anterior circulation syndrome; POCS, posterior circulation syndrome; RIGHT, Rapid intervention with glyceryl trinitrate in hypertensive stroke trial; TACS, total anterior circulation syndrome.

acute ischaemic stroke undergoing reperfusion therapies (>99% thrombolysis), but does not influence functional outcome at 90 days. GTN within 6 h of stroke onset in this setting was associated with tendencies toward improved functional outcome and fewer haemorrhagic complications. The timing of BP lowering in the context of reperfusion therapies warrants further investigation, particularly in regard to thrombectomy and recanalisation status, in ongoing and future studies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request.

AUTHOR CONTRIBUTIONS

JA performed the literature search, pooled analyses, statistical analysis and interpretation, manuscript drafting, and editing. LW performed pooling of databases, statistical interpretation, reviewed, and edited the manuscript. NS performed statistical interpretation, reviewed, and edited the manuscript. JW set up and co-ordinated all scan reading, including scan rating, training and data cleaning, reviewed and edited the manuscript. PB conceptualized the study, statistical interpretation, reviewed, and edited the manuscript, is corresponding author and has responsibility for submission. All authors contributed to the article and approved the submitted version.

FUNDING

There was no specific funding for the present study. ENOS was funded by the UK Medical Research Council (G0501797) and BUPA foundation. RIGHT was funded by Nottingham University Hospitals NHS Trust (R&D Pump Priming Competition) and by the Division of Stroke, University of Nottingham. RIGHT-2 was funded by the British Heart Foundation (CS/14/4/30972). JW declares funding from the Scottish Imaging Network, A Platform for Scientific Excellence (SINAPSE) and the UK Dementia Research Institute which receives funding from the UK Medical Research Council, Alzheimer's Society and Alzheimer's Research UK. The funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

ACKNOWLEDGMENTS

We thank the participants for taking part in the trials used in this analysis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.584038/full#supplementary-material

REFERENCES

1. Emberson J, Lees K, Lyden P, Blackwell L, Albers G, Bluhmki E, et al. Effect of treatment delay, age and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. *Lancet.* (2014) 384:1929–35. doi: 10.1016/S0140-6736(14)60584-5
2. Goyal M, Menon BK, van Zwam WH, Dippel DWJ, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet.* (2016) 397:1723–31. doi: 10.1016/S0140-6736(16)00163-X
3. Nogueira RG, Jadhav AP, Haussen DC, Bonafe A, Budzik RF, Bhuva P, et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. *N Engl J Med.* (2018) 378:11–21. doi: 10.1056/NEJMoa1706442
4. Yang P, Zhang Y, Zhang L, Zhang Y, Treurniet KM, Chen W, et al. Endovascular thrombectomy with or without intravenous alteplase in acute stroke. *N Engl J Med.* London (2020) 382:1981–93. doi: 10.1056/NEJMoa2001123
5. Anderson CS, Huang Y, Lindley RI, Chen X, Arima H, Chen G, et al. Intensive blood pressure reduction with intravenous thrombolysis therapy for acute ischaemic stroke (ENCHANTED): an international, randomised, open-label, blinded-endpoint, phase 3 trial. *Lancet.* (2019) 393:877–88. doi: 10.1016/S0140-6736(19)30038-8
6. Woodhouse L, Scutt P, Krishnan K, Berge E, Gommans J, Ntaios G, et al. Effect of hyperacute administration (within 6 hours) of transdermal glyceryl trinitrate, a nitric oxide donor, on outcome after stroke: subgroup analysis of the efficacy of nitric oxide in stroke (ENOS) trial. *Stroke.* (2015) 46:3194–201. doi: 10.1161/STROKEAHA.115.009647
7. Bath PM, Scutt P, Anderson CS, Appleton JP, Berge E, Cala L, et al. Prehospital transdermal glyceryl trinitrate in patients with ultra-acute presumed stroke (RIGHT-2): an ambulance-based, randomised, sham-controlled, blinded, phase 3 trial. *Lancet.* (2019) 393:1009–20. doi: 10.1016/S0140-6736(19)30194-1
8. Ankolekar S, Fuller M, Cross I, Renton C, Cox P, Sprigg N, et al. Feasibility of an ambulance-based stroke trial, and safety of glyceryl trinitrate in ultra-acute stroke: the rapid intervention with glyceryl trinitrate in hypertensive stroke trial (RIGHT, ISRCTN66434824). *Stroke.* (2013) 44:3120–8. doi: 10.1161/STROKEAHA.113.001301
9. Higgins JPT, Green S (editors). *Cochrane Handbook for Systematic Reviews of Interventions 2011.* Version 5.1.0. Available online at: http://www.handbook.cochrane.org
10. IST-3 collaborative group. Association between brain imaging signs, early and late outcomes, and response to intravenous alteplase after acute ischaemic stroke in the third international stroke trial (IST-3): secondary analysis of a randomised controlled trial. *Lancet Neurol.* (2015) 14:485–96. doi: 10.1016/S1474-4422(15)00012-5
11. The ENOS Trial Investigators. Efficacy of nitric oxide, with or without continuing antihypertensive treatment, for management of high blood pressure in acute stroke (ENOS): a partial-factorial randomised controlled trial. *Lancet.* (2015) 385:617–28. doi: 10.1016/S0140-6736(14)61121-1
12. Royal College of Physicians. *National Clinical Guideline for Stroke. Prepared by the Intercollegiate Stroke Working Party.* London: Fifth ed. Royal College of Physicians (2016).
13. Minhas J, Wang X, Chalmers J, Anderson C, Robinson T, ENCHANTED Investigators. The enhanced control of hypertension and thrombolysis stroke study (ENCHANTED) blood pressure (BP) arm: comparative effects of intensive- versus standard-BP lowering treatment in large-vessel occlusion. *Eur Stroke J.* (2019) 4:35. doi: 10.1177/2396987319845560
14. van den Berg SA, Mulder MJHL, Goldhoorn RB, Coutinho JM, Roozenbeek B, Dippel DWJ, et al. Blood pressure and functional outcome after endovascular treatment: results from the MR CLEAN registry. *Eur Stroke J.* (2018) 3:8–9. doi: 10.1177/2396987318770127

15. Bath PM, Krishnan K, Appleton JP. Nitric oxide donors (nitrates), L-arginine, or nitric oxide synthase inhibitors for acute stroke. *Cochrane Database Syst Rev.* (2017) 4:CD000398. doi: 10.1002/14651858.CD000398.pub2
16. O'Byrne S, Shirodaria C, Millar T, Stevens C, Blake D, Benjamin N. Inhibition of platelet aggregation with glyceryl trinitrate and xanthine oxidoreductase. *J Pharmacol Exp Ther.* (2000) 292:326–30.
17. Bath PMW, Pathansali R, Iddenden R, Bath FJ. The effect of transdermal glyceryl trinitrate, a nitric oxide donor, on blood pressure and platelet function in acute stroke. *Cerebrovasc Dis.* (2001) 11:265–72. doi: 10.1159/000047649
18. Butterworth RJ, Cluckie A, Jackson SH, Buxton-Thomas M, Bath PM. Pathophysiological assessment of nitric oxide (given as sodium nitroprusside) in acute ischaemic stroke. *Cerebrovasc Dis.* (1998) 8:158–65. doi: 10.1159/000015842
19. Periayah MH, Halim AS, Mat Saad AZ. Mechanism action of platelets and crucial blood coagulation pathways in hemostasis. *Int J Hematol Oncol Stem Cell Res.* (2017) 11:319–27.
20. Bath PM, Woodhouse LJ, Krishnan K, Appleton JP, Anderson CS, Berge E, et al. Prehospital transdermal glyceryl trinitrate for ultra-acute intracerebral hemorrhage: data from the RIGHT-2 trial. *Stroke.* (2019) 50:3064–71. doi: 10.1161/STROKEAHA.119.026389
21. van den Berg SA, Dippel DWJ, Hofmeijer J, Fransen PSS, Caminada K, Siegers A, et al. Multicentre randomised trial of acute stroke treatment in the ambulance with a nitroglycerin patch (MR ASAP): study protocol for a randomised controlled trial. *Trials.* (2019) 20:383. doi: 10.1186/s13063-019-3419-z

Conflict of Interest: PB is Stroke Association Professor of Stroke Medicine, is a National Institute of Health Research Emeritus Senior Investigator and was chief investigator for the ENOS, RIGHT, and RIGHT-2 trials.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

CORRECTION
published: 26 November 2020
doi: 10.3389/fneur.2020.625572

Corrigendum: Intracranial Bleeding After Reperfusion Therapy in Acute Ischaemic Stroke Patients Randomized to Glyceryl Trinitrate vs. Control: An Individual Patient Data Meta-Analysis

Jason P. Appleton[1,2], Lisa J. Woodhouse[1], Nikola Sprigg[1,3], Joanna M. Wardlaw[4] and Philip M. Bath[1,3] on behalf of the ENOS, RIGHT, and RIGHT-2 Investigators*

[1] Stroke Trials Unit, Division of Clinical Neuroscience, University of Nottingham, Nottingham, United Kingdom, [2] Stroke, University Hospitals Birmingham National Health Service Foundation Trust, Birmingham, United Kingdom, [3] Stroke, Nottingham University Hospitals National Health Service Trust, Nottingham, United Kingdom, [4] Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, United Kingdom

Keywords: bleeding, glyceryl trinitrate, ischaemic stroke, reperfusion, thrombolysis, thrombectomy, meta-analysis

A Corrigendum on

Intracranial Bleeding After Reperfusion Therapy in Acute Ischaemic Stroke Patients Randomized to Glyceryl Trinitrate vs. Control: An Individual Patient Data Meta-Analysis
by Appleton, J. P., Woodhouse, L. J., Sprigg, N., Wardlaw, J. M., and Bath, P. M. (2020). Front. Neurol. 11:584038. doi: 10.3389/fneur.2020.584038

Joanna M. Wardlaw was not included as an author in the published article. The corrected Author Contributions and Funding statements appear below.

AUTHOR CONTRIBUTIONS

JA performed the literature search, pooled analyses, statistical analysis and interpretation, manuscript drafting, and editing. LW performed pooling of databases, statistical interpretation, reviewed, and edited the manuscript. NS performed statistical interpretation, reviewed, and edited the manuscript. JW set up and co-ordinated all scan reading, including scan rating, training and data cleaning, reviewed and edited the manuscript. PB conceptualized the study, statistical interpretation, reviewed, and edited the manuscript, is corresponding author and has responsibility for submission. All authors contributed to the article and approved the submitted version.

FUNDING

There was no specific funding for the present study. ENOS was funded by the UK Medical Research Council (G0501797) and BUPA foundation. RIGHT was funded by Nottingham University Hospitals NHS Trust (R&D Pump Priming Competition) and by the Division of Stroke, University of Nottingham. RIGHT-2 was funded by the British Heart Foundation (CS/14/4/30972). JW declares funding from the Scottish Imaging Network, A Platform for Scientific Excellence (SINAPSE) and the UK Dementia Research Institute which receives funding from the UK Medical

OPEN ACCESS

Approved by:
Frontiers Editorial Office, Frontiers Media SA, Switzerland

****Correspondence:***
Philip M. Bath
philip.bath@nottingham.ac.uk

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *03 November 2020*
Accepted: *04 November 2020*
Published: *26 November 2020*

Citation:
Appleton JP, Woodhouse LJ, Sprigg N, Wardlaw JM and Bath PM (2020) Corrigendum: Intracranial Bleeding After Reperfusion Therapy in Acute Ischaemic Stroke Patients Randomized to Glyceryl Trinitrate vs. Control: An Individual Patient Data Meta-Analysis. Front. Neurol. 11:625572. doi: 10.3389/fneur.2020.625572

Research Council, Alzheimer's Society and Alzheimer's Research UK. The funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

frontiers in Neurology

ORIGINAL RESEARCH
published: 10 November 2020
doi: 10.3389/fneur.2020.582767

Added Prognostic Value of Hemorrhagic Transformation Quantification in Patients With Acute Ischemic Stroke

Katinka R. van Kranendonk[1], Kilian M. Treurniet[1,2], Anna M. M. Boers[3], Olvert A. Berkhemer[1,4,5], Jonathan M. Coutinho[6], Hester F. Lingsma[7], Wim H. van Zwam[8], Aad van der Lugt[5], Robert J. van Oostenbrugge[9], Diederik W. J. Dippel[4], Yvo B. W. E. M. Roos[6], Henk A. Marquering[1,10], Charles B. L. M. Majoie[1] and the MR CLEAN investigators*

[1] Department of Radiology and Nuclear Medicine, Amsterdam UMC, Location AMC, University of Amsterdam, Amsterdam, Netherlands, [2] Department of Radiology, Haaglanden Medical Center (HMC), Den Haag, Netherlands, [3] Nico.lab, Amsterdam, Netherlands, [4] Department of Neurology, Erasmus MC-University Medical Center Rotterdam, Rotterdam, Netherlands, [5] Department of Radiology & Nuclear Medicine, Erasmus MC-University Medical Center Rotterdam, Rotterdam, Netherlands, [6] Department of Neurology, Amsterdam UMC, Location AMC, University of Amsterdam, Amsterdam, Netherlands, [7] Department of Public Health, Center for Medical Decision Making, Erasmus MC-University Medical Center Rotterdam, Rotterdam, Netherlands, [8] Department of Radiology and Nuclear Medicine, Cardiovascular Research Institute Maastricht (CARIM), University Medical Center, Maastricht, Netherlands, [9] Department of Neurology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Center, Maastricht, Netherlands, [10] Department of Biomedical Engineering and Physics, Amsterdam UMC, Location AMC, University of Amsterdam, Amsterdam, Netherlands

OPEN ACCESS

Edited by:
Nishant K. Mishra,
University of California, Los Angeles, United States

Reviewed by:
Craig S. Anderson,
University of New South Wales, Australia
Mark William Parsons,
The University of Newcastle, Australia

***Correspondence:**
Katinka R. van Kranendonk
k.r.vankranendonk@amsterdamumc.nl

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: 14 July 2020
Accepted: 29 September 2020
Published: 10 November 2020

Citation:
van Kranendonk KR, Treurniet KM, Boers AMM, Berkhemer OA, Coutinho JM, Lingsma HF, van Zwam WH, van der Lugt A, van Oostenbrugge RJ, Dippel DWJ, Roos YBWEM, Marquering HA, Majoie CBLM and the MR CLEAN investigators (2020) Added Prognostic Value of Hemorrhagic Transformation Quantification in Patients With Acute Ischemic Stroke. Front. Neurol. 11:582767. doi: 10.3389/fneur.2020.582767

Introduction and Aim: Hemorrhagic transformation (HT) frequently occurs after acute ischemic stroke and negatively influences the functional outcome. Usually, HT is classified by its radiological appearance. Discriminating between the subtypes can be complicated, and interobserver variation is considerable. Therefore, we aim to quantify rather than classify hemorrhage volumes and determine the association of hemorrhage volume with functional outcome in comparison with the European Cooperative Acute Stroke Study II classification.

Patients and Methods: We included patients from the MR CLEAN trial with follow-up imaging. Hemorrhage volume was estimated by manual delineation of the lesion, and HT was classified according to the European Cooperative Acute Stroke Study II classification [petechial hemorrhagic infarction types 1 (HI1) and 2 (HI2) and parenchymal hematoma types 1 (PH1) and 2 (PH2)] on follow-up CT 24 h to 2 weeks after treatment. We assessed functional outcome using the modified Rankin Scale 90 days after stroke onset. Ordinal logistic regression with and without adjustment for potential confounders was used to describe the association of hemorrhage volume with functional outcome. We created regression models including and excluding total lesion volume as a confounder.

Results: We included 478 patients. Of these patients, 222 had HT. Median hemorrhage volume was 3.37 ml (0.80–12.6) and per HT subgroup; HI1: 0.2 (0.0–1.7), HI2: 3.2 (1.7–6.1), PH1: 6.3 (4.2–13), and PH2: 47 (19–101). Hemorrhage volume was associated with functional outcome [adjusted common odds ratio (acOR): 0.83, 95% CI: 0.73–0.95] but not anymore after adjustment for total lesion volume (acOR: 0.99, 95% CI: 0.86–1.15,

per 10 ml). Hemorrhage volume in patients with PH2 was significantly associated with functional outcome after adjusting total lesion volume (acOR: 0.70, 95% CI: 0.50–0.98).

Conclusion: HT volume is associated with functional outcomes in patients with acute ischemic stroke but not independent of total lesion volume. The extent of a PH2 was associated with outcome, suggesting that measuring hemorrhage volume only provides an additional benefit in the prediction of the outcome when a PH2 is present.

Keywords: ischemic stroke (IS), hemorrhagic transformation (HT), intracranial hemorrhage (ICH), endovascular therapy (EVT), hemorrhage volume, thrombolysis

INTRODUCTION

Hemorrhagic transformation (HT) commonly occurs as a natural progression or as a complication of reperfusion therapy for acute ischemic stroke (1, 2). Large, but also small HT subtypes were found to be associated with poor functional outcome (3). Incidence varies and differences in definition of HT between studies complicate comparisons between studies. Usually, HT is classified according to the European Cooperative Acute Stroke Study II (ECASS II) classification based on radiological appearance (4). This classification divides HT in four groups: hemorrhagic infarction type 1 (HI1), which is defined as small petechiae along the margins of the infarct; hemorrhagic infarction type 2 (HI2), defined as confluent petechiae within the infarcted area but no space-occupying effect; parenchymal hematoma type 1 (PH1) as blood clots in 30% or less of the infarcted area with some slight space-occupying effect; and parenchymal hematoma type 2 (PH2) as blood clots in more than 30% of the infarcted area with substantial space-occupying effect (4).

The ECASS classification only takes hemorrhage volume relative to the infarct volume into account when a PH is present, and therefore, small hemorrhages could be classified as PH2 when the infarct is small. The opposite is true when large hematomas develop within massive infarcts. These hematomas are not classified as PH2 when their relative size is $<$30% of the infarct while their objective size could be more than 40 ml. These hemorrhages might lead to symptomatic intracranial hemorrhage (sICH). However, according to the Heidelberg Bleeding classification, ICH other than PH2 might be symptomatic, but it is advised not to classify those hemorrhages as sICH (5).

Further, an agreement between observers for HT is only fair, as discriminating between HT subtypes can be challenging (6, 7). This limited agreement might contribute to a variation in the reported incidence of HT between studies.

As an alternative to the current rather crude classification of HT, we aim to quantify the hemorrhage volume of patients with HT and to assess its prognostic value by determining the association of hemorrhage volume with functional outcome in comparison with the ECASS II classification. Additionally, we determine whether hemorrhage volumes smaller than 30% of lesion volume might have been symptomatic.

METHODS

We included all patients with follow-up imaging from the MR CLEAN trial (8). The MR CLEAN trial was a multicenter randomized controlled trial that assessed the safety and efficacy of endovascular therapy compared with usual care after acute ischemic stroke due to large vessel occlusion. The MR CLEAN study protocol has been described previously (9).

We assessed potential HT on follow-up CT scans that were acquired ∼5 days after inclusion. When these scans were not available, 24-h follow-up CT scans were examined. Hemorrhage volume was measured by a trained observer (KRK) by manually delineating the hemorrhages using ITK-SNAP (version 3.4.0). Hemorrhage volume consists of all hemorrhage present on the CT scan, including concomitant intraventricular hemorrhage and subarachnoid hemorrhage. HT was classified according to the ECASS II classification (4). In the MR CLEAN trial, sICH was classified as neurologic deterioration with an increase of more than four points on the National Institute of Health Stroke Scale and hemorrhage visible on imaging (8).

The functional outcome was assessed at ∼90 days after stroke onset and attributed with a score according to the modified Rankin Scale (mRS). The mRS ranges from 0 to 6, where 0 indicates no symptoms and 6 indicates death.

Statistical Analysis

Mean and SD are used to summarize normally distributed variables; for non-normal distributed variables, the median and interquartile range are used. We compared hemorrhage volumes between all HT subtypes using a Kruskal–Wallis test. The association of hemorrhage volume with functional outcome was assessed using ordinal logistic regression analysis using the full mRS scale as the outcome measure. The association of hemorrhage volume with functional outcome was estimated as a common odds ratio (cOR) per 10-ml increase, expressing the relative risk of a shift in the direction of good outcomes for every 10 ml of hemorrhage. A cOR $<$ 1 indicates a shift toward worse outcomes on the mRS. Three models were made; in the first model, we assessed the association of hemorrhage volume with functional outcome. In the second model, we assessed the association of hemorrhage volume and all HT subgroups with functional outcome, and the third model described the association of hemorrhage volume and sICH with functional outcome. We adjusted every model for potential confounders: diabetes mellitus, systolic blood pressure (measured

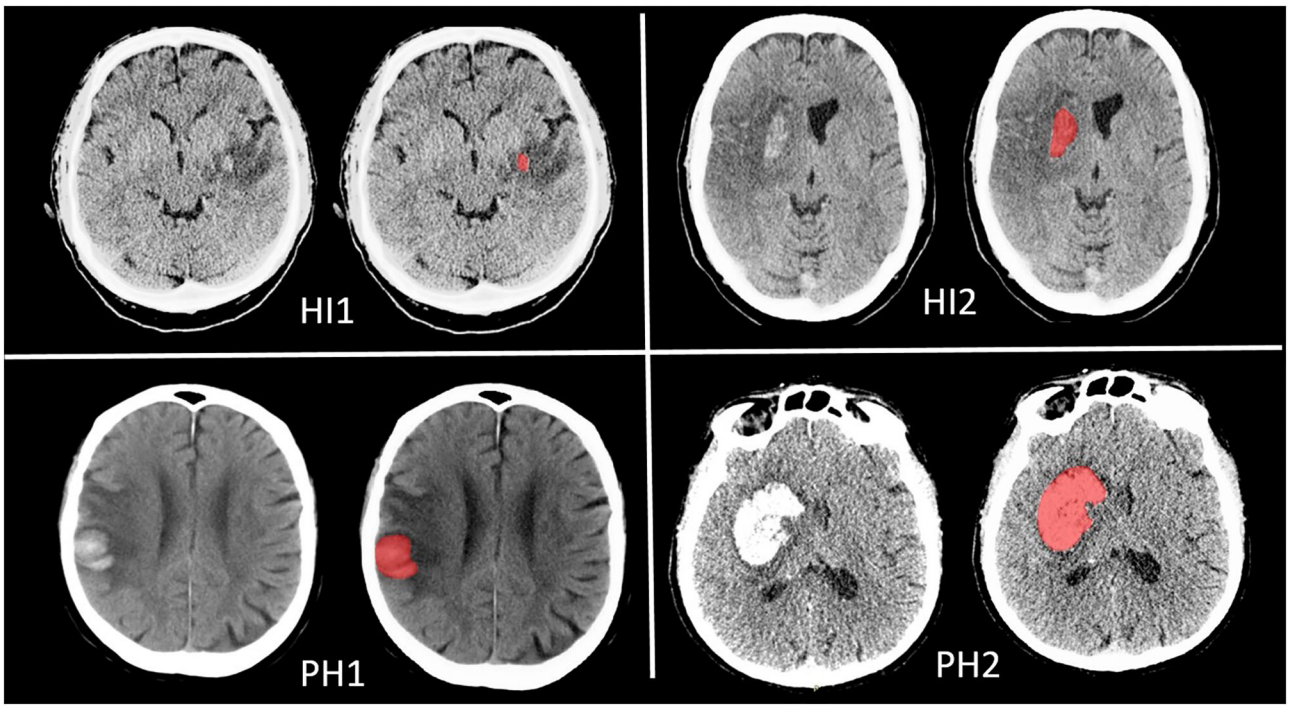


FIGURE 1 | Quantification of Hemorrhagic Transformation per subtype.

on admission), intravenous thrombolysis, endovascular therapy, time from onset to randomization, history of ischemic stroke, age, atrial fibrillation, and baseline National Institutes of Health Stroke Scale. We conducted an additional subgroup analysis to assess the association of hemorrhage volume with functional outcome per HT subgroup.

Follow-up lesion volume included both infarct and hemorrhage volume and was estimated using a validated automated measurement (10). In some patients with a large PH, the lesion volume is equal to the hemorrhage volume, and the actual infarct is masked by hemorrhage. Adjusting for follow-up lesion volume might result in an underestimation of the impact of hemorrhage volume. However, HT is more likely to occur within large infarcts, and not adjusting for lesion volume could overestimate the impact of HT. Therefore, we conducted analyses with additional adjustment for follow-up lesion volume. We conducted the statistical analysis using R {R Core Team [V.4.0.0 (2020)]; R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria; used packages rms (11), ggplot2 (12), and tableone (13)}.

RESULTS

Of all the patients with follow-up imaging ($n = 478$), 222 had HT. Of these 222 patients with HT, we measured hemorrhage volumes of 219 patients (**Figure 1**). Hemorrhage volumes of three patients could not be measured due to insufficient image quality.

Hemorrhage volumes differed between HT subgroups ($p < 0.001$). Patients with PH2 had the largest hemorrhage volumes [46.8 (interquartile range: 19–101) ml] (**Table 1**).

Hemorrhage volume was significantly associated with worse functional outcomes in the unadjusted and adjusted analyses [cOR 0.75, 95% confidence interval (CI) 0.67 to 0.83 and acOR 0.77, 95% CI: 0.69 to 0.87 per 10 ml] (**Figure 2**). After additional adjustment for follow-up lesion volume, the association was weaker (acOR 0.90, 95% CI 0.80 to 1.02) (**Table 2**, Model 1).

In model 2, the analysis that included hemorrhage volume and all HT subgroups, hemorrhage volume and all HT subgroups except PH2 with no HT as reference level were significantly and independently associated with functional outcome in the adjusted and unadjusted analyses. After additional adjustment for follow-up lesion volume, only HI2 and PH2 were associated with functional outcome (acOR 0.57, 95% CI 0.34 to 0.95 and acOR 0.36, 95% CI 0.14 to 0.97, respectively).

Subgroup Analysis

Hemorrhage volume in patients with PH2 was significantly associated with functional outcome in the adjusted analysis, including follow-up lesion volume (acOR 0.70, 95% CI 0.50 to 0.98). This association was not observed in the other HT subtypes (**Figure 3**).

Symptomatic Intracranial Hemorrhage

Thirty-five patients with HT were classified as sICH (example in **Figure 4**). The median hemorrhage volume of patients with sICH was 53 (24–106) ml. Of those patients with

TABLE 1 | Patient characteristics.

	No HT ($n = 256$)	HI1 ($n = 76$)	HI2 ($n = 71$)	PH1 ($n = 36$)	PH2 ($n = 39$)
Hemorrhage volume, ml—median (IQR)	0 (0–0)	0.17 (0–1.7)	3.2 (1.7–6.1)	6.3 (4.2–12.9)	46.8 (18.7–100.7)
EVT—no. (%)	111 (43.4)	28 (36.8)	35 (49.3)	21 (58.3)	17 (43.6)
Treatment with IV alteplase—no. (%)	228 (89.1)	66 (86.8)	65 (91.5)	33 (91.7)	36 (92.3)
Age—mean (SD)	64 (14.3)	65 (12.6)	66 (12.9)	64 (14.8)	68 (14.1)
Baseline NIHSS—mean (SD)	17 (5.7)	19 (6.1)	18 (4.4)	18 (4.3)	19 (4.8)
History of ischemic stroke—no. (%)	26 (10.2)	7 (9.2)	8 (11.3)	1 (2.8)	9 (23.1)
Atrial fibrillation—no. (%)	58 (22.7)	17 (22.4)	25 (35.2)	15 (41.7)	14 (35.9)
Diabetes mellitus—no. (%)	27 (10.5)	13 (17.1)	9 (12.7)	5 (13.9)	7 (17.9)
Systolic blood pressure—mean (SD)	143 (22.4)	144 (26)	142 (27.5)	153 (23.6)	160 (31.4)
Time from stroke onset to randomization per minute—median (IQR)	193 (147–254)	217 (148–258)	207 (158–281)	213 (165–278)	223 (181–265)
Follow-up lesion volume—median (IQR)	47 (18–118)	132 (58–207)	120 (78–243)	172 (97–274)	165 (93–323)

EVT, endovascular treatment; HT, hemorrhagic transformation; HI, hemorrhagic infarction; PH, parenchymal hematoma; NIHSS, National Institute Of Health Stroke Scale; IQR, interquartile range.

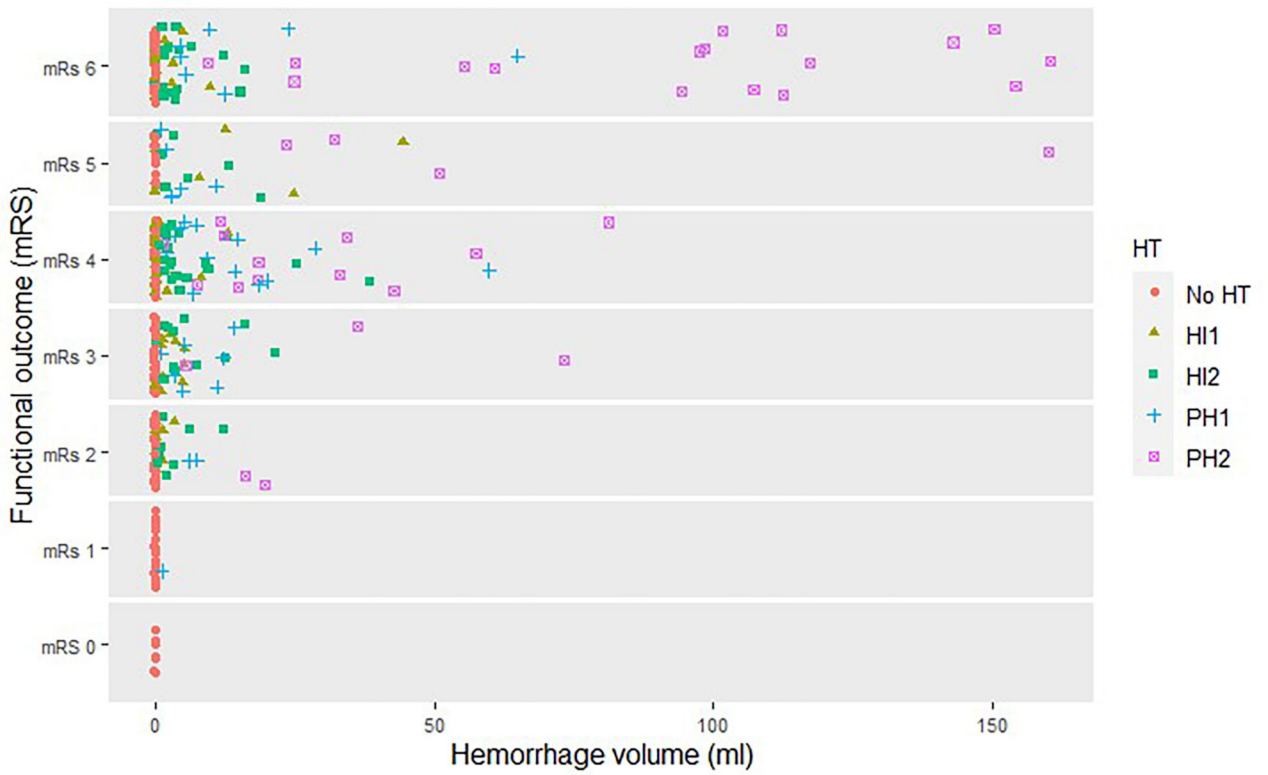


FIGURE 2 | Hemorrhage volume and functional outcome per HT subgroup.

sICH, 14 had hemorrhages <30% of lesion volume. Some of these hemorrhages likely caused symptoms, whereas in some sICH, not only hemorrhage would have caused symptoms but also infarct growth probably contributed to the neurological deterioration.

In model 3, hemorrhage volume and sICH were both significantly associated with functional outcome (cOR 0.83, 95% CI 0.73 to 0.95) and the unadjusted analysis (cOR 0.31, 95% CI 0.12 to 0.78). In the adjusted analyses, sICH was not significantly associated with functional outcome (acOR 0.45, 95% CI 0.17 to 1.17) (**Table 2**). After additional adjustment for follow-up lesion volume, the association of hemorrhagic volume with functional outcome was attenuated.

TABLE 2 | Adjusted and unadjusted OR's of the association of hemorrhage volume in ml with functional outcome.

Model		Unadjusted OR and 95% CI	Adjusted OR and 95% CI	Adjusted OR and 95% CI (with FLV)
1	Hemorrhage volume, per 10 ml	0.75 (0.67 to 0.83)	0.77 (0.69 to 0.87)	0.90 (0.80 to 1.02)
2	Hemorrhage volume, per 10 ml	0.79 (0.69 to 0.90)	0.83 (0.73 to 0.95)	0.99 (0.86 to 1.15)
	HI1	0.42 (0.27 to 0.65)	0.56 (0.36 to 0.89)	0.68 (0.42 to 1.06)
	HI2	0.40 (0.24 to 0.64)	0.44 (0.27 to 0.71)	0.58 (0.34 to 0.96)
	PH1	0.46 (0.25 to 0.85)	0.41 (0.22 to 0.78)	0.72 (0.37 to 1.41)
	PH2	0.55 (0.23 to 1.31)	0.50 (0.20 to 1.24)	0.37 (0.14 to 0.98)
3	Hemorrhage volume, per 10 ml	0.83 (0.73 to 0.95)	0.83 (0.73 to 0.95)	0.94 (0.80 to 1.09)
	sICH	0.31 (0.12 to 0.78)	0.45 (0.17 to 1.17)	0.69 (0.23 to 2.07)

The association of hemorrhage volume in milliliters with the full scale mRS score.
This table lists the association of hemorrhage volume with the full scale mRS score.
Adjusted for HT classification, atrial fibrillation, baseline NIHSS, intravenous thrombolysis, diabetes mellitus, time from stroke onset to randomization, age, endovascular therapy, previous stroke, systolic blood pressure (measured on admission). An additional analysis was conducted with follow-up lesion volume included in the adjusted analysis.
In models 2 and 3, no HT and no sICH were used as reference level when assessing the association of HT subgroups and sICH with functional outcome.
FLV, Follow-up lesion volume; HT, hemorrhagic transformation; HI, hemorrhagic infarction; PH, parenchymal hematoma; sICH, symptomatic intracranial hemorrhage; mRS, modified Rankin Scale.

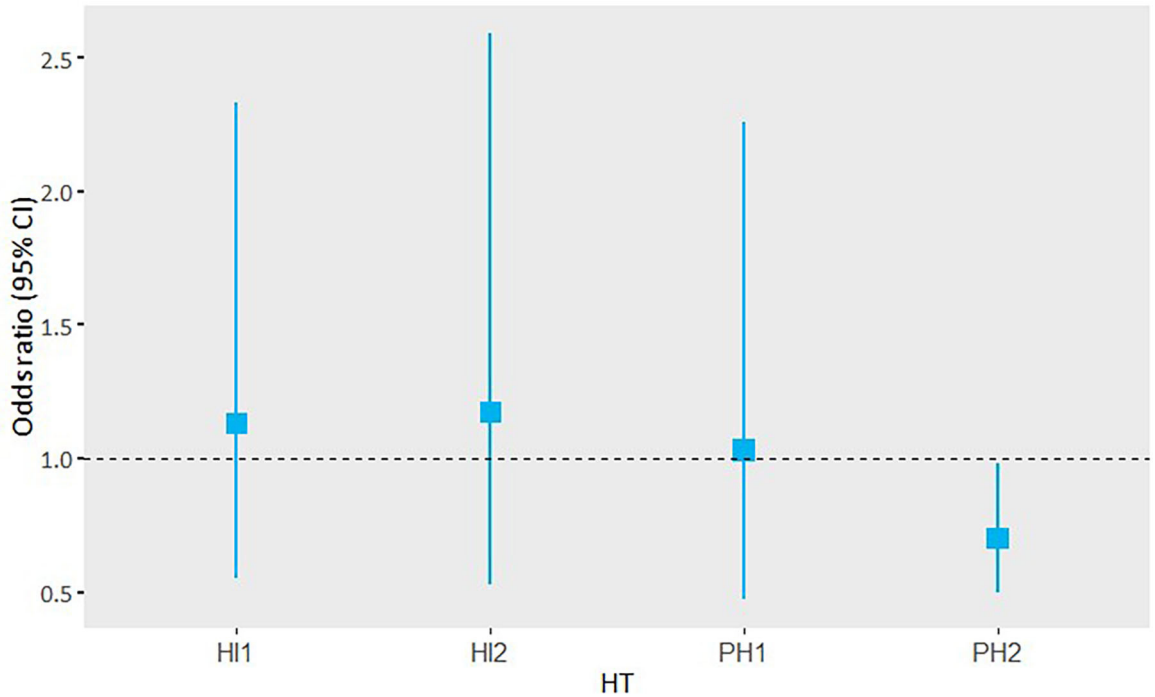


FIGURE 3 | Adjusted OR and 95% CI of the subgroup analysis of hemorrhage volume and its association with functional outcome per HT subgroup.

DISCUSSION

We have shown that hemorrhage volume and the ECASS classification are associated with functional outcomes independently of each other. In the adjusted analysis, hemorrhage volume was associated with functional outcome, and PH2 was not associated with functional outcome. This was also seen in the analysis with sICH. However, in the adjusted analysis that included follow-up lesion volume, hemorrhage volume was not associated with functional outcome. In the subgroup analysis, only hemorrhage volume of PH2 was associated with functional outcome.

Previous studies suggested that hemorrhage volume could be more appropriate than a radiological classification, as it gives a more objective description when assessing HT (14, 15). These studies had a relatively small sample size compared with the

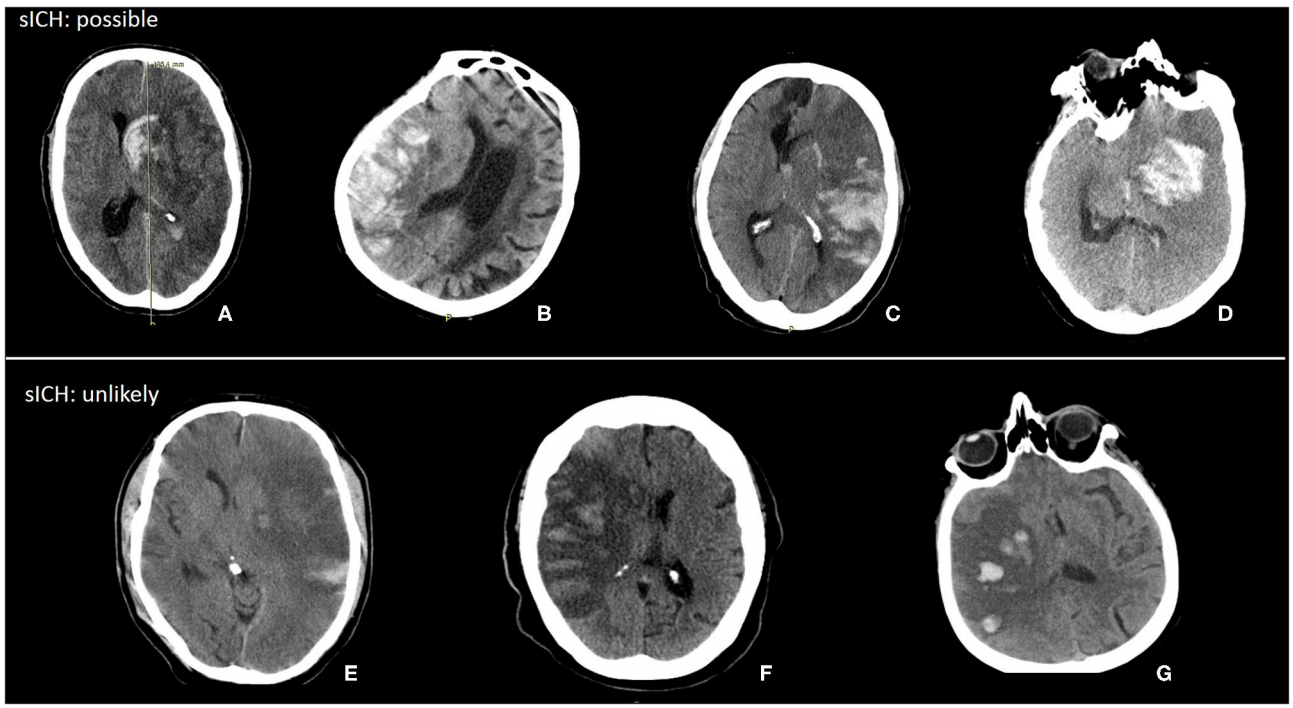


FIGURE 4 | Examples of possible and unlikely sICH with hemorrhage volume < 30% of lesion volume. **(A)** Hemorrhage volume: 65 ml, lesion volume: 279 ml, hemorrhage (%): 24%. **(B)** Hemorrhage volume: 61 ml, lesion volume: 275 ml, hemorrhage (%): 22%. **(C)** Hemorrhage volume: 102 ml, lesion volume: 423 ml, hemorrhage (%): 24%. **(D)** Hemorrhage volume: 51 ml, lesion volume: 347 ml, hemorrhage (%): 15%. **(E)** Hemorrhage volume: 5 ml, lesion volume: 411 ml, hemorrhage (%): 1%. **(F)** Hemorrhage volume: 13 ml, lesion volume: 215 ml, hemorrhage (%): 6%. **(G)** Hemorrhage volume: 12 ml, lesion volume: 191 ml, hemorrhage (%): 6%.

sample size of our study. Moreover, they only included patients with PH. In our study, we have shown that patients with large PH2 are more likely to have poor functional outcomes. This effect was not seen in the smaller HT subtypes (HI1, HI2, and PH1). This suggests that hemorrhage volume has only prognostic value in patients with a PH2.

Assessing the "true" effect of hemorrhage on outcome warrants adjustment for final infarct size due to their association. However, the definition of follow-up lesion volume as used in major studies (a combination of final infarct volume, swelling, edema, and hemorrhage) causes some difficulty (16). In patients with HI, adjustment for follow-up lesion volume will likely result in an accurate estimate of outcome. Quantifying these hemorrhages can be complicated, as the delineation of the hemorrhage is not clear. The brain tissue can be swollen and have petechial bleedings. Delineating the petechial bleedings results in small hemorrhage volumes, and the impact of the swollen brain tissue is not taken into account in this assessment. The lack of including a measure for swelling can result in a stronger observed association of the HI1, HI2, and PH1 classifications with the functional outcome than hemorrhage volume alone. However, large lesion volumes (incorporating both infarct and parenchymal swelling) are associated with HT and with a poor functional outcome, prompting us to include follow-up lesion volume in the analysis (17). As this measure includes hemorrhage, infarcted tissue, and edema while the proportion of hemorrhage is small, it will be correct to adjust for lesion volume. Conversely, for patients with a PH, hemorrhages can be large and tend to mask the infarct volume completely. In these cases, the value of the lesion volume is similar to the hemorrhage volume. Adding both values to the analysis will underestimate the association of large hemorrhage volumes with functional outcome.

Not all patients with sICH have a PH2 with a hematoma that consists of more than 30% of the infarct volume. When the infarct is very large, even a hemorrhage of 100 ml is <30% of the infarct volume, but it might cause symptoms and neurologic deterioration. However, some of the patients classified with sICH were unlikely to have symptoms due to hemorrhage. In almost all examples of sICH we showed, a midline shift was present. In four cases, the hemorrhage might have contributed to the midline shift leading to poor functional outcomes. In the other three cases, the midline shift was probably caused by infarct growth and not due to hemorrhage. For the classification of sICH, it is important to determine if it is likely that the hemorrhage is causing the symptoms as has been proposed in the Heidelberg Bleeding Classification (5).

An advantage of quantifying hemorrhage volume is that it might be less sensitive to interobserver variability than

classifying HT. Quantifying hemorrhage volume can be time-consuming. However, it may be possible to automatically quantify hemorrhage volume, as this is accomplished with subarachnoid hemorrhage and hemorrhagic stroke (18, 19). In some HT cases, it is difficult to distinguish petechial hemorrhage from remaining intact cortex throughout the infarct, also introducing a subjective element when performing a manual assessment. Making it more accessible and less sensitive to interobserver variability when classifying HT, it could be classified as HT or no HT and measure hemorrhage volume only when a PH is present.

This study had several limitations; some patients had diffuse brain swelling with hemorrhage, which is complicated to delineate and could have resulted in smaller hemorrhage volumes for those patients. Hemorrhage volumes were quantified by one observer, and therefore, the interobserver variability could not be assessed. However, measuring hemorrhage volume by one observer leads to less variation to assess its association with functional outcome, and eventually, hemorrhage volume might be assessed by an automated measurement.

In conclusion, hemorrhage volume is associated with functional outcomes but not independent of total lesion volume. However, hemorrhage volume could be useful for classifying HT particularly to measure the extent of a PH.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of their sensitive nature. Requests to access the datasets should be directed to the MR CLEAN executive committee (mrclean-trial.org).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by a central medical ethics committee and the research boards of all participating centers accepted the MR CLEAN trial. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

WZ, AL, RO, DD, YR, and CM designed the MR CLEAN trial. OB collected and prepared the data for the trial. KK, OB, AB, and KT prepared data for this study. KK performed the statistical analysis, interpreted the results, and drafted the paper. HM, KT, and CM assisted with the statistical analysis, interpretation of the results, and drafting the paper. OB, HL, WZ, AL, RO, DD, YR, and CM critically revised the paper. All authors contributed to the article and approved the submitted version.

FUNDING

The MR CLEAN trial (Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands) was partly funded by the Dutch Heart Foundation grant number 2008T30 and by unrestricted grants from AngioCare BV, Medtronic/Covidien/EV3, MEDAC Gmbh/LAMEPRO, Penumbra, Inc, Stryker, and Top Medical/Concentric.

MR CLEAN INVESTIGATORS

Olvert A. Berkhemer, Amsterdam UMC, location AMC, the Netherlands and Erasmus MC-University Medical Center Rotterdam, the Netherlands. Puck S.S. Fransen, Erasmus MC-University Medical Center Rotterdam, the Netherlands. Debbie Beumer, Erasmus MC-University Medical Center Rotterdam, the Netherlands and Maastricht University Medical Center and Cardiovascular Research Institute Maastricht (CARIM), the Netherlands. Berkhemer, Fransen, and Beumer contributed equally. Lucie A. van den Berg, Amsterdam UMC, location AMC, the Netherlands. Hester F. Lingsma, Erasmus MC-University Medical Center Rotterdam, the Netherlands. Albert J. Yoo, Massachusetts General Hospital, Boston, United States of America. Wouter J. Schonewille, Saint Antonius Hospital, Nieuwegein, the Netherlands. Jan Albert Vos, MD, Sint Antonius Hospital, Nieuwegein, the Netherlands. Paul J. Nederkoorn, Amsterdam UMC, location AMC, the Netherlands. Marieke J.H. Wermer and Marianne A.A. van Walderveen, Leiden University Medical Center, the Netherlands. Julie Staals, Maastricht University Medical Center and Cardiovascular Research Institute Maastricht (CARIM), the Netherlands. Jeannette Hofmeijer and Jacques A. van Oostayen, Rijnstate Hospital, Arnhem, the Netherlands. Geert J. Lycklama à Nijeholt and Jelis Boiten, MC Haaglanden, the Hague, the Netherlands. Patrick A. Brouwer and Bart J. Emmer, Erasmus MC-University Medical Center Rotterdam, the Netherlands. Sebastiaan F. de Bruijn and Lukas C. van Dijk, HAGA Hospital, the Hague, the Netherlands. L. Jaap Kappelle, University Medical Center Utrecht, the Netherlands. Rob H. Lo, University Medical Center Utrecht, the Netherlands. Ewoud J. van Dijk and Joost de Vries, Radboud University Medical Center, Nijmegen, the Netherlands. Paul L.M. de Kort and Willem Jan J. van Rooij, Sint Elisabeth Hospital, Tilburg, the Netherlands. Jan S.P. van den Berg and Boudewijn A.A.M. van Hasselt, Isala Klinieken, Zwolle, the Netherlands. Leo A.M. Aerden and René J. Dallinga, Reinier de Graaf Gasthuis, Delft, the Netherlands. Marieke C. Visser and Joseph C.J. Bot, Amsterdam UMC, location VU, Amsterdam, the Netherlands. Patrick C. Vroomen and Omid Eshghi, University Medical Center Groningen, the Netherlands. Tobien H.C.M.L. Schreuder and Roel J.J. Heijboer, Atrium Medical Center, Heerlen, the Netherlands. Koos Keizer and Alexander V. Tielbeek, Catharina Hospital, Eindhoven, the Netherlands. Heleen M. den Hertog and Dick G. Gerrits, Medical Spectrum Twente, Enschede, the Netherlands. Renske M. van den Berg-Vos and Giorgos B. Karas, Sint Lucas Andreas Hospital, Amsterdam, the Netherlands. Ewout W. Steyerberg, Erasmus MC-University Medical Center Rotterdam, the Netherlands. H. Zwenneke Flach, Isala Klinieken, Zwolle, the Netherlands. Henk A. Marquering and Marieke E.S. Sprengers, Amsterdam UMC, location AMC, the Netherlands. Sjoerd F.M. Jenniskens,

Radboud University Medical Center, Nijmegen, the Netherlands. Ludo F.M. Beenen and René van den Berg, Amsterdam UMC, location AMC, the Netherlands. Peter J. Koudstaal and Wim H. van Zwam, Erasmus MC-University Medical Center Rotterdam, the Netherlands. Yvo B.W.E.M. Roos, Amsterdam UMC, location AMC, the Netherlands. Aad van der Lugt, Erasmus MC-University Medical Center Rotterdam, the Netherlands. Robert J. van Oostenbrugge, Maastricht University Medical Center and Cardiovascular Research Institute Maastricht (CARIM), the Netherlands. Charles B.L.M. Majoie, Amsterdam UMC, location AMC, the Netherlands. Diederik W.J. Dippel, Erasmus MC-University Medical Center Rotterdam, the Netherlands. van Zwam, Roos, van der Lugt, van Oostenbrugge, Majoie and Dippel contributed equally.

REFERENCES

1. Hornig CR, Dorndorf W, Agnoli LA. Hemorrhagic cerebral infarction- a prospective study. *Stroke.* (1986) 17:179–85. doi: 10.1161/01.STR.17.2.179
2. Group TMAST—ES. Thrombolytic therapy with streptokinase in acute ischemic stroke. *N Engl J Med.* (1996) 335:145–50. doi: 10.1056/NEJM199607183350301
3. Kranendonk KR van, Treurniet KM, Boers AMM, Berkhemer OA, van den Berg LA, Chales V, et al. Hemorrhagic transformation is associated with poor functional outcome in patients with acute ischemic stroke due to a large vessel occlusion. *J Neurointerv Surg.* (2019) 11:464–8. doi: 10.1136/neurintsurg-2018-014141
4. Hacke W, Kaste M, Fieschi C, von Kummer R, Davalos A, Meier D, et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). *Lancet.* (1998) 352:1245–51. doi: 10.1016/S0140-6736(98)08020-9
5. von Kummer R, Broderick JP, Campbell BCV, Demchuk A, Goyal M, Hill MD, et al. The heidelberg bleeding classification. *Stroke.* (2015) 46:2981–6. doi: 10.1161/STROKEAHA.115.010049
6. Fahed R, Ben Maacha M, Ducroux C, Khoury N, Blanc R, Piotin M, et al. Agreement between core laboratory and study investigators for imaging scores in a thrombectomy trial. *J Neurointerv Surg.* (2018) 10:e30. doi: 10.1136/neurintsurg-2018-013867
7. Guenego A, Lecler A, Raymond J, Sabben C, Khoury N, Premal K, et al. Hemorrhagic transformation after stroke: inter- and intrarater agreement. *Eur J Neurol.* (2019) 26:476–82. doi: 10.1111/ene.13859
8. Berkhemer OA, Fransen PSS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med.* (2015) 372:11–20. doi: 10.1056/NEJMoa1411587
9. Fransen PSS, Beumer D, Berkhemer OA, van den Berg LA, Lingsma H, van der Lugt A, et al. MR CLEAN, a multicenter randomized clinical trial of endovascular treatment for acute ischemic stroke in the Netherlands: study protocol for a randomized controlled trial. *Trials.* (2014) 15:343. doi: 10.1186/1745-6215-15-343
10. Boers AM, Marquering HA, Jochem JJ, Besseling NJ, Berkhemer OA, van der Lugt A, et al. Automated cerebral infarct volume measurement in follow-up noncontrast CT scans of patients with acute ischemic stroke. *AJNR Am J Neuroradiol.* (2013) 34:1522–27. doi: 10.3174/ajnr.A3463
11. Harrell FE. *RMS: Regression Modeling Strategies. R package version 6.0-0.* (2017). Available online at: https://CRAN.R-project.org/package=rms (accessed 13 Jul 2020).
12. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer-Verlag (2009). doi: 10.1007/978-0-387-98141-3
13. Yoshida K, Bohn J. *Tableone: Create "Table 1" to Describe Baseline Charachteristics. R package version 0.11.1.* (2015). Available online at: https://CRAN.R-project.org/package=tableone (accessed July 13, 2020).
14. Christoforidis GA, Slivka A, Mohammad Y, Karakasis C, Avutu B, Yang M. Size matters. *Stroke.* (2007) 38:1799–804. doi: 10.1161/STROKEAHA.106.472282
15. Tao H, Ramadas G, Carrozzella J, Khatri P, Broderick J, Spilker J, et al. Parenchymal hematoma and total lesion volume in combined IV/IA revascularization stroke therapy. *J Neurointerv Surg.* (2012) 4:256–60. doi: 10.1136/neurintsurg-2011-010062
16. Boers AMM, Jansen IGH, Beenen LFM, Devlin TG, Roman LS, Heo JH, et al. Association of follow-up infarct volume with functional outcome in acute ischemic stroke: a pooled analysis of seven randomized trials. *J Neurointerv Surg.* (2018) 10:1137–42. doi: 10.1136/neurintsurg-2017-013724
17. Berger C, Fiorelli M, Steiner T, Schäbitz WR, Bozzao L, Bluhmki E, et al. Hemorrhagic transformation of ischemic brain tissue : asymptomatic or symptomatic? *Stroke.* (2001) 32:1330–5. doi: 10.1161/01.STR.32.6.1330
18. Boers AM, Zijlstra IA, Gathier CS, van den Berg R, Slump CH, Marquering HA, et al. Automatic quantification of subarachnoid hemorrhage on noncontrast CT. *Am J Neuroradiol.* (2014) 35:2279–86. doi: 10.3174/ajnr.A4042
19. Xue W, Vegunta S, Zwart CM, Aguilar MI, Patel AC, Hoxworth JM, et al. Retrospective validation of a computer-assisted quantification model of intracerebral hemorrhage volume on accuracy, precision, acquisition time compared with standard ABC/2 manual volume calculation. *AJNR Am J Neuroradiol.* (2017) 38:1536–42. doi: 10.3174/ajnr.A5256

Conflict of Interest: Academic medical center Amsterdam received funds from Stryker for consultations by CM, YR, and OB. CM received research grants from Cardiovasculair Onderzoek Nederland (CVON)/Dutch Heart Foundation, European Commission, Twin Foundation and Stryker (all paid to institution). HM, AB, CM, and YR are shareholders of Nico.lab, a company that focuses on the use of artificial intelligence for medical image analysis. Erasmus University Medical Center received funds from Bracco Imaging for consultations by DD. Erasmus University Medical Center received funds from CVON/Dutch Heart Foundation, European Commission, Stryker, Penumbra, and Medtronic for the execution of stroke trials by DD and AL. Maastricht University Medical Center received funds from Stryker and Cerenovus for consultations by WZ.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

PERSPECTIVE
published: 13 November 2020
doi: 10.3389/fneur.2020.594289

Selective Brain Hypothermia in Acute Ischemic Stroke: Reperfusion Without Reperfusion Injury

Jae H. Choi[1,2]*, *Sven Poli*[3], *Michael Chen*[4], *Thanh N. Nguyen*[5], *Jeffrey L. Saver*[6], *Charles Matouk*[7] *and John Pile-Spellman*[1,2]

[1] Neurovascular Center, Neurological Surgery, P.C., Lake Success, NY, United States, [2] Hybernia Medical, LLC, New Rochelle, NY, United States, [3] Department of Neurology & Stroke, Hertie-Institute for Clinical Brain Research, Eberhard-Karls University of Tübingen, Tübingen, Germany, [4] Stroke Center, Department of Neurosurgery, Rush University Medical Center, Chicago, IL, United States, [5] Interventional Neurology/Neuroradiology, Boston University School of Medicine, Boston, MA, United States, [6] Comprehensive Stroke Center and Department of Neurology, University of California, Los Angeles (UCLA), Los Angeles, CA, United States, [7] Neurovascular Surgery, Department of Neurosurgery, Yale University-New Haven Hospital, New Haven, CT, United States

In acute ischemic stroke, early recanalization of the occluded artery is crucial for best outcome to be achieved. Recanalization aims at restoring blood flow to the ischemic tissue (reperfusion) and is achieved with pharmacological thrombolytic drugs, endovascular thrombectomy (EVT) devices, or both. The introduction of modern endovascular devices has led to tremendous anatomical and clinical success with rates of substantial reperfusion exceeding 80% and proven clinical benefit in patients with anterior circulation large vessel occlusions (LVOs). However, not every successful reperfusion procedure leads to the desired clinical outcome. In fact, the rate of non-disabled outcome at 3 months with current EVT treatment is ∼1 out of 4. A constraint upon better outcomes is that reperfusion, though resolving ischemic stress, may not restore the anatomic structures and metabolic functions of ischemic tissue to their baseline states. In fact, ischemia triggers a complex cascade of destructive mechanisms that can sometimes be exacerbated rather than alleviated by reperfusion therapy. Such reperfusion injury may cause infarct progression, intracranial hemorrhage, and unfavorable outcome. Therapeutic hypothermia has been shown to have a favorable impact on the molecular elaboration of ischemic injury, but systemic hypothermia is limited by slow speed of attaining target temperatures and clinical complications. A novel approach is endovascular delivery of hypothermia to cool the affected brain tissue selectively and rapidly with tight local temperature control, features not available with systemic hypothermia devices. In this perspective article, we discuss the possible benefits of adjunctive selective endovascular brain hypothermia during interventional stroke treatment.

Keywords: stroke, reperfusion, reperfusion injury, hypothermia, brain cooling, selective endovascular brain cooling, neuroprotection

OPEN ACCESS

Edited by:
Richard Leigh,
National Institutes of Health (NIH), United States

Reviewed by:
Miao Chen,
University of Shanghai for Science and Technology, China
Yuchuan Ding,
Wayne State University, United States

***Correspondence:**
Jae H. Choi
jaehchoi0524@gmail.com

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *13 August 2020*
Accepted: *15 October 2020*
Published: *13 November 2020*

Citation:
Choi JH, Poli S, Chen M, Nguyen TN, Saver JL, Matouk C and Pile-Spellman J (2020) Selective Brain Hypothermia in Acute Ischemic Stroke: Reperfusion Without Reperfusion Injury. Front. Neurol. 11:594289. doi: 10.3389/fneur.2020.594289

INTRODUCTION

Several randomized controlled clinical trials have demonstrated the clinical benefit of endovascular thrombectomy (EVT) in selected patients with acute ischemic stroke due to large vessel occlusions (LVO-AIS) (1–7). The introduction of endovascular clot extraction has not only led to tremendous

improvements in reperfusion rates (often exceeding 80%) and clinical outcome (compared to intravenous rtPA alone), but also offered opportunities to further refine patient selection criteria and extend the treatment time window (8–10). Although a significant improvement in clinical outcome after LVO-AIS could be achieved, a wide gap remains between the success in restoring perfusion through the occluded artery (>4 out of 5) and the rate of excellent, non-disabled (modified Rankin Scale score 0-1) functional outcome achieved in only ∼1 out of 4 treated patients (11). Recently, Van Horn and colleagues evaluated 123 consecutive patients at a single German center from 2015 to 2019 who had complete TICI (Thrombolysis in Cerebral Infarction score) 3 reperfusion and still found 54.5% to have poor clinical outcomes at 90 days (12).

Brain ischemia triggers a cascade of molecular and cellular mechanisms many of which have been identified (13). Following the quick depletion of oxygen and energy carriers from brain tissue it comes to progressive failure of cellular ion pumps, NMDA (N-Methyl- d-aspartate) receptor activation, and anoxic depolarization that further lead to disturbance of ion homeostasis, excitotoxicity, acidification, and increasing cellular influx of Ca2+ (14–17). Activation of nitric oxide synthase and cyclooxygenase-2, generation of free radicals, upregulation of cell adhesion molecules, and increase in the production of proinflammatory cytokines follow (18–22). The resulting inflammatory reactions include recruitment of cell-mediated immunity, activation of protein kinases and matrix zinc-metalloproteinases, and neutrophil transmigration, among others (22–28). In addition, apoptosis is promoted by up-regulation of the BAX (Bcl-2 Associated X-protein) and calpain genes (29, 30).

As a result of these molecular pathways, functional and structural changes follow, such as impaired vasomotor regulation (31, 32), cytotoxic and vasogenic edema (33, 34), and breakdown of the blood-brain-barrier (35–37). With sustained activation of these pathways the risk for extensive neuronal cell death, infarct progression, and intracranial hemorrhage increases (38, 39).

Paradoxically, reperfusion of the ischemic brain tissue can exacerbate these destructive processes that have been triggered by stroke. This is called reperfusion injury and is thought to be the result of multiple pathways of tissue insult, oxidative stress, leukocyte infiltration, complement activation, mitochondrial dysfunction, platelet activation and aggregation, and blood-brain-barrier disruption, culminating in neuron death, brain edema or hemorrhagic transformation (13, 40–42). Reperfusion injury is a common biologic phenomenon across multiple organs and not limited to reperfusion procedures of the neurovasculature, also occurring following treatment of ischemic conditions of the limbs, gastrointestinal tract, and the heart (43–45). The most feared consequence of cerebral reperfusion injury is intracerebral hemorrhage (ICH) (46, 47).

EVT devices are well-suited to remove the target thrombus and anatomically clear the artery to restore blood flow, but do not offer direct therapy of metabolic consequences of ischema. For ameliorating metabolic disruptions, therapeutic hypothermia has been one of the most promising concepts based on its pleiotropic mechanisms of action (48, 49). In this perspective article we present the possible benefits of a novel form of therapeutic hypothermia: endovascular selective brain cooling, and how its adjunct application during endovascular stroke treatment could improve the outcome in LVO-AIS patients by reducing the deleterious impact of ischemia and reperfusion injury.

SUBSECTIONS

The Physiological Limits of Endovascular Clot Extraction

Although there are numerous endovascular devices available to remove a clot from the neurovasculature, in principle, they are of two main types (1–7). One has a tip with a stent-like mesh that lodges into the clot and allows its retrieval and the other, is an aspiration catheter that applies a suction force to the clot while it is removed from the vasculature. The success rate to clear the artery from a clot with endovascular devices is high (Thrombolysis in Cerebral Infarction Scale score 2b and 3) and ranges between 59 to 88% (1–7).

The clinical benefit of the combined treatment approach, i.e., systemic pharmacological therapy and endovascular clot extraction, over pharmacological therapy alone, is due to its high effectiveness to anatomically revascularize the occluded artery. In addition, endovascular catheters may be used to locally infuse fluids and various drugs. However, drugs that are considered neuroprotective and for intra-arterial use are rather limited (50), and currently, there are no intra-arterial agents with the FDA-approved indication to be used for the treatment of acute ischemic stroke.

The rate for symptomatic ICH, often seen with parenchymal hemorrhage, following the combined treatment of acute ischemic stroke varies and may be as high as 10% (1–10). The rate for asymptomatic ICH, often associated with hemorrhagic infarction-type hemorrhagic transformation, is generally higher and may involve as many as 1 out of 3 treated patients (36–39). Clinical factors, such as thrombolytic therapy, thromboembolism, and specific imaging markers, comorbid factors, and clinical work-flow performance markers are often considered risk factors for post-treatment ICH (13, 36–39, 51). As such, these factors represent parts of the puzzle that complete the picture to understanding how stroke evolves toward a critical level of impairment of cerebral autoregulation, edema, blood-brain-barrier disruption, and post-treatment reperfusion injury. Recanalization therapy, albeit necessary and often successful to restore the ischemic brain to baseline condition, does not directly modify these pathophysiologic mechanisms of stroke and reperfusion injury. Furthermore, almost all drugs with mechanisms thought to counter a specific part of the stroke injury cascade have failed to provide a conclusive neuroprotective effect in clinical studies (50, 52). Certainly, a treatment concept that can attenuate or prevent these pathophysiologic processes is desirable and would be an ideal candidate for adjunctive application.

The Physiological Limits of Systemic Hypothermia

Therapeutic hypothermia has been identified as one of the most promising neuroprotective methods. Systemic cooling

experiments in stroke models involving animals across various species have shown that hypothermia is neuroprotective in terms of reduction in infarct size and improvement in neurobehavioral testing scores (average effect size 44% [95% confidence interval, 40−47%]) without increasing the risk for ICH (48). The clinical translation of systemic hypothermia (mild to moderate hypothermia, reduction of body core temperature to ∼33°C −35°C) has been successful in comatose patients with out-of-hospital cardiac arrest in both shockable and non-shockable rhythms (53, 54) as well as in neonates with ischemic encephalopathy (55). In addition, cardiac surgery has been routinely performed with extracorporeal blood cooling or total exchange with cold fluids (profound hypothermia, reduction of body core temperature to ≤25°C) to protect the brain from ischemic injury during circulatory arrest or vascular clamping (56).

The neuroprotective effect of cooling is the result of hypothermia's pleiotropic mechanisms of action. Among a plethora of demonstrated effects that help to produce a physiological state of ischemic tolerance, cooling causes metabolic depression reducing the cellular demand for oxygen and energy, has anti-excitotoxic, anti-inflammatory, anti-edematous, and anti-apoptotic properties, and suppresses the breakdown of the blood-brain-barrier (49, 57, 58). Perhaps the most known endogenous feature of hypothermia is found in hibernating mammals that are able to survive prolonged periods of hypometabolism and reduced tissue perfusion under extreme hypothermic conditions (body temperature decreases even below the freezing point of water) (59).

What appears to be simply natural and is repeated year after year in hibernating mammals, in humans the process of inducing and maintaining systemic hypothermia are extremely difficult, complicated, prolonged, and painful due to the strong physiological counter mechanisms and frequent adverse events (60, 61) (**Figures 1A–C**). As such, this clinical process requires specialized intensive care resources, sedation, and muscle relaxation, machine ventilation, and co-treatment of the frequent adverse effects of body hypothermia. Despite the successes achieved in the clinical settings of out-of-hospital cardiac arrest and neonatal asphyxia, systemic hypothermia has been difficult to realize in acute stroke patients (62).

The Promises of Adjunct Endovascular Brain Hypothermia

Selective brain hypothermia is an attractive alternative to systemic hypothermia, providing focused cooling of the injured organ and avoiding the complications of systemic hypothermia, including intubation, shivering, pneumonia, altered coagulability, and cold-induced stress reactions (58). A variety of devices and strategies have been developed to induce selective brain hypothermia and tested in preclinical models and early human clinical trials, including intranasal selective hypothermia, transvenous endovascular cooling, extraluminal vascular cooling, and epidural cerebral cooling (63–72). However, advance of these devices in clinical development has been constrained by slow onset of cooling induced by external cerebral cooling techniques and procedural time delay needed to place internal nasopharyngeal cooling devices. A promising emerging approach that overcomes these limitations is selective endovascular brain cooling (SEBC; **Supplement Table**) through an interventional catheter system that is navigated to the common or internal carotid artery to perform an intravascular heat-exchange with the arterial blood before it enters the cerebral vasculature (**Figure 1D**). Different endovascular local heat-exchange concepts exist exploiting the physical forces of conduction or direct mixing of cold fluids with blood, and intravascular vs. extracorporeal heat-exchange methods (63, 72).

Experiments in animal stroke models, commonly with transient occlusion of the middle cerebral artery, have found a significant improvement in outcome (stroke volume, edema, behavioral scores) with brief endovascular selective brain cooling [average effect size 51% (95% CI, 38–64%)] (73, 74). Hereby, the risk for ICH was not increased in the cooling groups compared to control groups. The preferred method is direct intra-arterial infusion of cold fluids to cool the blood that enters the brain either during ischemia, post reperfusion, or during both phases. Although brain cooling was performed for only a short duration, the rapid induction of brain hypothermia led to the suppression of the pathophysiological mechanisms of ischemia and reperfusion injury to levels that allowed a significantly better restoration of the affected brain tissue when compared to the normothermia group.

Speed of brain cooling is one of the unique features of SEBC that distinguishes itself from any form of systemic hypothermia. This is because only a selected vascular territory of the brain is cooled, rather than the whole body, and a direct modification of the arterial input temperature is performed (63). The cooling performance with SEBC is in the range of minutes with the direct infusion method, not hours (**Figure 1D**). For instance, a brain temperature reduction of more than 3.5°C in 10 min was shown in a large pig model with SEBC using the direct intra-arterial cold infusion (IACI) method (75). In a canine transient occlusion model, a reduction in brain temperature of >10°C within 10 min was found using SEBC, with direct IACI during the phases of ischemia and post-reperfusion (76). The animals with brain cooling had smaller infarct sizes compared to controls. Extracorporeal blood cooling is a more invasive cooling concept primarily performed in the clinical setting of cardiac surgery. Blood from the femoral artery, aorta, or carotid artery is passed through an extracorporeal heat-exchanger where it is cooled to the desired temperature. This cooled and medically processed blood is infused back into the cerebral circulation, often via the carotid artery. In another swine stroke model experiment, extracorporeal blood cooling led to a brain temperature decrease of >6°C in ∼30 min (77). In contrast to SEBC with IACI (cold fluid or cold blood), local blood cooling via conduction, i.e., with a closed-loop intra-carotid catheter in which cold fluids circulate, is far less effective due to the limited dimensions of the parent artery and resulting limitations to the size and surface area of the heat-exchange catheter (78).

SEBC has also been studied in humans (**Table 1**). The feasibility and safety of SEBC with a brief intra-carotid infusion of cold fluid was demonstrated in a first-in-human study

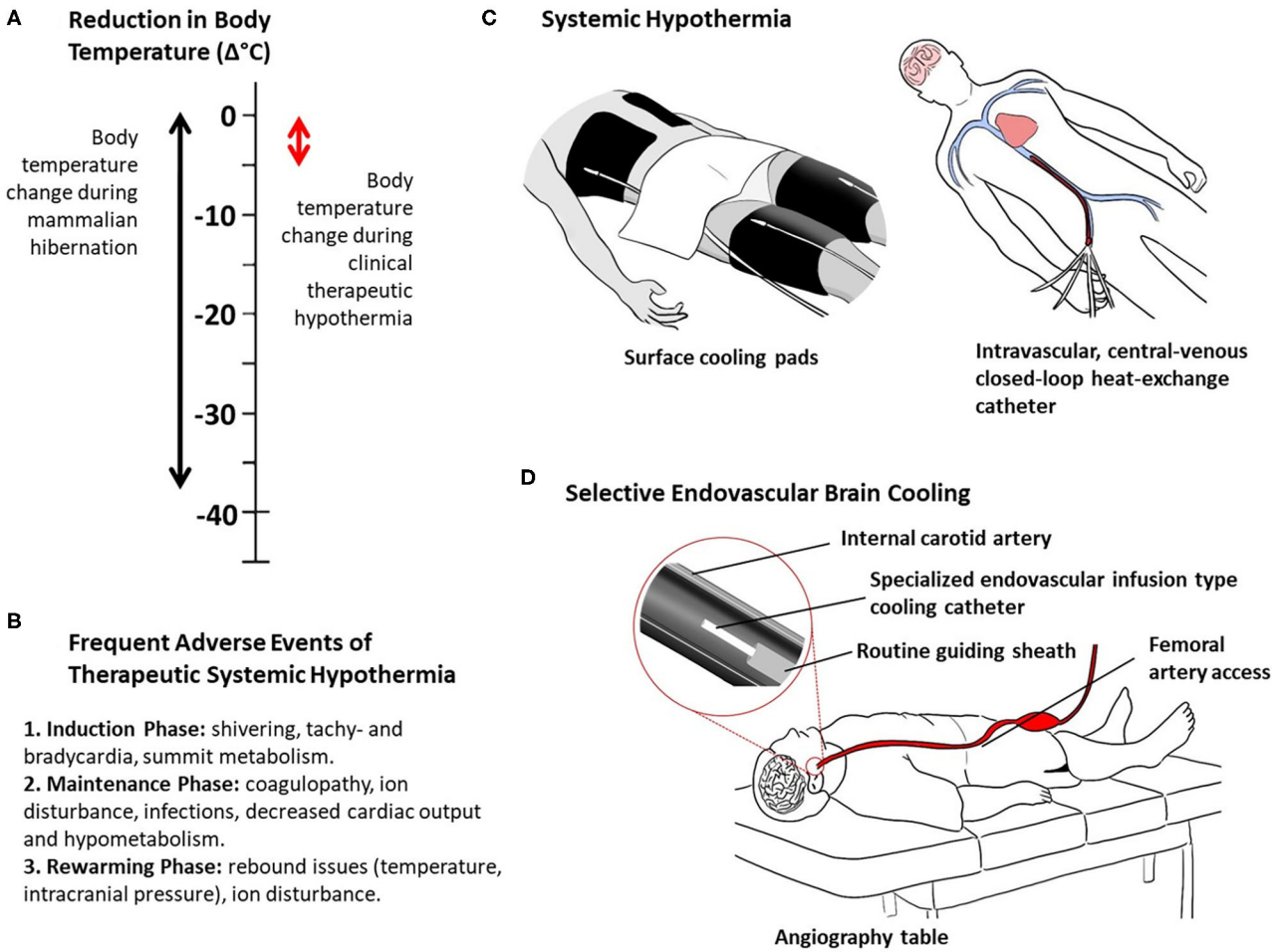


FIGURE 1 | Thermal characteristics of mammalian hibernation and clinical hypothermia **(A)**, frequent adverse events of systemic therapeutic hypothermia **(B)**, and methods of clinical therapeutic hypothermia **(C, D)**. Systemic hypothermia, examples of surface and intravascular conductive cooling **(C)**. Selective endovascular brain cooling, example of direct intra-arterial cold infusion **(D)**.

in 18 elective cerebrovascular patients undergoing follow-up cerebral angiograms (79). Blood temperature of the ipsilateral jugular venous bulb was monitored as a surrogate for local brain temperature. Clinical testing during the procedure in awake patients, and after the procedure in all patients, found that this selective cooling process was painless and produced no serious adverse effects. Transcranial Doppler monitoring and serial blood sample analyses showed stable parameters. In three following clinical studies, this direct infusion method of SEBC was applied, during ischemia and post-reperfusion, in acute ischemic stroke LVO patients (80–82). However, brain temperature or surrogates were not measured. Due to the limited number of patients and study design, the efficacy of SEBC in LVO patients remains to be determined. Nevertheless, SEBC with brief cold fluid infusion was found to be safe in LVO patients undergoing endovascular therapy and was not associated with serious adverse events. A phase II randomized controlled trial is currently being conducted to study brief SEBC with IACI in a larger group of LVO acute ischemic stroke patients undergoing clot extraction (83). In 2018, a single-arm explorative study investigating the safety of brief SEBC-IACI-induced brain cooling in anterior circulation acute ischemic stroke patients undergoing clot extraction (and refractory to tissue plasminogen activator therapy) was completed (84). The results are pending publication.

DISCUSSION

The advantages of endovascular selective brain cooling for the treatment of LVO stroke are mani-fold. One, with significantly reduced time to target temperature of the organ of interest, the brain, hypothermic neuroprotection can be achieved quickly. Hereby, the physical concept of SEBC is ideally suited to achieve brain cooling quickly (10–30 times faster than traditional systemic hypothermia) and with

TABLE 1 | Clinical studies of selective endovascular brain cooling with cold fluid infusion.

Year	Author	N (Controls)	Setting	IA infusion	Sequence	Cold inf volume	Brain temp	Blood flow	Outcome
2010	Choi*	18	Angiogram	ICA	Intra-proc	300 ml	YES	TCD	Clinical
2012	Neimark**	Data from*	Computer simulation	ICA	Intra-proc	300 ml	YES	NO	Clinical
2016	Peng	11 (15)	Acute ischemic stroke	CA	Pre-Revasc	500 ml	NO	NO	Infarct volume
2016	Chen	26	Acute ischemic stroke	CA	Pre/Post-Revasc	350 ml	NO	NO	Clinical
2018	Wu	45 (68)	Acute ischemic stroke	CA	Pre/Post-Revasc	350 ml	NO	NO	Infarct volume

**First-in-human proof of concept.*

***Data from clinical study inputted in computer simulated heat transfer model of the human head.*

IA, intra-arterial; Inf, infusion; Temp, temperature; ICA, internal carotid artery; TCD, transcranial Doppler; CA, cerebral artery; Intra-proc, intra-procedural; Pre-Revasc, Pre-Revascularization.

minimal invasiveness because it directly modifies the cerebral arterial input temperature and would be administered via the routine endovascular route. Two, selective brain cooling allows to reduce the impact of cooling on the body, thus minimizing or avoiding systemic hypothermia and its adverse consequences. Three, due to the endovascular route, SEBC is ideally suited to be applied as an adjunct treatment to endovascular recanalization procedures in LVO stroke. Four, due to its selective and immediate cooling features and endovascular route, SEBC would be capable to attenuate the destructive forces of reperfusion injury, locally and directly, following endovascular clot-extraction.

In practice, and taking the direct infusion method as an example, the SEBC catheter would be placed in the ipsilateral (ischemic hemisphere) internal carotid artery through the same intra-arterial access as used for clot extraction (**Figure 1D**). The catheter diameter would be small enough to fit through the regular guiding sheath. Selective brain cooling would be performed immediately following thrombectomy via exchange of catheters. Parallel use of thrombectomy and SEBC catheters is a possibility. In order to achieve brain hypothermia quickly, one could even attempt to cool the ischemic brain before thrombectomy is performed (intra-ischemic), as done in the explorative clinical studies (**Table 1**). However, this would undoubtedly delay reperfusion therapy and pose additional risks for distal embolization and cerebral vascular injury due to manipulations of the cooling catheter as it is pushed past the clot.

The key to reducing the impact of ischemia and reperfusion injury is the suppression of their pathophysiological mechanisms. While it is clear that SEBC-induced hypothermic neuroprotection would be delayed in LVO patients when they finally undergo endovascular clot-extraction, regardless of how quickly brain cooling can be achieved, the results from animal stroke models and our understanding of the molecular and cellular evolution of stroke suggest that brain cooling is neuroprotective, even when delayed for several hours. This is a plausible assumption. If the early stage excitotoxicity cannot be prevented, brain hypothermia may still suppress the later-stage inflammatory processes, reduce edema, and prevent vessels from becoming too "leaky." Disturbance of the structural integrity and leaky vessels are considered the basic preconditions for developing post stroke ICH.

The beneficial impact of SEBC on the potential harms of sudden reperfusion is more evident when considering SEBC is applied as an adjunct to endovascular clot-extraction. Furthermore, SEBC induced via the direct mixing method with IACI could offer additional benefit as the incoming blood would be diluted, therefore reducing the impact of inflammatory promoters and immune cells on the ischemic and reperfused brain tissue. Another important advantage of the infusion method is that the dimensions of the infusion-type cooling catheter would be small enough to fit through the guiding sheath that has already been placed to navigate the EVT catheter to remove the clot, requiring only an exchange of catheters or even allowing concomitant use of both catheter systems (**Figure 1D**).

There are limitations of SEBC. First, although hypothermia induction would be rapid, the duration of cooling would be limited by the routine times allowed for indwelling endovascular catheters to remain in the arterial system. Second, the direct mixing method with IACI, albeit the fastest cooling method in physical terms, would be limited by the volume that could be infused within a certain period. In contrast, the extracorporeal blood cooling concept would theoretically address the issue of hypervolemia as practically the same amount of blood is re-introduced into the cerebral circulation as it was removed from the system before exposing the blood to the external heat-exchanger. With this isovolemic cooling concept, long-term brain hypothermia would become feasible. However, this isovolemic method adds layers of invasiveness and complexity to the hyperacute workflow, such as a second arterial puncture (blood outflow), vascular reconstruction (e.g., temporary arterial occlusion), necessity of a perfusion specialist, and heparinization of blood in the external circulation. Third, potential adverse effects may occur from local exposure to cold, additional fluid volume (local hemodilution) and mechanical stress from the endovascular cooling catheter. Thus, careful monitoring of vitals, dilution, local temperature, and potential vascular injury and

spasm would be necessary. Lasty, there are yet no devices on the market for SEBC. Only routine catheters have been used to explore and investigate the safety and feasibility of brief SEBC (10–15 min) in elective and acute cerebrovascular patients. While the results have been promising and larger trials are underway, it is questionable whether brief and uncontrolled IACI will deliver the answers to the ideal depth, duration, and timing of brain hypothermia the determination of which should be based on physiological parameters and cerebral metabolic demand, such as changes in brain metabolism, cerebral blood flow, and infarct evolution. This could become more critical when infusing fluids distally to a cerebral artery occlusion (intra-ischemic brain cooling) without any information about the metabolic and hemodynamic condition of the ischemic tissue bed. As such, we believe that to provide a safe and most efficient SEBC, specialized catheter systems are necessary that offer excellent heat-exchange, mixing, and embedded safety and control mechanisms.

Given the growing and widespread utilization of endovascular clot-extraction in LVO stroke and lack of additional means to counter the deleterious mechanisms of ischemia and reperfusion injury (12, 85), SEBC is an appealing concept to reap the benefits of therapeutic hypothermia while minimizing the adverse effects of systemic hypothermia. Based on our current understanding of the mechanisms of stroke, reperfusion, and therapeutic (brain) hypothermia, it is reasonable to consider an improvement in outcome and reduction in the occurrence of post-stroke ICH may occur. Currently, technologies that would enable safe and controlled SEBC in acute ischemic stroke patients are in pre-clinical and explorative clinical development (**Supplement Table**). However, it is foreseeable that specialized medical devices for SEBC will be available in the near future. Ultimately, clinical investigations will show whether SEBC will be a safe, practical, and effective tool in the armamentarium of stroke treatment.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

JC and JP-S contributed conception of the article. JC wrote the first draft of the article. All authors have approved the final version of the manuscript, contributed critical review and revision of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.594289/full#supplementary-material

REFERENCES

1. Berkhemer OA, Fransen PS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med*. (2015) 372:11–20. doi: 10.1056/NEJMoa1411587
2. Jovin TG, Chamorro A, Cobo E, de Miquel MA, Molina CA, Rovira A, et al. Thrombectomy within 8 hours after symptom onset in ischemic stroke. *N Engl J Med*. (2015) 372:2296–306. doi: 10.1056/NEJMoa1503780
3. Saver JL, Goyal M, Bonafe A, Diener HC, Levy EI, Pereira VM, et al. Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. *N Engl J Med*. (2015) 372:2285–95. doi: 10.1056/NEJMoa1415061
4. Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. *N Engl J Med*. (2015) 372:1019–30. doi: 10.1056/NEJMoa1414905
5. Campbell BC, Mitchell PJ, Kleinig TJ, Dewey HM, Churilov L, Yassi N, et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N Engl J Med*. (2015) 372:1009–18. doi: 10.1056/NEJMoa1414792
6. Mocco J, Zaidat OO, von Kummer R, Yoo AJ, Gupta R, Lopes D, et al. Aspiration thrombectomy after intravenous alteplase vs. intravenous alteplase alone. *Stroke*. (2016) 47:2331–8. doi: 10.1161/STROKEAHA.116.013372
7. Martins SO, Mont'Alverne F, Rebello LC, Abud DG, Silva GS, Lima FO, et al. Thrombectomy for stroke in the public health care system of Brazil. *N Engl J Med*. (2020) 382:2316–26. doi: 10.1056/NEJMoa2000120
8. Saver JL, Goyal M, van der Lugt A, Menon BK, Majoie CBLM, Dippel DW, et al. Time to treatment with endovascular thrombectomy and outcomes from ischemic stroke: a meta-analysis. *JAMA*. (2016) 316:1279–88. doi: 10.1001/jama.2016.13647
9. Albers GW, Marks MP, Kemp S, Christensen S, Tsai JP, Ortega-Gutierrez S, et al. Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. *N Engl J Med*. (2018) 378:708–18. doi: 10.1056/NEJMoa1713973
10. Nogueira RG, Jadhav AP, Haussen DC, Bonafe A, Budzik RF, Bhuva P, et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. *N Engl J Med*. (2018) 378:11–21. doi: 10.1056/NEJMoa1706442
11. Badhiwala JH, Nassiri F, Alhazzani W, Selim MH, Farrokhyar F, Spears, et al. Endovascular thrombectomy for acute ischemic stroke: a meta-analysis. *JAMA*. (2015) 314:1832–43. doi: 10.1001/jama.2015.13767
12. van Horn N, Kniep H, Leischner H, McDonough R, Deb-Chatterji M, Broocks G, et al. Predictors of poor clinical outcome despite complete reperfusion in acute ischemic stroke patients. *J NeuroInterv Surg*. (2020). doi: 10.1136/neurintsurg-2020-015889. [Epub ahead of print].
13. Choi JH, Pile-Spellman J. Reperfusion changes after stroke and practical approaches for neuroprotection. *Neuroimag Clin N Am*. (2018) 28:663–82. doi: 10.1016/j.nic.2018.06.008
14. Lipton P, Raley PKM, Lobner D. Long-term inhibition of synaptic transmission and macromolecular synthesis following anoxia in the rat hippocampal slice: interaction between Ca and NMDA receptors. In: G. Somjen, editor. *Mechanisms of Cerebral Hypoxia and Stroke*. Boston, MA: Springer (1998). p. 229–49.
15. Lauritzen M, Hansen AJ. The effect of glutamate receptor blockade on anoxic depolarization and cortical spreading depression. *J Cerebral Blood Flow Metab*. (1992) 12:223–9. doi: 10.1038/jcbfm.1992.32
16. Grigg JJ, Anderson EG. Competitive and non-competitive N-methyl-D-aspartate antagonists modify hypoxia-induced membrane potential changes and protect rat hippocampal slices from functional failure: a quantitative comparison. *J Pharmacol Exp Ther*. (1990) 253:130–5.
17. Benveniste H, Drejer J, Schousboe A, Diemer N. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J Neurochem*. (1984) 43:1369–74. doi: 10.1111/j.1471-4159.1984.tb05396.x

18. Iadecola C, Xu X, Zhang F, El-Fakahany EE, Ross ME. Marked induction of calcium-independent nitric oxide synthase activity after focal ischemia. *J Cerebral Blood Flow Metabol.* (1995) 15:52–9. doi: 10.1038/jcbfm.1995.6
19. Nogawa S, Zhang F, Ross ME, Iadecola C. Cyclooxygenase-2 gene expression in neurons contributes to ischemic brain damage. *J Neurosci.* (1997) 17:2746–55. doi: 10.1523/JNEUROSCI.17-08-02746.1997
20. Degraba TJ. The role of inflammation after acute stroke. utility of pursuing anti-adhesion molecule therapy. *Neurology.* (1998) 51(Suppl. 3):S62–8. doi: 10.1212/WNL.51.3_Suppl_3.S62
21. Chamorro A, Hallenbeck J. The harms and benefits of inflammatory and immune responses in vascular disease. *Stroke.* (2006) 37:291–3. doi: 10.1161/01.STR.0000200561.69611.f8
22. Zheng Z, Yenari MA. Post-ischemic inflammation: molecular mechanisms and therapeutic implications. *Neurol Res.* (2004) 26:884–92. doi: 10.1179/016164104X2357
23. Nawashiro H, Tasaki K, Ruetzler A, Hallenbeck J. TNF-alpha pretreatment induces protective effects against focal cerebral ischemia in mice. *J Cerebral Blood Flow Metab.* (1997) 17:483–90. doi: 10.1097/00004647-199705000-00001
24. Allan SM, Rothwell NJ. Cytokines and acute neurodegeneration. *Nat Rev Neurosci.* (2001) 2:734–44. doi: 10.1038/35094583
25. Baeuerle PA, Henkel T. Function and activation of NF-kappa B in the immune system. *Annu Rev Immunol.* (1994) 12:141–79. doi: 10.1146/annurev.iy.12.040194.001041
26. Schneider A, Martin-Villalba A, Weih F, Vogel J, Wirth T, Schwaninger M. NF-kappaB is activated and promotes cell death in focal cerebral ischemia. *Nat Med.* (1999) 5:554–9. doi: 10.1038/8432
27. Irving EA, Bamford M. Role of mitogen- and stress-activated kinases in ischemic injury. *J Cerebral Blood Flow Metab.* (2002) 22:631–47. doi: 10.1097/00004647-200206000-00001
28. Rosenberg GA. Matrix metalloproteinases in neuroinflammation. *Glia.* (2002) 39:279–91. doi: 10.1002/glia.10108
29. Chen J, Zhu RL, Nakayama M. Expression of the apoptosis-effector gene, BAX, is upregulated in vulnerable hippocampal CA1 neurons following global ischemia. *J Neurochem.* (1996) 67:64–71. doi: 10.1046/j.1471-4159.1996.67010064.x
30. Squier MKT, Miller ACK, Malkinson AM, Cohen JJ. Calpain activation in apoptosis. *J Cell Physiol.* (1994) 159:229–37. doi: 10.1002/jcp.1041590206
31. Marchal G, Furlan M, Beaudouin V, Rioux P, Hauttement JL, Serrati C, et al. Early spontaneous hyperperfusion after stroke. a marker of favourable tissue outcome? *Brain.* (1996) 119:409–19. doi: 10.1093/brain/119.2.409
32. Macfarlane R, Moskowitz MA, Sakas DE, Tasdemiroglu E, Wei EP, Kontos HA. The role of neuroeffector mechanisms in cerebral hyperperfusion syndromes. *J Neurosurg.* (1991) 75:845–55. doi: 10.3171/jns.1991.75.6.0845
33. Schaefer PW, Gonzalez RG, Hunter G, Wang B, Koroshetz WJ, Schwamm LH. Diagnostic value of apparent diffusion coefficient hyperintensity in selected patients with acute neurologic deficits. *J Neuroimaging.* (2001) 11:369–80. doi: 10.1111/j.1552-6569.2001.tb00065.x
34. Ay H, Buonanno FS, Schaefer PW, Le DA, Wang B, Gonzalez RG, et al. Posterior leukoencephalopathy without severe hypertension: utility of diffusion-weighted MRI. *Neurology.* (1998) 51:1369–76. doi: 10.1212/WNL.51.5.1369
35. Wang X, Tsuji K, Lee SR, Ning M, Furie KL, Buchan AM, et al. Mechanisms of hemorrhagic transformation after tissue plasminogen activator reperfusion therapy for ischemic stroke. *Stroke.* (2004) 35:2726–30. doi: 10.1161/01.STR.0000143219.16695.af
36. Warach S, Latour LL. Evidence of reperfusion injury, exacerbated by thrombolytic therapy, in human focal brain ischemia using a novel imaging marker of early blood–brain barrier disruption. *Stroke.* (2004) 35(Suppl. I):2659–61. doi: 10.1161/01.STR.0000144051.32131.09
37. Leigh R, Christensen S, Campbell BCV, Marks MP, Albers GW, Lansberg MG. Pretreatment blood–brain barrier disruption and post-endovascular intracranial hemorrhage. *Neurology.* (2016) 87:263–9. doi: 10.1212/WNL.0000000000002862
38. Fiehler J, Remmele C, Kucinski T, Rosenkranz M, Thomalla G, Weiller C, et al. Reperfusion after severe local perfusion deficit precedes hemorrhagic transformation: An MRI study in acute stroke patient. *Cerebrovasc Dis.* (2005) 19:117–24. doi: 10.1159/000083180
39. Renu A, Laredo C, Tudela R, Urra X, Lopes-Rueda A, Llull L, et al. Brain hemorrhage after endovascular reperfusion therapy of ischemic stroke: a threshold-finding whole-brain perfusion CT study. *J Cerebral Blood Flow Metabol.* (2017) 37:153–65. doi: 10.1177/0271678X15621704
40. Pan J, Konstas AA, Bateman B, Ortolano G, Pile-Spellman J. Reperfusion injury following cerebral ischemia: pathophysiology, MR imaging, and potential therapies. *Neuroradiology.* (2007) 49:93–102. doi: 10.1007/s00234-006-0183-z
41. Karapanayioitides T, Meuli R, Devuyst G, Piechowski-Jozwiak B, Dewarrat A, Ruchat P, et al. Postcarotid endarterectomy hyperperfusion or reperfusion syndrome. *Stroke.* (2005) 36:21–6. doi: 10.1161/01.STR.0000149946.86087.e5
42. Lin L, Wang X, Yu Z. Ishemia-reperfusion injury in the brain: Mechanisms and potential therapeutic strategies. *Biochem Pharmacol.* (2016) 5:213. doi: 10.4172/2167-0501.1000213
43. Jennings RB, Sommers HM, Smyth GA, Flack HA, Linn H. Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. *Arch Pathol.* (1960) 70:68–78.
44. Beyersdorf F, Matheis G, Kruger S, Hanselmann A, Freisleben HG, Zimmer G, et al. Avoiding reperfusion injury after limb revascularization: experimental observations and recommendations for clinical application. *J Vase Surg.* (1989) 9:757–66. doi: 10.1016/0741-5214(89)90081-5
45. Gonzalez LM, Moeser AJ, Blikslager AT. Animal models of ischemia-reperfusion-induced intestinal injury: progress and promise for translational research. *Am J Physiol Gastrointest Liver Physiol.* (2015) 308:63–75. doi: 10.1152/ajpgi.00112.2013
46. Khatri P, Wechsler LR, Broderick JP. Intracranial hemorrhage associated with revascularization therapies. *Stroke.* (2007) 38:431–40. doi: 10.1161/01.STR.0000254524.23708.c9
47. Khatri R, McKinney AM, Swenson B, Janardhan V. Blood-brain barrier, reperfusion injury, and hemorrhagic transformation in acute ischemic stroke. *Neurology.* (2012) 79(13 Suppl. 1):S52–7. doi: 10.1212/WNL.0b013e3182697e70
48. van der Worp HB, Sena ES, Donnan GA, Howells DW, Macleod MR. Hypothermia in animal models of acute ischaemic stroke: a systematic review and meta-analysis. *Brain.* (2007) 130:3063–74. doi: 10.1093/brain/awm083
49. Polderman KH. Induced hypothermia and fever control for prevention and treatment of neurological injuries. *Lancet.* (2008) 371:1955–69. doi: 10.1016/S0140-6736(08)60837-5
50. Hill MD, Goyal MG, Menon BK, Nogueira RG, McTaggart RA, Demchuk AM, et al. Efficacy and safety or nerinetide for the treatment of acute ischaemic stroke (ESCAPE-NA1): a multicentre, double-blind, randomised controlled trial. *Lancet.* (2020) 395:878–87. doi: 10.1016/S0140-6736(20)30258-0
51. Nogueira RG, Gupta R, Jovin TG, Levy EI, Liebeskind DS, Zaidat OO, et al. Predictors and clinical relevance of hemorrhagic transformation after endovascular therapy for anterior circulation large vessel occlusion strokes: a multicenter retrospective analysis of 1122 patients. *J Neurointerv Surg.* (2015) 7:16–21. doi: 10.1136/neurintsurg-2013-010743
52. O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW. 1,026 experimental treatments in acute stroke. *Ann Neurol.* (2006) 59:467–77. doi: 10.1002/ana.20741
53. Bernard SA, Gray TW, Buist MD, Jones BM, Silvester W, Gutteridge G, et al. Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *N Engl J Med.* (2002) 346:557–63. doi: 10.1056/NEJMoa003289
54. Lascarrou JB, Merdji H, Le Gouge A, Colin G, Grillet G, Girardie P, et al. Targeted temperature management for cardiac arrest with nonshockable rhythm. *N Engl J Med.* (2019) 381:2327–37. doi: 10.1056/NEJMoa1906661
55. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med.* (2005) 353:1574–84. doi: 10.1056/NEJMcps050929
56. Harrington DK, Walker AS, Kukuntla H, Bracewell RM, Clutton-Brock TH, Faroqui M, et al. Selective antegrade cerebral perfusion attenuates brain metabolic deficit in aortic arch surgery. a prospective randomized trial. *Circulation.* (2004) 110(Suppl. II): 231–6. doi: 10.1161/01.CIR.0000138945.78346.9c
57. Erecinska M, Thoresen M, Silver IA. Effects of hypothermia on energy metabolism in mammalian central nervous system. *J Cerebral Blood*

Flow Metab. (2003) 23:513–30. doi: 10.1097/01.WCB.0000066287.21705.21
58. Zhao H, Steinberg G, Sapolsky RM. General vs. specific actions of mild-moderate hypothermia in attenuating cerebral ischemic damage. *J Cerebral Blood Flow Metab.* (2007) 27:1879–94. doi: 10.1038/sj.jcbfm.9600540
59. Drew KL, Buck CL, Barnes BM, Christian SL, Rasley BT, Harris MB. Central nervous system regulation of mammalian hibernation: implications for metabolic suppression and ischemia tolerance. *J Neurochem.* (2007) 102:1713–26. doi: 10.1111/j.1471-4159.2007.04675.x
60. Lyden P, Hemmen T, Grotta J, Rapp K, Ernstrom K, Rzesiewicz T, et al. Results of the ICTuS 2 Trial. *Stroke.* (2016) 47:2888–95.
61. Andresen M, Gazmuri JT, Marin A, Regueira T, Rovegno M. Therapeutic hypothermia for acute brain injuries. *Scand J Trauma Resusc.* (2015) 23:42. doi: 10.1186/s13049-015-0121-3
62. van der Worp HB, Macleod MR, Bath PMW, Bathula R, Christensen H, Colam B, et al. Therapeutic hypothermia for acute ischaemic stroke. results of a European multicentre, randomised, phase III clinical trial. *J Cerebral Blood Flow Metab.* (2019) 4:254–62. doi: 10.1177/2396987319844690
63. Choi JH, Pile-Spellman J. Selective brain hypothermia. In: AA. Romanovsky, editor. *Handbook of Clinical Neurology*, Vol. 157 (3rd series) Thermoregulation: From Basic Neuroscience to Clinical Neurology, Part II. (2018). p. 839–52.
64. Christian E, Zada G, Sung G, Giannotta SL. A review of selective hypothermia in the management of traumatic brain injury. *Neurosurg Focus.* (2008) 25:E9. doi: 10.3171/FOC.2008.25.10.E9
65. Hachimi-Idrissi S, Corne L, Ebinger G, Michotte Y, Huyghens L. Mild hypothermia induced by a helmet device: a clinical feasibility study. *Resuscitation.* (2001) 51:275–81. doi: 10.1016/S0300-9572(01)00412-9
66. Bayegan K, Janata A, Frossard M, Holzer M, Sterz F, Losert UM, et al. Rapid non-invasive external cooling to induce mild therapeutic hypothermia in adult human-sized swine. *Resuscitation.* (2007) 76:291–8. doi: 10.1016/j.resuscitation.2007.07.006
67. Gladen A, Iaizzo PA, Bischof JC, Erdman AG, Divani AA. A head and neck support device for inducing local hypothermia. *J Med Dev.* (2013) 8:110021–9. doi: 10.1115/1.4025448
68. Islam S, Hampton-Till J, Watson N, Mannakkara NN, Hamarneh A, Webber T, et al. Early targeted brain COOLing in the cardiac CATHeterisation laboratory following cardiac arrest (COOLCATH). *Resuscitation.* (2015) 95:61–7. doi: 10.1016/j.resuscitation.2015.09.386
69. Poli S, Purrucker J, Priglinger M, Ebner M, Sykora M, Diedler J, et al. Rapid induction of COOLing in stroke patients (iCOOL1): a randomised pilot study comparing cold infusions with nasopharyngeal cooling. *Crit Care.* (2014) 18:582. doi: 10.1186/s13054-014-0582-1
70. Sayedsaadat SM, Marasco SF, Daly DJ, McEgan R, Anderson J, Rodgers S, et al. Selective brain hypothermia: feasibility and safety study of a novel method in five patients. *Perfusion.* (2019) 35:96–103. doi: 10.1177/0267659119853950
71. Assis FR, Narasimhan B, Ziai W, Tandri H. From systemic to selective brain cooling – Methods in review. *Brain Circ.* (2019) 5:179–86. doi: 10.4103/bc.bc_23_19
72. Wu L, Wu D, Yang T, Xu J, Chen J, Wang L, et al. Hypothermic neuroprotection against acute ischemic stroke: The 2019 update. *J Cerebral Blood Flow Metab.* (2020) 40:461–81. doi: 10.1177/0271678X19894869
73. Esposito E, Ebner M, Ziemann, U, Poli S. In cold blood: intraarterial cold infusion for selective brain cooling in stroke. *J Cerebral Blood Flow Metab.* (2014) 34:743–52. doi: 10.1038/jcbfm.2014.29
74. Dumitrascu OM, Lamb J, Lyden PD. Still cooling after all these years: meta-analysis of pre-clinical trials of therapeutic hypothermia for acute ischemic stroke. *J Cerebral Blood Flow Metab.* (2016) 36:1157–64. doi: 10.1177/0271678X16645112
75. Choi JH, Mangla S, Barone FC, Novotney C, Pile-Spellman J. Rapid and selective brain cooling and maintenance of selective cooling with intra-carotid cold fluid infusion is feasible and safe. *Eur Stroke J.* (2016) 1:391. doi: 10.1177/2396987316642909
76. Caroff J, King RM, Mitchell JE, Marosfoi M, Licwinko JR, Gray-Edwards HL, et al. Focal cooling of brain parenchyma in a transient large vessel occlusion model: proo-of-concept. *J Neurointervent Surg.* (2020) 12:209–13. doi: 10.1136/neurintsurg-2019-015179
77. Solar RJ, Lownie SP. Neuroprotection by selective endovascular brain cooling-the TwinFlo catheter. *EuroIntervention.* (2020) 15:1291–6. doi: 10.4244/EIJ-D-19-00316
78. Merrill TL, Mitchell JE, Merrill D. Heat transfer analysis of catheters used for localized tissue cooling to attenuate reperfusion injury. *Med Engin Physics.* (2016) 38:75. doi: 10.1016/j.medengphy.2016.05.007
79. Choi JH, Marshall RS, Neimark MA, Konstas AA, Lin E, Chiang YT, et al. Selective brain cooling with endovascular intracarotid infusion of cold saline: a pilot feasibility study. *AJNR Am J Neuroradiol.* (2010) 31:928–34. doi: 10.3174/ajnr.A1961
80. Peng X, Wan Y, Liu W, Dan B, Lin L, Tang Z. Protective roles of intra-arterial mild hypothermia and arterial thrombolysis in acute cerebral infarction. *SpringerPlus.* (2016) 5:1988. doi: 10.1186/s40064-016-3654-7
81. Chen J, Liu L, Zhang H, Geng X, Jiao L, Li G, et al. Endovascular hypothermia in acute ischemic stroke. pilot study of selective intra-arterial cold saline infusion. *Stroke.* (2016) 47:1933–35. doi: 10.1161/STROKEAHA.116.012727
82. Wu C, Zhao W, An H, Wu L, Chen J, Hussain M, et al. Safety, feasibility, and potential efficacy of intraarterial selective cooling infusion for stroke patients treated with mechanical thrombectomy. *J Cerebral Blood Flow Metab.* (2018) 38:2251–60. doi: 10.1177/0271678X18790139
83. ClinicalTrials.gov Identifier: NCT03163459. Available online at: https://clinicaltrials.gov/ct2/show/NCT03163459 (accessed September 29, 2020).
84. UMIN-CTR Clinical Trial Identifier: UMIN000018255. Available online at: https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000021101 (accessed September 29, 2020).
85. Linfante I, Starosciak AK, Walker GR, Dabus G, Castonguay AC, Gupta R, et al. Predictors of poor outcome despite recanalization: a multiple regression analysis of the NASA registry. *J Neurointerv Surg.* (2016) 8:224–9. doi: 10.1136/neurintsurg-2014-011525

Conflict of Interest: JC and JS are co-founders of Hybernia Medical, LLC. SP and JS are consultants to Hybernia Medical, LLC.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 17 November 2020
doi: 10.3389/fneur.2020.582639

Association of Blood Pressure at Successful Recanalization and Parenchymal Hemorrhage After Mechanical Thrombectomy With General Anesthesia

Hui Cheng[1†], Chao Xu[2†], Xing Jin[1], Yigang Chen[1], Xu Zheng[1], Feina Shi[1], Xudong He[1], Yonggang Hao[1], Yun Jiang[1], Jinhua Zhang[1] and Zhicai Chen[3*]*

[1] Department of Neurology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, China, [2] Department of Neurology, Zhejiang Provincial People's Hospital, Hangzhou, China, [3] Department of Neurology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

OPEN ACCESS

Edited by:
Jean-Marc Olivot, Center Hospitalier Universitaire de Toulouse, France

Reviewed by:
Thomas Geeraerts, Université Toulouse III Paul Sabatier, France
Mikael Mazighi, Université de Paris, France

***Correspondence:**
Zhicai Chen
chenzhicai@zju.edu.cn
Jinhua Zhang
zhangjinhua@zju.edu.cn

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: 13 July 2020
Accepted: 08 October 2020
Published: 17 November 2020

Citation:
Cheng H, Xu C, Jin X, Chen Y, Zheng X, Shi F, He X, Hao Y, Jiang Y, Zhang J and Chen Z (2020) Association of Blood Pressure at Successful Recanalization and Parenchymal Hemorrhage After Mechanical Thrombectomy With General Anesthesia. Front. Neurol. 11:582639. doi: 10.3389/fneur.2020.582639

Background: This study aims to investigate the association between blood pressure (BP) at the time of recanalization and hemorrhagic transformation in large vessel occlusion (LVO) patients following mechanical thrombectomy (MT) with general anesthesia.

Methods: We retrospectively reviewed our data base for patients with acute ischemic stroke acute ischemic stroke (AIS) who received MT between January 2018 and December 2019. The BP at two adjacent time points immediately after successful recanalization was recorded for subsequent calculation of mean BP (BP_{mean}), maximum BP (BP_{max}), minimum BP (BP_{min}), range of BP (BP_{range}), and standard deviation of SP (BP_{SD}). Hemorrhagic transformation was identified on 24-h computerized tomography images according to the European Cooperative Acute Stroke Study (ECASS) III trial. We used binary logistic regression analysis to investigate the association of BP parameters and the incidence of parenchymal hemorrhage (PH) and PH-2.

Results: A total of 124 patients with anterior circulation LVO were finally included for analyses. After controlling for intravenous thrombolysis, procedure duration of mechanical thrombectomy, baseline National institutes of Health Stroke Scale (NIHSS), baseline ASPECTS, and number of device passes, the results showed that every increment of 10 mmHg in SBP_{range} (OR 1.559; 95% CI 1.027–2.365; $P = 0.037$) and SBP_{SD} (OR 1.998; 95% CI 1.017–3.925; $P = 0.045$) were independently associated with PH. After adjustment for intravenous thrombolysis, procedure duration of mechanical thrombectomy, baseline NIHSS, the results showed that every increment of 10 mmHg in SBP_{mean} (OR 1.973; 95% CI 1.190–3.271; $P = 0.008$), SBP_{max} (OR 1.838; 95% CI 1.199 to 2.815; $P = 0.005$), SBP_{range} (OR 1.908; 95% CI 1.161–3.136; $P = 0.011$) and SBP_{SD} (OR 2.573; 95% CI 1.170–5.675; $P = 0.019$) were independently associated with PH-2.

Conclusion: Patients with higher systolic BP and variability at the time of successful recanalization were more likely to have PH-2 in LVO patients following MT with general anesthesia.

Keywords: blood pressure, mechanical thrombectomy, hemorrhagic transformation, recanalization, large artery occlusion

INTRODUCTION

Mechanical thrombectomy (MT) for large vessel occlusion (LVO) has proved to be the new standard of therapy in acute ischemic stroke (AIS) (1). But more than 50% of patients still have unfavorable outcomes after early successful recanalization (2). The most severe complication is hemorrhagic transformation (HT), especially parenchymal hemorrhage (PH), which could result in early neurological deterioration and long-term outcomes (3). The current guidelines from the American Heart Association/American Stroke Association guidelines arbitrarily recommend blood pressure (BP) control of <180/105 mm Hg during and after MT. However, data regarding guidance for optimal BP management among patients treated with MT remain scarce (4). Theoretically, the target of BP control should be lower in patients following MT because of the high hemorrhagic transformation risk after clot removal (5).

Several studies have shown that blood pressure after MT is related to hemorrhagic transformation. Goyal et al. have found that elevated maximum systolic BP levels during the first 24 h following MT are independently correlated with worse functional outcomes in LVO patients (6). Another previous study involving 182 patients found that increased BP variability during the first 24 h predicts worse neurologic outcomes in AIS patients treated with intra-arterial therapies (7). In most of the previous studies on the relationship between BP and hemorrhagic transformation, blood pressure was taken after admission to the neurologic intensive care unit. There are few studies on BP at the time of recanalization and hemorrhagic transformation in LVO patients following MT with general anesthesia.

In light of these considerations, we aimed to investigate the relationship between BP at the time of recanalization and hemorrhagic transformation and hypothesized that patients with elevated BP had higher risk of hemorrhagic transformation.

METHODS AND MATERIALS

Study Subjects

MT under general anesthesia is our center's first choice standard procedure. Only in a few cases when the anesthesiologist could not arrive at the angio-suite in time is MT under conscious sedation chosen. Considering the difference of BP levels between patients under general anesthesia and conscious sedation, we excluded patients with conscious sedation in order to reduce the heterogeneity of study subjects. Propofol, sufentanil, and rocuronium were used to induce general anesthesia. Propofol was used for the maintenance of anesthesia. The BP control target in our center is to maintain BP at ≤180/105 mmHg during the MT procedure in patients who undergo mechanical thrombectomy. During the operation, BP can be regulated by using vasoactive drugs.

We retrospectively reviewed our data base for LVO patients who received MT from January 2018 to December 2019. This study included patients who (1) had internal carotid artery or middle cerebral artery occlusion, (2) received MT with general anesthesia, (3) achieved thrombolysis in myocardial infarction (TICI) 2b/3 recanalization after the procedure, and (4) had 24–36 h follow-up CT scan for evaluation of hemorrhagic transformation. Patients who had baseline systolic BP (SBP) ≥200 mmHg were excluded before the initial inclusion stage.

We retrieved data including age, gender, baseline National Institutes of Health Stroke Scale (NIHSS) score, baseline systolic BP (SBP), and diastolic BP (DBP) levels, vascular risk factors including atrial fibrillation, diabetes mellitus, hypertension, hyperlipidemia, smoking, and history of stroke/transient ischemic attack (TIA), time from onset to successful recanalization—which was defined by TICI scores of 2b or 3 after M (8) number of devices passes. Hemorrhagic transformation was identified as hemorrhagic infarction (HI) and parenchymal hemorrhage (PH) on 24–36 h CT images according to the European Cooperative Acute Stroke Study (ECASS) III trial. Hematoma occupying <30% of infarcted tissue and having no substantive mass effect was defined as PH-1, and hematoma occupying >30% or more of the infarcted tissue with obvious mass effect was defined as PH-2 (9). The etiologies of stroke were determined according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) (10).

Assessment of BP Parameters

Continuous arterial BP during the mechanical thrombectomy was automatically monitored by invasive BP monitoring using an arterial catheter in patients under general anesthesia. BP values were recorded every 15 min. Blood pressure at two adjacent time points immediately after successful recanalization was recorded for subsequent calculation. The maximum (max), minimum (min), average (mean), range (maximum–minimum), and standard deviation (SD) values of BP at the time of successful recanalization were calculated.

Statistical Analysis

Quantitative variables were presented as mean ± SD or median (interquartile range)—depending on the normality of the distribution—and categorical variables were presented as frequency (percentage). We use Fisher's exact test for dichotomous variables, an independent sample 2-tailed *t*-test, or Mann-Whitney *U*-test for continuous variables. Associations of each BP parameter with PH was determined using binary logistic regression models adjusted by baseline characteristics

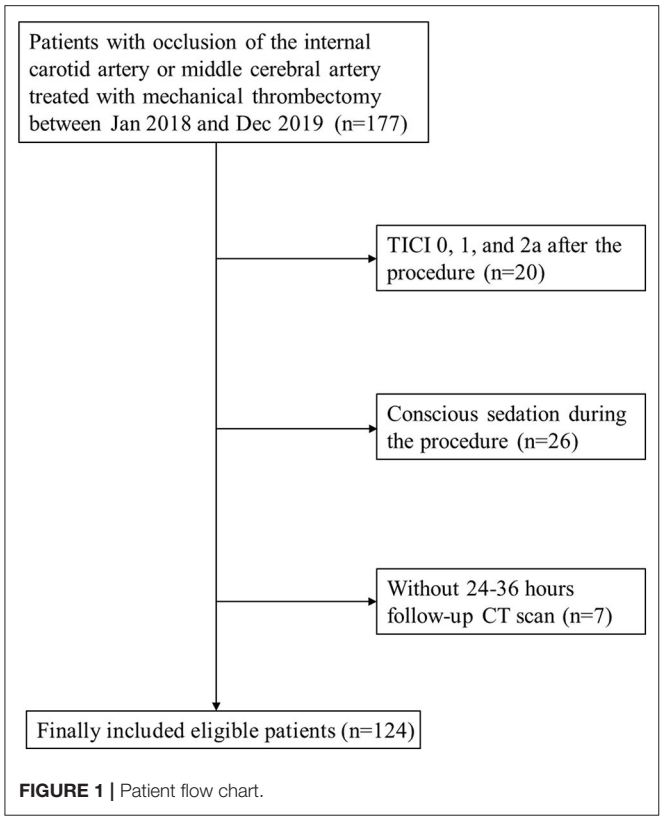


FIGURE 1 | Patient flow chart.

with a $P < 0.1$ in univariate analyses and some well-recognized confounders, respectively. The receiver operating characteristic (ROC) derived optimal cutoff was determined at the maximal Youden's Index. All statistical analyses were performed using SPSS, Version 23.0 (IBM, Armonk, New York). A $P < 0.05$ was considered statistically significant.

RESULTS

Initially, 177 patients with occlusion of the internal carotid artery or middle cerebral artery treated with MT were included. As shown in **Figure 1**, a total of 124 patients were finally enrolled after excluding patients due to TICI 0-2a after the procedure ($n = 20$), conscious sedation ($n = 26$), and no 24–36 h follow-up CT scan ($n = 7$).

Of the 124 analyzed patients, the mean age was 66 ± 12 years, and the median baseline NIHSS score was 15 (IQR 11–18) For this group, mean onset-to-reperfusion time was 415 (IQR 317–540) min. A total of 18 (14.5%) had PH at 24–36 h, and 10 of them were PH-2 (8.1%).

Associations of BP Parameters and Hemorrhagic Transformation

As shown in **Table 1**, patients with PH had higher baseline NIHSS scores (17 vs. 14, $P = 0.009$), a higher number of device passes, smaller baseline ASPECTS (6 vs. 8, $P = 0.023$), higher 24 h NIHSS (22 vs. 10.5, $P < 0.001$), and lower rate of 3 months mRS ≤2 (0 vs. 49, $P < 0.001$) than those without PH. The SBP_{max} ($P = 0.046$), SBP_{range} ($P = 0.037$), and SBP_{SD} ($P = 0.037$) were higher in the PH group. There were no significant differences in other variables, including baseline BP. After controlling for intravenous thrombolysis, procedure duration of mechanical thrombectomy, baseline NIHSS, baseline ASPECTS, and the number of device passes, the results showed that every increment of 10 mmHg in SBP_{range} (OR 1.559; 95% CI 1.027–2.365; $P = 0.037$) and SBP_{SD} (OR 1.998; 95% CI 1.017–3.925; $P = 0.045$) were independently associated with PH (**Table 2**).

Patients with PH-2 had a higher baseline NIHSS score (18 vs. 14, $P = 0.005$), higher 24-h NIHSS (25 vs. 11, $P = 0.005$), and lower rate of 3 months mRS ≤2 (0 vs. 45, $P = 0.006$) than those without PH-2. The SBP_{max} ($P < 0.001$), SBP_{mean} ($P = 0.001$), SBP_{range} ($P = 0.001$), SBP_{SD} ($P = 0.002$), DBP_{max} ($P = 0.006$), SBP_{mean} ($P = 0.001$), SBP_{min} ($P = 0.046$) were higher in the PH-2 group. After adjustment for intravenous thrombolysis, procedure duration of mechanical thrombectomy, and baseline NIHSS, the results showed that every increment of 10 mmHg in SBP_{mean} (OR 1.973; 95% CI 1.190–3.271; $P = 0.008$), SBP_{max} (OR 1.838; 95% CI 1.199–2.815; $P = 0.005$), SBP_{range} (OR 1.908; 95% CI 1.161–3.136; $P = 0.011$) and SBP_{SD} (OR 2.573; 95% CI 1.170–5.657; $P = 0.019$) were independently associated with PH-2 (**Table 2**).

The ROC curves of SBP_{mean}, SBP_{max}, SBP_{range}, and SBP_{SD} in predicting PH-2 are shown in **Figure 2**, and the areas under the curve (AUCs) were 0.796, 0.836, 0.729, and 0.732, respectively. The optimal cutoffs in predicting PH-2 were 126, 133, 10, and 7.5 mmHg for SBP_{mean}, SBP_{max}, SBP_{range}, and SBP_{SD}, respectively (**Table 3**). **Table 3** shows the diagnostic parameters including AUCs, sensitivity, and specificity at the maximal Youden's Index of SBP_{mean}, SBP_{max}, SBP_{range}, and SBP_{SD}. As shown in **Figure 3**, the probability of PH-2 increased with the increase in SBP_{max}.

DISCUSSION

Our data suggest that higher systolic BP, measured by SBP_{mean}/SBP_{max}, and higher BP variability measured by SBP_{range}/SBP_{SD} at the time of successful recanalization were consistently associated with a higher likelihood of PH-2 in LVO patients following MT with general anesthesia.

The recommendations of the American Heart Association/American Stroke Association guidelines for BP control in LVO patients treated with MT indicate that an optimal BP target that simultaneously avoids the risk of hemorrhagic transformation and impairment of cerebral perfusion remains unknown (4). Avoiding hypoperfusion injury in ischemic tissue and hyperperfusion injury in reperfused tissue are both essential for BP management after MT (11), indicating that the BP target following MT should not be too high or too low. Several previous studies had demonstrated a U-shaped relationship between admission SBP and mortality in AIS patients (12–14). Given that the overall recanalization rate of MT is high, it seems less important to maintain high BP levels to avoid hypoperfusion injury in ischemic tissue. For patients with successful recanalization after MT, higher BP levels during the first 24 h after MT was correlated with a higher likelihood

TABLE 1 | Univariate analyses of baseline characteristics.

	PH		*P*-value	PH-2		*P*-value
	Yes ($n = 18$)	No ($n = 106$)		Yes ($n = 10$)	No ($n = 114$)	
Age (years)	67.7 ± 13.1	65.5 ± 11.4	0.455	71.0 ± 12.2	65.4 ± 11.6	0.149
Male, *n* (%)	11 (61.1)	61 (57.5)	0.777	5 (50.0)	67 (58.8)	0.590
Risk factors						
Smoking, *n* (%)	3 (16.7)	25 (23.6)	0.516	2 (20.0)	26 (22.8)	0.839
Hypertension, *n* (%)	10 (55.6)	70 (66.0)	0.390	8 (80.0)	72 (63.2)	0.286
Diabetes mellitus, *n* (%)	3 (16.7)	17 (16.0)	0.947	2 (20.0)	18 (15.8)	0.729
Atrial fibrillation, *n* (%)	10 (55.6)	50 (47.1)	0.510	6 (60.0)	54 (47.4)	0.443
Hyperlipidaemia, *n* (%)	0 (0.0)	2 (1.9)	0.557	0 (0.0)	2 (1.8)	0.673
History of stroke/TIA, *n* (%)	3 (16.7)	17 (16.0)	0.947	2 (20.0)	18 (15.8)	0.729
Clinical variables						
Baseline NIHSS (IQR)	17 (13–19)	14 (10–17)	0.009	18 (16–20)	14 (11–17)	0.005
Onset-to-reperfusion time, min	488.0 ± 202.2	454.2 ± 203.7	0.517	445.4 ± 203.3	460.3 ± 203.8	0.824
Baseline SBP, mm Hg	143.1 ± 19.9	143.6 ± 23.2	0.932	140.8 ± 17.1	143.8 ± 23.1	0.687
Baseline DBP, mm Hg	87.1 ± 14.4	84.2 ± 15.9	0.474	87.2 ± 16.3	84.4 ± 15.7	0.602
Bridging thrombolysis, *n* (%)	7 (38.9)	40 (37.7)	0.926	5 (50.0)	42 (36.8)	0.411
Baseline ASPECTS (IQR)	6 (4–8)	8 (6–9)	0.023	7 (4–9)	8 (6–9)	0.537
TOAST classification			0.476			0.739
Cardioembolism, *n* (%)	10 (55.6)	55 (51.9)		6 (60.0)	59 (51.8)	
Large arterial atherosclerosis, *n* (%)	2 (11.1)	26 (24.5)		1 (10.0)	27 (23.7)	
Undetermined Etiology, *n* (%)	6 (33.3)	23 (21.7)		3 (30.0)	26 (22.8)	
Others, *n* (%)	0 (0.0)	2 (1.9)		0 (0.0)	2 (1.8)	
Number of device passes	3 (2–4)	2 (1–3)	0.017	2 (1–5)	2 (1–3)	0.170
Recanalization, *n* (%)	15 (83.3)	75 (70.8)	0.269	9 (90.0)	81 (71.1)	0.198
Procedure duration, min	87.5 ± 46.0	73.8 ± 48.1	0.264	75.7 ± 51.5	75.8 ± 47.8	0.995
BP parameters at successful recanalization						
SBP_{mean}	119.5 ± 17.6	114.2 ± 12.2	0.112	127.5 ± 15.6	113.8 ± 12.4	0.001
SBP_{max}	126.7 ± 70.9	118.7 ± 14.3	0.046	137.5 ± 15.5	118.3 ± 14.7	<0.001
SBP_{min}	111.8 ± 17.5	109.8 ± 12.2	0.548	116.7 ± 19.9	109.5 ± 12.2	0.099
SBP_{range}	14.8 ± 16.2	8.8 ± 10.0	0.037	20.8 ± 17.0	8.7 ± 10.1	0.001
SBP_{SD}	9.1 ± 9.7	5.4 ± 6.1	0.037	12.3 ± 9.7	5.4 ± 6.3	0.002
DBP_{mean}	68.4 ± 13.8	63.6 ± 9.5	0.067	72.3 ± 16.4	63.6 ± 19.4	0.001
DBP_{max}	71.8 ± 14.2	66.6 ± 10.8	0.076	76.9 ± 15.5	66.6 ± 10.8	0.006
DBP_{min}	64.7 ± 14.5	60.6 ± 10.8	0.114	67.4 ± 18.6	60.7 ± 8.9	0.046
DBP_{range}	7.1 ± 7.6	5.9 ± 6.2	0.474	9.6 ± 8.5	5.8 ± 6.1	0.077
DBP_{SD}	4.3 ± 4.0	3.6 ± 3.7	0.493	5.4 ± 4.1	3.6 ± 3.6	0.137
Outcome parameters						
sICH, *n* (%)	13 (72.2)	6 (5.7)	<0.001	8 (80.0)	11 (9.6)	<0.001
SAH, *n* (%)	6 (33.3)	5 (4.7)	<0.001	6 (60.0)	5 (4.4)	<0.001
24 hours NIHSS	22 (15–36)	10.5 (3–17)	<0.001	25 (15–36)	11 (4–19)	0.005
3 months mRS ≤2	0 (0)	49 (46.2)	<0.001	0 (0)	45 (43)	0.006

DBP, diastolic blood pressure; NIHSS, National Institutes of Health Stroke Scale; PH, parenchymal hemorrhage; SBP, systolic blood pressure; TIA, transient ischemic attack; TOAST, the Trial of Org 10172 in Acute Stroke Treatment (TOAST); SAH, subarachnoid hemorrhage; sICH, symptomatic intracranial hemorrhage.

of sICH and mortality (15). In patients with hyperdensity on immediate non-contrast CT following MT, which indicates risk of hyperperfusion injury, BP levels during the first 24 h were linearly correlated with PH (11). In the present study, we found that SBP_{mean} and SBP_{max} at the time of successful recanalization after MT with general anesthesia were associated with a higher risk of PH-2. Potential reperfusion injury to ischemic tissue may explain why PH-2 was more common in those patients who had higher SBP after MT. Cerebral perfusion pressure is linearly correlated with BP due to impaired cerebral autoregulation following ischemia (16). The increase of systemic BP may result in hyperperfusion injury in ischemic tissues and aggravate blood-brain barrier damage, causing subsequent hemorrhagic transformation (17).

TABLE 2 | Binary logistic regression analyses of associations between blood pressure parameters (per 10 mm Hg increase) and PH.

	PH			PH-2		
	OR	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
SBP_{mean}	1.261	0.861–1.847	0.234	**1.973**	**1.190–3.271**	**0.008**
SBP_{max}	1.303	0.953–1.780	0.097	**1.838**	**1.199–2.815**	**0.005**
SBP_{min}	1.034	0.689–1.552	0.871	1.443	0.871–2.389	0.154
SBP_{range}	**1.559**	**1.027–2.365**	**0.037**	**1.908**	**1.161–3.136**	**0.011**
SBP_{SD}	**1.998**	**1.017–3.925**	**0.045**	**2.573**	**1.170–5.657**	**0.019**
DBP_{mean}	1.261	0.773–2.058	0.354	1.659	0.913–3.014	0.097
DBP_{max}	1.256	0.804–1.962	0.317	1.720	0.990–2.986	0.054
DBP_{min}	1.172	0.715–1.923	0.529	1.397	0.770–2.538	0.272
DBP_{range}	1.393	0.637–3.048	0.406	2.289	0.924–5.669	0.074
DBP_{SD}	1.605	0.434–5.940	0.479	3.098	0.681–14.100	0.144

Bold type indicates statistical significance.
DBP, diastolic blood pressure; SBP, systolic blood pressure.
The associations of each BP parameter with PH were determined using binary logistic regression models adjusted for baseline NIHSS, baseline ASPECTS, and number of device passes, bridging thrombolysis and procedure duration. The associations of each BP parameter with PH-2 were determined using binary logistic regression models adjusted for baseline NIHSS, bridging thrombolysis, and procedure duration.

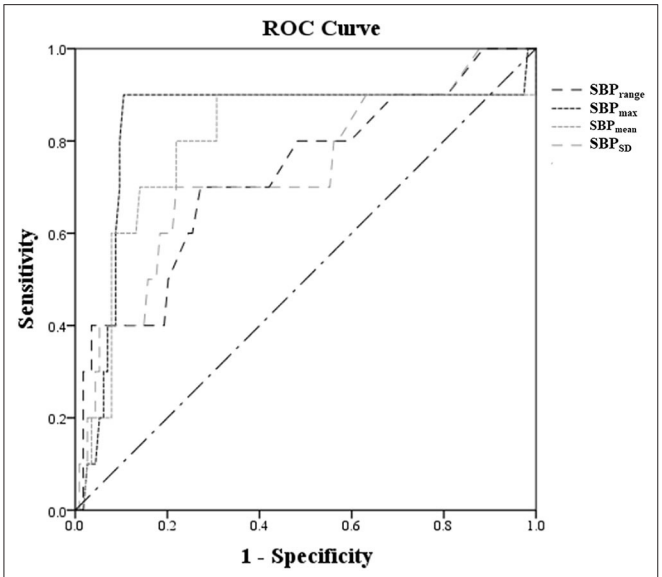


FIGURE 2 | ROC curves of SBP_{mean}, SBP_{max}, SBP_{range}, and SBP_{SD} at the time of reperfusion after IVT to predict PH-2.

Our study also found that patients with high BP variability measured by SBP_{range}/SBP_{SD} at the time of successful recanalization were prone to have PH-2, which is consistent with other related studies. Previous studies had found that blood pressure variability, reflecting the extent of blood pressure fluctuations, could predict unfavorable outcomes in AIS patients receiving intravenous rt-PA (18). Another study in patients treated by intra-arterial therapies showed that SBP variability within the first 24 h was correlated with poor outcomes at 3 months (7). Increased BP variability may lead to instability of cerebral perfusion due to the impairment in autoregulation. The instability of cerebral perfusion pressure in the setting of restoration of blood flow to ischemic tissues may cause disruption of blood–brain barrier permeability and reperfusion injury, resulting in hemorrhagic complications (17, 19). The results suggest that we should not only pay attention to the absolute value of BP control target after MT, but also the stability of BP.

The major difference between our study and other previous studies is that the observation index we focused on is the BP at the time of successful recanalization. Successful recanalization following MT basically solves the risk of hypoperfusion, avoiding reperfusion injury to reduce hemorrhagic transformation becoming more important. Our study suggests that BP has already affected hemorrhagic transformation at the time of successful recanalization in the angio-suite. It may be reasonable to keep BP to low levels and decrease fluctuations since the time of successful recanalization following MT. Interestingly, in line with a previous study (20), minimal BP after MT did not affect the rate of hemorrhagic transformation in our study, which supports the notion that an aggressive BP target is feasible for patients with successful recanalization following MT. The optimal BP control target varies in different studies, which may be related to different study subjects enrolled. In our study, controlling SBP below 120 seems to be beneficial for patients with successful recanalization.

Limitations include the small sample size of the study and its retrospective design, which might have potential for selection bias. Second, we only recorded BP values every 15 min after successful recanalization. It would be more informative to record blood pressure every 3 min or even every minute. Third, we assessed only the relationship between BP and hemorrhagic transformation in AIS patients with anterior circulation LVO occlusion. Therefore, the conclusion can't be simply extended to patients with posterior circulation LVO. Finally, because of

TABLE 3 | Predictive value of blood pressure parameters for PH-2.

	AUC	95% CI	*P*-value	Cutoff value	Sensitivity	Specificity
SBP_{mean}	0.796	0.619–0.974	0.002	126 mmHg	0.700	0.860
SBP_{max}	0.836	0.662–1.000	<0.001	133 mmHg	0.900	0.895
SBP_{range}	0.729	0.552–0.905	0.017	10 mmHg	0.700	0.728
SBP_{SD}	0.732	0.554–0.910	0.015	7.5 mmHg	0.700	0.781

AUC, area of the curve; DBP, diastolic blood pressure; SBP, systolic blood pressure.

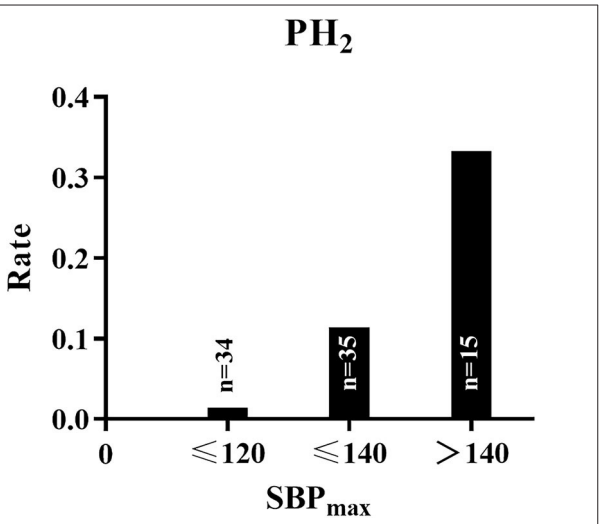


FIGURE 3 | The probability of PH-2 increased with the increase of SBP_{max}.

limitations of the observational design, the causal relationship between hemorrhagic transformation and blood pressure cannot be determined and prospective randomized controlled trials are needed to address this problem.

In conclusion, patients with higher systolic BP and variability at the time of successful recanalization were more likely to have PH-2 in LVO patients following MT with general anesthesia. Further research is needed to confirm this relationship and the optimal treatment target of blood pressure control after MT.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the human ethics committee of The Sir Run Run Shaw Hospital Affiliated with Zhejiang University approved the protocol of this study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HC and CX conducted the statistical analyses and drafted and revised the manuscript. XJ, YC, XZ, FS, XH, YH, and YJ participated in data acquisition and interpretation. ZC and JZ participated in study concept and design, data interpretation and made a major contribution to revising the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was funded by the foundation of the Science and Technology Department of Zhejiang Province (2017C33095) and Zhejiang Provincial Natural Science Foundation of China (LQ20H090007).

REFERENCES

1. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. 2018 guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the american heart association/american stroke association. *Stroke.* (2018) 49:e46–110. doi: 10.1016/j.jvs.2018.04.007
2. Goyal M, Menon BK, van Zwam WH, Dippel DW, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet*. (2016) 387:1723–31. doi: 10.1016/S0140-6736(16)00163-X
3. van Kranendonk KR, Treurniet KM, Boers AMM, Berkhemer OA, van den Berg LA, Chalos V, et al. Hemorrhagic transformation is associated with poor functional outcome in patients with acute ischemic stroke due to a large vessel occlusion. *J Neurointerv Surg*. (2019) 11:464–8. doi: 10.1136/neurintsurg-2018-014141
4. Malhotra K, Goyal N, Katsanos AH, Filippatou A, Mistry EA, Khatri P, et al. Association of blood pressure with outcomes in acute stroke thrombectomy. *Hypertension*. (2020) 75:730–9. doi: 10.1161/HYPERTENSIONAHA.119.14230
5. Goyal N, Tsivgoulis G, Pandhi A, Dillard K, Alsbrook D, Chang JJ, et al. Blood pressure levels post mechanical thrombectomy and outcomes in non-recanalized large vessel occlusion patients. *J Neurointerv Surg*. (2018) 10:925–31. doi: 10.1136/neurintsurg-2017-013581
6. Goyal N, Tsivgoulis G, Pandhi A, Chang JJ, Dillard K, Ishfaq MF, et al. Blood pressure levels post mechanical thrombectomy and outcomes in large vessel occlusion strokes. *Neurology*. (2017) 89:540–7. doi: 10.1212/WNL.0000000000004184
7. Bennett AE, Wilder MJ, McNally JS, Wold JJ, Stoddard GJ, Majersik JJ, et al. Increased blood pressure variability after endovascular thrombectomy for acute stroke is associated with worse clinical outcome. *J Neurointerv Surg*. (2018) 10:823–7. doi: 10.1136/neurintsurg-2017-013473

8. Kleine JF, Wunderlich S, Zimmer C, Kaesmacher J. Time to redefine success? Tici 3 versus tici 2b recanalization in middle cerebral artery occlusion treated with thrombectomy. *J Neurointerv Surg*. (2017) 9:117–21. doi: 10.1136/neurintsurg-2015-012218
9. Neuberger U, Mohlenbruch MA, Herweh C, Ulfert C, Bendszus M, Pfaff J. Classification of bleeding events: comparison of ecass III (european cooperative acute stroke study) and the new heidelberg bleeding classification. *Stroke*. (2017) 48:1983–5. doi: 10.1161/STROKEAHA.117.016735
10. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. Toast. Trial of org 10172 in acute stroke treatment. *Stroke*. (1993) 24:35–41. doi: 10.1161/01.STR.24.1.35
11. Ding X, Xu C, Zhong W, Gong X, Zhou Y, Chen Z, et al. Association of maximal systolic blood pressure with poor outcome in patients with hyperattenuated lesions on immediate ncct after mechanical thrombectomy. *Journal Neurointerv Surg*. (2020) 12:127–31. doi: 10.1136/neurintsurg-2019-014846
12. Castillo J, Leira R, Garcia MM, Serena J, Blanco M, Davalos A. Blood pressure decrease during the acute phase of ischemic stroke is associated with brain injury and poor stroke outcome. *Stroke*. (2004) 35:520–6. doi: 10.1161/01.STR.0000109769.22917.B0
13. Mulder M, Ergezen S, Lingsma HF, Berkhemer OA, Fransen PSS, Beumer D, et al. Baseline blood pressure effect on the benefit and safety of intra-arterial treatment in mr clean (multicenter randomized clinical trial of endovascular treatment of acute ischemic stroke in the netherlands). *Stroke*. (2017) 48:1869–76. doi: 10.1161/STROKEAHA.117.017996
14. Leonardi-Bee J, Bath PM, Phillips SJ, Sandercock PA, Group ISTC. Blood pressure and clinical outcomes in the international stroke trial. *Stroke*. (2002) 33:1315–20. doi: 10.1161/01.STR.0000014509.11540.66
15. Anadani M, Orabi MY, Alawieh A, Goyal N, Alexandrov AV, Petersen N, et al. Blood pressure and outcome after mechanical thrombectomy with successful revascularization. *Stroke*. (2019) 50:2448–54. doi: 10.1161/str.50.suppl_1.153
16. Delgado-Mederos R, Ribo M, Rovira A, Rubiera M, Munuera J, Santamarina E, et al. Prognostic significance of blood pressure variability after thrombolysis in acute stroke. *Neurology*. (2008) 71:552–8. doi: 10.1212/01.wnl.0000318294.36223.69
17. Liu K, Yan S, Zhang S, Guo Y, Lou M. Systolic blood pressure variability is associated with severe hemorrhagic transformation in the early stage after thrombolysis. *Transl Stroke Res*. (2016) 7:186–91. doi: 10.1007/s12975-016-0458-6
18. Endo K, Kario K, Koga M, Nakagawara J, Shiokawa Y, Yamagami H, et al. Impact of early blood pressure variability on stroke outcomes after thrombolysis: the samurai rt-pa registry. *Stroke*. (2013) 44:816–8. doi: 10.1161/STROKEAHA.112.681007
19. Zhang T, Wang X, Wen C, Zhou F, Gao S, Zhang X, et al. Effect of short-term blood pressure variability on functional outcome after intra-arterial treatment in acute stroke patients with large-vessel occlusion. *BMC Neurol*. (2019) 19:228. doi: 10.1186/s12883-019-1457-5
20. Mistry EA, Mistry AM, Nakawah MO, Khattar NK, Fortuny EM, Cruz AS, et al. Systolic blood pressure within 24 hours after thrombectomy for acute ischemic stroke correlates with outcome. *J Am Heart Assoc*. (2017) 6:e006167. doi: 10.1161/JAHA.117.006167

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 26 November 2020
doi: 10.3389/fneur.2020.594251

Oral Anticoagulation and Risk of Symptomatic Hemorrhagic Transformation in Stroke Patients Treated With Mechanical Thrombectomy: Data From the Nordictus Registry

María E. Ramos-Araque[1,2], Alba Chavarría-Miranda[2], Beatriz Gómez-Vicente[2], Elena López-Cancio Martínez[3], María Castañón Apilánez[3], Mar Castellanos[4], María López Fernández[4], Herbert Tejada Meza[5,6], Javier Marta Moreno[5,7], Javier Tejada García[8], Iria Beltrán Rodríguez[8], Patricia de la Riva[9], Noemi Díez[9], Susana Arias Rivas[10], María Santamaría Cadavid[10], Yolanda Bravo Anguiano[11], Mónica Bártulos Iglesias[11], Enrique Jesús Palacio Portilla[12], Marian Revilla García[12], Juan José Timiraos Fernández[13], Naroa Arenaza Basterrechea[13], José Luis Maciñeiras Montero[14], Pablo Vicente Alba[14], Francisco José Julián Villaverde[15], Ana Pinedo Brochado[16], Itxaso Azkune[16], Freijo M. Mar[17], Alain Luna[17] and Juan F. Arenillas[2,18] on behalf of NORDICTUS Investigators*

[1] Department of Neurology, Institute of Biomedical Research of Salamanca, Hospital Universitario de Salamanca, Salamanca, Spain, [2] Department of Neurology, Hospital Clínico Universitario de Valladolid, Valladolid, Spain, [3] Department of Neurology, Stroke Unit, Hospital Universitario Central de Asturias, Oviedo, Spain, [4] A Coruña Biomedical Research Institute/A Coruña University Hospital, A Coruña, Spain, [5] Department of Neurology, Stroke Unit, Hospital Universitario Miguel Servet, Zaragoza, Spain, [6] Department of Radiology, Neurointerventionism Unit, Hospital Universitario Miguel Servet, Zaragoza, Spain, [7] Instituto de Investigación Sanitaria de Aragón (IIS Aragón), Zaragoza, Spain, [8] Department of Neurology, Complejo Asistencial Universitario de León, León, Spain, [9] Department of Neurology, Donostia University Hospital, San Sebastián, Spain, [10] Department of Neurology, Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain, [11] Department of Neurology, Hospital Universitario de Burgos, Burgos, Spain, [12] Department of Neurology, Hospital Universitario Marqués de Valdecilla, Santander, Spain, [13] Department of Neurology, Stroke Unit, Hospital Universitario de Araba, Vitoria, Spain, [14] Department of Neurology, Complejo Hospitalario Universitario de Vigo, Vigo, Spain, [15] Department of Neurology, Hospital San Pedro, La Rioja, Spain, [16] Department of Neurology, Hospital Galdakao-Usansolo, Bizkaia, Spain, [17] Neurovascular Group, Neurology Department, Biocruces Research Health Institute, Cruces Hospital, Barakaldo, Spain, [18] Clinical Neurosciences Research Group, Department of Medicine, University of Valladolid, Valladolid, Spain

Introduction: We aimed to evaluate if prior oral anticoagulation (OAC) and its type determines a greater risk of symptomatic hemorrhagic transformation in patients with acute ischemic stroke (AIS) subjected to mechanical thrombectomy.

Materials and Methods: Consecutive patients with AIS included in the prospective reperfusion registry NORDICTUS, a network of tertiary stroke centers in Northern Spain, from January 2017 to December 2019 were included. Prior use of oral anticoagulants, baseline variables, and international normalized ratio (INR) on admission were recorded. Symptomatic intracranial hemorrhage (sICH) was the primary outcome measure. Secondary outcome was the relation between INR and sICH, and we evaluated mortality and functional outcome at 3 months by modified Rankin scale. We compared patients with and without previous OAC and also considered the type of oral anticoagulants.

OPEN ACCESS

Edited by:
Bruce Campbell,
The University of Melbourne, Australia

Reviewed by:
Johannes Kaesmacher,
Technical University of Munich, Germany
Kyriakos Lobotesis,
Imperial College London, United Kingdom

***Correspondence:**
María E. Ramos-Araque
ramosmaryesther@gmail.com

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *12 August 2020*
Accepted: *26 October 2020*
Published: *26 November 2020*

Citation:
Ramos-Araque ME, Chavarría-Miranda A, Gómez-Vicente B, López-Cancio Martínez E, Castañón Apilánez M, Castellanos M, López Fernández M, Tejada Meza H, Marta Moreno J, Tejada García J, Beltrán Rodríguez I, de la Riva P, Díez N, Arias Rivas S, Santamaría Cadavid M, Bravo Anguiano Y, Bártulos Iglesias M, Palacio Portilla EJ, Revilla García M, Timiraos Fernández JJ, Arenaza Basterrechea N, Maciñeiras Montero JL, Vicente Alba P, Julián Villaverde FJ, Pinedo Brochado A, Azkune I, Mar FM, Luna A and Arenillas JF (2020) Oral Anticoagulation and Risk of Symptomatic Hemorrhagic Transformation in Stroke Patients Treated With Mechanical Thrombectomy: Data From the Nordictus Registry. Front. Neurol. 11:594251. doi: 10.3389/fneur.2020.594251

Results: About 1.455 AIS patients were included, of whom 274 (19%) were on OAC, 193 (70%) on vitamin K antagonists (VKA), and 81 (30%) on direct oral anticoagulants (DOACs). Anticoagulated patients were older and had more comorbidities. Eighty-one (5.6%) developed sICH, which was more frequent in the VKA group, but not in DOAC group. OAC with VKA emerged as a predictor of sICH in a multivariate regression model (OR, 1.89 [95% CI, 1.01–3.51], $p = 0.04$) and was not related to INR level on admission. Prior VKA use was not associated with worse outcome in the multivariate regression model nor with mortality at 3 months.

Conclusions: OAC with VKA, but not with DOACs, was an independent predictor of sICH after mechanical thrombectomy. This excess risk was associated neither with INR value by the time thrombectomy was performed, nor with a worse functional outcome or mortality at 3 months.

Keywords: anticoagulants, stroke, thrombectomy, intracranial hemorrhage, vitamin K

INTRODUCTION

Symptomatic intracranial hemorrhage (sICH) is a life-threatening complication associated with reperfusion therapies after acute ischemic stroke (AIS) (1). Treatment with direct oral anticoagulants (DOACs), or vitamin K antagonists (VKA) with an international normalized ratio (INR) > 1.7 was a contraindication for the administration of intravenous thrombolysis due to the increased risk of intracranial bleeding (2). However, the risk of developing symptomatic hemorrhagic transformation in anticoagulated patients is not yet fully established after endovascular treatment (EVT) (3). Patients taking oral anticoagulation (OAC) were often underrepresented in pivotal clinical trials of mechanical thrombectomy, accounting for <5% of the total (4). To date, it has been a matter of controversy if EVT in AIS with prior anticoagulant treatment carries a major risk of bleeding. It is essential to select the best therapeutic approach in this subset of patients, because despite best medical treatment with anticoagulation 1–3% of patients will develop strokes annually (5).

In addition, there is little data regarding the possible influence of the type of anticoagulant (VKA vs. DOACs) and the risk of sICH after EVT in AIS. Recently, a systematic review and meta-analysis demonstrated that patients under VKA treatment had an increased risk of sICH and mortality after mechanical thrombectomy (1), although another observational study did not report differences in the rates of sICH between patients under VKA compared with DOACs (6).

Data are also limited and inconclusive regarding the relationship between the intensity of anticoagulation and the risk of sICH after EVT (7, 8). To date, neither INR level above a certain threshold nor DOAC activity values have been associated with an increased sICH or poor outcome risk. Thus, clarification of these scientific questions would satisfy considerable clinical need, since EVT is the only therapeutic option for anticoagulated AIS patients with a large vessel occlusion (9).

We aimed to study whether prior OAC, either assessed as a whole group or focusing on the type of oral anticoagulants used, was associated with an increased risk of sICH in AIS patients. As secondary aims, we studied the relationship between INR level at the time of treatment and sICH risk and the impact of prior OAC on functional outcome and predictors of mortality in all cohort.

MATERIALS AND METHODS

Study Design and Patient Selection

We performed a retrospective, observational, multicentric study based on a prospective registry of all consecutive AIS patients treated with reperfusion therapies in tertiary Stroke Centers in Spain included in the NORDICTUS Registry between 2017 and 2019. NORDICTUS is a research network in cerebrovascular diseases that brings together all public hospitals with stroke units in North-West Spain serving an area of 11.5 million people. Fourteen tertiary stroke centers participated in the investigation and the study was approved by their respective local institutional Clinical Research Ethics Committee. Written informed consent was obtained from all included patients or their relatives giving permission to enter their information into our reperfusion registry and to use of the data for scientific purposes, in accordance with the Spanish Personal Data Protection law.

Patients treated with mechanical thrombectomy included in the Nordictus Registry were selected for this study if they fulfilled the following criteria: (1) patients with disabling focal neurological deficit and ischemic stroke with demonstrated vessel occlusion, (2) time from symptom onset to groin puncture <24 h, including wake up strokes and unknown onset, (3) no intracranial hemorrhage at baseline cranial tomography (CT); (4) no extensive early ischemic signs as defined by an Alberta Stroke Program Early CT Score (ASPECTS) > 5 (10); (5) in patients with time from symptom onset to EVT >6 h, presence of a target mismatch profile on CT perfusion (11); and (6) absence of previous relevant disability evaluated by the modified Rankin Scale (mRS) (pre-stroke mRS score ≤2). If no contraindication existed (including INR <1.7 in those under VKA or DOAC in the prior 48 h), prior treatment with rt-PA was administered before EVT following regular guidelines.

Clinical Data and Baseline Variables

All patients included were clinically managed according to our institutional protocols, which are based on current international stroke guidelines. Our reperfusion registry includes the following baseline variables: age, sex, previous functional disability defined by mRS, information about the presence of arterial hypertension, diabetes, dyslipidemia, smoking, atrial fibrillation, history of coronary disease, or stroke prior to admission, previous treatment with antiplatelets, or anticoagulants and their type (VKA, DOACs, heparin). Anticoagulation status was recorded at the time of admission, and urgent INR was obtained in all patients prior to thrombectomy. Information regarding baseline clinical stroke severity according to National institute of Health Stroke Scale (NIHSS) score, location of artery occlusion and treatment, type of anesthesia during the procedure, and time intervals were also recorded. Stroke subtypes were categorized according to the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification (12).

Neuroimaging Protocol

A basal cranial noncontrast CT and CT angiography (CTA) scan was performed before endovascular treatment. Extent of early ischemic changes was evaluated by ASPECTS, and the arterial occlusion site was defined based on the CTA.

Target mismatch was established by a core volume of <70 cm^3, a penumbra/core ratio >1.8, and a mismatch volume >15 ml.

Recanalization after EVT was evaluated by the Thrombolysis in Cerebral Infarction (TICI) scale (13) and considered successful when TICI 2b-3 was achieved after the procedure.

A follow-up brain CT was performed after 24 h of treatment or earlier in case clinical deterioration occurred with the intention to assess the presence of hemorrhagic transformation. Hemorrhagic transformation subtypes were categorized according to the European Cooperative Acute Stroke Study (ECASS-2) definition as hemorrhagic infarction (HI) types 1 and 2 and parenchymal hematoma types 1 and 2 (14). In addition, sICH was defined according to Heidelberg criteria (15) as hemorrhagic transformation that was associated with significant neurological worsening, which was defined as an increase of four or more points in the NIHSS scale (15).

Primary and Secondary Outcome

The primary study outcome was the presence of sICH after endovascular treatment.

As secondary outcomes, we aimed to evaluate whether the INR value in patients on VKA at the time of treatment carries a higher risk of sICH. We also studied predictors of mortality and poor functional outcome at 3 months after the stroke, which was defined as a modified Rankin score >2.

Also, we compared outcomes in patients with and without previous use of OAC and according to the type of oral anticoagulants (VKA vs. DOACs).

Statistical Analysis

Data from each center were prospectively included in an anonymized online database of NORDICTUS research groups and statistical analysis after exporting the data was performed using SPSS statistics version 24 (Chicago, Illinois, USA).

Baseline quantitative continuous variables were expressed using their mean, standard deviation (SD) or median (interquartile range), as appropriate. Categorical variables were presented as number of cases and their percentage (%). Normality distribution of the data was assessed by using Kolmogorov-Smirnov test. Baseline characteristics of the study groups were compared using the Chi-square test for discrete variables, t-student test for quantitative variables with a normal distribution and Mann-Whitney U test for quantitative variables not following a normal distribution.

Bivariate analyses were performed to detect baseline variables associated with the occurrence of the primary and secondary endpoints. A logistic regression analysis was performed to identify independent predictors of sICH, poor functional outcome, and mortality. Adjustment was performed for all variables showing a $p < 0.1$ on the respective bivariate analysis. Age, arterial hypertension, dyslipidemia, and atrial fibrillation were also entered into the model as relevant potential confounding factors derived from recent literature (6) and from our bivariate analysis of variables associated with prior OAC. We performed a subgroup analysis to evaluate the influence of the type of anticoagulant (VKA vs. DOACs) on the outcomes. The logistic regression data are presented as adjusted odds ratio (OR) and respective 95% confidence intervals (CI) and statistical significance level was defined as a p value < 0.05. OR for ASPECTS and mRS was expressed as per 1-point increased in LR model. In the subgroup of VKA-treated patients, we performed a multivariate analysis to analyze the relationship between pretreatment INR level and sICH risk implementing continuous INR.

RESULTS

From January 2017 to December 2019, 1,710 consecutive patients treated with mechanical thrombectomy were included in the NORDICTUS stroke registry. From those patients who underwent EVT, 1,455 fulfilled our inclusion criteria. Reasons for exclusion of the remaining patients were treatment with heparin ($n = 14$), prestroke mRS >2 ($n = 172$), and unavailable follow-up data ($n = 69$).

The distribution of baseline variables of the study group is shown in **Table 1**. Six hundred and sixty-four patients (46%) were women, the mean age of the overall population was 72.42 years (SD: 12.84) and median NIHSS at admission was 16 (10–20). Two hundred and seventy-four patients (19%) were on oral anticoagulants, 193 on VKA (70%) and 81 (30%) on DOACs.

Regarding baseline characteristics according to anticoagulation status (**Table 1**), patients on OAC (DOACs and VKA) were older and had significantly higher prevalence of arterial hypertension, dyslipidemia, previous atrial fibrillation, ischemic cardiopathy, and previous stroke. VKA patients had higher punctuations on baseline NIHSS and lower ASPECTS compared with DOACs and non-OAC patients. Site of occlusion were not statistically different between both groups, except for

TABLE 1 | Baseline characteristics of whole study sample and bivariate analysis according to OAC status.

	All cohort (n = 1,455)	Non-OAC (n = 1,181)	DOACs (n = 81)	VKA (n = 193)	p Value[a]	P value[b]
Age (year, mean ± SD)	72.4 ± 12.8	71.5 ± 13.2	76.37 ± 9.79	76.66 ± 10.2	<0.001	0.73
Sex [women; *n* (%)]	664 (46)	525 (45)	38 (48)	101 (52)	0.13	0.50
Hypertension [*n* (%)]	927 (64)	729 (62)	59 (73)	139 (72)	0.005	0.53
Diabetes mellitus [*n* (%)]	285 (20)	221 (19)	19 (24)	45 (23)	0.22	1
Dyslipidemia [*n* (%)]	705 (49)	550 (47)	47 (58)	108 (57)	0.01	0.89
Atrial fibrillation [*n* (%)]	402 (28)	153 (13)	78 (96)	171 (89)	<0.001	0.11
History of coronary disease [*n* (%)]	197 (14)	140 (12)	17 (21)	40 (21)	<0.001	1
Previous stroke [*n* (%)]	188 (13)	121 (10)	18 (23)	49 (25)	<0.001	0.64
Smoking [current or past; *n* (%)]	463 (33)	398 (35)	19 (24)	46 (24)	<0.001	0.90
Prior mRS [0–1; *n* (%)]	1,282 (88)	1,065 (90)	60 (74)	157 (81)	<0.001	0.38
Antiplatelet treatment	343 (24)	323 (27)	5 (6)	15 (8)	<0.001	0.59
Acetylsalicylic acid [*n* (%)]	343 (24)	262 (22)	3 (4)	12 (6)	<0.001	0.56
Clopidogrel [*n* (%)]	277 (19)	33 (3)	2 (3)	2 (1)	0.35	0.58
Double antiplatelet therapy [*n* (%)]	37 (3)	17 (1)	0	1 (1)	0.32	1
Baseline NIHSS median (IQR)	16 (10–20)	16 (10–20)	15 (9–20)	18 (12–21)	0.027	0.05
Baseline ASPECTS median (IQR)	9 (8–10)	9 (8–10)	10 (8–10)	8 (7–10)	0.13	<0.001
MCA-M1 occlusion [*n* (%)]	792 (55)	625 (54)	49 (61)	118 (62)	0.09	0.34
Tandem occlusion [*n* (%)]	250 (17)	230 (20)	6 (7)	14 (7)	<0.001	1
TOAST [cardioembolic origin; *n* (%)]	682 (50)	438 (40)	70 (91)	174 (93)	<0.001	0.58
Known time of symptom onset (%)	964 (66)	785 (67)	53 (66)	126 (65)	0.94	1
Time intervals [median (IQR) min]						
Onset-to-door time	115 (228–61)	118 (234–61)	97 (54–236)	104 (60–216)	0.15	0.73
Onset-to-arterial puncture	218 (318–161)	225 (320–165)	197 (152–300)	201 (160–312)	0.07	0.30

OAC, oral anticoagulation; DOACs, direct oral anticoagulants; VKA, vitamin K antagonist; NIHSS, National Institute of Health Stroke Scale; TOAST, Trial of Org 10172 in acute stroke registry; ASPECTS, Alberta Stroke Program Early CT Score; MCA, middle cerebral artery; IQR, interquartile range; mRS, modified Rankin Scale.

[a] *p-values indicate comparisons between overall groups.*

[b] *p-values indicate comparisons between DOACs and VKA.*

a lower proportion of tandem occlusion in the OAC group. Prestroke functional status (mRS 0–1) was worse compared with patients who were not on oral anticoagulants. Also, patients on OAC tended to have more cardioembolic strokes.

In terms of safety, the proportion of patients with sICH was similar in patients with and without oral anticoagulants.

As shown in **Supplementary Table 1**, the proportion of patients treated with rt-PA differed between two groups. As expected, prior intravenous thrombolysis was less frequently given in the OAC group (p = <0.001). Successful and near to complete recanalization (TICI 2b or 3) was achieved in 86% patients of the overall sample and was similar in non-OAC vs. OAC patients.

Main Outcome: Symptomatic Intracerebral Hemorrhage

Bivariate analysis of baseline variables potentially associated with sICH is shown in **Table 2**. Patients who had a sICH were more often on VKA treatment, had worse prestroke functional status, and less often had a more proximal large vessel occlusion. Higher baseline NIHSS, lower ASPECT score, and longer onset to door and onset to groin puncture times were also associated with a higher sICH risk on bivariate analysis.

After multivariate analysis, prior VKA treatment (OR, 1.89 [95% CI, 1.01–3.51], p = 0.04), prior mRS (OR, 1.43 [95% CI, 1.03–1.99]), p = 0.03), and basal ASPECTS on admission (OR, 0.72 [95% CI, 0.61–0.84]), p < 0.001) emerged as independent predictors of sICH (**Table 3**).

Secondary Outcomes

In the group of patients on VKA, we evaluated the association between pre-treatment INR and sICH risk using INR as a continuous variable. The multivariate analysis did not disclose any significant association between these variables in an unadjusted (OR, 1.13 [95% CI, 0.52–2.46], p = 0.74) and after adjusted model (OR, 1.01 [95% CI, 0.42–2.41], p = 0.97).

Patients on oral anticoagulants had lower rates of functional independence at the third month (50 vs. 43%, p = 0.050). No significant differences in mortality within 90 days were observed in patients under oral anticoagulant treatment vs. non-anticoagulated patients.

Prior VKA treatment was associated with a worse outcome in bivariate analysis (p = 0.002) but not after multivariate adjustment (OR, 1.13 [95% CI, 0.76–1.67], p = 0.52) (**Table 4**).

As shown in **Supplementary Table 2**, after multivariate analysis, the predictors of mortality in all cohort were older age (OR, 1.03 [95% CI, 1.02–1.05], p < 0.001), worse prestroke

TABLE 2 | Baseline characteristics of whole study sample and bivariate analysis according to symptomatic intracranial hemorrhage.

	No sICH (n = 1,374)	sICH (n = 81)	p-value
Age (year, mean ± SD)	72.3 ± 12.9	73.4 ± 11.1	0.78
Sex [women; *n* (%)]	631 (46)	33 (42)	0.48
Hypertension [*n* (%)]	874 (64)	53 (65)	0.81
Diabetes mellitus [*n* (%)]	266 (20)	19 (24)	0.38
Dyslipidemia [*n* (%)]	668 (49)	37 (46)	0.64
Atrial fibrillation [*n* (%)]	376 (28)	26 (33)	0.47
History of coronary disease [*n* (%)]	187 (14)	10 (12)	0.86
Previous Stroke [*n* (%)]	172 (13)	16 (20)	0.08
Smoking (current or past) [*n* (%)]	437 (33)	26 (32)	0.49
Prior mRS 0–1 [*n* (%)]	1218 (89)	64 (79)	0.01
OAC [*n* (%)]	254 (19)	20 (25)	0.18
Treatment with VKA	174 (13)	19 (24)	0.01
Treatment with DOACs	80 (6)	1 (1.2)	0.08
Antiplatelet [any, *n* (%)]	329 (24)	14 (17)	0.5
Acetylsalicylic acid [*n* (%)]	264 (19)	13 (16)	0.56
Clopidogrel [*n* (%)]	36 (3)	1 (1)	0.7
Double antiplatelet therapy [*n* (%)]	18 (1)	0	0.61
Baseline NIHSS [median, (IQR)]	16 (10–20)	18 (13–21)	0.02
Baseline ASPECTS [median, (IQR)]	9 (8–10)	8 (9–7)	<0.001
MCA-M1 occlusion [*n* (%)]	755 (56)	37 (46)	0.04
Tandem occlusion [*n* (%)]	232 (17)	18 (22)	0.22
TOAST [cardioembolic origin; *n* (%)]	649 (50)	33 (45)	0.81
Known time of symptom onset (%)	912 (66)	52 (64)	0.71
Time intervals [median (IQR) min]			
Onset-to-door time	110 (60–224)	164 (90–265)	<0.001
Onset-to-arterial puncture	214 (160–315)	278 (217–356)	<0.001

NIHSS, National Institute of Health Stroke Scale; TOAST, Trial of Org 10172 in acute stroke registry; ASPECTS, Alberta Stroke Program Early CT Score; MCA, middle cerebral artery; IQR, interquartile range; mRS, modified Rankin Scale; OAC, oral anticoagulation; DOACs, direct oral anticoagulants; VKA, vitamin K antagonist.

TABLE 3 | Logistic regression: predictors of symptomatic intracranial hemorrhage.

	OR (IC 95%)	p-value
Prior mRS	1.43 (1.03–1.99)	0.03
Baseline NIHSS	1 (0.96–1.05)	0.72
ASPECTS	0.71 (0.61–0.84)	<0.001
Treatment with VKA	1.89 (1.01–3.51)	0.04
Treatment with DOACs	0.32 (0.04–2.42)	0.27
Vessel occlusion MCA-M1	0.91 (0.71–1.16)	0.46
Age	1.01 (0.99–1.04)	0.2
Arterial Hypertension	0.89 (0.51–1.55)	0.68
Hypercholesterolemia	0.82 (0.49–1.37)	0.46
Atrial fibrillation	0.79 (0.54–1.17)	0.25

mRS, modified Rankin Scale; NIHSS, National Institute of Health Stroke Scale; ASPECTS, Alberta Stroke Program Early CT Score; MCA, middle cerebral artery; VKA, vitamin K antagonist.

TABLE 4 | Logistic regression model: predictors of poor functional outcome at 3 months of all cohort.

	OR (IC 95%)	p-value
Age	1.04 (1.02–1.05)	<0.001
Sex (women)	1 (0.75–1.32)	0.99
Arterial hypertension	1.20 (0.89–1.60)	0.21
Diabetes mellitus	1.21 (0.86–1.70)	0.26
Prior mRS	1.61 (1.31–1.98)	<0.001
Baseline NIHSS	1.09 (1.07–1.11)	<0.001
Vessel occlusion MCA-M1	1.06 (0.95–1.17)	0.27
VKA treatment	1.13 (0.76–1.67)	0.52
ASPECTS	0.73 (0.66–0.81)	<0.001
Atrial fibrillation	0.92 (0.77–1.10)	0.39
Onset-to-door time	1 (0.99–1)	0.68

mRS, modified Rankin Scale; NIHSS, National Institute of Health Stroke Scale; MCA, middle cerebral artery; VKA, vitamin K antagonist; ASPECTS, Alberta Stroke Program Early CT Score.

functional status (OR, 1.37 [95% CI, 1.12–1.68], $p = 0.002$), higher punctuation on NIHSS on admission (OR, 1.07 [95% CI, 1.05–1.01], $p < 0.001$), and higher rates of proximal occlusion (OR, 1.11 [95% CI, 1–1.23], $p = 0.044$).

Subgroup Analysis in OAC Patients (VKA vs. DOACs)

We performed a separate multivariate-adjusted logistic regression model in the group of patients treated with oral anticoagulants. Prior anticoagulation with VKA was associated with a significant increase in sICH risk when compared with DOACs (OR, 8.41 [95% CI, 1.03–68.54]), $p = 0.04$).

DISCUSSION

In this observational study based on a prospective multicenter reperfusion registry, the most important finding was that prior anticoagulation with VKA emerged as an independent predictor of sICH in AIS patients undergoing EVT. This excess bleeding risk associated with VKA contrasts with the lack of association between the use of DOACs and sICH risk, which suggests that DOACs have a better safety profile than VKA in the setting of EVT for AIS. This different safety profile is another solid reason to prescribe DOACs over VKA to try to prevent ischemic stroke in patients with non-valvular atrial fibrillation.

Our main observation is in line with a recent systematic review and meta-analysis (1), that has reported higher sICH rates in VKA users but not in patients under DOACs. The authors of that study described a significant increase in mortality in patients anticoagulated with VKA, which we did not find in our series. This excess mortality associated with VKA has also been found by other studies. Taken together, all these findings contribute to raise the questions of whether there is a high-bleeding risk profile among patients treated with VKA undergoing EVT and if EVT is safe in all circumstances

for patients anticoagulated with VKA. Future research should address these issues (1, 16). Use of VKA was associated with worse functional outcome at 3 months in bivariate analysis but did not predict functional dependence in the multivariate regression model, in accordance with a recent study (6). This lack of association with poor functional outcome could be due to the role of confounding factors associated with the use of VKA, such as age, comorbidities, premorbid status, or higher incidence of atrial fibrillation (17) in patients under VKA treatment compared with nonanticoagulated patients.

In our study, we did not find an association between pre-EVT INR level and the risk of sICH among VKA patients. However, only seven patients had a value of INR >3, so this precludes any conclusion. On the other hand, our result is in agreement with previous works showing that increased INR was not associated with higher sICH risk (6–8, 18). Thus, pretreatment INR level may not reflect the risk of bleeding after EVT, and therefore exclusion of EVT candidates based on INR level, as done with intravenous thrombolysis patients, does not seem to be justified according to existing evidence. The fact that these patients had an ischemic stroke under anticoagulation and subsequently a severe hemorrhagic complication after EVT, might be related to high instability or fluctuations of the intensity of anticoagulation under VKA (3). In this setting, it would have been ideal to evaluate the risk associated with the percentage of time patients were in range of effective anticoagulation during the previous months, but this data was not available in our registry.

In our study, the rates of sICH between oral anticoagulated patients and those not taking oral anticoagulants was similar, which is also in agreement with previous reported series (18, 19). Moreover, recanalization rates were also similar in the two groups. Other series have reported better TICI scores in patients receiving OAC, raising the prospect that anticoagulation itself may reduce fibrin formation and facilitate EVT (6) or that thrombus from cardioembolic origin could be more prone to be removed (20), but this is unclear (21). According to our results, we can affirm that EVT is safe and feasible in the context of previous anticoagulation as previously reported (22). Our proportion of anticoagulated patients subjected to EVT (19%) is slightly higher than rates reported in most recently published data (6); this emphasizes the importance of the occurrence of stroke in previous anticoagulated patients who will further require reperfusion therapy (17). Our rate of sICH is 5.6%, which is also in line with the proportion reported in recent series of patients with AIS treated with mechanical thrombectomy (1, 6). Even with this higher risk of sICH in VKA patients, EVT should be performed if there are no other contraindications. Moreover, we have to identify a clinical profile that can predict the higher risk of bleeding, to determine if EVT is safe in all circumstances for VKA patients.

This study faces some limitations. First, this is a retrospective analysis from non-randomized, observational data. Second, the selection of patients for EVT was conducted at the discretion of each center and this could represent a confounding factor for the indication of EVT. Third, we could not estimate DOACs compliance or activity or INR therapeutic range of VKA patients, which perhaps facilitates the risk of sICH after EVT, even when IVT was withheld in 91% of OAC patients in our series. Finally, the small number of sICH events limited the number of variables entered in the multivariate regression model. Therefore, the influence of other nonconsidered potential confounding variables cannot be entirely ruled out.

CONCLUSIONS

Prior use of VKA, but not of DOACs, was associated with an increased risk of sICH in patients with AIS subjected to EVT. However, this was not related to worse outcome at 3 months. The risk of sICH was not associated with the intensity of anticoagulation as determined by the pre-EVT INR level in patients treated with VKA. Prior anticoagulation with DOACs appears to have a better safety profile when compared with VKAs in AIS patients undergoing thrombectomy.

DATA AVAILABILITY STATEMENT

The datasheets are available upon reasonable request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by fourteen tertiary stroke centers participated in the investigation and the study was approved by their respective local institutional Clinical Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MER-A drafted the manuscript and performed statistical analysis. AC-M partly drafted the manuscript. EL-CM, MC, JMar, JTG, PR, and EPP critically reviewed the manuscript. AC-M, BG-V, EL-CM, MCA, MC, MLF, HTM, JMar, JTG, IBR, PR, ND, SAR, MSC, YBA, MBI, MRG, JTF, NAB, JMac, PVA, FJV, APB, IA, FM, and AL acquired data. JFA conceived the study, acquired data, and critically reviewed the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

MER-A was funded by The Instituto de Salud Carlos III (JR19/00020). BG-V was funded by Junta de Castilla y León and Fondo Social Europeo. Research grant provided by Daiichi Sankyo to NORDICTUS. Investigators participating in this study belong to the RETICS INVICTUS PLUS financed by the Instituto de Salud Carlos III (RD1670019/0004, RD16/0019/0018, RD16/0019/0019, and RD16/0019/0020).

ACKNOWLEDGMENTS

List of all NORDICTUS investigators is available at the **Supplementary Annex**. Thanks to Dr. Javaad Ahmad for the revision of English grammar.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.594251/full#supplementary-material

REFERENCES

1. Meinel TR, Kniepert JU, Seiffge DJ, Gralla J, Jung S, Auer E, et al. Endovascular stroke treatment and risk of intracranial hemorrhage in anticoagulated patients. *Stroke.* (2020) 51:892–8. doi: 10.1161/STROKEAHA.119.026606
2. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke a guideline for healthcare professionals from the American Heart Association/American Stroke A. *Stroke.* (2019) 50:344–418. doi: 10.1161/STR.0000000000000211
3. L'allinec V, Ernest M, Sevin-Allouet M, Testard N, Delasalle-Guyomarch B, Guillon B, et al. Safety and efficacy of mechanical thrombectomy in acute ischemic stroke of anticoagulated patients. *J Neurointerv Surg.* (2018) 10:E29. doi: 10.1136/neurintsurg-2017-013714
4. Vidale S, Agostoni E. Endovascular treatment of ischemic stroke: an updated meta-analysis of efficacy and safety. *Vasc Endovascular Surg.* (2017) 51:215–9. doi: 10.1177/1538574417698905
5. Wong JWP, Churilov L, Dowling R, Mitchell P, Bush S, Kanesan L, et al. Safety of endovascular thrombectomy for acute ischaemic stroke in anticoagulated patients ineligible for intravenous thrombolysis. *Cerebrovasc Dis.* (2019) 46:193–9. doi: 10.1159/000493801
6. Goldhoorn R-JB, van de Graaf RA, van Rees JM, Lingsma HF, Dippel DWJ, Hinsenveld WH, et al. Endovascular treatment for acute ischemic stroke in patients on oral anticoagulants. *Stroke.* (2020) 51:1781–9. doi: 10.1161/STROKEAHA.119.028675
7. Mundiyanapurath S, Tillmann A, Möhlenbruch MA, Bendszus M, Ringleb PA. Endovascular stroke therapy may be safe in patients with elevated international normalized ratio. *J Neurointerv Surg.* (2017) 9:1187–90. doi: 10.1136/neurintsurg-2016-012757
8. Pandhi A, Tsivgoulis G, Ishfaq MF, Katsanos A, Magoufis G, Malhotra K, et al. Mechanical thrombectomy outcomes in large vessel stroke with high international normalized ratio. *J Neurol Sci.* (2019) 396:193–8. doi: 10.1016/j.jns.2018.11.019
9. Diener HC, Foerch C, Riess H, Röther J, Schroth G, Weber R. Treatment of acute ischaemic stroke with thrombolysis or thrombectomy in patients receiving anti-thrombotic treatment. *Lancet Neurol.* (2013) 12:677–88. doi: 10.1016/S1474-4422(13)70101-7
10. Pexman JH, Barber PA, Hill MD, Sevick RJ, Demchuk AM, Hudon ME, et al. Use of the Alberta Stroke Program Early CT Score (ASPECTS) for assessing CT scans in patients with acute stroke. *AJNR Am J Neuroradiol.* (2001) 22:1534–42.
11. Albers GW, Marks MP, Kemp S, Christensen S, Tsai JP, Ortega-Gutierrez S, et al. Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. *N Engl J Med.* (2018) 378:708–18. doi: 10.1056/NEJMoa1713973
12. Chung JW, Park SH, Kim N, Kim WJ, Park JH, Ko Y, et al. Trial of ORG 10172 in acute stroke treatment (TOAST) classification and vascular territory of ischemic stroke lesions diagnosed by diffusion-weighted imaging. *J Am Heart Assoc.* (2014) 3:1–8. doi: 10.1161/JAHA.114.001119
13. Zaidat OO, Yoo AJ, Khatri P, Tomsick TA, Von Kummer R, Saver JL, et al. Recommendations on angiographic revascularization grading standards for acute ischemic stroke: a consensus statement and for the cerebral angiographic revascularization, grading (CARG) Collaborators, STIR Revascularization working group, and STIR Thrombol. *Stroke.* (2013) 44:2630–63. doi: 10.1161/STROKEAHA.113.001972
14. Hacke W, Kaste M, Fieschi C, Von Kummer R, Davalos A, Meier D, et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). *Lancet.* (1998) 352:1245–51. doi: 10.1016/S0140-6736(98)08020-9
15. Von Kummer R, Broderick JP, Campbell BCV, Demchuk A, Goyal M, Hill MD, et al. The heidelberg bleeding classification: classification of bleeding events after ischemic stroke and reperfusion therapy. *Stroke.* (2015) 46:2981–6. doi: 10.1161/STROKEAHA.115.010049
16. Rebello LC, Haussen DC, Belagaje S, Anderson A, Frankel M, Nogueira RG. Endovascular treatment for acute ischemic stroke in the setting of anticoagulation. *Stroke.* (2015) 46:3536–9. doi: 10.1161/STROKEAHA.115.011285
17. Liu M, Zheng Y, Li G. Safety of recanalization therapy in patients with acute ischemic stroke under anticoagulation: a systematic review and meta-analysis. *J Stroke Cerebrovasc Dis.* (2018) 27:2296–305. doi: 10.1016/j.jstrokecerebrovasdis.2018.04.012
18. Uphaus T, Singer OC, Berkefeld J, Nolte CH, Bohner G, Niederkorn K, et al. Safety of endovascular treatment in acute stroke patients taking oral anticoagulants. *Int J Stroke.* (2017) 12:412–5. doi: 10.1177/1747493016677986
19. L'Allinec V, Sibon I, Mazighi M, Labreuche J, Kyheng M, Boissier E, et al. MT in anticoagulated patients: direct oral anticoagulants versus vitamin K antagonists. *Neurology.* (2020) 94:e842–50. doi: 10.1212/WNL.0000000000008873
20. Benavente L, Larrosa D, García-Cabo C, Pérez ÁI, Rico M, Vega P, et al. Safety and efficacy of mechanical thrombectomy in acute ischemic stroke of anticoagulated patients-a prospective observational study. *J Stroke Cerebrovasc Dis.* (2016) 25:2093–8. doi: 10.1016/j.jstrokecerebrovasdis.2016.06.006
21. Boeckh-Behrens T, Schubert M, Förschler A, Prothmann S, Kreiser K, Zimmer C, et al. The impact of histological clot composition in embolic stroke. *Clin Neuroradiol.* (2016) 26:189–97. doi: 10.1007/s00062-014-0347-x
22. Zapata-Wainberg G, Ximénez-Carrillo Á, Trillo S, Fuentes B, Cruz-Culebras A, Aguirre C, et al. Mechanical thrombectomy in orally anticoagulated patients with acute ischemic stroke. *J Neurointerv Surg.* (2018) 10:834–8. doi: 10.1136/neurintsurg-2017-013504

Conflict of Interest: JA reports having received speaker or consultant honoraria by: BI, Bayer, Daiichy Sankyo, Pfizer, Amgen, Medtronic. MC has received speaker honoraria from Pfizer-BristolMyers Squibb, Daichii-Sankyo, and Boehringer-Ingelheim, and for providing consultancy on advisory board meetings from Amgen and Allergan.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

BRIEF RESEARCH REPORT
published: 08 December 2020
doi: 10.3389/fneur.2020.582994

The Relationship Between Penumbral Tissue and Blood-Brain Barrier Disruption in Acute Stroke Patients Presenting in an Extended Time Window

*Parisa Heidari, Sarah Blayney, Jarrhett Butler, Emi Hitomi, Marie Luby and Richard Leigh**

National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, United States

Background: Penumbral brain tissue identified with multimodal imaging can be salvaged with reperfusion in an extended time window. The risk of severe hemorrhagic complications after reperfusion therapy increases with worsening disruption of the blood-brain barrier (BBB). The relationship between penumbral tissue and BBB disruption has not been previously studied.

Methods: Stroke patients presenting in an extended time window without a large vessel occlusion who underwent diffusion-perfusion MRI within 24 h of last-seen-normal were included. The volume of penumbral tissue was calculated using mismatch on MRI. Mean permeability derangement (MPD) of the BBB was measured within the ischemic lesion. A target profile (TP) for treatment was defined based on the EXTEND trial.

Results: 222 patients were included with a median age of 73 and 55% women. The median NIHSS was 6, the mean core volume was 14 ml, the mean ischemic volume was 47 mL and the mean mismatch volume was 33 mL. Higher MPD was significantly associated with less mismatch volume ($p = 0.001$). A target profile was associated with lower MPD (OR 0.97; CI 0.96:0.99; $p < 0.001$). Of the 105 patients who had a TP, 31 (30%) had a MPD > 20% suggesting an increased risk of hemorrhage. Thus, 33% (74/222) of patients had a favorable profile for benefit and safety.

Conclusions: Patients presenting in an extended time window with a favorable penumbral profile for treatment have less severe BBB disruption. Up to a third of patients who currently go untreated could be considered for enrollment in a clinical trial of thrombolysis in an extended time window.

Keywords: thrombolysis (tPA), extended time window, intracranial hemorrhage, blood-brain barrer, penumbra

OPEN ACCESS

Edited by:
Tae-Hee Cho,
Hospices Civils de Lyon, France

Reviewed by:
Klearchos Psychogios,
Metropolitan Hospital, Greece
Diana Amantea,
University of Calabria, Italy

****Correspondence:***
Richard Leigh
richard.leigh@nih.gov

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *13 July 2020*
Accepted: *19 November 2020*
Published: *08 December 2020*

Citation:
Heidari P, Blayney S, Butler J, Hitomi E, Luby M and Leigh R (2020) The Relationship Between Penumbral Tissue and Blood-Brain Barrier Disruption in Acute Stroke Patients Presenting in an Extended Time Window. Front. Neurol. 11:582994. doi: 10.3389/fneur.2020.582994

BACKGROUND

The goal of a clinical trial is to determine if an intervention is safe and effective at its pre-specified objective. In acute stroke trials of reperfusion therapy, safety is primarily determined by the risk of symptomatic hemorrhagic complications, while efficacy is primarily determined by the ability to avert disability. It is well established that reperfusion of hypoperfused tissue that has not infarcted

is an effective treatment for acute stroke (1). It has also been demonstrated that risk of hemorrhagic complications associated with reperfusion therapies increases with more severe disruption of the blood-brain barrier (BBB) (2, 3).

Currently there is no recommended reperfusion therapy for patients who present >4.5 h from symptom discovery in the absence of a large vessel occlusion. Recent trials using penumbral imaging have found that some of these patients may benefit from intravenous thrombolysis (4–6). However, it is not known if patients with a favorable pattern on penumbral imaging (favoring benefit) also have preserved integrity of their BBB (favoring safety). The purpose of this study was to investigate the relationship between penumbral profile and BBB disruption in patients presenting outside of the approved thrombolysis window who are ineligible for endovascular treatment.

METHODS

This research was conducted as a retrospective analysis of de-identified registry data, for which we obtained a determination of *Not Human Subjects Research* from the NIH Office of Human Subjects Research Protections (OHSRP).

Population

The details of this population have been described in a previous publication (7). Briefly, it includes patients presenting to two stroke centers over a 5 year period who were evaluated and underwent MRI with diffusion weighted imaging (DWI) and perfusion weighted imaging (PWI) within 24 h of being last seen normal. Patients were excluded if they presented within 4 h of being last seen normal or if they received any acute reperfusion therapy. Four hours was used instead of 4.5 h under the assumption that patients presenting with a narrow window for treatment might be excluded due to time constraints. Patients with a large vessel occlusion (LVO) were excluded since there are established treatments for LVO in the extended time window. Patients were excluded if they did not have an ischemic lesion which was defined on PWI using a time-to-peak (TTP) threshold of 4 s beyond normal.

Imaging

Details of the MRI protocol have been published previously (7). Ischemic lesions were identified on PWI using a TTP threshold of 4 s delay relative to the contralateral hemisphere. Relative delay in TTP has been found to be equivalent to other methods of identifying ischemia but does not require deconvolution of an arterial input function (AIF) making it less susceptible to errors introduced by AIF selection (8, 9). PWI lesions were superimposed on the apparent diffusion coefficient (ADC) maps after co-registration of the source images. The core infarct volume was defined as the portion of the ischemic lesion defined on PWI that had an ADC value < 620 μm/s on DWI (1). The mismatch ratio was defined as the ischemic volume from PWI divided by the infarct core volume from DWI. The mismatch volume was defined as the PWI volume minus the DWI volume.

The mean permeability derangement (MPD), which is a measure of BBB disruption, was calculated from the source images of the PWI scan in the same manner previously described (7). PWI is generated using a dynamic susceptibility contrast (DSC) image sequence. In the setting of BBB disruption, the recorded signal in these images represents both intravascular flow and intraparenchymal leakage of gadolinium through the BBB. These two signals can be separated using an arrival time correction (10). The resulting metric, K_2, is an index that reflects the fraction of the recorded signal that is due to gadolinium leakage through the BBB and can also be represented as a percent. Mean permeability derangement (MPD) is the average K_2 value of all voxels within the ischemic lesion that are 2 standard deviations above normal identified from the contralateral hemisphere. It has previously been found that an MPD > 20% is associated with severe hemorrhagic complications after treatment with IV thrombolysis (2).

Target profile (TP) was defined using the parameters from the EXTEND trial (5). To be considered to have a TP, the mismatch ratio had to be >1.2, the mismatch volume had to be >10 mL and the core infarct had to be <70 mL. Image analysis was performed in Matlab (Mathworks, Natick, MA).

Statistical Analysis

Mismatch volume was treated as an independent continuous variable and compared with MPD as a dependent continuous variable with linear regression. Mismatch ratio was not used in the linear regression due to its instability in certain situations (such as when it is infinite). TP was treated as a dichotomous variable and compared with MPD with logistic regression. Statistical analysis was performed in STATA 13 (StataCorp LLC, College Station, TX).

RESULTS

The cohort consisted of 222 patients with a median age of 73 of whom 55% were women. The median NIHSS was 6. The mean DWI core volume was 14 ml, the mean PWI ischemic volume was 47 mL and the mean mismatch penumbra volume was 33 mL. One hundred five patients (47%) met requirements for a TP. The median MPD was 18%.

Higher MPD was significantly associated with less mismatch volume ($p = 0.001$). **Figure 1** shows the 95% confidence intervals for this relationship. In multivariate analysis mismatch volume remained significantly associated with MPD ($p = 0.001$) independent of age ($p = 0.104$), sex ($p = 0.735$), NIHSS ($p = 0.868$), DWI volume ($p = 0.647$) and time from symptom discovery to MRI ($p = 0.724$). Mismatch volume and PWI volume correlated highly and were not independent of each other.

TP was associated with lower MPD (OR 0.97; CI 0.96:0.99; $p < 0.001$) such that for every 10% increase in MPD the chance of having a TP is reduced by 30%. **Figure 2** shows a boxplot of MPD for patients with and without a TP. Of the 105 patients who had a TP, 31 (30%) had a MPD > 20%. The 20% threshold has been associated with increased risk of parenchymal hematoma formation in patients treated with IV thrombolysis (2). If those patients were excluded, along with the patients who did not have a TP, the remaining 74 patients

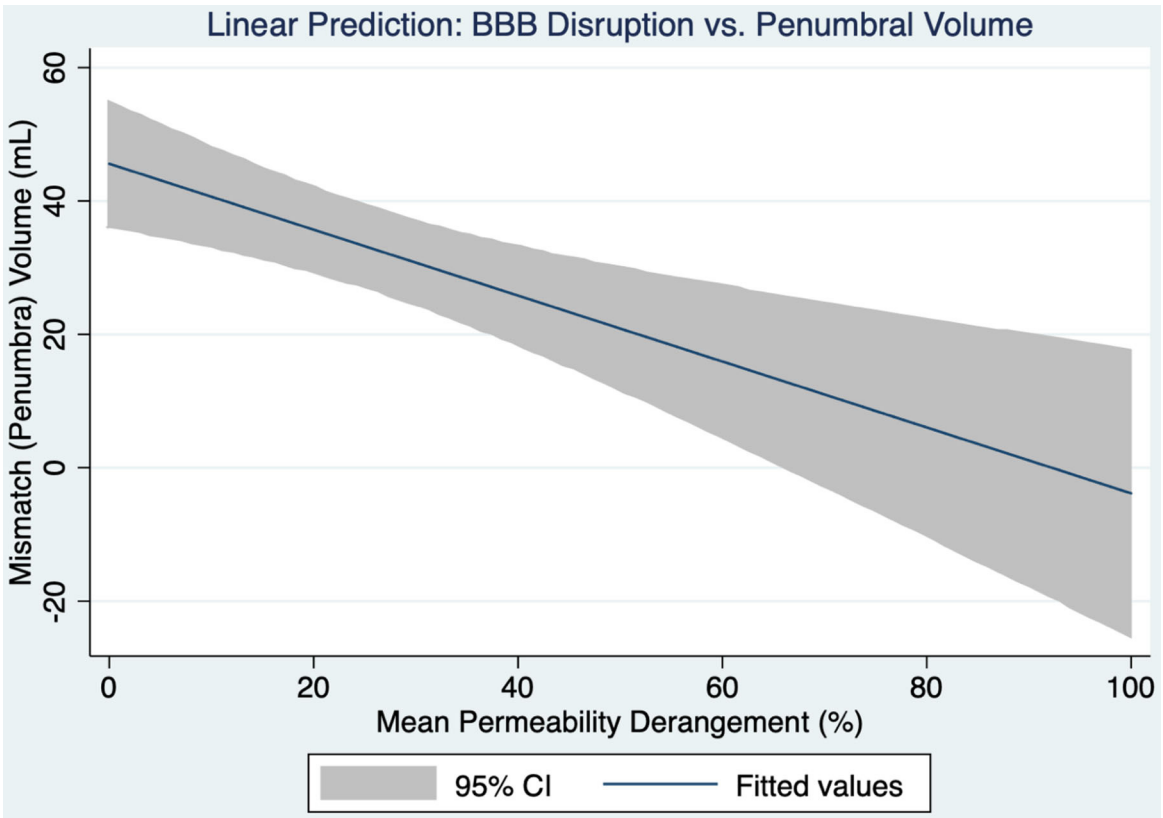


FIGURE 1 | A linear prediction model for the relationship between blood-brain barrier disruption and the volume of penumbral tissue is shown with 95% confidence intervals.

would potentially represent the population that would have maximum benefit while minimizing risk in a trial of extended time window thrombolysis. Taken over the 5-year period that this study was derived from, it implies that ∼1.2 patients per month would be eligible for enrollment in such a study at our institutions.

To further evaluate the role of time in TP and MPD, the cohort was divided into two groups based on whether their time from symptom onset to MRI was greater than or less than 9 h. Two hundred eighteen patients had a documented time of symptom discovery; of these 176 were imaged <9 h from symptom discovery, and 42 were imaged >9 h from symptom discovery. Comparing these groups with logistic regression found that patients presenting in a later time window had significantly lower NIHSS ($p = 0.001$), but no difference in DWI volume, PWI volume, penumbra volume or MPD (**Table 1**). However, when treating time-to-MRI as a continuous variable and dichotomizing by the presence of a TP, later presentation was associated with decreased likelihood of a TP (OR 0.99, CI 0.997:0.999, $p = 0.12$). Comparing time-to-MRI with MPD > 20 did not find an association ($p = 0.138$) which is in agreement with our previously published findings (7).

DISCUSSION

In a broad sense this study asked the question: In a population of patients who presented in an extended time window without acute treatment options, was the presence of an imaging target for treatment benefit associated with an imaging target for treatment safety? In a narrower sense this study asked the question: Is the presence of penumbra associated with preserved BBB integrity? We found that most patients with an imaging target for benefit also had an imaging target for safety. Furthermore, a larger amount of penumbral tissue was associated with less disruption of the BBB.

The ischemic penumbra was originally defined as loss of electrical activity in brain tissue in the setting of decreased cerebral blood flow below a threshold such that this activity could be restored in the setting of restoration of blood flow (11). This concept of penumbra was later modified to reflect tissue at risk of infarction in the absence of reperfusion, and thus a target for salvage with acute reperfusion therapies. The introduction of MRI led to the development of a biomarker for penumbral tissue, the diffusion-perfusion mismatch (12). Advances in technology, combined with real-time post-processing services, made penumbral imaging more widely available using CT

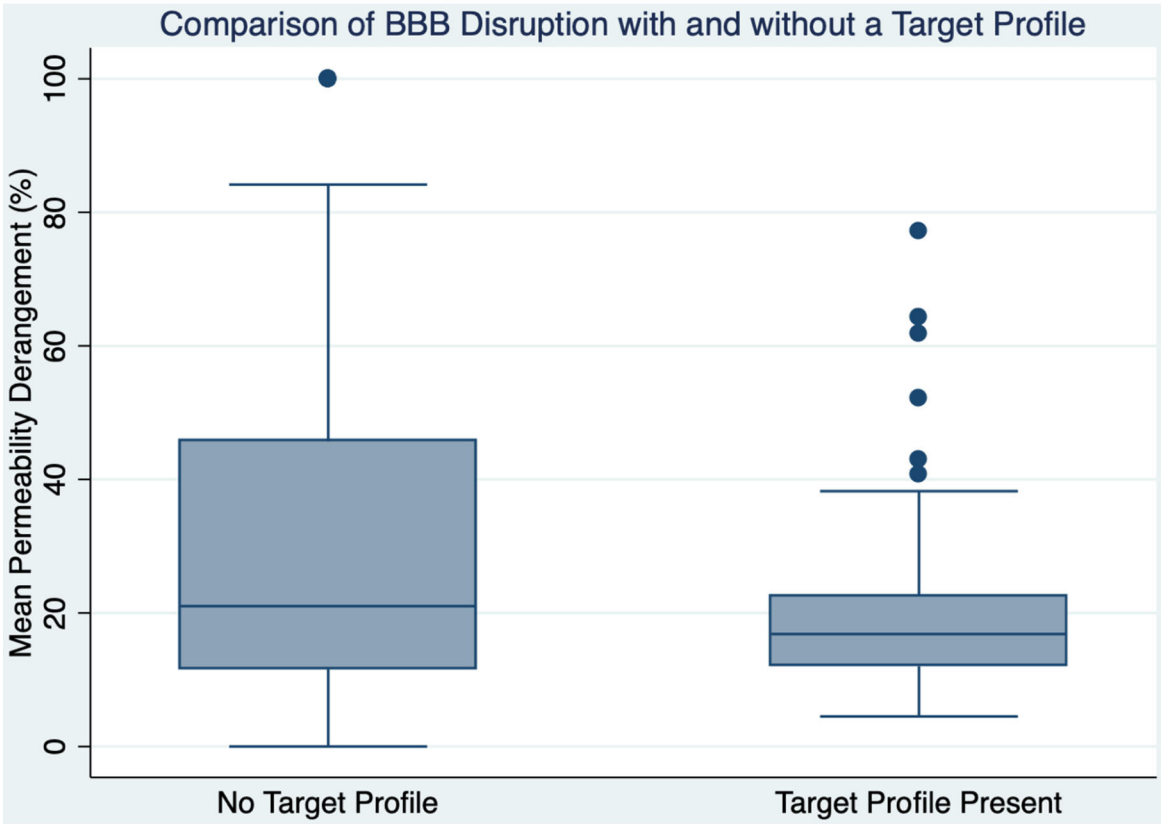


FIGURE 2 | A boxplot is shown comparing blood-brain barrier disruption between those with and without a target profile.

TABLE 1 | Population characteristics for all patients and the subgroups of patients imaged less than and greater than 9 h from the time of symptom discovery.

	All patients ($n = 222$)	<9 h ($n = 176$)	>9 hours ($n = 42$)	*p*-value
Median age	73	75	66.5	0.05
Sex (% female)	55	57	50	0.43
Median NIHSS	6	9	3	0.001
Mean DWI volume (mL)	14	15	11	0.5
Mean PWI volume (mL)	47	48	43	0.69
Mean penumbra volume (mL)	33	33	32	0.9
Median MPD (%)	18	18	18	0.87
Mean time from symptom discovery to MRI (minutes)	364	242	875	–

perfusion. Through a series of studies that culminated with the DEFUSE 3 study (1), penumbral imaging was validated not only as a way to select patients who would benefit from treatment, but also as a way to remove the restrictive time-based model for making treatment decisions, at least for mechanical thrombectomy (13).

Treatment with intravenous thrombolytics, however, remains time-based. Delay in arrival to the emergency room is the most common reason patients are not treated with thrombolysis (14). Recent advances using FLAIR MRI have expanded treatment of patients whose onset is unknown, such as wake-up stroke (15). However, using this approach, half of patients are still excluded due to being in an extended time window (16). Thus, recent studies have focused on using penumbral imaging to identify patients who would benefit from thrombolysis independent of time (5, 6). The ECASS 4 study was stopped early but found a trend to benefit when selecting patients based on a DWI/PWI mismatch. The EXTEND trial which was largely a CT-based trial found patients with penumbral tissue were more likely to have reduced disability when treated with thrombolysis. A meta-analysis of these studies strengthened the findings and reported a symptomatic hemorrhage rate of 5% (4).

Thus, despite treatment of patients out to 9 h, the hemorrhage rate remained modest. This could in part be due to the inclusion

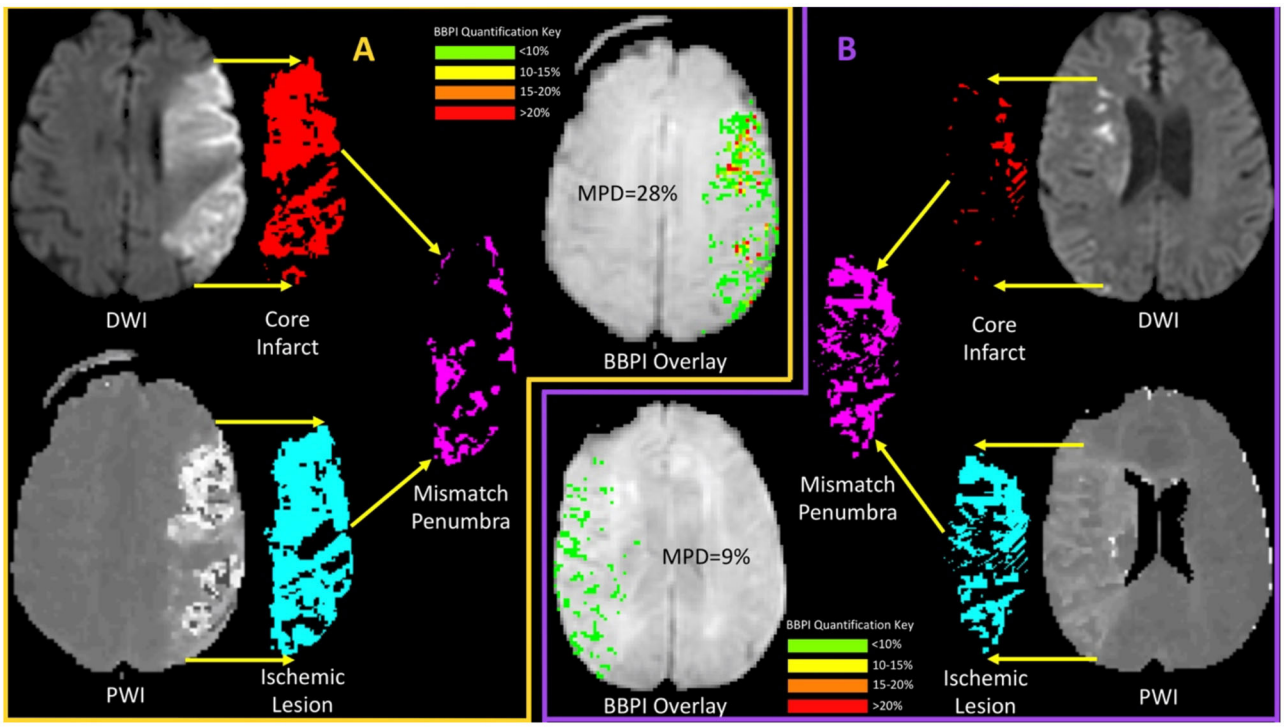


FIGURE 3 | A comparison of mean permeability derangement (MPD) for two patients, one with and one without a target profile, is shown. **(A)** (orange boarder) shows an example of a patient with a large ischemic core (red) which when compared with the ischemic lesions (light blue) results in a small mismatch (magenta) relative to the core and a non-target profile. The BBPI overlay for this patient shows multiple focal areas of red that indicate >20% leakage resulting in an MPD of 28%. **(B)** (purple boarder) shows an example of a patient with a small core (red) and a much larger ischemic lesion (light blue) resulting in a large mismatch (magenta) and the presence of a target profile. For this patient the BBPI overlay is entirely green (<10%) resulting in an MPD of 9%. These examples demonstrate the finding of less BBB disruption in patients with a better penumbral profile.

of wake-up strokes that were not actually in an extended time window. Another hypothesis is that patients with a favorable penumbral pattern at an extended time point have a similar hemorrhage risk compared to patients presenting in an early window. Our results support the latter. Specifically, patients with larger amounts of preserved ischemic tissue had less injury not only to that tissue, but the core infarct as well, when assessed by damage to the BBB. **Figure 3** shows an example of this by comparing the BBB profiles of two patients, one with a large core that is not a TP, and one with a small core and large mismatch that is a TP. The preservation of penumbral tissue into extended time windows is thought to be facilitated by collateral blood flow, the robustness of which appears to vary widely throughout the population. It appears that these collaterals not only delay the growth of the infarct but may also prevent rupture of the BBB.

We also found that one third of patents presenting in an extended time window with a favorable penumbral pattern were potentially at high risk for severe hemorrhagic complications based on BBB disruption. This could be in part because we extended the window out to 24 h; however, based on the DEFUSE 3 trial, we know that it is imaging and not time that should be guiding decisions (13). It also may be the case that not all severe hemorrhagic events are symptomatic, since MPD does not account for this, thus the actual number of patients with symptomatic intracranial hemorrhage might be lower than 30%. However, our results suggest that combining BBB imaging with penumbral imaging may be a way to identify a subset of patients who are most likely to benefit from thrombolysis in an extended time window.

There are several limitations to this study. It is a retrospective study of a deidentified dataset with minimal clinical information about the subjects. While prior studies suggest that BBB measurements are a good surrogate for hemorrhage risk, this has never been prospectively tested. Furthermore, the MPD threshold of 20% is an approximation and the true threshold may be different. This study also only focused on BBB disruption within the ischemic tissue and did not take into consideration BBB disruption that may occur in reperfused tissue. The results of this study only apply to MRI selected patients as the K_2 metric can only be extracted from MRI and not from CTP.

CONCLUSIONS

Patients presenting in an extended time window with a favorable penumbral profile for treatment have less severe BBB disruption. This may explain why hemorrhage rates in extended window

trials have been modest. The addition of BBB imaging to existing post-processing methods that calculate penumbra has the potential to improve safety. Future trials of extended time window thrombolysis are needed.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation upon reasonable request and after appropriate approval.

ETHICS STATEMENT

The studies involving human participants were reviewed and determined by NIH Office of Human Subjects Research Protections (OHSRP) to qualify as Not Human Subjects Research. Written informed consent from the patients/participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

PH, SB, JB, and EH: processed the data and revised the manuscript for intellectual content. ML: major role in data acquisition and revised the manuscript for intellectual content. RL: design and conceptualized study, analyzed the data and finalized the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This research was funded by the NINDS intramural research program of the NIH.

REFERENCES

1. Albers GW, Marks MP, Kemp S, Christensen S, Tsai JP, Ortega-Gutierrez S, et al. Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. *N Engl J Med.* (2018) 378:708–18. doi: 10.1056/NEJMoa1713973
2. Leigh R, Jen SS, Hillis AE, Krakauer JW, Barker PB, STIR, et al. Pretreatment blood-brain barrier damage and post-treatment intracranial hemorrhage in patients receiving intravenous tissue-type plasminogen activator. *Stroke.* (2014) 45:2030–5. doi: 10.1161/STROKEAHA.114.005249
3. Leigh R, Christensen S, Campbell BC, Marks MP, Albers GW, Lansberg MG, et al. Pretreatment blood-brain barrier disruption and post-endovascular intracranial hemorrhage. *Neurology.* (2016) 87:263–9. doi: 10.1212/WNL.0000000000002862
4. Campbell BCV, Ma H, Ringleb PA, Parsons MW, Churilov L, Bendszus M, et al. Extending thrombolysis to 4.5-9 h and wake-up stroke using perfusion imaging: a systematic review and meta-analysis of individual patient data. *Lancet.* (2019) 394:139–47. doi: 10.1016/S0140-6736(19)31053-0
5. Ma H, Campbell BCV, Parsons MW, Churilov L, Levi CR, Hsu C, et al. Thrombolysis guided by perfusion imaging up to 9 hours after onset of stroke. *N Engl J Med.* (2019) 380:1795–803. doi: 10.1056/NEJMoa1813046
6. Ringleb P, Bendszus M, Bluhmki E, Donnan G, Eschenfelder C, Fatar M, et al. Extending the time window for intravenous thrombolysis in acute ischemic stroke using magnetic resonance imaging-based patient selection. *Int J Stroke.* (2019) 14:483–90. doi: 10.1177/1747493019840938
7. Butler J, Heidari P, Blayney S, Hitomi E, Luby M, Leigh R. Blood-brain barrier integrity of stroke patients presenting in an extended time window. *BMC Neurol.* (2020) 20:54. doi: 10.1186/s12883-020-01634-2
8. Wouters A, Christensen S, Straka M, Mlynash M, Liggins J, Bammer R, et al. A comparison of relative time to peak and tmax for mismatch-based patient selection. *Front Neurol.* (2017) 8:539. doi: 10.3389/fneur.2017.00539
9. Zaro-Weber O, Moeller-Hartmann W, Heiss WD, Sobesky J. Maps of time to maximum and time to peak for mismatch definition in clinical stroke studies validated with positron emission tomography. *Stroke.* (2010) 41:2817–21. doi: 10.1161/STROKEAHA.110.594432
10. Leigh R, Jen SS, Varma DD, Hillis AE, Barker PB. Arrival time correction for dynamic susceptibility contrast mr permeability imaging in stroke patients. *PLoS.ONE.* (2012) 7:e52656. doi: 10.1371/journal.pone.0052656
11. Astrup J, Siesjo BK, Symon L. Thresholds in cerebral ischemia - the ischemic penumbra. *Stroke.* (1981) 12:723–5. doi: 10.1161/01.STR.12.6.723
12. Schlaug G, Benfield A, Baird AE, Siewert B, Lovblad KO, Parker RA, et al. The ischemic penumbra: operationally defined by diffusion and perfusion mri. *Neurology.* (1999) 53:1528–37. doi: 10.1212/WNL.53.7.1528
13. Albers GW. Late window paradox. *Stroke.* (2018) 49:768–71. doi: 10.1161/STROKEAHA.117.020200
14. Demaerschalk BM, Kleindorfer DO, Adeoye OM, Demchuk AM, Fugate JE, Grotta JC, et al. Scientific rationale for the inclusion and exclusion criteria for intravenous alteplase in acute ischemic stroke: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke.* (2016) 47:581–641. doi: 10.1161/STR.0000000000000086
15. Thomalla G, Simonsen CZ, Boutitie F, Andersen G, Berthezene Y, Cheng B, et al. Mri-guided thrombolysis for stroke with unknown time of onset. *N Engl J Med.* (2018) 379:611–22. doi: 10.1056/NEJMoa1804355
16. Thomalla G, Boutitie F, Fiebach JB, Simonsen CZ, Nighoghossian N, Pedraza S, et al. Stroke with unknown time of symptom onset: baseline clinical and magnetic resonance imaging data of the first thousand patients in wake-up. *Stroke.* (2017) 48:770–3. doi: 10.1161/STROKEAHA.116.015233

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 09 December 2020
doi: 10.3389/fneur.2020.585929

Intravenous Tissue Plasminogen Activator in Combination With Mechanical Thrombectomy: Clot Migration, Intracranial Bleeding, and the Impact of "Drip and Ship" on Effectiveness and Outcomes

Adam Chang[1†], Elham Beheshtian[2†], Edward J. Llinas[1], Oluwatoyin R. Idowu[2] and Elisabeth B. Marsh[1]*

[1] Department of Neurology, The Johns Hopkins School of Medicine, Baltimore, MD, United States, [2] Department of Radiology, The Johns Hopkins School of Medicine, Baltimore, MD, United States

OPEN ACCESS

Edited by:
Nishant K. Mishra,
University of California, Los Angeles,
United States

Reviewed by:
Philip M. Meyers,
Columbia University, United States
Alain Bonafe,
Hôpital Gui De Chauliac, France

***Correspondence:**
Elisabeth B. Marsh
ebmarsh@jhmi.edu

[†] These authors share first authorship

Specialty section:
This article was submitted to Stroke,
a section of the journal Frontiers in Neurology

Received: 22 July 2020
Accepted: 16 November 2020
Published: 09 December 2020

Citation:
Chang A, Beheshtian E, Llinas EJ, Idowu OR and Marsh EB (2020) Intravenous Tissue Plasminogen Activator in Combination With Mechanical Thrombectomy: Clot Migration, Intracranial Bleeding, and the Impact of "Drip and Ship" on Effectiveness and Outcomes. Front. Neurol. 11:585929. doi: 10.3389/fneur.2020.585929

Purpose: Intravenous tissue plasminogen activator (tPA) is indicated prior to mechanical thrombectomy (MT) to treat large vessel occlusion (LVO). However, administration takes time, and rates of clot migration complicating successful retrieval and hemorrhagic transformation may be higher. Given time-to-effectiveness, the benefit of tPA may vary significantly based on whether administration occurs at a thrombectomy-capable center or transferring hospital.

Methods: We prospectively evaluated 170 individuals with LVO involving the anterior circulation who underwent MT at our Comprehensive Stroke Center over a 3.5 year period. Two thirds ($n = 114$) of patients were admitted through our Emergency Department (ED). The other 33% were transferred from outside hospitals (OSH). Patients meeting criteria were bridged with IV tPA; the others were treated with MT alone. Clot migration, recanalization times, TICI scores, and hemorrhage rates were compared for those bridged vs. treated with MT alone, along with modified Rankin scores (mRS) at discharge and 90-day follow-up. Multivariable regression was used to determine the relationship between site of presentation and effect of tPA on outcomes.

Results: Patients presenting to an OSH had longer mean discovery to puncture/recanalization times, but were actually more likely to receive IV tPA prior to MT (70 vs. 42%). The rate of clot migration was low (11%) and similar between groups, though slightly higher for those receiving IV tPA. There was no difference in symptomatic ICH rate after tPA. TICI scores were also not significantly different; however, more patients achieved TICI 2b or higher reperfusion (83 vs. 67%, $p = 0.027$) after tPA, and TICI 0 reperfusion was seen almost exclusively in patients who were not treated with tPA. Those bridged at an OSH required fewer passes before successful recanalization (2.4 vs. 1.6, $p = 0.037$). Overall, mean mRS scores on discharge and at 90 days were significantly better for those receiving IV tPA (3.9 vs. 4.6, 3.4 vs. 4.4 respectively, $p \sim 0.01$) and differences persisted when comparing only patients recanalized in under 6 h.

Conclusion: Independent of site of presentation, IV tPA before MT appears to lead to better radiographic outcomes, without increased rates of clot migration or higher intracranial hemorrhage risk, and overall better functional outcomes.

Keywords: MCA occlusion, thrombectomy, stroke, IV tPA, hemorrhage

INTRODUCTION

In 1995, intravenous tissue plasminogen activator (tPA), a thrombolytic designed to recanalize occluded blood vessels, was shown to significantly improve outcomes and became the first FDA approved treatment for acute ischemic stroke (1). Subsequent studies have shown that following IV tPA with mechanical thrombectomy (MT) in patients with large vessel occlusion (LVO) [e.g., internal carotid (ICA) terminus or middle cerebral artery (MCA) lesions] can improve outcomes further (2–5). This has become the standard practice at most Comprehensive Stroke Centers.

In trials demonstrating the efficacy of MT for large vessel occlusion, the majority of eligible patients were bridged with IV tPA prior to intervention (2–5). Interestingly, in 2013, the SYNTHESIS Expansion Investigators compared treatment with IV tPA plus MT to MT alone and found them to be equivalent (6). It has been proposed that tPA, which works immediately on clot breakdown, may facilitate the use of MT and help to treat distal embolization (7, 8); however, a recent study published in *Stroke* suggested that the use of IV tPA prior to mechanical lysis may complicate the procedure by converting easy to reach clots located at the proximal M1 branch to distal M2 branches (9). This, phenomenon could account for the unanticipated results of the SYNTHESIS trial and were not evaluated as part of the study. Additionally, though not different in the SYNTHESIS trial, it is possible that in some cases IV tPA may increase the rate of other complications such as hemorrhagic transformation that worsen long-term outcomes.

In this study, we evaluate the positive and negative effects of IV tPA administration prior to MT for patients presenting with LVO of the anterior circulation, and their potential impact on long-term outcomes. Individuals who received IV tPA are compared to those who did not due to either a medical contraindication (e.g., on systemic anticoagulation) or because they presented outside the 4.5 h treatment window. We stratify individuals by site of presentation [our local Emergency Department (ED) vs. transfer from an outside hospital (OSH)] to account for the effect of time on tPA's efficacy, and determine the rate of distal clot migration after combined treatment (IV tPA and MT) compared to MT alone. We then evaluate the effect of IV tPA on the efficiency and success of mechanical thrombectomy (time to recanalization and degree of reperfusion [Thrombolysis in Cerebral Infarction (TICI) score)] and risk of hemorrhagic transformation. A subgroup analysis is performed comparing functional outcomes for only those recanalized in under 6 h, to account for potential bias due to treatment in later time windows. Results will further inform the risk-benefit discussion when considering treatment options for acute ischemic stroke.

MATERIALS AND METHODS

This study was approved by the Johns Hopkins Institutional Review Board, who waived the need for informed consent given the observational nature of the study. A cohort of patients presenting directly to the Emergency Department at our large, urban, Comprehensive Stroke Center, or transferred from an outside hospital, between January 2016 and June 2019 who underwent MT for acute stroke due to LVO was prospectively followed. Mechanical thrombolysis was performed using either mechanical aspiration (MAT) or a stentriever device (SMAT). Intra-arterial tPA was rarely administered during intervention. Similar to prior MT trials, only individuals with thrombus involving the internal carotid artery (ICA) terminus, middle cerebral artery (MCA), and anterior cerebral artery (ACA) were included in the final analysis. Eligible patients presenting within 4.5 h of symptom onset (10) were treated with IV tPA prior to MT based on current practice guidelines (11).

Patient demographics (age, race, sex), medical variables (baseline modified Rankin score (mRS) (12), history of atrial fibrillation, diabetes mellitus, smoking, home medications), stroke characteristics [time of stroke discovery, stroke etiology (by TOAST criteria) (13), infarct volume, admission NIH Stroke Scale (NIHSS) score (14)], intervention-related variables [time of groin puncture and recanalization, ASPECTS score (15), collateral grade (16), thrombolysis modality (aspiration vs. stentriever), number of passes to achieve reperfusion of TICI 2b or greater, final TICI score (17)], and outcomes (length of stay, mRS at discharge and 90 day follow-up, 90 day mortality) were recorded.

Clot Migration

Thrombus location was confirmed by a board certified neuroradiologist using the patient's initial MRA or CTA and compared to clot location on digital subtraction angiography at the time of thrombectomy. Clot migration was defined as movement of the thrombus: (1) to a more distal named vessel (e.g., ICA to MCA), (2) from the proximal to distal M1 branch of the MCA, or (3) to a distal branch of a major vessel (e.g., M1 to M2 segments).

Hemorrhagic Transformation and Infarct Volume

The clinical course was reviewed by a board-certified vascular neurologist. Evidence of blood on follow-up imaging (non-contrast head CT or MRI) within 36 h of initial treatment, along with a change in examination of 4 NIHSS points or more was considered a symptomatic intracranial hemorrhage (18). Final infarct volume was calculated from the patient's MRI using the

TABLE 1 | Patient characteristics.

	Total population (*N* = 170)	Emergency department (*N* = 114)	Outside hospital (*N* = 56)	*P*-value
Demographics				
Age, mean years (SD)	69.5 (16.7)	69.7 (15.9)	69.0 (18.4)	0.813
Sex, *n* male (%)	74 (44)	50 (44)	24 (43)	0.901
Race, *n* black (%)	52 (32)	36 (32)	16 (30)	0.773
Ethnicity, *n* Hispanic (%)	6 (4)	4 (4)	2 (4)	0.894
Medical characteristics				
Baseline mRS, mean (SD)	0.6 (0.8)	0.5 (0.8)	0.6 (0.7)	0.510
Diabetes, *n* (%)	55 (32)	34 (30)	21 (38)	0.315
Hyperlipidemia, *n* (%)	81 (48)	58 (51)	23 (41)	0.229
Hypertension, *n* (%)	141 (83)	96 (84)	45 (80)	0.530
Atrial fibrillation, *n* (%)	85 (50)	55 (49)	30 (54)	0.549
Tobacco use, *n* (%)	74 (45)	51 (45)	23 (43)	0.834
Antiplatelet use, *n* (%)	54 (40)	35 (39)	19 (41)	0.824
Anticoagulant use, *n* (%)	31 (23)	19 (22)	12 (24)	0.724
Stroke characteristics				
IV tPA, *n* (%)	87 (51)	48 (42)	39 (70)	**0.001**
NIHSS on presentation, mean (SD)	15.7 (6.9)	15.2 (7.0)	16.7 (6.6)	0.185
ASPECTS, mean (SD)	9.1 (1.2)	9.3 (1.0)	8.6 (1.5)	**<0.001**
Collateral grade, *n* (%)				0.168
0	57 (34)	35 (31)	22 (39)	
1	105 (62)	75 (66)	30 (54)	
2	4 (2)	1 (1)	3 (5)	
3	4 (2)	3 (3)	1 (2)	
Stroke volume, mean (SD)	62.7 (71.9)	55.8 (57.4)	78.2 (95.8)	0.082
Stroke etiology, *n* (%)				0.688
Large artery	42 (25)	28 (25)	14 (25)	
Cardioembolism	104 (62)	68 (60)	36 (65)	
Small vessel	2 (1)	1 (1)	1 (2)	
Other etiology	8 (5)	7 (6)	1 (2)	
Undetermined	12 (7)	9 (8)	3 (5)	
Intervention characteristics				
Clot location (scan), *n* (%)				0.271
ICA	51 (30)	33 (29)	18 (33)	
M1	88 (52)	57 (50)	31 (56)	
M2	30 (18)	24 (21)	6 (11)	
Location (angio), *n* (%)				0.765
None	3 (2)	3 (3)	0 (0)	
ICA	48 (28)	30 (26)	18 (32)	
M1	78 (46)	54 (47)	24 (43)	
M2	40 (24)	26 (23)	14 (25)	
Clot migration, *n* (%)	18 (11)	10 (9)	8 (15)	0.254
Discovery to puncture, mean min (SD)	258.1 (196.5)	232.8 (187.7)	314.2 (206.0)	**0.021**
Discovery to recanalization, mean min (SD)	322.8 (244.7)	306.7 (251.5)	359.3 (227.6)	0.278
Door to scan, mean min (SD)		74.8 (60.4)		
Door to puncture, mean min (SD)		170.3 (140.2)		
Door to recanalization, mean min (SD)		229.6 (156.0)		
Puncture to recanalization, mean min (SD)	53.0 (33.5)	55.6 (34.0)	47.7 (32.1)	0.201
Modality, *n* (%)				0.644
MAT	16 (9)	12 (11)	4 (7)	
SMAT	130 (76)	88 (77)	42 (75)	

(Continued)

TABLE 1 | Continued

	Total population (*N* = 170)	Emergency department (*N* = 114)	Outside hospital (*N* = 56)	*P*-value
No. passes, mean (SD)	2.0 (1.4)	2.1 (1.5)	1.9 (1.2)	0.429
TICI score, *n* (%)				0.544
0	9 (6)	7 (7)	2 (4)	
1	3 (2)	3 (3)	0 (0)	
2a	12 (8)	10 (9)	2 (4)	
2b	46 (30)	30 (28)	16 (36)	
3	82 (54)	57 (53)	25 (56)	
Symptomatic ICH, *n* (%)	23 (14)	14 (13)	9 (16)	0.540
Outcomes				
Length of stay,mean days (SD)	9.5 (8.3)	10.3 (8.8)	7.9 (6.9)	0.079
mRS at discharge, mean (SD)−120 people	4.3 (1.6)	4.3 (1.6)	4.4 (1.7)	0.800
mRS at 90 days, mean (SD)−124 people	3.9 (2.1)	3.8 (2.0)	4.3 (2.3)	0.172
Mortality	48 (28)	31 (27)	17 (30)	0.667

Bold values indicates statistical significance p < 0.05.

Generic Lesion Segmentation tool in Carestream Vue PACS, version 12 (Carestream Vue PACS, 2019).

Statistical Analysis

Analyses were performed using STATA version 14. Differences between patients presenting directly to our ED vs. an OSH were determined using Student's *t*-tests and chi square analysis for continuous and categorical variables, respectively. The groups were then analyzed separately and divided into those treated and not treated with IV tPA. Primary variables of interest included: rate of clot migration, hemorrhagic transformation, and mRS on discharge and at 90 days post-stroke. Functional outcomes were also reported as "good" (mRS 0-2) vs. "poor" (mRS 3-6). Groin puncture to recanalization time, number of passes, and final TICI scores were also compared. Following univariate analysis, the effect of bridging with IV tPA on long-term outcome was adjusted for age, race, sex, baseline mRS, site of presentation (ED vs. OSH), collateral grade, and time from symptom onset to recanalization in multivariable linear regression. Regression analyses were also performed to look at independent predictors of clot migration, sICH, and 90-day mortality. To account for potential time-to-treatment bias, a subsequent sub-group analysis was performed comparing discharge and 90 day outcomes for only those patients recanalized within the early (<6 h) time window.

RESULTS

One hundred ninety patients were admitted and underwent MT at our Comprehensive Stroke Center over the 3.5 year study period; 170 had occlusions involving the anterior circulation and were included in further analysis. Approximately half ($n = 87$) were eligible and bridged with IV tPA prior to MT while the other 49% presented outside of the 4.5 h treatment window ($n = 44$, 53%) or did not meet inclusion criteria (on anticoagulation: $n = 27$, 33%; other: $n = 12$, 14%) (1). The majority of patients had high ASPECTs scores, but relatively poor collaterals, and were treated with stentrievers. Characteristics of the entire cohort are displayed in **Table 1**. The average age of the entire cohort was 69.5 years (SD 16.7). Forty-four percent were male; 32% were black. The mean infarct volume was 62.7 cc (SD 71.9). The average NIHSS on admission was 15.7 (SD 6.9) and the majority of strokes were due either to large artery disease (25%) or cardioembolism (62%) (13). The mean mRS at discharge was 4.3 (SD 1.6), and at 90 days was 3.9 (SD 2.1).

Effect of Site of Presentation

Patients presenting to the ED were similar at baseline to those transferred from an OSH with the exception of longer times from discovery to scan, groin puncture, and recanalization (see **Table 1**). Despite this, they were more likely to be treated with IV tPA prior to MT (70 vs. 42%, $p = 0.001$). Outcomes following intervention were also similar between groups.

Effect of IV tPA

Though similar with respect to demographics and medical comorbidities, patients treated with IV tPA prior to thrombectomy were less likely to be on an anticoagulant prior to admission (7 vs. 38%, $p < 0.001$; see **Table 2**).

Clot Migration, Ease of Intervention, and Hemorrhagic Transformation

The overall rate of clot migration was low (11%). Distal migration did occur more frequently in patients after IV tPA (13 vs. 8%), though the difference did not reach statistical significance. For patients presenting directly to our ED, tPA administration did not increase door to groin puncture or recanalization times. These times were not calculated for those being transferred from an OSH given that they were taken immediately to the Interventional Radiology Suite for the procedure and had already received tPA. Data regarding the effect of tPA administration on transfer times were unavailable. There was also no significant difference in groin puncture to recanalization time for ED or

TABLE 2 | Effect of IV tPA.

	Emergency department			Outside hospital transfer		
	No tPA (N = 66)	**tPA (*N* = 48)**	***P*-value**	**No tPA (*N* = 17)**	**tPA (*N* = 39)**	***P*-value**
Demographics						
Age, mean years (SD)	71.1 (15.6)	67.6 (16.2)	0.249	68.5 (16.5)	69.2 (19.3)	0.897
Sex, *n* male (%)	28 (42)	22 (46)	0.717	6 (35)	18 (46)	0.450
Race, *n* black (%)	20 (31)	16 (35)	0.656	4 (24)	12 (33)	0.468
Ethnicity, *n* Hispanic (%)	2 (3)	2 (4)	0.740	0 (0)	2 (6)	0.300
Medical characteristics						
Baseline mRS, mean (SD)	0.7 (0.8)	0.4 (0.6)	0.078	0.5 (0.7)	0.7 (0.7)	0.521
Diabetes, *n* (%)	26 (39)	8 (17)	**0.009**	2 (12)	19 (49)	**0.009**
Hyperlipidemia, *n* (%)	38 (58)	20 (42)	0.093	7 (41)	16 (41)	0.992
Hypertension, *n* (%)	61 (92)	35 (73)	**0.005**	15 (88)	30 (77)	0.327
Atrial fibrillation, *n* (%)	36 (55)	19 (40)	0.097	10 (59)	20 (51)	0.603
Tobacco use, *n* (%)	31 (48)	20 (42)	0.525	10 (59)	13 (36)	0.119
Antiplatelet use, *n* (%)	24 (45)	11 (31)	0.163	7 (50)	12 (38)	0.428
Anticoagulant use, *n* (%)	18 (34)	1 (3)	**0.001**	8 (50)	4 (12)	**0.004**
Stroke characteristics						
NIHSS on admission, mean (SD)	16.1 (6.7)	13.9 (7.3)	0.093	17.6 (7.4)	16.3 (6.3)	0.493
ASPECTS, mean (SD)	9.1 (1.1)	9.6 (0.7)	**0.004**	8.5 (1.4)	8.7 (1.5)	0.609
Collateral grade, *n* (%)			0.376			0.492
0	23 (35)	12 (25)		6 (35)	16 (41)	
1	42 (64)	33 (69)		11 (65)	19 (49)	
2	0 (0)	1 (2)		0 (0)	3 (8)	
3	1 (2)	2 (4)		0 (0)	1 (3)	
Stroke volume, mean (SD)	58.2 (61.8)	52.7 (51.7)	0.636	79.1 (84.7)	77.7 (102.3)	0.965
Stroke etiology, *n* (%)			0.37			0.604
Large artery	16 (25)	12 (25)		4 (24)	10 (26)	
Cardioembolism	41 (63)	27 (56)		13 (76)	23 (61)	
Small vessel	0 (0)	1 (2)		0 (0)	1 (3)	
Other etiology	5 (8)	2 (4)		0 (0)	1 (3)	
Undetermined	3 (5)	6 (13)		0 (0)	3 (8)	
Intervention characteristics						
Clot location (scan), *n* (%)			0.721			0.943
ICA	21 (32)	12 (25)		6 (35)	12 (32)	
M1	32 (48)	25 (52)		9 (53)	22 (58)	
M2	13 (20)	11 (23)		2 (12)	4 (11)	
Location (angio), *n* (%)			0.739			0.945
None	0 (0)	3 (6)				
ICA	19 (29)	11 (23)		6 (35)	12 (31)	
M1	30 (45)	24 (50)		7 (41)	17 (44)	
M2	16 (24)	10 (21)		4 (24)	10 (26)	
Clot migration, *n* (%)	5 (8)	5 (10)	0.597	2 (12)	6 (16)	0.696
Discovery to puncture, mean min (SD)	234.9 (154.6)	229.8 229 (2)	0.895	431.8 (322.5)	255.4 (58.7)	**0.005**
Discovery to recanalization, mean min (SD)	291.1 (157.1)	326.4 (337.0)	0.527	483.6 (365.9)	299.6 (70.2)	**0.019**
Door to scan, mean min (SD)	75.9 (67.3)	73.3 (50.1)	0.828			
Door to puncture, mean min (SD)	181.2 (153.7)	155.6 (119.6)	0.366			
Door to recanalization, mean min (SD)	242.4 (165.4)	213.3 (143.4)	0.386			
Puncture to recanalization, mean min (SD)	53.0 (31.1)	58.9 (37.5)	0.418	53.8 (39.1)	45.0 (28.9)	0.417
Modality, *n* (%)			0.291			0.771
MAT	6 (9)	6 (13)		1 (6)	3 (8)	
SMAT	52 (79)	36 (75)		13 (76)	29 (74)	

(Continued)

TABLE 2 | Continued

	Emergency department			Outside hospital transfer		
	No tPA (N = 66)	**tPA (*N* = 48)**	***P*-value**	**No tPA (*N* = 17)**	**tPA (*N* = 39)**	***P*-value**
No. passes, mean (SD)	2.2 (1.5)	2.0 (1.4)	0.524	2.4 (1.5)	1.6 (0.9)	**0.037**
TICI score, *n* (%)			0.197			0.189
0	6 (10)	1 (2)		2 (13)	0 (0)	
1	2 (3)	1 (2)		0 (0)	0 (0)	
2a	8 (13)	2 (4)		1 (7)	1 (3)	
2b	15 (25)	15 (32)		4 (27)	12 (40)	
3	29 (48)	28 (60)		8 (53)	17 (57)	
Sympomatic ICH, *n* (%)	9 (14)	5 (10)	0.543	4 (24)	5 (13)	0.316
Outcomes						
Length of stay, mean days (SD)	11.1 (9.9)	9.3 (7.1)	0.282	9.2 (9.7)	7.3 (5.4)	0.372
mRS at discharge, mean (SD)−120 people	4.7 (1.4)	3.7 (1.7)	**0.004**	4.4 (1.6)	4.3 (1.8)	0.829
mRS at 90 days, mean (SD)−124 people	4.2 (1.9)	3.1 (2.1)	**0.009**	4.8 (1.9)	4.0 (2.5)	0.307
Mortality, *n* (%)	23 (35)	8 (17)	**0.031**	7 (41)	10 (26)	0.245

Bold values indicates statistical significance p < 0.05.

OSH patients regardless of bridging. However, the percentage of patients achieving a TICI score of 2b or better was substantially higher for those bridged with IV tPA (83 vs. 67%, $p = 0.027$), and TICI 0 perfusion was seen almost exclusively in patients who were not treated with IV tPA. None of our selected variables were predictive of clot migration in our cohort, likely because the rate was low. Rate of symptomatic hemorrhage was not statistically different between groups, but tended to be higher for transferred patients who did not receive IV tPA. Patients were significantly more likely to experience a sICH if they were white (18 vs. 6%, $p = 0.038$) and had larger infarct volumes (58 vs. 103 cc, $p = 0.018$). These variables approached statistical significance in multivariable models adjusting for age, time from symptom discovery, and admission NIHSS. Variables including advanced age, hypertension, atrial fibrillation, higher TICI scores, the use of stentrievers as opposed to aspiration, and longer times to recanalization led to higher rates of sICH, but did not meet statistical significance even in univariate analysis. Not surprisingly, 90-day mortality was significantly higher for those with sICH. Similar factors were associated with increased risk for mortality as with sICH; however, in multivariable regression, only baseline mRS and infarct volume remained significant.

Functional Outcomes

Patients treated with IV tPA plus MT experienced a better overall functional recovery, with significantly lower mRS scores at both discharge [3.9 (SD 1.7) vs. 4.6 (SD 1.4), $p = 0.011$] and follow-up [3.4 (SD 2.3) vs. 4.4 (SD 1.9), $p = 0.012$] compared to those only receiving MT. A Rankin Shift is displayed in **Figure 1** illustrating improved outcomes at both discharge and 90 days post-stroke for patients bridged with IV tPA prior to mechanical thrombectomy. When comparing good (mRS 0-2) vs. poor (mRS 3-6) functional outcomes, those treated with IV tPA were more likely to have a good outcome at both discharge (24 vs. 14%, $p = 0.167$) and 90 days (41 vs. 18%, $p = 0.006$), but only long-term results reached significance. While functional outcomes differed, final infarct volumes and length of stay were similar between the two groups. Results did not change when evaluating only patients recanalized within the early (<6 h) treatment window ($n = 73$). Patients treated with IV tPA prior to MT within this window continued to demonstrate lower mRS scores at discharge [3.9 (SD 1.8) vs. 4.9 (SD 1.3), $p = 0.010$] and 90 days [3.3 (SD 2.4 vs. 4.7 (SD 1.6), $p = 0.007$] compared to those treated with MT alone.

Results from the multivariable linear regression are displayed in **Table 3**. The group bridged with IV tPA was found to have better mRS scores at discharge and 90-day follow-up than those treated with MT alone even when age, race, sex, site of presentation, collateral grade, baseline mRS, and time to recanalization, were adjusted for. Age, collateral grade, and baseline mRS were also found to be independently associated with improved outcome. Results were most significant at 90-day follow-up.

DISCUSSION

Currently, IV tPA prior to mechanical thrombectomy (MT) is considered standard of care for eligible patients presenting with LVO within the accepted time window (4.5 h from last known normal) (10, 11). Nonetheless, the added benefit of combination therapy has been questioned and concerns raised regarding potential drawbacks. Several trials have recently attempted to address the role of tPA in MT with mixed results. DIRECT MT was a randomized controlled clinical trial of 41 Chinese centers that found no difference in 90 day mRS score for patients bridged with IV tPA vs. treatment with MT alone; (19) however all patients included presented directly to the thrombectomy-capable center, and a similar trial was unable to show non-inferiority of MT compared to the combined approach. (20) While we did not find major significant differences in radiographic or functional outcomes between patients presenting to the ED vs. an OSH, those transferred did

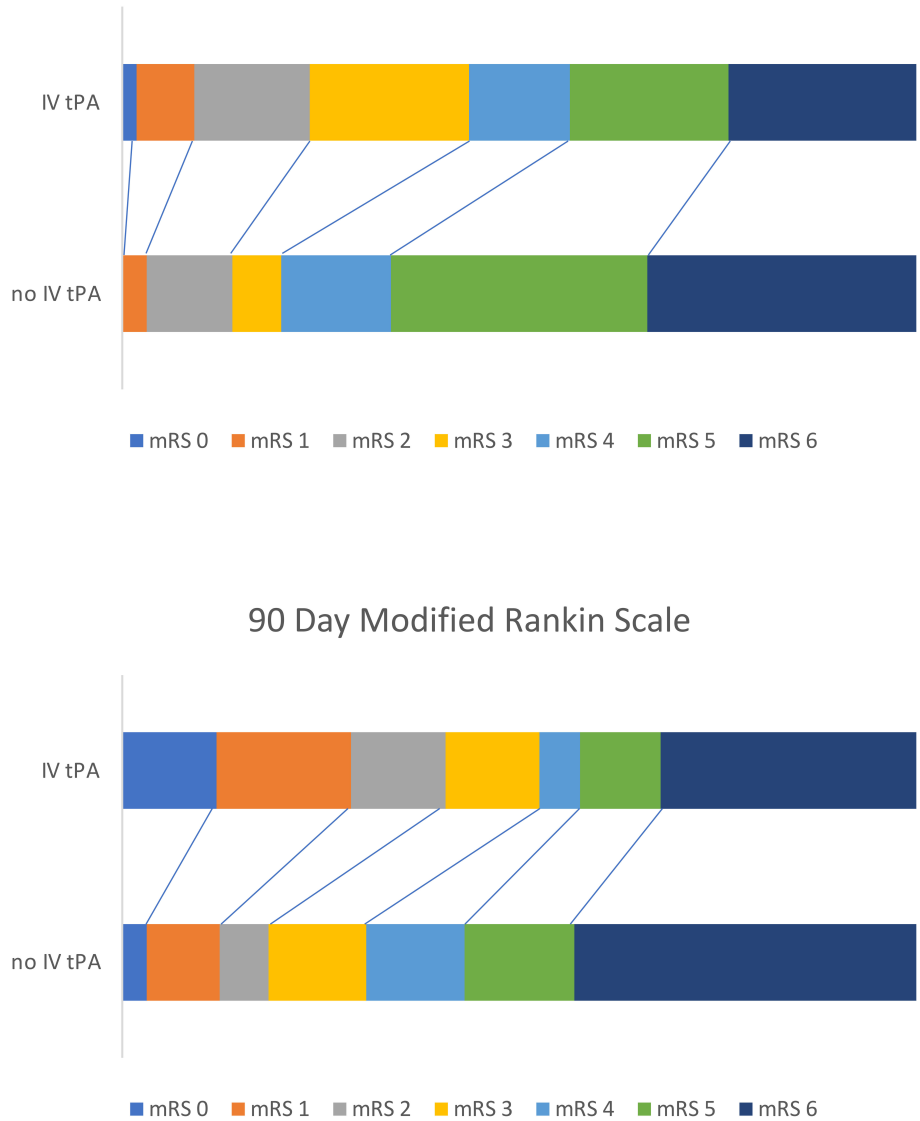


FIGURE 1 | Rankin shift illustrating improved outcomes for those bridged with IV tPA before mechanical thrombectomy at both discharge and 90 days post-stroke.

require fewer passes to achieve recanalization, which may have been in part due to the fact that there was a longer period of time for tPA to soften the clot. Previous studies have shown the rate of recanalization after IV tPA, negating the need for subsequent intervention, to be as high as 11% (21); however, a recent meta-analysis showed a lower rate of recanalization following IV tPA for ICA terminus and proximal MCA occlusions (22). More recently, Ren and colleagues reported that treatment with IV tPA prior to MT led to distal clot migration and a higher rate of unsuccessful clot removal in their patient population (9).

Given that the administration of IV tPA takes time, appears to have varying degrees of effectiveness, and theoretically increases the risk of clot migration and hemorrhagic transformation, some have raised the concern that using IV tPA as a bridge to MT may actually prolong recanalization times and be harmful to patients (23). Our data support that IV tPA does lead to a higher rate of distal clot migration; however, this overall rate is relatively small, and did not reach statistical significance in our population, consistent with at least one prior study failing to demonstrate a significant association between IV tPA and thrombus migration (23). Interestingly, at 13%, our rate of partial recanalization following tPA was lower than those reported by Seners and colleagues (up to 33%) (24), but more in line with the 7% rate reported by Mendez et al. (25). The variability may have in part been influenced by thrombus location, as the majority of our occlusions involved the distal ICA and proximal MCA. More importantly, despite a higher rate of clot migration with tPA, we show that taking the time to administer alteplase

TABLE 3 | Multivariable regression of discharge and 90 day post-stroke outcomes.

	Discharge mRS			90 day mRS		
	Coefficient	***P*-value**	**95% Confidence interval**	**Coefficient**	***P*-value**	**95% Confidence interval**
IV tPA	−0.514	0.069	−1.068 to 0.040	−0.775	**0.045**	−1.531 to −0.019
Age	0.036	**<0.001**	0.018–0.055	0.049	**<0.001**	0.024–0.073
Race	0.037	0.916	−0.666 to 0.741	0.337	0.475	−0.597 to 1.272
Sex	−0.234	0.448	−0.844 to 0.376	0.111	0.791	−0.718 to 0.939
Baseline mRS	0.629	**0.002**	0.236–1.022	0.575	**0.036**	0.039–1.111
Collateral grade	−0.668	**0.014**	−1.194 to −0.141	−0.785	**0.033**	−1.506 to −0.063
Discovery to recanalization	0.0003	0.555	−0.001 to 0.001	0.001	0.339	−0.001 to 0.002
Outside hospital	−0.356	0.294	−1.026 to 0.314	0.304	0.517	−0.624 to 1.231

Bold values indicates statistical significance $p < 0.05$.

neither significantly increased door to groin puncture times nor interfered with clot removal, and actually improved reperfusion (TICI scores), perhaps by softening the thrombus and making it more amenable to intervention. Time from groin puncture to recanalization was not affected by treatment with tPA, however the number of required passes to recanalize the vessel was lower, particularly in OSH transfers, perhaps because there was a longer period of time for tPA to take effect (26, 27).

Notably, the rate of symptomatic hemorrhage was not increased, even when times were longer, to potentially offset this advantage. One of the most feared complications, studies have shown that longer times to reperfusion are associated with higher bleeding rates (28), so this is an important finding when considering the risk/benefit profile of treatment. Our overall, rate of sICH (14%) is similar to that of other studies for mechanical thrombectomy (29) and is as expected higher than that for administration of IV tPA alone (1). Patients within our cohort were more likely to experience sICH if they were white and had larger infarct volumes, though other variables such as age, atrial fibrillation (which can lead to larger infarcts), hypertension, and longer times to recanalization trended toward higher hemorrhage rates and may have reached significance with a larger sample size. This is also consistent with the literature (29, 30) and did not vary based on whether they were bridged with IV tPA prior to thrombectomy. We did observe a slightly higher sICH rate in patients from an OSH who were not bridged with IV tPA. This may have been due to higher rates of systemic anticoagulation in this group. Interestingly, the use of stentrievers vs. mechanical aspiration led to more hemorrhages in our cohort. This difference did not reach statistical significance, but may be at least in part due to success of reperfusion and reinstating blood flow (more TICI 3 vs. 2b seen with stentrievers), as better TICI scores was also associated with higher hemorrhage risk, which theoretically could be due to increased risk of short-term reperfusion injury in those recanalized vs. those whose vessel remained closed. Importantly, adequate reperfusion (2b/3) was required in order to achieve a good outcome at 90 days.

In addition to clot migration and hemorrhage risk, we evaluated the effect of combined therapy vs. MT alone on long-term functional outcome (mRS). Similar to previous studies (31, 32) our data suggest a significant recovery benefit when tPA is given prior to MT. The difference persisted even when adjusting for time to recanalization and other differences between the two groups. While the underlying mechanism remains unclear, it has been consistently demonstrated. One possibility is that early administration of IV tPA leads to clot migration or partial recanalization that could contribute to earlier or increased perfusion to salvageable brain during the intervention period, allowing for a better long-term prognosis. More work is needed to elucidate the underlying mechanisms.

Our study is not without limitations. It is a relatively small cohort from a single institution and is not randomized, introducing the possibility that those not treated with IV tPA had worse outcomes because of additional comorbidities or circumstances that prevented them from being tPA candidates, including delayed presentation from symptom onset. To account for this, we adjusted for the most common exclusion criteria, time to reperfusion, and compared functional outcomes for only those treated within the early window (<6 h); however, there may be additional confounding factors. In addition, the average door to puncture time was >2 h. It is possible that centers with shorter times would find that IV tPA administration does prolong time to groin puncture. Door to scan, door to needle, and door to puncture times were only calculated and analyzed for patients presenting to our ED, rather than those transferred given the information available to us, so time from symptom onset to reperfusion was used evaluate the impact of time on risk of clot migration and sICH.

Despite these limitations, our data are consistent with other subgroup analyses indicating that administration of IV tPA improves MT outcomes, and we show that this does not come at the expense of prolonged treatment times, procedural difficulties, or higher hemorrhage rates. Notably, despite some differences, we did not find enough variance between individuals presenting directly to a thrombectomy-capable center vs. being transferred for the procedure to advocate for different treatment paradigms based on site location, and all patients appeared to benefit functionally from bridging with IV tPA prior to thrombectomy.

CONCLUSION

For patients undergoing MT for large vessel occlusion, the use of IV tPA to bridge to MT does not delay treatment times or result in increased clot migration leading to difficulty with clot extraction or higher rates of intracranial hemorrhage. Treatment with IV tPA at both thrombectomy-capable centers and transferring hospitals results in better overall TICI scores and long-term functional outcomes than those treated with MT alone. When possible, use of IV tPA in combination with MT should remain first line treatment for large vessel occlusions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors upon request, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Johns Hopkins Institutional Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AC and EB were responsible for data collection and drafting the initial manuscript. EL was responsible for data collection. OI was responsible for overall conceptualization of the project, data collection, and manuscript revision. EM was responsible for overall conceptualization of the project, oversight, data analysis, and manuscript revision. All authors contributed to the article and approved the submitted version.

REFERENCES

1. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med*. (1995) 333:1581–8. doi: 10.1056/NEJM199512143332401
2. Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. *N Engl J Med*. (2105) 372:1019–30. doi: 10.1056/NEJMoa1414905
3. Campbell BCV, Mitchell PJ, Kleinig TJ, Dewey HM, Churilov L, Yassi N, et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N Engl J Med*. (2015) 372:1009–18. doi: 10.1056/NEJMoa1414792
4. Berkhemer OA, Fransen PSS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med*. (2015) 372:11–20. doi: 10.1056/NEJMoa1411587
5. Jovin TG, Chamorro A, Cobo E, de Miquel MA, Molina CA, Rovira A, et al. Thrombectomy within 8 hours after symptom onset in ischemic stroke. *N Engl J Med*. (2015) 372:2296–306. doi: 10.1056/NEJMoa1503780
6. Ciccone A, Valvassori L, Nichelatti M, Sgoifo A, Ponzio M, Sterzi R, et al. Endovascular treatment for acute ischemic stroke. *N Engl J Med*. (2013) 368:904–13. doi: 10.1056/NEJMoa1213701
7. Guedin P, Larcher A, Decroix JP, Labreuche J, Dreyfus JF, Evrard S, et al. Prior IV thrombolysis facilitates mechanical thrombectomy in acute ischemic stroke. *J Stroke Cerebrovasc Dis*. (2015) 24:952–7. doi: 10.1016/j.jstrokecerebrovasdis.2014.12.015
8. Behme D, Kabbasch C, Kowoll A, Dorn F, Liebig T, Weber W, et al. Intravenous thrombolysis facilitates successful recanalization with stent-retriever mechanical thrombectomy in middle cerebral artery occlusions. *J Stroke Cerebrovasc Dis*. (2016) 25:954–9. doi: 10.1016/j.jstrokecerebrovasdis.2016.01.007
9. Ren Y, Churilov L, Mitchell P, Dowling R, Bush S, Yan B. Clot migration is associated with intravenous thrombolysis in the setting of acute ischemic stroke. *Stroke*. (2018) 49:3060–2. doi: 10.1161/STROKEAHA.118.022751
10. Hacke W, Kaste M, Bluhmki E, Brozman M, Dávalos A, Guidetti VL, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med*. (2008) 359:1317–29. doi: 10.1056/NEJMoa0804656
11. Jauch EC, Saver JL, Adams HP, Bruno A, Connors JJ, Demaerschalk BM, et al. Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. (2013) 44:870–947. doi: 10.1161/STR.0b013e318284056a
12. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke*. (1988) 19:604–7. doi: 10.1161/01.STR.19.5.604
13. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. definitions for use in a multicenter clinical trial. TOAST. Trial of ORG 10172 in acute stroke treatment. *Stroke*. (1993) 24:35–41. doi: 10.1161/01.STR.24.1.35
14. Brott T, Adams HP Jr, Olinger CP, Marler JR, Barsan WG, Biller J, et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke*. (1989) 20:864–70. doi: 10.1161/01.STR.20.7.864
15. Barber PA, Demchuk AM, Zhang J, Buchan AM. Validity and reliability of a quantitative computed tomography score in predicting outcome of hyperacute stroke before thrombolytic therapy. ASPECTS Study Group. Alberta Stroke Programme Early CT Score. *Lancet*. (2000) 355:1670–4. doi: 10.1016/S0140-6736(00)02237-6
16. Kim JJ, Fischbein NJ, Lu Y, Pham D, Dillon WP. Regional angiographic grading system for collateral flow correlation with cerebral infarction in patients with middle cerebral artery occlusion. *Stroke*. (2004) 35:1340–4. doi: 10.1161/01.STR.0000126043.83777.3a
17. Higashida RT, Furlan AJ, Roberts H, Tomsick T, Connors B, Barr J, et al. Trial design and reporting standards for intra-arterial cerebral thrombolysis for acute ischemic stroke. *Stroke*. (2003) 34:e109–37. doi: 10.1161/01.STR.0000082721.62796.09
18. ECASS, Larrue V, von Kummer R, Muller A, Bluhmki E. Risk factors for severe hemorrhagic transformation in ischemic stroke patients treated with recombinant tissue plasminogen activator. a secondary analysis of the European-Australian Acute Stroke Study (ECASS II). *Stroke*. (2001) 32:438–41. doi: 10.1161/01.STR.32.2.438
19. Yang P, Zhang Y, Zhang L, Zhang, Y, Treurniet KM, Chen W, et al. Endovascular thrombectomy with or without intravenous alteplase in acute stroke. *N Engl J Med*. (2020) 382:1981–93. doi: 10.1056/NEJMoa2001123
20. Suzuki K, Kimura K, Takeuchi M, Morimoto M, Kanazawa R, Kamiya Y, et al. The randomized study of endovascular therapy with versus without intravenous tissue plasminogen activator in acute stroke with ICA and M1 occlusion (SKIP study). *Int J Stroke*. (2019) 14:752–5. doi: 10.1177/1747493019840932
21. Tsivgoulis G, Katsanos AH, Schellinger PD, Köhrmann M, Varelas P, Magoufis G, et al. Successful reperfusion with intravenous thrombolysis preceding mechanical thrombectomy in large-vessel occlusions. *Stroke*. (2018) 49:232–5. doi: 10.1161/STROKEAHA.117.019261
22. Kaesmacher J, Giarrusso M, Zibold F, Mosimann PJ, Dobrocky T, Piechowiak E, et al. Rates and quality of preinterventional reperfusion in patients

with direct access to endovascular treatment. *Stroke*. (2018) 49:1924–32. doi: 10.1161/STROKEAHA.118.021579
23. Kaesmacher J, Maegerlein C, Kaesmacher M, Zimmer C, Poppert H, Friedrich B, et al. Thrombus migration in the middle cerebral artery: incidence, imaging signs, and impact on success of endovascular thrombectomy. *J Am Heart Assoc*. (2017) 6:e005149. doi: 10.1161/JAHA.116.005149
24. Seners P, Turc G, Maïer B, Mas JL, Oppenheim C, Baron JC. Incidence and predictors of early recanalization after intravenous thrombolysis. *Stroke*. (2016) 47:2409–12. doi: 10.1161/STROKEAHA.116.014181
25. Mendez B, Requena M, Aires A, Martins N, Boned S, Rubiera M, et al. Direct transfer to angio-suite to reduce workflow times and increase favorable clinical outcome: a case-control study. *Stroke*. (2018) 49:2723–7. doi: 10.1161/STROKEAHA.118.021989
26. Alexanderov AV, Molina CA, Grotta JC, Garami Z, Ford SR, Alvarez-Sabin J, et al. Ultrasound enhanced thrombolysis for stroke. *Int J Stroke*. (2006) 1:26–9. doi: 10.1111/j.1747-4949.2005.00012.x
27. Mueller L, Pult F, Meisterernst J, Heldner MR, Mono ML, Kurmann R, et al. Impact of intravenous thrombolysis on recanalization rates in patients with stroke treated with bridging therapy. *Eur J Neurol*. (2017) 24:1016–21. doi: 10.1111/ene.13330
28. Mazya M, Egido JA, Ford GA, Lees KR, Mikulik R, Toni D, et al. Predicting the risk of symptomatic intracerebral hemorrhage in ischemic stroke treated with intravenous alteplase. *Stroke*. (2012) 43:1524–31. doi: 10.1161/STROKEAHA.111.644815
29. Kidwell CS, Saver JL, Carneado J, Sayre J, Starkman S, Duckwiler G, et al. Predictors of hemorrhagic transformation in patients receiving intra-arterial thrombolysis. *Stroke*. (2002) 33:717–24. doi: 10.1161/hs0302.104110
30. Álvarez-Sabín J, Maisterra O, Santamarina E, Kase CS. Factors influencing haemorrhagic transformation in ischaemic stroke. *Lancet Neurol*. (2013) 12:689–705. doi: 10.1016/S1474-4422(13)70055-3
31. Kaesmacher J, Kleine J. Bridging therapy with i. v. rtPA in MCA occlusion prior to endovascular thrombectomy: a double-edged sword? *Clin Neuroradiol*. (2018) 28:81–9. doi: 10.1007/s00062-016-0533-0
32. Mistry E, Mistry A, Nakawah M, Chitale RV, James RF, Volpi, et al. Mechanical thrombectomy outcomes with and without intravenous thrombolysis in stroke patients: a meta-analysis. *Stroke*. (2017) 48:2450–6. doi: 10.1161/STROKEAHA.117.017320

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 09 December 2020
doi: 10.3389/fneur.2020.594672

Pathophysiology of Blood–Brain Barrier Permeability Throughout the Different Stages of Ischemic Stroke and Its Implication on Hemorrhagic Transformation and Recovery

Sara Bernardo-Castro [1†], João André Sousa [1†], Ana Brás [1], Carla Cecília [1], Bruno Rodrigues [1], Luciano Almendra [1], Cristina Machado [1], Gustavo Santo [1], Fernando Silva [1], Lino Ferreira [2], Isabel Santana [1,2] and João Sargento-Freitas [1,2]*

[1] Stroke Unit, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal, [2] Faculdade de Medicina da Universidade de Coimbra, Coimbra, Portugal

OPEN ACCESS

Edited by:
Richard Leigh,
National Institutes of Health (NIH),
United States

Reviewed by:
Whitney Freeze,
Maastricht University, Netherlands
Ozge Altintas Kadirhan,
Kirklareli University, Turkey

***Correspondence:**
João Sargento-Freitas
jsargentof@gmail.com

[†]These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: 14 August 2020
Accepted: 09 November 2020
Published: 09 December 2020

Citation:
Bernardo-Castro S, Sousa JA, Brás A, Cecília C, Rodrigues B, Almendra L, Machado C, Santo G, Silva F, Ferreira L, Santana I and Sargento-Freitas J (2020) Pathophysiology of Blood–Brain Barrier Permeability Throughout the Different Stages of Ischemic Stroke and Its Implication on Hemorrhagic Transformation and Recovery. Front. Neurol. 11:594672. doi: 10.3389/fneur.2020.594672

The blood–brain barrier (BBB) is a dynamic interface responsible for maintaining the central nervous system homeostasis. Its unique characteristics allow protecting the brain from unwanted compounds, but its impairment is involved in a vast number of pathological conditions. Disruption of the BBB and increase in its permeability are key in the development of several neurological diseases and have been extensively studied in stroke. Ischemic stroke is the most prevalent type of stroke and is characterized by a myriad of pathological events triggered by an arterial occlusion that can eventually lead to fatal outcomes such as hemorrhagic transformation (HT). BBB permeability seems to follow a multiphasic pattern throughout the different stroke stages that have been associated with distinct biological substrates. In the hyperacute stage, sudden hypoxia damages the BBB, leading to cytotoxic edema and increased permeability; in the acute stage, the neuroinflammatory response aggravates the BBB injury, leading to higher permeability and a consequent risk of HT that can be motivated by reperfusion therapy; in the subacute stage (1–3 weeks), repair mechanisms take place, especially neoangiogenesis. Immature vessels show leaky BBB, but this permeability has been associated with improved clinical recovery. In the chronic stage (>6 weeks), an increase of BBB restoration factors leads the barrier to start decreasing its permeability. Nonetheless, permeability will persist to some degree several weeks after injury. Understanding the mechanisms behind BBB dysregulation and HT pathophysiology could potentially help guide acute stroke care decisions and the development of new therapeutic targets; however, effective translation into clinical practice is still lacking. In this review, we will address the different pathological and physiological repair mechanisms involved in BBB permeability through the different stages of ischemic stroke and their role in the development of HT and stroke recovery.

Keywords: blood-brain barrier, permeability, stroke, hemorrhagic transformation, pathophysiology

INTRODUCTION

The blood–brain barrier (BBB) is a dynamic physiological structure that constitutes an interface between the vasculature system and the neural tissues, regulating diverse processes such as cerebral blood flow and angiogenesis, neuronal development, and synaptic activity (1). It also acts as a physical and metabolic (2) barrier that regulates the transport of substances in a bi-directional way (3) and protects the central nervous system (CNS) from unwanted compounds playing a crucial role in maintaining its homeostasis (2, 3). The precise knowledge of the structure and functioning mechanisms of the BBB on physiological conditions is key to understand how it reacts to different situations and pathologies (4). One rather complex condition is acute ischemic stroke (AIS). This pathology is characterized by different hemodynamic stages where BBB permeability (BBBP) can either be a friend or a foe, favoring hemorrhagic transformation (HT) on the one hand and enhancing neoangiogenesis and allowing the delivery of potentially therapeutic agents whose access to the CNS would be otherwise impossible on the other hand. In AIS, we can define hyperacute (<6 h), acute (6–72 h), subacute (>72 h), and chronic stages (>6 weeks). Each phase has its own particular BBB status, with distinct pathological backgrounds and often contradictory clinical consequences. Ultimately, BBB disruption plays a key modulator and precipitant role in HT, recognized as the most devastating complication after an ischemic stroke.

Structure of the Blood–Brain Barrier: Neurovascular Unit and the Junctional Complex

The selective and protective features of the BBB are due to its special structural composition; **Figure 1** shows a schematic representation. The main physical barrier between the blood and the CNS is composed of the BBB endothelial cells (BECs) of the blood vessels (5), but to fulfill all BBB characteristics, they function along with other components. BECs are surrounded by pericytes and astrocytes (through their foot processes), and the basement membrane is composed of extracellular matrix components (EMCs) (1, 4, 6–8), constituting a continuous stratum that separates the vasculature and the neural tissue (4). The relationship between all these components constitutes a dynamic functional unit called the neurovascular unit (NVU) (4, 6, 7).

BBB Endothelial Cells

BECs constitute the innermost luminal component of the BBB (4). Their unique properties make them distinguishable from other peripheral endothelial cells (4) and allow them to strictly regulate ion movement across CNS (9). These cells contain a higher number of mitochondria, allowing the generation of greater amounts of biological energy required to maintain BBB integrity (10) and augment its selective molecular permeability (4) BECs are polarized (8) and display numerous receptors, ion channels, and surface transport proteins along with limited vesicular transport (1, 4, 11). They also possess extremely low levels of leucocyte adhesion molecules which hamper the infiltration of immune cells into the CNS and a negative surface charge that repels negatively charged compounds (1). All these features strictly regulate solute permeability into the CNS, along with the presence of the junctional complex, which binds BECs to each other (12).

Junctional Complex

The junctional complex is compromised by tight junctions (TJs), adherens junctions (AJs), and GAP junctions (GJs) (12, 13). A schematic representation is given in **Figure 1**. TJs reduce the permeation of polar solutes into the brain extracellular fluids and limit the passage of proteins and lipids located at the apico-lateral membrane (13–15). They are composed of a series of transmembrane adhesion proteins, cytoplasmatic/scaffolding proteins, and an actin cytoskeleton. Transmembrane proteins compromise claudins, which are the primary sealing proteins of TJs, occludins, acting as regulators and as a platform for signaling processes, and junctional adhesion molecules, key proteins on tubule formation and in leukocyte adhesion and transmigration. The scaffolding proteins provide a link between the transmembrane proteins and the actin cytoskeleton and participate in intracellular signaling. This group of proteins is formed by the zonula occludes proteins, and they are essential for claudin strands, occludins, and JAM assembly and for anchoring these proteins to the actin cytoskeleton which delivers essential physical support for the complex. AJs hold the cells together, giving the tissue structural support (14), and are mainly composed of cadherins, transmembrane proteins responsible for the adhesion between cells, and catenins, cytoplasmatic proteins that support cadherin association and regulate out–in processes. GJs are crucial for intercellular communication and are composed of members of the connexin family (Cx) (12). Disruption of this junctional complex assembly or function directly affects the BBB characteristics, mainly its permeability (12).

Abbreviations: AIS, acute ischemic stroke; AJs, adherens junctions; AMT, adsorptive-mediated transcytosis; Ang, angiopoietin; ADC, apparent diffusion coefficient; ABC, ATP-dependent binding cassette; BM, basement membrane; BBB, blood–brain barrier; BECs, blood–brain barrier endothelial cells; BBBP, blood–brain barrier permeability; CMT, carrier-mediated transport; CNS, central nervous system; CBF, cerebral blood flow; cSVD, cerebral small-vessel disease; SDF-1, chemokine stromal cell-derived factor-1; Cx, connexin; DW-MRI, diffusion-weighted magnetic resonance imaging; DCE, dynamic contrast-enhanced; DSC, dynamic susceptibility contrast; EPCs, endothelial progenitor cells; EMCs, extracellular matrix components; GJs, gap junctions; GFAP, glial fibrillary acidic protein; HT, hemorrhagic transformation; HARM sign, hyperintense acute reperfusion marker; ICH, intracranial hemorrhage; JAMS, junctional adhesion molecules; LAT, large neutral amino acid transporters; MIP-1α or CCL3, macrophage inflammatory protein1-α; MMP, matrix metalloproteases; MSCs, mesenchymal stem cells; MT, mechanical thrombectomy; MCA, middle cerebral artery; MCP-1 or CCL2, monocyte chemotactic protein-1; NVU, neurovascular unit; OAPs, orthogonal arrays of particles; PWI, perfusion-weighted imaging; ROS, reactive oxygen species; RMT, receptor-mediated transcytosis; rTPA, recombinant tissue plasminogen activator; CCL5 or RANTES, regulated upon activation, normal T-cell expressed and secreted; SVZ, subventricular zone; TJs, tight junctions; VEGF, vascular endothelial growth factor; WMH, white matter hyperintensities.

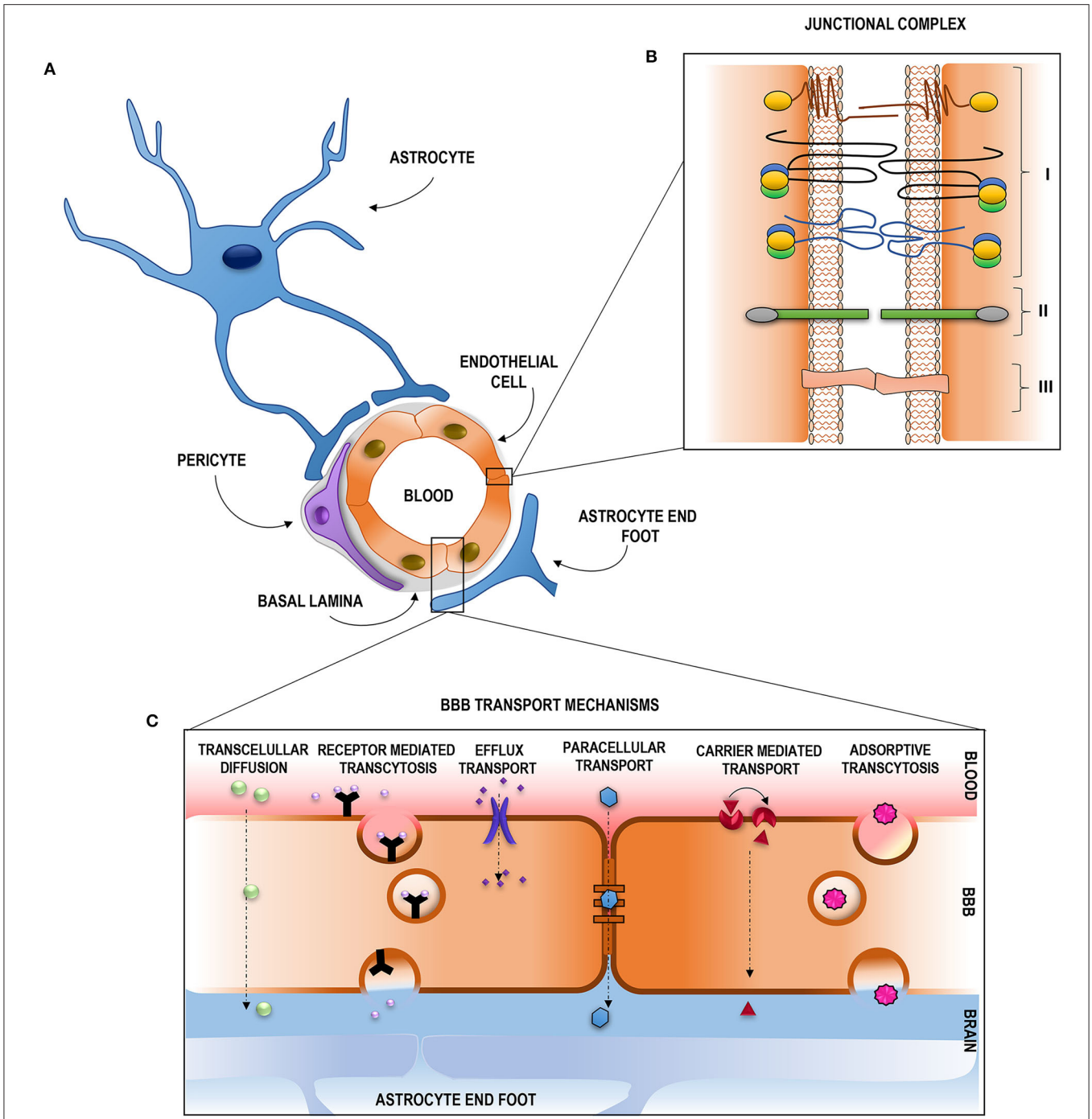


FIGURE 1 | Structural composition and transport pathways across the blood–brain barrier. **(A)** Representation of the distribution of the NVU components. **(B)** Junctional complex of the brain endotelial cells: (I) Tight junctions: composed by JAMS, caludins and occludins. ZO proteins are represented in yellow (ZO-1), green (ZO-2) and blue (ZO-3); (II) Adherens Junction, cadhenins represented in green and caterins in gray; (III) GAP junctions. **(C)** Schematic representation of the transport pathways across the BBB.

Basal Membrane

Surrounding BECs, we can find the basement membrane (BM). The BM is the acellular component of the NVU (1) compromised by a specialized layer of EMCs, including type IV collagen, laminin, nidogen, and heparin sulfate proteoglycans (9, 10). The BM provides an anchor for the rest of the cellular components, thus mediating in their crosstalk and providing microvascular stability (1, 9). The disruption of BM will lead to damaged TJs and compromised BBB integrity (1).

Pericytes

Embedded within the BM are pericytes (1, 9). Pericytes are critical for maintaining BBB integrity (8). They form peg–socket-type junctions with BECs (1, 9, 16) which prevent leucocyte infiltration through the BM into the CNS (1). Pericytes synthesize some important EMCs for BM formation (8) and are able to modulate capillary diameter through the expression of contractile proteins (1, 17). In addition, pericytes can play a similar role to macrophages (18), implying a phagocytic function able to degrade cell debris and erythrocytes following leakage after BBB disruption (1, 18).

Astrocytes

With their end-foots completely covering cerebral blood vessels (1, 9, 19) are astrocytes. Astrocytes are the major glial cells of the NVU (1, 9) proving a link between the neural system and vasculature (20) and assuming a central role in dynamic CNS signaling (1). Astrocyte end-foots express a high-density of orthogonal arrays of particles, among which we can find aquaporin IV, critical for regulating water homeostasis in the CNS (9, 21). This neuro-vascular coupling enables astrocytes to adjust CBF in response to local neurons by eliciting vasoconstriction and vasodilation of brain vessels (22) regulating the contraction and dilation of pericytes surrounding capillaries. Astrocytes also contribute to neuronal functions such as synaptic plasticity (23) and provision of energy substrate (19). Thus, the high relationship between astrocytes and vasculature is essential in maintaining BBB integrity (19, 24).

The NVU is a dynamic structure in close contact with, among others, immune cells. In this context, it is of special relevance to mention microglia cells.

Microglia

Microglia are tissue-resident macrophages (25) and are the most abundant immune cells in the CNS (26). They participate in the NVU by occasionally contacting with microvessels (24) and are the primary mediators of CNS inflammatory response (26). Microglia cells detect the very first signs of tissue damage (27) and are able to vary their phenotype in the presence of any threat. Along this, microglia have a pivotal role in the maintenance of CNS homeostasis (27), constantly surveying and screening the microenvironment within the NVU (28). Furthermore, microglia communicates in an active and dynamic way with neurons (28, 29), playing a crucial role in supporting their functions (28).

Recognition of the NVU is of high importance to reach a better understanding of brain physiology and its behavior in different pathologies such as ischemic brain injury (6). The close contact between the NVU, neurons, microglia, and other immune cells (10), along with the functional interactions and signaling between all the components (30), confers the BBB its unique characteristics, and in order to maintain its correct functioning, the neural environment must be preserved. This requires the precise regulation of molecule and ion transport between the blood and the brain (4).

Transport Across the Blood–Brain Barrier

Despite this efficient barrier function for preventing the entrance of non-desirable compounds, the ECs of a healthy BBB also act as a filter, allowing a selective exchange of solutes and regulatory factors between the blood and the brain through a number of highly controlled routes (30–32). These routes can be divided into two main pathways: paracellular and transcellular (33, 34) (**Figure 1**). The paracellular transport occurs by passive diffusion through the TJs, while the transcellular transport pathway occurs *via* the BECs *per se* (32) and can either be energy dependent (active) or not energy dependent (passive) (34). The passive transcellular pathway includes transcellular diffusion, while the active transcellular pathway includes receptor-mediated transcytosis (RMT), active efflux transport, and adsorptive-mediated transcytosis (32, 35). Carrier-mediated transport (CMT) can be either energy dependent or energy independent (34).

Paracellular Pathway

The paracellular pathway occurs between cells by the passive diffusion of low molecular mass hydrophilic molecules (32, 33, 36, 37) depending on the electrochemical, hydrostatic, and osmotic gradient (34, 38). Nonetheless, in the CNS, this transport is highly restricted and conditioned by the TJs (30, 38) and is, therefore, negligible (32). Thus, the vast majority of molecules have to use the transcellular pathway to cross the BBB.

Transcellular Pathway

Transcellular Diffusion

Transcellular diffusion takes place at the luminal and abluminal membrane of BECs (38) and is restricted to gases such O_2 and CO_2 (2) and small lipophilic molecules (36, 37) of <400 Da (8) and <8 hydrogen bonds (39).

Carrier-Mediated Transport

CMT can either be active or passive (34), and it allows the exchange of molecules between blood and CNS through the BBB *via* substrate-specific transporters (8, 34). GLUT-1, large neutral amino acid transporters, and nucleoside transporters are among the CMT proteins of great importance in BBB homeostasis maintenance (39).

Receptor-Mediated Transcytosis

RMT is the main pathway used for the uptake of molecules that do not possess a specific carrier (39), such as hormones and high molecular mass proteins (32, 34). These molecules bind to the specific receptor on the cell surface, resulting in the formation of endocytic vesicles that will cross the BBB to release the ligand, allowing the receptor to be recycled (30). The transferrin receptor and the low-density lipoprotein are good examples of this pathway (32).

Efflux Transport

Efflux pumps are responsible for the removal of substances out of the CNS into the systemic circulation in order to prevent the accumulation of compounds that have gone through the BBB (34, 38). Among these efflux pumps, we can find proteins belonging to the ATP-dependent binding cassette (ABC) transporter superfamily such as P-glycoprotein (P-gp or ABCB1) (37, 39).

Adsorptive Transcytosis

Adsorptive transcytosis relies on the non-specific transport of positively charged substrates, such as cationized albumin, when they react with the negatively charged surface of BECs (32, 38).

After briefly reviewing the physiological functions of BBB and NVU, it is clear that the strict regulation of the BBB plays a fundamental contribution to the maintenance of brain homeostasis, triggering a vast number of pathological consequences when any of its components is altered (6). Such alterations can lead to a disruption of the BBB and an increased BBBP which is linked to the pathophysiology of many neurological disorders including stroke (35).

Blood–Brain Barrier and Stroke

A total of 14 million people suffer from stroke worldwide; 5.5 million of them die and another 5 million stay permanently disabled (40, 41), placing this disease as the second leading cause of mortality and morbidity worldwide (40–43). Then, 86% of all strokes are of ischemic nature (11), and they occur as a consequence of the interruption or severe reduction of blood flow and oxygen in cerebral arteries (44). This initial occlusion causes a myriad of dynamically interconnected pathophysiological events (35) that start with the onset of the ischemic insult (45) and follow a time-dependent progression through the different stroke stages. These events normally overlap (46) and can eventually lead to the destruction and/or dysfunction of brain cells, causing neurological deficits (47). Nowadays, the only approved and effective treatment to try to avoid this situation is recanalization therapy to restore the normal blood flow, but the narrow therapeutic window of the disease limits its use to ∼5% of patients. Treating patients outside this window could contribute to additional tissue damage and an increase in the risk of HT (48, 49).

One of the major events taking place in this pathophysiological response is the disruption of the BBB (3). It appears soon after the onset of artery occlusion and continues for several days to weeks after stroke (6). BBB disruption can be associated with reperfusion and is consequently attributed to dysfunctional TJs and endothelial damage, leading to increased permeability of the affected vessels (50). But rather than being solely a consequence of injury, BBB disruption also contributes to it (51) and is usually associated with poor clinical prognosis (3, 42, 49). The increase in BBBP enables the passage of molecules, fluids, and blood into the brain (52) and follows a complex time-course progression mediated by complex pathophysiological processes (53) that go from initial to secondary injury and to tissue repair and later regenerative events (54). The concrete pathways underlining the BBB dysfunction and repair in stroke are yet unclear. Recent studies are accumulating evidence proposing that the fine-tuning of these complex pathways is regulated by the action of microRNAs (miRNAs). miRNAs are endogenous, single-stranded, non-coding RNAs which inhibit protein synthesis by either mRNA degradation or transient translational arrest, allowing them to regulate most biological processes from apoptosis, inflammation, or oxidative stress to angiogenesis and neurogenesis. In fact, it has been shown that miRNAs are key in the BBBP regulation (55, 56). **Figure 2** shows a schematic representation of the main processes driving BBBP.

Traditionally, the dynamics of the BBB were thought to occur in a biphasic "open–close–open" process (57, 58), but the inconsistency in the opening/closing times has lead recent literature to propose a more continuous opening with biphasic peaks but without the BBB closing in between (46, 59–62). The first opening has been documented to occur in the hyperacute stage of stroke within the first 6 h after onset in both animal (46, 60–62) and human studies (63–65). This initial permeability is thought to be due to the sudden hypoxia of different brain cells and early BBB disruption (11). During the next 72/96 h, in the acute phase of stroke, the neuroinflammation processes motivated by the first cytotoxic events will further rupture the BBB (54). It is at this stage where the second peak of permeability is usually observed (46, 60, 62). This greater increase in permeability will lead to a higher risk of HT motivated by several mechanisms, among which is reperfusion (47, 66). Further longitudinal studies in animal models of stroke have demonstrated that permeability remains increased up to weeks after stroke (61, 62, 67). Some human studies have also demonstrated elevated permeability after 1 week (63, 68, 69). This suggests that BBB stays opened during the subacute and the chronic stages of stroke. This late opening is believed to be correlated with regenerative processes that will improve recovery and wound healing rather than contribute to pathology.

This review aims to give a meticulous view on the complexity of the cellular and molecular mechanisms that lead to the increase of BBBP during the different phases of stroke and its clinical implication on the development of reperfusion injury and HT, as well as the recovery processes derived from it. This knowledge will hopefully offer guidance on future novel treatments that directly target BBB permeability either by preserving its integrity or as a vehicle for drug delivery as well as a better understanding of how the BBB status may influence acute stroke care decisions.

THE BLOOD–BRAIN BARRIER IN THE HYPERACUTE PHASE OF STROKE

As cerebral blood flow (CBF) is impaired, delivery of oxygen and glucose—two essential substances to brain metabolism—is compromised. Adenosine triphosphate (ATP) levels reduce in the ischemic brain tissue, and ionic transporters Na^+-K^+-ATPase and Ca^{2+}-ATPase lack the substrate for its normal functioning. Na^+ accumulates within the cell, driving the movement of fluid inwards and resulting in oncotic cell swelling termed cytotoxic edema (70). This occurs just as CBF is below 30/100 mg*min and can be detected immediately after an arterial occlusion through the decrease of the apparent diffusion coefficient of water which, in turn, is responsible for the increase of signal intensity in diffusion-weighted magnetic resonance imaging (71–73). At this phase, the BBB is mainly intact.

Uptake of Na^+ in endothelial ion transporters through Na–K–Cl cotransporter and Na/H exchanger that does not depend on ATP is not effectively counterbalanced by Na^+ secretion, leading to endothelial cell swelling and BBB breakdown (11).

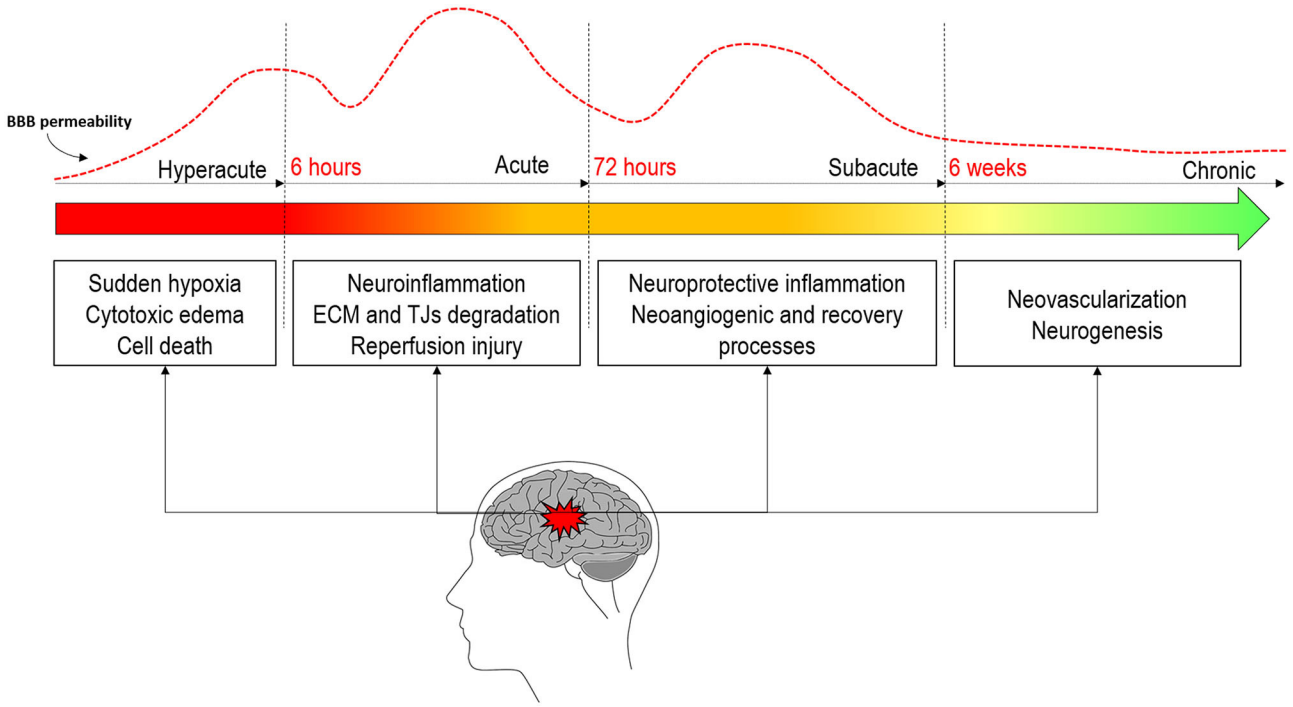


FIGURE 2 | Evolution of blood–brain barrier permeability throughout the different stages of stroke and the main pathophysiological processes in each stage.

An increase in intracellular Ca^{+} is promoted by a failing Ca^{2+}-ATPase coupled with a functioning Na^{+}/Ca^{2+}exchanger with plenty of intracellular Na^{+}. This intracellular Ca^{+} disturbs cellular mechanisms and accelerates cell death through the toxicity of high concentrations of glutamate and dopamine and the activation of several Ca^{2+}-dependent catalytic enzymes (74). Glutamate excitotoxicity coupled with cellular depolarization overstimulates metabotropic and ionotropic glutamate receptors, AMPA and NMDA receptors, disrupting calcium homeostasis even more. Ca^{2+}-induced mitochondria dysfunction leads to reactive oxygen species (ROS) generation such as superoxide anions (75); but not only ions are responsible for this early BBB disruption, other mediators such as aquaporins, matrix metalloproteases (MMP), inflammatory cells, bradykinin, vascular endothelial growth factor, and nitric oxide synthase appear to have a role (7, 76, 77). In fact, MMPs seem to be a key player in BBB disruption as they directly degrade TJ proteins and ECM components (53). As the BBB breakdown occurs, within minutes to hours of ischemic onset (78), macromolecules such as proteins exit the vasculature and enter the brain extracellular space, exerting an osmotic gradient, pulling water, and generating vasogenic edema. A phase of ionic edema is also sometimes referred to as an intermediate between cytotoxic and vasogenic edema, corresponding to the formation of extracellular edema with an undamaged BBB and impermeable BBB to larger molecules such as proteins (70, 71). Since white matter is more compliant than gray matter, vasogenic edema tends to affect the former more. This occurs in the first 4–6 h after the initial vascular insult and can be identified by conventional MRI T2-weighted images and fluid-attenuated inversion recovery sequences (79, 80). A larger volume of water is mobilized in vasogenic edema compared to cytotoxic edema, paving the way for brain swelling, increased intracranial pressure, herniation, and additional ischemic injuries due to an imbalance between brain pressure and capillary pressure. Brain edema is a life-threatening complication and a leading cause of early death after stroke (81). Such phenomenon is commonly associated with infarctions involving the whole middle cerebral artery (MCA) territory, a condition that accounts for 10% of all ischemic strokes (82). In those malignant MCA infarctions, edema generally peaks between 48 and 96 h after the onset, with a fatal outcome in 80% of patients in the first week (76, 82). Several anti-edematous and intracranial pressure-lowering therapies have been proposed, including osmotherapy, steroids, hyperventilation, and early decompressive craniectomy, the latter being effective in mortality reduction (83). The overall very poor prognosis has recently led investigators to evaluate, in randomized clinical trials, the potential benefit of endovascular recanalization in patients with acute ischemic stroke due to a large vessel occlusion in the distal internal carotid artery and MCA M1 segment who have already a large core of infarction [Alberta Stroke Program Early CT Score (ASPECTS) < 6]. Patients with low ASPECTS may still benefit from endovascular recanalization since it may reduce the risk of edema formation and malignant MCA infarction (84). SELECT-2 (NCT03876457), IN EXTREMIS-LASTE (85), TENSION (NCT03094715), and TESLA (NCT03805308) trials are currently underway to evaluate this hypothesis.

In this context, HT occurs as an end-stage endothelial dysfunction, with a compromise of capillary integrity and extravasation of blood into the brain parenchyma. It is the sum of ischemia plus reperfusion.

Reperfusion

The reestablishment of blood flow in a previously occluded vascular bed is a double-edged sword. It may warrant the survival of the surrounding penumbra, but at the same time, it might contribute to the flow of water and osmotic solutes through the ruptured BBB. Reperfusion is a three-stage process that may occur spontaneously and can be stimulated or anticipated by recanalization therapies with reopening of the occluded artery (78, 86). In this process, there is a first stage of reactive hyperemia with loss of cerebral vasoregulation associated with cytotoxic edema. Then, there is a following stage of hypoperfusion in relation to a reactive microvasculature obstruction through endothelial and astrocyte end-feet swelling, microvilli formation, and inflammatory activation that further aggravates BBB breakdown (86). This corresponds to a no-reflow effect and a phase of ischemic stunning of the brain, a term coined in cardiology to describe myocardium contractile depression after reperfusion of coronary occlusion (87, 88). Interestingly, it has been shown that, after ischemic injury, pericytes contract and remain like that even after the complete reopening of the occluded artery, this being one of the responsible factors for the non-reflow effect after recanalization therapy (89) that negatively affects tissue survival (90). After this hypoperfusion, there is an increase of paracellular permeability mediated by MMP which predominantly occurs between 3 to 8 h (MMP-2) and 18 to 96 h (MMP-3 and MMP-9) after the initial reperfusion. This latter period is associated with vasogenic edema and angiogenesis (86).

As described, BBB disruption is intrinsically part of the ischemia-reperfusion continuum and a fundamental but not sufficient factor to the most severe clinical presentation of reperfusion injury which is intracerebral hemorrhage. One important aspect here is timing. Although reperfusion is absolutely necessary for tissue survival, it can contribute to additional tissue damage (47, 78), and the later spontaneous recanalization is achieved, the higher is the risk of HT (91). The same is true to thrombolysis, as initiating recombinant tissue plasminogen activator (rTPA) treatment beyond the recommended 4.5 h from symptom has been associated with adverse effects, particularly HT (92). This situation will be discussed in the later sections of this review.

The hyperacute management of ischemic stroke goes beyond recanalization therapy. Common concurrent conditions and/or complications need to be tackled as a way of avoiding additional harm to the brain and thus promoting neuroprotection.

The Importance of Comorbidities in the Acute Management of Stroke

Glycemia, oxygen, blood pressure, and temperature need to be within certain thresholds. Hyperglycemia and hypoglycemia are both contributors to BBB dysfunction in infarct regions after reperfusion, mediated by elevations in the expression of MMP-2/-9 and decrease of TJ proteins including occludin, claudin-5, and ZO-1 (93). At this early stage of stroke, hyperglycemia also causes BBB disruption, again mediated by MMP-2/9 extracellular degradation, caveolin-1-mediated intracellular translocation, and autophagy-lysosome-mediated degradation of ZO-1 protein (94). Hypoxia is another insult that alters BBB integrity, increasing its permeability through MMP-9-dependent loss of tight junctions with disrupted continuity of occludin and ZO-1 (95) and through the generation of reactive oxygen species (96). Hyperthermia is another significant and independent contributor to BBBP (95, 97).

BLOOD–BRAIN BARRIER PERMEABILITY IN THE ACUTE PHASE OF STROKE AND ITS IMPLICATION ON HEMORRHAGIC TRANSFORMATION

After the first six hyperacute hours and during the next 72/96 h, the acute stage of stroke will take place. This stage is critical for saving the surrounding area of the ischemic core, known as peri-infarct tissue, as the cell death processes initiated in the hyperacute phase can expand and become a part of the ischemic core if not salvaged by the reperfusion therapy (54). The progression of brain ischemia over time from the infarct core to the penumbra and peri-infarct tissue involves secondary injury cascades. Delayed cell injury in the penumbra occurs with inflammation and free radical generation which will not only produce secondary damage and modify the extracellular matrix but also generate the signals for neural repair in later stages (54).

Over the course of days to weeks, the neuroinflammatory response takes place (98), becoming the main factor for increased BBBP (7). Recent evidence points that inflammatory processes in stroke are regulated by miRNAs. Neuroinflammation can be induced by miRNAs such as miR-155 or suppressed by miRNAs like miR-146a, miR-124, or miR-21. Other miRNAs can have the capacity of suppressing or promoting the inflammatory response, such as the case of the let-7 family (99). Ischemia-induced cell death, cell debris, and increased ROS produced in the hyperacute stage lead to neuroinflammation by activating resident microglia and astrocytes (100, 101). This activation has been shown to occur 4–6 h after occlusion in animal models of stroke (102). At 24 h, the microglial reaction is well developed (102), and it reaches its proliferation peak at 48–72 h after focal cerebral ischemia, lasting for several weeks (98). Of important note is that, upon activation, microglia can acquire two phenotypes depending on their polarization (103): M1 phenotype, the classically activated microglia that contributes to neuroinflammation and increased BBBP, and M2 phenotype, which will have an important role in recovery (6, 100, 101, 104). Pro-inflammatory microglia is capable of releasing cytotoxic compounds (101) such as nitric oxide and inflammatory cytokines like IL-1β, IL-1α, TNF-α, and IL-6, favoring BBB disruption and the increase of its permeability (27, 98, 100, 103). These pro-inflammatory cytokines play crucial roles in the neuroinflammatory cascade that will affect the disruption and permeability of the BBB. The activation of TNF-α receptors has a neurotoxic repercussion that leads, among other

things, to the activation of apoptotic factors and MMPs (105). Moreover, TNF-α can disrupt the BBB by reducing claudin-5, occluding, and ZO-1 expression, affecting the stability of TJs (35). On its side, IL-1 induces endothelial activation, leading to an increase in cytokines/chemokines and MMP-9 production, which comes along with BBB disruption and immune cell infiltration (105).

The production and the upregulation of MMP-9 are extremely important in stroke development and outcome. This metalloproteinase plays a crucial role in BBB breakdown. MMP-9 belongs to the gelatinases group in the metalloproteins family (106). It has a pivotal role in the proteolytic degradation of the ECM components of the BBB and is capable of digesting TJ proteins such as occludin and claudin, contributing to BBB disruption and permeability increase (107) since the degradation of essential components such as laminin, fibronectin, collagens, or proteoglycans destabilizes structural support for the BBB, producing leakage and breakdown (108). Its expression is rapidly upregulated in ischemic injury (109), reaching high peaks of activity at around 24–48 h (109, 110). The high activity of MMP-9 within the acute phase of ischemic stroke has been reported to increase the risk of secondary bleeding, and its presence in AIS patient's serum is correlated with worse clinical outcomes. In fact, MMP-9 degradation of the matrix is a major contributor to intracranial hemorrhage (108).

On its side, IL-6 plasma levels also correlate with stroke severity and poor clinical outcome (105). It can be detected in the first hours after stroke onset but reaches its peak at 24 h, remaining detectable up to 14 days (111). IL-6 produces gliosis, activates endothelial cells, and increases BBB damage in stroke (7). Furthermore, it actively contributes to the synthesis and release of some chemokines (105).

Chemokines are cytokines that, acting on its inflammatory function (103), are able to attract infiltrating leukocytes from circulating blood (112), contributing to aggravate this situation (100, 101). They are released in response to the action of the inflammatory cytokines by damaged CNS cells (105). Three of the most studied chemokines in the human neuroinflammatory response are the macrophage inflammatory protein1-α (MIP-1α or CCL3), the monocyte chemotactic protein-1 (MCP-1 or CCL2), and CCL5 or RANTES (103, 113) While CCL2 and CCL3 are associated with an enlarged ischemic territory, monocyte accumulation, and microglial activation in the injured brain tissue, respectively, CCL5 has been shown to be a potent pro-inflammatory chemokine linked to a greater BBB disruption, possibly by enhancing MMP-9 activity (103).

Another factor that has been shown to be key to the progression of ischemic brain damage is cyclooxygenase (COX)-2 from the COX inflammatory enzymes. It contributes to BBB damage as part of a secondary inflammatory response from 24 to 72 h after the initial insult (114).

All of these pathological neuroinflammatory responses will, therefore, lead to the rupture of the BBB components, and TJ dysfunction is perpetuated; thus, paracellular permeability increases (35), allowing the penetration of, among other molecules, thousands of peripheral immune cells into the brain (6, 100). These peripheral immune cells have the capacity of augmenting neuroinflammation by producing cytotoxic compounds that will join the damaged CNS cells and worsen BBB disruption (6). Among the infiltrating immune cells, neutrophils are of special relevance. Neutrophils are the primary responders after ischemic injury (115). The infiltration of neutrophils into the brain is aided by adhesion molecules *via* their attachment to the endothelial wall, thereby stimulating and facilitating diapedesis through the vessel wall to the site of ischemic brain injury (101). Neutrophils reach their peak around 2–4 days post-injury and then decrease (101). The presence of neutrophils in peripheral blood has been associated with worse clinical outcomes in AIS patients (116, 117). This may be associated with the fact that neutrophils show an important role in basal lamina degradation and hence in BBB disruption, most likely because they produce MMP-9 (117–120).

As all these processes are taking place, the BBB becomes more permeable and thus more likely to completely rupture. This scenario could lead to the extravasation of high amounts of blood into the brain, causing one of the most common and worst outcomes of ischemic stroke, HT.

HEMORRHAGIC TRANSFORMATION: THE ROLE OF BBB PERMEABILITY

HT is a common and serious complication of AIS occurring in a total of 30–40% of clinical cases (81, 121). It can happen spontaneously as a natural evolution of stroke or precipitated by reperfusion therapy (121, 122). In any of the cases, HT occurs when cerebral blood flow is restored to damaged blood vessels weakened by ischemic stroke (47, 66). In fact, the disruption and leakiness of the BBB on pretreatment imaging is correlated with the severity of HT (123). Furthermore, a higher BBBP is associated with not only the severity of but also the likelihood to develop HT (64, 124, 125). This association has been shown by two systematic reviews studying the prediction of HT with MRI (126) and computed tomography (CT) imaging (127). The pathophysiology of HT is multifactorial and has not yet been clearly elucidated, but it is linked to processes that alter the integrity of the BBB and basal lamina matrix (121). Among the factors that are implicated in this process, we can find matrix metalloproteinases, inflammation, vascular endothelial growth factor, nitric oxide synthase, and free oxygen radicals (81).

HT occurs in an undetermined period of time that varies from a few hours to even weeks after stroke. It has been hypothesized that the mechanisms that drive the early appearance of HT (<36 h) are different from those driving delayed HT (66). In short, early HT correlates with earlier BBB disruption which is often due to reperfusion treatment (128). In fact, HT appearing on the first 36 h after treatment is directly correlated to reperfusion (122). On the other hand, HT appearing after the first 36 h is correlated with a delayed BBB disruption. This is supported by the fact that a BBB disruption measured in the first hours of stroke does not predict the appearance of HT occurring later than 3 days (129). It is thought that late HT occurs due to increased BBB permeability and blood flow after cerebral edema reduction (130, 131). The hemorrhage appearance differs

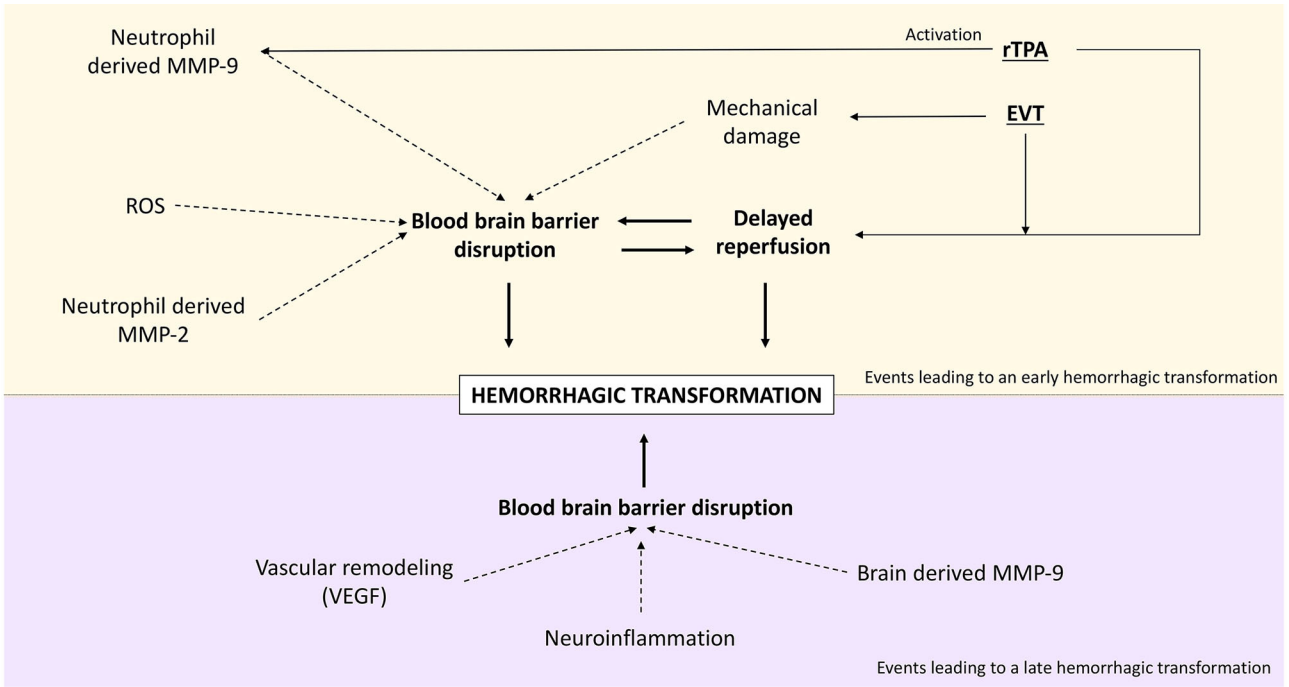


FIGURE 3 | Main events leading to hemorrhagic transformation after stroke.

according to the stage it occurs. While early HT tends to be a dense parenchymal hematoma, late HT more frequently takes the form of petechial hemorrhages (132). **Figure 3** shows a schematic representation of the main processes that lead to early and late HT.

Early BBB disruption and, therefore, early HT are driven by several molecules as expressed in the previous sections. Reperfusion-induced ROS can disrupt the neurovascular unit (66) which will lead to the entrance of neutrophils from the blood. Neutrophils have, as pointed out earlier in this review, the capacity to secrete MMP-9. Neutrophil-derived MMP-9 is therefore a pivotal mediator of early HT (133). MMP-2 is also a great determinant in early BBB disruption (134), as it has been shown in the rat model to mediate occludin and claudin-5 degradation from the cytoskeleton, causing early ischemic BBB disruption (135).

In contrast, delayed BBB disruption and HT (>36 h) are believed to be related to the activation of brain-derived proteases such as MMP-9, neuroinflammation, and factors that promote vascular remodeling such as vascular endothelial growth factor (VEGF) (66). A relationship between MMP-9 levels and the development of late HT has been established (107, 136), and some animal studies have shown that after 24 h, the major source of MMP-9 is brain cells and not neutrophils (137, 138). In parallel, vascular remodeling factors can also contribute to the development of delayed HT (66). Vascular remodeling is a key process for lesion recovery, but its initial phase requires the mobilization of progenitor endothelial cells motivated especially by VEGF. This process implies an immature BBB (139), hence the risk of developing HT. This mechanism will be further explained later in this manuscript.

HT Motivated by Reperfusion Treatments: Thrombolysis and Endovascular Treatment

As said, reperfusion is a crucial step for a favorable outcome after stroke. But although reperfusion treatment is absolutely necessary for tissue survival, it also contributes to additional tissue damage (47, 78) that can cause HT, which will lead to a worse clinical outcome and patient recovery. Mechanisms behind rTPA-induced HT include thrombolytic and non-thrombolytic actions. It has been suggested that after rTPA therapy, HT is motivated not only by reperfusion but also due to the dysregulation in extracellular proteolysis of the NVU matrix through tPA's effects on metalloproteinase activity (66). In fact, MMP-9 release from human neutrophils is highly induced by rTPA (7), which is in line with the previously described mechanism of early HT. In addition, the contrast used in imaging and therapeutic acute phase techniques may itself promote hemorrhage. Toxicity on basal lamina is thought to be the underlying mechanism (78).

Endovascular treatment (EVT) in stroke care poses additional challenges. Firstly, mechanical clot removing implies, at least partially, direct endothelial trauma and potential disruption. Devices used in mechanical thrombectomy (MT) lead to endothelial denudation, disruption of the internal elastic lamina, and edema in the intimal and medial layers (140). Secondly, it allows for rapid and sudden reperfusion. Not surprisingly, HT is a major complication of EVT. Symptomatic intracranial

hemorrhage (ICH) occurred in 4.4% of patients in HERMES meta-analysis (141), and Hao et al. meta-analysis (142), including 1,499 patients submitted to EVT who showed that 35% of them developed ICH. Several predictors of HT after EVT have been identified so far. At time to the procedure, low ASPECTS score and poor collateral status are among them (143–145). These have also been associated with edema formation (146). There is a close interaction between BBB and EVT. Hyperdensities visible on post-procedural computed tomography after EVT are common and may be secondary to contrast extravasation or ICH. Both are due to a state of increased BBB permeability or even disruption. The contrast usually clears up within 24 h, though some studies point out an association between HT and unfavorable outcome (147). Dual-energy head CT is currently the gold standard to distinguish both conditions in control CT post-EVT (147, 148). One study (123) found BBB disruption evaluated by non-contrast CT scan within 3 h of the procedure and defined as parenchymal hyperdensity on CT scan (including both blood and contrast medium), occurring in 61% of patients. Multiple thrombectomy passes have also been independently associated with a significant increase in BBB disruption as evaluated by hyperintense acute reperfusion marker (HARM) which is a hyperintensity on T2-weighted fluid-attenuated inversion recovery (FLAIR) MRI following an injection of gadolinium-based contrast agent (149). An interesting question raised by Renú et al. (150) regarding such an imagological finding is whether this is attributable to MT-induced damage to BBB at a microcirculatory level or by direct damage to the proximal vessel wall where the MT device is deployed. The same question can be raised to iodinated contrast extravasation and the associated BBB compromise mentioned above.

There is no firm evidence on the actual HT rates for each of the stroke phases, but there is a trend toward a higher rate in the acute phase, particularly when reperfusion therapies are administered and when reperfusion is achieved outside the desirable time window. In fact, study comparisons are significantly hampered by different methodologies, time-point assessments, classification, and imaging modalities used. However, it is consensual that the vast majority of symptomatic HT post-reperfusion seem to occur within 24 h, and only around 10–15% occur beyond this time, with the clear majority occurring within 36 h (151). In a study compromising 55 stroke patients, the rate of HT in the acute phase (first 60 h) was 62.5% for rtPA-treated patients and 33.3% for the non-treated group of patients. On the opposite, 37.5% of the treated patients developed HT beyond 60 h, this being at a rate of 66.67% of the non-treated patients (152). In line with this, another study of 30 AIS patients who developed HT showed that 40% of them presented with HT in the first week with a median of 24 h, and 60% developed HT beyond 1 week, with a median of 21 days. Of the patients presenting with HT in the acute phase, 25% have had received reperfusion therapy, while only 16.7% of the late HT patients did (131). In another cohort of 527 patients, 86.3% of patients with HT developed it in the first 48 h (130). Montaner et al. showed that 68.75% of patients with HT presented it in the first 48 h. Interestingly, seven of the 11 patients developing HT in the first 48 h had recanalization in the first 12 h, while none of the patients with HT beyond 48 h had early recanalization (110). In line with this work, Molina et al. showed, in a cohort of non-thrombolytic treated patients, that a spontaneous recanalization happening between 6 and 24 h directly affected the appearance of HT. In this study, 58% of patients that underwent recanalization between 6 and 12 h developed HT; this incidence was 50% for patients recanalizing at 12–24 h, while patients that had spontaneous recanalization in 6 h or less and between 24 and 48 h did not develop any HT (91). In another cohort of 407 patients with AIS not receiving thrombolytic treatment, the authors found spontaneous HT in 12.3% of the patients, 32% within the first 48 h and 40% between 4 and 7 days from the symptom onset (153).

An absolute HT rate for each stroke phase is difficult to establish considering the multitude of factors influencing its development, but it seems clear that recanalization occurring after the hyperacute phase is directly linked with the early appearance of HT, while the lack of recanalization may motivate a delayed appearance of HT. Nonetheless, patient and stroke characteristics such as stroke severity and infarct size, increasing age, baseline systolic blood pressure, hypertension, or serum glucose (66) are factors influencing the appearance of HT and its time-point.

BLOOD–BARRIER PERMEABILITY EVALUATION IN THE EARLY DETECTION OF HT

Due to the lack of effective treatment, the early prediction of HT has become one of the hot topics in stroke research aiming to find a better patient selection for recanalization therapies. As discussed before in this manuscript, BBB breakdown is considered to be the basic pathophysiology of HT, and BBBP is directly correlated with the appearance of subsequent HT, suggesting that the assessment of the BBB permeability should be the most promising predictor of HT (154). The most common tools to measure BBBP are imaging techniques due to their accessibility and capability to quantitatively assess permeability. Nonetheless, biomarkers evaluating BBBP may also enable the early assessment of BBB disruption and hence the early prediction of HT (154) in AIS patients. In this section, we will review the most used and promising tools for BBBP assessment and HT prediction, each of which has its own advantages and disadvantages (**Table 1**).

Imaging Tools

BBB integrity can be evaluated in a timely manner by MRI or CT imaging. Both modalities detect the extravasation of intravenously administered gadolinium or iodine-based contrasts, respectively. BBB status can be not only qualitatively (statically) but also quantitatively (dynamically) evaluated depending on the inclusion of time as a variable of interest and the attenuation or enhancement pattern following the administration of contrast agent as the nominators. Static imaging is limited to a binary evaluation of signs and patterns secondary to contrast extravasation. Such signs include parenchymal enhancement on post-contrast T1-weighted MRI,

TABLE 1 | Advantages and disadvantages of blood–brain barrier permeability assessment methods.

Method	Advantages	Disadvantages	References
IMAGING			
Dynamic contrast-enhanced mri	Quantitative measure No biological risk Not invasive Good temporal resolution	Expensive Contrast agent needed Not widely available Potential patient limitation (e.g., metal implants)	(42, 155–157)
Dynamic susceptibility contrast mri	No biological risk Not invasive Very short acquisition time	Similar to DCE-MRI	(42, 156, 158)
Computed tomography	Quantitative measure Cheap and fast Widely available Acquisition and processing simplicity High-resolution parametric images	Contrast agent needed Limited brain coverage Ionizing radiation Longer bolus duration than MRI	(42, 155–157)
Positron emission tomography	Quantitative hemodynamic data	Expensive Radioactive Highly inaccessible and time-consuming Complex procedures Poor spatial and temporal resolution	(159, 160)
BIOMARKERS			
mmp-9	High sensitivity for BBB damage Low cost Quantifiable	Single time-point Results are not reflected immediately Low specificity for acute ischemic stroke (AIS)	(161)
S100B	High sensitivity for BBB damage Low cost Quantifiable Stable in the bloodstream	Low specificity for AIS Not immediately reflected Single time-point	(161, 162)
Tight junction proteins	Prospective marker Low cost Quantifiable	Single time-point Results are not reflected immediately	(161)
Cerebrospinal fluid/plasma albumin ratio	Low-cost, method-independent measure: allows the same reference value for different institutions Quantifiable	Invasive Not commonly used	(161, 163)

which is highly specific but not a sensitive predictor of HT (155, 164), HARM sign, and hyperdensities on CT scan. These latter findings have been mentioned earlier in this manuscript. In short, HARM corresponds to the juxtacortical cerebrospinal fluid (CSF) enhancement on FLAIR imaging and has been associated with HT in AIS patients (128, 165). This association, however, has been contested (166, 167). Hyperdensities seen in control non-contrast CT scan after MT consist of early intraparenchymal hyperdense areas as a result of increased permeability and may predict HT after MT (123).

Currently, dynamic contrast-enhanced (DCE) MRI is considered the most widely used imaging technique for BBB research (168) since it provides quantitative estimates of contrast agent leakage at moderate spatial resolution. It allows for a quantitative BBBP assessment both in healthy and diseased vessels with an adequate spatial resolution. Predicting HT has been the main clinical research application of DCE-MRI studies in AIS. Its time-consuming protocols for image acquisition makes DCE-MRI still not part of the routine clinical practice (169, 170). In this sense, dynamic susceptibility contrast (DSC) MRI is one of the most appealing alternatives. DSC is frequently used in stroke imaging (170) and, like DCE, relies on the tracking of a paramagnetic intravenous contrast agent. It is based on the susceptibility changes after injecting the contrast agent and depends only on its first pass, therefore reducing the image acquisition time (158, 169). DSC-MRI can be referred to as perfusion-weighted imaging (PWI), providing information about the blood flow to the brain (169) and which can be used as a surrogate for BBBP able to predict HT (42).

Perfusion CT can also generate BBBP maps that can help in the identification of patients at risk of HT (65, 171, 172), but its relevance compared to other predictors has been contested (173).

Not so relevant in an acute stroke context due to its accessibility and time consumption, positron emission tomography scan allows the determination of BBBP by the uptake of tracers radiolabeled with short-lived positron-emitting isotopes with a lower spatial resolution compared with MRI but with higher sensitivity (174, 175).

So far, we are unable to withhold patients from reperfusion therapies based on BBB status evaluated from these imaging techniques, but imaging of the BBB in the early hours of stroke is a promising strategy to improve patient safety. Also promising

is the usage of these imaging modalities in the selection of patients that may benefit from an extended time window of intravenous thrombolysis (176) and in the early detection of patients that may develop severe stroke complications such as HT, which would be key in the specific guided treatment of AIS patients avoiding, therefore, poorer prognosis and adverse clinical developments (154).

A recent meta-analysis on the prognostic performance of MRI on HT prediction in AIS patients showed that MRI has a specificity of 79% and a sensitivity of 82%. These values increased when perfusion imaging was studied alone, with 92% specificity and 80% sensitivity in terms of HT prediction (126).

DSC-PWI permeability values have been used to try to establish a relation between BBBP and subsequent HT. Pretreatment MRI PWI with DSC imaging from a cohort of patients from DEFUSE 2 trial was studied. The investigators found a relationship between the degree of BBB disruption and the severity of ICH. A pre-specified BBBP disruption threshold of 21% resulted in a sensitivity of 37.5% and a specificity of 80% for the prediction of parenchymal hematoma, with a positive predictive value of 0.375 (177). Similarly, another study with PWI imaging showed pretreatment permeability derangements to have 29% sensitivity and 98% specificity in predicting HT (178).

Regarding CT, a meta-analysis on its predictive value revealed that a high BBBP derived from perfusion CT analysis is associated with the appearance of HT, with a pooled sensitivity of 84%, a pooled specificity of 74%, an estimated positive predictive value of 46%, and an estimated negative predictive value of 77% (127). In line with this, another meta-analysis on the predictive value of perfusion CT showed a pooled sensitivity of 85.9% and a pooled specificity of 73.9% (95% CI: 45–92%) and an accuracy with a negative predictive value of 92.9% (179).

Biomarkers

So far, many biomarkers have been reported to indicate BBB damage, but none of them meets all the characteristics for an ideal biomarker (high specificity, sensitivity, and reliability, easy and fast assessment, and minimal invasiveness) (161). Here we present a brief review of the most promising biomarkers in terms of BBBP assessment and HT prediction.

MMP-9

MMP-9 plasma concentration is a strong marker of BBB disruption and permeability in stroke. High plasma MMP-9 concentration levels have also been proven to be an independent predictor of HT. MMP-9 plasma levels of $\geq$140 ng/ml measured in AIS patients showed a sensitivity of 87% and a specificity of 90% for HT prediction, with positive and negative predictive values of 61 and 97%, respectively (180). Recently, it has been shown that the levels of MMP-9 over 181.7 ng/ml have 82.9% sensitivity and 81.3% specificity for the prediction of spontaneous HT in non-treated patients (181) and that values over 775 ng/ml measured at 6 h are independently associated with HT [OR 2.91 (1.14–7.42); $p = 0.03$] in patients treated with thrombectomy (182).

S100β

S100β is a low-molecular-weight glial protein (45) that does not circulate in the blood of healthy individuals, but it can be released and detected in the peripheral blood after BBB injury; hence, its concentration is related to the extent of BBB opening (161, 183). Its presence after AIS has been directly correlated with subsequent HT. A study performed in patients treated with thrombolytic therapy found that a cutoff value of 0.23 μg/L of S100β provided a sensitivity of 46% and a specificity of 82% in the independent prediction of HT after AIS (184). This is in line with another study presenting a cutoff value of >11.89 pg/ml of S100β as a predictor of HT in non-treated AIS patients, with 92.9% sensitivity and 48.1% specificity (185).

TJs Proteins

BBB components, particularly TJ proteins such as claudin 5 and occludins, can also serve as BBBP biomarkers due to their release into the blood circulation after stroke (161). Analyzing serum levels of BBB components may be an effective way to screen for subsequent HT (154). A great amount of the BBB's selective permeability has been attributed to claudins, with claudin-5 being the most abundant type in the BBB; therefore, the appearance of this protein in the serum of AIS patients is thought to be indicative of increased BBBP (186). In addition, significantly higher levels of claudin-5 have been found in patients with HT in comparison with those without HT, with a predictive cutoff value for HT of >1.601 ng/ml, sensitivity of 64.3%, and specificity of 53.9%. These predictive values are similar to occludins. With a cutoff value of >0.029 ng/ml, occludins yield 58.6% and 67.5% of sensitivity and specificity, respectively (185).

Cerebrospinal Fluid/Plasma Albumin Ratio

The CSF albumin concentration is minimal in non-pathologic conditions. After BBB disruption, albumin can enter CSF from the blood; consequently, the CSF/serum albumin ratio can be used as a reliable marker of BBBP (6, 161). It has been shown that AIS patients have higher values of this ratio than healthy people (163, 187, 188). The CSF/plasma albumin ratio has also been related to the severity of the injury, stroke evolution, and long-term outcome (163). In addition, this ratio has been shown to be associated with the appearance of HT in AIS patients (188). Despite that, the acquisition of this ratio requires obtaining both blood and CSF albumin, limiting the use of this BBBP biomarker (161).

The predictive value of protein biomarkers for HT varies widely; hence, it has been proposed that a panel of biomarkers could have greater discriminative power than any single biomarker alone (188).

THE BLOOD–BRAIN BARRIER IN THE SUBACUTE STAGE OF STROKE

The subacute stage takes place around 1 week post-stroke, being crucial in brain repair and patient recovery.

Brain recovery is dependent on neuroinflammation. As we have described in the acute phase, neuroinflammation is mediated in a great part by pro-inflammatory microglia, leading

to pathologic inflammation. Recently, it has been suggested that this harmful active microglia of the acute stage may have beneficial effects when it appears in delayed stages (100). The neuroinflammatory reaction will become a recovery pathway by changing microglia to its anti-inflammatory phenotype. Anti-inflammatory microglia contributes to stroke recovery by expressing anti-inflammatory cytokines, such as IL-10, IL-4, and some neurotrophic factors which prevent inflammation (100) and play important roles in tissue repair and wound healing (27).

All of these recovery processes lead to a stabilization of the permeability of the BBB, although several animal studies have shown increased permeability up to 1 (62) and 3 weeks (61). Along with this, some human studies have also shown increased BBB up to 1 week (63, 68) and further (189), suggesting that BBB remains open and permeable through this subacute stage. Nonetheless, the dynamics and the permeability range of this phase are extremely diverse and dependent on the stroke characteristics (47).

The regenerative response after stroke includes angiogenesis and the modification of the vascular tree (54), the former being one of the major events contributing to an increase in BBB permeability in the subacute stage. An association of subacute BBB permeability with the migratory and angiogenic capacities of endothelial progenitor cells (EPCs) at day 7 after stroke has been shown in humans (68).

The Importance of Angiogenesis in Stroke Recovery and BBB Permeability

Angiogenesis is a multi-step process that refers to the sprouting of new blood vessels from the already existing vasculature (190). It is a natural physiologic mechanism that restores blood flow and, hence, oxygen supply, and normal metabolism in the ischemic tissue (191) being fundamental for ischemic brain repair (192). Promoting angiogenesis is one of the most important strategies for functional recovery after stroke (193). It is closely associated with reduced cerebral infarction and improved neurological recovery (194) as shown in animal models of stroke (195) and AIS patients (136). In fact, higher angiogenesis has been associated with a longer survival of stroke patients (196) and BBB stability (136). However, its benefit is time dependent, as the premature promotion of angiogenesis after stroke (such as VEGF administration) can lead to enhanced vascular permeability and increased HT risk (197).

Angiogenesis is a complex process that involves several consecutive steps (198) from endothelial cell proliferation and migration to tube formation, branching, and anastomosis (192, 198). All these processes are modulated by the inflammatory microenvironment formed in previous stages (105). Therefore, the induction of angiogenesis after an ischemic injury is mediated by the same stimuli that cause the pathologic BBB disruption (198). BECs are the primary effectors of the angiogenic response after ischemic injury, followed by the pericytes and smooth muscle cells (139).

In the first moment, angiogenesis requires the mobilization of EPCs to the ischemic area (105). This needs vasodilatation and permeability increase of the existent vessels and happens subsequently after hypoxia, in response to the production of chemokines and factors such as VEGF and ANG-2 by hypoxic cells (199). BECs enlarge and produce proteases (collagenase and matrix metalloproteinase) that are capable of local degradation of the basement membrane (139). Pericytes detach from the vessel wall and liberate themselves from the basement membrane, thanks to proteolytic degradation (199). This implies the initial rupture of the BBB for cellular migration. Once this mobilization is completed, BECs return to a quiescent-like state that is associated with the formation of cell–cell junctions and vessel maturation (200). In animal models of stroke, endothelial proliferation has been reported as early as 12–24 h (139), while active angiogenesis, with the consecutive maturation and stabilization of blood vessels, has been proven to occur around 3–4 days after injury in AIS patients (196).

VEGF, from various cellular sources, binds to its receptors on nearby vascular endothelial cells to directly initiate an angiogenic response. This binding activates a series of downstream signals and tyrosine kinases that promote angiogenesis (194). Angiopoietin (Ang)-1 and Ang-2 and their receptors, Tie-1 and Tie-2, are also deeply involved in neoangiogenesis. In short, Ang-2 promotes angiogenesis, whereas Ang-1 inhibits it (194). The close regulation between VEGF and angiopoietins plays a pivotal role in neoangiogenesis. Zhang et al. demonstrated in a rat model of stroke that the early upregulation of VEGF receptors along with a downregulation on Ang-1 is linked to an increase in BBB leakage. On the contrary, the upregulation of VEGF receptors and Ang/Tie 2 in later stages was correlated with the increase in the number of capillaries and enlarged vessels in the penumbra (201). Interestingly, it has been shown that miRNA miR-210 is involved in this angiogenic regulation in response to ischemic injury since its upregulation is able to improve angiogenesis for brain tissue repair (202).

Recently, it has been suggested that MMP-9 may also play an important role in angiogenesis. Despite its huge involvement in BBB disruption and development of HT, MMP-9 seems to be beneficial in vascular remodeling (194). Zhao et al. demonstrated in a rat model of stroke that a second phase of increased MMP-9 at 7–14 days post-stroke was correlated with angiogenesis since the inhibition of MMP-9 resulted in, among other things, malformation of blood vessels (109).

BLOOD–BRAIN BARRIER PERMEABILITY IN THE CHRONIC PHASE OF STROKE

In a chronic post-stroke setting, >6 weeks after the event, the permeability of BBB is significantly less compared to those of the initial phases. There is a sealing of the barrier with an overexpression of TJ proteins and a *de novo* organization of junction proteins (3). As many of the factors responsible for the hyperpermeability tend to decrease in this stage, there is also an increase in factors that contribute to the restoration of BBB permeability, such as Ang-1, which keeps endothelial cells in a quiescent state and promotes cell–cell and cell–extracellular matrix interactions, sphingosine-1- phosphate, and activated protein C, both of which stabilize junctions and the cytoskeleton

(3, 203). In the long-term, angiogenesis occurring predominantly in the subacute stage allows blood flow restoration to previously ischemic areas and reduces BBB permeability by contributing to the reduction of brain-derived factors that increase BBB permeability (204). In the late phase of cerebral ischemic injury, recovery is dependent on the restoration of the NVU complex. Herein BBB development is intrinsically connected with neurogenesis. There are two endogenous neural progenitor cell hubs in the adult brain: in the subventricular zone (SVZ) of the lateral ventricle walls and in the sub-granular zones of the hippocampal dentate gyrus and circumventricular organs (205–207). Neurogenesis is linked with angiogenesis. Cells in SVZ have increased expression of VEGF and its receptor Flk. In fact, VEGF is both an angiogenic and a neurotrophic factor that contributes to the migration of neuronal progenitor cells (208, 209). Being the most abundant cell type within the brain parenchyma, astrocytes have a predominant role in the regulation of neurogenesis. They are the source of two major astrocytic intermediate filament proteins, glial fibrillary acidic protein and vimentin, whose absence would severely impact stroke recovery (210). Several neurotrophic factors are also secreted by astrocytes, including brain-derived neurotrophic factor, glia-derived neurotrophic factor, nerve growth factor, basic fibroblast growth factor, VEGF, ciliary neurotrophic factor, and erythropoietin (6). These astrocyte-derived factors not only protect neurons in the acute phase but also play a crucial role in promoting neuron survival, axonal sprouting, neurovascular unit remodeling, and functional recovery in the chronic phase by contributing to neural repair and neuroplasticity (3, 6). Astrocytes have shown *in vitro* to stimulate the migration and proliferation of adult neural stem cells through the secretion of the neuroblast attracting chemokine stromal cell-derived factor-1 (211). Synaptogenesis is controlled and enabled by astrocytes *via* thrombospondins 1 and 2, which are secreted glycoproteins that are activated after AIS. Its absence, in animal models, leads to defects in synaptogenesis and axonal sprouting post-stroke (212). Other cell types are deemed important. Progenitor cells are integrated directly in the damaged barrier, particularly in the endothelium and the surrounding tissue, and release mediators that contribute to barrier repair after stroke (6). Circulating EPC has a relevant role in the subacute phase as mentioned before. Mesenchymal stem cells (MSCs) are another category of progenitor cells and one of the leading restorative cell therapy candidates. VEGF, Ang-1, bFGF-2, placental growth factor, and insulin-like growth factor are among the variety of angiogenic factors expressed by MSC (6). Most studies have focused on MSC potential in the early stages of stroke (213), but a recent phase I/II clinical trial has evaluated the administration of intravenous allogenic MSC in chronic stroke, suggesting behavioral gains and laying the ground for a randomized controlled trial (214). Juxtavascular microglia, which are brain tissue-resident macrophages, enhance brain repair by removing toxic debris, reducing neuroinflammation and releasing trophic factors (215). This is true for the tissue-restorative microglia (M2) phenotype as opposed to the M1 phenotype. Microglial activation has been shown to occur in every phase of ischemic stroke. In the chronic phase, activated microglia are located in the peri-infarct region and distal areas, but generally the greatest amount of microglia peaks in the early stages (216). Beneficial and detrimental effects of microglia have been observed depending on the morphology and subtype of activated microglia (216). Chronic cerebral hypoperfusion induces microglial activation, polarized toward M1 (pro-inflammatory) phenotype, and the drug fingolimod has been shown, in an animal model, to attenuate such microglia-mediated neuroinflammation and to promote oligodendrocytogenesis by shifting microglia toward M2 subtype (217). Clopidogrel and other P2R2Y12-targeted antiplatelets which are frequently administered in a chronic stroke setting as means of secondary prevention affect G-protein coupled purinergic receptor P2Y. This receptor mediates the microglia-induced reversal of BBB opening after an injury (3, 218).

Even though several repair mechanisms exert its efficacy, in the long run, some degree of BBB dysfunction will persist with long-lasting leakage after stroke. Insufficient or disorganized TJ complex formation, an expression of claudin-1 with decreased levels of claudin-5 trans interaction, and claudin-5/ZO-1 interaction in leaky vessels (219) are among the proposed mechanisms behind an incomplete recovery of BBB permeability. In fact, the risk of intracranial hemorrhage after an ischemic stroke is the greatest in the first 30 days and decreases thereafter, but it remains higher than in the general population (220).

The incomplete closure of BBB, on the other hand, may benefit neurogenesis due to the link between these two processes mentioned before, and it can be seen as a window of opportunity for therapeutic delivery, as endogenous neural recovery after an ischemic stroke is often insufficient. This is the case for cell therapy in stroke recovery, more specifically a BBB permeation-mediated stem cell therapy, as the entrance of transplanted cells into the brain from the periphery is facilitated by a permeable BBB. Transplantation of allogeneic neural, mesenchymal, and endothelial stem cells has itself shown promising results in stroke models in stabilizing BBB and promoting BBB integrity and in vascular regeneration after stroke (8, 221).

Cerebral Small-Vessel Disease

BBB dysfunction is a hallmark of cerebral small-vessel disease (cSVD), a condition associated with cognitive impairment, lacunar strokes, microinfarcts, microbleeds, and widespread white matter injury (222–226). Patients with higher white matter hyperintensities have significantly higher BBB leakage as evaluated by DCE-MRI (227). Doubts arise on the directionality of the association between BBB and cSVD (cause *vs*. consequence) and the exact location of the dysfunction (capillaries? arterioles?), but its association is undisputable (226, 228). On the basis for such association might be altered cerebral hemodynamics with loss of cerebral autoregulation, higher arterial stiffness, and increased speed and pulsatility of flow in small vessels (arterioles and capillaries). The resultant sheer stress damages the endothelium and BBB (229). Endothelial dysfunction seems to play a crucial role in the pathogenesis of cSVD and BBB dysfunction. Overexpression of inflammation markers (*e.g.*, intracellular adhesion molecule)

(230), C-reactive protein (231), coagulation markers (232), and hyperhomocysteinemia (233) support that hypothesis. In the upstream, chronic hypertension and diabetes (both common in cSVD) have been shown to impair cerebral blood flow and oxygenation and promote an increase in BBB permeability. Hypoxia contributes to the death of oligodendrocytes and consequent gliosis, as hypoxia-inducible factor-1 has already been identified in pathological studies of affected white matter (234). Increased MMP has also been demonstrated in the white matter of patients with vascular dementia (235). Clinically, BBB derangements in this spectrum of conditions lead to a higher risk of remote cerebral hemorrhage and HT of the infarcted area after thrombolysis in patients with cSVD (178, 236–238). Leukoaraiosis itself is an imaging finding whose presence increases the risk of HT (178).

DISCUSSION

BBB disruption after AIS occurs predominantly in the hyperacute and acute stages where major complications and clinical deterioration may take place. Reperfusion therapy, the only approved stroke treatment, may potentiate BBB permeability and promote HT. In this review, we have tried to give thoughtful insight into the dynamic changes of BBB permeability across the timespan of an ischemic stroke, a topic in which there is still limited knowledge but a handful of therapeutic opportunities. **Table 2** summarizes the main processes driving BBB dynamics and HT after stroke. We performed a comprehensive review from bench to the bedside, linking pathophysiological processes to their therapeutic counterparts and showing how hyperacute, acute, subacute, and chronic stroke care interacts with BBB status and how clinical practice may change in the future in order to prevent HT.

Stroke care and the prognosis are highly dependent on intravenous thrombolysis or mechanical thrombectomy. These treatments are available to only a fraction of AIS patients due to their strict time and tissue window eligibility criteria. Knowledge of BBB status through current and under-development permeability imaging techniques could be an interesting strategy in AIS patients to extend such criteria safely, avoid hazardous recanalization, and reduce HT or to test other strategies such as tighter BP control in patients with BBB imaging-proven severe and extensive damage. Early prediction of HT could also be enabled by the evaluation of biomarkers related to the disruption of BBB.

If knowing BBB status could potentially help us guide acute stroke care decisions, understanding the exact mechanisms behind the BBB dysregulation and the pathophysiology leading to HT is key to come up with a direct therapeutic strategy to modulate BBB.

Avoiding excessive opening in the early stages of stroke is one of the major goals in experimental stroke treatment. Protection of the BBB in the early phases of a stroke may be useful in extending the recanalization therapy time window; hence, up-regulating of existing protective mechanisms is a possible way to achieve this. For example, a preclinical trial studying CD151, a member of the transmembrane four superfamilies that plays a key role in maintaining vascular stability, showed that its upregulation in the early phases of stroke is able to preserve BBB integrity (239), turning it into a possible therapeutic target.

Other promising targets in BBB protection include the reduction of ROS and the inhibition of MMPs in the acute phases (240). As said, the increased ROS that is produced by ischemia–reperfusion can disrupt the NVU and thus predispose the BBB to HT (240); thus, targeting ROS with free radical spin-trap agents seems a promising field in stroke treatment. The spin-trap agent NXY-059 has shown to reduce stroke impairment (241) and to confer protection toward tPA-induced hemorrhage (242) in animal models of stroke. Nonetheless, it turned out ineffective in treating clinical AIS in the first hours of stroke (243, 244). Another spin-trap agent that has recently shown clinical efficacy is edarvone. One retrospective observational study conducted in Japan has suggested that the early administration of this compound was associated with better functional outcomes and reduce HT after AIS (245), although there is data suggesting that the use of this compound may be related to increased HT (246); thus, more study is needed on this matter. Preserving mitochondrial function after stroke is another approach to inhibit ROS production and reduce ischemia/reperfusion damage. A therapeutic peptide called bendavia has recently appeared in animal models as a potential neuroprotective agent acting in the mitochondrial pathway (247). Another promising strategy in this field is the use of stanniocalcin-1 (STC-1). STC-1 is a glycoprotein-secreted hormone with antioxidant effects that may reduce the formation of ROS (248) and exert neuroprotective effects against cerebral ischemic injury (249). Recently, it has been shown in an animal model of stroke that STC-1 can exert its antioxidant activity by inhibiting brain edema and BBB permeability and consequently improving the neurological alterations following cerebral ischemia (250). It may represent a potential new strategy to target stroke pathophysiology.

Similarly, early administration of MMP inhibitors has shown to reduce BBB permeability and the rate of HT (66). Since MMPs are the primary mediators of BBB disruption and hence HT, its inhibition at the early stages of stroke is of great relevance. Nonetheless, delayed MMP inhibition was shown to increase brain injury and worsen outcomes (109). Most efforts are being put into MMP-9 inhibition as it is the key mediator in ECM degradation, BBB disruption, and HT development. Minocycline, a broad-spectrum antibiotic with anti-inflammatory and antiapoptotic effects (251), is a potential neuroprotective therapeutic compound that may limit BBB dysfunction, preventing TJP disruption through the reduction of microglia's pro-inflammatory cytokines (104) and the inhibition of MMP-9 action. It has been shown to reduce HT in rat stroke models (252). The translation to the bedside of this therapeutic compound has shown to be safe in humans and effective in lowering MMP-9 levels in treated patients (253, 254), with some neuroprotective effects (255), but it does not yield firm evidence on HT prevention.

Developing novel stroke therapies is essential as most patients are ineligible for reperfusion therapy. The brain represents

TABLE 2 | Main events driving blood–brain barrier (BBB) dynamics and hemorrhagic transformation after stroke.

Stage	Event	BBB status	Response
Hyperacute	Na^+ accumulation	Intact	Cytotoxic edema
Hyperacute	Ca^+ accumulation	Initial disruption	Glutamate excitotoxicity and mitochondria dysfunction
Hyperacute	MMPs action	Disrupted	Vasogenic edema
Hyperacute	Reperfusion treatments	Disrupted	Blood flow restoration
Hyperacute/acute	Mechanical BBB damage due to reperfusion treatments	Disrupted	Hemorrhagic transformation
Hyperacute/acute	rTPA activation of proteases	Disrupted	Hemorrhagic transformation
Acute	Neuroinflammatory response	Disrupted	Permeability increase and immune cell infiltration
Acute	MMP-9 degradation	Disrupted	Hemorrhagic transformation
Acute	Start of vascular remodeling	Disrupted	Hemorrhagic transformation
Subacute	Anti-inflammatory response	Start of the recovery	Stabilization of permeability
Subacute	Angiogenesis	Start of the recovery	Physiologic BBB increase and cerebral blood flow restoration
Chronic	Overexpression of tight junction proteins	Recovered	Sealing of the BBB
Chronic	Migration of neuronal progenitor cells	Recovered	Neural repair and neuroplasticity

a therapeutic "sanctuary," restricting the vast majority of therapeutic compounds from accessing it (256). Therefore, the selective regional BBB permeability after stroke may be used for therapeutic delivery. Nowadays, crossing the BBB remains one of the most challenging tasks for brain disease treatments and is a hot topic in the study of neurological disorders. Current strategies focus not only on targeting the BBB endothelium but also on the effective transport of specific compounds across it and the subsequent targeted drug release at appropriate targets in the brain (32).

Strategies under study to cross the BBB include viral vectors (257) as gene therapy delivery, enhancement of brain permeability (258), delivery through the pathologic permeable BBB, intranasal drug administration to bypass the BBB (259), nanoparticles (NPs) to actively target the brain, or delivery through active BBB transporters (39). These last two strategies are tightly related since transporter-mediated endocytosis of NPs through the BBB is one of the most promising pathways for drug delivery through the BBB (39). In fact, NP-mediated drug delivery is emerging as an effective and non-invasive system to treat brain diseases. Nanotechnology-based BBB crossing has been successfully demonstrated in preclinical animal studies of different neurological disorders such as Alzheimer's disease (260), brain cancer (261), and even stroke (262).

Along with NPs, using the BBB permeability as a gate to the brain is a promising tool for the delivery of targeted therapeutics in diseases related to damaged BBB, such as stroke. To reach that, further research is required to establish treatments directed to the maintenance and modulation of the BBB integrity through the inhibition/enhancement of the underlying biochemical processes.

Another important target in stroke therapy is neoangiogenesis occurring in the subacute stage. New vessels forming due to angiogenesis make the BBB leakier, and although such remodeling is necessary for brain recovery as already commented, as the vessel becomes leakier, they can be prone to HT due to this vascular unsteadiness (240). In fact, a study performed in an animal model of stroke showed that the treatment with anti-VEGF reduced the incidence of HT along with attenuated degradation of BBB components and MMP-9 activation even in the presence of tPA treatment (263), suggesting that the modulation of neoangiogenic factors could be an interesting strategy to avoid or lessen HT.

Although still in pre-clinical research, a very promising approach for stroke treatment is gene therapy through the use of miRNAs due to their ability to regulate large sets of genes related to different pathways of the ischemic stroke cascade (55). Several recent studies have shown the possibilities in this field. Bernstein et al. demonstrated that, in an animal model of stroke, the early administration of the let-7g* miRNA was capable of preserving neural tissue, diminishing BBBP, and ameliorating the neuroinflammatory immune response (264). Another recent study showed that the overexpression of the miRNAs miR-126-3p and−5p was able to reduce the expression of proinflammatory cytokines and adhesion molecules and hence attenuate the BBB disruption in the acute stage of AIS (265). Sun et al. demonstrated that the selective deletion of the miRNAs miR-15a/16-1 decreased BBBP, macrophage infiltration, and brain water content, suggesting that the pharmacological inhibition of this miRNA cluster could be a promising target in genic stroke therapy (266).

Modulation of the BBB and/or targeted delivery strategies, either taking advantage of this modulation or using the physiological transport pathways to deliver therapeutic compounds into specific parts of the brain, are under extensive study in a pre-clinical setting. Unfortunately, the majority of promising therapies in animals have failed to successfully translate into clinical therapies for HT (66), and we are still lacking applicable translational therapeutic options to reach the CNS.

What may be more within reach is the evaluation of BBB permeability through perfusion studies in AIS patients as a way of avoiding HT as well as including those that would otherwise be ineligible to reperfusion therapies, leaving aside

strict and rigid criteria such as time and moving toward a more personalized medicine.

AUTHOR CONTRIBUTIONS

JS-F was responsible for the conception of the general idea of this work. SB-C and JS contributed to the refinement and modulation of the manuscript structure, conducted the literature review, and wrote the manuscript. JS-F critically revised the work for intellectual content. The rest of the co-authors critically revised and corrected the final version of the manuscript.

FUNDING

This work was framed within the NANOSTEM project. This project has received funding from the European Union's Horizon 2020 Research and Innovation Program under grant agreement number 764958.

REFERENCES

1. Pandit R, Chen L, Götz J. The blood-brain barrier: physiology and strategies for drug delivery. *Adv Drug Deliv Rev*. (2019). doi: 10.1016/j.addr.2019.11.009. [Epub ahead of print].
2. Xie J, Shen Z, Anraku Y, Kataoka K, Chen X. Nanomaterial-based blood-brain-barrier (BBB) crossing strategies. *Biomaterials*. (2019) 224:119491. doi: 10.1016/j.biomaterials.2019.119491
3. Jiang X, Andjelkovic A V., Zhu L, Yang T, Bennett MVL, Chen J, et al. Blood-brain barrier dysfunction and recovery after ischemic stroke. *Prog Neurobiol*. (2018) 163–164:144–71. doi: 10.1016/j.pneurobio.2017.10.001
4. Serlin Y, Shelef I, Knyazer B, Friedman A. Anatomy and physiology of the blood-brain barrier. *Semin Cell Dev Biol*. (2015) 38:2–6. doi: 10.1016/j.semcdb.2015.01.002
5. Haley MJ, Lawrence CB. The blood-brain barrier after stroke: structural studies and the role of transcytotic vesicles. *J Cereb Blood Flow Metab*. (2017) 37:456–70. doi: 10.1177/0271678X16629976
6. Zhang W, Zhu L, An C, Wang R, Yang L, Yu W, et al. The blood brain barrier in cerebral ischemic injury – disruption and repair. *Brain Hemorrhages*. (2020) 1:34–53. doi: 10.1016/j.hest.2019.12.004
7. Yang C, Hawkins KE, Doré S, Candelario-Jalil E. Neuroinflammatory mechanisms of blood-brain barrier damage in ischemic stroke. *Am J Physiol Cell Physiol*. (2019) 316:C135–53. doi: 10.1152/ajpcell.00136.2018
8. Sweeney MD, Zhao Z, Montagne A, Nelson AR, Zlokovic B V. Blood-brain barrier: from physiology to disease and back. *Physiol Rev*. (2019) 99:21–78. doi: 10.1152/physrev.00050.2017
9. Daneman R, Prat A. The blood–brain barrier. *Cold Spring Harb Perspect Biol*. (2015) 7:a020412. doi: 10.1101/cshperspect.a020412
10. Blanchette M, Daneman R. Formation and maintenance of the BBB. *Mech Dev*. (2015) 138:8–16. doi: 10.1016/j.mod.2015.07.007
11. Abdullahi W, Tripathi D, Ronaldson PT. Blood-brain barrier dysfunction in ischemic stroke: targeting tight junctions and transporters for vascular protection. *Am J Physiol Cell Physiol*. (2018) 315:C343–56. doi: 10.1152/ajpcell.00095.2018
12. Stamatovic SM, Johnson AM, Keep RF, Andjelkovic AV. Junctional proteins of the blood-brain barrier: new insights into function and dysfunction. *Tissue Barriers*. (2016) 4:e1154641. doi: 10.1080/21688370.2016.1154641
13. Rusu AD, Georgiou M. The multifarious regulation of the apical junctional complex. *Open Biol*. (2020) 10:190278. doi: 10.1098/rsob.190278
14. Abbott NJ, Patabendige AAK, Dolman DEM, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. *Neurobiol Dis*. (2010) 37:13–25. doi: 10.1016/j.nbd.2009.07.030
15. Tietz S, Engelhardt B. Brain barriers : crosstalk between complex tight junctions and adherens junctions. *J Cell Biol*. (2015) 209:493–506. doi: 10.1083/jcb.201412147
16. Armulik A, Genové G, Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev Cell*. (2011) 21:193–215. doi: 10.1016/j.devcel.2011.07.001
17. Hall CN, Reynell C, Gesslein B, Hamilton NB, Mishra A, Sutherland BA, et al. Capillary pericytes regulate cerebral blood flow in health and disease. *Nature*. (2014) 508:55–60. doi: 10.1038/nature13165
18. Ferland-McCollough D, Slater S, Richard J, Reni C, Mangialardi G. Pericytes, an overlooked player in vascular pathobiology. *Pharmacol Ther*. (2017) 171:30–42. doi: 10.1016/j.pharmthera.2016.11.008
19. Liu CY, Yang Y, Ju WN, Wang X, Zhang HL. Emerging roles of astrocytes in neuro-vascular unit and the tripartite synapse with emphasis on reactive gliosis in the context of alzheimer's disease. *Front Cell Neurosci*. (2018) 12:193. doi: 10.3389/fncel.2018.00193
20. Iadecola C. The neurovascular unit coming of age: a journey through neurovascular coupling in health and disease. *Neuron*. (2017) 96:17–42. doi: 10.1016/j.neuron.2017.07.030
21. Abbott NJ, Rönnbäck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci*. (2006) 7:41–53. doi: 10.1038/nrn1824
22. Howarth C. The contribution of astrocytes to the regulation of cerebral blood flow. *Front Neurosci*. (2014) 8:103. doi: 10.3389/fnins.2014.00103
23. Brambilla R. The contribution of astrocytes to the neuroinflammatory response in multiple sclerosis and experimental autoimmune encephalomyelitis. *Acta Neuropathol*. (2019) 135:757–83. doi: 10.1007/s00401-019-01980-7
24. Baeten KM, Akassoglou K. Extracellular matrix and matrix receptors in blood-brain barrier formation and stroke. *Dev Neurobiol*. (2011) 71:1018–39. doi: 10.1002/dneu.20954
25. Hammond TR, Robinton D, Stevens B. Microglia and the brain: complementary partners in development and disease. *Annu Rev Cell Dev Biol*. (2018) 34:523–44. doi: 10.1146/annurev-cellbio-100616-060509
26. Thurgur H, Pinteaux E. Microglia in the neurovascular unit: blood–brain barrier–microglia interactions after central nervous system disorders. *Neuroscience*. (2019) 405:55–67. doi: 10.1016/j.neuroscience.2018.06.046
27. Kabba JA, Xu Y, Christian H, Ruan W, Chenai K, Xiang Y, et al. Microglia: housekeeper of the central nervous system. *Cell Mol Neurobiol*. (2018) 38:53–71. doi: 10.1007/s10571-017-0504-2
28. El Ali A, Rivest S. Microglia ontology and signaling. *Front Cell Dev Biol*. (2016) 4:72. doi: 10.3389/fcell.2016.00072
29. Nayak D, Roth T, McGavern D. Microglia development and function. *Annu Rev Immunol*. (2014) 32:367–402. doi: 10.1146/annurev-immunol-032713-120240.Microglia
30. Lalatsa A, Butt AM. Physiology of the blood-Brain barrier and mechanisms of transport across the BBB. *Nanotechnology*. (2018) 2018:49–74. doi: 10.1016/B978-0-12-812218-1.00003-8
31. Bazzoni G. Endothelial tightjunctions: permeable barriers of the vesselwall. *Thromb Haemost*. (2006) 95:36–42. doi: 10.1160/TH05
32. Georgieva JV, Hoekstra D, Zuhorn IS. Smuggling drugs into the brain: an overview of ligands targeting transcytosis for drug delivery across the blood–brain barrier. *Pharmaceutics*. (2014) 6:557–83. doi: 10.3390/pharmaceutics6040557
33. Laksitorini M, Prasasty VD, Kiptoo PK, Siahaan TJ. Pathways and progress in improving drug delivery through the intestinal mucosa and blood-brain barriers. *Ther Deliv*. (2014) 5:1143–63. doi: 10.4155/tde.14.67
34. Tajes M, Ramos-Fernández E, Weng-Jiang X, Bosch-Morató M, Guivernau B, Eraso-Pichot A, et al. The blood-brain barrier: structure, function and therapeutic approaches to cross it. *Mol Membr Biol*. (2014) 31:152–67. doi: 10.3109/09687688.2014.937468
35. Sarvari S, Moakedi F, Hone E, Simpkins JW, Ren X. Mechanisms in blood-brain barrier opening and metabolism-challenged cerebrovascular ischemia with emphasis on ischemic stroke. *Metab Brain Dis*. (2020) 35:851–68. doi: 10.1007/s11011-020-00573-8
36. Moura RP, Martins C, Pinto S, Sousa F, Sarmento B. Blood-brain barrier receptors and transporters: an insight on their function and how to exploit

them through nanotechnology. *Expert Opin Drug Deliv.* (2019) 16:271–85. doi: 10.1080/17425247.2019.1583205
37. Barar J, Rafi MA, Pourseif MM, Omidi Y. Blood-brain barrier transport machineries and targeted therapy of brain diseases. *BioImpacts.* (2016) 6:225–48. doi: 10.15171/bi.2016.30
38. Orthmann A, Fichtner I, Zeisig R. Improving the transport of chemotherapeutic drugs across the blood-brain barrier. *Expert Rev Clin Pharmacol.* (2011) 4:477–90. doi: 10.1586/ecp.11.26
39. Lombardo SM, Schneider M, Türeli AE, Türeli NG. Key for crossing the BBB with nanoparticles : the rational design. *Beilstein J Nanotechnol.* (2020) 11:866–83. doi: 10.3762/bjnano.11.72
40. GBD. (2016). Stroke Collaborators. Global, regional, and national burden of stroke, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* (2019) 18:439–58. doi: 10.1016/S1474-4422(19)30034-1
41. Rajsic S, Gothe H, Borba HH, Sroczynski G, Vujicic J, Toell T, et al. Economic burden of stroke: a systematic review on post-stroke care. *Eur J Heal Econ.* (2019) 20:107–34. doi: 10.1007/s10198-018-0984-0
42. Hoffmann A, Zhu G, Wintermark M. Advanced neuroimaging in stroke patients: prediction of tissue fate and hemorrhagic transformation. *Expert Rev Cardiovasc Ther.* (2012) 10:515–24. doi: 10.1586/erc.12.30
43. Matta R, Gonzalez AL. Stroke repair via biomimicry of the subventricular zone. *Front Mater.* (2018) 5:15. doi: 10.3389/fmats.2018.00015
44. Allen CLÃ, Bayraktutan U. Topical reviews oxidative stress and its role in the pathogenesis of ischaemic stroke. *World Stroke Organ Int J Stroke.* (2009) 4:461–70. doi: 10.1111/j.1747-4949.2009.00387.x
45. Saenger AK, Christenson RH. Stroke biomarkers : progress and challenges for diagnosis, prognosis, differentiation, and treatment. *Clin Chemestry.* (2010) 56:21–33. doi: 10.1373/clinchem.2009.133801
46. Pillai DR, Pharm M, Dittmar MS, Baldaranov D, Robin M, Henning EC, et al. Cerebral ischemia-reperfusion injury in rats – a 3 T MRI study on biphasic blood-brain barrier opening and the dynamics of edema formation. *J Cereb Blood Flow Metab.* (2009) 29:1846–55. doi: 10.1038/jcbfm.2009.106.CEREBRAL
47. Sandoval KE, Witt KA. Blood-brain barrier tight junction permeability and ischemic stroke. *Neurobiol Dis.* (2008) 32:200–19. doi: 10.1016/j.nbd.2008.08.005
48. Kassner A, Merali Z. Assessment of blood–brain barrier disruption in stroke. *Stroke.* (2015) 46:3310–5. doi: 10.1161/STROKEAHA.115.008861
49. Avsenik J, Bisdas S, Popovic KS. Blood-brain barrier permeability imaging using perfusion computed tomography. *Radiol Oncol.* (2015) 49:107–14. doi: 10.2478/raon-2014-0029
50. Krueger M, Bechmann I, Immig K, Reichenbach A, Härtig W, Michalski D. Blood-brain barrier breakdown involves four distinct stages of vascular damage in various models of experimental focal cerebral ischemia. *J Cereb Blood Flow Metab.* (2015) 35:292–303. doi: 10.1038/jcbfm.2014.199
51. Balkaya M, Kim I, Shakil F, Cho S. CD36 deficiency reduces chronic BBB dysfunction and scar formation and improves activity, hedonic and memory deficits in ischemic stroke. *J Cereb Blood Flow Metab.* (2020). doi: 10.1177/0271678x20924099
52. Wong AD, Ye M, Levy AF, Rothstein JD, Bergles DE, Searson PC. The blood-brain barrier: an engineering perspective. *Front Neuroeng.* (2013) 6:7. doi: 10.3389/fneng.2013.00007
53. Yang Y, Rosenberg GA. Blood-brain barrier breakdown in acute and chronic cerebrovascular disease. *Stroke.* (2011) 42:3323–8. doi: 10.1161/STROKEAHA.110.608257
54. Carmichael ST. The 3 Rs of stroke biology: radial, relayed, and regenerative. *Neurotherapeutics.* (2016) 13:348–59. doi: 10.1007/s13311-015-0408-0
55. Heydari E, Alishahi M, Ghaedrahmati F, Winlow W, Khoshnam SE, Anbiyaiee A. The role of non-coding RNAs in neuroprotection and angiogenesis following ischemic stroke. *Metab Brain Dis.* (2020) 35:31–43. doi: 10.1007/s11011-019-00485-2
56. Lopez-ramirez MA, Reijerkerk A, Vries HE De, Romero IA. Regulation of brain endothelial barrier function by microRNAs in health and neuroinflammation. *FASEB J.* (2016) 30:2662–72. doi: 10.1096/fj.201600435RR
57. Rosenberg GA, Estrada EY, Dencoff JE. Matrix metalloproteinases and TIMPs are associated with blood-brain barrier opening after reperfusion in rat brain. *Stroke.* (1998) 29:2189–95. doi: 10.1161/01.STR.29.10.2189
58. Huang ZG, Xue D, Preston E, Karbalai H, Buchan AM. Biphasic opening of the blood-brain barrier following transient focal ischemia: effects of hypothermia. *Can J Neurol Sci.* (1999) 26:298–304. doi: 10.1017/S0317167100000421
59. Liu P, Zhang R, Liu D, Wang J, Yuan C, Zhao X, et al. Time-course investigation of blood–brain barrier permeability and tight junction protein changes in a rat model of permanent focal ischemia. *J Physiol Sci.* (2018) 68:121–7. doi: 10.1007/s12576-016-0516-6
60. Lin CY, Chang C, Cheung WM, Lin MH, Chen JJ, Hsu CY, et al. Dynamic changes in vascular permeability, cerebral blood volume, vascular density, and size after transient focal cerebral ischemia in rats: evaluation with contrast-enhanced magnetic resonance imaging. *J Cereb Blood Flow Metab.* (2008) 28:1491–501. doi: 10.1038/jcbfm.2008.42
61. Strbian D, Durukan A, Pitkonen M, Marinkovic I, Tatlisumak E, Pedrono E, et al. The blood-brain barrier is continuously open for several weeks following transient focal cerebral ischemia. *Neuroscience.* (2008) 153:175–81. doi: 10.1016/j.neuroscience.2008.02.012
62. Durukan A, Marinkovic I, Strbian D, Pitkonen M, Pedrono E, Soinne L, et al. Post-ischemic blood-brain barrier leakage in rats: one-week follow-up by MRI. *Brain Res.* (2009) 1280:158–65. doi: 10.1016/j.brainres.2009.05.025
63. Merali Z, Huang K, Mikulis D, Silver F, Kassner A. Evolution of blood-brain-barrier permeability after acute ischemic stroke. *PLoS ONE.* (2017) 12:e0171558. doi: 10.1371/journal.pone.0171558
64. Aviv RI, D'Esterre CD, Murphy BD, Hopyan JJ, Buck B, Mallia G, et al. Hemorrhagic transformation of ischemic stroke: prediction with CT perfusion. *Radiology.* (2009) 250:867–77. doi: 10.1148/radiol.2503080257
65. Kim T, Koo J, Kim S hoon, Song IU, Chung SW, Lee KS. Blood-brain barrier permeability assessed by perfusion computed tomography predicts hemorrhagic transformation in acute reperfusion therapy. *Neurol Sci.* (2018) 39:1579–84. doi: 10.1007/s10072-018-3468-1
66. Jickling GC, Liu D, Stamova B, Ander BP, Zhan X, Lu A, et al. Hemorrhagic transformation after ischemic stroke in animals and humans. *J Cereb Blood Flow Metab.* (2014) 34:185–99. doi: 10.1038/jcbfm.2013.203
67. Abo-Ramadan U, Durukan A, Pitkonen M, Marinkovic I, Tatlisumak E, Pedrono E, et al. Post-ischemic leakiness of the blood-brain barrier: a quantitative and systematic assessment by Patlak plots. *Exp Neurol.* (2009) 219:328–33. doi: 10.1016/j.expneurol.2009.06.002
68. Sargento-Freitas J, Aday S, Nunes C, Cordeiro M, Gouveia A, Silva F, et al. Endothelial progenitor cells enhance blood-brain barrier permeability in subacute stroke. *Neurology.* (2018) 90:e127–34. doi: 10.1212/WNL.0000000000004801
69. Villringer K, Sanz Cuesta B, Ostwaldt AC, Grittner U, Brunecker P, Khalil AA, et al. DCE-MRI blood–brain barrier assessment in acute ischemic stroke. *Neurology.* (2017) 88:433–40. doi: 10.1212/WNL.0000000000003566
70. Stokum JA, Gerzanich V, Simard JM. Molecular pathophysiology of cerebral edema. *J Cereb Blood Flow Metab.* (2016) 36:513–38. doi: 10.1177/0271678X15617172
71. von Kummer R, Dzialowski I. Imaging of cerebral ischemic edema and neuronal death. *Neuroradiology.* (2017) 59:545–53. doi: 10.1007/s00234-017-1847-6
72. Hossmann KA. Viability thresholds and the penumbra of focal ischemia. *Ann Neurol.* (1994) 36:557–65. doi: 10.1002/ana.410360404
73. Hoehn-Berlage M, Norris DG, Kohno K, Mies G, Leibfritz D, Hossmann KA. Evolution of regional changes in apparent diffusion coefficient during focal ischemia of rat brain: the relationship of quantitative diffusion NMR imaging to reduction in cerebral blood flow and metabolic disturbances. *J Cereb Blood Flow Metab.* (1995) 15:1002–11. doi: 10.1038/jcbfm.1995.126
74. Vazana U, Veksler R, Pell GS, Prager O, Fassler M, Chassidim Y, et al. Glutamate-mediated blood–brain barrier opening: implications for neuroprotection and drug delivery. *J Neurosci.* (2016) 36:7727–39. doi: 10.1523/JNEUROSCI.0587-16.2016
75. Iadecola C, Cho S, Feuerstein GZ, Hallenbeck J. Cerebral Ischemia and Inflammation. In: Grotta J, editor. *Stroke: Pathophysiology, Diagnosis, and Management*. London: Churchill Livingstone (2004). p. 883–93. doi: 10.1016/B0-44-306600-0/50052-3

76. Bardutzky J, Schwab S. Antiedema therapy in ischemic stroke. *Stroke.* (2007) 38:3084–94. doi: 10.1161/STROKEAHA.107.490193
77. Simard JM, Kent TA, Chen M, Tarasov KV, Gerzanich V. Brain oedema in focal ischaemia: molecular pathophysiology and theoretical implications. *Lancet Neurol.* (2007) 6:258–68. doi: 10.1016/S1474-4422(07)70055-8
78. Khatri R, McKinney AM, Swenson B, Janardhan V. Blood-brain barrier, reperfusion injury, and hemorrhagic transformation in acute ischemic stroke. *Neurology.* (2012) 79 (Suppl. 13):S52–7. doi: 10.1212/WNL.0b013e3182697e70
79. Dijkhuizen RM, Nicolay K. Magnetic resonance imaging in experimental models of brain disorders. *J Cereb Blood Flow Metab.* (2003) 23:1383–402. doi: 10.1097/01.WCB.0000100341.78607.EB
80. Giraud M, Cho TH, Nighoghossian N, Maucort-Boulch D, Deiana G, Østergaard L, et al. Early blood brain barrier changes in acute ischemic stroke: a sequential MRI study. *J Neuroimaging.* (2015) 25:959–63. doi: 10.1111/jon.12225
81. Balami JS, Chen RL, Grunwald IQ, Buchan AM. Neurological complications of acute ischaemic stroke. *Lancet Neurol.* (2011) 10:357–71. doi: 10.1016/S1474-4422(10)70313-6
82. Hacke W, Schwab S, Horn M, Spranger M, von De Georgia MKR. "Malignant" middle cerebral artery territory infarction. *Arch Neurol.* (1996) 54:309–415. doi: 10.1001/archneur.1996.00550040037012
83. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American heart association/American stroke association. *Stroke.* (2018) 49:e46–110. doi: 10.1161/STR.0000000000000158
84. Broocks G, Hanning U, Flottmann F, Schönfeld M, Faizy TD, Sporns P, et al. Clinical benefit of thrombectomy in stroke patients with low ASPECTS is mediated by oedema reduction. *Brain.* (2019) 142:1399–407. doi: 10.1093/brain/awz057
85. Arquizan C, Lapergue B, Jovin T, Costalat V. *MOSTE LASTE | In Extremis Study - MOSTE LASTE.* Available online at: https://www.inextremis-study.com/ (accessed June 20, 2020).
86. Lin L, Wang X, Yu Z. Ischemia-reperfusion injury in the brain: mechanisms and potential therapeutic strategies. *Biochem Pharmacol.* (2016) 5:213. doi: 10.4172/2167-0501.1000213
87. Alexandrov AV, Hall CE, Labiche LA, Wojner AW, Grotta JC. Ischemic stunning of the brain: early recanalization without immediate clinical improvement in acute ischemic stroke. *Stroke.* (2004) 35:449–52. doi: 10.1161/01.STR.0000113737.58014.B4
88. Maytin M, Colucci WS. Molecular and cellular mechanisms of myocardial remodeling. *J Nucl Cardiol.* (2002) 9:319–27. doi: 10.1067/mnc.2002.123207
89. Yemisci M, Gursoy-Ozdemir Y, Vural A, Can A, Topalkara K, Dalkara T. Pericyte contraction induced by oxidative-nitrative stress impairs capillary reflow despite successful opening of an occluded cerebral artery. *Nat Med.* (2009) 15:1031–7. doi: 10.1038/nm.2022
90. Dalkara T, Gursoy-Ozdemir Y, Yemisci M. Brain microvascular pericytes in health and disease. *Acta Neuropathol.* (2011) 122:1–9. doi: 10.1007/s00401-011-0847-6
91. Molina CA, Montaner J, Abilleira S, Ibarra B, Romero F, Arenillas JF, et al. Timing of spontaneous recanalization and risk of hemorrhagic transformation in acute cardioembolic stroke. *Stroke.* (2001) 32:1079–84. doi: 10.1161/01.STR.32.5.1079
92. The NINDS t-PA Stroke Study Group. Intracerebral hemorrhage After Intravenous t-PA therapy for ischemic stroke. *Stroke.* (1997) 28:2109–18. doi: 10.1161/01.str.28.11.2109
93. Zhang S, Song XY, Xia CY, Ai Q Di, Chen J, Chu SF, et al. Effects of cerebral glucose levels in infarct areas on stroke injury mediated by blood glucose changes. *RSC Adv.* (2016) 6:93815–25. doi: 10.1039/c6ra19715a
94. Zhang S, An Q, Wang T, Gao S, Zhou G. Autophagy- and MMP-2/9-mediated reduction and redistribution of ZO-1 contribute to hyperglycemia-increased blood–brain barrier permeability during early reperfusion in stroke. *Neuroscience.* (2018) 377:126–37. doi: 10.1016/j.neuroscience.2018.02.035
95. Bauer AT, Bürgers HF, Rabie T, Marti HH. Matrix metalloproteinase-9 mediates hypoxia-induced vascular leakage in the brain via tight junction rearrangement. *J Cereb Blood Flow Metab.* (2010) 30:837–48. doi: 10.1038/jcbfm.2009.248
96. Al Ahmad A, Gassmann M, Ogunshola OO. Involvement of oxidative stress in hypoxia-induced blood-brain barrier breakdown. *Microvasc Res.* (2012) 84:222–5. doi: 10.1016/j.mvr.2012.05.008
97. Kiyatkin EA, Sharma HS. Permeability of the blood-brain barrier depends on brain temperature. *Neuroscience.* (2009) 161:926–39. doi: 10.1016/j.neuroscience.2009.04.004
98. Xing C, Arai K, Lo EH, Hommel M. Pathophysiologic cascades in ischemic stroke. *Int J Stroke.* (2012) 7:378–85. doi: 10.1111/j.1747-4949.2012.00839.x
99. Slota JA, Booth SA. MicroRNAs in neuroinflammation : implications in therapeutic applications. *Non-Coding RNA.* (2019) 5:35. doi: 10.3390/ncrna5020035
100. Liu R, Pan M-X, Tang J-C, Zhang Y, Liao H-B, Zhuang Y, et al. Role of neuroinflammation in ischemic stroke. *Neuroimmunol Neuroinflammation.* (2017) 4:158. doi: 10.20517/2347-8659.2017.09
101. Kim JY, Park J, Chang JY, Kim SH, Lee JE. Inflammation after Ischemic stroke: the role of leukocytes and glial cells. *Exp Neurobiol.* (2016) 25:241–51. doi: 10.5607/en.2016.25.5.241
102. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci.* (1999) 22:391–7. doi: 10.1016/S0166-2236(99)01401-0
103. Chengrui A, Shi Y, Li P, Hu X, Gan Y, Stetler RA, et al. Molecular dialogs between the ischemic brain and the peripheral immune system: dualistic roles in injury and repair. *Prog Neurobiol.* (2013) 115:6–24. doi: 10.1016/j.pneurobio.2013.12.002
104. Ronaldson PT, Davis TP. Regulation of blood–brain barrier integrity by microglia in health and disease: a therapeutic opportunity. *J Cereb Blood Flow Metab.* (2020) 40 (Suppl. 1):S61–24. doi: 10.1177/0271678X20951995
105. Ballesteros I, Cuartero MI, Pradillo JM, Moro MA, Lizasoain I. Cytokines and chemokines in stroke. *Prim Cerebrovasc Dis Second Ed.* (2017) 2017:280–4. doi: 10.1016/B978-0-12-803058-5.00058-8
106. Kurzepa J, Kurzepa J, Golab P, Czerska S, Bielewicz J. The significance of matrix metalloproteinase (MMP)-2 and MMP-9 in the ischemic stroke. *Int J Neurosci.* (2014) 124:707–16. doi: 10.3109/00207454.2013.872102
107. Turner RJ, Sharp FR. Implications of MMP9 for blood brain barrier disruption and hemorrhagic transformation following ischemic stroke. *Front Cell Neurosci.* (2016) 10:56. doi: 10.3389/fncel.2016.00056
108. Rosell A, Foerch C, Murata Y, Lo EH. Mechanisms and markers for hemorrhagic transformation after stroke. *Acta Neurochir Suppl.* (2008) 105:173–8. doi: 10.1007/978-3-211-09469-3_34
109. Zhao BQ, Wang S, Kim HY, Storrie H, Rosen BR, Mooney DJ, et al. Role of matrix metalloproteinases in delayed cortical responses after stroke. *Nat Med.* (2006) 12:441–5. doi: 10.1038/nm1387
110. Montaner J, Alvarez-Sabín J, Molina CA, Anglés A, Abilleira S, Arenillas J, et al. Matrix metalloproteinase expression is related to hemorrhagic transformation after cardioembolic stroke. *Stroke.* (2001) 32:2762–7. doi: 10.1161/hs1201.99512
111. Block F, Peters M, Nolden-Koch M. Expression of IL-6 in the ischemic penumbra. *Neuroreport.* (2000) 11:963–7. doi: 10.1097/00001756-200004070-00013
112. García-Berrocoso T, Giralt D, Llombart V, Bustamante A, Penalba A, Flores A, et al. Chemokines after human ischemic stroke: from neurovascular unit to blood using protein arrays. *Transl Proteomics.* (2014) 3:1–9. doi: 10.1016/j.trprot.2014.03.001
113. Zaremba J, Ilkowski J, Losy J. Serial measurements of levels of the chemokines CCL2, CCL3 and CCL5 in serum of patients with acute ischaemic stroke. *Folia Neuropathol.* (2006) 44:282–9.
114. Eduardo Candelario-Jalil, Armando González-Falcón, Michel García-Cabrera OSL BLF. Postischemic treatment with the cyclooxygenase-2 inhibitor nimesulide reduces blood- brain. *J Neurochem.* (2007) 100:1097–118. doi: 10.1037/a0015864
115. Schofield ZV, Woodruff TM, Halai R, Wu MCL, Cooper MA. Neutrophils - a key component of ischemia-reperfusion injury. *Shock.* (2013) 40:463–70. doi: 10.1097/SHK.0000000000000044
116. Kim J, Song TJ, Park JH, Lee HS, Nam CM, Nam HS, et al. Different prognostic value of white blood cell subtypes in patients

with acute cerebral infarction. *Atherosclerosis.* (2012) 222:464–7. doi: 10.1016/j.atherosclerosis.2012.02.042
117. Maestrini I, Tagzirt M, Gautier S, Dupont A, Mendyk A-M, Susen S, et al. Analysis of the association of MPO and MMP-9 with stroke severity and outcome. *Neurology.* (2020) 95:e97–e108. doi: 10.1212/wnl.0000000000009179
118. Rosell A, Cuadrado E, Ortega-Aznar A, Hernández-Guillamon M, Lo EH, Montaner J. MMP-9-positive neutrophil infiltration is associated to blood-brain barrier breakdown and basal lamina type IV collagen degradation during hemorrhagic transformation after human ischemic stroke. *Stroke.* (2008) 39:1121–6. doi: 10.1161/STROKEAHA.107.500868
119. Chou SHY, Feske SK, Simmons SL, Konigsberg RGJ, Orzell SC, Marckmann A, et al. Elevated peripheral neutrophils and matrix metalloproteinase 9 as biomarkers of functional outcome following subarachnoid hemorrhage. *Transl Stroke Res.* (2011) 2:600–7. doi: 10.1007/s12975-011-0117-x
120. Li P, Gan Y, Sun B-L, Zhang F, Lu B, Gao Y, et al. Adoptive regulatory T-cell therapy protects against cerebral ischemia. *Ann Neurol.* (2012) 74:458–71. doi: 10.1002/ana.23815
121. Wang X, Lo EH. Triggers and mediators of hemorrhagic transformation in cerebral ischemia. *Mol Neurobiol.* (2003) 28:229–44. doi: 10.1385/MN:28:3:229
122. Thanvi BR, Treadwell S, Robinson T. Haemorrhagic transformation in acute ischaemic stroke following thrombolysis therapy: classification, pathogenesis and risk factors. *Postgrad Med J.* (2008) 84:361–7. doi: 10.1136/pgmj.2007.067058
123. Shi ZS, Duckwiler GR, Jahan R, Tateshima S, Szeder V, Saver JL, et al. Early blood-brain barrier disruption after mechanical thrombectomy in acute ischemic stroke. *J Neuroimaging.* (2018) 28:283–8. doi: 10.1111/jon.12504
124. Ozkul-Wermester O, Guegan-Massardier E, Triquenot A, Borden A, Perot G, Gérardin E. Increased blood-brain barrier permeability on perfusion computed tomography predicts hemorrhagic transformation in acute ischemic stroke. *Eur Neurol.* (2014) 72:45–53. doi: 10.1159/000358297
125. Li Q, Gao X, Yao Z, Feng X, He H, Xue J, et al. Permeability surface of deep middle cerebral artery territory on computed tomographic perfusion predicts hemorrhagic transformation after stroke. *Stroke.* (2017) 48:2412–8. doi: 10.1161/STROKEAHA.117.017486
126. Suh CH, Jung SC, Cho SJ, Woo DC, Oh WY, Lee JG, et al. MRI for prediction of hemorrhagic transformation in acute ischemic stroke: a systematic review and meta-analysis. *Acta Radiol.* (2019) 18:284185119887593. doi: 10.1177/0284185119887593
127. Suh CH, Jung SC, Cho SJ, Kim D, Lee JGJ Bin, Woo DC, et al. Perfusion CT for prediction of hemorrhagic transformation in acute ischemic stroke: a systematic review and meta-analysis. *Eur Radiol.* (2019) 29:4077–87. doi: 10.1007/s00330-018-5936-7
128. Latour LL, Kang DW, Ezzeddine MA, Chalela JA, Warach S. Early blood-brain barrier disruption in human focal brain ischemia. *Ann Neurol.* (2004) 56:468–77. doi: 10.1002/ana.20199
129. Neumann-Haefelin C, Brinker G, Uhlenküken U, Pillekamp F, Hossmann KA, Hoehn M. Prediction of hemorrhagic transformation after thrombolytic therapy of clot embolism: an MRI investigation in rat brain. *Stroke.* (2002) 33:1392–8. doi: 10.1161/01.STR.0000014619.59851.65
130. Pande SD, Win MM, Khine AA, Zaw EM, Manoharraj N, Lolong L, et al. Haemorrhagic transformation following ischaemic stroke: a retrospective study. *Sci Rep.* (2020) 10:5319. doi: 10.1038/s41598-020-62230-5
131. Maeshima S, Okamoto S, Okazaki H, Mizuno S, Asano N, Tsunoda T, et al. Hemorrhagic transformation in patients with cerebral infarction referred to a rehabilitation hospital. *Interv Neurol.* (2015) 4:69–74. doi: 10.1159/000439548
132. Hornig CR, Dorndorf W, Agnoli AL. Hemorrhagic cerebral infarction - a prospective study. *Stroke.* (1986) 17:179–85. doi: 10.1161/01.STR.17.2.179
133. Gidday JM, Gasche YG, Copin JC, Shah AR, Perez RS, Shapiro SD, et al. Leukocyte-derived matrix metalloproteinase-9 mediates blood-brain barrier breakdown and is proinflammatory after transient focal cerebral ischemia. *Am J Physiol Heart Circ Physiol.* (2005) 289:558–68. doi: 10.1152/ajpheart.01275.2004
134. Chang D Il, Hosomi N, Lucero J, Heo JH, Abumiya T, Mazar AP, et al. Activation systems for latent matrix metalloproteinase-2 are upregulated immediately after focal cerebral ischemia. *J Cereb Blood Flow Metab.* (2003) 23:1408–19. doi: 10.1097/01.WCB.0000091765.61714.30
135. Liu J, Jin X, Liu KJ, Liu W. Matrix metalloproteinase-2-mediated occludin degradation and caveolin-1-mediated claudin-5 redistribution contribute to blood-brain barrier damage in early ischemic stroke stage. *J Neurosci.* (2012) 32:3044–57. doi: 10.1523/JNEUROSCI.6409-11.2012
136. Navarro-Sobrino M, Rosell A, Hernández-Guillamon M, Penalba A, Boada C, Domingues-Montanari S, et al. A large screening of angiogenesis biomarkers and their association with neurological outcome after ischemic stroke. *Atherosclerosis.* (2011) 216:205–11. doi: 10.1016/j.atherosclerosis.2011.01.030
137. Maier CM, Hsieh L, Yu F, Bracci P, Chan PH. Matrix metalloproteinase-9 and myeloperoxidase expression: quantitative analysis by antigen immunohistochemistry in a model of transient focal cerebral ischemia. *Stroke.* (2004) 35:1169–74. doi: 10.1161/01.STR.0000125861.55804.f2
138. Harris AK, Ergul A, Kozak A, Machado LS, Johnson MH, Fagan SC. Effect of neutrophil depletion on gelatinase expression, edema formation and hemorrhagic transformation after focal ischemic stroke. *BMC Neurosci.* (2005) 6:49. doi: 10.1186/1471-2202-6-49
139. Prakash R, Carmichael ST. Blood-brain barrier breakdown and neovascularization processes after stroke and traumatic brain injury. *Curr Opin Neurol.* (2015) 28:556–64. doi: 10.1097/WCO.0000000000000248
140. Teng D, Pannell JS, Rennert RC, Li J, Li YS, Wong VW, et al. Endothelial trauma from mechanical thrombectomy in acute stroke: *in vitro* live-cell platform with animal validation. *Stroke.* (2015) 46:1099–106. doi: 10.1161/STROKEAHA.114.007494
141. Goyal M, Menon BK, Van Zwam WH, Dippel DWJ, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet.* (2016) 387:1723–31. doi: 10.1016/S0140-6736(16)00163-X
142. Hao Y, Zhang Z, Zhang H, Xu L, Ye Z, Dai Q, et al. Risk of intracranial hemorrhage after endovascular treatment for acute ischemic stroke: systematic review and meta-analysis. *Interv Neurol.* (2017) 6:57–64. doi: 10.1159/000454721
143. Lee Y Bin, Yoon W, Lee YY, Kim SK, Baek BH, Kim JT, et al. Predictors and impact of hemorrhagic transformations after endovascular thrombectomy in patients with acute large vessel occlusions. *J Neurointerv Surg.* (2019) 11:469–73. doi: 10.1136/neurintsurg-2018-014080
144. Nogueira RG, Gupta R, Jovin TG, Levy EI, Liebeskind DS, Zaidat OO, et al. Predictors and clinical relevance of hemorrhagic transformation after endovascular therapy for anterior circulation large vessel occlusion strokes: a multicenter retrospective analysis of 1122 patients. *J Neurointerv Surg.* (2015) 7:16–21. doi: 10.1136/neurintsurg-2013-010743
145. Boisseau W, Fahed R, Lapergue B, Desilles JP, Zuber K, Khoury N, et al. Predictors of parenchymal hematoma after mechanical thrombectomy: a multicenter study. *Stroke.* (2019) 50:2364–70. doi: 10.1161/STROKEAHA.118.024512
146. Galego O, Jesus-Ribeiro J, Baptista M, Sargento-Freitas J, Martins AI, Silva F, et al. Collateral pial circulation relates to the degree of brain edema on CT 24 hours after ischemic stroke. *Neuroradiol J.* (2018) 31:456–63. doi: 10.1177/1971400918769912
147. Chen Z, Zhang Y, Su Y, Sun Y, He Y, Chen H. Contrast extravasation is predictive of poor clinical outcomes in patients undergoing endovascular therapy for acute ischemic stroke in the anterior circulation. *J Stroke Cerebrovasc Dis.* (2020) 29:104494. doi: 10.1016/j.jstrokecerebrovasdis.2019.104494
148. Phan CM, Yoo AJ, Hirsch JA, Nogueira RG, Gupta R. Differentiation of hemorrhage from iodinated contrast in different intracranial compartments using dual-energy head CT. *Am J Neuroradiol.* (2012) 33:1088–94. doi: 10.3174/ajnr.A2909
149. Luby M, Hsia AW, Nadareishvili Z, Cullison K, Pednekar N, Adil MM, et al. Frequency of blood-brain barrier disruption post-endovascular therapy and multiple thrombectomy passes in acute ischemic stroke patients. *Stroke.* (2019) 50:2241–4. doi: 10.1161/STROKEAHA.119.025914
150. Renú A, Laredo C, Amaro S. Letter by Renú et al. Regarding Article, Frequency of blood-brain barrier disruption post-endovascular therapy and multiple thrombectomy passes in acute ischemic stroke patients. *Stroke.* (2019) 50:2241–4.

151. Yaghi S, Willey JZ, Cucchiara B, Goldstein JN, Gonzales NR, Khatri P, et al. Treatment and outcome of hemorrhagic transformation after intravenous alteplase in acute ischemic stroke a scientific statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke.* (2017) 48:e343–61. doi: 10.1161/STR.0000000000000152
152. Seidel G, Cangür H, Albers T, Burgemeister A, Meyer-Wiethe K. Sonographic evaluation of hemorrhagic transformation and arterial recanalization in acute hemispheric ischemic stroke. *Stroke.* (2009) 40:119–23. doi: 10.1161/STROKEAHA.108.516799
153. Tan S, Wang D, Liu M, Zhang S, Wu B, Liu B. Frequency and predictors of spontaneous hemorrhagic transformation in ischemic stroke and its association with prognosis. *J Neurol.* (2014) 261:905–12. doi: 10.1007/s00415-014-7297-8
154. Lu G, He Q, Shen Y, Cao F. Potential biomarkers for predicting hemorrhagic transformation of ischemic stroke. *Int J Neurosci.* (2018) 128:79–89. doi: 10.1080/00207454.2017.1349766
155. Kassner A, Mandell DM, Mikulis DJ. Measuring permeability in acute ischemic stroke. *Neuroimaging Clin N Am.* (2011) 21:315–25. doi: 10.1016/j.nic.2011.01.004
156. O'Connor JPB, Tofts PS, Miles KA, Parkes LM, Thompson G, Jackson A. Dynamic contrast-enhanced imaging techniques: CT and MRI. *Br J Radiol.* (2011) 84:S112–20. doi: 10.1259/bjr/55166688
157. Kidwell CS, Hsia AW. Imaging of the brain and cerebral vasculature in patients with suspected stroke: advantages and disadvantages of CT and MRI. *Curr Neurol Neurosci Rep.* (2006) 6:9–16. doi: 10.1007/s11910-996-0003-1
158. Jahng GH, Li KL, Ostergaard L, Calamante F. Perfusion magnetic resonance imaging: a comprehensive update on principles and techniques. *Korean J Radiol.* (2014) 15:554–77. doi: 10.3348/kjr.2014.15.5.554
159. Donahue MJ, Achten E, Cogswell PM, De Leeuw FE, Derdeyn CP, Dijkhuizen RM, et al. Consensus statement on current and emerging methods for the diagnosis and evaluation of cerebrovascular disease. *J Cereb Blood Flow Metab.* (2018) 38:1391–417. doi: 10.1177/0271678X17721830
160. Wey HY, Desai VR, Duong TQ. A review of current imaging methods used in stroke research. *Neurol Res.* (2013) 35:1092–102. doi: 10.1179/1743132813Y.0000000250
161. Li W, Pan R, Qi Z, Liu K. Current progress in searching for clinically useful biomarkers of blood–brain barrier damage following cerebral ischemia. *Brain Circ.* (2018) 4:145–52. doi: 10.4103/bc.bc_11_18
162. Miao Y, Liao JK. Potential serum biomarkers in the pathophysiological processes of stroke. *Expert Rev Neurother.* (2014) 14:173–85. doi: 10.1586/14737175.2014.875471
163. Brouns R, Wauters A, De Surgeloose D, Mariën P, De Deyn PP. Biochemical markers for blood-brain barrier dysfunction in acute ischemic stroke correlate with evolution and outcome. *Eur Neurol.* (2011) 65:23–31. doi: 10.1159/000321965
164. Hjort N, Wu O, Ashkanian M, Sølling C, Mouridsen K, Christensen S, et al. MRI detection of early blood-brain barrier disruption: parenchymal enhancement predicts focal hemorrhagic transformation after thrombolysis. *Stroke.* (2008) 39:1025–8. doi: 10.1161/STROKEAHA.107.497719
165. Warach S, Latour LL. Evidence of reperfusion injury, exacerbated by thrombolytic therapy, in human focal brain ischemia using a novel imaging marker of early blood-brain barrier disruption. *Stroke.* (2004) 35:2659–61. doi: 10.1161/01.STR.0000144051.32131.09
166. Rozanski M, Ebinger M, Schmidt WU, Hotter B, Pittl S, Heuschmann PU, et al. Hyperintense acute reperfusion marker on FLAIR is not associated with early haemorrhagic transformation in the elderly. *Eur Radiol.* (2010) 20:2990–6. doi: 10.1007/s00330-010-1881-9
167. Ostwaldt AC, Rozanski M, Schaefer T, Ebinger M, Jungehülsing GJ, Villringer K, et al. Hyperintense acute reperfusion marker is associated with higher contrast agent dosage in acute ischaemic stroke. *Eur Radiol.* (2015) 25:3161–6. doi: 10.1007/s00330-015-3749-5
168. Thrippleton MJ. MRI measurement of blood–brain barrier leakage: minding the gaps. *J Physiol.* (2019) 597:667–8. doi: 10.1113/JP277425
169. Leigh R, Jen SS, Varma DD, Hillis AE, Barker PB. Arrival time correction for dynamic susceptibility contrast MR permeability imaging in stroke patients. *PLoS ONE.* (2012) 7:e52656. doi: 10.1371/journal.pone.0052656
170. Yuan L, Yu X, Zhang M, Jiang Q, Du PY. Correction of T1 effects in calculation of relative recirculation in ischemic stroke patients. *J Med Biol Eng.* (2016) 36:740–50. doi: 10.1007/s40846-016-0167-3
171. Hom J, Dankbaar JW, Soares BP, Schneider T, Cheng SC, Bredno J, et al. Blood-brain barrier permeability assessed by perfusion ct predicts symptomatic hemorrhagic transformation and malignant edema in acute ischemic stroke. *Am J Neuroradiol.* (2011) 32:41–8. doi: 10.3174/ajnr.A2244
172. Puig J, Blasco G, Daunis-I-Estadella P, Van Eendendburg C, Carrillo-García M, Aboud C, et al. High-permeability region size on perfusion CT predicts hemorrhagic transformation after intravenous thrombolysis in stroke. *PLoS ONE.* (2017) 12:e0188238. doi: 10.1371/journal.pone.0188238
173. Horsch AD, Bennink E, Van Seeters T, Kappelle LJ, Van Der Graaf Y, Mali WPTM, et al. Computed tomography perfusion derived blood-brain barrier permeability does not yet improve prediction of hemorrhagic transformation. *Cerebrovasc Dis.* (2018) 45:26–32. doi: 10.1159/000485043
174. Okada M, Kikuchi T, Okamura T, Ikoma Y, Tsuji AB, Wakizaka H, et al. *In-vivo* imaging of bloodbrain barrier permeability using positron emission tomography with 2-amino-[3-11C] isobutyric acid. *Nucl Med Commun.* (2015) 36:1239–48. doi: 10.1097/MNM.0000000000000385
175. Venneti S, Lopresti BJ, Wiley CA. Molecular imaging of microglia/macrophages in the brain. *Glia.* (2013) 61:10–23. doi: 10.1002/glia.22357
176. Butler J, Heidari P, Blayney S, Hitomi E, Luby M, Leigh R. Blood-brain barrier integrity of stroke patients presenting in an extended time window. *BMC Neurol.* (2020) 20:54. doi: 10.1186/s12883-020-01634-2
177. Leigh R, Christensen S, Campbell BCV, Marks MP, Albers GW, Lansberg MG. Pretreatment blood-brain barrier disruption and post-endovascular intracranial hemorrhage. *Neurology.* (2016) 87:263–9. doi: 10.1212/WNL.0000000000002862
178. Bang OY, Saver JL, Alger JR, Shah SH, Buck BH, Starkman S, et al. Patterns and predictors of blood-brain barrier permeability derangements in acute ischemic stroke. *Stroke.* (2009) 40:454–61. doi: 10.1161/STROKEAHA.108.522847
179. Adebayo OD, Culpan G. Diagnostic accuracy of computed tomography perfusion in the prediction of haemorrhagic transformation and patient outcome in acute ischaemic stroke: a systematic review and meta-analysis. *Eur Stroke J.* (2020) 5:4–16. doi: 10.1177/2396987319883461
180. Castellanos M, Leira R, Serena J, Pumar JM, Lizasoain I, Castillo J, et al. Plasma metalloproteinase-9 concentration predicts hemorrhagic transformation in acute ischemic stroke. *Stroke.* (2003) 34:40–5. doi: 10.1161/01.STR.0000046764.57344.31
181. Yuan R, Tan S, Wang D, Wu S, Cao X, Zhang S, et al. Predictive value of plasma matrix metalloproteinase-9 concentrations for spontaneous haemorrhagic transformation in patients with acute ischaemic stroke: a cohort study in Chinese patients. *J Clin Neurosci.* (2018) 58:108–12. doi: 10.1016/j.jocn.2018.09.014
182. Mechtouff L, Bochaton T, Paccalet A, Crola Da Silva C, Buisson M, Amaz C, et al. Matrix metalloproteinase-9 relationship with infarct growth and hemorrhagic transformation in the era of thrombectomy. *Front Neurol.* (2020) 11:473. doi: 10.3389/fneur.2020.00473
183. Marchi N, Rasmussen P, Kapural M, Fazio V, Kight K, Mayberg MR, et al. Peripheral markers of brain damage and blood-brain barrier dysfunction. *Restor Neurol Neurosci.* (2003) 21:109–21.
184. Foerch C, Wunderlich MT, Dvorak F, Humpich M, Kahles T, Goertler M, et al. Elevated serum S100B levels indicate a higher risk of hemorrhagic transformation after thrombolytic therapy in acute stroke. *Stroke.* (2007) 38:2491–5. doi: 10.1161/STROKEAHA.106.480111
185. Kazmierski R, Michalak S, Wencel-Warot A, Nowinski WL. Serum tight-junction proteins predict hemorrhagic transformation in ischemic stroke patients. *Neurology.* (2012) 79:1677–85. doi: 10.1212/WNL.0b013e31826e9a83
186. Lv J, Hu W, Yang Z, Li T, Jiang S, Ma Z, et al. Focusing on claudin-5: a promising candidate in the regulation of BBB to treat ischemic stroke. *Prog Neurobiol.* (2018) 161:79–96. doi: 10.1016/j.pneurobio.2017.12.001
187. Hornig CR, Busse O, Buettner T, Dorndorf W, Agnoli A, Akengin Z. Ct contrast enhancement on brain scans and blood-csf barrier disturbances in cerebral ischemic infarction. *Stroke.* (1985) 16:268–73. doi: 10.1161/01.STR.16.2.268
188. Jiao X, He P, Li Y, Fan Z, Si M, Xie Q, et al. The role of circulating tight junction proteins in evaluating blood brain barrier disruption following intracranial hemorrhage. *Dis Markers.* (2015) 2015:860120. doi: 10.1155/2015/860120

189. Liu H-S, Chung H-W, Chou M-C, Liou M, Wang C-Y, Kao H-W, et al. Effects of microvascular permeability changes on contrast-enhanced T1 and pharmacokinetic MR imagings after ischemia. *Stroke.* (2013) 44:1872–7. doi: 10.1161/STROKEAHA.113.001558
190. Seto S-W, Chang D, Jenkins A, Bensoussan A, Kiat H. Angiogenesis in ischemic stroke and angiogenic effects of chinese herbal medicine. *J Clin Med.* (2016) 5:56. doi: 10.3390/jcm5060056
191. Beck H, Plate KH. Angiogenesis after cerebral ischemia. *Acta Neuropathol.* (2009) 117:481–96. doi: 10.1007/s00401-009-0483-6
192. Zhang ZG, Chopp M. Neurorestorative therapies for stroke: underlying mechanisms and translation to the clinic. *Lancet Neurol.* (2009) 8:491–500. doi: 10.1038/jid.2014.371
193. Ozaki T, Nakamura H, Kishima H. Therapeutic strategy against ischemic stroke with the concept of neurovascular unit. *Neurochem Int.* (2019) 126:246–51. doi: 10.1016/j.neuint.2019.03.022
194. Yin K-J, Hamblin M, Chen E. Angiogenesis-regulating microRNAs and ischemic stroke. *Curr Vasc Pharmacol.* (2015) 13:352–65. doi: 10.6004/jnccn.2014.0005
195. Rust R, Grönnert L, Gantner C, Enzler A, Mulders G, Weber RZ, et al. Nogo-A targeted therapy promotes vascular repair and functional recovery following stroke. *Proc Natl Acad Sci USA.* (2019) 116:14270–9. doi: 10.1073/pnas.1905309116
196. Krupinski J, Kaluza J, Kumar P, Kumar S, Wang JM. Role of angiogenesis in patients with cerebral ischemic stroke. *Stroke.* (1994) 25:1794–8. doi: 10.1161/01.STR.25.9.1794
197. Rust R. Insights into the dual role of angiogenesis following stroke. *J Cereb Blood Flow Metab.* (2020) 40:1167–71. doi: 10.1177/0271678X20906815
198. Shi Y, Shu H, Nomi A, Lei-lei Z, Zhang B, Qian H. Mesenchymal stem cell–derived extracellular vesicles: a new impetus of promoting angiogenesis in tissue regeneration. *Cytotherapy.* (2019) 21:497–508. doi: 10.1016/j.jcyt.2018.11.012
199. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. *Nature.* (2011) 473:298–307. doi: 10.1038/nature10144
200. Fournier P, Viallard C, Dejda A, Sapieha P, Larrivée B, Royal I, et al. The protein tyrosine phosphatase PTPRJ / DEP-1 contributes to the regulation of the Notch-signaling pathway and sprouting angiogenesis. *Angiogenesis.* (2020) 23:145–57. doi: 10.1007/s10456-019-09683-z
201. Zhang ZG, Zhang L, Tsang W, Soltanian-Zadeh H, Morris D, Zhang R, et al. Correlation of VEGF and angiopoietin expression with disruption of blood-brain barrier and angiogenesis after focal cerebral ischemia. *J Cereb Blood Flow Metab.* (2002) 22:379–92. doi: 10.1097/00004647-200204000-00002
202. Zhang H, Wu J, Wu J, Fan Q, Zhou J, Wu J, et al. Exosome - mediated targeted delivery of miR - 210 for angiogenic therapy after cerebral ischemia in mice. *J Nanobiotechnology.* (2019) 17:29. doi: 10.1186/s12951-019-0461-7
203. Rodrigues SF, Granger DN. Blood cells and endothelial barrier function. *Tissue Barriers.* (2015) 3:37–41. doi: 10.4161/21688370.2014.978720
204. Nag S, Kapadia A, Stewart DJ. Review: molecular pathogenesis of blood-brain barrier breakdown in acute brain injury. *Neuropathol Appl Neurobiol.* (2011) 37:3–23. doi: 10.1111/j.1365-2990.2010.01138.x
205. Lin R, Cai J, Nathan C, Wei X, Schleidt S, Rosenwasser R, et al. Neurogenesis is enhanced by stroke in multiple new stem cell niches along the ventricular system at sites of high BBB permeability. *Neurobiol Dis.* (2015) 74:229–39. doi: 10.1016/j.nbd.2014.11.016
206. Zhang RL, Chopp M, Roberts C, Liu X, Wei M, Nejad-Davarani SP, et al. Stroke increases neural stem cells and angiogenesis in the neurogenic niche of the adult mouse. *PLoS ONE.* (2014) 9:e0113972. doi: 10.1371/journal.pone.0113972
207. Sanin V, Heeß C, Kretzschmar HA, Schüller U. Recruitment of neural precursor cells from circumventricular organs of patients with cerebral ischaemia. *Neuropathol Appl Neurobiol.* (2013) 39:510–8. doi: 10.1111/j.1365-2990.2012.01301.x
208. Gotts JE, Chesselet MF. Vascular changes in the subventricular zone after distal cortical lesions. *Exp Neurol.* (2005) 194:139–50. doi: 10.1016/j.expneurol.2005.02.001
209. Schänzer A, Wachs F, Wilhelm D, Cooper-Kuhn C, Beck H. Direct stimulation of adult neural stem cells by vascular endothelial growth factor. *Brain Pathol.* (2004) 14:237–48. doi: 10.1111/j.1750-3639.2004.tb00060.x
210. Liu Z, Li Y, Cui Y, Roberts C, Lu M, Wilhelmsson U, et al. Beneficial effects of gfap/vimentin reactive astrocytes for axonal remodeling and motor behavioral recovery in mice after stroke. *Glia.* (2014) 62:2022–33. doi: 10.1002/glia.22723
211. Imitola J, Raddassi K, Park KI, Mueller FJ, Nieto M, Teng YD, et al. Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1α/CXC chemokine receptor 4 pathway. *Proc Natl Acad Sci USA.* (2004) 101:18117–22. doi: 10.1073/pnas.0408258102
212. Liauw J, Hoang S, Choi M, Eroglu C, Choi M, Sun GH, et al. Thrombospondins 1 and 2 are necessary for synaptic plasticity and functional recovery after stroke. *J Cereb Blood Flow Metab.* (2008) 28:1722–32. doi: 10.1038/jcbfm.2008.65
213. Vu Q, Xie K, Eckert M, Cramer SC. Meta-analysis of preclinical studies of mesenchymal stromal cells for ischemic stroke. *Neurology.* (2014) 82:1277–86. doi: 10.1212/WNL.0000000000000278
214. Levy ML, Crawford JR, Dib N, Verkh L, Tankovich N, Cramer SC. Phase I/II study of safety and preliminary efficacy of intravenous allogeneic mesenchymal stem cells in chronic stroke. *Stroke.* (2019) 50:2835–41. doi: 10.1161/STROKEAHA.119.026318
215. Zhao LR, Willing A. Enhancing endogenous capacity to repair a stroke-damaged brain: an evolving field for stroke research. *Prog Neurobiol.* (2018) 163–164:5–26. doi: 10.1016/j.pneurobio.2018.01.004
216. Qin C, Zhou LQ, Ma XT, Hu ZW, Yang S, Chen M, et al. Dual functions of microglia in ischemic stroke. *Neurosci Bull.* (2019) 35:921–33. doi: 10.1007/s12264-019-00388-3
217. Qin C, Fan WH, Liu Q, Shang K, Murugan M, Wu LJ, et al. Fingolimod protects against ischemic white matter damage by modulating microglia toward M2 polarization via STAT3 pathway. *Stroke.* (2017) 48:3336–46. doi: 10.1161/STROKEAHA.117.018505
218. Lou N, Takano T, Pei Y, Xavier AL, Goldman SA, Nedergaard M. Purinergic receptor P2RY12-dependent microglial closure of the injured blood-brain barrier. *Proc Natl Acad Sci USA.* (2016) 113:1074–9. doi: 10.1073/pnas.1520398113
219. Sladojevic N, Stamatovic SM, Johnson AM, Choi J, Hu A, Dithmer S, et al. Claudin-1-dependent destabilization of the blood-brain barrier in chronic stroke. *J. Neurosci.* (2019) 39:743–57. doi: 10.1523/JNEUROSCI.1432-18.2018
220. Ögren J, Irewall AL, Bergström L, Mooe T. Intracranial hemorrhage after ischemic stroke: incidence, time trends, and predictors in a Swedish Nationwide Cohort of 196 765 Patients. *Circ Cardiovasc Qual Outcomes.* (2015) 8:413–20. doi: 10.1161/CIRCOUTCOMES.114.001606
221. Geng J, Wang L, Qu M, Song Y, Lin X, Chen Y, et al. Endothelial progenitor cells transplantation attenuated blood-brain barrier damage after ischemia in diabetic mice via HIF-1α. *Stem Cell Res Ther.* (2017) 8:163. doi: 10.1186/s13287-017-0605-3
222. Zhang CE, Wong SM, Van De Haar HJ, Staals J, Jansen JFA, Jeukens CRLPN, et al. Blood-brain barrier leakage is more widespread in patients with cerebral small vessel disease. *Neurology.* (2017) 88:426–32. doi: 10.1212/WNL.0000000000003556
223. Huisa BN, Caprihan A, Thompson J, Prestopnik J, Qualls CR, Rosenberg GA. Long-term blood-brain barrier permeability changes in binswanger disease. *Stroke.* (2015) 46:2413–8. doi: 10.1161/STROKEAHA.115.009589
224. Topakian R, Barrick TR, Howe FA, Markus HS. Blood-brain barrier permeability is increased in normal-appearing white matter in patients with lacunar stroke and leucoaraiosis. *J Neurol Neurosurg Psychiatry.* (2010) 81:192–7. doi: 10.1136/jnnp.2009.172072
225. Wardlaw JM, Doubal F, Armitage P, Chappell F, Carpenter T, Muñoz Maniega S, et al. Lacunar stroke is associated with diffuse blood-brain barrier dysfunction. *Ann Neurol.* (2009) 65:194–202. doi: 10.1002/ana.21549
226. Li Y, Li M, Zuo L, Shi Q, Qin W, Yang L, et al. Compromised blood-brain barrier integrity is associated with total magnetic resonance imaging burden of cerebral small vessel disease. *Front Neurol.* (2018) 9:221. doi: 10.3389/fneur.2018.00221
227. Li Y, Li M, Zhang X, Shi Q, Yang S, Fan H, et al. Higher blood–brain barrier permeability is associated with higher white matter hyperintensities burden. *J Neurol.* (2017) 264:1474–81. doi: 10.1007/s00415-017-8550-8

228. Hainsworth AH, Fisher MJ. A dysfunctional blood-brain barrier and cerebral small vessel disease. *Neurology.* (2017) 88:420–1. doi: 10.1212/WNL.0000000000003561
229. Cuadrado-Godia E, Dwivedi P, Sharma S, Santiago AO, Gonzalez JR, Balcells M, et al. Cerebral small vessel disease: a review focusing on pathophysiology, biomarkers, and machine learning strategies. *J Stroke.* (2018) 20:302–20. doi: 10.5853/jos.2017.02922
230. Markus HS, Hunt B, Palmer K, Enzinger C, Schmidt H, Schmidt R. Markers of endothelial and hemostatic activation and progression of cerebral white matter hyperintensities: longitudinal results of the Austrian stroke prevention study. *Stroke.* (2005) 36:1410–4. doi: 10.1161/01.STR.0000169924.60783.d4
231. Van Dijk EJ, Prins ND, Vermeer SE, Vrooman HA, Hofman A, Koudstaal PJ, et al. C-reactive protein and cerebral small-vessel disease: the Rotterdam scan study. *Circulation.* (2005) 112:900–5. doi: 10.1161/CIRCULATIONAHA.104.506337
232. Knottnerus ILH, Winckers K, Ten Cate H, Hackeng TM, Lodder J, Rouhl RPW, et al. Levels of heparin-releasable TFPI are increased in first-ever lacunar stroke patients. *Neurology.* (2012) 78:493–8. doi: 10.1212/WNL.0b013e318246d6b7
233. Vermeer SE, Van Dijk EJ, Koudstaal PJ, Oudkerk M, Hofman A, Clarke R, et al. Homocysteine, silent brain infarcts, and white matter lesions: The Rotterdam scan study. *Ann Neurol.* (2002) 51:285–9. doi: 10.1002/ana.10111
234. Fernando MS, Simpson JE, Matthews F, Brayne C, Lewis CE, Barber R, et al. White matter lesions in an unselected cohort of the elderly: molecular pathology suggests origin from chronic hypoperfusion injury. *Stroke.* (2006) 37:1391–8. doi: 10.1161/01.STR.0000221308.94473.14
235. Rosenberg GA, Sullivan N, Esiri MM. White matter damage is associated with matrix metalloproteinases in vascular dementia. *Stroke.* (2001) 32:1162–7. doi: 10.1161/01.STR.32.5.1162
236. Pantoni L, Fierini F, Poggesi A. Thrombolysis in acute stroke patients with cerebral small vessel disease. *Cerebrovasc Dis.* (2014) 37:5–13. doi: 10.1159/000356796
237. Charidimou A, Pasi M, Fiorelli M, Shams S, Von Kummer R, Pantoni L, et al. Leukoaraiosis, cerebral hemorrhage, and outcome after intravenous thrombolysis for acute ischemic stroke: a meta-analysis (v1). *Stroke.* (2016) 47:2364–72. doi: 10.1161/STROKEAHA.116.014096
238. Charidimou A, Turc G, Oppenheim C, Yan S, Scheitz JF, Erdur H, et al. Microbleeds, cerebral hemorrhage, and functional outcome after stroke thrombolysis: individual patient data meta-analysis. *Stroke.* (2017) 48:2084–90. doi: 10.1161/STROKEAHA.116.012992
239. Xu W, Gao C, Wu J. CD151 alleviates early blood – brain barrier dysfunction after experimental focal brain ischemia in rats. *Cell Mol Neurobiol.* (2020). doi: 10.1007/s10571-020-00842-1. [Epub ahead of print].
240. Kanazawa M, Takahashi T, Nishizawa M, Shimohata T. Therapeutic strategies to attenuate hemorrhagic transformation after tissue plasminogen activator treatment for acute ischemic stroke. *J Atheroscler Thromb.* (2017) 24:240–53. doi: 10.5551/jat.RV16006
241. Marshall JWB, Cummings RM, Bowes LJ, Ridley RM, Green AR. Functional and histological evidence for the protective effect of NXY-059 in a primate model of stroke when given 4 hours after occlusion. *Stroke.* (2003) 34:2228–33. doi: 10.1161/01.STR.0000087790.79851.A8
242. Lapchak PA, Araujo DM, Song D, Wei J, Purdy R, Zivin JA. Effects of the spin trap agent disodium-[(tert-butylimino)methyl]benzene-1,3-disulfonate N-oxide (generic NXY-059) on intracerebral hemorrhage in a rabbit large clot embolic stroke model: combination studies with tissue plasminogen activator. *Stroke.* (2002) 33:1665–70. doi: 10.1161/01.STR.0000017145.22806.AA
243. Antonic A, Dottori M, Macleod MR, Donnan GA, Howells DW. NXY-059, a failed stroke neuroprotectant, offers no protection to stem cell-derived human neurons. *J Stroke Cerebrovasc Dis.* (2018) 27:2158–65. doi: 10.1016/j.jstrokecerebrovasdis.2018.03.015
244. Shuaib A, Lees KR, Lyden P, Grotta J, Davalos A, Davis SM, et al. NXY-059 for the treatment of acute ischemic stroke. *N Engl J Med.* (2007) 357:562–71. doi: 10.1056/NEJMoa070240
245. Enomoto M, Endo A, Yatsushige H, Fushimi K, Otomo Y. Clinical effects of early edaravone use in acute ischemic stroke patients treated by endovascular reperfusion therapy. *Stroke.* (2019) 50:652–8. doi: 10.1161/STROKEAHA.118.023815
246. Mishina M, Komaba Y, Kobayashi S, Kominami S, Fukuchi T, Mizunari T, et al. Administration of free radical scavenger edaravone associated with higher frequency of hemorrhagic transformation in patients with cardiogenic embolism. *Neurol Med Chir.* (2008) 48:292–7. doi: 10.2176/nmc.48.292
247. Imai T, Matsubara H, Nakamura S, Hara H, Shimazawa M. The mitochondria- targeted peptide, bendavia, attenuated ischemia/reperfusion-induced stroke damage. *Neuroscience.* (2020) 443:110–9. doi: 10.1016/j.neuroscience.2020.07.044
248. Liu D, Huang L, Wang Y, Wang W, Wehrens XHT, Belousova T, et al. Human stanniocalcin-1 suppresses angiotensin II-induced superoxide generation in cardiomyocytes through UCP3-mediated anti-oxidant pathway. *PLoS ONE.* (2012) 7:e036994. doi: 10.1371/journal.pone.0036994
249. Durukan Tolvanen A, Westberg JA, Serlachius M, Chang ACM, Reddel RR, Andersson LC, et al. Stanniocalcin 1 is important for poststroke functionality, but dispensable for ischemic tolerance. *Neuroscience.* (2013) 229:49–54. doi: 10.1016/j.neuroscience.2012.10.062
250. Bonfante S, Della Giustina A, Danielski LG, Denicol T, Joaquim L, Biehl E, et al. Stanniocalcin-1 ameliorates cerebral ischemia by decrease oxidative stress and blood brain barrier permeability. *Microvasc Res.* (2020) 128:103956. doi: 10.1016/j.mvr.2019.103956
251. Majid A. Neuroprotection in stroke: past, present, and future. *ISRN Neurol.* (2014) 2014:515716. doi: 10.1155/2014/515716
252. Fan X, Lo EH, Wang X. Effects of minocycline plus tissue plasminogen activator combination therapy after focal embolic stroke in type 1 diabetic rats. *Stroke.* (2013) 44:745–52. doi: 10.1161/STROKEAHA.111.000309
253. Chang JJ, Kim-Tenser M, Emanuel BA, Jones GM, Chapple K, Alikhani A, et al. Minocycline and matrix metalloproteinase inhibition in acute intracerebral hemorrhage: a pilot study. *Eur J Neurol.* (2017) 24:1384–91. doi: 10.1111/ene.13403
254. Switzer JA, Hess DC, Ergul A, Waller JL, MacHado LS, Portik-Dobos V, et al. Matrix metalloproteinase-9 in an exploratory trial of intravenous minocycline for acute ischemic stroke. *Stroke.* (2011) 42:2633–5. doi: 10.1161/STROKEAHA.111.618215
255. Fouda AY, Newsome AS, Spellicy S, Waller JL, Zhi W, Hess DC, et al. Minocycline in acute cerebral hemorrhage an early phase randomized trial. *Stroke.* (2017) 48:2885–7. doi: 10.1161/STROKEAHA.117.018658
256. Heye AK, Culling RD, Valdés Hernández MDC, Thrippleton MJ, Wardlaw JM, Valdes Hernandez MDC, et al. Assessment of blood-brain barrier disruption using dynamic contrast-enhanced MRI a systematic review. *NeuroImage Clin.* (2014) 6:262–74. doi: 10.1016/j.nicl.2014.09.002
257. Gray SJ, Woodard KT, Samulski RJ. Viral vectors and delivery strategies for CNS gene therapy. *Ther Deliv.* (2010) 1:517–34. doi: 10.4155/tde.10.50
258. Liang J, Gao C, Zhu Y, Ling C, Wang Q, Huang Y, et al. Natural brain penetration enhancer-modified albumin nanoparticles for glioma targeting delivery. *ACS Appl Mater Interfaces.* (2018) 10:30201–13. doi: 10.1021/acsami.8b11782
259. Crowe TP, Greenlee MHW, Kanthasamy AG, Hsu WH. Mechanism of intranasal drug delivery directly to the brain. *Life Sci.* (2018) 195:44–52. doi: 10.1016/j.lfs.2017.12.025
260. Carradori D, Balducci C, Re F, Brambilla D, Le Droumaguet B, Flores O, et al. Antibody-functionalized polymer nanoparticle leading to memory recovery in Alzheimer's disease-like transgenic mouse model. *Nanomedicine.* (2018) 14:609–18. doi: 10.1016/j.nano.2017.12.006
261. Hua H, Zhang X, Mu H, Meng Q, Jiang Y, Wang Y, et al. RVG29-modified docetaxel-loaded nanoparticles for brain-targeted glioma therapy. *Int J Pharm.* (2018) 543:179–89. doi: 10.1016/j.ijpharm.2018.03.028
262. Amani H, Habibey R, Shokri F, Hajmiresmail SJ, Akhavan O, Mashaghi A, et al. Selenium nanoparticles for targeted stroke therapy through modulation of inflammatory and metabolic signaling. *Sci Rep.* (2019) 9:6044. doi: 10.1038/s41598-019-42633-9
263. Kanazawa M, Igarashi H, Kawamura K, Takahashi T, Kakita A, Takahashi H, et al. Inhibition of VEGF signaling pathway attenuates hemorrhage after tPA treatment. *J Cereb Blood Flow Metab.* (2011) 31:1461–74. doi: 10.1038/jcbfm.2011.9

264. Bernstein DL, Gajghate S, Reichenbach NL, Winfield M, Persidsky Y, Heldt NA, et al. Let-7G counteracts endothelial dysfunction and ameliorating neurological functions in mouse ischemia/reperfusion stroke model. *Brain Behav Immun.* (2020) 87:543–55. doi: 10.1016/j.bbi.2020.01.026
265. Pan J, Qu M, Li Y, Wang L, Zhang L, Wang Y, et al. MicroRNA-126-3p/-5p overexpression attenuates blood-brain barrier disruption in a mouse model of middle cerebral artery occlusion. *Stroke.* (2020) 51:619–27. doi: 10.1161/STROKEAHA.119.027531
266. Ma F, Sun P, Zhang X, Hamblin MH, Yin K. Endothelium-targeted deletion of the miR-15a / 16-1 cluster ameliorates blood-brain barrier dysfunction in ischemic stroke. *Sci Signal.* (2020) 13:eaay5686. doi: 10.1126/scisignal.aay5686

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 11 December 2020
doi: 10.3389/fneur.2020.599908

Intracranial Hemorrhage After Reperfusion Therapies in Acute Ischemic Stroke Patients

Benjamin Maïer[1,2,3], *Jean Philippe Desilles*[1,2,3] *and Mikael Mazighi*[1,2,3]*

[1] Interventional Neuroradiology Department, Hôpital Fondation Adolphe de Rothschild, Paris, France, [2] Université de Paris, Paris, France, [3] Laboratory of Vascular Translational Science, INSERM U1148, Paris, France

Reperfusion therapies are the mainstay of acute ischemic stroke (AIS) treatments and overall improve functional outcome. Among the established complications of intravenous (IV) tissue-type plasminogen activator (tPA), intracranial hemorrhage (ICH) is by far the most feared and has been extensively described by seminal works over the last two decades. Indeed, IV tPA is associated with increased odds of any ICH and symptomatic ICH responsible for increased mortality rate during the first week after an AIS. Despite these results, IV tPA has been found beneficial in several pioneering randomized trials and improves functional outcome at 3 months. Endovascular therapy (EVT) combined with IV tPA for AIS patients consecutive to an anterior circulation large-vessel occlusion does not increase ICH occurrence. Of note, EVT following IV tPA leads to significantly higher rates of early reperfusion than with IV tPA alone, with no difference in ICH, which challenges the paradigm of reperfusion as a major prognostic factor for ICH complications. However, several blood biomarkers (glycemia, platelet and neutrophil count), clinical factors (age, AIS severity, blood pressure management, diabetes mellitus), and neuroradiological factors (cerebral microbleeds, infarct size) have been identified as risk factors for ICH after reperfusion therapy. In the years to come, the ultimate goal will be to further improve either reperfusion rates and functional outcome, while reducing hemorrhagic complications. To this end, various approaches being investigated are discussed in this review, such as blood-pressure control after reperfusion or the use of new antiplatelet agents as an adjunct to IV tPA and exhibit reduced hemorrhagic potential during the early phase of AIS.

Keywords: acute ischemic stroke, intracranial hemorrhage, thrombolysis, blood pressure, endovascular treatment, thrombectomy, disabilility, mortality

OPEN ACCESS

Edited by:
Bruce Campbell,
The University of Melbourne, Australia

Reviewed by:
Michael V. Mazya,
Karolinska University Hospital, Sweden
Craig S. Anderson,
University of New South Wales, Australia

***Correspondence:**
Mikael Mazighi
mmazighi@for.paris

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *28 August 2020*
Accepted: *17 November 2020*
Published: *11 December 2020*

Citation:
Maïer B, Desilles JP and Mazighi M (2020) Intracranial Hemorrhage After Reperfusion Therapies in Acute Ischemic Stroke Patients. Front. Neurol. 11:599908. doi: 10.3389/fneur.2020.599908

INTRODUCTION

To date, reperfusion therapies represent the mainstay of acute ischemic stroke (AIS) treatments (1, 2). Reperfusion can be performed pharmacologically by the use of intravenous recombinant human tissue-type plasminogen activator (IV tPA; alteplase, Boehringer Ingelheim, Germany) within the first 4.5 h after stroke onset (3), and since 2015, by endovascular therapy (EVT) in case of an anterior circulation large-vessel occlusion (LVO) (4). These different treatments have been found effective in reducing 3 month neurological disability. Indeed, in a meta-analysis of individual data from randomized controlled trials (RCTs), at 3 months, 32.9% of patients with IV tPA within 3 h after AIS onset had a modified Rankin Scale score 0–1 vs. 23.1% of the placebo group (5). Similarly,

EVT following IV tPA vs. IV tPA alone in a setting of LVO of the anterior circulation significantly reduced disability at 3 months (4).

However, these treatments are by no means entirely free of complications, with intracranial hemorrhage (ICH) the most feared. Hemorrhagic complications after reperfusion therapies include a broad spectrum of severity between small petechial hemorrhagic infarcts (HIs) to parenchymal hematomas (PHs) (6–8). ICH, especially with PH, is associated with increased morbidity and mortality (9, 10). This explains in part why ICH and especially symptomatic ICH (sICH) are mandatory safety outcomes of most AIS RCTs. Nevertheless, the ICH description remains challenging, given its several different classifications and timeframe assessment (8). sICH and asymptomatic ICH after AIS have been differentiated, but the prognostic significance of asymptomatic ICH remains largely unknown. In this review, we discuss the implications of ICH after IV tPA and EVT. After examining the different clinical, radiological, and biological baseline characteristics associated with increased ICH occurrence, we review possible modifiable factors and future therapeutic approaches.

ICH AFTER INTRAVENOUS TPA THERAPY

Background

The serine protease tPA is mainly synthesized in endothelial cells and scarcely present in blood in the physiological condition. It converts the proenzyme plasminogen into plasmin. The active form of plasmin cleaves fibrin strands into small fibrin degradation products leading to fibrinolysis. This fibrinolysis cascade is tightly regulated under physiological conditions. Especially, at a physiological concentration, tPA can only activate plasminogen into plasmin when associated with fibrin, thus forming the ternary fibrinolysis complex responsible for a highly local fibrinolysis activation (11). However, numerous studies of IV tPA therapy for acute myocardial infarction have found that with tPA in the therapeutic concentration range, plasminogen can also be converted to plasmin in contact with circulating fibrinogen owing to the incomplete fibrin specificity of tPA. Therefore, plasmin can degrade fibrinogen, thus leading to a corresponding fibrinogen consumption and the formation of fibrinogen products (12, 13). The formation of fibrinogen degradation products with anticoagulant properties, called fibrinogen degradation coagulopathy, was linked to a surplus of hemorrhagic complications in acute myocardial infarction studies (14). In a recent study of AIS patients receiving IV tPA, 20% of patients showed fibrinogen degradation coagulopathy defined as a fibrinogen level decrease $\geq$200 mg/dL at 6 h after IV tPA infusion, which was associated with increased rate of hemorrhagic complications (15).

Abbreviations: AIS, acute ischemic stroke; ASPECTS, Alberta Stroke Program Early CT Score; EVT, endovascular therapy; CT, computed tomography; BP, blood pressure; SBP, systolic blood pressure; ICH, intracranial hemorrhage; sICH, symptomatic intracranial hemorrhage; HI, hemorrhagic infarctions; PH, parenchymal hematoma; NIHSS, National Institutes of Health Stroke Scale; mTICI, modified Thrombolysis In Cerebral Ischemia; RCT, randomized controlled trial; IV tPA, intravenous recombinant tissue plasminogen activator.

The natural history of AIS by itself is associated with the occurrence of delayed ICH, usually called hemorrhagic transformation or hemorrhagic infarction. In the European Cooperative Acute Stroke Study (ECASS) 3 trial, the frequency of any ICH in the placebo group reached 17.6%, with 3.5% being sICH according to the National Institute of Neurological Disorders and Stroke (NINDS) definition (3). Notably, the ICH risk in the setting of AIS without IV tPA therapy remains low, with mainly small petechial HIs associated with baseline stroke severity (16) but with limited, if any, impact on 3 month functional outcome in the ECASS 1 and 2 trials (17–19). These HIs were statistically independent of tPA use in the ECASS 1 and 2 trials and had a paradoxical lower proportion in the tPA group as compared with the placebo group (8). Thus, this ICH could be mainly due to the natural history of AIS and not the IV tPA use (19–22). In the late 1980s and early 1990s, when IV tPA was not approved for AIS and primary management was restricted to clinical monitoring, these HIs were frequently described (43%) and usually associated with severe AIS criteria such as brain edema, mass effect, blood–brain barrier disruption, and baseline neurological severity (22). In the setting of LVO AIS, the proportion of these HIs could even reach 50%, and they were associated with early hypodensities on baseline computed tomography (CT) scans (20). The pathophysiological mechanisms underlying HI development are still not elucidated. Reperfusion has long been cited as a main causal factor for the occurrence of HIs or ICH in general, along with blood pressure or hyperglycemia (23). However, this paradigm is now clearly questioned because of the lack of increased ICH risk observed after EVT-induced reperfusion, as discussed below.

ICH and sICH Definitions

As the most severe complication of IV tPA, ICH defined in the setting of AIS is crucial because it involves key safety outcomes in RCTs and may help neurologists in the everyday management of AIS. ICH can be defined clinically (symptomatic vs. asymptomatic), radiologically (HIs vs. PHs) but also within the timeframe of assessment (early vs. late) (8). The notion of sICH in the setting of AIS was first introduced by Levy et al., requiring contemporaneous neurological worsening or a new mass effect on brain CT scan (24, 25). In the NINDS trial (parts 1 and 2), the clinical aspect of ICH was taken into account by the notion of a "temporally related neurologic deterioration," without further details (6, 8). The Prolyse in Acute Cerebral Thromboembolism (PROACT) II trial, assessing the efficacy of intra-arterial prourokinase for AIS, was the first trial to specifically define the meaning of clinical deterioration after treatment, as an increase from baseline of four points in the National Institutes of Health Stroke Scale (NIHSS) score (8, 26). Because several conditions may increase the NIHSS score in the setting of AIS (e.g., epilepsy, mass effect, ischemic lesion growth), further trials specified ICH as the presumed responsible cause of neurological deterioration ($\geq$4 points in NIHSS score). The rationale for distinguishing sICH and asymptomatic ICH is prognosis. First, and as mentioned above, asymptomatic ICH usually involves HI lesions as opposed to PH lesions, without any impact on 3 month functional outcome (19). However, this

last point must be considered with caution in light of recent studies highlighting the association between asymptomatic ICH after EVT and worse clinical outcome (27, 28). Second, such distinction provides a practical and useful tool for RCTs to assess the safety related to IV tPA use (8).

The radiological distinction was introduced in the early 1990s and first distinguished HI and PH on follow-up CT scan (7). These lesions were used in the NINDS study as "acute infarction with punctate or variable hypodensity/hyperdensity, with an indistinct border within the vascular territory" for HIs and "typical homogeneous, hyperdense lesion with a sharp border with or without edema or mass effect" for PHs (29). In the ECASS trials, these definitions were implemented and improved with the distinction between HI1 and HI2 and PH1 and PH2 (**Table 1**). Therefore, the ECASS definition includes the ICH mass effect that was not considered in the NINDS definition (<30% or ≥30% of the infarct area with or without space-occupying effect) depending on the PH type. PH1 is associated with risk of early neurological deterioration but not disability or death (19). In contrast, PH2 is strongly associated with early neurological deterioration but also with 3 month disability and death (19). In the ECASS 1 trial, PH1 was not associated with neurological deterioration, disability, or death, but PH2 was strongly associated with early neurological deterioration [odds ratio (OR) = 32.3, 95% confidence interval (CI) = 13.4–77.7] and 3 month death (OR = 18, 95% CI = 8.05–40.1) (30). As discussed below, PH2 is strongly associated with tPA use and increases with tPA dose (3-fold for 0.9 mg/kg and 3.6-fold for 1.1 mg/kg) (8). Given its tremendous clinical significance, the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST) definition used local or remote PH2 on the 22 to 36 h posttreatment CT scan as a surrogate marker for sICH (**Table 1**) (31). More recently, the ECASS 4, the Extending the Time for Thrombolysis in Emergency Neurological Deficits (EXTEND), and the Echoplanar Imaging Thrombolytic Evaluation trials also used PH2 for defining sICH, when associated with an increase of four points in NIHSS score (32). Finally, the ICH and sICH classifications may differ regarding the timeframe of evaluation. In brief, the NINDS, PROACT II, and ECASS 1 trials assessed early ICH within 36 h after stroke onset (6, 8, 17, 26). In contrast, other trials considered a delayed timeframe for ICH assessment: within 7 days (3, 33). As discussed, delayed assessment could result in poorer correlations with tPA use, notably owing to its known short half-life and also in the context of EVT (8). In this context, the Heidelberg bleeding classification was developed during the XII Thrombolysis Symposium on Thrombolysis, Thrombectomy and Ischemic Stroke treatment with the aim to propose a revised classification that specifies, in a single format, ICH topography (intraparenchymal, subdural, and subarachnoid, to take into account EVT devices complications); localization (within the infarct or remote); symptomatic or asymptomatic aspects; and type of ICH (HI1, 2; PH1, 2) to harmonize everyday practice and RCT designs (**Tables 1**, **2**) (25). In the Heidelberg Classification, PH2 is considered a distinct entity from other ICHs owing to its pronounced impact on functional outcome and death. Details are also provided in the original publication to help the clinician distinguish the types of PH (25).

In summary, several classifications exist for ICH and sICH assessment, often originating from a dedicated RCT. Depending on the aforementioned definitions, these classifications are more or less inclusive and may capture varying degrees of bleeding events after AIS treated with IV tPA. For instance, the NINDS and International Stroke Trial 3 (IST-3) definitions are considered inclusive because they assess ICH within 7 days, with all types of ICH considered. In contrast, the SITS-MOST definition is more restrictive (PH2), with specific clinical deterioration definitions and shorter timeframe (22–36 h). These discrepancies regarding ICH definitions may explain, at least in part, the variations in ICH rates seen among trials, which is discussed below.

Results From IV tPA RCTs

IV tPA Therapy Increases ICH Rates and Severity After an AIS

With now 25 years since the original publication of the NINDS trial and the consequent publication of results of several large RCTs evaluating different tPA molecules in the setting of AIS, we have a large amount of data regarding ICH rates after IV tPA therapy.

In the NINDS trial, 3% of the tPA group had asymptomatic ICH as compared with 2% in the placebo group (6). However, 6% of patients had sICH during the first 36 h in the tPA group, 50% of whom died, as compared with none in the placebo group ($p < 0.001$).

In the ECASS 3 trial, ICH rates (i.e., with the radiological definitions described above) reached 27% in the tPA group vs. 17.6% in the placebo group ($p = 0.001$) (3). sICH occurred in 2.4% vs. 0.2%, respectively, with tPA treatment leading to an increase in sICH rate as compared with the placebo group (OR = 9.85, 95% CI = 1.26–77.32, $p = 0.008$) (3). Of note, the ECASS 3 investigators also assessed sICH according to different validated definitions used in prior trials. This additional analysis is instructive because it highlights the previously discussed issue of ICH and sICH definitions. Therefore, in ECASS 3, sICH occurred in 5.3% and 2.2% of the tPA and placebo groups, respectively, according to the ECASS 2 definition (OR = 2.43, 95% CI = 1.11–5.35, $p = 0.02$), 1.9 and 0.2% according to the SITS-MOST definition (OR = 7.84, 0.98–63, $p = 0.02$), and 7.9 and 3.5% according to the NINDS definition (OR = 2.38, 1.25–4.52, $p = 0.006$) (3). All sICH cases in the ECASS 3 trial occurred within 22 to 36 h.

In the SITS-MOST study, PH1 and PH2 on the 22 to 36 h posttreatment CT scan occurred in 2.6 and 2.5% of patients, respectively (31). According to the SITS-MOST definition, sICH occurred in 1.6 and 1.7% of patients in experienced and new SITS-MOST centers, respectively (0.28% fatal within 24 h) and reached 2.2% within 7 days (31). According to the NINDS definition, 7.3% had sICH, which underlines the higher ICH rates with the NINDS definition (31).

In the IST-3 trial, tPA treatment yielded a 7-fold significantly increased number of sICH cases and an 8-fold increase in fatal sICH cases (33).

TABLE 1 | Intracranial hemorrhage (ICH) and symptomatic ICH (sICH) rates in intravenous recombinant tissue plasminogen activator (IV tPA) randomized controlled trials.

RCT	Year	Treatment	Dose	Timeframe	Safety Outcomes	Results
NINDS part 1	1995	Alteplase	0.9 mg/kg	<3 h 50% within 90 min	CT required at 24 h and 7 to 10 days or when clinically necessary sICH: if not seen on previous CT and any decline in neurologic status	**Asymptomatic ICH** tPA: $N = 5$ (3%); placebo: $N = 3$ (2%) **Symptomatic ICH during the first 36 h** tPA: $N = 8$ (6%); placebo: $N = 0$; $p < 0.001$ *Fatal:* tPA: $N = 4$; placebo: $N = 0$ *Non-fatal:* tPA: $N = 4$; placebo: $N = 0$
NINDS part 2	1995	Alteplase	0.9 mg/kg	<3 h 50% within 90 min	CT required at 24 h and 7 to 10 days or when clinically necessary sICH: if not seen on previous CT and any decline in neurologic status	**Asymptomatic ICH** tPA: $N = 5$ (3%); placebo: $N = 3$ (2%) **Symptomatic ICH during the first 36 h** tPA: $N = 8$ (6%); placebo: $N = 0$; $p < 0.001$ *Fatal:* tPA: $N = 4$; placebo: $N = 0$ *Nonfatal:* tPA: $N = 4$; placebo: $N = 0$
ECASS 1	1995	Alteplase	1.1 mg/kg	0–360 min	CT at 24 h and between day 6 and 8 HI I/II and PH I/II HI I: small petechiae along the margins of the infarct HI II: more confluent petechiae within the infarct area, without space-occupying effect PH I: blood clot <30% of the infarct area with mild space-occupying effect PH II: dense blood clot >30% of the infarct volume with significant space-occupying effect	**Deaths from ICH:** 6.3% in tPA vs. 2.4% in placebo, $p = 0.02$ (ITT analysis) **Overall rates of ICH in ITT analysis:** $N = 134$ in the tPA vs. $N = 113$ in placebo **Overall HI:** (ITT) $N = 72$ in the tPA vs. $N = 93$ in placebo, $p < 0.001$ **Overall PH:** (ITT) $N = 62$ in the tPA vs. $N = 20$ in placebo, $p < 0.001$
ECASS 2	1998	Alteplase	0.9 mg/kg	0–360 min	CT 22–36 h after treatment and at day 7 HI1, HI2, PH1, PH2 Symptomatic and asymptomatic	*Up to day 7* tPA (0–3 h) vs. placebo: HI1: 19% vs. 29% HI2: 12% vs. 16% PH1: 1% vs. 4% **PH2: 7% vs. 1%** tPA (3–6 h) vs. placebo: HI1: 19.9% vs. 23.3% HI2: 16% vs. 11.3% PH1: 4.3% vs. 1.9% **PH2: 8.3% vs. 0.6%** Total rt-PA vs. placebo: HI1: 19.6% vs. 24.3% HI2: 15.2% vs. 12.2% **PH: 11.8% vs. 3.1% (x4 for tPA)** PH1: 3.7% vs. 2.3% **PH2: 8.1% vs. 0.8%** **Frequency of sICH: 2.5-fold excess with tPA**
ATLANTIS A	2000	Alteplase	0.9 mg/kg	0–360 min	CT at 24 h and any CT within 10 days Symptomatic or asymptomatic according to the blinded investigator	Asymptomatic ICH (day 10): tPA 12.7% vs. Placebo 4.3% Symptomatic ICH/fatal ICH (day 10): tPA: 11.3% vs. 0%
ATLANTIS B	1999	Alteplase	0.9 mg/kg	0–300 min	CT at baseline, 18–30 h (sooner if deterioration) Symptomatic or asymptomatic according to the blinded investigator ICH: presence of any blood seen on a brain CT	Asymptomatic ICH: tPA 11.4% vs. placebo 4.7%, $p = 0.004$ Symptomatic ICH: tPA 7% vs. placebo 1.1%, $p < 0.001$ Fatal ICH: tPA 3% vs. placebo 0%, $p = 0.005$

(Continued)

TABLE 1 | Continued

RCT	Year	Treatment	Dose	Timeframe	Safety Outcomes	Results
SITS-MOST	2007	Alteplase	0.9 mg/kg	0–180 min	Local or remote PH2 on the 22–36 h posttreatment scan, combined with a neurological deterioration of ≥4 in NIHSS score from baseline, or from the lowest NIHSS score between baseline and 24 h, or leading to death Also, sICH according to the NINDS, ECASS, definitions ICH also assessed on additional scans if needed	At 22–36 h scans - HI1: 5.4% - HI2: 4.0% - PH1: 2.6% - PH2: 2.5% - Remote PH1: 1.7% - Remote PH2: 1.1% sICH rates according to the SITS-MOST definition: - Experienced centers: 1.6% - New centers: 1.7% sICH rates according to the NINDS definition and Cochrane reviews: - Experienced centers: 7.3% - New centers: 7.3%
ECASS 3	2008	Alteplase	0.9 mg/kg	180–270 min	CT or MRI at 22–36 h. Additional CT studies at the discretion of the investigators HI1, HI2, PH1, PH2 sICH: any apparently extravascular blood in the brain or within the cranium associated with clinical deterioration (≥4 increase in NIHSS score or death)	ICH: tPA vs. placebo 27% vs. 17.6%, $p = 0.001$ sICH rates tPA: <3 cases per 100 patients, (2.4), higher compared to placebo: OR = 9.85 (95% CI = 1.26–77.32), $p = 0.008$ *According to ECASS II* definition: OR = 2.43 (1.11–5.35), $p = 0.02$ *According to SITS-MOST:* OR = 7.84 (0.98–63), $p = 0.02$ *According to NINDS:* OR = 2.38 (1.25–4.52), $p = 0.006$ All sICH occurred within 22–36 h
EPITHET	2008	Alteplase	0.9 mg/kg	180–360 min	sICH: ICH with significant clinical deterioration of ≥4 in NIHSS score within 36 h of treatment, AND parenchymal hemorrhage of grade 2 on CT (PH2), SITS-MOST definition	Rates of sICH: tPA 7.7% vs. placebo 0% Median baseline DWI volumes did not differ between patients with without sICH
IST-3	2012	Alteplase	0.9 mg/kg	Within 360 min	sICH: "Clinically important worsening on a valid stroke scale or occurrence of a clinical syndrome suggesting recurrent stroke with ICH on CT/MR performed within 7 days Asymptomatic ICH: ICH within 7 days" Asymptomatic ICH: "presence of any ICH on CT/MR performed within 7 days without any clinical deterioration"	sICH (tPA vs. placebo): Total: OR = 6.94 (4.07–11.8), $p < 0.0001$ Non-fatal: OR = 5.56 (2.72–11.4), $p < 0.0001$ Fatal: OR = 8.12 (3.68–17.9), $p < 0.0001$
ENCHANTED	2016	Alteplase	0.9 vs. 0.6 mg/kg	Within 270 min	Deterioration in neurologic symptoms (≥4 in NIHSS score) and the rates of PH2 on MRI 22 to 36 h after treatment	sICH by SITSMOST definition: low-dose vs. standard dose 1% vs. 2.1%, OR = 0.48 (0.27–0.86), $p = 0.01$ As exploratory analysis, according to the NINDS definition: low-dose vs. standard dose 5.9% vs. 8.0%, OR = 0.73 (0.55–0.95), $p = 0.02$
Wake-Up	2018	Alteplase	0.9 mg/kg	Mismatch DWI/FLAIR	Deterioration in neurologic symptoms (≥4 in NIHSS score) and rates of PH2 on MRI 22 to 36 h after treatment	PH2: tPA vs. placebo 4% vs. 0.4%, OR = 10.46 (1.32–82.77), $p = 0.03$ sICH (SITS-MOST): tPA vs. placebo: 2% vs. 0.4%, OR = 4.95 (0.57–42.87), $p = 0.15$ sICH (ECASS 2): 2.8% vs. 1.2%, OR = 2.40 (0.60–9.53), $p = 0.21$

(Continued)

TABLE 1 | Continued

RCT	Year	Treatment	Dose	Timeframe	Safety Outcomes	Results
						sICH (ECASS III): 2.4% vs. 0.4%, OR = 6.04 (0.72–50.87), $p = 0.10$ sICH (NINDS): 8% vs. 4.9%, OR = 1.78 (0.84–3.71), $p = 0.13$
ECASS 4/ EXTEND/ EPITHET	2019	Alteplase	0.9 mg/kg	270–540 min	sICH: PH2 within 36 h after intervention with ≥4 increase in NIHSS score from baseline	sICH alteplase: 5% vs. sICH placebo: <1% OR = 9.70 (1.23–76.55), $p = 0.03$
EXTEND	2019	Alteplase	0.9 mg/kg	270–540 min	sICH: PH2 within 36 h after intervention with ≥4 increase in NIHSS score from baseline	sICH tPA vs. placebo: 6.2% vs. 0.9%, aOR = 7.22 (0.97–53.54), $p = 0.053$

95% CI, 95% confidence interval; ATLANTIS, Alteplase Thrombolysis for Acute Non-interventional Therapy in Ischemic Stroke; CT, computed tomography; DWI, diffusion-weighted imaging imaging; ECASS, European Cooperative Acute Stroke Study; EXTEND, Extending the Time for Thrombolysis in Emergency Neurological Deficits; FLAIR, fluid-attenuated inversion recovery; HI, hemorrhagic infarct; ITT, intention to treat; ICH, intracranial hemorrhage; IV tPA, intravenous recombinant tissue plasminogen activator; IST 3, International Stroke Trial; MRI, magnetic resonance imaging; NIHSS, National Institutes of Health Stroke Scale; NINDS, National Institute of Neurological Disorders and Stroke; OR, odds ratio; PH, parenchymal hematoma; RCT, randomized controlled trial; sICH, symptomatic intracranial hemorrhage; SITS-MOST, Safe Implementation of Thrombolysis in Stroke-Monitoring Study.

In 2004, a first pooled analysis of the Alteplase Thrombolysis for Acute Non-interventional Therapy in Ischemic Stroke (ATLANTIS), ECASS and NINDS trials showed a substantial increase in PH2 rates in the tPA group as compared with the placebo group (5.9% vs. 1.1%), 60% being fatal within 3 months (34). These hematomas were associated with tPA treatment and age but not baseline NIHSS score or time from stroke onset to IV tPA infusion (34). Of note, and as previously described, substantial differences existed between the trials in the definition of ICH.

In 2012, Wardlaw et al. performed an additional updated systematic review and meta-analysis of all evidence from RCTs for IV tPA in AIS (35). This study yielded 12 RCTs including more than 7,000 patients with IV tPA given within 6 h from stroke onset. Data for fatal ICHs were available from eight trials and ICHs occurred in 3.6% of the tPA group vs. 0.6% of the placebo group, with an absolute increase of 29 deaths/1,000 patients (35). Moreover, tPA use did not increase the number of deaths within 7 days from causes other than ICH, which underlines a strong association between early deaths in the tPA group and fatal ICH (35). All 12 trials assessed sICH, which occurred in 7.7% of the tPA group vs. 1.8% of the placebo group (35). This resulted in a crude estimate of the absolute excess of sICH with tPA of 58/1,000 patients treated (35). Finally, in 2014, a meta-analysis of individual patient data from nine trials (5) evaluated sICH with the ECASS 3 and SITS-MOST definitions: tPA significantly increased the likelihood of sICH. Among the 3,391 patients receiving tPA, 231 (6.8%) had a PH2 within 7 days vs. 44 (1.3%) in the control group (OR = 5.5, 95% CI = 4.01–7.70) (5). Similar results were obtained for SITS-MOST–criteria PH2 within 36 h (OR = 6.67, 95% CI = 4.11–10.84) (5). These PH2 cases were fatal within 7 days for 2.7% patients in the tPA group vs. 0.4% in the control group. Importantly, this early excess mortality caused by ICH in the tPA group did not result in increased overall mortality at 3 months and did not limit tPA effectiveness on 3 month functional outcomes (5).

In 2016, the Enhanced Control of Hypertension and Thrombolysis Stroke Study (ENCHANTED) evaluated the non-inferiority of a lower dose of IV tPA (0.6 mg/kg) compared to the standard dose (0.9 mg/kg) (36). Although ENCHANTED did not show the non-inferiority of low-dose IV tPA to standard dose with respect to death and functional outcomes at 3 months, the low-dose group had significantly fewer sICHs. With the SITS-MOST definition, the frequency of sICH was lower in the low-dose than standard-dose group (1% vs. 2.1%; OR = 0.48, 95% CI = 0.27–0.86) (36). sICH rates with the NINDS definition were also lower in the low-dose group. In addition, fatal ICH rates were lower in the low-dose than standard-dose group (1.3% vs. 2.5%) (36). These results are particularly relevant and support the impact of IV tPA dose as a risk factor for sICH. Nevertheless, given the non-significant results, current North American and European guidelines recommend a standard dose for IV tPA treatment, considered as the best benefit–risk ratio (1, 2).

The Efficacy and Safety of MRI-based Thrombolysis in wake-up Stroke trial evaluated magnetic resonance imaging (MRI)–guided thrombolysis for stroke with unknown time onset (37). sICH occurred in 2% of patients in the IV tPA group and 0.4% in the placebo group (adjusted OR = 4.95, 95% CI = 0.57–42.87), according to the SITS-MOST definition (37). These proportions remained similar according to the ECASS 2, ECASS 3 and NINDS definitions (37). Recently, the EXTEND trial assessed the effectiveness of IV tPA initiated 4.5 to 9.0 h after stroke onset in patients with a favorable perfusion-imaging profile detected by automated perfusion imaging (38). sICH occurred in 6.2% of patients in the IV tPA group and 0.9% in the placebo group (adjusted relative risk=7.22, 95% CI = 0.97–53.54) according to the SITS-MOST definition (38). A recent systematic review and meta-analysis of individual data from the EXTEND, ECASS-4 and EPITHET trials, all evaluating the effectiveness of IV tPA beyond 4.5 h based on perfusion mismatch, found a frequency of 5% sICH in the IV tPA group vs. <1% in the placebo group (OR = 9.7, 95% CI = 1.23–76.55) (32), with the SITS-MOST definition used for this study. Hence, the frequency of

TABLE 2 | ICH and sICH rates in endovascular therapy (EVT) randomized controlled trials.

RCT	Year	ICH and sICH definitions	Results EVT+IV tPA vs. IV tPA alone
MRCLEAN	2015	sICH defined as neurologic deterioration (increase of ≥4 NIHSS score) and evidence of ICH on imaging study Follow-up CT scan at 24 h and 5 days (ECASS)	- Any type of sICH: 7.7% vs. 6.4% - PH2: 6% vs. 5.2% - SAH: 0.9% vs. 0%
ESCAPE	2015	sICH defined as new ICH (ICH, SAH, IVH, or SDH), associated with clinical evidence of neurological worsening, in which the hemorrhage is judged to be the most important cause of the neurological worsening. Clinical worsening guided by the NIHSS score of a minimum of two or more points different from baseline	- sICH: 3.6% vs. 2.7% - Perforation of the middle cerebral artery: 0.6% vs. 0%
EXTEND-IA	2015	- sICH: Any intracranial, including any subarachnoid hemorrhage, associated with clinical symptoms - Symptomatic intracerebral hemorrhage: parenchymal hematoma type 2 (PH2) within 36 h after treatment combined with an increase of ≥4 in NIHSS score from baseline	- sICH: 0% vs. 6% - PH: 11% vs. 9%
REVASCAT	2015	sICH according to the SITS-MOST and ECASS 2 definitions: - PH-2 on follow-up imaging (within 36 h) and neurologic deterioration of ≥4 in NIHSS score. - Any type of intracerebral hemorrhage on posttreatment imaging with an increase of ≥4 in NIHSS score	- sICH according to the SITS-MOST definition: 1.9% vs. 1.9% - sICH according to the ECASS 2 definition: 4.9% vs. 1.9% - Asymptomatic ICH: 16.5% vs. 10.7% - SAH: 4.9% vs. 1.9% - PH2: 2.9% vs. 1.9% - Arterial perforation: 4.9% vs. 0% - Arterial dissection: 3.9% vs. 0%
SWIFTPRIME	2015	sICH assessed at 27 ± 6 h and defined as any PH1, PH2, remote ICH, SAH, IVH associated with ≥4 points worsening in NIHSS score within 24 h	- sICH at 27 hours: 0% vs. 3% - Parenchymal hematoma: 5% vs. 7% - PH1: 4% vs. 3% - PH2: 1% vs. 4% - SAH: 4% vs. 1%
THRACE	2016	sICH at 24 h: visible intracranial bleeding on CT or MRI plus an increase of ≥4 in NIHSS score Asymptomatic ICH on CT or MRI at 24 h	- sICH: 2% vs. 2% - HI1: 10% vs. 12% - HI2: 13% vs. 11% - PH1: 6% vs. 5% - PH2: 7% vs. 4% - SAH: 4% vs. 1% - IVH: 4% vs. 2% - Remote ICH: 2% vs. 3% - Perforation: 1% vs. 0%
DEFUSE 3	2018	sICH defined as an increase of ≥4 in NIHSS score that was associated with brain hemorrhage on imaging within 36 h after stroke onset	*In this trial, the control group did not receive alteplase* - sICH: 7% vs. 4% - PH2: 9% vs. 3%
DAWN	2017	sICH defined as the presence of extravascular blood in the cranium associated with an increase of ≥4 in NIHSS score or death and judged to be the predominant cause of neurologic deterioration within 24 h after randomization (ECASS 3 definition)	*In this trial, the control group did not receive alteplase* - sICH: 6% vs. 3% - Perforation: 0%

ATLANTIS, Alteplase Thrombolysis for Acute Non-interventional Therapy in Ischemic Stroke; CT, computed tomography; DWI, diffusion-weighted imaging; ECASS, European Cooperative Acute Stroke Study; EVT, endovascular therapy; FLAIR, fluid-attenuated inversion recovery; EXTEND, Extending the Time for Thrombolysis in Emergency Neurological Deficits; HI, hemorrhagic infarct; ITT, intention to treat; ICH, intracranial hemorrhage; IV tPA, intravenous recombinant tissue plasminogen activator; IVH, intraventricular hemorrhage; IST 3, International Stroke Trial; MRI, magnetic resonance imaging; NIHSS, National Institutes of Health Stroke Scale; NINDS, National Institute of Neurological Disorders and Stroke; OR, odds ratio; PH, parenchymal hematoma; RCT, randomized controlled trial; SAH, subarachnoid hemorrhage; SDH, subdural hematoma; sICH, symptomatic intracranial hemorrhage; SITS-MOST, Safe Implementation of Thrombolysis in Stroke-Monitoring Study.

sICH beyond 4.5 h in patients receiving IV tPA seems similar to that with the 0 to 4.5 h window, with a 3.4% sICH frequency according to the SITS-MOST definition in the meta-analysis of individual data published by Whiteley et al. (39). Fatal ICH occurred in 2.3% of patients receiving IV tPA beyond 4.5 h (32) and 2.6% receiving IV tPA from 0 to 4.5 h (39). Despite indirect

comparisons between the 0 to 4.5 and 4.5 to 9.0 h time windows, these data seem to show the overall safety of IV tPA beyond 4.5 h in patients selected with a favorable perfusion-imaging profile regarding sICH rates. Similarly, the safety profile regarding sICH proportion in "wake-up stroke" patients receiving IV tPA is also reassuring and should favor IV tPA implementation, if indicated.

In summary, the results from RCTs and meta-analyses including individual pooled patient data point in the same direction and demonstrate an increased risk of PH2 and sICH after tPA treatment by 6-fold [depending on the definition used (39)], responsible for excess early mortality caused by ICH. Despite indirect comparisons, these results seem similar for the 4.5 to 9.0 h time window (32, 38). Nevertheless, and despite these concerning findings, the beneficial effect of tPA on 3 month functional outcome remained significant among these studies. From a practical viewpoint, it seems crucial to evaluate the ICH risk in different subgroups of patients and to assess the impact of different suspected or known risk factors for ICH after IVT. This analysis, discussed below, could pave the way toward individualized management.

Predictive Factors of ICH Occurrence

A recent meta-analysis of individual patient data found a proportional increase in ICH risk in patients receiving tPA regardless of pre-specified subgroups such as age (≤80, >80 years old), treatment delay (≤3, 3–4.5, >4.5 h) and baseline stroke severity (NIHSS score 0–4, 5–10, 11–15, 16–21, ≥22), with non-significant interaction (39). Several previous publications attempted to outline risks factors for PHs or sICH. However, these works are difficult to interpret given the different ICH definitions used (16, 17, 40). In the ECASS 1 trial, risk factors for PHs increased with age and IV tPA treatment (17, 40). In the NINDS trial, factors associated with sICH were baseline neurological severity, the presence of early ischemic changes on pretreatment brain CT scan and IV tPA treatment (6). In the ECASS 2 trial, significant risk factors for PHs were IV tPA treatment, extent of parenchymal hypodensity on baseline CT scan, history of congestive heart failure, increasing age and a high baseline systolic blood pressure (40). Of note, two interactions were detected: IV tPA with age and aspirin, meaning that PHs after IV tPA treatment was more frequent in older patients and in patients receiving aspirin at the time of stroke (40). The evidence that aspirin could increase the sICH risk is of interest and was confirmed by the Early Administration of Aspirin In patients treated with Alteplase for Acute Ischemic Stroke (ARTIS) trial (41). In this trial, sICH occurred more often in the aspirin than control group (4.3 and 1.6%) and was responsible for the poor outcome in the aspirin vs. control group (41). This finding was also confirmed by a *post hoc* analysis showing that aspirin increased the risk of early neurologic deterioration due to sICH by a factor of 3.7 in patients receiving tPA (42). In the SITS-IST registry (SITS-ISTR), baseline anti-thrombotic treatment was the most pronounced risk factor, with the association of aspirin and clopidogrel increasing by 3-fold and aspirin alone by 2-fold the odds of sICH occurrence (43). Baseline neurological severity with NIHSS score ≥13 was independently associated with sICH (OR = 2.2, 95% CI = 1.7–3.0), as was NIHSS score 7 to 12 (OR = 1.6, 95% CI = 1.1–2.1). Other factors were baseline glucose level (≥180 mg/dL), age ≥72 years, systolic blood pressure ≥146 mmHg, weight ≥95 kg, onset-to-treatment time ≥180 min and history of hypertension (43). Baseline neurological severity was also associated with absolute excess risk of PH2, fatal ICH, and sICH according to the SITS-MOST definition in a secondary analysis of individual data from a meta-analysis (39). In this analysis, with baseline NIHSS score 0 to 4, the absolute excess risk of ICH in the tPA group over the control group was 1.5% (95% CI = 0.8–2.6) as compared with 3.7% (95% CI = 2.1–6.3) with baseline NIHSS score ≥22 (39).

As illustrated above, several risk factors for ICH after IV tPA seem to emerge across studies and deserve specific attention.

Age

Evidence regarding the impact of age as a risk factor for ICH are conflicting. Several studies have showed such associations, as in the ECASS 1 trial, in which each 10 year increase in age predicted PHs (16). In the pooled analysis of the ATLANTIS, ECASS, and NINDS trials, secondary analysis showed age is strongly associated with the occurrence of PHs ($p = 0.0002$) (34). In a meta-analysis of 55 studies, Whiteley et al. found an association between older age and significantly increased risk of sICH after IV tPA; however, this study did not include the IST-3 trial data (44). Of note, other studies seemed to show the opposite, especially at the 80 year-old threshold (45, 46). Mishra et al. found that the rate of sICH according to the SITS-MOST definition was 2.5% vs. 1.9% with age ≥80 vs. <80 years and thus not significantly higher (OR = 1.3, 95% CI = 0.96–1.8, $p = 0.07$) (47). According to the NINDS definition (any increase in NIHSS score from baseline and any parenchymal ICH), the corresponding rate of sICH was significantly higher (11% vs. 8.3%), thus highlighting the importance of the sICH definition, as mentioned previously (47). Likewise, Ford et al. found that the unadjusted sICH rate did not differ significantly between patients >80 and ≤80 years old with the SITS-MOST definition (1.8% vs. 1.7%) but was again significantly increased according to the NINDS definition (48). Multivariate analysis adjusting for several baseline prognostically important factors found no difference in sICH rates between patients >80 and ≤80 years old (OR = 0.90, 95% CI = 0.73–1.09 according to the SITS-MOST definition and OR = 0.96, 95% CI = 0.87–1.06 according to the NINDS definition) (48). In the Multicenter rt-PA Acute Stroke Survey, age (per 10 years) was associated with sICH (OR = 1.32, 95% CI = 1.03–1.69) but did not remain significant on adjustment for clinical, biological, and radiological cofounders (49). In a systematic review, Engelter et al. found a similar likelihood of sICH in patients ≥80 and <80 years old (50). Unfortunately, limited data from RCTs are available because age ≥80 years was usually considered an exclusion criterion. In the IST-3 trial, almost 50% of the randomized patients were >80 years old, including 7% who were >90 years old, thus providing important data regarding IV tPA safety in this population (33). An updated meta-analysis found no heterogeneity regarding fatal ICH ($p = 0.4$) and sICH ($p = 0.1$) in the IST-3 trial and other trials included in the study (35). Patients >80 years old seemed to benefit from IV tPA, especially up to 3 h after stroke onset (35). Altogether,

these findings highlight the importance of sICH definition (e.g., SITS-MOST vs. NINDS definition). In addition, the coexistence of numerous comorbidities in older people could explain the increased risk of sICH in some studies. Thus, age could become an indirect marker of other relevant factors associated with sICH, notably, the coexistence of a microangiopathy, such as cerebral amyloid angiopathy, highly prevalent in this population. This issue is discussed below with radiological predictive factors of ICH.

Stroke Severity

Baseline neurological severity was one of the three risk factors independently associated with sICH in the NINDS trial (five NIHSS categories, OR = 1.8, 95% CI = 1.2–2.9) (29). In the Multicenter t-PA Acute Stroke Survey, baseline NIHSS (per one-category increase) was also a risk factor for all tPA-related ICH and remained an independent predictor in each multivariate statistical model (49). These results were also confirmed in an individual-patient data meta-analysis, which found an absolute excess risk of PH2, fatal ICH, and sICH according to the SITS-MOST definition with increasing baseline stroke severity (39). Because NIHSS scores are closely related to infarct volume, these findings could also result from the association between sICH risk and baseline extensive infarct lesions (51). In addition, risk for sICH is non-linear with NIHSS score $\geq$20 and may actually decrease with NIHSS score >25 (52). This finding is likely explained by "ceiling effects" of the NIHSS, particularly for basilar artery occlusion, which may be associated with severe disturbance of consciousness and high NIHSS score while causing ischemia in a tissue volume less than that of a complete middle cerebral artery territory (52). Recently, the risk of sICH after IV tPA in posterior circulation strokes was found to be half of that of anterior circulation strokes, albeit with higher risk of death, which illustrates the limitations of the NIHSS score for stroke severity assessment (53).

Blood Pressure

Hypertension has major consequences both during the early and late phases of AIS (54). The IST found no association between baseline systolic blood pressure and sICH (55). However, in the same year, mean blood pressure (per 25 mmHg) was found independently associated with all tPA-related ICH in the multicenter rt-PA Acute Stroke Survey (49). Prebolus systolic blood pressure >185 mmHg and diastolic blood pressure >110 mmHg were independently associated with increased odds of sICH (OR = 2.59, 95% CI = 1.07–6.25) (56). These results were also confirmed within the first 24 h after IV tPA in the SITS-ISTR (57). Indeed, high systolic blood pressure 2 to 24 h after IV tPA as a categorical variable was linearly associated with sICH occurrence (57). The EPITHET investigators also found a strong association between post-tPA blood pressure and PH rates (58). The 24 h weighted average systolic blood pressure predicted PH, and for every 10 mmHg increase in blood pressure post-treatment, the odds of PHs increased by 59% (58). In the IST-3, the odds of sICH also increased by 10% with each 10 mmHg increase in baseline systolic blood pressure (59). In this study, systolic blood pressure variability (i.e., standard deviation) was associated with 3 month death but not sICH. This concept of blood pressure variability after IV tPA is critical and has been reported to have an independent association with infarct growth, early clinical course, and 3 month functional outcome (60). The SITS investigators confirmed these findings and found a strong association between blood pressure variability defined by successive variation within the first 24 h and the occurrence of sICH according to either the SITS-MOST or ECASS definitions (61).

The ENCHANTED trial also assessed intensive blood pressure–lowering (target systolic blood pressure 130–140 mmHg within 60 min of randomization) as compared with guideline-recommended blood pressure lowering (target systolic blood pressure <180 mmHg) in patients receiving IV tPA for AIS (62). In this study, the frequency of any ICH (within 7 days after randomization) was lower in the intensive blood pressure–lowering group than the guideline-recommended blood pressure group (14.8% vs. 18.7%, $p = 0.01$). However, sICH defined by the SITSMOST, NINDS, ECASS 2, ECASS 3, and IST-3 definitions did not significantly differ among the two groups, despite a trend for lower frequency in the intensive blood-pressure management group (62). Similarly, rates of PH2 and fatal ICH within 7 days were lower but not significantly in the intensive blood pressure–lowering group as compared with the guideline-recommended group (62). Overall, these findings suggest that the blood-pressure control strategy in the early management of AIS may affect the occurrence of hemorrhagic complications. However, because of statistically non-significant results, the optimal blood pressure target for preventing ICH after IV tPA is currently not defined, and further studies are needed to answer this question.

Baseline Glycemia and Diabetes Mellitus

Hyperglycemia and diabetes mellitus are known risk factors for ICH and sICH. Since the early 2000s, the Multicenter rt-PA Stroke Survey Group showed a nearly 4-fold increase in risk of sICH after IV tPA in diabetic patients (49). As well, glucose level (per 50 mg/dL increase) was strongly associated with all ICHs in this study (OR = 1.36, 95% CI = 1.11–1.67) (49). SITS investigators confirmed these findings and found a 2-fold increase in sICH risk for patients with baseline glucose level $\geq$180 mg/dL (43). In a systematic review and meta-analysis of 54 studies, diabetes was associated with increased odds of sICH in unadjusted analysis but not multivariate analysis (63). Increased admission glucose level was also associated with a 9% increase in sICH on multivariate analysis (63). Of note, pathophysiological processes triggered by hyperglycemia and responsible for ICH seem mediated by microvascular thromboinflammation exacerbation, with an increased activation of platelets and neutrophils (64).

Ethnicity

Ethnicity could also be a risk factor for sICH after reperfusion therapy as is clearly the case for acute ST segment elevation myocardial infarction (65). Numerous publications have assessed the effectiveness and safety of IV tPA in the Asian population, which presents unique aspects of AIS, such as higher frequency of intracranial atheroma and intracranial dissection (66). Of note,

the official dose of IV tPA in Japan and several countries in Eastern and Southern Asia is 0.6 mg/kg, which complicates the comparison of sICH with European or North American rates (66). However, Toyoda et al. recently compared sICH rates after IV tPA among different registries. The sICH frequency was 1.7% in the European SITS-MOST registry, 1.9% in the SITS-NEW registry (including Korea, China, India and Singapore), 3.5% in the J-MARS registry (Japan), and 1.3% in the SAMURAI r-tPA registry (Japan) (66). Overall, these results are reassuring regarding the safety of IV tPA in the Asian population. Recently, the RADIANT study assessed the impact of racial disparities in ICH occurrence after IV tPA (67). Two ethnic groups (non-Hispanics and Hispanics) and four racial groups [Asian, Black, White, and other (Native Americans, Pacific Islanders)] were identified, and sICH rates did not differ among the racial or ethnic groups (67). Using the Get With the Guidelines Stroke Program, Mehta et al. assessed in 54,334 patients the impact of race/ethnic-related differences on safety outcomes after IV tPA (68). This study found a higher rate of hemorrhagic complications after IV tPA in Black (OR = 1.14, 95% CI = 1.04–1.28) and Asian patients (OR = 1.36, 95% CI = 1.14–1.61). This increased risk was due to sICH in Asian patients but risk of other bleeding in Black patients (68). Several reasons may contribute to this result: differences in health care access regarding vascular risk factor prevention and genetic variants among these populations.

Neuroradiological Findings

This section raises the question of the imaging modality performed before IV tPA therapy in the setting of AIS. As the validated imaging modality, brain CT, is fast and provides the required information needed for the medical management in the acute phase but ultimately provides few imaging ICH predictive factors, only the early CT changes >33% of the middle cerebral artery territory before IV tPA were found a predictor of sICH and increased the odds of sICH by 6.7 (49). Among the different MRI patterns possibly associated with increased odds of sICH, several studies highlighted the prognostic value of baseline apparent diffusion coefficient and the diffusion-weighted lesion volume (69–71). Still, the presence of cerebral microbleeds (CMBs) seems to be the main radiological finding strongly associated with risk of sICH. A recent meta-analysis showed that the presence of CMBs and high CMB burden on baseline MRI were independently associated with sICH (72). In a recent individual-patient data meta-analysis, patients with CMB showed increased risk of PHs and remote PHs but not sICH (73). CMB burden was associated with sICH and PH, and >10 CMBs independently predicted poor functional outcome (73).

Blood Biomarkers

Several studies assessed the association between baseline blood cell count and sICH occurrence in AIS patients receiving IV tPA. Baseline neutrophil count and neutrophil-to-lymphocyte ratio were independently associated with increased risk of sICH (74, 75). Other studies found baseline low platelet count associated with increased sICH risk (76, 77).

Finally, two studies assessed the impact of early fibrinogen degradation coagulopathy after IV tPA therapy. Both found an independent increased risk of ICH associated with an early decrease in fibrinogen level. These last results support IV tPA–induced fibrinogen degradation, with its biological consequences on hemostasis and platelet aggregation, as one of the main factors in post-AIS ICH severity (15, 78).

ICH AFTER ENDOVASCULAR THERAPY

Since 2015, the association of EVT and IV tPA is the gold-standard treatment of AIS consecutive to LVO of the anterior circulation (4). This approach has revolutionized AIS management, leading to >80% successful reperfusion after treatment and improved 3 month functional outcomes (4). The assessment of hemorrhagic complications in the context of EVT is recent and must acknowledge certain features that were irrelevant for IV tPA alone, such as the high rates of reperfusion, but also possible complications due to EVT devices and navigation in the intracranial arteries.

Results From RCT and Observational Studies

As illustrated in **Table 2**, the association of EVT and IV tPA did not increase the rate of PH2 or sICH vs. IV tPA alone. However, comparing results of trials is challenging, given the different definitions of sICH used. In 2016, a meta-analysis of individual patient data from five RCTs evaluated the proportion of patients with sICH as defined by each trial and PH2 rates within 5 days (4). Overall, sICH rates did not differ between the intervention group (EVT and IV tPA, 4.4%) and control group (IV tPA alone, 4.3%) (4). These results were also confirmed for the rate of PH2 (5.1% in the intervention group vs. 5.3% in the control group) (4). Another recent meta-analysis of patient-level data assessed the safety features of EVT from seven RCTs and confirmed no difference between sICH and PH2 rates among EVT and control groups (79). PH2 rates showed no treatment effect modification by baseline imaging features. However, EVT was associated with increased odds of sICH for patients with baseline large AIS defined as early ischemic change in >33% of the middle cerebral artery territory (adjusted OR = 4.17, 95% CI = 1.30–13.44) as compared with controls (79). Onset-to-reperfusion time seems a strong predictor of ICH in EVT-treated patients (80). Indeed, in this study, which pooled data from previous EVT trials of first-generation EVT devices, the adjusted OR for each 30 min increase in time was 1.21 (95% CI = 1.10–1.33) for ICH (according to the ECASS 2 definition) (80). These results were also confirmed in the recent meta-analysis of individual patient data from five RCTs, in which the onset-to-reperfusion time was significantly associated with PH2 occurrence (OR per 1 h delay: 1.43 (1.09–1.88) (81).

Observational studies, reflecting real-life practice, have confirmed these data. In a large observational study from the Endovascular Treatment for Ischemic Stroke (ETIS) registry, independent predictors of PHs after EVT included age (per 1 year

increase), current smoking, admission ASPECTS (per 1-point decrease), general anesthesia, angiographic poor collaterals, and embolization in a new territory (9). The Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands (MRCLEAN) investigators performed a similar study and found only increased systolic blood pressure and atrial fibrillation associated with PH occurrence (82). Of note, increased systolic blood pressure and antiplatelet use were also associated with increased odds of sICH (82). Thus, consistent with IV tPA, systolic blood pressure appears to be a predictor of sICH after EVT as well and needs to be taken into account, being an eligible modifiable risk factor (discussed below).

Recently, Nogueira et al., in a multicenter retrospective analysis of 1,122 patients receiving EVT for anterior circulation strokes, assessed the predictors and clinical relevance of hemorrhagic transformation (83). PH occurred in 8.5% and was associated with atrial fibrillation (OR = 1.61, 95% CI = 1.01–2.55), a finding already described in patients receiving IV tPA (83). The use of the MERCI thrombectomy device (a first-generation EVT device) was associated with increased rates of HIs (OR = 1.47, 95% CI = 1.02–2.12), potentially implicating the type of device used during EVT in the occurrence of sICH (83). In the Contact Aspiration vs. Stent Retriever for Successful Revascularization trial, PH2 frequency was greater in the stent retriever group than aspiration group (7.6% vs. 3.7%), but sICH rates were not statistically different (6.5 and 5.3%) (84). In addition, subarachnoid hemorrhages, a known complication of EVT, occurred in 7.1 and 6.9% of the stent retriever and aspiration groups (84). These results were confirmed by the Aspiration thrombectomy vs. stent retriever thrombectomy as first-line approach for LVO trial, with similar sICH rates according to the SITS-MOST definition (3%) (85). Altogether, these results suggest that sICH rates are not increased by EVT and that last-generation devices (i.e., stent retriever and aspiration) have similar sICH and subarachnoid hemorrhage rates.

Although most trials assessed the effectiveness of EVT for LVO (intracranial internal carotid artery, M1 segment of the middle cerebral artery), EVT for M2 occlusion is increasingly performed and raises the question of safety outcomes. In a multicenter retrospective cohort with isolated M2 occlusions, sICH rates did not differ between the EVT and medical groups (86). As compared with M1 occlusions treated with EVT, rates of sICH were lower in the M2 group, notably regarding PH2 rates (87). Overall, these data are reassuring and illustrate the safety of EVT for M2 occlusions in terms of sICH rates.

Finally, because increased time from stroke onset to reperfusion is associated with ICH (80), the "drip and ship" paradigm (i.e., patient transfer from a primary to a comprehensive stroke center for EVT) could be associated with increased risk of sICH as compared with the "mothership" paradigm (primary management in the comprehensive stroke center with EVT). However, despite longer onset-to-recanalization time in the former vs. latter group (292 vs. 240 min), a recent study found similar sICH rates among the two groups (2% vs. 3%, $p = 0.6$) (88). sICH rates were also recently evaluated between directly admitted and transferred patients with data from a national database (8,533 patients) (89): hemorrhage rates did not differ between the two groups, despite longer times from stroke onset to reperfusion (89).

The Issue of Blood Pressure in EVT-Treated Patients

Numerous studies have recently highlighted the impact of systolic blood pressure in the early phase of AIS treated by EVT (90–93). The MRCLEAN investigators have found higher systolic BP before EVT associated with increased probability of sICH (94). The odds of sICH increased 21% for every 10 mmHg increase in blood pressure (94). In the ETIS registry, baseline systolic blood pressure was also strongly associated with 3 month mortality and functional outcomes (93). sICH rates were not significantly related to baseline systolic or diastolic blood pressure (93). Systolic, pulse pressure and mean arterial BP variability during EVT seem to be critically associated with functional outcome (91, 92, 95–98), but data are scarce regarding the association of blood pressure variability during EVT and sICH risk. After EVT, and notably after complete reperfusion, blood pressure variability seems associated with increased rates of sICH (99, 100). Recently, Matusevicius et al. assessed the association between blood pressure after EVT and sICH based on the recanalization status in a large international registry (101). Increased systolic blood pressure was associated with sICH in patients with unsuccessful recanalization, and mean 24 h systolic blood pressure >160 mmHg was associated with sICH, regardless of the recanalization status (101). These studies are limited by the heterogeneity in definition of sICH, as discussed above, and further research is needed to fully understand the mechanisms underlying these associations.

FUTURE DIRECTIONS: HOW TO DECREASE HEMORRHAGIC COMPLICATIONS AFTER REPERFUSION THERAPY

Clinical Studies to Come

In the hemodynamic field, the upcoming results of the BP TARGET trial (NCT03160677) are much awaited (102). This RCT with blinded endpoint evaluated the impact on ICH rates of an intensive blood pressure–lowering strategy (systolic blood pressure <130 mmHg) for 24 h after successful reperfusion in AIS patients receiving EVT (102). With ICH rates as the primary outcome, the BP TARGET trial should provide new insights into the impact of blood-pressure management on ICH occurrence. Other RCTs evaluating blood pressure–lowering strategies after reperfusion therapies are currently in progress or are about to begin and include the BEST II trial (NCT04116112), OPTIMAL-BP (NCT04205305), and ENCHANTED 2 (NCT04140110).

In the antithrombotic field, current strategies aim at optimizing cerebral reperfusion pharmacologically as an adjunct to current recommended therapies with IVT and EVT. In this

context, ACT017, an antibody fragment targeting platelet GPVI receptor, seems to be a new antiplatelet agent that safely improves reperfusion (103). After showing its safety profile in preclinical and phase I studies, the ACTIMIS trial (NCT03803007) is currently evaluating this first-in-class antiplatelet agent following tPA within 4.5 h from stroke onset. The ACTIMIS trial will be an opportunity to assess the benefit/risk ratio of this new antiplatelet molecule.

Several trials are evaluating the effectiveness and safety of EVT in large ischemic core at presentation. As previously stated, large ischemic core (>33% of the middle cerebral artery territory, low ASPECTS) may expose the patient to increased risk of sICH after reperfusion therapy. The Large Stroke Therapy Evaluation study from the INEXTREMIS Trial (NCT03811769) is evaluating EVT in large ischemic core (ASPECT <5 or 3 to 5 for patients >80 years old). Other studies, such as the Efficacy and Safety of Thrombectomy in Stroke With Extended Lesion and Extended Time Window (TENSION; NCT03094715) and Thrombectomy for Emergent Salvage of Large Anterior Circulation Ischemic Stroke (TESLA; NCT03805308) trials, are also evaluating these outcomes in large ischemic core at presentation. The results of these trials are awaited because they will provide valuable insights regarding the safety of reperfusion treatments in these settings and the impact of reperfusion in patients presenting low ASPECTS.

Finally, because IV tPA is strongly associated with hemorrhagic complications such as sICH (despite its effectiveness) and because EVT results in high (>90%) recanalization rates, several trials are evaluating the non-inferiority of EVT alone vs. the association of IV tPA and EVT in patients directly transferred to a comprehensive stroke center. These trials are based on the hypothesis that for directly admitted patients, IV tPA may increase intracranial bleeding complications without improving reperfusion rates. Two trials [MRCLEAN NO-IV and Bridging Thrombolysis vs. Direct Mechanical Thrombectomy in Acute Ischemic Stroke (SWIFTDIRECT)] are underway and should answer these issues. Results of the Direct Intra-arterial Thrombectomy in Order to Revascularize AIS patients With LVO Efficiently in Chinese Tertiary Hospitals (DIRECT MT) trial were published in 2020 and revealed that EVT alone was non-inferior to EVT after IV tPA (77). The trial found lower, although not significant, sICH rates according to the Heidelberg definition in the direct EVT group than the IV tPA and EVT group (4.3% vs. 6.1%) (77). The results of the other trials are expected, in order to compare the sICH rates between EVT alone and the association of IV tPA and EVT.

CONCLUDING REMARKS

Overall, this review illustrates how ICH is the most feared complication in the early management of AIS. sICH is strongly associated with 3 month unfavorable outcomes and mortality. Importantly, because of the many different definitions of sICH, comparison between studies is difficult. In addition, many sICH risk factors have been identified and include blood biomarkers and clinical and neuroradiological factors, some of which are used in predictive scores (104, 105). However, these scores have so far limited impact on clinical practice, notably because of the lack of effective treatments of sICH. Moreover, despite this known inherent risk of sICH after IV tPA, the benefit–risk ratio favors its use, and no score should restrict its use if indicated. Current ongoing trials aim at extending the indications of EVT (timeframes, occlusion sites, infarct core volume, etc.), improving recanalization rates, while reducing the risk of sICH. From this perspective, many RCTs are of interest because they address the issue of important risk factors of sICH such as blood pressure or antithrombotic treatments. Still, an effort must be made in the design of future RCTs regarding sICH definitions, to improve the implementation of the results in clinical practice and allow for their comparison.

AUTHOR CONTRIBUTIONS

BM, JPD, and MM contributed to the review, concept and design, the analysis and interpretation of data from the literature search, drafted the manuscript, made critical revision of the manuscript for important intellectual content. MM supervised this work. All authors read and approved the final manuscript.

FUNDING

MM declares institutional fees for teaching presentations from Boehringer Ingelheim, Medtronic, Amgen, and consulting fees from Boehringer Ingelheim, Acticor Biotech, and Air liquide. MM received a grant from the French National Research Agency (ANR) as part of the Investments for the future program (PIA) (grant agreement no. ANR-18-RHUS-0001) for the BOOSTER (Brain clOt persOnalized therapeutic Strategies for sTroke Emergent Reperfusion) consortium.

ACKNOWLEDGMENTS

We thank Laura Smales for editing the manuscript.

REFERENCES

1. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the early management of patients with acute ischemic stroke: 2019. Update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. (2019) 50:e344–418. doi: 10.1161/STR.0000000000000211
2. Turc G, Bhogal P, Fischer U, Khatri P, Lobotesis K, Mazighi M, et al. European Stroke Organisation (ESO) - European Society for Minimally Invasive Neurological Therapy (ESMINT) guidelines on mechanical thrombectomy in acute ischemic stroke. *J Neurointerv Surg*. (2019) 11:535–8. doi: 10.1136/neurintsurg-2018-014569
3. Hacke W, Kaste M, Bluhmki E, Brozman M, Davalos A, Guidetti D, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med*. (2008) 359:1317–29. doi: 10.1056/NEJMoa0804656

4. Goyal M, Menon BK, van Zwam WH, Dippel DW, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet.* (2016) 387:1723–31. doi: 10.1016/S0140-6736(16)00163-X
5. Emberson J, Lees KR, Lyden P, Blackwell L, Albers G, Bluhmki E, et al. Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. *Lancet.* (2014) 384:1929–35. doi: 10.1016/S0140-6736(14)60584-5
6. National Institute of Neurological D, Stroke rt PASSG. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med.* (1995) 333:1581–7. doi: 10.1056/NEJM199512143332401
7. Pessin MS, Del Zoppo GJ, Estol CJ. Thrombolytic agents in the treatment of stroke. *Clin Neuropharmacol.* (1990) 13:271–89. doi: 10.1097/00002826-199008000-00001
8. Trouillas P, von Kummer R. Classification and pathogenesis of cerebral hemorrhages after thrombolysis in ischemic stroke. *Stroke.* (2006) 37:556–61. doi: 10.1161/01.STR.0000196942.84707.71
9. Boisseau W, Fahed R, Lapergue B, Desilles JP, Zuber K, Khoury N, et al. Predictors of parenchymal hematoma after mechanical thrombectomy: a multicenter study. *Stroke.* (2019) 50:2364–70. doi: 10.1161/STROKEAHA.118.024512
10. Paciaroni M, Agnelli G, Corea F, Ageno W, Alberti A, Lanari A, et al. Early hemorrhagic transformation of brain infarction: rate, predictive factors, and influence on clinical outcome: results of a prospective multicenter study. *Stroke.* (2008) 39:2249–56. doi: 10.1161/STROKEAHA.107.510321
11. Hoylaerts M, Rijken DC, Lijnen HR, Collen D. Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin. *J Biol Chem.* (1982) 257:2912–9. doi: 10.1055/s-0038-1652446
12. Stangl K, Laule M, Tenckhoff B, Stangl V, Gliech V, Dubel P, et al. Fibrinogen breakdown, long-lasting systemic fibrinolysis, and procoagulant activation during alteplase double-bolus regimen in acute myocardial infarction. *Am J Cardiol.* (1998) 81:841–7. doi: 10.1016/S0002-9149(98)00018-6
13. Tanswell P, Tebbe U, Neuhaus KL, Glasle-Schwarz L, Wojcik J, Seifried E. Pharmacokinetics and fibrin specificity of alteplase during accelerated infusions in acute myocardial infarction. *J Am Coll Cardiol.* (1992) 19:1071–5. doi: 10.1016/0735-1097(92)90297-Z
14. Mueller HS, Rao AK, Forman SA. Thrombolysis in myocardial infarction (TIMI): comparative studies of coronary reperfusion and systemic fibrinogenolysis with two forms of recombinant tissue-type plasminogen activator. *J Am Coll Cardiol.* (1987) 10:479–90. doi: 10.1016/S0735-1097(87)80188-2
15. Matosevic B, Knoflach M, Werner P, Pechlaner R, Zangerle A, Ruecker M, et al. Fibrinogen degradation coagulopathy and bleeding complications after stroke thrombolysis. *Neurology.* (2013) 80:1216–24. doi: 10.1212/WNL.0b013e3182897015
16. Larrue V, von Kummer R, del Zoppo G, Bluhmki E. Hemorrhagic transformation in acute ischemic stroke. Potential contributing factors in the European Cooperative Acute Stroke Study. *Stroke.* (1997) 28:957–60. doi: 10.1161/01.STR.28.5.957
17. Hacke W, Kaste M, Fieschi C, Toni D, Lesaffre E, von Kummer R, et al. Intravenous thrombolysis with recombinant tissue plasminogen activator for acute hemispheric stroke. The European Cooperative Acute Stroke Study (ECASS). *JAMA.* (1995) 274:1017–25. doi: 10.1001/jama.274.13.1017
18. Hacke W, Kaste M, Fieschi C, von Kummer R, Davalos A, Meier D, et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). Second European-Australasian Acute Stroke Study Investigators. *Lancet.* (1998) 352:1245–51. doi: 10.1016/S0140-6736(98)08020-9
19. Berger C, Fiorelli M, Steiner T, Schabitz WR, Bozzao L, Bluhmki E, et al. Hemorrhagic transformation of ischemic brain tissue: asymptomatic or symptomatic? *Stroke.* (2001) 32:1330–5. doi: 10.1161/01.STR.32.6.1330
20. Bozzao L, Angeloni U, Bastianello S, Fantozzi LM, Pierallini A, Fieschi C. Early angiographic and CT findings in patients with hemorrhagic infarction in the distribution of the middle cerebral artery. *Am J Neuroradiol.* (1991) 12:1115–21.
21. Toni D, Fiorelli M, Bastianello S, Sacchetti ML, Sette G, Argentino C, et al. Hemorrhagic transformation of brain infarct: predictability in the first 5 hours from stroke onset and influence on clinical outcome. *Neurology.* (1996) 46:341–5. doi: 10.1212/WNL.46.2.341
22. Hornig CR, Dorndorf W, Agnoli AL. Hemorrhagic cerebral infarction–a prospective study. *Stroke.* (1986) 17:179–85. doi: 10.1161/01.STR.17.2.179
23. Moulin T, Crepin-Leblond T, Chopard JL, Bogousslavsky J. Hemorrhagic infarcts. *Eur Neurol.* (1994) 34:64–77. doi: 10.1159/000117012
24. Levy DE, Brott TG, Haley EC Jr, Marler JR, Sheppard GL, et al. Factors related to intracranial hematoma formation in patients receiving tissue-type plasminogen activator for acute ischemic stroke. *Stroke.* (1994) 25:291–7. doi: 10.1161/01.STR.25.2.291
25. von Kummer R, Broderick JP, Campbell BC, Demchuk A, Goyal M, Hill MD, et al. The Heidelberg bleeding classification: classification of bleeding events after ischemic stroke and reperfusion therapy. *Stroke.* (2015) 46:2981–6. doi: 10.1161/STROKEAHA.115.010049
26. Furlan A, Higashida R, Wechsler L, Gent M, Rowley H, Kase C, et al. Intra-arterial prourokinase for acute ischemic stroke. The PROACT II study: a randomized controlled trial. Prolyse in Acute Cerebral Thromboembolism. *JAMA.* (1999) 282:2003–11. doi: 10.1001/jama.282.21.2003
27. Hao Y, Liu W, Wang H, Zi W, Yang D, Wang W, et al. Prognosis of asymptomatic intracranial hemorrhage after endovascular treatment. *J Neurointerv Surg.* (2019) 11:123–6. doi: 10.1136/neurintsurg-2018-013848
28. Jiang F, Zhao W, Wu C, Zhang Z, Li C, Che R, et al. Asymptomatic intracerebral hemorrhage may worsen clinical outcomes in acute ischemic stroke patients undergoing thrombectomy. *J Stroke Cerebrovasc Dis.* (2019) 28:1752–8. doi: 10.1016/j.jstrokecerebrovasdis.2019.02.006
29. Intracerebral hemorrhage after intravenous t-PA therapy for ischemic stroke. The NINDS t-PA Stroke Study Group. *Stroke.* (1997) 28:2109–18. doi: 10.1161/01.STR.28.11.2109
30. Fiorelli M, Bastianello S, von Kummer R, del Zoppo GJ, Larrue V, Lesaffre E, et al. Hemorrhagic transformation within 36 hours of a cerebral infarct: relationships with early clinical deterioration and 3-month outcome in the European Cooperative Acute Stroke Study I (ECASS I) cohort. *Stroke.* (1999) 30:2280–4. doi: 10.1161/01.STR.30.11.2280
31. Wahlgren N, Ahmed N, Davalos A, Ford GA, Grond M, Hacke W, et al. Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST): an observational study. *Lancet.* (2007) 369:275–82. doi: 10.1016/S0140-6736(07)60149-4
32. Campbell BCV, Ma H, Ringleb PA, Parsons MW, Churilov L, Bendszus M, et al. Extending thrombolysis to 4.5-9 h and wake-up stroke using perfusion imaging: a systematic review and meta-analysis of individual patient data. *Lancet.* (2019) 394:139–47. doi: 10.1016/S0140-6736(19)31053-0
33. IST-3 collaborative group, Sandercock P, Wardlaw JM, Lindley RI, Dennis M, Cohen G, et al. The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. *Lancet.* (2012) 379:2352–63. doi: 10.1016/S0140-6736(12)60768-5
34. Hacke W, Donnan G, Fieschi C, Kaste M, von Kummer R, Broderick JP, et al. Association of outcome with early stroke treatment: pooled analysis of ATLANTIS, ECASS, and NINDS rt-PA stroke trials. *Lancet.* (2004) 363:768–74. doi: 10.1016/S0140-6736(04)15692-4
35. Wardlaw JM, Murray V, Berge E, del Zoppo G, Sandercock P, Lindley RL, et al. Recombinant tissue plasminogen activator for acute ischaemic stroke: an updated systematic review and meta-analysis. *Lancet.* (2012) 379:2364–72. doi: 10.1016/S0140-6736(12)60738-7
36. Anderson CS, Robinson T, Lindley RI, Arima H, Lavados PM, Lee TH, et al. low-dose versus standard-dose intravenous alteplase in acute ischemic stroke. *N Engl J Med.* (2016) 374:2313–23. doi: 10.1056/NEJMoa1515510
37. Thomalla G, Simonsen CZ, Boutitie F, Andersen G, Berthezene Y, Cheng B, et al. MRI-guided thrombolysis for stroke with unknown time of onset. *N Engl J Med.* (2018) 379:611–22. doi: 10.1056/NEJMoa1804355
38. Ma H, Campbell BCV, Parsons MW, Churilov L, Levi CR, Hsu C, et al. Thrombolysis guided by perfusion imaging up to 9 hours after onset of stroke. *N Engl J Med.* (2019) 380:1795–803. doi: 10.1056/NEJMoa1813046
39. Whiteley WN, Emberson J, Lees KR, Blackwell L, Albers G, Bluhmki E, et al. Risk of intracerebral haemorrhage with alteplase after acute ischaemic stroke: a secondary analysis of an individual patient data meta-analysis. *Lancet Neurol.* (2016) 15:925–33. doi: 10.1016/S1474-4422(16)30076-X

40. Larrue V, von Kummer RR, Muller A, Bluhmki E. Risk factors for severe hemorrhagic transformation in ischemic stroke patients treated with recombinant tissue plasminogen activator: a secondary analysis of the European-Australasian Acute Stroke Study (ECASS II). *Stroke.* (2001) 32:438–41. doi: 10.1161/01.STR.32.2.438
41. Zinkstok SM, Roos YB, investigators A. Early administration of aspirin in patients treated with alteplase for acute ischaemic stroke: a randomised controlled trial. *Lancet.* (2012) 380:731–7. doi: 10.1016/S0140-6736(12)60949-0
42. Zinkstok SM, Beenen LF, Majoie CB, Marquering HA, de Haan RJ, Roos YB. Early deterioration after thrombolysis plus aspirin in acute stroke: a *post hoc* analysis of the antiplatelet therapy in combination with recombinant t-PA thrombolysis in ischemic stroke trial. *Stroke.* (2014) 45:3080–2. doi: 10.1161/STROKEAHA.114.006268
43. Mazya M, Egido JA, Ford GA, Lees KR, Mikulik R, Toni D, et al. Predicting the risk of symptomatic intracerebral hemorrhage in ischemic stroke treated with intravenous alteplase: safe Implementation of Treatments in Stroke (SITS) symptomatic intracerebral hemorrhage risk score. *Stroke.* (2012) 43:1524–31. doi: 10.1161/STROKEAHA.111.644815
44. Whiteley WN, Slot KB, Fernandes P, Sandercock P, Wardlaw J. Risk factors for intracranial hemorrhage in acute ischemic stroke patients treated with recombinant tissue plasminogen activator: a systematic review and meta-analysis of 55 studies. *Stroke.* (2012) 43:2904–9. doi: 10.1161/STROKEAHA.112.665331
45. Tanne D, Gorman MJ, Bates VE, Kasner SE, Scott P, Verro P, et al. Intravenous tissue plasminogen activator for acute ischemic stroke in patients aged 80 years and older: the tPA stroke survey experience. *Stroke.* (2000) 31:370–5. doi: 10.1161/01.STR.31.2.370
46. Sylaja PN, Cote R, Buchan AM, Hill MD, Canadian Alteplase for Stroke Effectiveness Study I. Thrombolysis in patients older than 80 years with acute ischaemic stroke: Canadian alteplase for stroke effectiveness study. *J Neurol Neurosurg Psychiatry.* (2006) 77:826–9. doi: 10.1136/jnnp.2005.086595
47. Mishra NK, Ahmed N, Andersen G, Egido JA, Lindsberg PJ, Ringleb PA, et al. Thrombolysis in very elderly people: controlled comparison of SITS international stroke thrombolysis registry and virtual international stroke trials archive. *BMJ.* (2010) 341:c6046. doi: 10.1136/bmj.c6046
48. Ford GA, Ahmed N, Azevedo E, Grond M, Larrue V, Lindsberg PJ, et al. Intravenous alteplase for stroke in those older than 80 years old. *Stroke.* (2010) 41:2568–74. doi: 10.1161/STROKEAHA.110.581884
49. Tanne D, Kasner SE, Demchuk AM, Koren-Morag N, Hanson S, Grond M, et al. Markers of increased risk of intracerebral hemorrhage after intravenous recombinant tissue plasminogen activator therapy for acute ischemic stroke in clinical practice: the Multicenter rt-PA Stroke Survey. *Circulation.* (2002) 105:1679–85. doi: 10.1161/01.CIR.0000012747.53592.6A
50. Engelter ST, Bonati LH, Lyrer PA. Intravenous thrombolysis in stroke patients of > or = 80 versus < 80 years of age–a systematic review across cohort studies. *Age Ageing.* (2006) 35:572–80. doi: 10.1093/ageing/afl104
51. Lansberg MG, Albers GW, Wijman CA. Symptomatic intracerebral hemorrhage following thrombolytic therapy for acute ischemic stroke: a review of the risk factors. *Cerebrovasc Dis.* (2007) 24:1–10. doi: 10.1159/000103110
52. Mazya MV, Lees KR, Collas D, Rand VM, Mikulik R, Toni D, et al. IV thrombolysis in very severe and severe ischemic stroke: results from the SITS-ISTR registry. *Neurology.* (2015) 85:2098–106. doi: 10.1212/WNL.0000000000002199
53. Keselman B, Gdovinova Z, Jatuzis D, Melo TPE, Vilionskis A, Cavallo R, et al. Safety and outcomes of intravenous thrombolysis in posterior versus anterior circulation stroke: results from the safe implementation of treatments in stroke registry and meta-analysis. *Stroke.* (2020) 51:876–82. doi: 10.1161/STROKEAHA.119.027071
54. Maier B, Kubis N. Hypertension and its impact on stroke recovery: from a vascular to a parenchymal overview. *Neural Plast.* (2019) 2019:6843895. doi: 10.1155/2019/6843895
55. Leonardi-Bee J, Bath PM, Phillips SJ, Sandercock PA, Group ISTC. Blood pressure and clinical outcomes in the International Stroke Trial. *Stroke.* (2002) 33:1315–20. doi: 10.1161/01.STR.0000014509.11540.66
56. Tsivgoulis G, Frey JL, Flaster M, Sharma VK, Lao AY, Hoover SL, et al. Pre-tissue plasminogen activator blood pressure levels and risk of symptomatic intracerebral hemorrhage. *Stroke.* (2009) 40:3631–4. doi: 10.1161/STROKEAHA.109.564096
57. Ahmed N, Wahlgren N, Brainin M, Castillo J, Ford GA, Kaste M, et al. Relationship of blood pressure, antihypertensive therapy, and outcome in ischemic stroke treated with intravenous thrombolysis: retrospective analysis from Safe Implementation of Thrombolysis in Stroke-International Stroke Thrombolysis Register (SITS-ISTR). *Stroke.* (2009) 40:2442–9. doi: 10.1161/STROKEAHA.109.548602
58. Butcher K, Christensen S, Parsons M, De Silva DA, Ebinger M, Levi C, et al. Postthrombolysis blood pressure elevation is associated with hemorrhagic transformation. *Stroke.* (2010) 41:72–7. doi: 10.1161/STROKEAHA.109.563767
59. Berge E, Cohen G, Lindley RI, Sandercock P, Wardlaw JM, Sandset EC, et al. Effects of blood pressure and blood pressure-lowering treatment during the first 24 hours among patients in the third International Stroke Trial of thrombolytic treatment for acute ischemic stroke. *Stroke.* (2015) 46:3362–9. doi: 10.1161/STROKEAHA.115.010319
60. Delgado-Mederos R, Ribo M, Rovira A, Rubiera M, Munuera J, Santamarina E, et al. Prognostic significance of blood pressure variability after thrombolysis in acute stroke. *Neurology.* (2008) 71:552–8. doi: 10.1212/01.wnl.0000318294.36223.69
61. Kellert L, Hametner C, Ahmed N, Rauch G, MacLeod MJ, Perini F, et al. Reciprocal interaction of 24-hour blood pressure variability and systolic blood pressure on outcome in stroke thrombolysis. *Stroke.* (2017) 48:1827–34. doi: 10.1161/STROKEAHA.117.016876
62. Anderson CS, Huang Y, Lindley RI, Chen X, Arima H, Chen G, et al. Intensive blood pressure reduction with intravenous thrombolysis therapy for acute ischaemic stroke (ENCHANTED): an international, randomised, open-label, blinded-endpoint, phase 3 trial. *Lancet.* (2019) 393:877–88. doi: 10.1016/S0140-6736(19)30038-8
63. Desilles JP, Meseguer E, Labreuche J, Lapergue B, Sirimarco G, Gonzalez-Valcarcel J, et al. Diabetes mellitus, admission glucose, and outcomes after stroke thrombolysis: a registry and systematic review. *Stroke.* (2013) 44:1915–23. doi: 10.1161/STROKEAHA.111.000813
64. Desilles JP, Syvannarath V, Ollivier V, Journe C, Delbosc S, Ducroux C, et al. Exacerbation of thromboinflammation by hyperglycemia precipitates cerebral infarct growth and hemorrhagic transformation. *Stroke.* (2017) 48:1932–40. doi: 10.1161/STROKEAHA.117.017080
65. Mehta RH, Stebbins A, Lopes RD, Rao SV, Bates ER, Pieper KS, et al. Race, bleeding, and outcomes in STEMI patients treated with fibrinolytic therapy. *Am J Med.* (2011) 124:48–57. doi: 10.1016/j.amjmed.2010.07.028
66. Toyoda K, Koga M, Hayakawa M, Yamagami H. Acute reperfusion therapy and stroke care in Asia after successful endovascular trials. *Stroke.* (2015) 46:1474–81. doi: 10.1161/STROKEAHA.115.008781
67. Chiang CC, Meyer DM, Meyer BC, Agrawal K, Modir R. RAcial Disparities in Ich after IV-tPA and Neurointerventional Treatment (RADIANT). *J Stroke Cerebrovasc Dis.* (2020) 29:104474. doi: 10.1016/j.jstrokecerebrovasdis.2019.104474
68. Mehta RH, Cox M, Smith EE, Xian Y, Bhatt DL, Fonarow GC, et al. Race/Ethnic differences in the risk of hemorrhagic complications among patients with ischemic stroke receiving thrombolytic therapy. *Stroke.* (2014) 45:2263–9. doi: 10.1161/STROKEAHA.114.005019
69. Selim M, Fink JN, Kumar S, Caplan LR, Horkan C, Chen Y, et al. Predictors of hemorrhagic transformation after intravenous recombinant tissue plasminogen activator: prognostic value of the initial apparent diffusion coefficient and diffusion-weighted lesion volume. *Stroke.* (2002) 33:2047–52. doi: 10.1161/01.STR.0000023577.65990.4E
70. Tong DC, Adami A, Moseley ME, Marks MP. Prediction of hemorrhagic transformation following acute stroke: role of diffusion- and perfusion-weighted magnetic resonance imaging. *Arch Neurol.* (2001) 58:587–93. doi: 10.1001/archneur.58.4.587
71. Lansberg MG, Thijs VN, Bammer R, Kemp S, Wijman CA, Marks MP, et al. Risk factors of symptomatic intracerebral hemorrhage after tPA therapy for acute stroke. *Stroke.* (2007) 38:2275–8. doi: 10.1161/STROKEAHA.106.480475
72. Tsivgoulis G, Zand R, Katsanos AH, Turc G, Nolte CH, Jung S, et al. Risk of symptomatic intracerebral hemorrhage after intravenous thrombolysis in patients with acute ischemic stroke and high cerebral

microbleed burden: a meta-analysis. *JAMA Neurol.* (2016) 73:675–83. doi: 10.1001/jamaneurol.2016.0292
73. Charidimou A, Turc G, Oppenheim C, Yan S, Scheitz JF, Erdur H, et al. Microbleeds, cerebral hemorrhage, and functional outcome after stroke thrombolysis. *Stroke.* (2017) 48:2084–90. doi: 10.1161/STROKEAHA.116.012992
74. Maestrini I, Strbian D, Gautier S, Haapaniemi E, Moulin S, Sairanen T, et al. Higher neutrophil counts before thrombolysis for cerebral ischemia predict worse outcomes. *Neurology.* (2015) 85:1408–16. doi: 10.1212/WNL.0000000000002029
75. Boisseau W, Desilles JP, Fahed R, Kyheng M, Zuber K, Sabben C, et al. Neutrophil count predicts poor outcome despite recanalization after endovascular therapy. *Neurology.* (2019) 93:e467–e75. doi: 10.1212/WNL.0000000000007859
76. Gensicke H, Al Sultan AS, Strbian D, Hametner C, Zinkstok SM, Moulin S, et al. Intravenous thrombolysis and platelet count. *Neurology.* (2018) 90:e690–7. doi: 10.1212/WNL.0000000000004982
77. Yang Y, Liu B, Wu L, Guan X, Luo Y, Jiang C, et al. Safety of intravenous thrombolysis for acute ischemic stroke in patients with thrombocytopenia. *Cerebrovasc Dis.* (2019) 48:157–64. doi: 10.1159/000504214
78. Trouillas P, Derex L, Philippeau F, Nighoghossian N, Honnorat J, Hanss M, et al. Early fibrinogen degradation coagulopathy is predictive of parenchymal hematomas in cerebral rt-PA thrombolysis: a study of 157 cases. *Stroke.* (2004) 35:1323–8. doi: 10.1161/01.STR.0000126040.99024.cf
79. Roman LS, Menon BK, Blasco J, Hernandez-Perez M, Davalos A, Majoie C, et al. Imaging features and safety and efficacy of endovascular stroke treatment: a meta-analysis of individual patient-level data. *Lancet Neurol.* (2018) 17:895–904. doi: 10.1016/S1474-4422(18)30242-4
80. Mazighi M, Chaudhry SA, Ribo M, Khatri P, Skoloudik D, Mokin M, et al. Impact of onset-to-reperfusion time on stroke mortality: a collaborative pooled analysis. *Circulation.* (2013) 127:1980–5. doi: 10.1161/CIRCULATIONAHA.112.000311
81. Saver JL, Goyal M, van der Lugt A, Menon BK, Majoie CB, Dippel DW, et al. Time to treatment with endovascular thrombectomy and outcomes from ischemic stroke: a meta-analysis. *JAMA.* (2016) 316:1279–88. doi: 10.1001/jama.2016.13647
82. Kranendonk KRv, Treurniet KM, Boers AMM, Berkhemer OA, Berg LAvd, Chalos V, et al. Clinical and imaging markers associated with hemorrhagic transformation in patients with acute ischemic stroke. *Stroke.* (2019) 50:2037–43. doi: 10.1161/STROKEAHA.118.024255
83. Nogueira RG, Gupta R, Jovin TG, Levy EI, Liebeskind DS, Zaidat OO, et al. Predictors and clinical relevance of hemorrhagic transformation after endovascular therapy for anterior circulation large vessel occlusion strokes: a multicenter retrospective analysis of 1122 patients. *J Neurointerv Surg.* (2015) 7:16–21. doi: 10.1136/neurintsurg-2013-010743
84. Lapergue B, Blanc R, Gory B, Labreuche J, Duhamel A, Marnat G, et al. Effect of endovascular contact aspiration vs stent retriever on revascularization in patients with acute ischemic stroke and large vessel occlusion: the ASTER randomized clinical trial. *JAMA.* (2017) 318:443–52. doi: 10.1001/jama.2017.9644
85. Turk AS III, Siddiqui A, Fifi JT, De Leacy RA, Fiorella DJ, Gu E, et al. Aspiration thrombectomy versus stent retriever thrombectomy as first-line approach for large vessel occlusion (COMPASS): a multicentre, randomised, open label, blinded outcome, non-inferiority trial. *Lancet.* (2019) 393:998–1008. doi: 10.1016/S0140-6736(19)30297-1
86. Sarraj A, Sangha N, Hussain MS, Wisco D, Vora N, Elijovich L, et al. Endovascular therapy for acute ischemic stroke with occlusion of the middle cerebral artery M2 segment. *JAMA Neurol.* (2016) 73:1291–6. doi: 10.1001/jamaneurol.2016.2773
87. Sheth SA, Yoo B, Saver JL, Starkman S, Ali LK, Kim D, et al. M2 occlusions as targets for endovascular therapy: comprehensive analysis of diffusion/perfusion MRI, angiography, and clinical outcomes. *J Neurointerv Surg.* (2015) 7:478–83. doi: 10.1136/neurintsurg-2014-011232
88. Gerschenfeld G, Muresan IP, Blanc R, Obadia M, Abrivard M, Piotin M, et al. Two paradigms for endovascular thrombectomy after intravenous thrombolysis for acute ischemic stroke. *JAMA Neurol.* (2017) 74:549–56. doi: 10.1001/jamaneurol.2016.5823
89. Rinaldo L, Brinjikji W, McCutcheon BA, Bydon M, Cloft H, Kallmes DF, et al. Hospital transfer associated with increased mortality after endovascular revascularization for acute ischemic stroke. *J Neurointerv Surg.* (2017) 9:1166–72. doi: 10.1136/neurintsurg-2016-012824
90. Maïer B, Dargazanli C, Bourcier R, Kyheng M, Labreuche J, Mosimann PJ, et al. Effect of steady and dynamic blood pressure parameters during thrombectomy according to the collateral status. *Stroke.* (2020) 51:1199–206. doi: 10.1161/STROKEAHA.119.026769
91. Maïer B, Fahed R, Khoury N, Guenego A, Labreuche J, Taylor G, et al. Association of blood pressure during thrombectomy for acute ischemic stroke with functional outcome a systematic review. *Stroke.* (2019) 50:2805–12. doi: 10.1161/STROKEAHA.119.024915
92. Maier B, Turc G, Taylor G, Blanc R, Obadia M, Smajda S, et al. Prognostic significance of pulse pressure variability during mechanical thrombectomy in acute ischemic stroke patients. *J Am Heart Assoc.* (2018) 7:e009378. doi: 10.1161/JAHA.118.009378
93. Maier B, Gory B, Taylor G, Labreuche J, Blanc R, Obadia M, et al. Mortality and disability according to baseline blood pressure in acute ischemic stroke patients treated by thrombectomy: a collaborative pooled analysis. *J Am Heart Assoc.* (2017) 6:e006484. doi: 10.1161/JAHA.117.004193
94. Mulder MJHL, Ergezen S, Lingsma HF, Berkhemer OA, Fransen PSS, Beumer D, et al. Baseline blood pressure effect on the benefit and safety of intra-arterial treatment in MR CLEAN (Multicenter Randomized Clinical Trial of Endovascular Treatment of Acute Ischemic Stroke in the Netherlands). *Stroke.* (2017) 48:1869–76. doi: 10.1161/STROKEAHA.117.017996
95. Valent A, Sajadhoussen A, Maïer B, Lapergue B, Labeyrie MA, et al. A 10% blood pressure drop from baseline during the procedure is strongly associated with worse neurological outcomes after thrombectomy for stroke. *J Neurointerv Surg.* (2019) 12:363–9. doi: 10.1136/neurintsurg-2019-015247
96. Petersen NH, Ortega-Gutierrez S, Wang A, Lopez GV, Strander S, Kodali S, et al. Decreases in blood pressure during thrombectomy are associated with larger infarct volumes and worse functional outcome. *Stroke.* (2019) 50:1797–804. doi: 10.1161/STROKEAHA.118.024286
97. Schönenberger S, Uhlmann L, Ungerer M, Pfaff J, Nagel S, Klose C, et al. Association of blood pressure with short- and long-term functional outcome after stroke thrombectomy: *post hoc* analysis of the SIESTA trial. *Stroke.* (2018) 49:1451–6. doi: 10.1161/STROKEAHA.117.019709
98. Rasmussen M, Schonenberger S, Henden PL, Valentin JB, Espelund US, Sorensen LH, et al. Blood pressure thresholds and neurologic outcomes after endovascular therapy for acute ischemic stroke: an analysis of individual patient data from 3 randomized clinical trials. *JAMA Neurol.* (2020) 77:622–31. doi: 10.1001/jamaneurol.2019.4838
99. Kim TJ, Park HK, Kim JM, Lee JS, Park SH, Jeong HB, et al. Blood pressure variability and hemorrhagic transformation in patients with successful recanalization after endovascular recanalization therapy: a retrospective observational study. *Ann Neurol.* (2019) 85:574–81. doi: 10.1002/ana.25434
100. Anadani M, Orabi MY, Alawieh A, Goyal N, Alexandrov AV, Petersen N, et al. Blood pressure and outcome after mechanical thrombectomy with successful revascularization: a multicenter study. *Stroke.* (2019) 50:2448–54. doi: 10.1161/str.50.suppl_1.153
101. Matusevicius M, Cooray C, Bottai M, Mazya M, Tsivgoulis G, Nunes AP, et al. Blood pressure after endovascular thrombectomy: modeling for outcomes based on recanalization status. *Stroke.* (2020) 51:519–25. doi: 10.1161/STROKEAHA.119.026914
102. Mazighi M, Labreuche J, Richard S, Gory B, Lapergue B, Sibon I, et al. Blood pressure target in acute stroke to reduce hemorrhage after endovascular therapy: the randomized BP TARGET Study protocol. *Front Neurol.* (2020) 11:480. doi: 10.3389/fneur.2020.00480
103. Renaud L, Lebozec K, Voors-Pette C, Dogterom P, Billiald P, Jandrot Perrus M, et al. Population pharmacokinetic/pharmacodynamic

modeling of glenzocimab (ACT017) a glycoprotein VI inhibitor of collagen-induced platelet aggregation. *J Clin Pharmacol.* (2020) 60:1198–208. doi: 10.1002/jcph.1616

104. Strbian D, Engelter S, Michel P, Meretoja A, Sekoranja L, Ahlhelm FJ, et al. Symptomatic intracranial hemorrhage after stroke thrombolysis: the SEDAN score. *Ann Neurol.* (2012) 71:634–41. doi: 10.1002/ana.23546
105. Zhang X, Xie Y, Wang H, Yang D, Jiang T, Yuan K, et al. Symptomatic intracranial hemorrhage after mechanical thrombectomy in Chinese ischemic stroke patients: the ASIAN score. *Stroke.* (2020) 51:2690–6. doi: 10.1161/STROKEAHA.120.030173

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

BRIEF RESEARCH REPORT
published: 22 December 2020
doi: 10.3389/fneur.2020.593098

Is This Contrast? Is This Blood? An Agreement Study on Post-thrombectomy Computed Tomography Scans

*Ronda Lun[1], Gregory B. Walker[1,2], Adrien Guenego[3], Mohammed Kassab[4,5], Eduardo Portela[5], Vignan Yogendrakumar[1], George Medvedev[2], Ken Wong[6], Michel Shamy[1,7], Dar Dowlatshahi[1,7] and Robert Fahed[1,5,7]**

[1] Ottawa Stroke Program, Department of Medicine (Neurology), University of Ottawa, Ottawa, ON, Canada, [2] Division of Neurology, Fraser Health Authority, Royal Columbian Hospital, New Westminster, BC, Canada, [3] Department of Interventional Neuroradiology, Erasme University Hospital, Brussels, Belgium, [4] Division of Neurosurgery, Department of Surgery, University of Ottawa, Ottawa, ON, Canada, [5] Interventional Neuroradiology, Department of Medical Imaging, University of Ottawa, Ottawa, ON, Canada, [6] Division of Medical Imaging, Fraser Health Authority, Royal Columbian Hospital, New Westminster, BC, Canada, [7] Ottawa Hospital Research Institute, Ottawa, ON, Canada

OPEN ACCESS

Edited by:
Bruce Campbell,
The University of Melbourne, Australia

Reviewed by:
Johannes Pfaff,
Heidelberg University Hospital, Germany
Teddy Y. Wu,
Christchurch Hospital, New Zealand

***Correspondence:**
Robert Fahed
robert.fahed@hotmail.fr

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: 09 August 2020
Accepted: 30 November 2020
Published: 22 December 2020

Citation:
Lun R, Walker GB, Guenego A, Kassab M, Portela E, Yogendrakumar V, Medvedev G, Wong K, Shamy M, Dowlatshahi D and Fahed R (2020) Is This Contrast? Is This Blood? An Agreement Study on Post-thrombectomy Computed Tomography Scans. Front. Neurol. 11:593098. doi: 10.3389/fneur.2020.593098

Background: Hemorrhagic transformation after acute ischemic stroke is a dreaded and severe complication of thrombolysis and thrombectomy. However, its detection on post-thrombectomy conventional non-contrast computed tomography (CT) scan can be complicated by the frequent (and sometimes concomitant) presence of contrast, resulting in changes in management.

Aims: Our objective was to assess the inter- and intra-rater reliability for the detection of blood and/or contrast on day-1 post-thrombectomy CT scans.

Methods: A total of 18 raters across 3 different specialties independently examined 30 post-thrombectomy CT scans selected from the Aspiration vs. STEnt-Retriever (ASTER) trial. They were asked to judge the presence of blood and contrast. Thirty days later, the same 18 raters again independently judged the 30 scans, in randomized order. Agreement was measured with Fleiss' and Cohen's *K* statistics.

Results: Overall agreement on blood and/ or contrast presence was only fair, $k = 0.291$ (95% CI = 0.273–0.309). There were 0 scans with consensus among the 18 readers on the presence of blood and/or contrast. However, intra-rater global agreement across all 18 physicians was relatively high, with a median kappa value of 0.675. This intra-rater consistency was seen across all specialties, regardless of level of training.

Conclusion: Physician judgment for the presence of blood and/or contrast on day-1 post-thrombectomy non-contrast CT scan shows limited inter-observer reliability. Advanced imaging modalities may then be warranted for challenging clinical cases.

Keywords: stroke, ischemic stroke, thrombectomy, hemorrhage, cerebral, agreement, reliability

INTRODUCTION

Endovascular thrombectomy (EVT) has become the standard of care for patients with acute ischemic stroke (AIS) secondary to large vessel occlusions (1). One of the major complications after AIS is hemorrhagic transformation (HT), reported to be up to 35% after EVT (2). However, arterial injection of iodine contrast during EVT may mimic the appearance of HT, due to its hyper-dense appearance on follow-up conventional non-contrast CT (NCCT) (3). Inaccurate identification of HT could delay necessary treatments such as antiplatelet or anticoagulant therapy, and potentially result in misdiagnosis of HT in future EVT trials. In this study, we aimed to evaluate inter-rater and intra-rater reliability for detection of HT and contrast staining (CS) on NCCT in EVT patients.

METHODS

We analyzed imaging data from the "Aspiration vs. STEnt-Retriever" (ASTER) trial (4). Access to the data can be obtained through formal proposal to the authors of the study.

A total of 30 NCCT scans performed 24–36 h after EVT were selected from the ASTER database with a roughly equal distribution of scans with HT, scans with CS, scans with both HT and CS, and scans with no HT or CS (i.e., approximately 6–8 scans in each subcategory). The studies were identified as such in the core laboratory of the ASTER study, which is composed of four attending physicians with 5–20 years of experience in neuroradiology. All selected cases of HT were parenchymal; we did not assess for the detection of subarachnoid or extra-cranial hemorrhage. Additional clinical details of the study are outlined elsewhere (5).

Eighteen raters from 3 tertiary stroke centers were recruited for independent interpretation of studies: 6 stroke neurologists, 6 interventional neuroradiologists (INR), and 6 diagnostic neuroradiologists (DNR), as they are all involved in multidisciplinary decisions for stroke patients. Within each specialty, there were 3 junior level physicians and 3 senior level physicians. Junior physicians were defined as fellows with <2 years of experience, and senior physicians were defined as staff physicians with more than 5 years of independent practice experience. To evaluate inter-rater reliability, the raters were asked to independently evaluate each NCCT for (1) presence of hemorrhage and (2) presence of contrast. To evaluate intra-rater reliability, the same independent raters were then asked to repeat the study assessments 1 month later, with the study order randomized. The raters had access to basic clinical information, including basic demographics, treatment status with thrombolysis, final thrombolysis in cerebral infarction recanalization score, time from symptom onset to recanalization, and day-1 National Institutes of Health Stroke Scale score (5).

The raters' dichotomized (yes/no) answers were transformed into a "global judgment" score of whether there was blood, contrast, or a combination of both. Fleiss' kappa was run to determine if there was inter-rater agreement. Intra-rater reliability was also assessed with Cohen's unweighted kappa

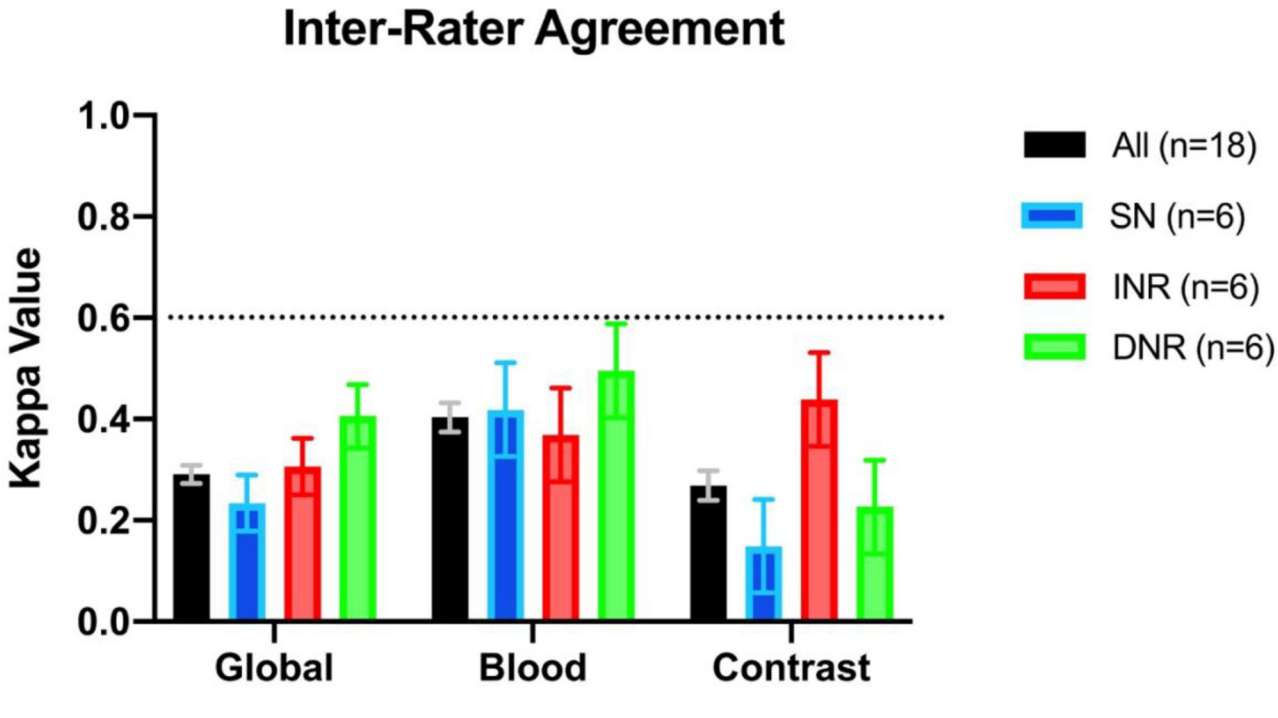


FIGURE 1 | Interrater agreement displayed as kappa values across specialties for assessment of the presence vs. absence of blood, contrast, or presence of both (i.e., global assessment). SN, stroke neurologist; INR, interventional neuroradiologist; DNR, diagnostic neuroradiologist.

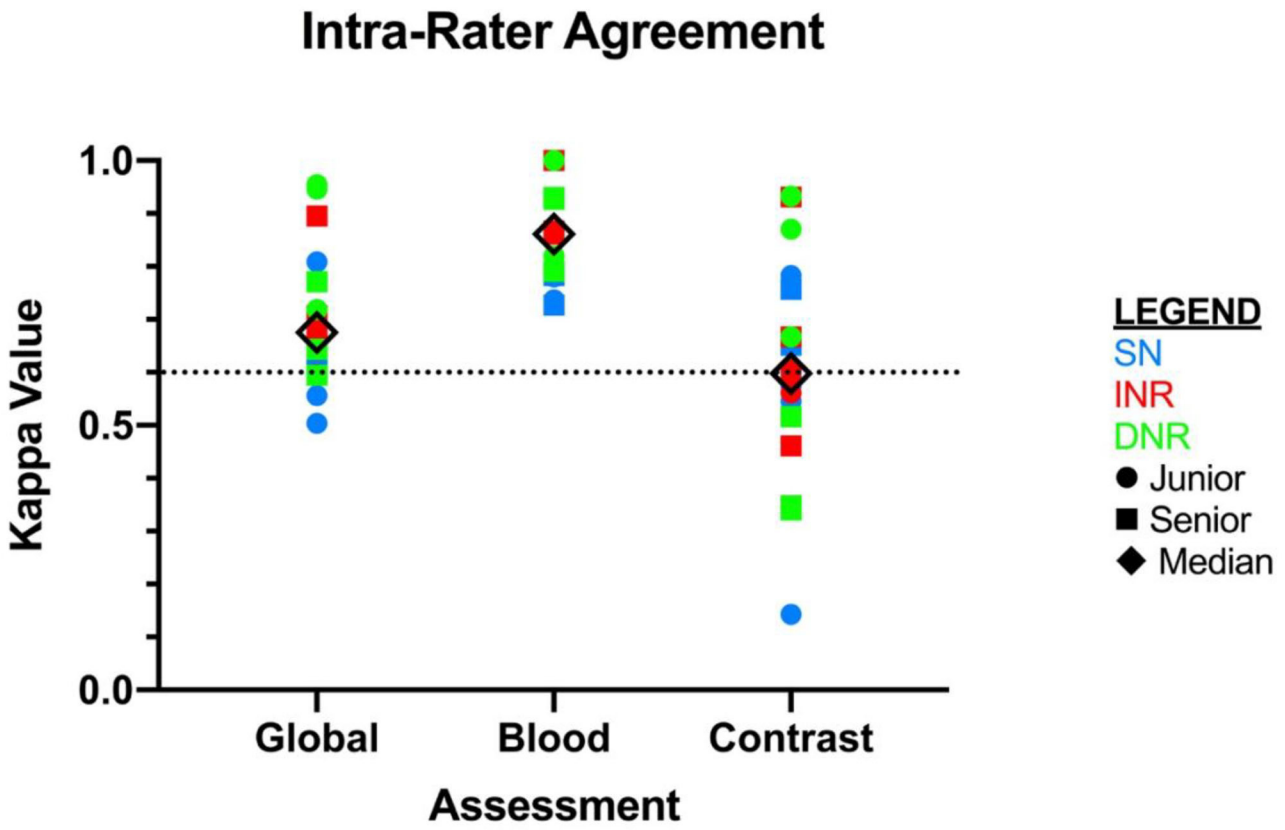


FIGURE 2 | Intra-rater agreement displayed as kappa values for each independent rater. *X*-axis is displayed by type of assessment: global, blood, and contrast. SN, stroke neurologist; INR, interventional neuroradiologist; DNR, diagnostic neuroradiologist.

TABLE 1 | Inter-rater agreement across specialties, displayed as Fleiss' Kappa values, with 95% confidence intervals.

	Inter-Rater Agreement (1st Reading)		
	Global judgment	**Blood (Yes/No)**	**Contrast (Yes/No)**
All ($n = 18$)	0.291 [0.273–0.309]	0.404 [0.375–0.432]	0.269 [0.240–0.298]
Stroke neurologists ($n = 6$)	0.234 [0.179–0.290]	0.418 [0.326–0.511]	0.149 [0.057–0.241]
Interventional neuroradiologists ($n = 6$)	0.306 [0.250–0.362]	0.369 [0.276–0.461]	0.439 [0.346–0.531]
Diagnostic neuroradiologists ($n = 6$)	0.406 [0.343–0.468]	0.495 [0.403–0.588]	0.227 [0.134–0.319]

values. The median number of times a rater's answer changed between the two assessments was calculated. All statistics were performed using SPSS v26.0 (IBM, Armonk, NY). Graphs were generated using GraphPad Prism v8.3.1. Levels of agreement were defined according to Landis and Koch: slight (0.00–0.20), fair (0.20–0.40), moderate (0.40–0.60), substantial (0.60–0.80), and excellent (>0.80) (6).

RESULTS

Inter-rater agreement on the detection of blood was moderate, $k = 0.404$ (95% CI = 0.375–0.432) (see **Figure 1**). There were 3/30 scans on which the 18 raters agreed upon the presence of blood, and 2/30 scans on which there was unanimous agreement on the absence of blood. Intra-rater agreement was at least substantial for the detection of blood across all specialties and levels of training, and 3 raters had perfect intra-rater agreement (median k value of 0.861) (**Figure 2**). The median number of changes of judgment between both readings was 2 (**Table 1**).

The inter-rater agreement for the detection of CS was only fair, $k = 0.269$ (95% CI = 0.240–0.298) (**Figure 1**). There were 0 scans on which all raters agreed upon the presence of contrast, and only 2/30 scans on which they unanimously agreed on the absence of contrast. The INR physicians collectively had the highest level of agreement, which nevertheless remained moderate ($k = 0.439$, 95% CI = 0.346–0.531) (**Table 1**). Intra-rater agreement was below substantial for 9/18 raters, and no rater reached perfect intra-rater agreement (median k value of 0.598) (**Figure 2**). The

TABLE 2 | Intra-rater agreements for 18 independent raters, displayed as Fleiss' Kappa values, with 95% confidence intervals.

	Intra-Rater Agreement		
	Global judgment	**Blood (Yes/No)**	**Contrast (Yes/No)**
Junior stroke neurologist #1	0.504 [0.271–0.767]	**0.737 [0.497–0.978]**	0.545 [0.251–0.840]
Junior stroke neurologist #2	**0.809 [0.604–1.000]**	**0.789 [0.562–1.000]**	**0.783 [0.364–1.000]**
Junior stroke neurologist #3	0.556 [0.293–0.820]	**0.780 [0.545–1.000]**	0.143 [−0.471–0.757]
Senior stroke neurologist #1	**0.610 [0.378–0.841]**	**0.783 [0.549–1.000]**	0.590 [0.262–0.918]
Senior stroke neurologist #2	**0.667 [0.450–0.883]**	**0.727 [0.478–0.976]**	**0.757 [0.496–1.000]**
Senior stroke neurologist #3	**0.668 [0.452–0.883]**	**0.867 [0.666–1.000]**	**0.651 [0.372–0.930]**
Junior interventional neuroradiologist #1	**0.681 [0.474–0.888]**	**0.862 [0.677–1.000]**	**0.605 [0.323–0.888]**
Junior interventional neuroradiologist #2	**0.707 [0.497–0.917]**	**0.861 [0.675–1.000]**	0.561 [0.210–0.912]
Junior interventional neuroradiologist #3	0.596 [0.375–0.817]	**0.795 [0.574–1.000]**	0.587 [0.292–0.883]
Senior interventional neuroradiologist #1	**0.895 [0.754–1.000]**	**0.861 [0.675–1.000]**	**0.931 [0.799–1.000]**
Senior interventional neuroradiologist #2	**0.707 [0.497–0.917]**	**0.867 [0.688–1.000]**	**0.667 [0.363–0.971]**
Senior Interventional Neuroradiologist #3	**0.657 [0.435–0.880]**	**1**	0.461 [0.112–0.811]
Junior diagnostic neuroradiologist #1	**0.955 [0.868–1.000]**	**1**	**0.933 [0.805–1.000]**
Junior diagnostic neuroradiologist #2	**0.946 [0.840–1.000]**	**1**	**0.870 [0.618–1.000]**
Junior diagnostic neuroradiologist #3	**0.719 [0.494–0.944]**	**0.830 [0.601–1.000]**	**0.667 [0.309–1.000]**
Senior diagnostic neuroradiologist #1	**0.644 [0.388–0.899]**	**0.791 [0.566–1.000]**	0.348 [−0.352–1.000]
Senior diagnostic neuroradiologist #2	0.595 [0.354–0.835]	**0.930 [0.794–1.000]**	0.340 [−0.089–0.768]
Senior diagnostic neuroradiologist #3	**0.771 [0.561–0.980]**	**0.927 [0.786–1.000]**	0.516 [−0.003–1.000]

Bolded values indicate kappa values > 0.6, suggesting substantial to excellent agreement.

median number of changes of judgment per physician was 4.5. Detailed kappa values for each rater are outlined in **Table 2**.

Overall global judgment for the presence of blood and/or contrast across all specialties was fair, $k = 0.291$ (95% CI = 0.273–0.309) (**Figure 1**). There were 0 scans where the raters unanimously agreed on the presence of blood, contrast, neither, or both.

DISCUSSION

Our study shows that the overall inter-rater agreement about the presence of hemorrhage and/ or contrast was limited across all specialties. While DNR physicians seemed to have the highest degree of inter-rater agreement on the presence of hemorrhage, a k value of 0.495 still only represents a "moderate" level of agreement, and is usually considered "weak" in the context of health care research (7). However, intra-rater agreement for the presence of blood was consistently high across all specialties and levels of training, including junior physicians with <2 years of experience. This is in contrast with agreement on CS, where inter-rater agreement was only fair, and 100% of the physicians disagreed with themselves on their second reading at least once. Even though INR physicians were the most consistent in their judgment of contrast, their agreement was only deemed "moderate." The lack of consensus on HT has been previously reported, and affects even simple dichotomized classifications such as hemorrhagic infarction vs. parenchymal hematoma (5), which are often used as outcome measures in clinical trials settings. While there are newly proposed rigorous classification systems for grading HT after ischemic stroke/reperfusion therapy, their reliability has not been assessed and they fail to address the issue of distinguishing CS from HT (8). The overall unreliable interdisciplinary interpretation of scans therefore may be attributed to unclear diagnostic criteria for CS and lack of additional imaging techniques to differentiate concurrent presence of both (9).

It is well-established that the phenomenon of CS can be seen after EVT, and is thought to relate to disruption of blood–brain barrier integrity in established ischemic infarct (10). Factors such as prolonged procedure time and multiple passes in the same vessel have been associated with higher risk for CS (11). The incidence of cerebral hyper-dense lesions after revascularization is high, and has been reported to be between 23 and 84%, depending on the definitions used and timing of follow-up imaging (10, 12). CS itself has been postulated to be associated with increased risk for HT and symptomatic ICH, although this is likely confounded by similar risk factors, such as large infarct size (13). Unfortunately, inaccurate detection of hemorrhage can lead to delayed initiation of anti-thrombotics, erroneous prognostication, and unnecessary investigations (9). It may be necessary to perform advanced imaging such as dual-energy CT (DECT) or gradient-recalled echo (GRE) sequence MRI for definitive diagnosis of hemorrhage vs. contrast (9, 14). However, MRI may be inaccessible to many centers in a timely fashion, and can still lead to false positive hemorrhage detection or false negative contrast extravasation if performed too soon after administration of contrast (14). DECT utilizes two distinctive voltage acquisitions to discriminate between materials with various attenuation properties, such as iodine vs. calcium or hemorrhage, but its availability is currently limited

across centers. Future studies may look at the combination of concurrent SWI with CT and compare them to plain CT images for reference.

Our study has important limitations. Imaging assessments were done in controlled settings with no time constraints and therefore results may differ from real-time clinical assessments. Accuracy analysis was not performed because of the extensive disagreements revealed between each rater, thereby defeating the relevance of such. While diagnostic accuracy was not the goal of the study, one potential way to address the lack of a "gold standard" would be the use of advanced imaging (i.e., MRI susceptibility based images or dual-energy CT scans). We recognize that the pragmatic approach to resolving disagreements and addressing uncertainty in imaging interpretation is effective communication between specialties. Lastly, this case series of patients was artificially constructed to minimize paradoxes of k statistics, and the exact results might not be reproducible in a different case series of patients.

CONCLUSION

There is a lack of agreement between physicians on the interpretation of post-EVT conventional CT scans for the presence/absence of both hemorrhage and contrast. Standardized definitions and clear diagnostic criteria for the two entities are warranted. Advanced imaging modalities such as DECT may be helpful in differentiating the two, if clinically indicated.

DATA AVAILABILITY STATEMENT

Access to the data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Fondation Rothschild Ethics Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

RL was responsible for performing the statistical analysis and writing the manuscript. RF was responsible for study design and data collection. All authors contributed to the writing and editing of the manuscript for intellectual content.

ACKNOWLEDGMENTS

DD has received grant funding from the Heart & Stroke foundation of Canada, and honoraria from Bayer and Apopharma unrelated to the submitted work. The corresponding author has full access to all the data in the study and has final responsibility for the decision to submit for publication.

REFERENCES

1. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. (2019) 50:e344–418. doi: 10.1161/STR.0000000000000211
2. Hao Y, Zhang Z, Zhang H, Xu L, Ye Z, Dai Q, et al. Risk of intracranial hemorrhage after endovascular treatment for acute ischemic stroke: systematic review and meta-analysis. *Interv Neurol*. (2017) 6:57–64. doi: 10.1159/000454721
3. Dekeyzer S, Nikoubashman O, Lutin B, De Groote J, Vancaester E, De Blauwe S, et al. Distinction between contrast staining and hemorrhage after endovascular stroke treatment: one CT is not enough. *J NeuroInterventional Surg*. (2017) 9:394–8. doi: 10.1136/neurintsurg-2016-012290
4. Lapergue B, Blanc R, Gory B, Labreuche J, Duhamel A, Marnat G, et al. Effect of endovascular contact aspiration vs. stent retriever on revascularization in patients with acute ischemic stroke and large vessel occlusion: the ASTER Randomized Clinical Trial. *JAMA*. (2017) 318:443–52. doi: 10.1001/jama.2017.9644
5. Guenego A, Lecler A, Raymond J, Sabben C, Khoury N, Premat K, et al. Hemorrhagic transformation after stroke: inter- and intrarater agreement. *Eur J Neurol*. (2019) 26:476–82. doi: 10.1111/ene.13859
6. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. (1977) 33:159–74.
7. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Med*. (2012) 22:276–82.
8. von Kummer R, Broderick JP, Campbell Bruce CV, Demchuk A, Goyal M, Hill MD, et al. The Heidelberg bleeding classification: classification of bleeding events after ischemic stroke and reperfusion therapy. *Stroke*. (2015) 46:2981–6. doi: 10.1161/STROKEAHA.115.010049
9. Almqvist H, Holmin S, Mazya MV. Dual energy CT after stroke thrombectomy alters assessment of hemorrhagic complications. *Neurology*. (2019) 93:e1068–75. doi: 10.1212/WNL.0000000000008093
10. Puntonet J, Richard M-E, Edjlali M, Ben Hassen W, Legrand L, Benzakoun, et al. Imaging findings after mechanical thrombectomy in acute ischemic stroke. *Stroke*. (2019) 50:1618–25. doi: 10.1161/STROKEAHA.118.024754
11. Chen Z, Zhang Y, Su Y, Sun Y, He Y, Chen H. Contrast extravasation is predictive of poor clinical outcomes in patients undergoing endovascular therapy for acute ischemic stroke in the anterior circulation. *J Stroke Cerebrovasc Dis*. (2020) 29:104494. doi: 10.1016/j.jstrokecerebrovasdis.2019.104494
12. Parrilla G, García-Villalba B, de Rueda ME, Zamarro J, Carrión E, Hernández-Fernández F, et al. Hemorrhage/contrast staining areas after mechanical intra-arterial thrombectomy in acute ischemic stroke: imaging findings and clinical significance. *AJNR Am J Neuroradiol*. (2012) 33:1791–6. doi: 10.3174/ajnr.A3044
13. Boisseau W, Fahed R, Lapergue B, Desilles J-P, Zuber K, Khoury N, et al. Predictors of parenchymal hematoma after mechanical thrombectomy. *Stroke*. (2019) 50:2364–70. doi: 10.1161/STROKEAHA.118.024512
14. Yedavalli V, Sammet S. Contrast extravasation versus hemorrhage after thrombectomy in patients with acute stroke. *J Neuroimaging*. (2017) 27:570–6. doi: 10.1111/jon.12446

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

SYSTEMATIC REVIEW
published: 21 January 2021
doi: 10.3389/fneur.2020.594613

Blood–Brain Barrier Disruption and Hemorrhagic Transformation in Acute Ischemic Stroke: Systematic Review and Meta-Analysis

Francesco Arba[1], Chiara Rinaldi[2], Danilo Caimano[2], Federica Vit[2], Giorgio Busto[3] and Enrico Fainardi[4]*

[1] Stroke Unit, AOU Careggi, Florence, Italy, [2] NEUROFARBA Department, University of Florence, Florence, Italy, [3] Neuroradiology, AOU Careggi, Florence, Italy, [4] Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

Introduction: Hemorrhagic transformation (HT) is a complication of reperfusion therapy for acute ischemic stroke. Blood–brain barrier (BBB) disruption is a crucial step toward HT; however, in clinical studies, there is still uncertainty about this relation. Hence, we conducted a systematic review and meta-analysis to summarize the current evidence.

Methods: We performed systematic review and meta-analysis of observational studies from January 1990 to March 2020 about the relation between BBB disruption and HT in patients with acute ischemic stroke with both computed tomography (CT) and magnetic resonance (MR) assessment of BBB. The outcome of interest was HT at follow-up imaging evaluation (within 48 h from symptom onset). We pooled data from available univariate odds ratios (ORs) in random-effects models with DerSimonian–Laird weights and extracted cumulative ORs.

Results: We included 30 eligible studies (14 with CT and 16 with MR), $N = 2{,}609$ patients, with 88% and 70% of patients included in CT and MR studies treated with acute stroke therapy, respectively. The majority of studies were retrospective and had high or unclear risk of bias. BBB disruption was measured with consistent methodology in CT studies, whereas in MR studies, there was more variability. All CT studies provided a BBB disruption cutoff predictive of HT. Four CT and 10 MR studies were included in the quantitative analysis. We found that BBB disruption was associated with HT with both CT (OR = 3.42; 95%CI = 1.62–7.23) and MR (OR = 9.34; 95%CI = 3.16–27.59). There was a likely publication bias particularly for MR studies.

Conclusion: Our results confirm that BBB disruption is associated with HT in both CT and MR studies. Compared with MR, CT has been more uniformly applied in the literature and has resulted in more consistent results. However, more efforts are needed for harmonization of protocols and methodology for implementation of BBB disruption as a neuroradiological marker in clinical practice.

Keywords: blood-brain-barrier, ischemic stroke, hemorrhagic transformation (HT), perfusion tomography, magnetic resoance imaging, intravenous thrombolysis, endovascular treatment (EVT)

OPEN ACCESS

Edited by:
Richard Leigh, Johns Hopkins University, United States

Reviewed by:
Kersten Villringer, Charité – Universitätsmedizin Berlin, Germany
Zurab Nadareishvili, George Washington University, United States

****Correspondence:***
Francesco Arba
francesco.arba@unifi.it

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *13 August 2020*
Accepted: *07 December 2020*
Published: *21 January 2021*

Citation:
Arba F, Rinaldi C, Caimano D, Vit F, Busto G and Fainardi E (2021) Blood–Brain Barrier Disruption and Hemorrhagic Transformation in Acute Ischemic Stroke: Systematic Review and Meta-Analysis. Front. Neurol. 11:594613. doi: 10.3389/fneur.2020.594613

INTRODUCTION

Ischemic stroke is a major cause of death and disability all over the world. In the last decades, acute treatments aiming to recanalize the occluded vessel demonstrated efficacy in reducing the functional burden of the disease; however, reperfusion of the ischemic tissue brings some risk, with the most feared being hemorrhagic transformation (HT). HT is a common phenomenon after brain ischemia, occurs in up to 40% of patients treated with acute stroke therapy (1), and is fatal in around 3% of patients (2). Identification of factors predictive of HT is therefore important to stratify the hemorrhagic risk of patients and for management of the hyperacute stroke phase.

In vitro and *in vivo* models suggested failure of endothelial integrity and loss of neurovascular homeostasis as the cellular mechanisms underlying blood extravasation (3, 4) and, from a structural point of view, disruption of the blood–brain barrier (BBB) as the pathophysiological step that leads to HT (5, 6). *In vivo* visualization and measurement of BBB disruption in the acute stroke setting before reperfusion therapy may represent a useful marker to identify patients more prone to develop HT. BBB disruption can be evaluated with either computed tomography (CT) or magnetic resonance (MR). Within each imaging technique, BBB disruption can be investigated using different algorithms that mainly measure contrast extravasation through microcirculation, with either qualitative or quantitative methods (7).

Although diverse studies have provided data about the link between BBB disruption and HT, no conclusive evidence is available, and BBB disruption, although potentially a useful biomarker, has not yet been adopted in clinical practice with regard to acute ischemic stroke. To investigate the effect of BBB disruption on HT and provide more precise estimate of this effect, we performed a systematic review of studies that evaluated BBB disruption with either CT or MR in acute ischemic stroke setting and subsequent HT.

METHODS

This review was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (8) and Meta-analysis Of Observational Studies in Epidemiology (MOOSE) (9) recommendations and the Cochrane Handbook for Systematic Review of Interventions (https://training.cochrane.org/handbook). Data search, extraction, analysis, and interpretation were performed following a pre-specified study protocol developed by the investigators (not registered or published).

Search Strategy and Selection Criteria

Potentially eligible studies were identified using PubMed and EMBASE databases by two independent investigators (FA and CR). Discrepancies were solved by consensus of all authors. We searched for eligible published studies in English, from January 1990 to March 2020, using the following search strategy: ("acute ischemic stroke" OR ((stroke OR "Acute Cerebrovascular Accident" OR "Acute Cerebrovascular Accidents") AND (ischaemic OR ischemic))) AND ((hemorrhag* OR haemorrhag* OR "parenchymal hematoma" OR "Cerebral Hemorrhage"[Mesh]) AND (Transform* OR Petechia* OR subsequent OR infarct*)) AND ("computed tomography perfusion" OR "CT perfusion" OR "perfusion computed tomography" OR "perfusion CT" OR "computed tomographic perfusion" OR (("Perfusion"[Mesh] OR "Perfusion Imaging"[MeSH Terms])AND "Tomography, X-Ray Computed"[Mesh])) for CT studies in PubMed; ("acute ischemic stroke" OR ((stroke OR "Acute Cerebrovascular Accident" OR "Acute Cerebrovascular Accidents") AND (ischaemic OR ischemic))) AND ((hemorrhag* OR haemorrhag* OR "parenchymal hematoma" OR "Cerebral Hemorrhage"[Mesh]) AND (Transform* OR Petechia* OR subsequent OR infarct*)) AND (("Magnetic Resonance Imaging"[Mesh] OR "NMR Imaging" OR "MR Tomography" OR "NMR Tomography" OR "Steady State Free Precession MRI" OR Zeugmatography OR "Proton Spin Tomography" OR "MRI Scans" OR "MRI Scan" OR "Spin Echo Imaging" OR "magnetization transfer" OR "magnetic resonance imaging" OR "magnetic resonance tomography" OR "mr imaging" OR MRI OR "magnetic resonance") AND (permeability OR "blood-brain-barrier" OR BBB)).

The reference list of eligible studies was screened to identify additional publications suitable for our purposes not included in the original list. We applied the following inclusion criteria: (1) English-written articles; (2) patients with acute ischemic stroke; (3) studies with observational (retrospective or prospective) design; (4) patients treated or not with acute stroke therapy (i.e., intravenous thrombolysis, intra-arterial procedures, or both); (5) assessment of BBB disruption with CT or MR scan before any acute stroke treatment; (6) assessment of HT at the follow-up CT or MR scan within 48 h from the first scan; and (7) studies with more than 10 patients. Case reports, conference abstracts, study protocols, and unpublished studies were not included. We also excluded experimental or animal studies. Where studies had overlapping cohorts, only the study with the largest sample size was included. We included studies that evaluated BBB disruption as either continuous or categorical (dichotomized) variable. Assessment of BBB disruption was defined either as quantitative when a numerical value within a continuous scale was provided or as qualitative when a visual rating (e.g., presence vs. absence of contrast parenchymal enhancement) was provided. Localization of BBB disruption was defined as follows: "focal" when the BBB disruption was detected and measured only in a restricted area of the whole ischemic tissue and "global" when the BBB disruption was detected and measured in the whole ischemic tissue.

Risk of Bias Assessment

Three investigators (CR, DC, and FV) independently extracted data from relevant studies using a predefined form including the following sections: (1) year of publication and study period; (2) study design; (3) inclusion and exclusion criteria; (4) clinical and radiological data; (5) definition and measurement of BBB; and (6) definition of HT. The same three investigators assessed study quality and risk of bias using the Newcastle-Ottawa Scale for cohort studies and the Cochrane "Tool to Assess Risk of Bias

in Cohort Studies" (https://methods.cochrane.org/). In case of uncertainty, the final decision was taken by an expert (FA).

Outcome

Our main outcome of interest was HT evaluated with CT or MR scan within 48 h from the first scan. We included studies with the following definitions of HT: (1) presence/absence; (2) ECASS-2 (European Co-operative Acute Stroke Study-II) (3) (10) NINDS (National Institute of Neurological Disorders and Stroke) (11) criteria; and (4) SITS-MOST (Safe Implementation of Thrombolysis in Stroke: Monitoring Study) (12) criteria.

Statistical Analysis

Data were pooled in the meta-analysis when at least two studies had available data on the main outcome of interest, i.e., HT. In all analyses, we used a random-effects model with DerSimonian–Laird weights. The direction and strength of the association between BBB permeability and HT were quantified using crude (i.e., unadjusted) odds ratio (OR) and their corresponding 95% confidence intervals (CIs), with the inverse variance method for weighting. We therefore included in the quantitative analysis (i.e., meta-analysis) only studies with available unadjusted OR. Statistical heterogeneity was assessed with I^2 statistics and visual inspection of forest plots. Values of $\leq$25, 25 to 50, and $\geq$50% were defined as low, moderate, and high degrees of heterogeneity, respectively. Publication bias was explored on funnel plots. All the analyses were performed in May 2020 using the meta-analysis software RevMan 5 (https://community.cochrane.org/).

Data Availability

Requests to access the dataset from qualified researchers trained in human subject confidentiality protocols may be sent to the corresponding author.

RESULTS

The initial search retrieved 656 results. After removing 189 duplicates, we screened 467 titles and abstracts and excluded 434 articles. We therefore examined 33 full-text articles and excluded two studies for sample size <10 patients, one study for overlapping cohort, two studies for an inadequate follow-up rate, and one study for missing HT assessment. We retrieved three studies from reference snowballing; we therefore included in the systematic review 30 articles. Of these, 14 had BBB assessment with CT (13–26) and 16 with MR (27–42), with a total of 2,609 patients (1,510 with CT and 1,099 with MR). The study selection process is illustrated in **Figure 1**. Data for the quantitative analysis (meta-analysis) were available for 1,298 (50%) patients, from 4/14 studies with CT (794 patients, 53% of patients included in CT studies) and 9/16 MR studies (504 patients, 45% of patients included in MR studies). Clinical data of studies included in the meta-analysis are shown in **Table 1**.

Computed Tomography Studies

Five studies were found as having a low risk of bias (15, 21, 23, 24, 26), seven a high risk (13, 17–20, 22, 25), and two an unclear risk (14, 16) (**Supplemental Table 1**). The general clinical characteristics of the included studies evaluating BBB with CT are summarized in the **Supplemental Table 2**. Studies were performed between 2004 and 2020; were from Europe (5), Asia (5), and North America (4); and were mainly single-center clinical cohorts, and five were with prospective design. Four studies had a sample size larger than 100 patients. Among 1,510 patients, a total of 359 (24%) had HT of any grade. The great majority of included patients (1,328/1,510; 88%) were treated with acute stroke therapy, including intravenous thrombolysis (N = 921), mechanical thrombectomy (N = 145), both (N = 58), and intra-arterial procedures (other than mechanical thrombectomy, N = 204); 150 (10%) patients received no treatment. For 32 (2%) patients, treatment type was not available. BBB assessment was performed within 24 h from symptom onset in all included studies. BBB disruption was measured with quantitative models in all studies: five studies used Ktrans, eight studies permeability surface (PS) products, and one study both parameters. Seven studies assessed focal BBB disruption, two studies global BBB disruption, and three studies both; in two studies, the localization of BBB assessment was not available. The radiological characteristics of the included studies are shown in **Supplemental Table 3**. Vendor largely varied across and within studies, slice number of scan acquisition ranged from 40 to 320, coverage ranged from 2.4 to 9 cm (five studies did not provide coverage), images were obtained with single-phase acquisition protocols in 10 studies and with a two-phase acquisition protocol in three studies, and acquisition time ranged across studies from 40 to 1,092 s. All CT studies performed a quantitative BBB disruption evaluation, which was made with the Patlak model in seven studies, with the non-linear-regression model in two studies and the Johnson–Wilson model in two studies, whereas in two studies, the model was not stated. Eleven studies provided a cutoff of BBB disruption predictive of HT (**Supplemental Table 3**), which ranged from 0.33 to 7 ml/100 mg/min in studies with Ktrans and from 0.23 to 6 ml/100 mg/min in studies with PS.

Univariate ORs were present in four studies (N = 794)—two prospective (24, 26) and two retrospective (15, 23)—other studies provided only multivariate or did not provide ORs. In the meta-analysis of the four aforementioned studies, BBB disruption was associated with HT (OR = 3.42; 95%CI = 1.62–7.23). We found moderate statistical heterogeneity across studies (I^2 = 51%; p = 0.001) (**Figure 2**). Visual inspection of funnel plots showed slight asymmetry, suggesting possible presence of publication bias **Supplemental Figure 1**; we did not perform further tests to explore publication bias since we pooled <10 studies.

Magnetic Resonance Studies

Five studies were found as having a low risk of bias (27, 38, 39, 41, 42), eight a high risk (28–31, 33, 34, 37, 40), and three an unclear risk (32, 35, 36) (**Supplemental Table 4**). The general clinical characteristics of the included studies evaluating BBB with MR are summarized in the **Supplemental Table 5**. Studies were performed between 1997 and 2018 and were from North America (7), Europe (4), Asia (2), or international cohorts (2) and were mainly single-center clinical cohorts; all but one are with retrospective design. Three studies had a sample size larger than

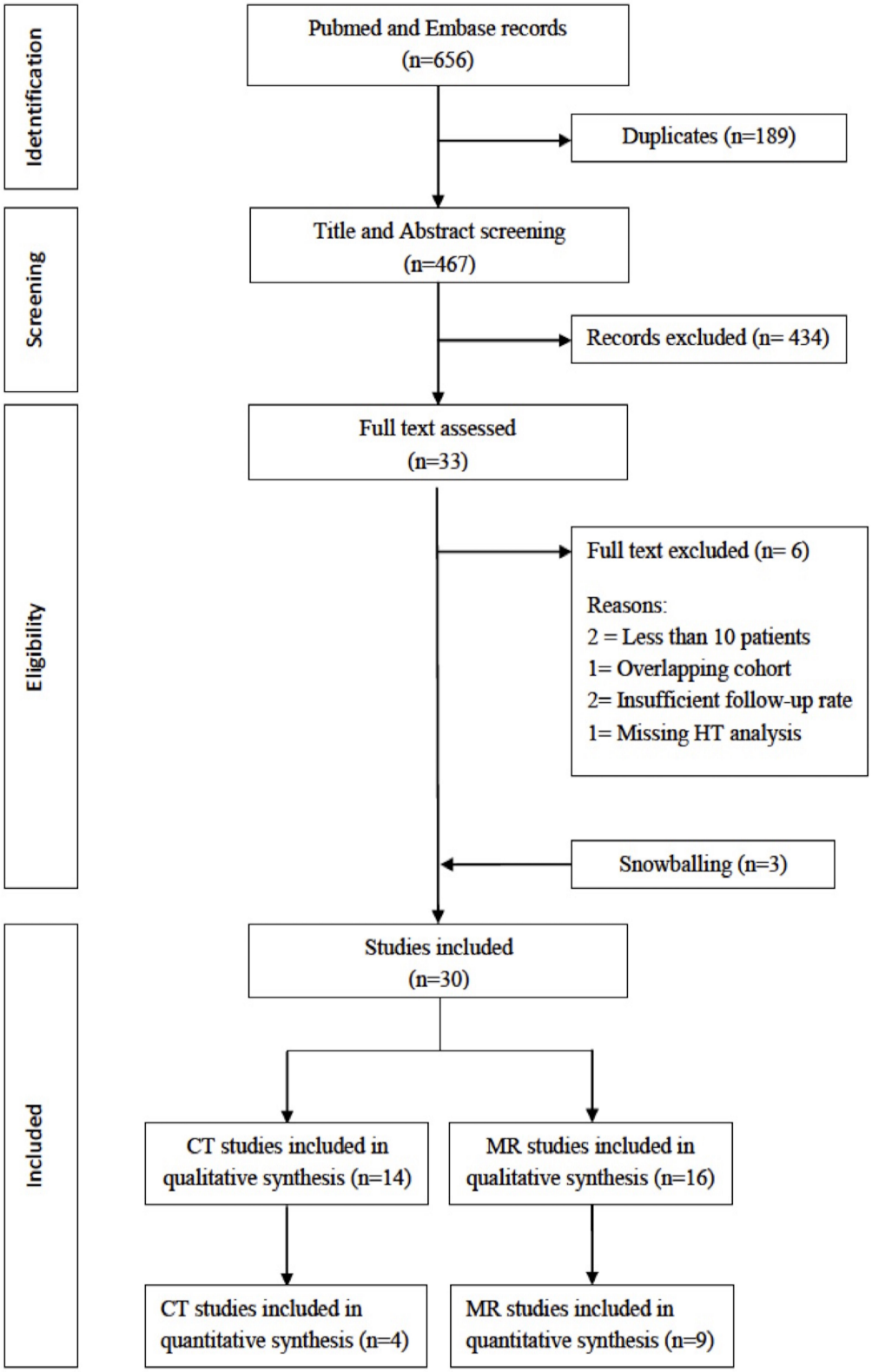


FIGURE 1 | Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flowchart of study selection.

TABLE 1 | Summary of clinical data of studies included in meta-analysis.

References	Design	Country and inclusion period	Sample size, *N*	Acute treatment, *N*	Stroke location, time from onset	Imaging type	BBB measurement type	BBB localization	Age, mean (±SD)	NIHSS, median (IQR) or mean (±SD)	HT, *N* (type)
Arba et al. (26)	Prospective, SC	Italy, 2015–2018	171	32 i.v. rt-PA, 102 MT, 37 both	AC, <12 h	TC	Ktrans	F, G	76 (±12)	18 (12–23)	31 (HI-2, PH-1, PH-2), 12 sICH
Hom et al. (15)	Retrospective, SC	USA, 2006–2009	32	NA	AC, <12 h	TC	PS	G	72 (65–85)	14 (10–17)	3 (PH-2 = 3)
Horsch et al. (23)	Retrospective, MC	Netherlands, 2009–2013	545	501 i.v. rt-PA, 44 IAT and/or MT	NA, <9 h	TC	PS	NA	68 (58–77)	8 (4–13)	57 (HI-1 = 12, HI-2 = 17, PH-1 = 15, PH-2 = 13)
Kim et al. (24)	Prospective, SC	South Korea, 2013–2015	46	22 i.v. rt-PA, 12 MT, 12 both	AC, <6 h	TC	Ktrans	NA	66 (±12)	11 (8–16)	15 (HI-1 = 6, HI-2 = 2, PH-1 = 3, PH-2 = 4)
Bang et al. (29)	Retrospective, SC	USA, 2004–2006	32	13 i.v. rt-PA, 1 i.v. rt-PA+ IAT, 12 MT, 6 i.v. rt-PA+MT	MCA territory, NA	MRI	Gd enhancement	F	67 (±20)	NA	12 (HI-1 = 1, HI-2 = 2, PH-1 = 1, PH-2 = 5, SAH = 1, remote ICH = 1)
Hjort et al. (30)	Prospective, SC	Denmark, 2004–2006	33	33 i.v. rt-PA	MCA territory, <3 h	MRI	Gd enhancement	F	68 (±8)	11 (±6)	16 (HI = 13, PH = 3)
Kastrup et al. (32)	Retrospective, SC	Germany, NA	100	100 i.v. rt-PA	NA, (treated before<6 h)	MRI	Gd enhancement	F	67 (±14)	11 (7.5–15.5)	9 (PH-1 = 5, PH-2 = 4)
Kim et al. (28)	Retrospective, SC	Korea, 1997–2003	55	15 i.v. rt-PA, 40 no treatment	MCA territory, <6 h	MRI	Gd enhancement	G	68.8 (±10.8)	15.0 (±5.6)	19 (HI = 14, PH = 5)
Latour et al. (27)	Retrospective, SC	USA, 2000–2002	119	28 i.v. rt-PA, 1 i.a. rt-PA, 90 no treatment	NA, <24 h	MRI	Gd enhancement	F	72.3 (±13.5)	7.85 (±8.62)	22 (NA)
Lee et al. (35)	Retrospective, SC	USA, 2001–2009	14	1 i.v. rt-PA, 1 IAT, 8 MT, 4 both	PC, NA	MRI	Gd enhancement	F	71.1 (NA)	20.5 (range 0–36)	5 (HI-1 = 1, HI-2 = 2, PH2 = 1, intra-ventricular = 1)
Liu et al. (36)	Retrospective, SC	China, 2000–2004	26	26 no treatment	AC, NA	MRI	Ktrans	F	56.10 (±17.48)	NA	10 (NA)
Nael et al. (42)	Retrospective, MC	USA, 2004–2012	83	13 i.v. rt-PA, 23 MT, 18 both, 29 no treatment	AC, <8 h	MRI	K2	G	66 (±15.2)	17 (13–21)	20 (PH = 20)
Rozanski et al. (34)	Retrospective, SC	Germany, 2008	47	10 i.v. rt-PA, 37 no treatment	NA, <24 h	MRI	Gd enhancement	F	83.9 (NA)	5 (range 0–20)	8 (HI-1 = 0, HI-2 = 4, PH-1 = 1, PH-2 = 1, sICH = 2)
Leigh et al. (39)	Retrospective, MC	USA, Austria 2008–2011	100	47 MT, 53 MT+ i.v. rt-PA	NA, <12 h	MRI	K2	F	65.6 (NA)	15.1 (±NA)	57 (HI = 33, PH = 24)

BB, blood–brain barrier; SC, single center; MC, multicenter; AC, anterior circulation; PC, posterior circulation; MCA, middle cerebral artery; rt-PA, recombinant tissue-plasminogen activator; i.v., intravenous; i.a., intra-arterial; IAT, intra-arterial thrombolysis; MT, mechanical thrombectomy; Gd, gadolinium; IQR, interquartile range; F, focal; G, global; SD, standard deviation; NIHSS, National Institute of Health Stroke Scale; HT, hemorrhagic transformation; HI, hemorrhagic infarction; PH, parenchymal hematoma; SAH, subarachnoid hemorrhage; ICH, intracranial hemorrhage; sICH, symptomatic intracranial hemorrhage; Ktrans, volume transfer constant; PS, permeability surface area products; rR, relative recirculation; %Recovery, percentage recovery; PB, post-bolus area; MPB, mean post-bolus intensity; CS, contrast slope; FC, final contrast; K2, tissue-to-blood transfer constant.

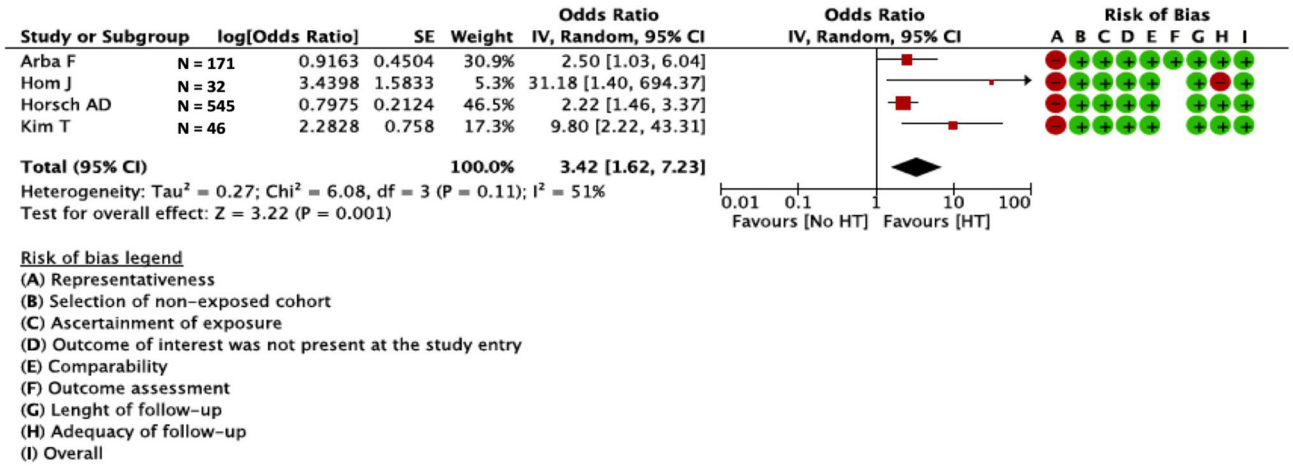


FIGURE 2 | Relation between BBB and HT in CT studies. BBB, blood–brain barrier; HT, hemorrhagic transformation; CT, computed tomography.

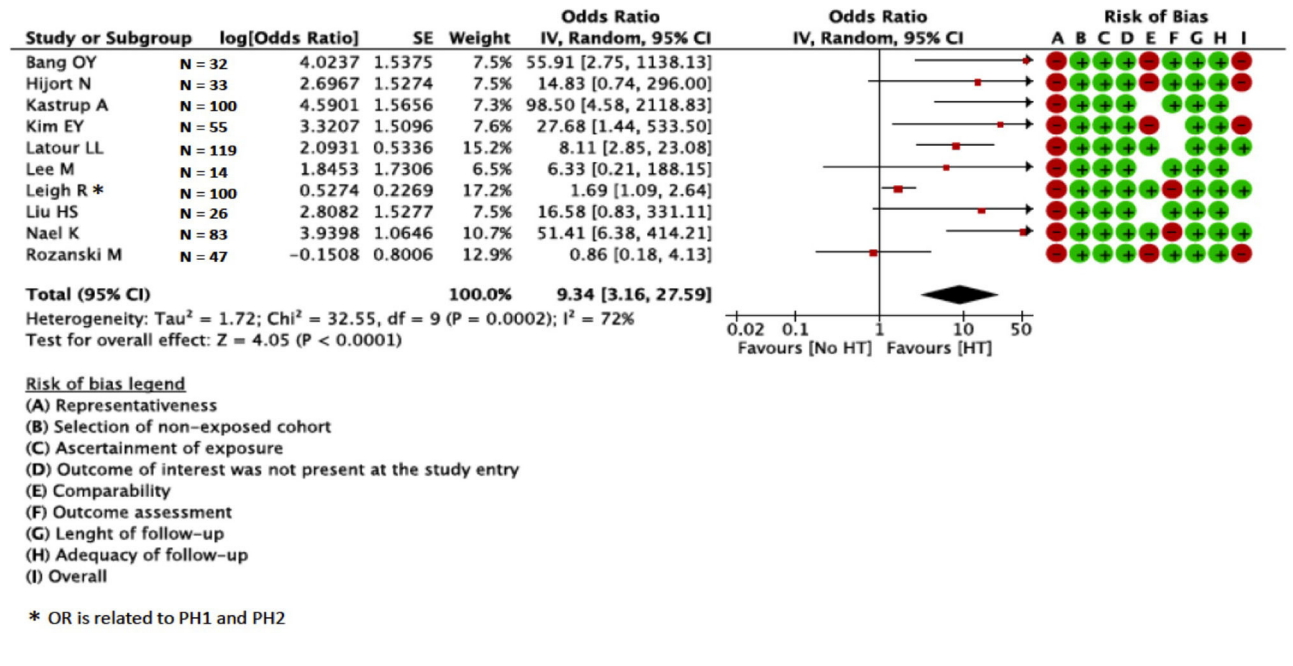


FIGURE 3 | Relation between BBB and HT in MR studies. BBB, blood–brain barrier; HT, hemorrhagic transformation; MR, magnetic resonance.

100 patients. Among 1,099 patients, a total of 326 (29.7%) had HT of any grade. The majority of included patients (759/1099; 69%) were treated with acute stroke therapy, including intravenous thrombolysis ($N = 502$), mechanical thrombectomy ($N = 146$), both ($N = 109$), and intra-arterial procedures (not specified, $N = 2$); 340 (31%) patients received no acute treatment. In nine studies, BBB assessment was performed within 24 h from symptom onset, whereas in seven studies, information about time of BBB assessment was not available. Twelve studies assessed focal, and four studies global BBB disruption. The radiological characteristics of the included studies are shown in the **Supplemental Table 6**. Compared with CT studies, there was less variability in vendor type, but three studies did not state the vendor type. Imaging studies were performed mainly on 1.5-T scanners; in two studies, magnetic field was not available. Section thickness was 5 mm in the majority of studies, although it was not specified in five studies; acquisition time ranged from more than 60 s to nearly 5 min and was not stated in 10 studies. Six studies measured BBB disruption with qualitative parameters such as parenchymal enhancement or extravasation of contrast, whereas seven studies provided quantitative measurements of BBB disruption. Of the latter studies, two studies used the Patlak

model, and five studies used first-pass T2* method. Only four studies, using different BBB measurements, provided a BBB disruption cutoff predictive of HT.

Univariate ORs were present in 10 studies ($N = 609$)—one was prospective (30), nine were retrospective (27–29, 32–36, 39, 42), and three studies provided quantitative ($N = 209$) and seven qualitative ($N = 400$) BBB disruption assessment. Other studies provided only multivariate or did not provide OR. In the meta-analysis, BBB disruption was associated with HT (OR = 9.34; 95%CI = 3.16–27.59) (**Figure 3**). We found high statistical heterogeneity across studies ($I^2 = 72\%$; $p < 0.00001$). The association between BBB disruption and HT was confirmed in the sensitivity analysis for studies with qualitative assessment of BBB (OR = 9.96; 95%CI = 2.82–35.20; **Supplemental Figure 2**), whereas the association was not confirmed for studies with quantitative assessment (OR = 9.33; 95%CI = 0.76–114.39; **Supplemental Figure 3**). Visual inspection of funnel plots showed asymmetry suggesting presence of publication bias (**Supplemental Figure 4**); we did not perform further tests to explore publication bias since we pooled 10 studies.

DISCUSSION

In this systematic review, we brought together available observational studies regarding the relation between BBB disruption and HT in acute ischemic stroke patients. Overall, our results confirmed the association between BBB disruption and HT. While CT studies showed a three-fold increased risk of HT, MR studies showed a nine-fold increased risk of HT, with around six-fold increased difference in MR studies. Among studies included in the meta-analysis, only three (36, 39, 42) out of 10 MR studies had quantitative assessment of BBB disruption; MR studies had smaller sample size compared with CT studies, as reflected by the wider confidence intervals in the pooled MR analysis. Furthermore, in the sensitivity analysis for MR, quantitative BBB disruption was not associated with HT, whereas the association was confirmed in qualitative studies. It is important to note that the majority of the included studies were retrospective, with a high risk of bias and a likely publication bias; thus, our conclusions have limitations.

We found more MR studies with assessment of BBB, and the rate of HT was higher in MR studies, perhaps alluding to the higher sensitivity of MR in diagnosis of HT. By contrast, the pool of patients included in studies with CT was larger, and methodology and protocols were generally more consistent with each other. Patients enrolled in CT studies were more frequently treated with acute stroke therapy than those enrolled in MR studies. Given that acute stroke treatment increases the odds of HT occurrence, the results from CT studies mainly apply to patients treated with acute stroke therapy. Conversely, results from MR studies, although more generalizable to all ischemic stroke patients, included more heterogeneity with regard to treatment, with around a third of patients not treated with acute stroke treatment. While all CT studies provided a time frame for the study inclusion, around a half of MR had missing information about the time from onset of stroke to enrollment in the study. This is an important limitation, because extent of BBB disruption is thought to be time-dependent (43–45), and the timing of BBB assessment is therefore a pivotal information. Finally, occlusion site is fundamental for stroke therapy, since endovascular therapy proved efficacy only in anterior circulation (46), whereas in the posterior circulation, there is no conclusive evidence (47). Evaluation of BBB disruption may be challenging with CT in the posterior circulation due to the limits of perfusion technique in this area (48), whereas it is feasible with MR with dynamic contrast-enhanced sequences. However, the majority of CT studies enrolled patients with ischemic stroke in the anterior circulation, whereas the site of occlusion was not stated in many MR studies. As confirmation of the methodological variability of MR studies, we found a high statistical heterogeneity for MR studies compared with moderate heterogeneity for CT studies. Although we acknowledge that the precision of MR in detecting BBB disruption and HT is likely superior to CT, all those methodological pitfalls of MR studies limit considerably direct transferability of results into clinical practice.

There are diverse methods for evaluation of BBB disruption. BBB was measured with Ktrans or PS in CT studies, whereas MR studies adopted qualitative and quantitative methods, although some study did not specify how BBB was measured. Remarkably, all CT studies provided quantitative measurements with continuous values for BBB disruption, whereas around a half of MR studies provided qualitative evaluation of BBB, i.e., presence/absence of contrast leakage. BBB disruption is a dynamic process that varies with age, vascular risk factors such as diabetes and hypertension, and pre-existing characteristics of the brain (49, 50). Some degree of BBB disruption may be present up to 95% of patients with acute stroke within the ischemic area (51); thus, quantitative measurement of BBB disruption is useful to provide a precise estimate of HT risk. In order to differentiate pathologic from physiologic BBB leakage, it is also important to identify a cutoff value predictive of HT, since qualitative assessment of BBB disruption (i.e., presence/absence), although easily detectable, may not provide enough information to accurately estimate risk of subsequent HT. However, we observed a large inconsistency among cutoffs provided among diverse studies. Two meta-analyses were attempted to provide diagnostic accuracy of CT parameters, including BBB disruption, in predicting HT (52, 53). Although both studies found that BBB disruption has good sensitivity and specificity for HT prediction, there was high heterogeneity across studies due to several reasons, particularly differences in protocols and methods of CT perfusion for BBB evaluation. This is in keeping with our results that confirmed moderate and high statistical heterogeneity for CT and MR studies, respectively, and showed relevant differences across studies in qualitative analysis, highlighting the need of standardized and replicable protocols for BBB assessment in clinical setting.

Evaluation of BBB disruption may help in early stratification of hemorrhagic risk in patients with ischemic stroke, particularly those treated with intravenous thrombolysis and/or endovascular procedures (5, 40). HT extent may range from single-blood petechiae with few clinical consequences to symptomatic HT

with a high rate of mortality and disability. While most studies reported the association between BBB disruption and HT, only few studies investigated the relation with unfavorable outcomes such as symptomatic intracranial hemorrhage (sICH) (20, 26, 34); therefore, this association, although potentially useful for clinicians, is still unclear and needs to be further clarified.

Our study has limitations that need to be addressed. Our quantitative analysis is based on unadjusted pooled estimates, and therefore, the association was not adjusted for other covariates and predictors of HT, such as age, stroke severity, and time from symptom onset to imaging. However, we extracted from the studies mean age of patients, stroke severity, site of occlusion, and time from onset to enrolment, and we observed that particularly in CT studies, such variables were similar, whereas in MR studies, there was more difference. It should be noted that only 4/14 CT studies (15, 23, 24, 26) and 10/16 MR studies (27–30, 32, 34–36, 42) reported univariate associations between BBB disruption and HT and were included in the meta-analysis; consequently, the ORs were found to represent a gross estimate of the relation between BBB and HT and should be interpreted with caution. Many CT and MR studies reported only adjusted associations; however, the sets of covariates largely differed across studies; thus, pooling-adjusted ORs were potentially inappropriate. This was also reflected by the number of studies with high or unclear risk of bias, mainly due to the adjusted analysis that often included covariates not relevant for the outcome of interest. Furthermore, funnel plots of studies included in the quantitative analysis suggested the presence of publication bias particularly for MR studies, possibly inflating the magnitude of effect of the association between BBB disruption and HT. Again, we examined studies from 1990 to 2020, and this may be a limitation due to the evolution in methods and technology for imaging in such a large time span. However, with a 30-year period of evaluation, our qualitative analysis is a comprehensive synthesis of available studies relevant for the topic, and the quantitative analysis attempted to provide ORs useful for future research. As a further limitation, we included only English-written studies.

More data are needed to overcome the limitations of current evidence about BBB disruption and HT. Our results suggest that protocols for BBB assessment need to increase consistency, BBB disruption evaluation should be quantitative, and future studies should provide a cutoff predictive of HT, preferably sICH or relevant HT. Furthermore, methodology and workflow of the studies should be easily reproducible and accessible. In this regard, as previously suggested (54, 55), CT seems to represent a fair trade-off between diagnostic detail and feasibility in acute stroke setting due to availability and few contraindications; however, MR is likely more accurate in detection of both BBB disruption and HT. Use of machine learning algorithms for MR (56) may help standardization of acquisition protocols and assessment of BBB in acute stroke; however, there is still no available evidence in this regard. Our results from the meta-analysis show that the association between BBB and HT is confirmed with both CT and MR, although with relevant limitations. Results from the systematic review suggest ease of standardized acquisition protocols, similar methodology, and similar characteristics of study population, which are the strengths of CT studies over MR, whereas the lack of standardized measurements for BBB disruption and quantitative cutoffs predictive of HT are the pitfalls of both CT studies and MR studies. Future studies need to define feasibility of protocols for BBB assessment and whether BBB disruption may serve as an adjunctive marker to identify patients at risk of HT, thus helping decision making and management of acute ischemic stroke patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

FA conceived the study, contributed to the critical analysis of data, performed the statistical analysis, and drafted the paper. CR, DC, and FV contributed to the critical analysis of data, selected the studies, and drafted the paper. GB and EF contributed to the critical analysis of data and drafted the paper. All authors reviewed and approved the manuscript.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Tessa Piazzini for her invaluable help in developing the search strategy.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.594613/full#supplementary-material

REFERENCES

1. Balami JS, Chen RL, Grunwald IQ, Buchan AM. Neurological complications of acute ischaemic stroke. *Lancet Neurol.* (2011) 10:357–71. doi: 10.1016/S1474-4422(10)70313-6
2. Whiteley WN, Emberson J, Lees KR, Blackwell L, Albers G, Bluhmki E, et al. Risk of intracerebral haemorrhage with alteplase after acute ischaemic stroke: a secondary analysis of an individual patient data meta-analysis. *Lancet Neurol.* (2016) 15:925–33. doi: 10.1016/S1474-4422(16)30076-X
3. Del Zoppo GJ. The neurovascular unit in the setting of stroke. *J Intern Med.* (2010) 267:156–71. doi: 10.1111/j.1365-2796.2009.02199.x
4. Warach S, Latour LL. Evidence of reperfusion injury, exacerbated by thrombolytic therapy, in human focal brain ischemia using a novel imaging marker of early blood-brain barrier disruption. *Stroke.* (2004) 35:2659–61. doi: 10.1161/01.STR.0000144051.32131.09
5. Khatri R, McKinney AM, Swenson B, Janardhan V. Blood-brain barrier, reperfusion injury, and hemorrhagic transformation in acute ischemic stroke. *Neurology.* (2012) 79:S52–S7. doi: 10.1212/WNL.0b013e3182697e70

6. Álvarez-Sabín J, Maisterra O, Santamarina E, Kase CS. Factors influencing haemorrhagic transformation in ischaemic stroke. *Lancet Neurol.* (2013) 12:689–705. doi: 10.1016/S1474-4422(13)70055-3
7. Kassner A, Merali Z. Assessment of blood-brain barrier disruption in stroke. *Stroke.* (2015) 46:3310–5. doi: 10.1161/STROKEAHA.115.008861
8. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med.* (2009) 6:e1000100. doi: 10.1371/journal.pmed.1000100
9. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. *JAMA.* (2000) 283:2008–2012. doi: 10.1001/jama.283.15.2008
10. Hacke W, Kaste M, Fieschi C, Von Kummer R, Davalos A, Meier D, et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). *Lancet.* (1998) 352:1245–51. doi: 10.1016/S0140-6736(98)08020-9
11. National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med.* (1995) 333:1581–8. doi: 10.1056/NEJM199512143332401
12. Wahlgren N, Ahmed N, Eriksson N, Aichner F, Bluhmki E, Dávalos A, et al. Multivariable analysis of outcome predictors and adjustment of main outcome results to baseline data profile in randomized controlled trials: safe implementation of thrombolysis in Stroke-MOnitoring STudy (SITS-MOST). *Stroke.* (2008) 39:3316–22. doi: 10.1161/STROKEAHA.107.510768
13. Lin K, Kazmi KS, Law M, Babb J, Peccerelli N, Pramanik BK. Measuring elevated microvascular permeability and predicting hemorrhagic transformation in acute ischemic stroke using first-pass dynamic perfusion CT imaging. *AJNR Am J Neuroradiol.* (2007) 28:1292–8. doi: 10.3174/ajnr.A0539
14. Aviv RI, D'Esterre CD, Murphy BD, Hopyan JJ, Buck B, Mallia G, et al. Hemorrhagic transformation of ischemic stroke: prediction with CT perfusion. *Radiology.* (2009) 250:867–77. doi: 10.1148/radiol.2503080257
15. Hom J, Dankbaar JW, Soares BP, Schneider T, Cheng SC, Bredno J, et al. Blood-brain barrier permeability assessed by perfusion ct predicts symptomatic hemorrhagic transformation and malignant edema in acute ischemic stroke. *AJNR Am J Neuroradiol.* (2011) 32:41–8. doi: 10.3174/ajnr.A2244
16. Ozkul-Wermester O, Guegan-Massardier E, Triquenot A, Borden A, Perot G, Gérardin E. Increased blood-brain barrier permeability on perfusion computed tomography predicts hemorrhagic transformation in acute ischemic stroke. *Eur Neurol.* (2014) 72:45–53. doi: 10.1159/000358297
17. Bennink E, Horsch AD, Dankbaar JW, Velthuis BK, Viergever MA, de Jong HW. CT perfusion analysis by nonlinear regression for predicting hemorrhagic transformation in ischemic stroke. *Med Phys.* (2015) 42:4610–8. doi: 10.1118/1.4923751
18. Yen P, Cobb A, Shankar JJS. Does computed tomography permeability predict hemorrhagic transformation after ischemic stroke? *World J Radiol.* (2016) 8:594. doi: 10.4329/wjr.v8.i6.594
19. Chen H, Liu N, Li Y, Wintermark M, Jackson A, Wu B, et al. Mismatch of low perfusion and high permeability predicts hemorrhagic transformation region in acute ischemic stroke patients treated with intra-arterial thrombolysis. *Sci Rep.* (2016) 6:27950. doi: 10.1038/srep27950
20. Li Y, Xia Y, Chen H, Liu N, Jackson A, Wintermark M, et al. Focal low and global high permeability predict the possibility, risk, and location of hemorrhagic transformation following intra-arterial thrombolysis therapy in acute stroke. *AJNR Am J Neuroradiol.* (2017) 38:1730–6. doi: 10.3174/ajnr.A5287
21. Li Q, Gao X, Yao Z, Feng X, He H, Xue J, et al. Permeability surface of deep middle cerebral artery territory on computed tomographic perfusion predicts hemorrhagic transformation after stroke. *Stroke.* (2017) 48:2412–8. doi: 10.1161/STROKEAHA.117.017486
22. Puig J, Blasco G, Daunis-I-Estadella P, Van Eendendburg C, Carrillo-García M, Aboud C, et al. High-permeability region size on perfusion CT predicts hemorrhagic transformation after intravenous thrombolysis in stroke. *PLoS ONE.* (2017) 12:e0188238. doi: 10.1371/journal.pone.0188238
23. Horsch AD, Bennink E, Van Seeters T, Kappelle LJ, Van Der Graaf Y, Mali WPTM, et al. Computed tomography perfusion derived blood-brain barrier permeability does not yet improve prediction of hemorrhagic transformation. *Cerebrovasc Dis.* (2018) 45:26–32. doi: 10.1159/000485043
24. Kim T, Koo J, Kim SH, Song IU, Chung SW, Lee KS. Blood-brain barrier permeability assessed by perfusion computed tomography predicts hemorrhagic transformation in acute reperfusion therapy. *Neurol Sci.* (2018) 39:1579–84. doi: 10.1007/s10072-018-3468-1
25. Li X, Liu H, Zeng W, Liu X, Wen Y, Xiong Q, et al. The value of whole-brain perfusion parameters combined with multiphase computed tomography angiography in predicting hemorrhagic transformation in ischemic stroke. *J Stroke Cerebrovasc Dis.* (2020) 29:104690. doi: 10.1016/j.jstrokecerebrovasdis.2020.104690
26. Arba F, Piccardi B, Palumbo V, Biagini S, Galmozzi F, Iovene V et al. Blood-brain-barrier leakage and reperfusion injury: results from the reperfusion injury in ischemic stroke (RISK) study. *Eur Stroke J.* (2019) 4(1 Suppl):96–49. AS10–63. doi: 10.1177/2396987319845578
27. Latour LL, Kang DW, Ezzeddine MA, Chalela JA, Warach S. Early blood-brain barrier disruption in human focal brain ischemia. *Ann Neurol.* (2004) 56:468–77. doi: 10.1002/ana.20199
28. Kim EY, Na DG, Kim SS, Lee KH, Ryoo JW, Kim HK. Prediction of hemorrhagic transformation in acute ischemic stroke: role of diffusion-weighted imaging and early parenchymal enhancement. *AJNR Am J Neuroradiol.* (2005) 26:1050–5.
29. Bang OY, Buck BH, Saver JL, Alger JR, Yoon SR, Starkman S, et al. Prediction of hemorrhagic transformation after recanalization therapy using T2*-permeability magnetic resonance imaging. *Ann Neurol.* (2007) 62:170–6. doi: 10.1002/ana.21174
30. Hjort N, Wu O, Ashkanian M, Sølling C, Mouridsen K, Christensen S, et al. MRI detection of early blood-brain barrier disruption: parenchymal enhancement predicts focal hemorrhagic transformation after thrombolysis. *Stroke.* (2008) 39:1025–8. doi: 10.1161/STROKEAHA.107.497719
31. Kassner A, Roberts TPL, Moran B, Silver FL, Mikulis DJ. Recombinant tissue plasminogen activator increases blood-brain barrier disruption in acute ischemic stroke: an MR imaging permeability study. *Am J Neuroradiol.* (2009) 30:1864–9. doi: 10.3174/ajnr.A1774
32. Kastrup A, Gröschel K, Ringer TM, Redecker C, Cordesmeyer R, Witte OW, et al. Early disruption of the bloodbrain barrier after thrombolytic therapy predicts hemorrhage in patients with acute stroke. *Stroke.* (2008) 39:2385–7. doi: 10.1161/STROKEAHA.107.505420
33. Thornhill RE, Chen S, Rammo W, Mikulis DJ, Kassner A. Contrast-enhanced MR imaging in acute ischemic stroke: T2* measures of blood-brain barrier permeability and their relationship to T1 estimates and hemorrhagic transformation. *Am J Neuroradiol.* (2010) 31:1015–22. doi: 10.3174/ajnr.A2003
34. Rozanski M, Ebinger M, Schmidt WU, Hotter B, Pittl S, Heuschmann PU, et al. Hyperintense acute reperfusion marker on FLAIR is not associated with early haemorrhagic transformation in the elderly. *Eur Radiol.* (2010) 20:2990–6. doi: 10.1007/s00330-010-1881-9
35. Lee M, Saver JL, Alger JR, Hao Q, Starkman S, Ali LK, et al. Blood-brain barrier permeability derangements in posterior circulation ischemic stroke: frequency and relation to hemorrhagic transformation. *J Neurol Sci.* (2012) 313:142–6. doi: 10.1016/j.jns.2011.08.048
36. Liu HS, Chung HW, Chou MC, Liou M, Wang CY, Kao HW, et al. Effects of microvascular permeability changes on contrast-enhanced T1 and pharmacokinetic MR imagings after ischemia. *Stroke.* (2013) 44:1872–7. doi: 10.1161/STROKEAHA.113.001558
37. Scalzo F, Alger JR, Hu X, Saver JL, Dani KA, Muir KW, et al. Multi-center prediction of hemorrhagic transformation in acute ischemic stroke using permeability imaging features. *Magn Reson Imaging.* (2013) 31:961–9. doi: 10.1016/j.mri.2013.03.013
38. Leigh R, Jen SS, Hillis AE, Krakauer JW, Barker PB. Pretreatment blood-brain barrier damage and post-treatment intracranial hemorrhage in patients receiving intravenous tissue-type plasminogen activator. *Stroke.* (2014) 45:2030–5. doi: 10.1161/STROKEAHA.114.005249
39. Leigh R, Christensen S, Campbell BCV, Marks MP, Albers GW, Lansberg MG. Pretreatment blood-brain barrier disruption and post-endovascular intracranial hemorrhage. *Neurology.* (2016) 87:263–9. doi: 10.1212/WNL.0000000000002862

40. Simpkins AN, Dias C, Leigh R. Identification of reversible disruption of the human blood-brain barrier following acute ischemia. *Stroke.* (2016) 47:2405–8. doi: 10.1161/STROKEAHA.116.013805
41. Villringer K, Sanz Cuesta BE, Ostwaldt AC, Grittner U, Brunecker P, Khalil AA, et al. DCE-MRI blood-brain barrier assessment in acute ischemic stroke. *Neurology.* (2017) 88:433–40. doi: 10.1212/WNL.0000000000003566
42. Nael K, Knitter JR, Jahan R, Gornbein J, Ajani Z, Feng L, et al. Multiparametric magnetic resonance imaging for prediction of parenchymal hemorrhage in acute ischemic stroke after reperfusion therapy. *Stroke.* (2017) 48:664–70. doi: 10.1161/STROKEAHA.116.014343
43. Nadareishvili Z, Simpkins AN, Hitomi E, Reyes D, Leigh R. Post-stroke blood-brain barrier disruption and poor functional outcome in patients receiving thrombolytic therapy. *Cerebrovasc Dis.* (2019) 47:135–42. doi: 10.1159/000499666
44. Morgan CA, Mesquita M, Ashioti M, Beech JS, Williams SCR, Irving E, et al. Late changes in blood–brain barrier permeability in a rat tMCAO model of stroke detected by gadolinium-enhanced MRI. *Neurol Res.* (2020) 2020:1–9. doi: 10.1080/01616412.2020.1786637
45. Butler J, Heidari P, Blayney S, Hitomi E, Luby M, Leigh R. Blood-brain barrier integrity of stroke patients presenting in an extended time window. *BMC Neurol.* (2020) 20:54. doi: 10.1186/s12883-020-01634-2
46. Goyal M, Menon BK, Van Zwam WH, Dippel DWJ, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: A meta-analysis of individual patient data from five randomised trials. *Lancet.* (2016) 387:1723–31. doi: 10.1016/S0140-6736(16)00163-X
47. Liu X, Dai Q, Ye R, Zi W, Liu Y, Wang H, et al. Endovascular treatment versus standard medical treatment for vertebrobasilar artery occlusion (BEST): an open-label, randomised controlled trial. *Lancet Neurol.* (2019) 2019:115–22. doi: 10.1016/S1474-4422(19)30395-3
48. Vagal A, Wintermark M, Nael K, Bivard A, Parsons M, Grossman AW, et al. Automated CT perfusion imaging for acute ischemic stroke: Pearls and pitfalls for real-world use. *Neurology.* (2019) 93:888–98. doi: 10.1212/WNL.0000000000008481
49. Farrall AJ, Wardlaw JM. Blood-brain barrier: ageing and microvascular disease - systematic review and meta-analysis. *Neurobiol Aging.* (2009) 30:337–52. doi: 10.1016/j.neurobiolaging.2007.07.015
50. Yang Y, Rosenberg GA. Blood–brain barrier breakdown in acute and chronic cerebrovascular disease. *Stroke.* (2011) 42:3323–8. doi: 10.1161/STROKEAHA.110.608257
51. Arba F, Leigh R, Inzitari D, Warach SJ, Luby M, Lees KR. Blood-brain barrier leakage increases with small vessel disease in acute ischemic stroke. *Neurology.* (2017) 89:2143–50. doi: 10.1212/WNL.0000000000004677
52. Adebayo OD, Culpan G. Diagnostic accuracy of computed tomography perfusion in the prediction of haemorrhagic transformation and patient outcome in acute ischaemic stroke: a systematic review and meta-analysis. *Eur Stroke J.* (2020) 5:4–16. doi: 10.1177/2396987319883461
53. Suh CH, Jung SC, Cho SJ, Kim D, Lee J Bin, Woo DC, et al. Perfusion CT for prediction of hemorrhagic transformation in acute ischemic stroke: a systematic review and meta-analysis. *Eur Radiol.* (2019) 29:4077–87. doi: 10.1007/s00330-018-5936-7
54. Campbell BCV, Christensen S, Levi CR, Desmond PM, Donnan GA, Davis SM, et al. Comparison of computed tomography perfusion and magnetic resonance imaging perfusion-diffusion mismatch in ischemic stroke. *Stroke.* (2012) 43:2648–53. doi: 10.1161/STROKEAHA.112.660548
55. Xin Y, Han FG. Diagnostic accuracy of computed tomography perfusion in patients with acute stroke: a meta-analysis. *J Neurol Sci.* (2016) 360:125–30. doi: 10.1016/j.jns.2015.11.046
56. Bang OY, Chung JW, Son JP, Ryu WS, Kim DE, Seo WK, et al. Multimodal MRI-based triage for acute stroke therapy: challenges and progress. *Front Neurol.* (2018) 9:586. doi: 10.3389/fneur.2018.00586

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 09 February 2021
doi: 10.3389/fneur.2020.629920

Intracranial Bleeding After Reperfusion Therapy in Acute Ischemic Stroke

Guillaume Charbonnier[1,2,3]*, *Louise Bonnet*[1], *Alessandra Biondi*[2,4] *and Thierry Moulin*[1,3,4]

[1] Neurology Department, Besançon University Hospital, Besançon, France, [2] Interventional Neuroradiology Department, Besançon University Hospital, Besançon, France, [3] EA 481 Neurosciences laboratory, Franche-Comté University, Besançon, France, [4] CIC-1431 Inserm, Besançon, France

Intracranial hemorrhage is one of the most feared complications following brain infarct. Ischemic tissues have a natural tendency to bleed. Moreover, the first recanalization trials using intravenous thrombolysis have shown an increase in mild to severe intracranial hemorrhage. Symptomatic intracerebral hemorrhage is strongly associated with poor outcomes and is an important factor in recanalization decisions. Stroke physicians have to weigh the potential benefit of recanalization therapies, first, with different risks of intracranial hemorrhage described in randomized controlled trials, and second with numerous risk markers that have been found to be associated with intracranial hemorrhage in retrospective series. These decisions have become quite complex with different intravenous thrombolytics and mechanical thrombectomy. This review aims to outline some elements of the pathophysiological mechanisms and classifications, describe most of the risk factors identified for each reperfusion therapy, and finally suggest future research directions that could help physicians dealing with these complications.

Keywords: stroke, intracranial bleeding, brain hemorrhage, hemorrhagic transformation, reperfusion, intravenous thrombolysis, mechanical thrombectomy

OPEN ACCESS

Edited by:
Nishant K. Mishra, University of California, Los Angeles, United States

Reviewed by:
Rossana Tassi, Siena University Hospital, Italy
Johannes Pfaff, Heidelberg University Hospital, Germany

***Correspondence:**
Guillaume Charbonnier
guillaume.charbonnier@univ-fcomte.fr

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *16 November 2020*
Accepted: *29 December 2020*
Published: *09 February 2021*

Citation:
Charbonnier G, Bonnet L, Biondi A and Moulin T (2021) Intracranial Bleeding After Reperfusion Therapy in Acute Ischemic Stroke. Front. Neurol. 11:629920. doi: 10.3389/fneur.2020.629920

INTRODUCTION

Recent advances in reperfusion therapies for acute ischemic stroke have required stroke physicians to deepen their understanding of cerebral hemorrhagic complications. Although the overall risks have been well-documented in various randomized controlled trials (RCT) of reperfusion therapies, the mechanisms underlying cerebral bleeding for an individual patient are still poorly understood. Intracranial bleeding following acute ischemic stroke has a major impact on patient outcomes, and controlling the risk of bleeding plays an important role in recanalization decisions. Intracranial bleeding can take a wide range of different forms, from extraparenchymal (subdural hematoma and subarachnoid hemorrhage) to intraparenchymal. The latter can be small suffusions to large hematomas with mass effect within infarction, all of which are called hemorrhagic transformation (HT). Large parenchymal hematomas are the most feared due to a high mortality rate and are present in ∼6% of patients after intravenous thrombolysis (IVT). In addition, infarct evolution and/or HT can cause dramatic neurologic deterioration. The frequency of HT is dependent on very different factors, including epidemiological factors (age, pre-stroke treatment, and conditions, etc.), characteristics of the infarct (size of ischemic core, timing of follow-up), reperfusion techniques at the acute phase (intravenous thrombolysis, mechanical thrombectomy, etc.), radiological

diagnosis (computed tomography, magnetic resonance imaging), and antithrombotics following the acute phase. This review aims to outline some elements of the pathophysiological mechanisms and classifications of HT, describe most of the risk factors identified for each reperfusion therapy, and finally suggest future research directions that could help physicians dealing with these complications.

PATHOPHYSIOLOGICAL MECHANISMS OF INTRACRANIAL BLEEDING FOLLOWING BRAIN INFARCT

Cerebral infarcts occur when a diminution of cerebral blood flow reaches a minimum threshold at which it cannot ensure a sufficient amount of oxygen and glucose. Like in many other organs, brain ischemic parenchyma have a tendency to bleed, and brain hemorrhage can lead to severe neurological deterioration. Mechanisms involved in hemorrhagic transformation can be considered from various points of view, including histological changes, vascular occlusion, collateral circulation, blood-brain barrier disruption, and infarct size.

Acute cerebral ischemia leads to the death of capillary cells, which causes vascular permeability and extravasation of blood in the brain parenchyma. The two main factors described in this process are oxidative stress and reperfusion injury. These factors lead to various mechanisms such as inflammation, leukocyte infiltration, vascular activation, and extracellular proteolysis (1, 2). The consequences are destruction of the basal lamina and endothelial tight junctions. Among the molecular processes involved, matrix metalloproteinase 9 (MMP-9) has been shown to play an important role in the destruction of basal lamina type IV collagen (1). Destruction of the basal lamina leads to leakage of macromolecules into the brain interstitial fluids. The resulting ionic gradient induces interstitial edema, known as vasogenic edema (as opposed to cytotoxic edema, which is associated with cell death). Vasogenic edema can lead to lesions on adjacent tissues. In the case of large infarcts, this mechanism can worsen and cause a malignant infarct, with catastrophic outcomes and high risk of hemorrhagic transformation. Hemorrhage seems to be very prevalent in ischemic lesions. In fact, early CT studies found that 65% of "pale infarcts," defined as negative CT for blood after ischemic stroke, showed petechial hemorrhage on microscopic sections (3). Reperfusion is involved in various pathways leading to cerebral injuries (4). First, it worsens ischemic injuries such as oxidative stress, suppression of protein synthesis, platelet activation, activation of the complement system, leukocyte infiltration, basal lamina disruption, and cerebral cell death. Moreover, reperfusion could induce specific mechanisms such as secondary hyperperfusion and hypoperfusion. The quantitative roles of thrombotic and inflammatory processes vs. reperfusion injuries are debated (5). Microvascular thromboinflammation could be hard to differentiate from reperfusion injuries.

Although ischemic lesions seem to be enough to cause hemorrhagic injuries, it has been suggested that late fragmentation of a thrombus, especially a larger one, could be the cause of late hemorrhagic complications. Fragmentation of a large thrombus could lead to distal migration and could damage the vascular bed (3).

It has been questioned if an occluded artery that causes a reduction of blood flow could decrease the risk of HT by reducing reperfusion injuries. The possibility of hemorrhagic complication with persistence of a proximal occlusion has led to discussion about the role of collateral circulation from the leptomeningeal network. A first cadaveric study demonstrated the presence of hemorrhagic infarcts with persistent proximal occlusion and possible involvement of the leptomeningeal network in reperfusion injuries (6). Another study, which used computed tomography to show hemorrhagic transformation and repeated angiography to demonstrate persistent occlusion and collateral development, highlighted a case of cortical HT in relation to collateral development (7).

On the other hand, reperfusion of the cerebral infarct could reduce ischemia and therefore reduce the risk of hemorrhage. Thus, higher prevalence of bleeding in the lenticulostriate artery territories could be linked to the absence of collateral anastomosis. It takes the form of a hemorrhage inside the infarcted tissue corresponding to intra-infarct hematoma or parenchymal hematoma. Profound ischemia leads to endothelial necrosis and hematomas (3). Additionally, increased risk of hemorrhage is well-documented in large infarcts, which supports the hypothesis of necrosis having a preponderant role in hemorrhage risk.

Another possible mechanism could be an abnormal response of the arterial wall to brain ischemia. Vasospasm is a complex phenomenon that is observed after the smooth muscular fibers of the vascular wall have been damaged. Hemorrhagic infarcts have been documented following vasospasm, so this could be another mechanism for hemorrhage following reperfusion (8). Furthermore, intimal lesions have been observed on occluded arteries. It has been debated whether these lesions were direct damage from the clot or consequences of secondary vasospasm (9).

Finally, hemorrhage in infarcted tissues seems to appear in a relatively short time window after stroke. Indeed, this complication occurs mostly within 24 h of thrombolysis (10).

REPERFUSION THERAPIES AND HEMORRHAGIC TRANSFORMATION: LEARNING FROM INTRAVENOUS THROMBOLYSIS, INTRA-ARTERIAL THROMBOLYSIS, MECHANICAL THROMBECTOMY, AND SONOTHROMBOLYSIS

Before reperfusion therapies, hemorrhagic transformation of acute ischemic stroke was not recognized and was badly classified. In fact, HT was usually described in the context of available treatments, mostly antithrombotics including anticoagulants. Use of reperfusion techniques in the acute phase of stroke has led to new needs for classification in RCTs.

Brief Historical Insight Into Hemorrhagic Transformation Related to Reperfusion Therapies

Before describing intracranial bleeding, it is necessary to review each reperfusion therapy and its latest developments. The hemorrhagic risk of these therapies is detailed in these therapies is detailed further. In the mid-1990s, the MAST-E and MAST-I trials failed to demonstrate the efficacy of intravenous streptokinase (11, 12) in the 6-h therapeutic window and showed an increased risk of HT and death in the intervention group. The ECASS I and II trials using tPA in the 6-h therapeutic window were also negative, with an increased rate of HT (13). The NINDS (14) trial showed the benefit of intravenous infusion of tPA in the 3-h therapeutic window, but with an increased risk of intracerebral hemorrhage in the intervention group. The large retrospective study SITS-MOST confirmed randomized data with real-life experience (15). Superiority of tPA in terms of functional independence at 3 months was then further demonstrated in the ECASS III (16) trial for the 4.5-h therapeutic window. Although negative on its primary outcome, IST-3 showed a significant shift on the Oxford Handicap Score using tPA in the 6-h therapeutic window, including in patients over 80 years old (17). The intervention was also associated with an increased risk of HT. Interestingly, mortality increased for the 7 days following IVT, but the rate was reversed between 7 days and 6 months so that similar rates were observed at 6 months. The therapy consisted of a 1-h intravenous infusion of alteplase, a fibrinolytic therapy, in order to achieve clot lysis and brain reperfusion. In 1998, a first phase II trial used intra-arterial thrombolysis as a new reperfusion technique (18). The investigators tried to achieve thrombus lysis by infusing recombinant pro-urokinase through a microcatheter placed in the thrombus or the M1 segment of the middle cerebral artery. Increased intracerebral hemorrhage was observed in the intervention group. In 2004, the first RCT using sonothrombolysis for cerebral recanalisation using transcranial 2-MHz Doppler Ultrasound showed a similar hemorrhagic risk. In 2015, five RCTs demonstrated the superiority of MT in anterior circulation acute ischemic stroke caused by large vessel occlusion (19), followed by two others (20, 21). The intervention consisted of an endovascular procedure, with catheterization of the cervical carotid and then the middle cerebral artery. The clot was removed mostly with the use of a stent retriever device. This consists of deploying a temporary stent delivered by a microcatheter to the site of occlusion. The clot is captured by the stent strands and then the stent is removed with the clot, achieving good recanalization in 80% of cases. The intervention group did not experience a higher incidence of intracranial bleeding. There are specific causes of bleeding after MT, as MT devices could led to endothelial lesions. Vessel wall components are found on histological analyses of thrombi (22), and vessel wall enhancement is found on the follow-up MRI after MT (23). The procedure may require microcatheterization with microwire, which could cause direct endothelial lesions and, rarely, perforation (24).

Diagnosis and Classifications

Radiological Screening of Hemorrhage After Cerebral Infarct

Surprisingly, there are no recommendations for the type of brain imaging or delay after reperfusion therapy. The ECASS III protocol performed a CT or MRI 22–36 h after IVT (16). In addition, there are no recommendations for a clear screening protocol for HT in the subacute phase for patients receiving different antithrombotic treatments. Also, no radiological patterns have been identified that could guide patient management. For now, only neurological deterioration is a clear indication for emergency HT screening.

Neuro-Imaging Classifications

Intracranial bleeding after acute ischemic stroke can follow various radiological patterns. A few classifications have been proposed, first every ischemic stroke (**Table 1**) (3), and then simplified for thrombolysis RCTs (13). The ECASS grading system (**Table 2**) differentiates Parenchymal Hematoma (PH) from Hemorrhagic Infarction (HI). PH1 was differentiated from PH2 by a bleeding volume of <30% of the underlying ischemia (25). A few examples of radiological patterns classified by different methods can be seen in **Figure 1**. Even if the different radiological classifications are quite similar, particularly

TABLE 1 | Adapted from Moulin et al. (3).

Hemorrhagic Infarct (HI)		
Cortical	HI c1	Petechial aspect located in the cortex
	HI c2	Confluent aspect involving 50–75% of the vascular territory
Deep	HI d1	Punctiform hemorrhage in the deep vascular territory
	HI d2	Confluent hemorrhage involving 50–75% of the vascular territory
Intra-Infarct Hematoma (IIH)		
Cortical	IIH c1	Homogeneous aspect involving 75–100% of the vascular territory without mass effect
	IIH c2	Hemorrhage in the whole vascular territory, liquid level, or mass effect
Deep	IIH d1	Homogeneous aspect involving 75–100% of the deep vascular territory with moderate mass effect
	IIH d2	Massive deep hemorrhage with severe mass effect

TABLE 2 | ECASS classification, adapted from Larrue et al. (25).

PH1	Blood clots in ≤30% of the infarcted area with some slight space-occupying effect
PH2	Blood clots in >30% of the infarcted area with a substantial space-occupying effect
HI1	Small petechiae along the margins of the infarct
HI2	Confluent petechiae within the infarcted area but no space-occupying effect

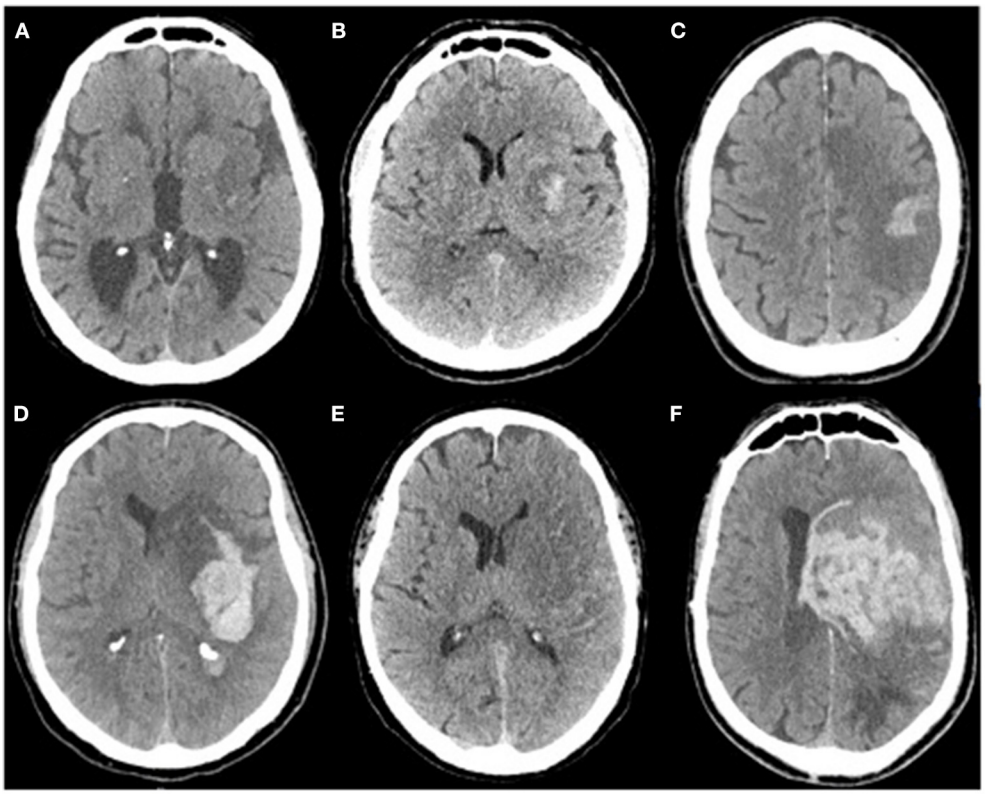


FIGURE 1 | (A) Isolated petechia of the posterior part of the lenticular nucleus: Moulin Hi d1, ECASS HI1, Heidelberg HI1, **(B)** Confluent petechial of the lenticular nucleus: Moulin HI d2, ECASS HI2, Heidelberg HI 2, **(C)** Cortical parenchymal hemorrhage: Moulin HI c1, ECASS HI1, Heidelberg HI1, **(D)** Deep parenchymal hemorrhage with mass effect and intraventricular hemorrhage: Moulin IIH d2, ECASS PH2, Heidelberg PH2 + class 3b, **(E)** isolated subarachnoid hemorrhage: Heidelberg class 3c, **(F)** Massive parenchymal hematoma Moulin IIH d2, ECASS, and Heidelberg PH2.

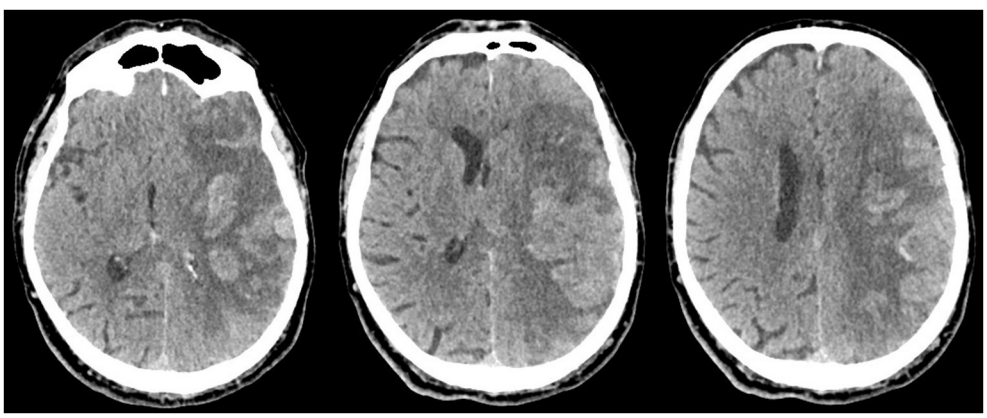


FIGURE 2 | Massive cortical hemorrhage with mass effect mostly caused by the infarcted tissue, which could be classified as Moulin HI c2, ECASS HI2, or Heidelberg HI2.

regarding the difference between PH and HI, some situations can be more challenging to classify (**Figure 2**).

The latter classification, which is used mostly for retrospective MT studies, was re-defined by a team of experts including investigators from the first MT RCTs, who proposed the Heidelberg Bleeding Classification (HBC) (26) (**Table 3**). A definite symptomatic intracerebral hemorrhage (sICH) is defined as a PH2 hematoma with a significant clinical deterioration (four points on NIHSS or two points in one category) if the bleeding is the main cause of deterioration. In this case, there

TABLE 3 | Heidelberg bleeding classification, adapted from von Kummer et al. (26).

Class	Type	Description
1	Hemorrhagic transformation of infarcted brain tissue	
1a	HI1	Scattered small petechiae, no mass effect
1b	HI2	Confluent petechiae, no mass effect
1c	PH1	Hematoma within infarcted tissue, occupying <30%, no substantive mass effect
2	Intracerebral hemorrhage within and beyond infarcted brain tissue	
	PH2	Hematoma occupying 30% or more of the infarcted brain tissue, with obvious mass effect
3	Intracerebral hemorrhage outside the infarcted brain tissue or intracranial-extracerebral hemorrhage	
3a	Parenchymal hematoma remote from infarcted brain tissue	
3b	Intraventricular hemorrhage	
3c	Subarachnoid hemorrhage	
3d	Subdural hemorrhage	

is no other dominant cause to explain the clinical deterioration (e.g., a partially hemorrhagic malignant infarct). Moreover, the HBC introduced new categories for previously non-classified intracerebral hemorrhages (intraventricular, subarachnoid, and subdural hemorrhages) and provided a formal approach to classifying those hemorrhages. These additions mean that substantially more hemorrhages can be diagnosed using the HBC compared to the ECASS classification (27).

Prognosis and Management of Hemorrhagic Transformation

sICH has been shown to be a catastrophic complication of acute ischemic stroke, with poorer clinical outcomes at 3 months (28–30). Only PH2 hemorrhages were initially associated with poor outcomes in ECASS. Recent retrospective studies also show an association with every ICH other than subarachnoid hemorrhage (SAH) in a MT cohort (31, 32). A retrospective study found an association between poor outcome and type 2 SAH, but not type 1 SAH, after endovascular thrombectomy (33). In that study, type 2 SAH was defined as SAH with isolated intra-parenchymal hematoma or other intracerebral hemorrhage.

Management of hemorrhagic transformation was discussed in the American Heart Association 2017 guidelines (34), which are summarized in **Table 4**. For alteplase-induced HT, cryoprecipitates, fresh frozen plasma, and tranexamic acid seem to have potential benefits. Blood pressure (BP) objectives should be guided by the recanalization status. In a fully recanalized patient, reduction of BP should be aggressive. Decompressive hemicraniectomy may be considered in select patients with sICH for whom surgery may improve outcome despite the ischemic injury.

INTRACRANIAL BLEEDING AFTER REPERFUSION THERAPY: RISK FACTORS AND RISK MARKERS

A wide range of risk factors and markers have been demonstrated as being associated with hemorrhagic transformation after reperfusion therapy. We will first describe the risk factors for cerebral hemorrhage demonstrated in RCTs for different reperfusion techniques (IV thrombolysis with alteplase tenecteplase, desmoteplase), MT, intra-arterial thrombolysis, sonothrombolysis. In a first part, we will focus on the hemorrhagic risk reported for each trial, without detailing the efficacy of these different trials. In a second part, we will describe the main risk markers for bleeding that have been described from retrospective studies or large cohorts. Most of the relevant risk factors and markers are summarized in **Table 5** with their quantitative association with HT and the population for which this association is applicable.

Risk Factors From IV and IA Thrombolysis RCTs

The NINDS and ECASS III trials were the first to demonstrate the clinical efficacy of IVT. Since the beginning of recanalization trials in acute ischemic stroke, it has been crucial to establish a clear and reproducible definition of cerebral hemorrhage. The most commonly used measure since the ECASS trial has been sICH, defined mostly as a PH2 hematoma with clinical deterioration (usually four points on the NIHSS scale). In the first thrombolysis trials, it seems that alongside the clinical efficacy, patients receiving thrombolysis treatment had a slightly higher risk of developing sICH compared to patients receiving placebo. Biological explanation for this risk seems complex. As previously mentioned, various mechanisms have been described as being involved in reperfusion injuries (4). Moreover, a possible specific explanation for intracerebral hemorrhage after IV alteplase is its role in the upregulation of MMP-9, which has been shown to play an important role in the destruction of the basal lamina (1). In the following section we review HT risks associated with each of the reperfusion therapies used in these trials (**Table 5A**).

Alteplase

Patients included in NINDS (14) received 0.9 mg/kg alteplase (10% as a bolus, max 90 mg), within 3 h of symptom onset. sICH was higher in the tPA group, 6.4 vs. 0.6% ($p = 0.001$). Patients included in ECASS III (13) received 0.9 mg/kg alteplase (10% as a bolus, max 90 mg) within 4.5 h of symptom onset. Intracranial hemorrhage was higher in the alteplase group (27.0 vs. 17.6%, $p = 0.001$). sICH was also higher in the intervention group but

TABLE 4 | Suggestions for reversal agents that may be considered on the basis of the mechanisms of action of the agent and alteplase in patients with sICH occurring within 36 h after alteplase infusion, adapted from Yaghi et al. (34).

Reversal agent	Suggested dose	Potential for benefit	Adverse effects
Cryoprecipitate	Consider sending a fibrinogen level immediately and empirically transfusing with 10 U cryoprecipitate, and anticipate giving more cryoprecipitate as needed to achieve a normal fibrinogen level of ≥150 mg/dL (10 U cryoprecipitate increases fibrinogen by nearly 50 mg/dL)	Potential for benefit in all sICH	Transfusion reaction and transfusion-related lung injury
Platelets	2 donors (8–10 U)	Potential for benefit is unclear except in patients with thrombocytopenia (platelets <100,000/μL), who may possibly benefit	Transfusion reaction, transfusion-related lung injury, volume overload
FFP	12 mL/kg	Potential for benefit is unclear except in patients on warfarin, in whom FFP may be considered	Transfusion reaction, transfusion-related lung injury, volume overload
PCC	25–50 U/kg (based on INR level)	Potential for benefit is unclear except in patients on warfarin, in whom PCC may be considered and is the preferred adjunctive treatment	Thrombotic complications
Vitamin K	10 mg intravenously	Potential for benefit is unclear except in patients on warfarin, in whom vitamin K may be used as an adjunctive treatment	Anaphylaxis
rFVIIa	20–160 μg/kg	Potential for benefit is unclear	Thrombotic complications
Antifibrinolytic agents	Aminocaproic acid: 4 g IV during first hour followed by 1 g/h for 8 h Tranexamic acid: 10 mg/kg 3–4 times/d (adjustment based on kidney function may be necessary)	Potential for benefit in all patients with sICH, particularly when blood products are contraindicated or declined by patient/family or if cryoprecipitate is not available	Thrombotic complications

FFP, fresh-frozen plasma; INR, international normalized ratio; PCC, prothrombin complex concentrate; rFVIIa, recombinant factor VIIa; and sICH, symptomatic intracranial hemorrhage.

was nevertheless low: 2.4 vs. 0.3%, odds ratio, 9.85; 95% CI, 1.26–77.32; $p = 0.008$).

The WAKE UP (36) trial included patients who had an unknown time of symptom onset but a radiological MRI mismatch. Patients received the same dose of tPA as in previous alteplase RCTs. The rate of sICH was 2.0% in the alteplase group vs. 0.4% in the placebo group ($p = 0.15$). This "tissue-based approach," opposed to "time-based approach," is consistent with previous retrospective studies that identified early CT signs as strong risk factors of HT in the first IVT trials (78).

The ENCHANTED trial aimed to assess whether low-dose alteplase could reach the same clinical efficacy as a standard dose of alteplase with a decreased hemorrhagic risk (53). Patients were randomized within 4.5 h of symptom onset to receive either a standard 0.9 mg/kg dose or 0.6 mg/kg. In the low-dose group, 1.0% had sICH vs. 2.1% in the standard group ($p = 0.01$). Sixty-three percent of the patients enrolled were Asian [previous studies have shown that Asian people are more likely to present cerebral hemorrhage (79)].

A recent meta-analysis combined five RCTs that compared the efficacy of sonothrombolysis as an adjuvant therapy to IVT (48). The incidence of sICH was 3.8% for the sonothrombolysis group vs. 2.6% for the IVT-alone group with no statistically significant difference.

Other IV Thrombolytics

In the NOR-TEST trial (50), patients were randomized to receive alteplase 0.9 mg/kg or tenecteplase 0.25 mg/kg in the 4.5-h window. sICH occurred in 3% of patients in the tenecteplase group vs. 2% of patients in the alteplase group, with no significant difference. These results were confirmed in a recent meta-analysis of five RCTs using tenecteplase vs. alteplase (2.9 vs. 2.6%, not significant) (80). A recent sub-analysis of the NOR-TEST trial confirmed similar risks of hemorrhage among three subgroups: elderly population (37), patients with severe to moderate strokes (38), and patients treated in the 3–4.5 h treatment window (39). Among the RCTs using tenecteplase, the recent EXTEND-IA TNK study (40) is of particular interest because it showed a higher rate of recanalization with tenecteplase in patients with large vessel occlusion addressed for thrombectomy. In this trial, the incidence of sICH was 1% in each group. A recent meta-analysis was conducted to assess the efficacy of intravenous desmoteplase vs. placebo in treating acute ischemic stroke (41). They included six RCTs, including patients 3–9 h after symptom onset. Studies used 62.5 to 125 μg/kg of desmoteplase. sICH was observed in 3.2% of patients in the desmoteplase groups and in 2.1% of placebo-treated patients (no significant difference). Three trials aimed to demonstrate the efficacy of intra-arterial therapy in the 6-h window for patients with middle cerebral

TABLE 5 | Main risk factors and risk markers associated with HT.

	Study population	Bleeding type	Odds ratio
A. Risk factors from RCT			
IV thrombolysis (0.9 mg/kg Alteplase) vs. placebo (14, 16)	Any ischemic stroke	sICH	From 9.9* to 10.7*
IA prourokinase or urokinase (35)	Large vessel occlusion	sICH	4.4*
IV thrombolysis (0.9 mg/kg) vs. placebo (36)	Any ischemic stroke with unknown onset and FLAIR/DWI mismatch on MRI	sICH	5*
Mechanical thrombectomy 6-24 h (35, 37–47)	Large vessel occlusion and radiological mismatch	sICH	From 1.75 to 2
Sonothrombolysis + IVT vs. IVT (48)	Any ischemic stroke	sICH	1.47
Use of stent retriever vs. direct aspiration (49)	Large vessel occlusion referred for thrombectomy	sICH	1.23
IV Tenecteplase (0.4 mg/kg) vs. Alteplase (0.9 mg/kg) (50)	Any ischemic stroke	sICH	1.16
IV Tenecteplase (0.25 mg/kg) vs. Alteplase (0.9 mg/kg) (40)	Large vessel occlusion referred for thrombectomy	sICH	1
IV Desmoteplase (62.5–125 μg/kg) vs. Alteplase (0.9 mg/kg) (41)	Any ischemic stroke	sICH	1.5
Mechanical thrombectomy within 6 h (19)	Large vessel occlusion	sICH	1.06
MT + Neretinide vs. MT (51)	Large vessel occlusion	sICH	0.8
MT alone vs. IVT+MT (52)	Large vessel occlusion	sICH	0.7
IVT (0.6 mg/kg) vs. IVT (0.9 mg/kg) (53)	Any ischemic stroke	sICH	0.5*
B. Risk markers from retrospective studies: clinical and biological markers and pre-stroke medication			
Baseline			
Renal Impairment (54, 55)	Any ischemic stroke treated by IVT	Heterogeneous definitions of intracerebral hemorrhage and neurological deterioration	2.79*; 2.08*
Any antiplatelet agent (54)			2.08*
Congestive heart failure (54)			1.96*
Atrial fibrillation (54)			1.86*
Age (54)			1.78*
Statin (54)			1.72*
NIHSS (54)			1.55*
Diabetes (54)			1.54*
Ischemic heart disease (54)			1.54*
Prior hypertension (54)			1.5*
Glucose (54)			1.10*
Smoking (54)			0.7*
Triiodothyronine (56)	Any ischemic stroke	ICH	3.46*
MMP-9 (57)	Ischemic stroke treated by MT	ICH	2.48*
Neutrophil-to-lymphocyte ratio (58)	Any ischemic stroke treated by IVT	sICH	2.08*
Fibrinogen decrease (59)	Any ischemic stroke treated by IVT	sICH	1.92*
Bilirubin (60)	Ischemic stroke treated by MT	sICH	1.36*
NIHSS (61)	Ischemic stroke treated by MT	sICH	1.089* per point
Age (61)			1.028* per year
Onset-to-end procedure time (61)			1.00* per min
Platelet count (54, 62)	Any ischemic stroke treated by IVT	sICH, ICH	From 0.47* to 0.86
Uric acid (63, 64)	Ischemic stroke treated by MT; any ischemic stroke	ICH	From 0.33* to 0.43*
Absolute Eosinophil Count $\geq 0.11 \times 10^9$/l (65)	Any ischemic stroke treated by IVT	ICH	0.22*
Subacute phase			
Clinical-genetic (66)	Any ischemic stroke treated by IVT	Parenchymal hemorrhage	5.16*
24 h arterial stiffness index (67)	Any ischemic stroke treated by OVT or MT	ICH	1.9*
24 h blood pressure variability (68)	Large vessel occlusion referred for MT	sICH	1.71*

(Continued)

TABLE 5 | Continued

	Study population	Bleeding type	Odds ratio
C. Risk markers from retrospective studies: imaging			
Blood-brain barrier permeability (69)	Ischemic stroke treated by IVT or MT	ICH	45.4*
Blood-brain barrier disruption after MT (70)	Ischemic stroke treated by MT	ICH	25.3*
>10 microbleeds on MRI (71)	Any ischemic stroke treated by IVT	sICH	5.55*
MT with ASPECTS 0–4 (72)	Ischemic stroke treated by MT in HERMES	sICH	3.94
Lower ASPECTS score (54)	Any ischemic stroke treated by IVT	Heterogeneous definitions of intracerebral hemorrhage and neurological deterioration	3.46*
Visible lesion on CT (54)			2.39*
Leukoaraiosis (54)			2.45*
ASPECTS score (73)	Ischemic stroke treated by MT	PH	1.87*
Intracranial calcifications on CT (74)	Non-cardiogenic ischemic strokes treated by IVT	ICH	1.504*
Procedure time (MT) (75)	Ischemic stroke treated by MT	sICH	1.43* per 30 mn
D. Risk markers from retrospective studies: composite scores			
	Study population	Bleeding type	Area under the curve
IER-SICH nomogram (61)	Ischemic stroke treated by MT	sICH	0.733*
TAG score (76)			0.79*
HAT score (77)	Any ischemic stroke treated by IVT without MT	sICH	0.769*

ASPECTS: Alberta Stroke Program Early CT score; CT: Computed Tomography; HAT: Hemorrhage After Stroke; ICH: Intracerebral Hemorrhage; IER-SICH: Italian Registry of Endovascular Stroke Treatment in Acute Stroke Symptomatic Intracerebral Hemorrhage score; IV: Intravenous; IVT: Intravenous Thrombolysis; MRI: Magnetic Resonance Imaging; MT: Mechanical Thrombectomy; PH: Parenchymal Hemorrhage; sICH: symptomatic intracerebral hemorrhage; TAG: TICI-ASPECTS-glucose.
Risk markers and risk factors are ordered by odds ratio.
**Statistically significant.*

artery occlusion. They used recombinant pro-urokinase 6 mg in PROACT I (18), 9 mg in PROACT II (42), and urokinase (maximal dose of 600,000 UI) in the MELT trial (43). A meta-analysis of the three trials (35) demonstrated a higher incidence of sICH in the intervention group: 10.5 vs. 2.4% ($p = 0.02$).

Risk Factors From Mechanical Thrombectomy RCTs

MT has recently shown great clinical efficacy in acute ischemic stroke with large vessel occlusion (LVO). When describing intracerebral hemorrhage, it is important to understand that the population of patients with LVO may differ from the patient populations included in the IVT trials seen above. IVT trials mainly select patients based on a non-contrast CT scan in order to exclude hemorrhage. This selection means that IVT trials may recruit patients with LVO, but not every patient in an IVT trial has LVO, unlike in MT trials. Patients with LVO present with poorer prognosis and high risk of spontaneous hemorrhage. No MT RCTs have demonstrated a significant increased risk of sICH.

In 2015, the first five RCTs to study MT demonstrated its clinical efficacy (44, 45, 81–83). These first trials were pooled into a meta-analysis that included patients presenting with LVO with various "symptom-onset to randomization" windows (19). They received either MT plus standard of care or standard of care alone (which included IVT in the majority of cases). The rate of sICH was 4.4% in the intervention group vs. 4.3% in the control group (not significant).

Two studies have been conducted to assess the efficacy of MT beyond the 6-h window in patients selected on a radiological mismatch measure. The DAWN trial (46) included patients who presented in the 6–24 h window with a radiological mismatch defined as a maximum diffusion volume on MRI or ischemia parameter on CT perfusion, which differs according to age. sICH occurred in 6% of the MT group vs. 3% in the control group (no significant difference). DEFUSE 3 enrolled patients in the 6–16 h window with a radiological mismatch assessed by perfusion imaging (CT or MRI). The rate of sICH was 7% in the MT group vs. 4% in the control group (not significant) (47).

A recent MT trial aimed to assess the efficacy of a neuroprotective agent (intravenous Neretinide) as an adjuvant therapy to MT in LVO (51). The trial was negative but showed a clinical effect on the subgroup of patients who did not receive alteplase. The rate of sICH was not statistically significantly different, with a rate of 3.5% in the intervention group vs. 4.3% in the control group.

The DIRECT MT trial (52) aimed to demonstrate the non-inferiority of MT alone vs. MT plus IVT in LVO. The incidence of sICH was 4.3% in the MT-alone group vs. 6.1% in the MT plus IVT group, not significant.

Risk Markers for Bleeding From Retrospective Studies

Apart from revascularization RCTs, a wide range of risk markers have been described as being associated with sICH. These

markers were mostly described for retrospective cohorts. They can help to guide further research protocols, but they should not lead to a change in practices based on recommendations following clinical data from RCTs. As retrospective studies reflect current practices, these risk markers were mostly described for IVT or MT procedures, in line with current guidelines (84). Because of the numerous risk marker studies in literature, we chose to report only the main ones in **Table 5B**. We also discuss a selection of the studies in the following section.

Epidemiological Markers

Older age has been associated with increased rates of sICH after IVT (54). Interestingly, reperfusion after MT in non-agenarians does not seem to lead to higher sICH rates than control groups of LVO (85).

Blood pressure following recanalization is a complex variable. In non-recanalized patients, high blood pressure could maintain the efficacy of arterial collaterals. However, higher systolic BP is associated with intracerebral hemorrhage and poor outcome after MT (86). Apart from the "raw" BP measure, it seems that BP variability is another important factor. Time rate of systolic blood pressure variation is independently associated with sICH (68). Furthermore, arterial stiffness has been demonstrated to be associated with sICH after IVT (67).

It has been proposed that patients of Asian ethnicity have an increased risk of intracerebral hemorrhage generally, which would put them even more at risk after recanalization. The RADIANT study looked at 916 patients from different ethnicities who underwent different types of recanalization. Chinese ethnicity was not associated with increased intracerebral hemorrhage, except for patients treated with IVT tPA only, with a prediction model from the logistic regression analysis, in association with age, international normalized value, and partial thromboplastin time (87). One meta-analysis evaluated all usual predictive markers of hemorrhagic transformation after IVT, specifically in the Chinese population (88). These markers were age, male sex, diabetes, atrial fibrillation (AF), previous stroke, onset-to-treatment time, NIHSS, infarct size, and ischemic signs of CT. A recent analysis of 1,324 genotypes of patients undergoing IVT led to the development of a clinical-genetic score using two genetic polymorphisms, which was validated on a MT cohort (66) in a Spanish population. These kinds of studies bring hope for more personalized decision making in the future.

Even if diabetes has been associated with poorer outcome after acute ischemic stroke, it has been debated whether these patients experience more sICH after IVT. Diabetes seems to increase hemorrhagic complications in an experimental stroke model (89). However, current clinical data does not support this idea (90), although one retrospective study including patients treated by MT described an association between sICH and diabetes (91).

Renal impairment has been strongly associated with HT after IVT (54), particularly in severe renal impairment (glomerular filtration rate <30 mL/mn) (92). A recent meta-analysis on a very large population of patients confirmed that chronic kidney disease is associated with sICH with either the NINDS or ECASS definition. The association remains for the <30 mL/mn and the <60 mL/mn subgroups (55). Although alteplase is metabolized by the liver, patients with chronic kidney disease present a higher risk of any hemorrhage, caused by endothelial and platelet dysfunction.

Smoking is a debated marker in HT. The apparent reduction of HT after IVT among smokers could be due to a younger age of the population and less advanced atherosclerosis at the time of presentation (54).

Stroke etiology could play an important role in HT. AF has been described as being a risk factor for hemorrhagic transformation (88, 93, 94), possibly because of larger thrombi (3). Infective endocarditis is a cause of spontaneous hemorrhagic transformation (95) and a possible cause of hemorrhage after recanalization. A recent meta-analysis showed better outcomes in these patients when treated by MT compared to IVT (96), with a risk of hemorrhage 4.14 times higher in the IVT group (96). In addition, retrospective data showed that patients with endocarditis who were treated by MT did not experience more sICH than patients with AF treated by MT (8.0 vs. 5.2%). Interestingly, a retrospective study showed different risk factors associated with sICH between large artery atherosclerosis and cardioembolic subtypes of stroke. Lower LDL-C and higher blood glucose were independent risk factors of large artery atherosclerosis, while lower albumin and platelet counts were independent risk factors of cardioembolic stroke (97).

Blood Test Markers

Early decrease of fibrinogen after IVT is associated with hemorrhagic sICH (59). Baseline blood glucose level has been described as an independent predictor of hemorrhagic transformation after IVT (54, 57). Postoperative hyperglycemia is associated with sICH after MT (98). A high neutrophil-to-lymphocyte ratio has been described as a predictor of hemorrhagic transformation after IVT (58). It has been demonstrated that an absolute eosinophil count $\geq 0.11 \times 10^9$/L was independently associated with a 78% reduction in the odds of developing hemorrhagic transformation (65). A low platelet count was associated with hemorrhagic transformation in a recent study (62). A previous larger cohort had a similar result; however, it did not reach statistical significance (54). A lower uric acid level is associated with sICH (63, 64). A recent study found that elevated bilirubin is an independent risk factor of sICH after MT (60). A case-control study found that low triiodothyronine syndrome was independently associated with the risk of hemorrhagic transformation, sICH, and severe parenchymal hematoma in patients with ischemic stroke (56). As described above, blood-brain barrier (BBB) permeability is a key factor of HT. A few biological markers that could affect BBB have been described. This is the case for metalloproteinase 9 (MMP-9) (57).

Mild Stroke Risk Markers

In mild stroke, defined by initial clinical stroke severity of NIHSS 0–5, two studies found that IVT was not associated with higher rates of sICH (99, 100). Two other studies observed no significant association between MT and sICH in cohorts of patients with mild stroke (101, 102).

Baseline Treatments

Ischemic stroke patients receiving anticoagulation treatment have been described as being at risk of hemorrhagic transformation (103). Direct oral anticoagulants have shown to be less associated with sICH than Vitamin K antagonists (104).

Neuro Imaging Markers

Computed Tomography

As described above, infarct size plays a key role in HT. Infarct core volume measurement can be challenging, which led to the development of a simple quantitative CT score called the Alberta Stroke Program Early CT Score (ASPECTS) (105). The score ranges from 0 to 10 and divides each hemisphere into 10 regions. Each region with early CT sign of ischemia loses 1 point on the scale. The scale has largely been used in RCTs and in retrospective series, even though its intra-rater and inter-rater reliability could not be sufficient (106). It is not surprising that ischemic core graded by ASPECTS is associated with sICH after MT (73). Furthermore, white matter lesions (leukoaraiosis) visible on CT, which can be a marker of small vessel disease, are associated with sICH (107). Calcification volume on CT is a predictor of sICH (74).

CT perfusion at baseline has been used to predict sICH. The authors used various parameters and thresholds, mostly with the aim of assessing blood-brain barrier disruption (108–111). Blood-brain barrier rupture is radiologically assessed by contrast enhancement of ischemic lesions. This radiological finding is associated with sICH (69, 70, 112). Also, post-procedural contrast accumulation after MT is associated with sICH (70, 113). Assessment of cerebral hemorrhage can be difficult after MT because the intra-arterial contrast can be seen in brain parenchyma for 24 h following the procedure. With regard to this problem, hemorrhage can be more accurately diagnosed using MRI (114) or dual energy CT (115, 116).

MRI

Conventional MRI. Conventional MRI can reveal several radiological signs associated with sICH. An initial study found that hemorrhagic transformation is associated with high permeability, hypoperfusion, low apparent diffusion coefficient (ADC), and FLAIR hyperintensity (117). The pooled sensitivity was 82% (95% confidence interval 61–93%) and the pooled specificity was 79% (95% confidence interval 71–85%). On the other hand, a second study found that vascular hyperintensities, old infarcts, and diffusion volume abnormalities were associated with sICH, but the only variable with an acceptable discrimination was volume of DWI abnormality. Other studies seem to show that the most predictive parameter is diffusion lesion volume, assessed by volume or DWI-ASPECTS (72, 118, 119).

Remote hemorrhage after ischemic stroke is a difficult question. In a recent MRI study, the authors demonstrated that there was a pre-existing lesion in half of patients presenting with a remote hemorrhage (120).

Perfusion MR. A recent study used arterial spin labeling to analyze perfusion MRI at 24 h post treatment. This technique can assess the relative cerebral blood flow of the ischemic tissue. Using the 25th percentile of this parameter, the authors obtained an independent predictor of PH and PH2 (121).

Microbleeds visualized on the baseline MRI are associated with an increased risk of sICH (122). A retrospective study demonstrated an association with a significant risk of sICH using a cut-off of 10 microbleeds (71).

Digital Subtracted Angiography (DSA)

In a retrospective study of MT, the visualization of angiographic blush was associated with sICH (123). In another study, early visualization of the internal cerebral vein on the lateral projection of the DSA was associated with sICH (124).

Predictive Scores From Multiple Risk Markers

It is clear that sICH is associated with various causes, and several authors have tried to establish composite scores including the main markers to obtain more precise predictions of hemorrhage: Cappellari (61), TAG Score (76), and Nisar (77).

Technical Considerations Associated With Mechanical Thrombectomy

MT is performed with different practices regarding peri-operative antithrombotics depending on the treatment center protocol. Some centers use peri-procedural heparin, a translation from other endovascular procedures, in order to avoid ischemic complications secondary to catheterization. This practice does not seem to be associated with higher rates of sICH according to a retrospective study (125).

In the case of tandem occlusion, defined as an extracranial occlusion in addition to the intracranial occlusion, the surgeon can be forced to treat the extracranial lesion by emergent stenting. Internal carotid stenting is usually performed under double antiplatelet therapy, which is continued for a few months, depending on the center, in order to prevent intra-stent stenosis. Intra-stent stenosis can lead to distal ischemic emboli or proximal occlusions. In the case of emergent carotid artery stenting during a MT procedure, most surgeons start a single antiplatelet therapy by aspirin and add a second antiplatelet agent after the CT at 24 h, in the absence of hemorrhage. This situation has led to the question of an increased risk of hemorrhage in these procedures.

A first study showed that the sICH rate in patients undergoing MT for tandem occlusion was similar to the sICH rate in the MT RCTs (126). Extracranial stenting and use of antiplatelet therapy was not associated with PH or HI. Another study from the same tandem occlusion cohort showed no increased risk of sICH in the case of antiplatelet therapy use during an MT procedure (127).

An ongoing RCT will demonstrate if emergent carotid stenting in tandem lesions is superior to not treating the extracranial lesion (128). Data regarding safety will assess the hemorrhagic risk of this procedure.

Complete recanalization is associated with less risk of sICH (76) and HI (73), probably due to smaller infarcts, as increased infarct size is associated with more HT.

The incidence of sICH was not higher in the stent retriever group vs. the direct aspiration group in the RCT ASTER I (49). However, stent retriever use was associated with higher hemorrhage in a retrospective series (91).

Multiple studies have investigated the link between the number of attempts performed by the surgeon and the rate of sICH; one study found that an increased number of attempts was associated with an increased risk (129), but two others did not find this association (130, 131). In addition, a longer procedure time seems to be associated with sICH (32, 75).

In the case of MT failure, the surgeon may use an adjuvant therapy that has not been tested for this indication by a RCT. A few of these therapies have been reported in small series: intra-arterial tPA (132), intra-arterial Urokinase (133), and intravenous Tirofiban (134, 135). These series did not show significant association between these adjunctive therapies and sICH.

One study investigated patients treated with repeated MT and showed that they had no increased risk of sICH (136).

FUTURE DIRECTIONS

Blood Pressure Management

A recent RCT investigated treating patients who were given IV thrombolysis by intensive blood pressure reduction. The idea was to decrease the incidence of hemorrhagic transformation. The trial was negative for the primary outcome, which was the reduction of disability at 3 months, assessed by mRS scale (137). On the other hand, the intervention group had a significant reduction in the secondary outcome: "any intracranial hemorrhage." An ongoing RCT (138) will demonstrate if aggressive reduction of BP (<140/90 mmHg) is effective after MT in reducing risk of intracranial hemorrhage. A recent review described hemodynamic parameters following recanalization and pointed out the fact that BP targets may be dependent on individual parameters such as autoregulatory limits and BP trajectories. In light of this data, chronic high BP in patients with severe carotid stenosis may not require treatment, whereas an unusual increase in BP may represent a risk of reperfusion injury (139).

Development of Low Hemorrhagic Risk Antithrombotic Therapy and Adjunctive Neuroprotective Therapies

As described above, blood-brain barrier disruption carries a high risk of HT. Several molecular processes have been described and could be potential therapeutic targets. MMP-9 has a key role, and a new phosphodiesterase-III inhibitor called cilostazol has been shown to ameliorate tight junction disruption *in vitro* (140). Cilostazol is a new antiplatelet therapy, and its use in a mouse model showed no increase in cerebral hemorrhage (141). Minocyclin can also target MMP-9 and could be an effective neuroprotective agent (142).

Another approach is to target cytotoxic injuries. Glycyrrhizin could inhibit peroxynitrin production and therefore has a neuroprotective effect on the HT cascade (143, 144).

A new antiplatelet therapy called ACT017 is currently being tested and shows a promising antithrombotic effect with no increased risk of hemorrhage (145).

Neurosurgery

Several trials are testing minimally invasive surgery for spontaneous intracerebral hematomas (146). If these techniques show efficacy, we can hope for translation to hemorrhagic transformation. Decompressive hemicraniectomy has demonstrated superiority in terms of morbidity and morbidity in middle cerebral artery occlusion complicated by malignant infarct (147), but some studies excluded HT causing mass effect. Despite no trials specifically investigating decompressive surgery for HT, the ongoing SWITCH trial aims to evaluate the safety and efficacy of hemicraniectomy in spontaneous intracerebral hemorrhage.

DISCUSSION

HT after recanalization therapy can have a wide range of outcomes in terms of severity. The most feared consequence is sICH, which is associated with catastrophic outcomes. It seems crucial in current practice to better characterize hemorrhagic transformation, based on clear pathophysiological mechanisms. It is only with a correct understanding of these mechanisms that stroke physicians will be able to prevent HT, make the most effective recanalization decisions, and try to control HT. Among the current recanalization therapies, IVT can increase the rate of HT, particularly in large infarcts. MT has not been shown to increase HT for the indications used in RCTs. Many clinical, biological, and radiological factors have been described as associated with HT. Developments in neuroimaging and use of composite scores could lead to a more personalized approach for HT prediction. The treatment options for sICH are still disappointing, as it seems very difficult to change its clinical course, which leads almost inevitably to a poor outcome.

AUTHOR CONTRIBUTIONS

GC, TM, and LB wrote the manuscript. AB provided radiological resources.

ACKNOWLEDGMENTS

The authors are grateful to Jennifer Dobson for proof reading the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.629920/full#supplementary-material

REFERENCES

1. Bar B, Biller J. Select hyperacute complications of ischemic stroke: cerebral edema, hemorrhagic transformation, and orolingual angioedema secondary to intravenous Alteplase. *Expert Rev Neurother.* (2018) 18:749–59. doi: 10.1080/14737175.2018.1521723
2. Sussman ES, Connolly ES. Hemorrhagic transformation: a review of the rate of hemorrhage in the major clinical trials of acute ischemic stroke. *Front Neurol.* (2013) 4:69. doi: 10.3389/fneur.2013.00069
3. Moulin T, Crépin-Leblond T, Chopard JL, Bogousslavsky J. Hemorrhagic Infarcts. *ENE.* (1994) 34:64–77. doi: 10.1159/000117012
4. Bai J, Lyden PD. Revisiting cerebral postischemic reperfusion injury: new insights in understanding reperfusion failure, hemorrhage, and edema. *Int J Stroke.* (2015) 10:143–52. doi: 10.1111/ijs.12434
5. Desilles JP, Mazighi M, Ho-Tin-Noé B. Letter by Desilles et al. regarding article, "ischemia-reperfusion injury after endovascular thrombectomy for ischemic stroke." *Stroke.* (2019) 50:e98. doi: 10.1161/STROKEAHA.118.024391
6. Ogata J, Yutani C, Imakita M, Ishibashi-Ueda H, Saku Y, Minematsu K, et al. Hemorrhagic infarct of the brain without a reopening of the occluded arteries in cardioembolic stroke. *Stroke.* (1989) 20:876–83. doi: 10.1161/01.str.20.7.876
7. Bozzao L, Angeloni U, Bastianello S, Fantozzi LM, Pierallini A, Fieschi C. Early angiographic and CT findings in patients with hemorrhagic infarction in the distribution of the middle cerebral artery. *AJNR Am J Neuroradiol.* (1991) 12:1115–21.
8. Terada T, Komai N, Hayashi S, Moriwaki H, Hyoutani G, Uematsu Y, et al. Hemorrhagic infarction after vasospasm due to ruptured cerebral aneurysm. *Neurosurgery.* (1986) 18:415–8. doi: 10.1227/00006123-198604000-00004
9. Brucher JM, De Smet YD, Gonsette RE. Rupture of the arterial wall in two cases of recent cerebral embolism: morphological expression of cerebral vasospasm? *J Neurol.* (1986) 233:267–70. doi: 10.1007/BF00314157
10. The NINDS t-PA Stroke Study Group null. Intracerebral hemorrhage after intravenous t-PA therapy for ischemic stroke. *Stroke.* (1997) 28:2109–118. doi: 10.1161/01.STR.28.11.2109
11. Multicenter Acute Stroke Trial–Europe Study Group, Hommel M, Cornu C, Boutitie F, Boissel JP. Thrombolytic therapy with streptokinase in acute ischemic stroke. *N Engl J Med.* (1996) 335:145–50. doi: 10.1056/NEJM199607183350301
12. Group MAST-I. Randomised controlled trial of streptokinase, aspirin, and combination of both in treatment of acute ischaemic stroke. Multicentre acute stroke trial–Italy (MAST-I) group. *Lancet.* (1995) 346:1509–14. doi: 10.5555/uri:pii:S0140673695920498
13. Hacke W, Kaste M, Fieschi C, Toni D, Lesaffre E, von Kummer R, et al. Intravenous thrombolysis with recombinant tissue plasminogen activator for acute hemispheric stroke: the European cooperative acute stroke study (ECASS). *JAMA.* (1995) 274:1017–25. doi: 10.1001/jama.1995.03530130023023
14. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med.* (1995) 333:1581–8. doi: 10.1056/NEJM199512143332401
15. Wahlgren N, Ahmed N, Dávalos A, Ford GA, Grond M, Hacke W, et al. Thrombolysis with alteplase for acute ischaemic stroke in the safe implementation of thrombolysis in stroke-monitoring study (SITS-MOST): an observational study. *Lancet.* (2007) 369:275–82. doi: 10.1016/S0140-6736(07)60149-4
16. Hacke W, Kaste M, Bluhmki E, Brozman M, Dávalos A, Guidetti D, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med.* (2008) 359:1317–29. doi: 10.1056/NEJMoa0804656
17. IST-3 collaborative group, Sandercock P, Wardlaw JM, Lindley RI, Dennis M, Cohen G, et al. The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. *Lancet.* (2012) 379:2352–63. doi: 10.1016/S0140-6736(12)60768-5
18. del Zoppo GJ, Higashida RT, Furlan AJ, Pessin MS, Rowley HA, Gent M. PROACT: a phase II randomized trial of recombinant pro-urokinase by direct arterial delivery in acute middle cerebral artery stroke. *Stroke.* (1998) 29:4–11. doi: 10.1161/01.STR.29.1.4
19. Goyal M, Menon BK, Zwam WH van, Dippel DWJ, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet.* (2016) 387:1723–31. doi: 10.1016/S0140-6736(16)00163-X
20. Bracard S, Ducrocq X, Mas JL, Soudant M, Oppenheim C, Moulin T, et al. Mechanical thrombectomy after intravenous alteplase versus alteplase alone after stroke (THRACE): a randomised controlled trial. *Lancet Neurol.* (2016) 15:1138–47. doi: 10.1016/S1474-4422(16)30177-6
21. Martins SO, Mont'Alverne F, Rebello LC, Abud DG, Silva GS, Lima FO, et al. Thrombectomy for stroke in the public health care system of Brazil. *N Engl J Med.* (2020) 382:2316–26. doi: 10.1056/NEJMoa2000120
22. Funatsu N, Hayakawa M, Hashimoto T, Yamagami H, Satow T, Takahashi JC, et al. Vascular wall components in thrombi obtained by acute stroke thrombectomy: clinical significance and related factors. *J Neurointerv Surg.* (2019) 11:232–6. doi: 10.1136/neurintsurg-2018-014041
23. Abraham P, Scott Pannell J, Santiago-Dieppa DR, Cheung V, Steinberg J, Wali A, et al. Vessel wall signal enhancement on 3-T MRI in acute stroke patients after stent retriever thrombectomy. *Neurosurg Focus.* (2017) 42:E20. doi: 10.3171/2017.1.FOCUS16492
24. Moshayedi P, Desai SM, Jadhav AP. Extravasation control with preserved vessel patency after wire perforation during neurothrombectomy: case report and literature review. *J Clin Neurosci.* (2019) 65:151–3. doi: 10.1016/j.jocn.2019.03.024
25. Larrue V, von Kummer RR, Müller A, Bluhmki E. Risk factors for severe hemorrhagic transformation in ischemic stroke patients treated with recombinant tissue plasminogen activator: a secondary analysis of the European-Australasian acute stroke study (ECASS II). *Stroke.* (2001) 32:438–41. doi: 10.1161/01.str.32.2.438
26. von Kummer R, Broderick JP, Campbell BCV, Demchuk A, Goyal M, Hill MD., et al., Majoie Charles B.L.M., Marquering Henk A., Mazya Michael V., et al. The Heidelberg bleeding classification. Classification of bleeding events after ischemic stroke and reperfusion therapy *Stroke.* (2015) 46:2981–6. doi: 10.1161/STROKEAHA.115.010049
27. Neuberger U, Möhlenbruch MA, Herweh C, Ulfert C, Bendszus M, Pfaff J. Classification of bleeding events: comparison of ECASS III (European cooperative acute stroke study) and the new Heidelberg bleeding classification. *Stroke.* (2017) 48:1983–5. doi: 10.1161/STROKEAHA.117.016735
28. Khatri P, Wechsler LR, Broderick JP. Intracranial hemorrhage associated with revascularization therapies. *Stroke.* (2007) 38:431–40. doi: 10.1161/01.STR.0000254524.23708.c9
29. Fiorelli M, Bastianello S, von Kummer R, del Zoppo GJ, Larrue V, Lesaffre E, et al. Hemorrhagic transformation within 36 hours of a cerebral infarct: relationships with early clinical deterioration and 3-month outcome in the European cooperative acute stroke study I (ECASS I) cohort. *Stroke.* (1999) 30:2280–4. doi: 10.1161/01.str.30.11.2280
30. van Kranendonk KR, Treurniet KM, Boers AMM, Berkhemer OA, van den Berg LA, Chalos V, et al. Hemorrhagic transformation is associated with poor functional outcome in patients with acute ischemic stroke due to a large vessel occlusion. *J Neurointerv Surg.* (2019) 11:464–8. doi: 10.1136/neurintsurg-2018-014141
31. Jiang F, Zhao W, Wu C, Zhang Z, Li C, Che R, et al. Asymptomatic intracerebral hemorrhage may worsen clinical outcomes in acute ischemic stroke patients undergoing thrombectomy. *J Stroke Cerebrovasc Dis.* (2019) 28:1752–8. doi: 10.1016/j.jstrokecerebrovasdis.2019.02.006
32. Lee YB, Yoon W, Lee YY, Kim SK, Baek BH, Kim JT, et al. Predictors and impact of hemorrhagic transformations after endovascular thrombectomy in patients with acute large vessel occlusions. *J Neurointerv Surg.* (2019) 11:469–73. doi: 10.1136/neurintsurg-2018-014080
33. Qureshi AI, Saleem MA, Aytac E. Postprocedure subarachnoid hemorrhage after endovascular treatment for acute ischemic stroke. *J Neuroimaging.* (2017) 27:493–8. doi: 10.1111/jon.12430
34. Yaghi S, Willey JZ, Cucchiara B, Goldstein JN, Gonzales NR, Khatri P, et al. Treatment and outcome of hemorrhagic transformation after intravenous alteplase in acute ischemic stroke: a scientific statement for healthcare professionals from the American heart association/American stroke association. *Stroke.* (2017) 48:e343–61. doi: 10.1161/STR.0000000000000152

35. Fields JD, Khatri P, Nesbit GM, Liu KC, Barnwell SL, Lutsep HL, et al. Meta-analysis of randomized intra-arterial thrombolytic trials for the treatment of acute stroke due to middle cerebral artery occlusion. *J Neurointerv Surg.* (2011) 3:151–5. doi: 10.1136/jnis.2010.002766
36. Thomalla G, Simonsen CZ, Boutitie F, Andersen G, Berthezene Y, Cheng B, et al. MRI-guided thrombolysis for stroke with unknown time of onset. *N Engl J Med.* (2018) 379:611–622. doi: 10.1056/NEJMoa1804355
37. Thommessen B, Næss H, Logallo N, Kvistad CE, Waje-Andreassen U, Ihle-Hansen H, et al. Tenecteplase versus alteplase after acute ischemic stroke at high age. *Int J Stroke.* (2020). doi: 10.1177/1747493020938306
38. Kvistad CE, Novotny V, Kurz MW, Rønning OM, Thommessen B, Carlsson M, Waje-Andreassen U, et al. Safety and outcomes of tenecteplase in moderate and severe ischemic stroke. *Stroke.* (2019) 50:1279–81. doi: 10.1161/STROKEAHA.119.025041
39. Rønning OM, Logallo N, Thommessen B, Tobro H, Novotny V, Kvistad CE, et al. Tenecteplase versus alteplase between 3 and 4.5 hours in low national institutes of health stroke scale. *Stroke.* (2019) 50:498–500. doi: 10.1161/STROKEAHA.118.024223
40. Campbell BCV, Mitchell PJ, Churilov L, Yassi N, Kleinig TJ, Dowling RJ, et al. Tenecteplase versus alteplase before thrombectomy for ischemic stroke. *N Engl J Med.* (2018) 378:1573–82. doi: 10.1056/NEJMoa1716405
41. Li X, Ling L, Li C, Ma Q. Efficacy and safety of desmoteplase in acute ischemic stroke patients: a systematic review and meta-analysis. *Medicine.* (2017) 96:e6667. doi: 10.1097/MD.0000000000006667
42. Furlan A, Higashida R, Wechsler L, Gent M, Rowley H, Kase C, et al. Intra-arterial prourokinase for acute ischemic stroke: the PROACT II study: a randomized controlled trial. *JAMA.* (1999) 282:2003–11. doi: 10.1001/jama.282.21.2003
43. Ogawa A, Mori E, Minematsu K, Taki W, Takahashi A, Nemoto S, et al. Randomized trial of intraarterial infusion of urokinase within 6 hours of middle cerebral artery stroke. *Stroke.* (2007) 38:2633–9. doi: 10.1161/STROKEAHA.107.488551
44. Saver JL, Goyal M, Bonafe A, Diener HC, Levy EI, Pereira VM, et al. Stent-Retriever Thrombectomy after Intravenous t-PA vs. t-PA alone in stroke. *N Engl J Med.* (2015) 372:2285–95. doi: 10.1056/NEJMoa1415061
45. Jovin TG, Chamorro A, Cobo E, de Miquel MA, Molina CA, Rovira A, et al. Thrombectomy within 8 hours after symptom onset in ischemic stroke. *N Engl J Med.* (2015) 372:2296–306. doi: 10.1056/NEJMoa1503780
46. Nogueira RG, Jadhav AP, Haussen DC, Bonafe A, Budzik RF, Bhuva P, et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. *N Engl J Med.* (2018) 378:11–21. doi: 10.1056/NEJMoa1706442
47. Albers GW, Marks MP, Kemp S, Christensen S, Tsai JP, Ortega-Gutierrez S, et al. Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. *N Engl J Med.* (2018) 378:708–18. doi: 10.1056/NEJMoa1713973
48. Li X, Du H, Song Z, Wang H, Tan Z, Xiao M, et al. Efficacy and safety of sonothrombolysis in patients with acute ischemic stroke: a systematic review and meta-analysis. *J Neurol Sci.* (2020) 416:116998. doi: 10.1016/j.jns.2020.116998
49. Lapergue B, Blanc R, Gory B, Labreuche J, Duhamel A, Marnat G, et al. Effect of endovascular contact aspiration vs stent retriever on revascularization in patients with acute ischemic stroke and large vessel occlusion: the ASTER randomized clinical trial. *JAMA.* (2017) 318:443–52. doi: 10.1001/jama.2017.9644
50. Logallo N, Novotny V, Assmus J, Kvistad CE, Alteheld L, Rønning OM, et al. Tenecteplase versus alteplase for management of acute ischaemic stroke (NOR-TEST): a phase 3, randomised, open-label, blinded endpoint trial. *Lancet Neurol.* (2017) 16:781–8. doi: 10.1016/S1474-4422(17)30253-3
51. Hill MD, Goyal M, Menon BK, Nogueira RG, McTaggart RA, Demchuk AM, et al. Efficacy and safety of nerinetide for the treatment of acute ischaemic stroke (ESCAPE-NA1): a multicentre, double-blind, randomised controlled trial. *Lancet.* (2020) 395:878–87. doi: 10.1016/S0140-6736(20)30258-0
52. Yang P, Zhang Y, Zhang L, Zhang Y, Treurniet KM, Chen W, et al. Endovascular thrombectomy with or without intravenous alteplase in acute stroke. *N Engl J Med.* (2020) 382:1981–93. doi: 10.1056/NEJMoa2001123
53. Anderson CS, Robinson T, Lindley RI, Arima H, Lavados PM, Lee TH, et al. Low-dose versus standard-dose intravenous alteplase in acute ischemic stroke. *N Engl J Med.* (2016) 374:2313–23. doi: 10.1056/NEJMoa1515510
54. Whiteley WN, Slot KB, Fernandes P, Sandercock P, Wardlaw J. Risk factors for intracranial hemorrhage in acute ischemic stroke patients treated with recombinant tissue plasminogen activator. *Stroke.* (2012) 43:2904–9. doi: 10.1161/STROKEAHA.112.665331
55. Malhotra K, Katsanos AH, Goyal N, Tayal A, Gensicke H, Mitsias PD, et al. Intravenous thrombolysis in patients with chronic kidney disease: a systematic review and meta-analysis. *Neurology.* (2020) 95:e121–30. doi: 10.1212/WNL.0000000000009756
56. Huang GQ, Zeng YY, Cheng QQ, Cheng HR, Ruan YT, Yuan CX, et al. Low triiodothyronine syndrome is associated with hemorrhagic transformation in patients with acute ischaemic stroke. *Aging.* (2019) 11:6385–97. doi: 10.18632/aging.102195
57. Mechtouff L, Bochaton T, Paccalet A, Crola Da Silva C, Buisson M, Amaz C, et al. Matrix metalloproteinase-9 relationship with infarct growth and hemorrhagic transformation in the era of thrombectomy. *Front Neurol.* (2020) 11:473. doi: 10.3389/fneur.2020.00473
58. Liu YL, Lu JK, Yin HP, Xia PS, Qiu DH, Liang MQ, et al. High neutrophil-to-lymphocyte ratio predicts hemorrhagic transformation in acute ischemic stroke patients treated with intravenous thrombolysis. *Int J Hypertens.* (2020) 2020:5980261. doi: 10.1155/2020/5980261
59. Yan S, Zhang X, Zhang R, Xu J, Lou M. Early fibrinogen depletion and symptomatic intracranial hemorrhage after reperfusion therapy. *Stroke.* (2019) 50:2716–21. doi: 10.1161/STROKEAHA.119.025711
60. Jian Y, Zhao L, Wang H, Li T, Zhang L, Sun M, et al. Bilirubin: a novel predictor of hemorrhagic transformation and symptomatic intracranial hemorrhage after mechanical thrombectomy. *Neurol Sci.* (2020) 41:903–9. doi: 10.1007/s10072-019-04182-x
61. Cappellari M, Mangiafico S, Saia V, Pracucci G, Nappini S, Nencini P, et al. IER-SICH nomogram to predict symptomatic intracerebral hemorrhage after thrombectomy for stroke. *Stroke.* (2019) 50:909–16. doi: 10.1161/STROKEAHA.118.023316
62. Wang R, Zeng J, Wang F, Zhuang X, Chen X, Miao J. Risk factors of hemorrhagic transformation after intravenous thrombolysis with rt-PA in acute cerebral infarction. *QJM.* (2019) 112:323–6. doi: 10.1093/qjmed/hcy292
63. Chen Z, Chen H, Zhang Y, He Y, Su Y. Lower uric acid level may be associated with hemorrhagic transformation but not functional outcomes in patients with anterior circulation acute ischemic stroke undergoing endovascular thrombectomy. *Metab Brain Dis.* (2020) 35:1157–64. doi: 10.1007/s11011-020-00601-7
64. Song Q, Wang Y, Cheng Y, Liu J, Wei C, Liu M. Serum uric acid and risk of hemorrhagic transformation in patients with acute ischemic stroke. *J Mol Neurosci.* (2020) 70:94–101. doi: 10.1007/s12031-019-01404-x
65. Jucevičiute N, MikuŽis P, Balnyte R. Absolute blood eosinophil count could be a potential biomarker for predicting haemorrhagic transformation after intravenous thrombolysis for acute ischaemic stroke. *BMC Neurol.* (2019) 19:127. doi: 10.1186/s12883-019-1359-6
66. Carrera C, Cullell N, Torres-Águila N, Muiño E, Bustamante A, Dávalos A, et al. Validation of a clinical-genetics score to predict hemorrhagic transformations after rtPA. *Neurology.* (2019) 93:e851–63. doi: 10.1212/WNL.0000000000007997
67. Acampa M, Camarri S, Lazzerini PE, Guideri F, Tassi R, Valenti R, et al. Increased arterial stiffness is an independent risk factor for hemorrhagic transformation in ischemic stroke undergoing thrombolysis. *Int J Cardiol.* (2017) 243:466–70. doi: 10.1016/j.ijcard.2017.03.129
68. Kim TJ, Park HK, Kim JM, Lee JS, Park SH, Jeong HB, et al. Blood pressure variability and hemorrhagic transformation in patients with successful recanalization after endovascular recanalization therapy: a retrospective observational study. *Ann Neurol.* (2019) 85:574–81. doi: 10.1002/ana.25434
69. Kim T, Koo J, Kim SH, Song IU, Chung SW, Lee KS. Blood-brain barrier permeability assessed by perfusion computed tomography predicts hemorrhagic transformation in acute reperfusion therapy. *Neurol Sci.* (2018) 39:1579–84. doi: 10.1007/s10072-018-3468-1
70. Shi ZS, Duckwiler GR, Jahan R, Tateshima S, Szeder V, Saver JL, et al. Early blood-brain barrier disruption after mechanical thrombectomy in acute ischemic stroke. *J Neuroimaging.* (2018) 28:283–8. doi: 10.1111/jon.12504

71. Schlemm L, Endres M, Werring DJ, Nolte CH. Benefit of intravenous thrombolysis in acute ischemic stroke patients with high cerebral microbleed burden. *Stroke.* (2020) 51:232–9. doi: 10.1161/STROKEAHA.119.027633
72. Román LS, Menon BK, Blasco J, Hernández-Pérez M, Dávalos A, Majoie CBLM, et al. Imaging features and safety and efficacy of endovascular stroke treatment: a meta-analysis of individual patient-level data. *Lancet Neurol.* (2018) 17:895–904. doi: 10.1016/S1474-4422(18)30242-4
73. Kaesmacher J, Kaesmacher M, Maegerlein C, Zimmer C, Gersing AS, Wunderlich S, et al. Hemorrhagic transformations after thrombectomy: risk factors and clinical relevance. *CED.* (2017) 43:294–304. doi: 10.1159/000460265
74. Yu Y, Zhang FL, Qu YM, Zhang P, Zhou HW, Luo Y, et al. Intracranial calcification is predictive for hemorrhagic transformation and prognosis after intravenous thrombolysis in non-cardioembolic stroke patients. *J Atheroscler Thromb*. (2020) 27. doi: 10.5551/jat.55889
75. Huang X, Cai Q, Xiao L, Gu M, Liu Y, Zhou Z, et al. Influence of procedure time on outcome and hemorrhagic transformation in stroke patients undergoing thrombectomy. *J Neurol.* (2019) 266:2560–70. doi: 10.1007/s00415-019-09451-5
76. Montalvo M, Mistry E, Chang AD, Yakhkind A, Dakay K, Azher I, et al. Predicting symptomatic intracranial haemorrhage after mechanical thrombectomy: the TAG score. *J Neurol Neurosurg Psychiatry.* (2019) 90:1370–4. doi: 10.1136/jnnp-2019-321184
77. Nisar T, Hanumanthu R, Khandelwal P. Symptomatic intracerebral hemorrhage after intravenous thrombolysis: predictive factors and validation of prediction models. *J Stroke Cerebrovasc Dis.* (2019) 28:104360. doi: 10.1016/j.jstrokecerebrovasdis.2019.104360
78. Jaillard A, Cornu C, Durieux A, Moulin T, Boutitie F, Lees KR et al. Hemorrhagic transformation in acute ischemic stroke. *Stroke.* 1999) 30:1326–32. doi: 10.1161/01.STR.30.7.1326
79. Menon BK, Saver JL, Prabhakaran S, Reeves M, Liang L, Olson DM, et al. Risk score for intracranial hemorrhage in patients with acute ischemic stroke treated with intravenous tissue-type plasminogen activator. *Stroke.* (2012) 43:2293–99. doi: 10.1161/STROKEAHA.112.660415
80. Kheiri B, Osman M, Abdalla A, Haykal T, Ahmed S, Hassan M, et al. Tenecteplase versus alteplase for management of acute ischemic stroke: a pairwise and network meta-analysis of randomized clinical trials. *J Thromb Thrombolysis.* (2018) 46:440–50. doi: 10.1007/s11239-018-1721-3
81. Berkhemer OA, Fransen PSS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med.* (2015) 372:11–20. doi: 10.1056/NEJMoa1411587
82. Campbell BCV, Mitchell PJ, Kleinig TJ, Dewey HM, Churilov L, Yassi N, et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N Engl J Med.* (2015) 372:1009–18. doi: 10.1056/NEJMoa1414792
83. Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. *N Engl J Med.* (2015) 372:1019–30. doi: 10.1056/NEJMoa1414905
84. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American heart association/American stroke association. *Stroke.* 50:e344–418. doi: 10.1161/str.0000000000000211
85. Drouard-de Rousiers E, Lucas L, Richard S, Consoli A, Mazighi M, Labreuche J, et al. Impact of reperfusion for nonagenarians treated by mechanical thrombectomy: insights from the ETIS registry. *Stroke.* (2019) 50:3164–9. doi: 10.1161/STROKEAHA.119.026448
86. Mistry EA, Mistry AM, Nakawah MO, Khattar NK, Fortuny EM, Cruz AS, et al. Systolic blood pressure within 24 hours after thrombectomy for acute ischemic stroke correlates with outcome. *J Am Heart Assoc.* (2017) 6:e006167. doi: 10.1161/JAHA.117.006167
87. Chiang CC, Meyer DM, Meyer BC, Agrawal K, Modir R. RAcial disparities in ich after IV-tPA and neurointerventional treatment (RADIANT). *J Stroke Cerebrovasc Dis.* (2020) 29:104474. doi: 10.1016/j.jstrokecerebrovasdis.2019.104474
88. Wen L, Zhang S, Wan K, Zhang H, Zhang X. Risk factors of haemorrhagic transformation for acute ischaemic stroke in Chinese patients receiving intravenous thrombolysis: a meta-analysis. *Medicine.* (2020) 99:e18995. doi: 10.1097/MD.0000000000018995
89. Mishiro K, Imai T, Sugitani S, Kitashoji A, Suzuki Y, Takagi T, et al. Diabetes mellitus aggravates hemorrhagic transformation after ischemic stroke via mitochondrial defects leading to endothelial apoptosis. *PLoS ONE.* (2014) 9:e103818. doi: 10.1371/journal.pone.0103818
90. Fuentes B, Cruz-Herranz A, Martínez-Sánchez P, Rodríguez-Sanz A, Ruiz Ares G, Prefasi D, et al. Acute ischemic stroke patients with diabetes should not be excluded from intravenous thrombolysis. *J Thromb Thrombolysis.* (2014) 38:522–7. doi: 10.1007/s11239-014-1110-5
91. Enomoto M, Shigeta K, Ota T, Amano T, Ueda M, Matsumaru Y, et al. Predictors of intracranial hemorrhage in acute ischemic stroke after endovascular thrombectomy. *Interv Neuroradiol.* (2020) 26:368–75. doi: 10.1177/1591019920926335
92. Tütüncü S, Ziegler AM., Scheitz JF, Slowinski T, Rocco A, Endres M, et al. Severe Renal impairment is associated with symptomatic intracerebral hemorrhage after thrombolysis for ischemic stroke. *Stroke.* (2013) 44:3217–9. doi: 10.1161/STROKEAHA.113.002859
93. Dang H, Ge WQ, Zhou CF, Zhou CY. The correlation between atrial fibrillation and prognosis and hemorrhagic transformation. *Eur Neurol.* (2019) 82:9–14. doi: 10.1159/000504191
94. Ge WQ, Chen J, Pan H, Chen F, Zhou CY. Analysis of risk factors increased hemorrhagic transformation after acute ischemic stroke. *J Stroke Cerebrovasc Dis.* (2018) 27:3587–90. doi: 10.1016/j.jstrokecerebrovasdis.2018.08.028
95. Ott BR, Zamani A, Kleefield J, Funkenstein HH. The clinical spectrum of hemorrhagic infarction. *Stroke.* (1986) 17:630–7. doi: 10.1161/01.STR.17.4.630
96. Bettencourt S, Ferro JM. Acute ischemic stroke treatment in infective endocarditis: systematic review. *J Stroke Cerebrovasc Dis.* (2020) 29:104598. doi: 10.1016/j.jstrokecerebrovasdis.2019.104598
97. Yang C, Zhang J, Liu C, Xing Y. Comparison of the risk factors of hemorrhagic transformation between large artery atherosclerosis stroke and cardioembolism after intravenous thrombolysis. *Clin Neurol Neurosurg.* (2020) 196:106032. doi: 10.1016/j.clineuro.2020.106032
98. Li F, Ren Y, Cui X, Liu P, Chen F, Zhao H, et al. Postoperative hyperglycemia predicts symptomatic intracranial hemorrhage after endovascular treatment in patients with acute anterior circulation large artery occlusion. *J Neurol Sci.* (2020) 409:116588. doi: 10.1016/j.jns.2019.116588
99. Tsivgoulis G, Goyal N, Katsanos AH, Malhotra K, Ishfaq MF, Pandhi A, et al. Intravenous thrombolysis for large vessel or distal occlusions presenting with mild stroke severity. *Eur J Neurol.* (2020) 27:1039–47. doi: 10.1111/ene.14199
100. Majidi S, Luby M, Lynch JK, Hsia AW, Benson RT, Kalaria CP, et al. MRI-based thrombolytic therapy in patients with acute ischemic stroke presenting with a low NIHSS. *Neurology.* (2019) 93:e1507–13. doi: 10.1212/WNL.0000000000008312
101. Wang GF, Zhao X, Liu SP, Xiao YL, Lu ZN. Efficacy and safety of mechanical thrombectomy for acute mild ischemic stroke with large vessel occlusion. *Med Sci Monit.* (2020) 26:e926110. doi: 10.12659/MSM.926110
102. Chin F, Waqas M, Chou R, Gerace PG, Rai HH, Vakharia K, et al. Impact of endovascular reperfusion on low national institutes of health stroke scale score large-vessel occlusion stroke. *J Stroke Cerebrovasc Dis.* (2020) 29:104836. doi: 10.1016/j.jstrokecerebrovasdis.2020.104836
103. Álvarez-Sabín J, Maisterra O, Santamarina E, Kase CS. Factors influencing haemorrhagic transformation in ischaemic stroke. *Lancet Neurol.* (2013) 12:689–705. doi: 10.1016/S1474-4422(13)70055-3
104. Meinel TR, Kniepert JU, Seiffge DJ, Gralla J, Jung S, Auer E, et al. Endovascular stroke treatment and risk of intracranial hemorrhage in anticoagulated patients. *Stroke.* (2020) 51:892–8. doi: 10.1161/STROKEAHA.119.026606
105. Barber PA, Demchuk AM, Zhang J, Buchan AM. Validity and reliability of a quantitative computed tomography score in predicting outcome of hyperacute stroke before thrombolytic therapy. ASPECTS study group. Alberta stroke programme early CT score. *Lancet.* (2000) 355:1670–4. doi: 10.1016/s0140-6736(00)02237-6
106. Kallmes DF, Brinjikji W, Boccardi E, Ciceri E, Diaz O, Tawk R, et al. Aneurysm study of pipeline in an observational registry (ASPIRe). *Interv Neurol.* (2016) 5:89–99. doi: 10.1159/000446503

107. Liu X, Zhang J, Tian C, Wang J. The relationship of leukoaraiosis, haemorrhagic transformation and prognosis at 3 months after intravenous thrombolysis in elderly patients aged ≥ 60 years with acute cerebral infarction. *Neurol Sci.* (2020) 41:3195–200. doi: 10.1007/s10072-020-04398-2
108. Adebayo OD, Culpan G. Diagnostic accuracy of computed tomography perfusion in the prediction of haemorrhagic transformation and patient outcome in acute ischaemic stroke: a systematic review and meta-analysis. *Eur Stroke J.* (2020) 5:4–16. doi: 10.1177/2396987319883461
109. Cao R, Ye G, Wang R, Xu L, Jiang Y, Wang G, et al. Collateral vessels on 4D CTA as a predictor of hemorrhage transformation after endovascular treatments in patients with acute ischemic stroke: a single-center study. *Front Neurol.* (2020) 11:60. doi: 10.3389/fneur.2020.00060
110. Suh CH, Jung SC, Cho SJ, Kim D, Lee JB, Woo DC, et al. Perfusion CT for prediction of hemorrhagic transformation in acute ischemic stroke: a systematic review and meta-analysis. *Eur Radiol.* (2019) 29:4077–87. doi: 10.1007/s00330-018-5936-7
111. Li Q, Gao X, Yao Z, Feng X, He H, Xue J, et al. Permeability surface of deep middle cerebral artery territory on computed tomographic perfusion predicts hemorrhagic transformation after stroke. *Stroke.* (2017) 48:2412–8. doi: 10.1161/STROKEAHA.117.017486
112. Bivard A, Kleinig T, Churilov L, Levi C, Lin L, Cheng X, et al. Permeability measures predict haemorrhagic transformation after ischemic stroke. *Ann Neurol.* (2020) 88:466–76. doi: 10.1002/ana.25785
113. Kim JM, Park KY, Lee WJ, Byun JS, Kim JK, Park MS, et al. The cortical contrast accumulation from brain computed tomography after endovascular treatment predicts symptomatic hemorrhage. *Eur J Neurol.* (2015) 22:1453–8. doi: 10.1111/ene.12764
114. You SH, Kim B, Kim BK, Suh SI. MR imaging for differentiating contrast staining from hemorrhagic transformation after endovascular thrombectomy in acute ischemic stroke: phantom and patient study. *AJNR Am J Neuroradiol.* (2018) 39:2313–9. doi: 10.3174/ajnr.A5848
115. Almqvist H, Almqvist NS, Holmin S, Mazya MV. Dual-energy CT follow-up after stroke thrombolysis alters assessment of hemorrhagic complications. *Front Neurol.* (2020) 11:357. doi: 10.3389/fneur.2020.00357
116. Choi Y, Shin NY, Jang J, Ahn KJ, Kim BS. Dual-energy CT for differentiating acute intracranial hemorrhage from contrast staining or calcification: a meta-analysis. *Neuroradiology.* (2020) 62:1617–26. doi: 10.1007/s00234-020-02486-w
117. Suh CH, Jung SC, Cho SJ, Woo DC, Oh WY, Lee JG, et al. MRI for prediction of hemorrhagic transformation in acute ischemic stroke: a systematic review and meta-analysis. *Acta Radiol.* (2020) 61:964–72. doi: 10.1177/0284185119887593
118. El Nawar R, Yeung J, Labreuche J, Chadenat ML, Duong DL, De Malherbe M, et al. MRI-based predictors of hemorrhagic transformation in patients with stroke treated by intravenous thrombolysis. *Front Neurol.* (2019) 10:897. doi: 10.3389/fneur.2019.00897
119. Panni P, Gory B, Xie Y, Consoli A, Desilles JP, Mazighi M, et al. Acute stroke with large ischemic core treated by thrombectomy. *Stroke.* (2019) 50:1164–71. doi: 10.1161/STROKEAHA.118.024295
120. Drelon A, Kuchcinski G, Caparros F, Dequatre-Ponchelle N, Moulin S, Cordonnier C, et al. Remote brain hemorrhage after IV thrombolysis: role of preexisting lesions. *Neurology.* (2020) 94:e961–7. doi: 10.1212/WNL.0000000000008874
121. Yu S, Ma SJ, Liebeskind DS, Qiao XJ, Yan L, Saver JL, et al. Reperfusion into severely damaged brain tissue is associated with occurrence of parenchymal hemorrhage for acute ischemic stroke. *Front Neurol.* (2020) 11:586. doi: 10.3389/fneur.2020.00586
122. Arca KN, Demaerschalk BM, Almader-Douglas D, Wingerchuk DM, O'Carroll CB. Does high cerebral microbleed burden increase the risk of intracerebral hemorrhage after intravenous tissue plasminogen activator for acute ischemic stroke? *Neurologist.* (2019) 24:40–3. doi: 10.1097/NRL.0000000000000228
123. Salehi OS, Boddu SR, Gusdon AM, Kummer B, Baradaran H, Patel P, et al. Angiographic blush after mechanical thrombectomy is associated with hemorrhagic transformation of ischemic stroke. *J Stroke Cerebrovasc Dis.* (2018) 27:3124–30. doi: 10.1016/j.jstrokecerebrovasdis.2018.07.004
124. Cartmell SCD, Ball RL, Kaimal R, Telischak NA, Marks MP, Do HM, et al. Early cerebral vein after endovascular ischemic stroke treatment predicts symptomatic reperfusion hemorrhage. *Stroke.* (2018) 49:1741–6. doi: 10.1161/STROKEAHA.118.021402
125. van de Graaf RA, Chalos V, van Es ACGM, Emmer BJ, Lycklama À, Nijeholt GJ, et al. Periprocedural intravenous heparin during endovascular treatment for ischemic stroke: results from the MR CLEAN registry. *Stroke.* (2019) 50:2147–55. doi: 10.1161/STROKEAHA.119.025329
126. Zhu F, Labreuche J, Haussen DC, Piotin M, Steglich-Arnholm H, Taschner C, et al. Hemorrhagic transformation after thrombectomy for tandem occlusions. *Stroke.* (2019) 50:516–9. doi: 10.1161/STROKEAHA.118.023689
127. Zhu F, Anadani M, Labreuche J, Spiotta A, Turjman F, Piotin M, et al. Impact of antiplatelet therapy during endovascular therapy for tandem occlusions: a collaborative pooled analysis. *Stroke.* (2020) 51:1522–9. doi: 10.1161/STROKEAHA.119.028231
128. Zhu F, Hossu G, Soudant M, Richard S, Achit H, Beguinet M, et al. Effect of emergent carotid stenting during endovascular therapy for acute anterior circulation stroke patients with tandem occlusion: a multicenter, randomized, clinical trial (TITAN) protocol. *Int J Stroke.* (2020). doi: 10.1177/1747493020929948
129. Bourcier R, Saleme S, Labreuche J, Mazighi M, Fahed R, Blanc R, et al. More than three passes of stent retriever is an independent predictor of parenchymal hematoma in acute ischemic stroke. *J Neurointerv Surg.* (2019) 11:625–9. doi: 10.1136/neurintsurg-2018-014380
130. Hassan AE, Kotta H, Shariff U, Preston L, Tekle W, Qureshi A. There is no association between the number of stent retriever passes and the incidence of hemorrhagic transformation for patients undergoing mechanical thrombectomy. *Front Neurol.* (2019) 10:818. doi: 10.3389/fneur.2019.00818
131. Tonetti DA, Desai SM, Casillo S, Stone J, Brown M, Jankowitz B, et al. Successful reperfusion, rather than number of passes, predicts clinical outcome after mechanical thrombectomy. *J Neurointerv Surg.* (2020) 12:548–51. doi: 10.1136/neurintsurg-2019-015330
132. Anadani M, Ajinkya S, Alawieh A, Vargas J, Chatterjee A, Turk A, et al. Intra-arterial tissue plasminogen activator is a safe rescue therapy with mechanical thrombectomy. *World Neurosurg.* (2019) 123:e604–8. doi: 10.1016/j.wneu.2018.11.232
133. Kaesmacher J, Bellwald S, Dobrocky T, Meinel TR, Piechowiak EI, Goeldlin M, et al. Safety and efficacy of intra-arterial urokinase after failed, unsuccessful, or incomplete mechanical thrombectomy in anterior circulation large-vessel occlusion stroke. *JAMA Neurol.* (2019) 77:318–26. doi: 10.1001/jamaneurol.2019.4192
134. Cheng Z, Geng X, Gao J, Hussain M, Moon SJ, Du H, et al. Intravenous administration of standard dose tirofiban after mechanical arterial recanalization is safe and relatively effective in acute ischemic stroke. *Aging Dis.* (2019) 10:1049–57. doi: 10.14336/AD.2018.0922
135. Yi HJ, Sung JH, Lee DH. Safety and efficacy of intra-arterial tirofiban injection during mechanical thrombectomy for large artery occlusion. *Curr Neurovasc Res.* (2019) 16:416–24. doi: 10.2174/1567202616666191023154956
136. Pirson FAV, van Oostenbrugge RJ, van Zwam WH, Remmers MJM, Dippel DWJ, van Es ACGM, et al. Repeated endovascular thrombectomy in patients with acute ischemic stroke: results from a nationwide multicenter database. *Stroke.* (2020) 51:526–32. doi: 10.1161/STROKEAHA.119.027525
137. Anderson CS, Huang Y, Lindley RI, Chen X, Arima H, Chen G, et al. Intensive blood pressure reduction with intravenous thrombolysis therapy for acute ischaemic stroke (ENCHANTED): an international, randomised, open-label, blinded-endpoint, phase 3 trial. *Lancet.* (2019) 393:877–88. doi: 10.1016/S0140-6736(19)30038-8
138. Mazighi M, Labreuche J, Richard S, Gory B, Lapergue B, Sibon I, et al. Blood pressure target in acute stroke to reduce hemorrhage after endovascular therapy: the randomized BP TARGET study protocol. *Front Neurol.* (2020) 11:480. doi: 10.3389/fneur.2020.00480
139. Silverman A, Kodali S, Sheth KN, Petersen NH. Hemodynamics and hemorrhagic transformation after endovascular therapy for ischemic stroke. *Front Neurol.* (2020) 11:728. doi: 10.3389/fneur.2020.00728
140. Nan D, Jin H, Deng J, Yu W, Liu R, Sun W, et al. Cilostazol ameliorates ischemia/reperfusion-induced tight junction disruption in brain endothelial cells by inhibiting endoplasmic reticulum stress. *FASEB J.* (2019) 33:10152–64. doi: 10.1096/fj.201900326R
141. Bieber M, Schuhmann MK, Volz J, Kumar GJ, Vaidya JR, Nieswandt B, et al. Description of a novel phosphodiesterase (PDE)-3 inhibitor protecting

mice from ischemic stroke independent from platelet function. *Stroke.* (2019) 50:478–86. doi: 10.1161/STROKEAHA.118.023664
142. Sheng Z, Liu Y, Li H, Zheng W, Xia B, Zhang X, et al. Efficacy of minocycline in acute ischemic stroke: a systematic review and meta-analysis of rodent and clinical studies. *Front Neurol.* (2018) 9:1103. doi: 10.3389/fneur.2018.01103
143. Chen H, Guan B, Wang B, Pu H, Bai X, Chen X, et al. Glycyrrhizin prevents hemorrhagic transformation and improves neurological outcome in ischemic stroke with delayed thrombolysis through targeting peroxynitrite-mediated HMGB1 signaling. *Transl Stroke Res.* (2019) 11:967–82. doi: 10.1007/s12975-019-00772-1
144. Chen H, Chen X, Luo Y, Shen J. Potential molecular targets of peroxynitrite in mediating blood-brain barrier damage and haemorrhagic transformation in acute ischaemic stroke with delayed tissue plasminogen activator treatment. *Free Radic Res.* (2018) 52:1220–39. doi: 10.1080/10715762.2018.1521519
145. Voors-Pette C, Lebozec K, Dogterom P, Jullien L, Billiald P, Ferlan P, et al. Safety and tolerability, pharmacokinetics, and pharmacodynamics of ACT017, an antiplatelet GPVI (glycoprotein VI) fab. *Arterioscler Thromb Vasc Biol.* (2019) 39:956–64. doi: 10.1161/ATVBAHA.118.312314
146. Zhao W, Wu C, Stone C, Ding Y, Ji X. Treatment of intracerebral hemorrhage: current approaches and future directions. *J Neurol Sci.* (2020) 416:117020. doi: 10.1016/j.jns.2020.117020
147. Das S, Mitchell P, Ross N, Whitfield PC. Decompressive hemicraniectomy in the treatment of malignant middle cerebral artery infarction: a meta-analysis. *World Neurosurg.* (2019) 123:8–16. doi: 10.1016/j.wneu.2018.11.176

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

BRIEF RESEARCH REPORT
published: 10 March 2021
doi: 10.3389/fneur.2021.649079

Early Venous Filling Following Thrombectomy: Association With Hemorrhagic Transformation and Functional Outcome

Sophie Elands[1], Pierre Casimir[1], Thomas Bonnet[2], Benjamin Mine[2], Boris Lubicz[2], Martin Sjøgård[3], Noémie Ligot[1] and Gilles Naeije[1]*

[1] Department of Neurology, Erasmus Hospital, Université Libre de Bruxelles, Brussels, Belgium, [2] Department of Interventional Neuroradiology, Erasmus Hospital, Université Libre de Bruxelles, Brussels, Belgium, [3] Laboratoire de Cartographie Fonctionnelle du Cerveau, Neuroscience Institute (ULB-Neuroscience Institute), Université Libre de Bruxelles, Brussels, Belgium

Background and Purpose: Previous studies have noted the angiographic appearance of early venous filling (EVF) following recanalisation in acute ischemic stroke. However, the prognostic implications of EVF as a novel imaging biomarker remain unclear. We aimed to evaluate the correlation between EVF with (i) the risk of subsequent reperfusion hemorrhage (RPH) and (ii) the association of EVF on both the NIHSS score at 24 h and functional outcome as assessed with the Modified Rankin Scale (mRS) score at 90 days.

Methods: We conducted a retrospective cohort study of patients presenting with an acute ischemic stroke due to a proximal large-vessel occlusion of the anterior circulation treated by thrombectomy. Post-reperfusion digital subtraction angiography was reviewed to look for EVF as evidenced by the contrast opacification of any cerebral vein before the late arterial phase.

Results: EVF occurred in 22.4% of the 147 cases included. The presence of EVF significantly increased the risk of RPH ($p = 0.0048$), including the risk of symptomatic hemorrhage ($p = 0.0052$). The presence of EVF ($p = 0.0016$) and the absence of RPH ($p = 0.0021$) were independently associated with a better outcome as defined by the NIHSS difference at 24 h, most significantly in the $EVF^{+}RPH^{-}$ group. No significant relationship was however found between either EVF or RPH and a mRS score ≤ 2 at 90 days.

Conclusion: Early venous filling on angiographic imaging is a potential predictor of reperfusion hemorrhage. The absence of subsequent RPH in this sub-group is associated with better outcomes at 24 h post-thrombectomy than in those with RPH.

Keywords: acute stroke, angiography digital subtraction, early venous filling, cerebral hemorrhage, thrombectomy, reperfusion after ischemia

OPEN ACCESS

Edited by:
Nishant K. Mishra, University of California, Los Angeles, United States

Reviewed by:
Jeremy Josef Heit, Stanford University, United States
Steffen Tiedt, LMU Munich University Hospital, Germany

****Correspondence:***
Sophie Elands
sophie.elands@erasme.ulb.ac.be

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *03 January 2021*
Accepted: *18 February 2021*
Published: *10 March 2021*

Citation:
Elands S, Casimir P, Bonnet T, Mine B, Lubicz B, Sjøgård M, Ligot N and Naeije G (2021) Early Venous Filling Following Thrombectomy: Association With Hemorrhagic Transformation and Functional Outcome. Front. Neurol. 12:649079. doi: 10.3389/fneur.2021.649079

INTRODUCTION

Stroke is the second most common cause of death and the main cause of acquired disability worldwide (1). Over sixty percent of morbidity and mortality related to stroke is due to large vessel occlusion (LVO) (2), which in itself accounts for about 30% of all ischemic strokes (3). The primary therapeutic aim is to rapidly recanalize the occluded vessel in order to restore blood flow and salvage

cerebral tissue so as to improve patient outcome. In that context, endovascular thrombectomy (EVT) with or without intravenous thrombolysis substantially reduces disability in selected cases of LVO (4). The benefit of recanalizing treatments must be balanced with procedural risks and LVO stroke complications such as hemorrhagic transformation and reperfusion hemorrhage (RPH), with hemorrhagic transformation occurring in up to 43% of patients (5). These hemorrhagic complications tend to be classified based on their radiological appearance according to the European Cooperative Acute Stroke Study (ECASS II) into parenchymal hematomas (PH) and hemorrhagic infarctions (HI). The incidence of PH after EVT was recently reported to be 6% (6), with PH strongly correlating with early neurological deterioration and poor clinical outcome (7).

Although time is of the essence in achieving recanalization, there has been a recent paradigm shift whereby neuroimaging is gaining center stage in EVT decision-making. It provides an invaluable insight that is both patient-specific and dynamic into the physiological effects of the vessel occlusion, the penumbra at stake and RPH risks. Neuroimaging thus plays a key role in providing a tailored-made risk-benefit calculation for recanalization intervention and prediction of treatment response (8).

Current pre-treatment evaluation techniques include perfusion imaging derived from either computer tomographic (CT) or magnetic resonance imaging (MRI), which allow a quantitative assessment of cerebral blood flow (CBF), cerebral blood volume (CBV), and mean transit time (MTT). These measures help to evaluate the degree of salvageable "penumbra," in other words the area of brain tissue surrounding the irreversibly damaged "infarcted core" that is at risk of infarction but may still be saved if reperfused. As such, the infarcted core is usually defined on CT as a CBF <30% of normal brain blood flow or on MRI as an apparent diffusion coefficient $<620\ \mu m^2/s$, whereas the area of critical hypoperfusion is identified as MTT of >6 s. The estimated penumbra, otherwise known as mismatch volume, is derived from the difference between these two values. However, in LVO, EVT decision currently relies on perfusion characteristics only when symptom onset exceeds 6 h. There, a favorable mismatch allows to extend the therapeutic window to as far as 24 h post-symptom onset (8). Similarly, perfusion imaging helps to assess the risk of bleeding following EVT, with an increased risk of hemorrhagic transformation in cases with a large ischemic core volume, severe blood flow restriction, blood-brain barrier disruption and poor collateral status (9).

However, within 6 h of symptoms onset in LVO, perfusion imaging is not warranted, preventing its use as prognostic tool for clinical outcome or complications in most of cases. In that context, digital subtraction angiography (DSA) could provide valuable information. As such, there is scarce evidence about the post-recanalization imaging biomarkers available on digital subtraction angiography (DSA). Prominent brain vascularity in the form of capillary blush, arteriovenous shunting and early venous filling (EVF) have been noted immediately after EVT (9). EVF, defined as the contrast opacification of any cerebral vein before the late arterial phase on post-reperfusion DSA, has previously been shown to be associated with an increased risk of subsequent infarction (10–12), a higher rate of reperfusion hemorrhage (RPH) and worse clinical outcomes (10, 13). However, these findings were limited by either outdated recanalisation techniques or small cohort size.

Here, we investigated the association between EVF and RPH, together with its impact on functional prognosis and physiopathological correlations by conducting a retrospective study on the largest cohort to date of patients undergoing thrombectomy for a proximal anterior circulation occlusion.

MATERIALS AND METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Patient Cohort

Ethics approval was obtained from our local institutional review board to conduct a retrospective cohort study with data collected from the medical records of patients presenting at Erasmus Hospital in Brussels with an acute ischemic stroke treated by thrombectomy between December 2014 and September 2019. Inclusion criteria were patients presenting with (1) a proximal occlusion of the anterior circulation including the internal carotid artery, M1 or M2 divisions of the Middle Cerebral Artery (MCA), (2) baseline Modified Rankin Scale (mRS) score of ≤ 2, (3) treated by mechanical thrombectomy within 24 h of symptom onset, (4) with a reperfusion score of Thrombolysis in Cerebral Infarction (TICI) equal or > II, and (5) imaging by CT within 24 h and/or MRI within 7 days of revascularization. The procedures followed were in accordance with institutional guidelines. Exclusion criteria included those with missing clinical data for statistical analysis.

Imaging

Pre-interventional imaging included non-contrast CT and CT angiography. Additional CT perfusion imaging was done if the patient presented >6 h after symptom onset as per current guidelines. Occasionally MRI with FLAIR, time-of-flight MR angiography, as well as diffusion and perfusion imaging were used if there was a contra-indication to CT imaging. Early Venous Filling (EVF) is defined as the contrast opacification of any cerebral vein before the late arterial phase on post-reperfusion DSA and rated as either present or absent (**Figure 1** depicts four illustrative cases). Reperfusion hemorrhage (RPH) was noted on CT or MRI, and classified based on their radiological appearance according to the European Cooperative Acute Stroke Study (ECASS II) into hemorrhagic infarction (HI) and parenchymal hematoma (PH), with HI1 defined as small petechiae along the ischemic margins, HI2 as confluent petechiae within the infarcted zone, PH1 as blood clots in <30% of the ischemic area with mild mass effect, and PH2 as blood clots in >30% of the ischemia zone with marked mass effect (5). A symptomatic hemorrhage was defined as RPH associated with an increase in NIHSS score of >2. All imaging was reviewed by two independent interventional neuroradiologists blinded to clinical outcome without concertation. EVF was considered present when identified by both neuroradiologists.

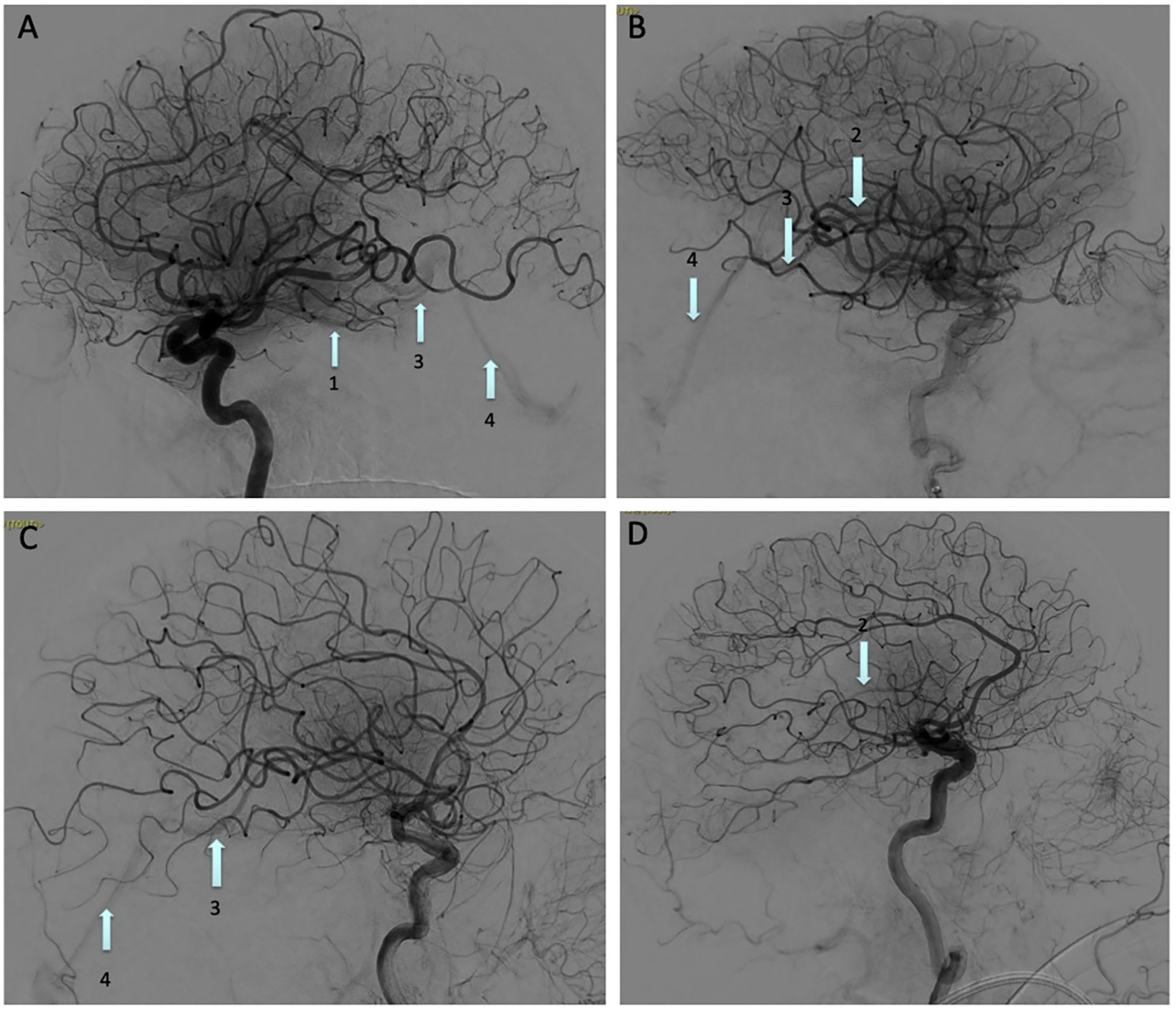


FIGURE 1 | Digital subtraction angiography images showing an Early Venous Filling (EVF) following recanalization of: **(A)** M1 occlusion, **(B)** M2 temporal branch occlusion, **(C)** M1 occlusion, and **(D)** Terminal portion of the internal carotid artery occlusion. Basal vein of Rosenthal (arrow 1), internal cerebral vein (arrow 2), great vein of Galen (arrow 3), straight sinus (arrow 4).

Outcomes

The primary outcome measure was the occurrence of RPH, recorded as present or absent. The secondary outcome measure was the functional outcome, using (1) the National Institutes of Health Stroke Scale (NIHSS) score difference between hospital admission and 24 h post-thrombectomy, and (2) the Modified Rankin Scale (mRS) score at 90 days with a good clinical outcome defined as a score ≤ 2.

Statistical Analysis

To determine whether EVF status was significantly related to the probability of (i) reperfusion hemorrhage and (ii) symptomatic hemorrhage, we used independent multiple logistic regression models with RPH or symptomatic hemorrhage as the dependent variables and EVF as regressor alongside the following potential confounding variables—age, sex, NIHSS score at admission, time to recanalization, site of occlusion and the presence of prior antiplatelet or anticoagulant therapy—to control for the effect of these on the EVF-RPH relationship.

In order to investigate the relationship between the functional outcome and (i) EVF and (ii) RPH, we included both in separate regression analyses with the two functional outcomes as the dependent variable in separate models. Specifically, we performed a multiple linear regression to investigate the relationship between the NIHSS difference (between admission and 24 h post-thrombectomy) and the regressors EVF and RPH, whilst also controlling for the other confounding variables listed above. In addition, we performed the same analysis using

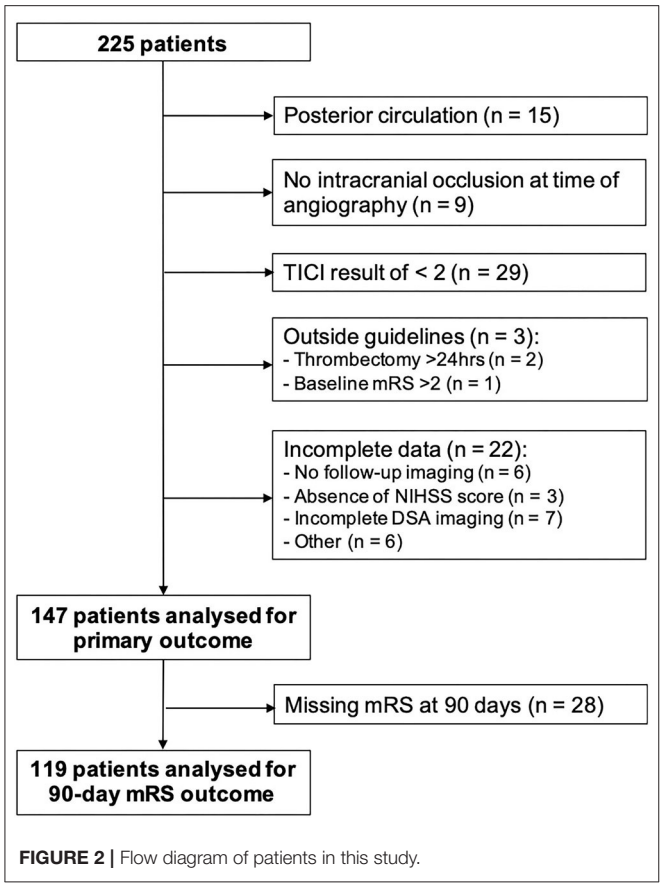


FIGURE 2 | Flow diagram of patients in this study.

symptomatic RPH. Similarly, we performed a multiple logistic regression analysis to investigate the relationship between the binarized mRS outcome (with a good outcome being a mRS $\leq$ 2) as dependent variable and the same regressors listed above.

To investigate whether the presence of symptomatic hemorrhage or any of the subtypes of RPH were found in significantly different proportions in the EVF^+ and EVF^- groups, we conducted five independent chi square tests for the difference in proportion of HI1, HI2, PH1, PH2, and symptomatic hemorrhage between the groups.

RESULTS

We identified 225 patients who underwent a thrombectomy. Seventy-eight met exclusion criteria (**Figure 2**), yielding a total of 147 patients. Seven patients had missing mRS scores at 90 days (4 EVF^-RPH^- and 3 EVF^+RPH^-), so only 140 patients were included in the analysis of these outcomes.

Of the 147 cases, 39 and 33 cases were noted to have an EVF, respectively, by neuroradiologist 1 (BM) and neuroradiologist 2 (TB) of which 33 were congruent and considered to have definite EVF (22.4%) (Cohen Kappa coefficient for EVF 0.23, 66% of agreement). The baseline clinical characteristics of both groups (EVF^- and EVF^+) are summarized in **Table 1**. There was no significant difference between these groups in terms of demographics, cardiovascular risk factors, prior antiplatelet/anticoagulation treatment, pre-therapeutic NIHSS, occlusion site, door-to-needle time or TICI result.

TABLE 1 | Clinical characteristics.

Characteristics	All (%)	EVF^- (%)	EVF^+ (%)
Number of patients	147	114 (77.6)	33 (22.4)
Age (average)	72.8	71.2	78
Female	101 (68.7)	79 (69.3)	22 (66.7)
Hypertension	105 (71.4)	79 (69.3)	26 (78.8)
Hypercholesterolemia	79 (53.7)	57 (50)	22 (66.7)
Diabetes	24 (16.3)	20 (17.5)	4 (12.1)
Coronary artery disease	43 (29.3)	33 (28.9)	10 (30.3)
Atrial fibrillation	66 (44.9)	48 (42.1)	18 (54.5)
Chronic kidney disease	24 (16.3)	17 (14.9)	7 (21.2)
Smoking (Prior/Active)	49 (33.3)	43 (37.7)	6 (18.2)
Prior stroke	18 (12.2)	14 (12.3)	4 (12.1)
Prior intracerebral hemorrhage	3 (2.0)	3 (2.6)	0 (0)
Anticoagulant/Antiplatelet therapy			
Aspirin	48 (32.7)	39 (34.2)	9 (27.3)
Clopidogrel	7 (4.8)	6 (5.2)	1 (3.0)
Warfarin	13 (8.8)	10 (8.8)	3 (9.1)
Heparin	1 (0.7)	1 (0.9)	0 (0)
Direct oral anticoagulant	16 (10.9)	14 (12.3)	2 (6.1)
Baseline scores			
Baseline NIHSS (average)	15.5	15.0	17.2
Baseline mRS (average)	0.5	0.5	0.5
Thrombolysis			
IV tPA	36 (24.5)	26 (22.8)	10 (30.3)
Time to IV tPA (min), average	110	110	111
Occlusion site			
Internal carotid artery	49 (33.3)	35 (30.7)	14 (42.4)
M1	81 (55.1)	66 (57.9)	15 (45.5)
M2	17 (11.6)	13 (11.4)	4 (12.1)
TICI score			
2a	15 (10.2)	8 (7.0)	7 (21.2)
2b	52 (35.4)	41 (36.0)	11 (33.3)
3	80 (54.4)	65 (57.0)	15 (45.5)
Time to recanalisation (min), average	293	291	299
Post-treatment imaging modality			
CT	145 (98.6)	113 (99.1)	32 (97.0)
MRI	93 (63.3)	70 (61.4)	23 (69.7)
Both	92 (62.3)	70 (61.4)	22 (66.7)
Reperfusion hemorrhage			
Any hemorrhage	42 (28.6)	28 (24.6)	14 (42.4)
HI1	13 (8.8)	8 (7.0)	5 (15.2)
HI2	7 (4.8)	6 (5.3)	1 (3.0)
PH1	15 (10.2)	10 (8.8)	5 (15.2)
PH2	7 (4.8)	4 (3.5)	3 (9.1)

EVF, Early Venous Filling; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; IV tPA, Intra-Venous tissue Plasminogen Activator; TICI, Thrombolysis in Cerebral Infarction; CT, Computed Tomography; MRI, Magnetic Resonance Imaging; HI, Hemorrhagic Infarction; PH, Parenchymal Hematoma.

Of those with RPH, 6 were symptomatic in the EVF^- group (3 PH1 and 3 PH2) compared to 1 PH2 in those EVF^+. Neither symptomatic hemorrhage nor any of the four types of RPH (HI1, HI2, PH1, PH2) were found in significantly different proportions in the EVF^+/EVF^- groups ($\chi^2 < 1.21$, $p > 0.271$).

In the primary outcome analysis, the risk of RPH was significantly increased by the presence of EVF (odds ratio 6.68, $p = 0.0048$) when controlling for other confounding factors. Similarly, the presence of EVF significantly increased the risk of *symptomatic* RPH (odds ratio 5.8, $p = 0.0052$).

With regards to the secondary outcome, a multiple regression analysis ($R^2 = 0.153$, $F = 4.82$, $p = 8.0 \times 10^{-5}$) showed that the NIHSS difference had a significant inverse relationship to EVF ($\beta = -4.75$, $t = -2.93$, $p = 0.0016$) and a significant positive relationship to the existence of RPH ($\beta = 4.19$, $t = 2.87$, $p = 0.0021$) when controlling for the known confounding factors. In other words, the presence of EVF was associated with an improvement (i.e., decrease) in NIHSS score independently of RPH, whereas the occurrence of RPH was associated with a worsening (i.e., increase) in NIHSS. The same analysis using the existence of a *symptomatic* RPH instead showed that this had an even more significant positive relationship with NIHSS difference ($\beta = 13.57$, $t = 5.75$, 6.56×10^{-8}).

These regression analyses show that the presence of EVF (EVF^+) and the absence of RPH (RPH^-) are independently associated with a better outcome as defined by the NIHSS difference. In addition, there was no significant effect of the interaction between EVF and RPH on the outcome. For these reasons, we would expect the EVF^+RPH^- group to be associated with the best outcome compared to the other three combinations, which is indeed what we observe (see **Table 2**).

In contrast, when looking at the mRS at 90 days, a multiple logistic regression analysis showed no significant relationship between mRS (binarized as either good mRS $\leq$ 2 or bad $>$2) and either EVF, RPH or symptomatic RPH, when controlled for the same confounding variables described previously (coefficient $p > 0.061$).

TABLE 2 | Functional outcomes by EVF and RPH subgroups.

NIHSSdiff	EVF^+	EVF^-	mRS90	EVF^+	EVF^-
RPH^+	5.2	3.3	RPH^+	3 (23.1%)	6 (20.1%)
RPH^-	10.2	6.1	RPH^-	10 (66.7%)	35 (51.5%)

EVF, Early Venous Filling; RPH, Reperfusion Hemorrhage; NIHSSdiff, National Institutes of Health Stroke Scale difference at 24 h; mRS90, Modified Rankin Scale score at 90 days.

DISCUSSION

In our study, EVF occurred in almost 25% of cases and was associated with both increased rate of RPH and improved functional outcome at 24 h in those combining EVF and no RPH. This double-edged sword effect may reflect a state of hyperperfusion, beneficial in preserving the penumbra, but also nefarious by increasing blood flow to a brain area made more fragile by ischemia.

In comparison to other large cohorts of LVO ischemic strokes (14), ours had a relatively higher proportion of women 68.7% (vs. 47%), hypertension 71.4% (vs. 57.5%), hyperlipidemia 53.7% (35.2%), and atrial fibrillation 44.9% (32.9%). Thus, care must be drawn before extrapolating the findings to a larger population. However, EVF occurrence in 22.4% of successful EVT cases aligns with the incidence found in previous studies using similar definitions of EVF (10, 13).

EVF was associated with a higher risk of both RPH and symptomatic hemorrhage, which is in line with the seminal study by Ohta et al. (10) where the rate of RPH and symptomatic hemorrhage (defined as massive hematoma with neurological worsening) was 61.3 and 32.3%, respectively, in their EVF^+ group. This was in the context of a prospective study on superselective local angiography via microcatheter before and during intra-arterial reperfusion therapy for acute MCA occlusion. In comparison to our study, the observed increased bleeding risk if EVF^+ was confounded by the use of microcatheter injections and intra-arterial thrombolysis, both independently associated with RPH. Similarly, Cartmell et al. (13) identified EVF to be a strong predictor of symptomatic parenchymal hematoma associated with a NIHSS decline of at least 3 points following revascularization therapy, with a sensitivity and specificity of both 0.83, albeit in a relatively small cohort size of 59 patients, similar in demographics to our population.

Independently of RPH, prior studies have suggested EVF to not be associated with a significant NIHSS shift (13), and to carry a less favorable long-term outcome as defined by an mRS$>$2 at 90 days (10, 13). However, our study suggests that EVF is associated with an initial functional improvement in terms of NIHSS difference at 24 h, especially so for those with EVF but no subsequent RPH. However, this functional improvement does not seem to be reflected in the long-term as we found no significant relationship between EVF and a mRS $\leq$ 2 at 90 days, even if there was a trend for higher rate of mRS score $<$2 (52.6 vs. 40.7%) at 90 days in subjects with EVF. The lack of significant long-term improvement is probably explained by the limited sample of our cohort and by the fact that after the first week, most complications relate to systemic rather than cerebrovascular complications clouding initial improvement results (15).

We hypothesize that EVF reflects a state of hyperemia, otherwise known as "luxury perfusion" (16), that could account for the better outcome in some and RPH in others. Indeed, diagnostic angiographic reports of EVF date back to the 1950s where EVF was thought to relate to an increasing circulatory rate or vasodilatation through the infarcted area, thereby enabling contrast material to reach the venous system more rapidly, especially once the occlusion has been relieved (17). This zone of focal hyperemia seems to correspond to a higher cerebral blood flow (CBF) and a reduced mean transit time (18, 19). Although hyperemia may equally be seen in the perifocal border zone around infarcts as an *angiographic blush,* EVF seems to reflect the presence of an ischemic core. Indeed, several studies showed that EVF was a predictor for irreversible regional tissue damage and subsequent infarction despite successful recanalization (10),

with an estimated sensitivity of 88–90.3% and specificity of 63–81% (11, 12). In contrast, the angiographic blush is probably in part due to the leaking out of vasodilating substances from the infarcted area.

Post-ischemic hyperemia following recanalisation has been observed in up to 30–40% of patients on different imaging modalities including CT perfusion (20), diffusion-perfusion MRI (21), as well as arterial spin labeled perfusion MRI (22–24). The hyperperfusion tends to be confined to the ischemic brain territory and is associated with an increased risk of hemorrhagic transformation (22–24), as well as an improved NIHSS score at 24 h and mRS $\leq$2 at 90 days (24). Similarly, PET imaging has demonstrated early focal hyperperfusion <48 h after onset may be a harmless and perhaps even a beneficial phenomenon (25, 26). These results parallel our findings and support EVF as an angiographic marker of hyperperfusion.

The exact pathophysiological basis of cerebral hyperemia is incompletely understood and probably multifactorial. Lassen (16) in 1966 suggested that an acute vessel occlusion may lead to focal hypoxia, with an increase in pCO_2 and a lowering of the pH, resulting in the release of vasoactive substances that stimulate capillary dilatation. Others have postulated the opening of arteriovenous shunts that bypass blocked capillary beds (27). Both are likely to lead to an increased regional cerebral blood flow. This is accompanied by damage to the cerebrovascular endothelium and disruption of the blood-brain barrier (28), all contributing to a loss of cerebral autoregulation.

Better understanding could come from recent advances that enable perfusion measurements to be derived quantitatively from DSA data acquired during EVT, offering the possibility of a more dynamic visualization of the cerebral hemodynamics during an acute ischemic stroke. Using this technique, Kosior et al. (19) demonstrated focal areas of hyperperfusion in 10 out of 50 patients shortly after recanalisation with focal areas of hyperemia corresponding to perfusion maps with a short T_{Max} and MTT. A U-shaped relationship between MTT and RPH was found, with the risk of hemorrhage greatest at both the lowest and highest extremes of MTT, reflecting the phenomena of hyperperfusion and no-reflow at either end of the spectrum. Their findings were however limited due to the lack of data on patient outcome. Nevertheless, it would be interesting to correlate their perfusion measurements with EVF on DSA to determine whether it corresponds to these visualized areas of focal hyperemia, thereby supporting our hypothesis.

EVF may thus reflect a state of hyperperfusion localized to the ischemic territory. This may be beneficial in preserving the penumbra, thereby leading to a better short-term functional outcome. It may however also bear an increased risk of reperfusion hemorrhage by increasing blood flow to an area made more fragile by ischemic damage. These findings may have implications on subsequent blood-pressure control and the initiation of antiplatelet or anticoagulant therapy post EVT.

CONCLUSION

In patients undergoing thrombectomy for a proximal anterior circulation occlusion, EVF is associated with both RPH and an improved NIHSS score at 24 h, reflecting a state of hyperperfusion localized to the ischemic territory. These findings bring new insights on post-ischemic cerebral blood flow regulation and have a potential impact on blood pressure management and decision-making around the timing of subsequent antiplatelet/anticoagulation treatment. EVF is thus a novel angiographic biomarker offering a personalized and dynamic insight into the individual patient's EVT treatment response.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Erasmus Hospital, Brussels, Belgium. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SE and GN designed the study. SE, PC, TB, and BM collected the data. SE, MS, and GN analyzed the data. SE wrote the manuscript. BL, NL, and GN revised the article critically. All authors approved the version to be published.

ACKNOWLEDGMENTS

We thank the reviewers for their constructive comments and suggestions.

REFERENCES

1. Feigin VL, Roth GA, Naghavi M, Parmar P, Krishnamurthi R, Chugh S, et al. Global burden of diseases, injuries and risk factors study 2013 and stroke experts writing group. Global burden of stroke and risk factors in 188 countries, during 1990-2013: a systematic analysis for the global burden of disease study 2013 *Lancet Neurol.* (2016) 15:913–24. doi: 10.1016/S1474-4422(16)30073-4
2. Malhotra K, Gornbein J, Saver JL. Ischemic strokes due to large-vessel occlusions contribute disproportionately to stroke-related dependence and death: a review. *Front Neurol.* (2017) 8:651. doi: 10.3389/fneur.2017.00651
3. Lakomkin N, Dhamoon M, Carroll K, Singh IP, Tuhrim S, Lee J, et al. Prevalence of large vessel occlusion in patients presenting with acute ischemic stroke: a 10-year systematic review of the literature. *J Neurointerv Surg.* (2019) 11:241–5. doi: 10.1136/neurintsurg-2018-014239

4. Román LS, Menon BK, Blasco J, Hernández-Pérez M, Dávalos A, Majoie CBLM, et al. HERMES collaborators. Imaging features and safety and efficacy of endovascular stroke treatment: a meta-analysis of individual patient-level data. *Lancet Neurol.* (2018) 17:895–904. doi: 10.1016/S1474-4422(18)30242-4
5. Heit JJ, Iv M, Wintermark M. Imaging of intracranial hemorrhage. *J Stroke.* (2017) 19:11–27. doi: 10.5853/jos.2016.00563
6. Elsaid N, Saied A, Joshi K, Nelson J, Baumgart J, Lopes D. 2D parametric parenchymal blood flow as a predictor of the hemorrhagic events after endovascular treatment of acute ischemic stroke: a single-center retrospective study. *Interv Neurol.* (2018) 7:522–32. doi: 10.1159/000491762
7. Fiorelli M, Bastianello S, Kummer von R, del Zoppo GJ, Larrue V, Lesaffre E, et al. Hemorrhagic transformation within 36 hours of a cerebral infarct: relationships with early clinical deterioration and 3-month outcome in the European Cooperative Acute Stroke Study I (ECASS I) cohort. *Stroke.* (1999) 30:2280–4. doi: 10.1161/01.STR.30.11.2280
8. Puig J, Shankar J, Liebeskind D, Terceño M, Nael K, Demchuk AM, et al. From "Time is Brain" to "Imaging is Brain": a paradigm shift in the management of acute ischemic stroke. *J Neuroimaging.* (2020) 30:562–71. doi: 10.1111/jon.12693
9. Elsaid N, Mustafa W, Saied A. Radiological predictors of haemorrhagic transformation after acute ischemic stroke: An evidence-based analysis. *Neuroradiol J.* (2020) 33:1971400919900275. doi: 10.1177/1971400919900275
10. Ohta H, Nakano S, Yokogami K, Iseda T, Yoneyama T, Wakisaka S. Appearance of early venous filling during intra-arterial reperfusion therapy for acute middle cerebral artery occlusion: a predictive sign for haemorrhagic complications. *Stroke.* (2004) 35:893–98. doi: 10.1161/01.STR.0000119751.92640.7F
11. Dorn F, Kuntze-Soderqvist A, Popp S, Lockau H, Haller B, Zimmer CT, et al. Early venous drainage after successful endovascular recanalization in ischemic stroke — a predictor for final infarct volume? *Neuroradiology.* (2012) 54:745–51. doi: 10.1007/s00234-011-0966-8
12. Fritzch D, Reiss-Zimmermann M, Lobsien D, Quäschling U, Hoffman KT. Arteriovenous shunts and capillary blush as an early sign of basal ganglia infarction after successful mechanical intra-arterial thrombectomy in ischaemic stroke. *Eur Radiol.* (2015) 25:3060–5. doi: 10.1007/s00330-015-3704-5
13. Cartmell SCD, Ball RL, Kaimal R, Telischak NA, Marks MP, Do HM, et al. Early cerebral vein after endovascular ischemic stroke treatment predicts symptomatic reperfusion hemorrhage. *Stroke.* (2018) 49:1741–6. doi: 10.1161/STROKEAHA.118.021402
14. Saver JL, Goyal M, van der Lugt A, Menon BK, Majoie CB, Dippel DW, et al. HERMES collaborators. time to treatment with endovascular thrombectomy and outcomes from ischemic stroke: a meta-analysis. *JAMA*. (2016) 316:1279–88. doi: 10.1001/jama.2016.13647
15. Viitanen M, Winblad B, Asplund K. Autopsy-verified causes of death after stroke. *Acta Med Scand.* (1987) 222:401–8. doi: 10.1111/j.0954-6820.1987.tb10956.x
16. Lassen NA. The luxury-perfusion syndrome and its possible relation to acute metabolic acidosis localised within the brain. *Lancet.* (1966) 2:1113–5. doi: 10.1016/S0140-6736(66)92199-4
17. Taveras JM, Gilson JM, Davis DO, Kilgore B, Rumbaugh CL. Angiography in cerebral infarction. *Radiology.* (1969) 93:549–58. doi: 10.1148/93.3.549
18. Olsen TS, Skriver EB, Herning M. Radiologic manifestations of focal cerebral hyperemia in acute stroke. *Acta Radiol.* (1991) 32:100–4. doi: 10.1177/028418519103200202
19. Kosior JC, Buck B, Wannamaker R, Kate M, Liapounova NA, Rempel JL, et al. Exploring reperfusion following endovascular thrombectomy. *Stroke.* (2019) 50:2389–95. doi: 10.1161/STROKEAHA.119.025537
20. Nguyen TB, Lum C, Eastwood JD, Stys PK, Hogan M, Goyal M. Hyperperfusion on perfusion computed tomography following revascularization for acute stroke. *Acta Radiol.* (2005) 46:610–5. doi: 10.1080/02841850510021607
21. Kidwell CS, Saver JL, Mattiello J, Starkman S, Vinuela F, Duckwiler G, et al. Diffusion-perfusion MRI characterization of post-recanalization hyperperfusion in humans. *Neurology.* (2001) 57:2015–21. doi: 10.1212/WNL.57.11.2015
22. Yu S, Liebeskind DS, Dua S, Wilhalme H, Elashoff D, Qiao XJ, et al. UCLA Stroke Investigators. Postischemic hyperperfusion on arterial spin labeled perfusion MRI is linked to hemorrhagic transformation in stroke. *J Cereb Blood Flow Metab*. (2015) 35:630–7. doi: 10.1038/jcbfm.2014.238
23. Okazaki S, Yamagami H, Yoshimoto T, Morita Y, Yamamoto H, Toyoda K, et al. Cerebral hyperperfusion on arterial spin labeling MRI after reperfusion therapy is related to hemorrhagic transformation. *J Cereb Blood Flow Metab.* (2017) 37:3087–90. doi: 10.1177/0271678X17718099
24. Shimonaga K, Matsushige T, Hosogai M, Hashimoto Y, Mizoue T, Ono C, et al. Hyperperfusion after endovascular reperfusion therapy for acute ischemic stroke. *J Stroke Cerebrovasc Dis.* (2019) 28:1212–8. doi: 10.1016/j.jstrokecerebrovasdis.2019.01.007
25. Fink GR, Herholz K, Pietrzyk U, Huber M, Heiss WD. Peri-Infarct perfusion in human ischemia: its relation to tissue metabolism, morphology, and clinical outcome. *J Stroke Cerebrovasc Dis.* (1993) 3:123–31. doi: 10.1016/S1052-3057(10)80239-3
26. Marchal G, Furlan M, Beaudouin V, Rioux P, Hauttement JL, Serrati C, et al. Early spontaneous hyperperfusion after stroke. A marker of favourable tissue outcome? *Brain.* (1996) 119 (Pt 2):409–19. doi: 10.1093/brain/119.2.409
27. Rowed DW, Stark VJ, Hoffer PB, Mullan S. Cerebral arteriovenous shunts re-examined. *Stroke.* (1972) 3:592–600. doi: 10.1161/01.STR.3.5.592
28. Desilles JP, Rouchaud A, Labreuche J, Meseguer E, Laissy JP, Serfaty JM, et al. Blood-brain barrier disruption is associated with increased mortality after endovascular therapy. *Neurology.* (2013) 80:844–51. doi: 10.1212/WNL.0b013e31828406de

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 14 May 2021
doi: 10.3389/fneur.2021.661955

Hemorrhagic Transformation in Ischemic Stroke and the Role of Inflammation

Elena Spronk[1]*, *Gina Sykes*[1], *Sarina Falcione*[1], *Danielle Munsterman*[1], *Twinkle Joy*[1], *Joseph Kamtchum-Tatuene*[2] and *Glen C. Jickling*[1]

[1] Division of Neurology, Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada, [2] Neuroscience and Mental Health Institute, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada

Hemorrhagic transformation (HT) is a common complication in patients with acute ischemic stroke. It occurs when peripheral blood extravasates across a disrupted blood brain barrier (BBB) into the brain following ischemic stroke. Preventing HT is important as it worsens stroke outcome and increases mortality. Factors associated with increased risk of HT include stroke severity, reperfusion therapy (thrombolysis and thrombectomy), hypertension, hyperglycemia, and age. Inflammation and the immune system are important contributors to BBB disruption and HT and are associated with many of the risk factors for HT. In this review, we present the relationship of inflammation and immune activation to HT in the context of reperfusion therapy, hypertension, hyperglycemia, and age. Differences in inflammatory pathways relating to HT are discussed. The role of inflammation to stratify the risk of HT and therapies targeting the immune system to reduce the risk of HT are presented.

Keywords: ischemic stroke, hemorrhagic transformation, inflammation, hypertension, diabetes, aging, reperfusion therapy

OPEN ACCESS

Edited by:
Nishant K. Mishra, University of California, Los Angeles, United States

Reviewed by:
Georgios Tsivgoulis, National and Kapodistrian University of Athens, Greece
Hansen Chen, Stanford University, United States

***Correspondence:**
Elena Spronk
espronk@ualberta.ca

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *31 January 2021*
Accepted: *29 March 2021*
Published: *14 May 2021*

Citation:
Spronk E, Sykes G, Falcione S, Munsterman D, Joy T, Kamtchum-Tatuene J and Jickling GC (2021) Hemorrhagic Transformation in Ischemic Stroke and the Role of Inflammation. Front. Neurol. 12:661955. doi: 10.3389/fneur.2021.661955

INTRODUCTION

Hemorrhagic transformation (HT) is a common complication of ischemic stroke that is often exacerbated by reperfusion with alteplase (recombinant tissue plasminogen activator) or endovascular therapy (EVT) (1). It occurs when the blood-brain barrier (BBB) is sufficiently disrupted to permit extravasation of peripheral blood into the brain. When HT occurs, it increases stroke morbidity and mortality and thus is important to prevent (2).

HT can be identified in 3–40% of patients with ischemic stroke, depending on the definition used and the characteristics of the cohort studied. HT is classified using both clinical and radiological criteria as summarized in **Table 1**. Clinical classification distinguishes symptomatic intracranial hemorrhage (sICH) from asymptomatic intracranial hemorrhage (aICH). sICH is defined as a worsening of the National Institutes of Health Stroke Scale (NIHSS) by $\geq$4 points within the first 36 h of stroke onset that is attributable to HT. A limitation of the clinical classification is that HT may be missed if it does not result in a major worsening of the neurological status. This may be the case when reperfusion therapy improves the NIHSS and offsets potential worsening caused by HT. The radiological classification of HT arose from the European Cooperative Acute Stroke Study (ECASS) and distinguishes small petechial hemorrhagic infarction (HI1), confluent petechial hemorrhagic infarction (HI2), small parenchymal hemorrhage (PH1) (<30% of infarct, mild mass effect), and large parenchymal hemorrhage (PH2, >30% of infarct, marked mass effect) (3). The ECASS definition has fairly

TABLE 1 | Classification systems of HT in ischemic stroke.

HT classification system	Type of HT	Criteria
Clinical	Symptomatic ICH (sICH)	Increase in the NIHSS by >4 points within the first 36 h of stroke onset
	Asymptomatic ICH (aICH)	Increase in the NIHSS by ≤4 points within the first 36 h of stroke onset
ECASS	HI1	Small petechial hemorrhagic infarction
	HI2	Confluent petechial hemorrhagic infarction
	PH1	Small parenchymal hemorrhage (<30% of infarct, mild mass effect)
	PH2	Large parenchymal hemorrhage (>30% of infarct, marked mass effect)
Heidelberg Bleeding Classification	1a HI1	Scattered small petechiae, no mass effect
	1b HI2	Confluent petechiae, no mass effect
	1c PH1	Hematoma within infarcted tissue, occupying <30%, no substantive mass effect
	2 PH2	Hematoma occupying 30% or more of the infarcted tissue, with obvious mass effect
	3	Intracerebral hemorrhage outside the infarcted brain tissue or intracranial-extracerebral hemorrhage
	3a	Parenchymal hematoma remote from infarcted brain tissue
	3b	Intraventricular hemorrhage
	3c	Subarachnoid hemorrhage
	3d	Subdural hemorrhage

poor intra- and inter-rater agreement for all types/categories except large PH2 (4). A Heidelberg Bleeding Classification scale has been proposed to address some of the challenges and limitations of the ECASS classification.

A challenge with clinical and radiological classifications of HT is that they lack specificity to the underlying mechanism of BBB disruption and HT. Details of the mechanism of BBB disruption and the cause of HT might be included in future classification systems as our understanding of the processes involved in HT evolves. **Figure 1** provides a schematic of the discussed immune pathways contributing to HT in ischemic stroke.

Abbreviations: A2M, a-2-macroglobulin; aICH, asymptomatic intracranial hemorrhage; AIS, acute ischemic stroke; Ang, angiopoietins; BBB, blood-brain barrier; c-Fn, cellular-fibronectin; DAMPs, damage associated molecular patterns; DM, diabetes mellitus; E-selectin, endothelial-leukocyte adhesion molecule 1; ECASS, European Cooperative Acute Stroke Study; eNOS, endothelial NO synthase; EVT, endovascular therapy; FGF, fibroblast growth factor; HgA1c, hemoglobin A1c; HI1, small petechial hemorrhagic infarction; HI2, confluent petechial hemorrhagic infarction; HIF-1, hypoxia-inducible factor 1; HMGB1, high mobility group box protein 1; HT, hemorrhagic transformation; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IGF-1, insulin-like growth factor 1; IL, interleukin; iNOS, inducible nitric oxide synthase; IV-tPA, intravenous tissue plasminogen activator; LPR, lipoprotein receptor; MCP-1, monocyte chemoattractant protein 1; MMP, matrix metalloproteinase; mRS, modified Rankin Scale; NETs, neutrophil extracellular traps; NFκB, nuclear factor-κB; NIHSS, National Institutes of Health Stroke Scale; NIL, neutrophil to leukocyte ratio; NLRP3, NOD-like receptor protein 3; NO, nitric oxide; NOX2, NADPH oxidase 2; NVU, neurovascular unit NXY-059, disodium 2,4-sulphophenyl-N-tert-butylnitrone; $ONOO^-$, peroxynitrite; PAI-1, plasminogen activator inhibitor-1; PAR-1, protease activated receptor 1; PDGF-CC, platelet derived growth factor-CC; PDGFRα, platelet derived growth factor receptor alpha; PH1, small parenchymal hemorrhage; PH2, large parenchymal hemorrhage; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; RNS, reactive nitrogen species; SBP, systolic blood pressure; SHR, spontaneously hypertensive rat; sICAM, soluble intercellular adhesion molecule; sICH, symptomatic intracranial hemorrhage; TAFI, thrombin-activated fibrinolysis inhibitor; TGF-β, transforming growth factor-beta; TJP, tight junction proteins; TLR, toll like receptor; TNF, tumor necrosis factor; TNK, Tenecteplase; tPa, tissue plasminogen activator; TXNIP, thioredoxin interacting protein; VAP-1,vascular adhesion molecule-1 VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

INFLAMMATION AND HT

Inflammation and immune system activation are important contributors to BBB disruption and HT (5). In acute stroke activated neutrophils and monocytes produce reactive oxygen species (ROS) and matrix metalloproteinases (MMP-9, MMP-2) that contribute to BBB disruption and HT (1). Microglia, astrocytes, and endothelial cells also contribute to BBB disruption and HT through the production of MMPs, proteases, vascular remodeling, and neuroinflammation (**Table 2**). In the following sections, we discuss how the immune system contributes to HT specifically in the context of clinical factors related to HT, notably stroke severity, reperfusion, hypertension, diabetes, and age.

Several trials have focused on improving stroke outcomes through targeting immune factors. There has yet to be immunomodulator identified to reduce the risk of HT. A summary of discussed treatments and their molecular targets is provided in **Table 3**.

CLINICAL RISK FACTORS FOR HT

Clinical features associated with an increased risk of HT in patients with ischemic stroke include advanced age, stroke severity (NIHSS), hypertension, hyperglycemia, poor collaterals, early infarction on brain imaging, low platelet count, use of antithrombotic drugs, and reperfusion therapy (15). Many of these factors are associated with inflammation and activation of the immune system, as discussed below. The clinical features have been summarized into scores to estimate the risk of HT and guide reperfusion therapy (**Table 4**). However, clinical characteristics and risk scores fail to identify all patients who experience HT, highlighting that a gap remains in our understanding of the pathophysiology of HT. Variables most commonly included in HT risk scores are stroke severity (admission NIHSS), hyperglycemia, hypertension, and age.

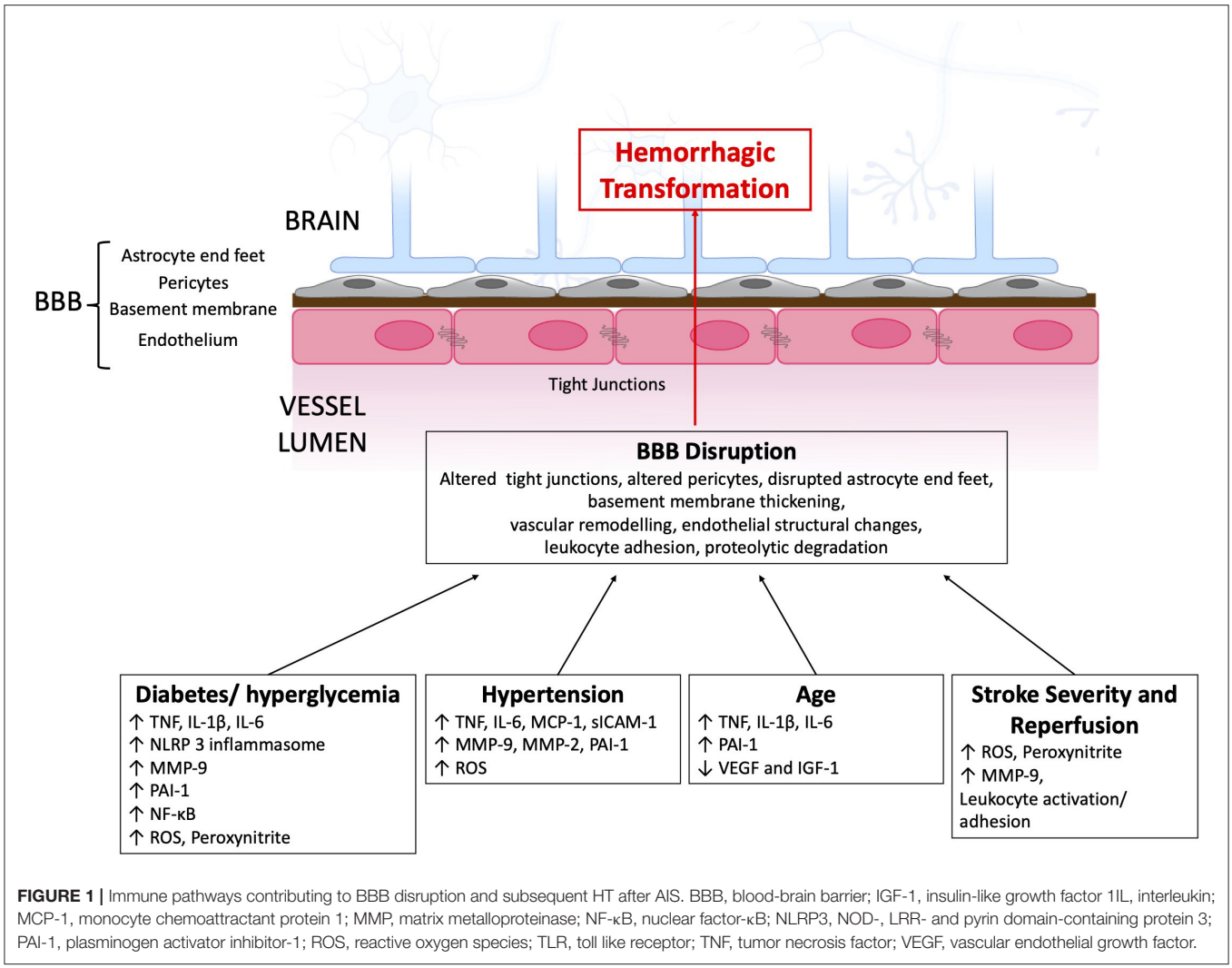


FIGURE 1 | Immune pathways contributing to BBB disruption and subsequent HT after AIS. BBB, blood-brain barrier; IGF-1, insulin-like growth factor 1IL, interleukin; MCP-1, monocyte chemoattractant protein 1; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; TLR, toll like receptor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

HEMORRHAGIC TRANSFORMATION AND STROKE SEVERITY

There is a strong relationship between duration and severity of brain ischemia and the risk of HT in both patients with stroke and experimental stroke models. Increased time from stroke onset is associated with larger core volumes, a higher degree of vascular disruption, and therefore a higher risk of HT. Increased time from ischemia onset to reperfusion has been associated with an increased risk of HT whether they received thrombolytic therapy or not (1). Recanalization beyond 6 h of stroke onset is an independent predictor of HT in human stroke, while early reperfusion is associated with a reduced risk of HT. Increased NIHSS is also associated with risk of HT. Patients with an NIHSS < 10 had $<13\%$ rate of HT. In comparison, patients with NIHSS > 15 had $>50\%$ rate of HT (22). Activation of the immune system is related to severity of infarction. Whether the greater immune activation from a larger infarct increases risk of HT remains unclear and warrants further evaluation.

THROMBOLYTIC THERAPY AND HT

Treatment with alteplase is associated with a 6–8% risk of sICH (23–27). This is mediated in part by the fact that thrombolytics breakdown blood clots and recanalize occluded cerebral vasculature. Recanalization of an occluded blood vessel can promote BBB disruption, contributing to reperfusion injury and increased risk of HT (1, 28). Activation of platelets, coagulation factors, and the innate and adaptive immune systems also contribute to injury following restoration of blood flow (29, 30).

Alteplase can also promote HT through non-fibrinolytic mechanisms, including activation of the immune system (31). Alteplase promotes neutrophil degranulation and release of MMP-9 (32). It acts on protease-activated receptor 1 (PAR-1) to increase MMP-9 expression and disrupt the BBB (33). Additionally, alteplase activates platelet-derived growth factor-CC (PDGF-CC), an agonist of platelet-derived growth factor receptor alpha (PDGFRa) on astrocyte end-feet (34). This can promote BBB disruption and HT, as PDGFRa activates MMPs

TABLE 2 | Inflammatory markers associated with HT.

Early HT (first 18 h of stroke onset)		
Inflammatory marker	**Source/mechanism**	**Role in HT**
ROS (increase)	Intracellular mitochondria, nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase xanthine oxidases, cellular membrane receptors inflammatory mediators, astrocytes	Disrupt the NVU (endothelial-pericyte-astrocyte) leading to increase BBB degradation
HMGB1	Microglia, astrocytes	Upregulates MMP-9 via TLR4. TNF, IL-1β
Peroxynitrite	Micro vessels, neurons and astrocytic end feet	Activate MMPs, disrupting vascular integrity
NF-κB	Astrocytes, microglia, and endothelial cells	Part of neutrophil infiltration pathway, upregulate cell adhesion molecules and inflammatory cytokines.
Leukocytes (increase)	Circulating leukocytes adhere to vascular endothelial cells following ischemia. Leukocyte adhesion and migration across the vasculature activates a number of signaling cascades (protein kinase C, focal adhesion kinase) that increase BBB permeability	Signaling cascade increased BBB permeability through ROS and MMP-9 expression.
MMP-9 (blood derived) (increase)	Leukocytes (Neutrophils) (not primary source). Mechanisms for MMP-9 activation following ischemia include: (1) ROS (2) TNF, IL-1β, and other cytokines that activate MMP-3 which converts proMMP-9 to active MMP-9 (3) actions of high mobility group box protein1 (HMGB1) on TLR4 receptors that then induce MMP-9 or (4) NF-κB induction of MMP-9	Luminal side: acts on TJP (tight junction proteins) (claudin-5, occludin, ZO-1) and basal lamina proteins (fibronectin, lamina, collagen), taken into endothelial cells or acts on basal lamina to open the BBB. Inside brain: Neutrophils can release MMPs that act directly on TJPs and/or basal lamina to disrupt the NVU (endothelial-pericyte-astrocyte)
MMP-2 (brain derived) (increase) remains elevated for days post-stroke	Astrocytes, endothelial cells and leukocytes	MMP-2 mediates degradation of occludin (tight junction protein)
Leukocyte gene expression	Six genes identified through mRNA expression: amphiregulin (AREG), membrane-associated ring finger (C3HC4) 7 (MARCH7), SMAD family member 4 (SMAD4), inositol polyphosphate-5-phosphatase (INPP5D), multiple coagulation factor deficiency 2 (MCFD2) and vascular endothelial growth inhibitor (VEGI)	
Late HT (18–24 h of stroke onset)		
	Source	**Role**
MMP-9 (Brain derived) (Increase)	MMP-9: astrocytes, neurons, microglia and endothelial cells. Activated by from ROS, TNF and IL-1β, HMGB1, NF-κB induction	Disruption of BBB
MMP-3 (Brain derived) (Increase)	MMP-3: pericytes and endothelial cells. MMP-3 acts on proMMP-9 to produce active MMP-9 and thus may promote HT	Disruption of BBB
Role of Vascular Remodeling	• A number of growth factors, MMPs and other molecules form new vessels and NVU • VEGF plays an important role in vascular remodeling and angiogenesis	VEGF: early, promotes BBB disruption; later promotes BBB integrity and vascular function. MMPs: Promote new vessel formation and increased pericyte/endothelial expression of tight junction proteins (ZO-1, occludin, claudin-5) HMGB1: acts on endothelial progenitor cells to promote peri-infarct angiogenesis (beneficial role)
ROS		Act as signaling molecules to regulate cell growth, differentiation and angiogenesis.

BBB, blood-brain barrier; HMGB1, high mobility group box protein 1; IL, interleukin; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; NVU, neurovascular unit; ROS, reactive oxygen species; TLR, toll like receptor; TNF, tumor necrosis factor; TJP, tight junction protein; VEGF, vascular endothelial growth factor.

(33). Alteplase also binds the lipoprotein receptor (LRP) on neurons and perivascular astrocytes to induce MMP-3 and MMP-6 expression (33). While inhibition of MMPs can reduce the risk of HT in animal stroke models, evidence in humans is lacking. Minocycline inhibits MMP-9 and has been evaluated in the MINOS trial (10). A phase 4 trial was later terminated due to lack of effect. MMP-9 inhibition, while potentially beneficial to reduce the risk of HT, may also impair post-stroke vascular remodeling and impair recovery. Indeed, inhibition of MMP-9 at 24–48 h after stroke worsens outcome in animal models (35). A phase 2 study, SAFE-TPA, evaluated different doses of Otaplimastat, a neuroprotectant inhibiting the MMP pathway (14). In animal models, Otaplimastat in combination with alteplase helped to reduce edema and HT. While Kim et al. (14) found the drug to

TABLE 3 | Therapeutic targets in acute ischemic stroke focused on modulating HT.

Therapy	Target	Effect on ischemic stroke	References
3K3A-APC (modified active protein C)	Protease-activated receptor 1	Possible benefit of reduced and smaller hemorrhages.	(6)
Enlimomab	ICAM-1	Associated with poor outcomes and increased HT	(7)
Edaravone	ROS	No benefit in reducing HT occurrence	(8)
Glycyrrhizin	HMGB1	• In rat model decreased HT • No human trial yet	(9)
Minocycline	MMP-9	No benefit	(10)
N-tert-butyl-α-phenylnitrone (PBN)	ROS	• In rat and rabbit models helped to decrease HT • No human trial yet	(11, 12)
NXY-059	ROS	Found to be ineffective for preventing HT	(13)
Otaplimastat	MMP	Further investigation required	(14)

HMGB1, high mobility group box protein 1; ICAM-1, intercellular adhesion molecule-1; MMP, matrix metalloproteinase; ROS, reactive oxygen species.

TABLE 4 | Risk scores proposed for HT prediction with alteplase administration.

Score	Name of score	Components	References
HTI – 0 to 6 points	Hemorrhagic transformation index score	ASPECTS (Alberta Stroke Program Early CT score), NIHSS, hyperdense middle cerebral artery sign, and presence of atrial fibrillation on ECG at admission	(16)
GRASPS	Glucose, Race, Age, Sex, Systolic blood Pressure, Severity	Glucose at presentation, race [Asian], age, sex [male], systolic blood pressure at presentation, and severity of stroke at presentation [NIH Stroke Scale]	(17)
HAT	Hemorrhage after thrombolysis	NIHSS score, hypodensity on CT scan (initial), serum glucose at baseline, and history of diabetes	(18)
HeRS	Hemorrhage Risk Stratification	Age, infarct volume, eGFR	(19)
HeRS plus	Hemorrhage Risk Stratification Plus	Addition of serum glucose, WBC count, and warfarin use on admission	(19)
SITS-sICH	Safe Implementation of Treatment in Stroke – Symptomatic IntraCerebral Hemorrhage risk	NIHSS score, serum glucose, systolic blood pressure, age, body weight, stroke onset to treatment time, aspirin or combined aspirin and clopidogrel, and history of hypertension	(20)
SEDAN Score	Hemorrhage risk after thrombolysis	Blood Sugar, Early infarct sign, Dense artery signs, Age, and NIHSS score	(21)

NIHSS, National Institutes of Health Stroke Scale.

be safe in patients, further investigation is required to determine effect in stroke.

Evidence is emerging regarding the use of Tenecteplase (TNK) in acute ischemic stroke (36, 37), a current treatment for acute myocardial infarction. TNK promotes the conversion of plasminogen to plasmin which degrades fibrin-based clots. It is a variant of recombinant tPA (tissue plasminogen activator), with higher resistance to plasminogen activator inhibitor-1 (PAI-1) and enhanced fibrin specificity (38). Studies of TNK in stroke are ongoing. In the EXTEND-IA TNK Trial, recanalization rates before thrombectomy were higher in the TNK group (22%, $n = 101$) compared with the alteplase group (10%, $n = 101$) with improved functional outcomes and similar risk of HT (1% sICH and 5–6% HT PH2) (37). As with alteplase, TNK may promote HT through several mechanisms that warrant further investigation.

Experimental evidence supports a role of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in early HT. Superoxide and peroxynitrite can disrupt microvascular integrity and may contribute to HT (1). Cerbral ischemia and reperfusion produce reactive oxygen and nitrogen species including superoxide, nitric oxide (NO), and peroxynitrite ($ONOO^-$) in microvessels, neurons and astrocyte end feet (39). Peroxynitrite can activate MMPs further disrupting vascular integrity. Hyperglycemia, as described in the section Hyperglycemia and HT can also enhance ROS and peroxynitrite.

High-mobility group box 1 (HMGB1) is released from injured cells of the central nervous system such as microglia and astrocytes (40). HMGB1 binds to its receptors (RAGE, toll-like receptor 2 (TLR2), and TLR4) and augments inflammation via the upregulation of tumor necrosis factor (TNF), interleukin 1β (IL-1β), and other cytokines (41). HMGB1 upregulates MMP-9 in neurons and astrocytes via TLR4. This could be a proposed target to help dampen the inflammatory response in cerebral ischemia and prevent HT as discussed in the section Hyperglycemia and HT.

Nuclear factor-κB (NF-κB) acts as a complex regulator of the innate immune system leading to activation of cellular adhesion molecules such as ICAM-1, VCAM-1 and E-selectin and inflammatory cytokines, mediating the neutrophil

infiltration pathway (42, 43). A study targeting HIF-1, hypoxia-inducible factor 1, a transcription factor that works to maintain homeostasis under hypoxia, found that BBB integrity was protected through suppressing the HMGB1/TLR4/NF-κB pathway to reduce the risk of alteplase-induced HT (44).

In experimental studies, Enlimomab, an ICAM-1 antibody, reduced leukocyte adhesion and infarct size (7). In the Enlimomab Acute Stroke Trial, it was associated with poor outcomes and increased HT.

In the RHAPSODY phase 2 trial, 3K3A-APC (a modified APC), a recombinant variant of human activated protein C (part of the coagulation pathway), was evaluated as a neuroprotectant in acute ischemic stroke treated with alteplase and or mechanical thrombectomy (6). 3K4A-APC acts on the protease-activated receptor 1 (PAR1) to promote vascular integrity and reduce neurological injury. The study showed a trend toward reduced HT, but further confirmation is required. However, targeting the PAR1 receptor shows promise as a strategy to reduce HT in stroke patients treated with reperfusion therapy.

ENDOVASCULAR THERAPY AND HT

Endovascular thrombectomy is of benefit in patients with acute large vessel occlusion and salvageable brain tissue (45). In a metanalysis of SWIFT PRIME, ESCAPE, EXTEND-IA, and REVASCAT the risk of sICH was 2.5% in EVT treated compared to 2.8% in non-EVT treated patients ($p = 0.76$), and the risk of PH was 8% in both EVT-treated patients and non-EVT patients ($p = 0.96$) (46). In the HERMES pooled analysis of MR CLEAN, ESCAPE, REVASCAT, SWIFT PRIME, and EXTEND IA stroke trials, the rate of sICH was 4.4% in the EVT group compared to 4.3% in the medical therapy group (45). Thus, the rate of HT is not significantly higher in the patients enrolled in EVT trials compared to controls receiving alteplase alone.

The DIRECT-MT trial compared EVT-only to EVT plus alteplase (47). The rates of HT were comparable between groups, with sICH occurring 4.3% in EVT group vs. 6.1% in EVT + alteplase ($p = 0.30$) and aICH occurring in 33.3% of the EVT group 36.2% in the EVT + alteplase group (p0.45). Similar rates of HT in EVT + alteplase vs. EVT alone have been reported by others (45, 48).

Thus, reperfusion with EVT may have lower rate of sICH than might be expected. EVT can provide a more rapid recanalization of large vessel occlusions which may reduce severity of ischemic brain injury, and thus reduce risk of HT. This is particularly true when EVT-treated patients are selected for small core and large penumbra by imaging. Core size does relate to risk of HT, with HT being higher in EVT-treated patients with larger core on baseline imaging (49). EVT may also produce differences in the type BBB disruption compared to alteplase. In an animal model, alteplase produced a more diffuse pattern of BBB disruption, whereas EVT had a more concentrated BBB disruption in central areas of ischemia (50). Additional evaluation is needed to understand HT in EVT-treated patients including differences in BBB disruption and potential benefits and risks of rt-PA (alteplase) use before EVT.

TABLE 5 | Summary of factors in hyperglycemia that promote BBB disruption and risk of HT.

Marker	Effect on BBB/consequences/contribution to risk of HT	References (for further reading)
Inflammation associated with diabetes		
Increase in chronic inflammatory cytokines: TNF, IL-1β, IL-6, and PAI-1	Impacting inflammation response (BBB permeability) PAI-1 interferes with tPa/alteplase degradation	(60)
NLRP3 Inflammasome	Associated with chronic inflammation in T2DM	(61)
Inflammation associated with hyperglycemia and ischemia		
Activation of NF-κB. Inflammatory response: includes TNF, IL-1β, IL- 6, sICAM-1, ICAM-1, VCAM-1 and E-selectin activation. Source: astrocytes	Inflammatory cascade attracting leukocytes to the ischemic area.	(42)
Increase in MMP-9 (associated with ischemia)	Disruption of BBB	(54, 55)
Increased adhesion of neutrophils	NVU disruption	(62)
Increased superoxide production, NADPH	Disruption of BBB	(63)
Peroxynitrite	• HMGB1 leading to activating leukocytes and inflammatory cytokines. • Activation of NLRP3 leading to neutrophil recruitment and BBB disruption	(9) (64)
Mechanical cellular changes due to chronic hyperglycemia		
Capillary basement membrane thickening and enhanced microvascular permeability	Diabetes complications impacting the BBB structure	(60)
Decrease in pericytes	Diabetes complications impacting the BBB structure (NVU)	(65)
Decreased tight junction proteins (occludin and claudin-5)	Disruption of BBB	(66)

BBB, blood-brain barrier; E-selectin, endothelial-leukocyte adhesion molecule 1; HMGB1, high mobility group box protein 1; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; NVU, neurovascular unit; PAI-1, plasminogen activator inhibitor-1; sICAM, soluble intercellular adhesion molecule; T2DM, type 2 diabetes; TNF, tumor necrosis factor; tPa, tissue plasminogen activator; VCAM-1, vascular cell adhesion molecule 1.

HYPERGLYCEMIA AND HT

Hyperglycemia is present in approximately 28–40% of patients with acute ischemic stroke (51–53). It can relate to pre-existing diabetes mellitus (DM) or a stress response leading to a rise in cortisol, glucose and catecholamines. For both diabetic and non-diabetic patients, hyperglycemia increases the risk of HT and is associated with worse clinical outcomes (51). There are conflicting reports concerning infarct growth in a hyperglycemic setting relative to normal glycemic levels (54–56). For the most part, hyperglycemia is associated with more rapid infarct growth and worse outcomes. In a review of 426 patients, higher values of HbA1c were associated with a higher risk of HT in patients with fasting blood glucose > 7.8 mmol/L (27% higher risk) and in those with fasting blood glucose < 7.8 mmol/L (36% higher risk) (57). Higher levels of HbA1c were also associated with a 48% higher risk of poor functional outcome. Treatment of hyperglycemia is recommended by stroke guidelines for the management of patients with acute ischemic stroke (58). Hyperglycemia is a risk factor for HT in both alteplase-treated patients and those not receiving thrombolysis. This association might be driven by hyperglycemia's effects on brain vasculature and the inflammatory response.

Hyperglycemia can potentiate inflammation resulting in increased BBB disruption and HT (59). Factors related to hyperglycemia that can increase the risk of HT are presented in **Table 5**. Diabetes is associated with an increase in plasma TNF, IL-1β, interleukin 6 (IL-6), interferon-g (IFNγ), and PAI-1, as well as alteration in immune cell response leading to chronic low-grade inflammation (60). An increase in PAI-1 may decrease the efficacy of alteplase and increase infarct size (67). This may result in increased risk of HT (68, 69). Hyperglycemia also activates nuclear factor-κB (NF-κB) in astrocytes, and enhances leukocyte adhesion through higher expression of cell adhesion molecules in cerebral blood vessels (ICAM1, intracellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1 and E-selectin) and the production of pro-inflammatory cytokines (TNF, IL-6, and IL-1β) (42, 70).

Neutrophils from patients with diabetes have enhanced capacity for endothelial adhesion (62). In stroke models using diabetic rats, hyperglycemia increases neutrophil adhesion and transmigration, and also increases expression of TNF, IL-1β, E-selectin, soluble intercellular adhesion molecules (sICAMs), endothelial NO synthase (eNOS) and inducible nitric oxide synthase (iNOS) (54, 71). In a rat model, hyperglycemia increases infarct volume, worsens outcome and increases risk of HT (55). Additionally, hyperglycemia increased the activity of MMP-9. Risk of HT is increased by glucose injection regardless of the time of ischemia (54, 55). Hyperglycemia can also increase superoxide production through NADPH oxidase, resulting in greater BBB disruption and HT. Apocynin, an NADPH oxidase inhibitor, prevents BBB disruption and HT in animal models of stroke (63).

In a rat model of stroke, hyperglycemia enhanced release of HMGB1 and the rate of alteplase related HT (9, 40, 56). Treatment with glycyrrhizin, a direct HMBG1 inhibitor, downregulated the expression of NADPH. This decreased superoxide and peroxynitrite($ONOO^-$), reduced TLR2, and MMP-9, and preservation of type IV collagen and claudin-5, thus attenuating HT and BBB damage. The role of an HMGB1 inhibitor in patients with stroke remains to be determined.

NLRP3 (NOD-like receptor protein 3) inflammasome can also promote BBB disruption and HT. In a MCAO rat model with acute hyperglycemia and increased expression of NADPH oxidase, iNOS induction lead to the production of superoxide, nitric oxide and peroxynitrite (56). It was suggested that peroxynitrite could activate NLRP3 inflammasome in hyperglycemia, a mediator in HT following reperfusion. Inhibiting the NLRP3 inflammasome minimized HT *in vivo*, and could potentially offer a treatment option.

NLRP3 inflammasome is also involved in chronic inflammation associated with type 2 diabetes (T2DM) (61). Primary activation of NLRP3 leads to transcription of pro-IL-1β and pro-IL-18 into mature IL-1β and IL-18. Active IL-1β is then responsible for increased expression of adhesion molecules, leading to neutrophil recruitment and contributing to BBB disruption (64). Inhibiting NLRP3 may reduce the impact of stroke in patients with diabetes by regulating neutrophil recruitment. Hyperglycemia may upregulate TXNIP-NLRP3 inflammasome causing alteplase induced BBB disruption and HT (72). Thioredoxin interacting protein (TXNIP) mediates hyperglycemia-induced oxidative damage and inflammation in the brain, while reducing cerebral glucose uptake/utilization.

Other mechanisms by which hyperglycemia can promote BBB disruption and cause HT include a decrease in tight junction proteins occludin and claudin-5 (66), chronic oxidative stress and thickening of microvascular basement membrane, increase of microvascular permeability (60), and loss of pericytes (65).

Effect of Hyperglycemia on Treatment With Alteplase

Hyperglycemia impairs the fibrinolytic activity of alteplase, particularly in the acute phase of treatment, which may result in larger infarct size with more severe ischemia and thus, higher risk of HT (63, 73–75). Type 2 DM is associated with increased blood levels of PAI-1, an inhibitor of tPA (endogenous and exogenous) (76). Hyperglycemia also affects the coagulation system by increasing thrombin production and stimulating the intrinsic tissue factor pathway, thus affecting reperfusion and contributing to HT (77). In rats with Type 1 DM, treatment of acute stroke with alteplase increased MMP-9 activity, BBB breakdown, brain hemorrhage, and worsened functional outcomes (75).

Treatment of Hyperglycemia to Prevent HT

Several stroke trials have evaluated the benefit of treating hyperglycemia to reduce the risk of HT. Control of hyperglycemia through insulin infusion was tested in patients in the UK Glucose Insulin in Stroke Trial (GIST UK) (78) and the SHINE trial (51). In the SHINE trial, 80% of the patients had Type 2 DM. In the intensive treatment group, the average blood glucose was 6.6 mmol/L compared to 9.9 mmol/L in the standard treatment group. No net improvement in stroke outcome was identified, despite a significant reduction in blood glucose achieved with insulin infusion. Potential acute lowering of glucose does not fully mitigate downstream effects already

TABLE 6 | Summary of factors related to hypertension that promote BBB disruption and risk of HT.

Marker	Effect on BBB/consequences/contribution to risk of HT	References (for further reading)
Changes to the immune system and response		
Elevated levels of proinflammatory cytokines as TNF, IL-6, MCP-1, and sICAM-1 in the vasculature	Impact endothelial cell ability to regulate. Leukocyte interaction and infiltration, disruption of BBB	(80)
Response to Angiotensin II due to chronic hypertension		
Increased MMP-9 and MMP-2	Activated by the renin-angiotensin system in the vasculature, leading to vessel remodeling and disruption of the BBB	(81)
Increase in serum PAI-1	Activated by Angiotensin II, interferes with the tPa/alteplase degradation	(67)
Increased ROS through NADPH oxidases	Macrophages in response to Angiotensin II Additionally, acute hypertension may attenuate the ability to cope with ROS	(82)
Mechanical cellular changes due to chronic hypertension		
Remodeling of microvasculature: Decreased lumen diameter, increased vascular resistance, increased wall to lumen diameter.	Decreased endothelial cell function and reduced autoregulation ability	(80)

BBB, Blood brain barrier; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; NADPH, Nicotinamide adenine dinucleotide phosphate; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; sICAM, soluble intercellular adhesion molecule; TNF, tumor necrosis factor; tPa, tissue plasminogen activator.

initiated by hyperglycemia such as enhanced inflammation, endothelial cell dysfunction, neutrophil adhesion, MMP-9 activity, and ROS production. There is an ongoing secondary analysis of the effect of acute insulin therapy on the risk of HT, which may provide further insights into the link between hyperglycemia and HT from the SHINE trial.

A systematic review of 274 research articles aimed to identify the ideal target and strategy for blood glucose management (79). Glucose control was recommended to start within the first few hours of stroke onset. Tight glucose control was not recommended due to the risk of hypoglycemic events. Glycemic variability is to be avoided, as it is associated with adverse outcomes.

HYPERTENSION AND HT

An elevated blood pressure can increase the risk of HT through a variety of mechanisms, including direct mechanical effects on the cerebral/brain vasculature, exacerbated inflammation, and vascular remodeling affecting collateral circulation, endothelial function, and autoregulation (80). A summary of factors in hypertension that impacted HT through BBB modification is shown in **Table 6**.

Hypertension is common in patients with ischemic stroke and associated with increased risk of HT (20). Blood pressure is elevated in over 60% of patients with acute stroke (83). The elevated blood pressure may be caused by several components, including pre-existing or untreated hypertension, stress response (transient response), increased intracranial pressure, disrupted central autonomic regulation, activation of the neuroendocrine system, or inflammatory responses.

Impact of Hypertension on Inflammation and BBB Permeability

Hypertension is associated with disruption of the BBB which could increase risk of HT (84). In the spontaneous hypertensive rat, BBB disruption can be observed as early as 3 months of age (85). The renin-angiotensin system is involved in hypertension and contributes to BBB disruption. It activates MMPs, causing an increase in circulating and tissular MMP-2 and MMP-9 (81, 86). The increase of angiotensin II in hypertension induces perivascular macrophages to produce ROS and contribute to BBB disruption (82, 84). Hypertension is associated with an increase in blood levels of pro-inflammatory cytokines such as TNF, IL-6, monocyte chemoattractant protein-1(MCP-1), and sICAM-1 which can contribute to BBB disruption (80). Hypertension is associated with a decreased expression of cerebral endothelial tight junction proteins (claudin-3, claudin-5, and claudin-12), thus enhancing BBB permeability (84). Angiotensin II causes an increase in serum PAI-1 levels, thus reducing the effectiveness of alteplase (69).

Hypertension Related Vascular Remodeling

Chronic hypertension alters the cerebral vasculature, with microvascular rarefaction, arterial remodeling, and increased BBB permeability (80). Hypertension is associated with a decrease in lumen diameter, increase in vascular resistance, and impairment of endothelial function. These effects impair collateral circulation, reducing capacity to maintain adequate oxygenation when a cerebral artery occlusion occurs. Poor collateral circulation, which sustains the penumbra, results in faster expansion of infarct core, associated with risk of HT (87, 88).

Impact of Hypertension on HT Following Reperfusion Therapy

In a rabbit clot model, hypertension induced before and after alteplase administration increases the risk of HT (89). The size of HT also relates to the peak mean arterial blood pressure. Decreasing pre-stroke blood pressure in rats reduced the risk of HT (90). BBB changes may be mechanical or result from acute changes related to endothelial or inflammatory activation.

TABLE 7 | Summary of factors related to age that impact HT and BBB.

Marker	Effect on BBB/consequences/contribution to risk of HT	References (for further reading)
Changes to the immune system and response		
Inflammation: low grade chronic inflammation. Elevated levels of proinflammatory mediator's TNF, IL-1β, IL-6	Effect the inflammatory response and BBB permeability/ function	(110–112)
Increase in PAI-1	Interferes with the tPa/alteplase degradation	(113)
Decrease in VEGF and IGF-1	Alter the angiogenesis response	(114)
Changes to the inflammatory response		
Enhanced neutrophil response/recruitment	Increased MMP-9 and ROS	(106)
Enhanced microglial response	Enhanced inflammation and BBB permeability	(115)
Mechanical cellular changes due to aging		
Endothelial cells change in structure and adopt a senescence phenotype	Increased ROS which reduces NO activity	(116)
Decrease in pericytes	Mechanical disruption to BBB permeability	(105)
Astrocytes change structure	Mechanical disruption to BBB permeability	(105, 115)

BBB, Blood brain barrier; IGF-1, insulin-like growth factor 1; IL, interleukin; NO, nitric oxide; PAI-1, plasminogen activator inhibitor-1; TNF, tumor necrosis factor; tPa, tissue plasminogen activator; VEGF, vascular endothelial growth factor.

Maintaining the blood pressure below 185/110 mmHg is recommended to reduce the risk of HT in patients treated with alteplase (91). The risk of HT increases with each 10 mmHg rise in systolic blood pressure from 140 to 180 mmHg (92). Observational studies suggest lower blood pressure within the first 24 h is associated with more favorable outcomes and less frequent sICH (93). In addition, the risk of HT following alteplase is higher in patients with greater blood pressure variability, particularly in the first 6 h (94), and in patients with elevated systolic blood pressure (95). An elevated systolic blood pressure (SBP) is also associated with worse outcomes and higher risks of HT in patients treated with EVT (96, 97).

Hypertension Treatment to Prevent HT

The ENCHANTED trial compared the benefit of intensive blood pressure control (SBP target of 130–140 mmHg) to that of the standard of care (SBP < 180 mmHg) (98). Intensive lowering of blood pressure was safe and decreased the incidence of HT from 18.7 to 14.8% ($p = 0.0137$). However, despite a reduction in HT, no net improvement in the 90-day Modified Rankin Scale (mRS) was observed. The best stroke outcomes are observed when BP is maintained between 140 and 180 mmHg (99). It is challenging to understand the ideal blood pressure for stroke treatment. In an acute setting, a rise in BP may be beneficial for preservation of brain perfusion in case of large vessel occlusion (opening of collateral vessels) while intensive BP lowering in the hyperacute phase (<48 h) may be detrimental in the context of impaired regulation of the cerebral circulation. The RIGHT-2 trial showed that functional outcomes were not improved with blood pressure reduction prior to stroke treatment (100). The Dutch ThRombolysis in Uncontrolled Hypertension (TRUTH) trial is an ongoing study evaluating whether actively lowering blood pressure below 185/110 mmHg affects stroke outcome in alteplase treated patients (101).

The BP-TARGET aimed to evaluate intense systolic blood pressure targets (100–129 mm Hg) and standard care systolic targets (130–185 mm Hg) for 24 h after reperfusion (102). Intensive blood pressure control was not superior to standard of care for the prevention of HT. A comment letter suggest that alternative variables should also be considered, such as collateral circulation, blood pressure variability at baseline and during monitoring (103).

AGE AND RISK OF HT

Advancing age is associated with an increased risk of HT (20, 104, 105), as well as worse stroke outcomes (106, 107). Infarct size is not consistent between sources; however, most authors suggest an increase in infarct size. Despite an increased risk, patients of older age derive similar benefit from thrombolysis (108, 109). The rationale for age to increase the risk of HT is multifactorial, including an increase in systemic inflammation and BBB permeability. Additionally, elderly patients are also more likely to be on antithrombotic treatment, have a greater burden of cerebrovascular disease, and are more likely to have comorbid diseases such as hypertension and diabetes. These comorbidities contribute to inflammation, notably atherosclerosis, diabetes, hypertension, and hyperlipidemia. When stroke occurs, this age-associated inflammation may contribute to BBB disruption leading to an increased risk of HT. **Table 7** summarizes the mechanisms through which age affects the risk of HT.

Effect of Age on the Immune System

Advanced age is characterized by immunosenescence, gradual immune dysregulation and changes to cell function, leading to a reduced capacity to respond to antigen stimulation (110). Aging is also associated with inflammaging: chronic inflammation with elevated plasma concentrations of TNF, IL-1β, and IL-6 predictive of fragility, mortality, and functional disability (110–112). This chronic low-grade inflammation state alters the innate immunity of the brain and may contribute to the risk of HT.

Aging impacts both the innate and adaptive immune systems (110). Over time, the availability of hematopoietic stem cells declines, leading to decreased capacity to renew peripheral immune cells. Fewer naive B-lymphocytes are produced from the bone marrow, and T cell diversity is also reduced after age 70. Cells of the myeloid lineage are also affected by age, neutrophils have decreased phagocytic capacity and a reduced oxidative burst, leading to an increased inflammatory time period (110, 112). Moreover, there is reduced production of cytokines from macrophages which have decreased phagocytic capacity (106, 110). During stroke, young mice tend to recruit monocytes, whereas aged mice have greater recruitment of neutrophils. Additionally, higher levels of ROS and MMP-9 are associated with increased age. As discussed in the section Inflammatory Biomarkers and Prediction of HT, an increased neutrophil to leukocyte (NIL) ratio was independently associated with HT (117). Furthermore, with advancing age, microglia may become more prone to generate an inflammatory response when stroke occurs (115). With advancing age, there is a dysregulation in microglia pathways leading to a prolonged and amplified immune response, thus potentially increasing the risk of HT.

Age and the Neurovascular Unit

The neurovascular unit also undergoes age-related changes which may contribute to the risk of HT (104, 105). Aged cerebral endothelial cells have a smaller cytoplasm and dysfunctional mitochondria that adopt a senescent phenotype, promoting low-grade inflammation. Endothelial cells also produce more ROS with age, which reduces NO activity and limits the vasodilation capacity (116). Moreover, the number of pericytes, vital for BBB integrity, decreases with age, with a negative impact on the microcirculation, reducing the ability to maintain the BBB integrity after stroke (105). Astrocytes can become more inflammatory with age, which may also contribute to the risk of HT (115). Additionally, astrocytes undergo clasmatodendrosis with age and retract their end-feet, thus altering BBB integrity (105, 115). Finally, with aging, miRNA and RNA expression for genes associated with vascular tone, tight junction protein expression and cell adhesion are downregulated, impacting the BBB.

Age and Cerebral Vasculature

Angiogenesis is a necessary process to reduce infarct volume and support recovery after stroke. The most critical factors for supporting capillary angiogenesis include fibroblast growth factor (FGF), TNF, transforming growth factor-beta (TGF-β), and the angiopoietins (Ang) (118). With age, there is an attenuated pro-angiogenic response, decreased expression of vascular endothelial growth factor (VEGF) and insulin-like growth factor 1 (IGF-1) possibly contributing to the risk of HT (114). VEGF has an important role in vascular remodeling and angiogenesis and is associated with delayed HT and vascular remodeling (1). VEGF has a biphasic role in stroke. Early after stroke, VEGF promotes BBB disruption and HT but supportes vascular function and BBB integrity at a later stage. In rats, VEGF signal inhibition attenuates alteplase-related HT (119). Age is associated with a decrease in the number and diameter of native collaterals as well as impaired collateral remodeling in the brain (120). Collaterals influence the rate of HT after thrombolytic treatment (121). Thus, the increased risk of HT with age may also relate to an age-associated reduction in collaterals.

INFLAMMATORY BIOMARKERS AND PREDICTION OF HT

Several protein and transcription biomarkers have been evaluated to predict the risk of HT. This includes plasma cellular-fibronectin (c-Fn), a major component of extracellular matrix involved in wound healing and cell adhesion (122); MMPs (122, 123); neutrophil and lymphocyte counts (117, 123); ferritin (123), vascular adhesion molecule−1 (VAP-1), and IL-10 that are associated with alteplase induced HT (124, 125). Additionally, inflammatory markers such as TNF, C-reactive protein and homocysteine are associated with the risk and severity of HT (126). Fibrinolysis inhibitors [PAI-1, lipoprotein(a), thrombin-activated fibrinolysis inhibitor (TAFI), and homocysteine] may help to predict sICH after thrombolysis (69, 122, 124).

Ischemic stroke elicits a strong local and peripheral inflammatory response. There is an increase in inflammatory cytokines (e.g., TNF, IL-1β, IL-6), cell adhesion molecules (e.g., ICAM-1, VCAM, P-selectin), damage-associated molecular patterns [DAMPs, e.g., high mobility group box protein 1 (HMGB1), peroxiredoxin, mitochondrial DNA], MMPs and ROS (127). Microglia, cerebral endothelial cells, and peripheral leukocytes are also activated during the immune response. This results in the recruitment, adhesion, and infiltration of the ischemic brain by peripheral leukocytes. Aspects of the inflammatory response alters the structure of the BBB, reorganizing tight junction proteins and the actin cytoskeleton, and contributing to HT (128).

A few trials have targeted ROS. NXY-059 targeting ROS was trialed to reduced alteplase-related intracranial hemorrhages (13). In animal studies, NXY-059 was associated with improved functional recovery and reduced infarction size. In the human trial, NXY-059 was found to be ineffective for preventing HT. Additionally, as part of a retrospective study in Japan, Edaravone administration with alteplase (rtPA) found that there may be a benefit in early stroke outcome (8). Edaravone suppresses ROS, inhibits vascular endothelial injuries, and protects the neurovascular unit. No benefit was found for HT occurrence.

Neutrophils are one of the first innate immune cells to reach the ischemic brain tissue (129). Neutrophils contribute to injury through inflammatory mediators and damage the BBB through the release of proteolytic enzymes, ROS, and interactions with the endothelium of cerebral blood vessels. In addition, neutrophils accumulate in higher numbers in regions of HT and correlate with disruption of the basal lamina. In experimental stroke studies, depletion of circulating neutrophils reduces thrombolysis-related hemorrhage (130). In 846 patients treated with thrombolysis, an increased neutrophil to leukocyte (NIL) ratio was independently associated with HT (117).

Neutrophils produce neutrophil extracellular traps (NETs), which can promote clot formation associated with resistance to

TABLE 8 | Leukocyte genes associated with HT in patients with ischemic stroke.

Gene	Function	Direction of expression
SMAD4	Codes for a member of the Smad family of signal transduction proteins, which are activated by TGF-β signaling to regulate the of target genes	Increased
INPP5D	Regulates proliferation and programming of myeloid cell	Increased
VEGI	Codes for a cytokine in the tumor necrosis factor ligand family	Decreased
AREG	Codes for a ligand for the epidermal growth factor receptor	Increased
MCFD2	Involved in the transport of coagulation factors V and VIII from the endoplasmic reticulum to the Golgi apparatus	Decreased
MARCH7	Regulates membrane receptor expression in several tissues, including leukocytes	Increased

thrombolysis (131). In cultured human neutrophils, incubation with alteplase induced neutrophil degranulation and an increase in MMP-9 activity in a dose-dependent manner (132). This may elevate the number of activated neutrophils, thus exacerbating the detrimental effects of neutrophils on the BBB to promote HT (129, 131). In mice with circulating leukocytes from MMP-9 null bone marrow, BBB disruption was decreased (133).

Leukocytes enter the parenchyma and adhere to the cells largely through adhesion molecules ICAM-1, P-selectin, E-selectin, and VCAM-1 (134). Cytokines released by cerebral ischemia such as TNF, IL-1, and IFN-γ up-regulate ICAM-1 on both cerebral endothelial cells and leukocytes. Leukocytes adhering the endothelium can block erythrocytes in the microvasculature, leading to further injury (135). Activated leukocytes can also lead to secondary injury by producing proteases, MMPs, and ROS that can disrupt blood vessels. Leukocyte derived MMP-9s are mediators of early HT (1). Leukocyte adhesion and migration across the vasculature activates a number of signaling cascades (protein kinase C, focal adhesion kinase) that increase the BBB permeability. Once in the CNS, activated leukocytes release inflammatory cytokines at the site of injury such as TNF, IL-1β, and IL-6 (136).

In both rat (11) and rabbit models (12), treated with alteplase plus N-tert-butyl-α-phenylnitrone (PBN) vs. alteplase alone, PBN was found to attenuate alteplase-induced HT. PBN is a spin trap agent, targeting ROS from leukocytes.

INFLAMMATORY GENES AND HT

In both patient and animal studies, the peripheral immune response in ischemic stroke can be characterized by RNA expression in circulating leukocytes (128). Using leukocyte RNA obtained before thrombolysis treatment in 44 patients with acute stroke (33 with HT), microarray gene expression studies have identified 6 genes that predict the occurrence of HT with 80% sensitivity and 70% specificity. The six-gene panel in **Table 8** highlights differences in inflammation and coagulation processes. Factors involved in HT of human stroke include a shift in growth factor-beta signaling involving SMAD4, INPP5D, and IRAK3, a disruption of coagulation factors V and VIII, and amphiregulin, a growth factor-beta that regulates MMP-9. Additionally, increased expression of AKAP7 in peripheral lymphocytes is linked to post stroke complications and BBB permeability (137). Genes associated with increased HT and mortality risk include two single nucleotide polymorphisms rs669 in α-2-macroglobulin (A2M) and rs1801020 in coagulation Factor XII (138). Factors associated with the efficacy of alteplase include low fibrin levels and polymorphism of Factor XIII V/V genotype (137).

The Genot-PA score, to predict HT in patients treated with alteplase, considered both genetics and clinical variables to establish patient risk (137). In the trial, 1,324 patients in a Spanish population, were risk-stratified and treated with either alteplase or a combination of alteplase and mechanical thrombectomy. The study was able to predict HT in patients treated with alteplase. External validation of the score is pending.

CONCLUSIONS

Inflammation contributes to BBB disruption and risk of HT, through an increase in ROS, MMPs and neutrophil activation. Clinical factors associated with inflammation and HT include hypertension, hyperglycemia, and age. Diabetes and hypertension lead to structural and functional changes of the neurovascular unit, alter collaterals, and elevate MMP-9, ROS, and proinflammatory cytokines (TNF, IL-1β, and IL-6). Advancing age is associated with a chronic elevation of inflammatory cytokine levels and changes in inflammatory response. These inflammatory markers provide insight to how clinical factors may increase the risk of HT. They may represent novel targets to reduce the risk of HT.

Further studies of the immune system and inflammation will be useful to better understand the risk of HT associated with reperfusion following thrombolysis and EVT and determine if modulation of the immune system could be useful to prevent HT. With increased use of reperfusion therapy and extension of eligibility criteria (e.g., inclusion of older patients, extended time windows), the importance of preventing HT will become more apparent. An improved safety profile with lower risk of HT could permit more widespread use of reperfusion therapy and greatly affect patients with stroke. Incorporating clinical features, genetics, biomarkers, and imaging to identify patients at risk for HT may help develop novel therapies to prevent HT in stroke.

AUTHOR CONTRIBUTIONS

All authors meet the authorship criteria and contributed substantially to conception and design or acquisition of data (ES and GJ), or analysis and interpretation of data (all authors), drafted (ES) or revised (all authors) and gave final approval of the version to be published (all authors).

FUNDING

GJ received research grant support from CIHR, Heart and Stroke Foundation, University Hospital Foundation, Hypertension Canada, CFI, and NIH. JK-T was supported by a Faculty of Medicine and Dentistry Motyl Graduate Studentship in Cardiac Sciences and an Alberta Innovates Graduate Student Scholarship.

REFERENCES

1. Jickling GC, Liu D, Stamova B, Ander BP, Zhan X, Lu A, et al. Hemorrhagic transformation after ischemic stroke in animals and humans. *J Cereb Blood Flow Metab.* (2014) 34:185–99. doi: 10.1038/jcbfm.2013.203
2. Kastrup A, Gröschel K, Ringer TM, Redecker C, Cordesmeyer R, Witte OW, et al. Early disruption of the bloodbrain barrier after thrombolytic therapy predicts hemorrhage in patients with acute stroke. *Stroke.* (2008) 39:2385–7. doi: 10.1161/STROKEAHA.107.505420
3. Fiorelli M, Bastianello S, von Kummer R, del Zoppo GJ, Larrue V, Lesaffre E, et al. Hemorrhagic transformation within 36 hours of a cerebral infarct: relationships with early clinical deterioration and 3-month outcome in the European Cooperative Acute Stroke Study I (ECASS I) cohort. *Stroke.* (1999) 30:2280–4. doi: 10.1161/01.STR.30.11.2280
4. Guenego A, Lecler A, Raymond J, Sabben C, Khoury N, Premat K, et al. Hemorrhagic transformation after stroke: inter- and intrarater agreement. *Eur J Neurol.* (2019) 26:476–82. doi: 10.1111/ene.13859
5. Kulik T, Kusano Y, Aronhime S, Sandler AL, Winn HR. Regulation of cerebral vasculature in normal and ischemic brain. *Neuropharmacology.* (2008) 55:281–8. doi: 10.1016/j.neuropharm.2008.04.017
6. Lyden, Pryor KE, Coffey CS, Cudkowicz M, Conwit R, Jadhav A, et al. Final results of the RHAPSODY trial: a multi-center, phase 2 trial using a continual reassessment method to determine the safety and tolerability of 3K3A-APC, a recombinant variant of human activated protein C, in combination with tissue plasminogen activator, mechanical thrombectomy or both in moderate to severe acute ischemic stroke. *Ann Neurol.* (2019) 85:125–36. doi: 10.1002/ana.25383
7. Sherman D, Bes A, J E. Use of anti-ICAM-1 therapy in ischemic stroke: results of the enlimomab acute stroke trial. *Neurology.* (2001) 57:1428–34. doi: 10.1212/WNL.57.8.1428
8. Wada T, Yasunaga H, Inokuchi R, Horiguchi H, Fushimi K, Matsubara T, et al. Effects of edaravone on early outcomes in acute ischemic stroke patients treated with recombinant tissue plasminogen activator. *J Neurol Sci.* (2014) 345:106–11. doi: 10.1016/j.jns.2014.07.018
9. Chen H, Guan B, Wang B, Pu H, Bai X, Chen X, et al. Glycyrrhizin prevents hemorrhagic transformation and improves neurological outcome in ischemic stroke with delayed thrombolysis through targeting peroxynitrite-mediated HMGB1 signaling. *Transl Stroke Res.* (2020) 11:967–82. doi: 10.1007/s12975-019-00772-1
10. Fagan SC, Waller JL, Nichols FT, Edwards DJ, Pettigrew LC, Clark WM, et al. Minocycline to improve neurologic outcome in stroke (MINOS): a dose-finding study. *Stroke.* (2010) 41:2283–7. doi: 10.1161/STROKEAHA.110.582601
11. Asahi M, Asahi K, Wang X, Lo EH. Reduction of tissue plasminogen activator-induced hemorrhage and brain injury by free radical spin trapping after embolic focal cerebral ischemia in rats. *J Cereb Blood Flow Metab.* (2000) 20:452–7. doi: 10.1097/00004647-200003000-00002
12. Lapchak PA, Chapman DF, Zivin JA. Pharmacological effects of the spin trap agents N-t-butyl-phenylnitrone (PBN) and 2,2,6, 6-tetramethylpiperidine-N-oxyl (TEMPO) in a rabbit thromboembolic stroke model: combination studies with the thrombolytic tissue plasminogen activator. *Stroke.* (2001) 32:147–53. doi: 10.1161/01.STR.32.1.147
13. Shuaib A, Lees KR, Lyden P, Grotta J, Davalos A, Davis SM, et al. NXY-059 for the treatment of acute ischemic stroke. *N Engl J Med.* (2007) 357:562–71. doi: 10.1056/NEJMoa070240
14. Kim, Lee KB, Park J-H, Sung SM, Oh K, Kim E-G, et al. Safety and efficacy of otaplimastat in patients with acute ischemic stroke requiring tPA (SAFE-TPA): a multicenter, randomized, double-blind, placebo-controlled phase 2 study. *Ann Neurol.* (2020) 87:233–45. doi: 10.1002/ana.25644
15. Whiteley WN, Slot KB, Fernandes P, Sandercock P, Wardlaw J. Risk factors for intracranial hemorrhage in acute ischemic stroke patients treated with recombinant tissue plasminogen activator: a systematic review and meta-analysis of 55 studies. *Stroke.* (2012) 43:2904–9. doi: 10.1161/STROKEAHA.112.665331
16. Kalinin MN, Khasanova DR, Ibatullin MM. The hemorrhagic transformation index score: a prediction tool in middle cerebral artery ischemic stroke. *BMC Neurol.* (2017) 17:177. doi: 10.1186/s12883-017-0958-3
17. Menon BK, Saver JL, Prabhakaran S, Reeves M, Liang L, Olson DM, et al. Risk score for intracranial hemorrhage in patients with acute ischemic stroke treated with intravenous tissue-type plasminogen activator. *Stroke.* (2012) 43:2293–9. doi: 10.1161/STROKEAHA.112.660415
18. Lou M, Safdar A, Mehdiratta M, Kumar S, Schlaug G, Caplan L, et al. The HAT score: a simple grading scale for predicting hemorrhage after thrombolysis. *Neurology.* (2008) 71:1417–23. doi: 10.1212/01.wnl.0000330297.58334.dd
19. Marsh EB, Llinas RH, Schneider ALC, Hillis AE, Lawrence E, Dziedzic P, et al. Predicting hemorrhagic transformation of acute ischemic stroke: prospective validation of the HeRS score. *Medicine.* (2016) 95:e2430. doi: 10.1097/MD.0000000000002430
20. Mazya M, Egido JA, Ford GA, Lees KR, Mikulik R, Toni D, et al. Predicting the risk of symptomatic intracerebral hemorrhage in ischemic stroke treated with intravenous alteplase: safe implementation of treatments in stroke (SITS) symptomatic intracerebral hemorrhage risk score. *Stroke.* (2012) 43:1524–31. doi: 10.1161/STROKEAHA.111.644815
21. Lyden PD. Stroke: Haemorrhage risk after thrombolysis–the SEDAN score. *Nat Rev Neurol.* (2012) 8:246–7. doi: 10.1038/nrneurol.2012.66
22. Kidwell CS, Saver JL, Carneado J, Sayre J, Starkman S, Duckwiler G, et al. Predictors of hemorrhagic transformation in patients receiving intra-arterial thrombolysis. *Stroke.* 33:717–24 (2002). doi: 10.1161/hs0302.104110
23. Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke t-PA Stroke Study Group. *J Neurosurg Anesthesiol.* (1996) 333:1581–7.
24. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and stroke statistics-2019 update: a report from the American Heart Association. *Circulation.* (2019) 139:e56–528.
25. Miller DJ, Simpson JR, Silver B, Silver B. Safety of thrombolysis in acute ischemic stroke: a review of complications, risk factors, and newer technologies. *Neurohospitalist.* (2011) 1:138–47. doi: 10.1177/1941875211408731
26. Sandercock P, Wardlaw JM, Lindley RI, Dennis M, Cohen G, Murray G, et al. The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. *Lancet.* (2012) 379:2352–63. doi: 10.1016/S0140-6736(12)60768-5
27. Whiteley WN, Emberson J, Lees KR, Blackwell L, Albers G, Bluhmki E, et al. Risk of intracerebral haemorrhage with alteplase after acute ischaemic stroke: a secondary analysis of an individual patient data meta-analysis. *Lancet Neurol.* (2016) 15:925–33. doi: 10.1016/S1474-4422(16)30076-X
28. Yaghi S, Willey JZ, Cucchiara B, Goldstein JN, Gonzales NR, Khatri P, et al. Treatment and outcome of hemorrhagic transformation after intravenous alteplase in acute ischemic stroke a scientific statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke.* (2017) 48:e343–61. doi: 10.1161/STR.0000000000000152
29. Nour M, Scalzo F, Liebeskind DS. Ischemia-reperfusion injury in stroke. *Interv Neurol.* (2013) 1:185–99. doi: 10.1159/000353125
30. Khatri R, McKinney AM, Swenson B, Janardhan V. Blood-brain barrier, reperfusion injury, and hemorrhagic transformation in acute ischemic stroke. *Neurology.* (2012) 79:S52–7. doi: 10.1212/WNL.0b013e3182697e70

31. Kaur J, Zhao Z, Klein GM, Lo EH, Buchan AM. The neurotoxicity of tissue plasminogen activator? *J Cereb Blood Flow Metab.* (2004) 24:945–63. doi: 10.1097/01.WCB.0000137868.50767.E8
32. Cuadrado E, Ortega L, Hernández-Guillamon M, Penalba A, Fernández-Cadenas I, Rosell A, et al. Tissue plasminogen activator (t-PA) promotes neutrophil degranulation and MMP-9 release. *J Leukoc Biol.* (2008) 84:207–14. doi: 10.1189/jlb.0907606
33. Lakhan SE, Kirchgessner A, Tepper D, Leonard A. Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. *Front Neurol.* (2013) 4 APR:1–15. doi: 10.3389/fneur.2013.00032
34. Su EJ, Fredriksson L, Geyer M, Folestad E, Cale J, Andrae J, et al. Activation of PDGF-CC by tissue plasminogen activator impairs blood-brain barrier integrity during ischemic stroke. *Nat Med.* (2008) 14:731–7. doi: 10.1038/nm1787
35. Kyung-Pil P, Anna R, Christian F, Changhong X, Jean KW, Seoul L, et al. Plasma and brain matrix metalloproteinase-9 after acute focal cerebral ischemia in rats. *Stroke.* (2009) 40:2836–42. doi: 10.1161/STROKEAHA.109.554824
36. Huang X, MacIsaac R, Thompson JL, Levin B, Buchsbaum R, Haley ECJ, et al. Tenecteplase versus alteplase in stroke thrombolysis: an individual patient data meta-analysis of randomized controlled trials. *Int J Stroke.* (2016) 11:534–43. doi: 10.1177/1747493016641112
37. Campbell BCV, Mitchell PJ, Churilov L, Yassi N, Kleinig TJ, Dowling RJ, et al. Tenecteplase versus alteplase before thrombectomy for ischemic stroke. *N Engl J Med.* (2018) 378:1573–82. doi: 10.1056/NEJMoa1716405
38. Adivitiya KY. The evolution of recombinant thrombolytics: current status and future directions. *Bioengineered.* (2017) 8:331–58. doi: 10.1080/21655979.2016.1229718
39. Gürsoy-Özdemir Y, Can A, Dalkara T. Reperfusion-induced oxidative/nitrative injury to neurovascular unit after focal cerebral ischemia. *Stroke.* (2004) 35:1449–53. doi: 10.1161/01.STR.0000126044.83777.f4
40. Huang J, Liu B, Yang C, Chen H, Eunice D, Yuan Z. Acute hyperglycemia worsens ischemic stroke-induced brain damage via high mobility group box-1 in rats. *Brain Res.* (2013) 1535:148-—155. doi: 10.1016/j.brainres.2013.08.057
41. Qiu J, Xu J, Zheng Y, Wei Y, Zhu X, Eng HL, et al. High-mobility group box 1 promotes metalloproteinase-9 upregulation through toll-like receptor 4 after cerebral ischemia. *Stroke.* (2010) 41:2077–82. doi: 10.1161/STROKEAHA.110.590463
42. Martini SR, Kent TA. Hyperglycemia in acute ischemic stroke: a vascular perspective. *J Cereb Blood Flow Metab.* (2006) 27:435–51. doi: 10.1038/sj.jcbfm.9600355
43. Harari OA, Liao JK. NF-κB and innate immunity in ischemic stroke. *Ann N Y Acad Sci.* (2010) 1207:32–40. doi: 10.1111/j.1749-6632.2010.05735.x
44. Kong L, Ma Y, Wang Z, Liu N, Ma G, Liu C, et al. Inhibition of hypoxia inducible factor 1 by YC-1 attenuates tissue plasminogen activator induced hemorrhagic transformation by suppressing HMGB1/TLR4/NF-κB mediated neutrophil infiltration in thromboembolic stroke rats. *Int Immunopharmacol.* (2021) 94:107507. doi: 10.1016/j.intimp.2021.107507
45. Goyal M, Menon BK, van Zwam WH, Dippel DWJ, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet.* (2016) 387:1723–31. doi: 10.1016/S0140-6736(16)00163-X
46. Campbell BC, Hill MD, Rubiera M, Menon BK, Demchuk A, Donnan GA, et al. Safety and efficacy of solitaire stent thrombectomy. *Stroke.* (2016) 47:798–806. doi: 10.1161/STROKEAHA.115.012360
47. Yang P, Zhang Y, Zhang L, Zhang Y, Treurniet KM, Chen W, et al. Endovascular thrombectomy with or without intravenous alteplase in acute stroke. *N Engl J Med.* (2020) 382:1981–93. doi: 10.1056/NEJMoa2001123
48. Chalos V, LeCouffe NE, Uyttenboogaart M, Lingsma HF, Mulder MJHL, Venema E, et al. Endovascular treatment with or without prior intravenous alteplase for acute ischemic stroke. *J Am Heart Assoc.* (2019) 8:e011592. doi: 10.1161/JAHA.118.011592
49. Langel C, Popovic KS. Infarct-core CT perfusion parameters in predicting post-thrombolysis hemorrhagic transformation of acute ischemic stroke. *Radiol Oncol.* (2018) 53:25–30. doi: 10.2478/raon-2018-0048
50. Aoki T, Sumii T, Mori T, Wang X, Lo EH. Blood-brain barrier disruption and matrix metalloproteinase-9 expression during reperfusion injury. *Stroke.* (2002) 33:2711–7. doi: 10.1161/01.STR.0000033932.34467.97
51. Johnston KC, Bruno A, Pauls Q, Hall CE, Barrett KM, Barsan W, et al. Intensive vs standard treatment of hyperglycemia and functional outcome in patients with acute ischemic stroke: the SHINE randomized clinical trial. *J Am Med Assoc.* (2019) 322:326–35. doi: 10.1001/jama.2019.9346
52. Garg R, Chaudhuri A, Munschauer F, Dandona P. Hyperglycemia, insulin, and acute ischemic stroke: a mechanistic justification for a trial of insulin infusion therapy. *Stroke.* (2006) 37:267–73. doi: 10.1161/01.STR.0000195175.29487.30
53. Williams LS, Rotich J, Qi R, Fineberg N, Espay A, Bruno A, et al. Effects of admission hyperglycemia on mortality and costs in acute ischemic stroke. *Neurology.* (2002) 59:67–71. doi: 10.1212/WNL.59.1.67
54. Ritter L, Davidson L, Henry M, Davis-Gorman G, Morrison H, Frye JB, et al. Exaggerated neutrophil-mediated reperfusion injury after ischemic stroke in a rodent model of type 2 diabetes. *Microcirculation.* (2011) 18:552–61. doi: 10.1111/j.1549-8719.2011.00115.x
55. Elgebaly MM, Ogbi S, Li W, Mezzetti EM, Prakash R, Johnson MH, et al. Neurovascular injury in acute hyperglycemia and diabetes: a comparative analysis in experimental stroke. *Transl Stroke Res.* (2011) 2:391–8. doi: 10.1007/s12975-011-0083-3
56. Chen H, Guan B, Chen S, Yang D, Shen J. Peroxynitrite activates NLRP3 inflammasome and contributes to hemorrhagic transformation and poor outcome in ischemic stroke with hyperglycemia. *Free Radic Biol Med.* (2021) 165:171–83. doi: 10.1016/j.freeradbiomed.2021.01.030
57. Zhang G, He M, Xu Y, Li X, Cai Z, Guo Z, et al. Hemoglobin A1c predicts hemorrhagic transformation and poor outcomes after acute anterior stroke. *Eur J Neurol.* (2018) 25:1432.e122. doi: 10.1111/ene.13747
58. Powers WJ, Alejandro R, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. 2018 guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke.* (2018) 49:e46–99. doi: 10.1161/STR.0000000000000172
59. Desilles JP, Meseguer E, Labreuche J, Lapergue B, Sirimarco G, Gonzalez-Valcarcel J, et al. Diabetes mellitus, admission glucose, and outcomes after stroke thrombolysis: a registry and systematic review. *Stroke.* (2013) 44:1915–23. doi: 10.1161/STROKEAHA.111.000813
60. Dokken BB. The pathophysiology of cardiovascular disease and diabetes: beyond blood pressure and lipids. *Diabetes Spectr.* (2008) 21:160–5. doi: 10.2337/diaspect.21.3.160
61. Hong P, Gu R-N, Li F-X, Xiong X-X, Liang W-B, You Z-J, et al. NLRP3 inflammasome as a potential treatment in ischemic stroke concomitant with diabetes. *J Neuroinflam.* (2019) 16:121. doi: 10.1186/s12974-019-1498-0
62. Desilles JP, Syvannarath V, Ollivier V, Journé C, Delbosc S, Ducroux C, et al. Exacerbation of thromboinflammation by hyperglycemia precipitates cerebral infarct growth and hemorrhagic transformation. *Stroke.* (2017) 48:1932–40. doi: 10.1161/STROKEAHA.117.017080
63. Won SJ, Tang XN, Suh SW, Yenari MA, Swanson RA. Hyperglycemia promotes tissue plasminogen activator-induced hemorrhage by increasing superoxide production. *Ann Neurol.* (2011) 70:583–90. doi: 10.1002/ana.22538
64. Guo Z, Yu S, Chen X, Zheng P, Hu T, Duan Z, et al. Suppression of NLRP3 attenuates hemorrhagic transformation after delayed rtPA treatment in thromboembolic stroke rats: involvement of neutrophil recruitment. *Brain Res Bull.* (2018) 137:229–40. doi: 10.1016/j.brainresbull.2017.12.009
65. Price TO, Eranki V, Banks WA, Ercal N, Shah GN. Topiramate treatment protects blood-brain barrier pericytes from hyperglycemia-induced oxidative damage in diabetic mice. *Endocrinology.* (2012) 153:362–72. doi: 10.1210/en.2011-1638
66. Yoo DY, Yim HS, Jung HY, Nam SM, Kim JW, Choi JH, et al. Chronic type 2 diabetes reduces the integrity of the blood-brain barrier by reducing tight junction proteins in the hippocampus. *J Vet Med Sci.* (2016) 78:957–62. doi: 10.1292/jvms.15-0589
67. Tjärnlund-Wolf A, Brogren H, Lo EH, Wang X. Plasminogen activator inhibitor-1 and thrombotic cerebrovascular diseases. *Stroke.* (2012) 43:2833–9. doi: 10.1161/STROKEAHA.111.622217

68. Dohgu S, Takata F, Matsumoto J, Oda M, Harada E, Watanabe T, et al. Autocrine and paracrine up-regulation of blood–brain barrier function by plasminogen activator inhibitor-1. *Microvasc Res.* (2011) 81:103–7. doi: 10.1016/j.mvr.2010.10.004
69. Ribo M, Montaner J, Molina CA, Arenillas JF, Santamarina E, Quintana M, et al. Admission fibrinolytic profile is associated with symptomatic hemorrhagic transformation in stroke patients treated with tissue plasminogen activator. *Stroke.* (2004) 35:2123–7. doi: 10.1161/01.STR.0000137608.73660.4c
70. Shukla V, Shakya AK, Perez-Pinzon MA, Dave KR. Cerebral ischemic damage in diabetes: an inflammatory perspective. *J Neuroinflam.* (2017) 14:21. doi: 10.1186/s12974-016-0774-5
71. Couret D, Bourane S, Catan A, Nativel B, Planesse C, Dorsemans AC, et al. A hemorrhagic transformation model of mechanical stroke therapy with acute hyperglycemia in mice. *J Comp Neurol.* (2018) 526:1006–16. doi: 10.1002/cne.24386
72. Ismael S, Nasoohi S, Yoo A, Ahmed HA, Ishrat T. Tissue plasminogen activator promotes TXNIP-NLRP3 inflammasome activation after hyperglycemic stroke in mice. *Mol Neurobiol.* (2020) 57:2495–508. doi: 10.1007/s12035-020-01893-7
73. Pandolfi A, Giaccari A, Cilli C, Alberta MM, Morviducci L, De Filippis EA, et al. Acute hyperglycemia and acute hyperinsulinemia decrease plasma fibrinolytic activity and increase plasminogen activator inhibitor type 1 in the rat. *Acta Diabetol.* (2001) 38:71–6. doi: 10.1007/s005920170016
74. Ribo M, Molina C, Montaner J, Rubiera M, Delgado-Mederos R, Arenillas JF, et al. Acute hyperglycemia state is associated with lower tPA-induced recanalization rates in stroke patients. *Stroke.* (2005) 36:1705–9. doi: 10.1161/01.STR.0000173161.05453.90.9f
75. Ning R, Chopp M, Yan T, Zacharek A, Zhang C, Roberts C, et al. Tissue plasminogen activator treatment of stroke in type-1 diabetes rats. *Neuroscience.* (2012) 222:326–32. doi: 10.1016/j.neuroscience.2012.07.018
76. Schneider DJ, Sobel BE. PAI-1 and diabetes: a journey from the bench to the bedside. *Diabetes Care.* (2012) 35:1961–7. doi: 10.2337/dc12-0638
77. Hafez S, Coucha M, Bruno A, Fagan SC, Ergul A. Hyperglycemia, acute ischemic stroke, and thrombolytic therapy. *Transl Stroke Res.* (2014) 5:442–53. doi: 10.1007/s12975-014-0336-z
78. Gray CS, Hildreth AJ, Sandercock PA, O'Connell JE, Johnston DE, Cartlidge NE, et al. Glucose-potassium-insulin infusions in the management of post-stroke hyperglycaemia: the UK glucose insulin in stroke trial (GIST-UK). *Lancet Neurol.* (2007) 6:397–406. doi: 10.1016/S1474-4422(07)70080-7
79. Palaiodimou L, Lioutas V-A, Lambadiari V, Paraskevas GP, Voumvourakis K, Tsivgoulis G. Glycemia management in acute ischemic stroke: current concepts and novel therapeutic targets. *Postgrad Med.* (2019) 131:423–37. doi: 10.1080/00325481.2019.1651206
80. Pires PW, Dams Ramos CM, Matin N, Dorrance AM. The effects of hypertension on the cerebral circulation. *Am J Physiol Circ Physiol.* (2013) 304:H1598–614. doi: 10.1152/ajpheart.00490.2012
81. Castro MM, Tanus-Santos JE, Gerlach RF. Matrix metalloproteinases: targets for doxycycline to prevent the vascular alterations of hypertension. *Pharmacol Res.* (2011) 64:567–72. doi: 10.1016/j.phrs.2011.04.002
82. Faraco G, Sugiyama Y, Lane D, Garcia-Bonilla L, Chang H, Santisteban MM, et al. Perivascular macrophages mediate the neurovascular and cognitive dysfunction associated with hypertension. *J Clin Invest.* (2016) 126:4674–89. doi: 10.1172/JCI86950
83. Qureshi AI. Acute hypertensive response in patients with stroke. *Circulation.* (2008) 118:176–87. doi: 10.1161/CIRCULATIONAHA.107.723874
84. Mohammadi MT, Dehghani GA. Acute hypertension induces brain injury and blood-brain barrier disruption through reduction of claudins mRNA expression in rat. *Pathol Res Pract.* (2014) 210:985–90. doi: 10.1016/j.prp.2014.05.007
85. Ueno M, Sakamoto H, Liao Y-J, Onodera M, Huang C-L, Miyanaka H, et al. Blood-brain barrier disruption in the hypothalamus of young adult spontaneously hypertensive rats. *Histochem Cell Biol.* (2004) 122:131–7. doi: 10.1007/s00418-004-0684-y
86. Flamant M, Placier S, Dubroca C, Esposito B, Lopes I, Chatziantoniou C, et al. Role of matrix metalloproteinases in early hypertensive vascular remodeling. *Hypertens.* (2007) 50:212–8. doi: 10.1161/HYPERTENSIONAHA.107.089631
87. Campbell, Christensen S, Tress BM, Churilov L, Desmond PM, Parsons MW, et al. Failure of collateral blood flow is associated with infarct growth in ischemic stroke. *J Cereb Blood Flow Metab.* (2013) 33:1168–72. doi: 10.1038/jcbfm.2013.77
88. Zhang, Yang Y, Sun H, Xing Y. Hemorrhagic transformation after cerebral infarction: current concepts and challenges. *Ann Transl Med.* (2014) 2:81. doi: 10.3978/j.issn.2305-5839.2014.08.08
89. Fagan SC, Bowes MP, Lyden PD, Zivin JA. Acute hypertension promotes hemorrhagic transformation in a rabbit embolic stroke model: effect of labetalol. *Exp Neurol.* (1998) 150:153–8. doi: 10.1006/exnr.1997.6756
90. Tejima E, Katayama Y, Suzuki Y, Kano T, Lo EH. Hemorrhagic transformation after fibrinolysis with tissue plasminogen activator. *Stroke.* (2001) 32:1336–40. doi: 10.1161/01.STR.32.6.1336
91. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke. *Stroke.* (2019) 50:e344–418. doi: 10.1161/STR.0000000000000211
92. Ahmed N, Wahlgren N, Brainin M, Castillo J, Ford GA, Kaste M, et al. Relationship of blood pressure, antihypertensive therapy, and outcome in ischemic stroke treated with intravenous thrombolysis: retrospective analysis from safe implementation of thrombolysis in stroke-international stroke thrombolysis register (SITS-IST). *Stroke.* (2009) 40:2442–9. doi: 10.1161/STROKEAHA.109.548602
93. Wu W, Huo X, Zhao X, Liao X, Wang C, Pan Y, et al. Relationship between blood pressure and outcomes in acute ischemic stroke patients administered lytic medication in the TIMS-China study. *PLoS ONE.* (2016) 11:e0144260. doi: 10.1371/journal.pone.0144260
94. Liu K, Yan S, Zhang S, Guo Y, Lou M. Systolic blood pressure variability is associated with severe hemorrhagic transformation in the early stage after thrombolysis. *Transl Stroke Res.* (2016) 7:186–91. doi: 10.1007/s12975-016-0458-6
95. Perini F, De Boni A, Marcon M, Bolgan I, Pellizzari M, Dionisio L. Systolic blood pressure contributes to intracerebral haemorrhage after thrombolysis for ischemic stroke. *J Neurol Sci.* (2010) 297:52–4. doi: 10.1016/j.jns.2010.06.025
96. Mulder MJHL, Ergezen S, Lingsma HF, Berkhemer OA, Fransen PSS, Beumer D, et al. Baseline blood pressure effect on the benefit and safety of intra-arterial treatment in MR CLEAN (multicenter randomized clinical trial of endovascular treatment of acute ischemic stroke in the Netherlands). *Stroke.* (2017) 48:1869–76. doi: 10.1161/STROKEAHA.116.016225
97. Matusevicius M, Cooray C, Bottai M, Mazya M, Tsivgoulis G, Nunes AP, et al. Blood pressure after endovascular thrombectomy: modeling for outcomes based on recanalization status. *Stroke.* (2020) 51:519–25. doi: 10.1161/STROKEAHA.119.026914
98. Anderson CS, Huang Y, Lindley RI, Chen X, Arima H, Chen G, et al. Intensive blood pressure reduction with intravenous thrombolysis therapy for acute ischaemic stroke (ENCHANTED): an international, randomised, open-label, blinded-endpoint, phase 3 trial. *Lancet.* (2019) 393:877–88. doi: 10.1016/S0140-6736(19)30038-8
99. Tikhonoff V, Zhang H, Richart T, Staessen JA. Blood pressure as a prognostic factor after acute stroke. *Lancet Neurol.* (2009) 8:938–48. doi: 10.1016/S1474-4422(09)70184-X
100. Bath PM, Scutt P, Anderson CS, Appleton JP, Berge E, Cala L, et al. Prehospital transdermal glyceryl trinitrate in patients with ultra-acute presumed stroke (RIGHT-2): an ambulance-based, randomised, sham-controlled, blinded, phase 3 trial. *Lancet.* (2019) 393:1009–20. doi: 10.1016/S0140-6736(19)30194-1
101. Zonneveld TP, Algra A, Dippel DWJ, Kappelle LJ, van Oostenbrugge RJ, Roos YBWEM, et al. The ThRombolysis in UnconTrolled Hypertension (TRUTH) protocol: an observational study on treatment strategy of elevated blood pressure in stroke patients eligible for IVT. *BMC Neurol.* (2015) 15:241. doi: 10.1186/s12883-015-0493-z
102. Mazighi M, Richard S, Lapergue B, Sibon I, Gory B, Berge J, et al. Safety and efficacy of intensive blood pressure lowering after successful endovascular therapy in acute ischaemic stroke (BP-TARGET): a multicentre, open-label, randomised controlled trial. *Lancet Neurol* (2021).

103. Ziai WC, Al-Kawaz M. Blood pressure management after endovascular therapy. *Lancet Neurol.* (2021) 20:248–49. doi: 10.1016/S1474-4422(21)00058-2
104. Sohrabji F, Bake S, Lewis DK. Age-related changes in brain support cells: implications for stroke severity. *Neurochem Int.* (2013) 63:291–301. doi: 10.1016/j.neuint.2013.06.013
105. Erickson MA, Banks WA. Age-associated changes in the immune system and blood¯brain barrier functions. *Int J Mol Sci.* (2019) 20:1632. doi: 10.3390/ijms20071632
106. Ritzel RM, Lai Y-J, Crapser JD, Patel AR, Schrecengost A, Grenier JM, et al. Aging alters the immunological response to ischemic stroke. *Acta Neuropathol.* (2018) 136:89–110. doi: 10.1007/s00401-018-1859-2
107. Manwani B, Liu F, Scranton V, Hammond MD, Sansing LH, McCullough LD. Differential effects of aging and sex on stroke induced inflammation across the lifespan. *Exp Neurol.* (2013) 249:120–31. doi: 10.1016/j.expneurol.2013.08.011
108. Emberson J, Lees KR, Lyden P, Blackwell L, Albers G, Bluhmki E, et al. Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. *Lancet.* (2014) 384:1929–35. doi: 10.1016/S0140-6736(14)60584-5
109. Costello CA, Campbell BC V, Perez de la Ossa N, Zheng TH, Sherwin JC, Weir L, et al. Age over 80 years is not associated with increased hemorrhagic transformation after stroke thrombolysis. *J Clin Neurosci.* (2012) 19:360–3. doi: 10.1016/j.jocn.2011.08.014
110. Weiskopf D, Weinberger B, Grubeck-Loebenstein B. The aging of the immune system. *Transpl Int.* (2009) 22:1041–50. doi: 10.1111/j.1432-2277.2009.00927.x
111. Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. *Nat Rev Cardiol.* (2018) 15:505–22. doi: 10.1038/s41569-018-0064-2
112. Shaw AC, Joshi S, Greenwood H, Panda A, Lord JM. Aging of the innate immune system. *Curr Opin Immunol.* (2010) 22:507–13. doi: 10.1016/j.coi.2010.05.003
113. Chan SL, Bishop N, Li Z, Cipolla MJ. Inhibition of PAI (plasminogen activator inhibitor)-1 improves brain collateral perfusion and injury after acute ischemic stroke in aged hypertensive rats. *Stroke.* (2018) 49:1969–76. doi: 10.1161/STROKEAHA.118.022056
114. Petcu EB, Smith RA, Miroiu RI, Opris MM. Angiogenesis in old-aged subjects after ischemic stroke: a cautionary note for investigators. *J Angiogenes Res.* (2010) 2:26. doi: 10.1186/2040-2384-2-26
115. Norden DM, Godbout JP. Review: microglia of the aged brain: primed to be activated and resistant to regulation. *Neuropathol Appl Neurobiol.* (2013) 39:19–34. doi: 10.1111/j.1365-2990.2012.01306.x
116. Cui H, Kong Y, Zhang H. Oxidative stress, mitochondrial dysfunction, and aging. *J Signal Transduct.* (2012) 2012:646354. doi: 10.1155/2012/646354
117. Maestrini I, Strbian D, Gautier S, Haapaniemi E, Moulin S, Sairanen T, et al. Higher neutrophil counts before thrombolysis for cerebral ischemia predict worse outcomes. *Neurology.* (2015) 85:1408–16. doi: 10.1212/WNL.0000000000002029
118. Ucuzian AA, Gassman AA, East AT, Greisler HP. Molecular mediators of angiogenesis. *J Burn Care Res.* (2010) 31:158–75. doi: 10.1097/BCR.0b013e3181c7ed82
119. Kanazawa M, Igarashi H, Kawamura K, Takahashi T, Kakita A, Takahashi H, et al. Inhibition of VEGF signaling pathway attenuates hemorrhage after tPA treatment. *J Cereb Blood Flow Metab.* (2011) 31:1461–74. doi: 10.1038/jcbfm.2011.9
120. Faber JE, Zhang H, Lassance-Soares RM, Prabhakar P, Najafi AH, Burnett MS, et al. Aging causes collateral rarefaction and increased severity of ischemic injury in multiple tissues. *Arterioscler Thromb Vasc Biol.* (2011) 31:1748–56. doi: 10.1161/ATVBAHA.111.227314
121. Bang OY, Saver JL, Kim SJ, Kim G-M, Chung C-S, Ovbiagele B, et al. Collateral flow averts hemorrhagic transformation after endovascular therapy for acute ischemic stroke. *Stroke.* (2011) 42:2235–9. doi: 10.1161/STROKEAHA.110.604603
122. Ng GJL, Quek AML, Cheung C, Arumugam T V, Seet RCS. Stroke biomarkers in clinical practice: a critical appraisal. *Neurochem Int.* (2017) 107:11–22. doi: 10.1016/j.neuint.2017.01.005
123. Lu G, He Q, Shen Y, Cao F. Potential biomarkers for predicting hemorrhagic transformation of ischemic stroke. *Int J Neurosci.* (2018) 128:79–89. doi: 10.1080/00207454.2017.1349766
124. Piccardi B, Biagini S, Iovene V, Palumbo V. Blood biomarkers of parenchymal damage in ischemic stroke patients treated with revascularization therapies. *Biomark Insights.* (2019) 14:1177271919888225. doi: 10.1177/1177271919888225
125. Castellanos M, Leira R, Serena J, Blanco M, Pedraza S, Castillo J, et al. Plasma cellular-fibronectin concentration predicts hemorrhagic transformation after thrombolytic therapy in acute ischemic stroke. *Stroke.* (2004) 35:1671–6. doi: 10.1161/01.STR.0000131656.47979.39
126. Bernstein JE, Savla P, Dong F, Zampella B, Wiginton JG 4th, Miulli DE, et al. Inflammatory markers and severity of intracerebral hemorrhage. *Cureus.* (2018) 10:e3529. doi: 10.7759/cureus.3529
127. Shichita T, Ito M, Yoshimura A. Post-ischemic inflammation regulates neural damage and protection. *Front Cell Neurosci.* (2014) 8:1–8. doi: 10.3389/fncel.2014.00319
128. Jickling GC, Ander BP, Stamova B, Zhan X, Liu D, Rothstein L, et al. RNA in blood is altered prior to hemorrhagic transformation in ischemic stroke. *Ann Neurol.* (2013) 74:232–40. doi: 10.1002/ana.23883
129. Planas AM. Role of immune cells migrating to the ischemic brain. *Stroke.* (2018) 49:2261–7. doi: 10.1161/STROKEAHA.118.021474
130. Yenari MK MA. Inflammatory responses in brain ischemia. *Curr Med Chem.* (2015) 22:1258–77. doi: 10.2174/0929867322666150209154036
131. Ouk T, Potey C, Maestrini I, Petrault M, Mendyk A-M, Leys D, et al. Neutrophils in tPA-induced hemorrhagic transformations: main culprit, accomplice or innocent bystander? *Pharmacol Ther.* (2019) 194:73–83. doi: 10.1016/j.pharmthera.2018.09.005
132. Carbone F, Vuilleumier N, Bertolotto M, Burger F, Galan K, Roversi G, et al. Treatment with recombinant tissue plasminogen activator (r-TPA) induces neutrophil degranulation in vitro via defined pathways. *Vascul Pharmacol.* (2015) 64:16–27. doi: 10.1016/j.vph.2014.11.007
133. Wang G, Guo Q, Hossain M, Fazio V, Zeynalov E, Janigro D, et al. Bone marrow-derived cells are the major source of MMP-9 contributing to blood–brain barrier dysfunction and infarct formation after ischemic stroke in mice. *Brain Res.* (2009) 1294:183–92. doi: 10.1016/j.brainres.2009.07.070
134. Ramiro L, Simats A, García-Berrocoso T, Montaner J. Inflammatory molecules might become both biomarkers and therapeutic targets for stroke management. *Ther Adv Neurol Disord.* (2018) 11:1756286418789340. doi: 10.1177/1756286418789340
135. Jayaraj RL, Azimullah S, Beiram R, Jalal FY, Rosenberg GA. Neuroinflammation: friend and foe for ischemic stroke. *J Neuroinflam.* (2019) 16:142. doi: 10.1186/s12974-019-1516-2
136. Kim JY, Park J, Chang JY, Kim S-H, Lee JE. Inflammation after ischemic stroke: the role of leukocytes and glial cells. *Exp Neurobiol.* (2016) 25:241–51. doi: 10.5607/en.2016.25.5.241
137. O'Connell GC, Treadway MB, Petrone AB, Tennant CS, Lucke-Wold N, Chantler PD, et al. Peripheral blood AKAP7 expression as an early marker for lymphocyte-mediated post-stroke blood brain barrier disruption. *Sci Rep.* (2017) 7:1172. doi: 10.1038/s41598-017-01178-5
138. del Río-Espínola A, Fernández-Cadenas I, Giralt D, Quiroga A, Gutiérrez-Agulló M, Quintana M, et al. A predictive clinical–genetic model of tissue plasminogen activator response in acute ischemic stroke. *Ann Neurol.* (2012) 72:716–29. doi: 10.1002/ana.23664

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 25 May 2021
doi: 10.3389/fneur.2021.663058

Subarachnoid Hemorrhage in Mechanical Thrombectomy for Acute Ischemic Stroke: Analysis of the STRATIS Registry, Systematic Review, and Meta-Analysis

Hubert Lee[1,2], *Ayman M. Qureshi*[1,3], *Nils H. Mueller-Kronast*[4], *Osama O. Zaidat*[5], *Michael T. Froehler*[6], *David S. Liebeskind*[7] *and Vitor M. Pereira*[1,8]*

[1] Division of Neuroradiology, Joint Department of Medical Imaging, Toronto Western Hospital, Toronto, ON, Canada, [2] Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA, United States, [3] Lysholm Department of Neuroradiology, National Hospital for Neurology and Neurosurgery, UCLH NHS Foundation Trust, London, United Kingdom, [4] Advanced Neuroscience Network/Tenet South Florida, Delray Beach, FL, United States, [5] Neuroscience Institute, St Vincent Mercy Medical Center, Toledo, OH, United States, [6] Department of Neurology, Vanderbilt University Medical Center, Nashville, TN, United States, [7] Department of Neurology, University of California, Los Angeles, Los Angeles, CA, United States, [8] Therapeutic Neuroradiology & Neurosurgery, St. Michael's Hospital, Toronto, ON, Canada

OPEN ACCESS

Edited by:
Richard Leigh,
Johns Hopkins University,
United States

Reviewed by:
Shahram Majidi,
Mount Sinai Hospital, United States
Marina Diomedi,
University of Rome Tor Vergata, Italy
Kyle Kern,
National Institute of Neurological Disorders and Stroke (NINDS),
United States

***Correspondence:**
Vitor M. Pereira
vitor.pereira@unityhealth.to

Specialty section:
This article was submitted to Stroke,
a section of the journal Frontiers in Neurology

Received: 02 February 2021
Accepted: 13 April 2021
Published: 25 May 2021

Citation:
Lee H, Qureshi AM, Mueller-Kronast NH, Zaidat OO, Froehler MT, Liebeskind DS and Pereira VM (2021) Subarachnoid Hemorrhage in Mechanical Thrombectomy for Acute Ischemic Stroke: Analysis of the STRATIS Registry, Systematic Review, and Meta-Analysis.
Front. Neurol. 12:663058.
doi: 10.3389/fneur.2021.663058

Background: The indications for mechanical thrombectomy in acute ischemic stroke continue to broaden, leading neurointerventionalists to treat vessel occlusions at increasingly distal locations farther in time from stroke onset. Accessing these smaller vessels raises the concern of iatrogenic subarachnoid hemorrhage (SAH) owing to increasing complexity in device navigation and retrieval. This study aims to determine the prevalence of SAH following mechanical thrombectomy, associated predictors, and resulting functional outcomes using a multicenter registry and compare this with a systematic review and meta-analysis of the literature.

Methods: Data from STRATIS (The Systematic Evaluation of Patients Treated with Neurothrombectomy Devices for Acute Ischemic Stroke) registry were analyzed dichotomized by the presence or absence of SAH after thrombectomy. Only patients with 24-h post-procedural neuroimaging were included ($n = 841$). Multivariable logistic regression was performed to identify significant predictors of SAH. A systematic review and random-effects meta-analysis was also conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) protocol.

Results: The prevalence of post-thrombectomy SAH was 5.23% in STRATIS with 15.9% (1.84% overall) experiencing neurological decline. Distal location of vessel occlusion (OR 3.41 [95% CI: 1.75–6.63], $p < 0.001$) and more than 3 device passes (OR 1.34 [95% CI: 1.09–1.64], $p = 0.01$) were associated with a higher probability of SAH in contrast to a reduction with administration of intravenous tissue plasminogen activator (tPA) (OR 0.48 [95% CI: 0.26–0.89], $p = 0.02$). There was a trend toward a higher discharge NIHSS (8.3 ± 8.7 vs. 5.3 ± 6.6, $p = 0.07$) with a significantly

reduced proportion achieving functional independence at 90 days (modified Rankin Score 0–2: 32.5% vs. 57.8%, $p = 0.002$) in SAH patients. Pooled analysis of 10,126 patients from 6 randomized controlled trials and 64 observational studies demonstrated a prevalence of 5.85% [95% CI: 4.51–7.34%, I^2: 85.2%]. Only location of vessel occlusion was significant for increased odds of SAH at distal sites (OR 2.89 [95% CI: 1.14, 7.35]).

Conclusions: Iatrogenic SAH related to mechanical thrombectomy is more common with treatment of distally-situated occlusions and multiple device passes. While low in overall prevalence, its effect is not benign with fewer patients reaching post-procedural functional independence, particularly if symptomatic.

Keywords: subarachnoid hemorrhage, endovascular therapy, thrombectomy, stent retriever, direct aspiration, large vessel occlusion, ischemic stroke

INTRODUCTION

Mechanical thrombectomy is well-established as the standard of care for treatment of acute ischemic stroke secondary to a large vessel occlusion (1–5). Despite demonstrating higher rates of revascularization compared to best medical management, these procedures harbor a small, but real risk, of subarachnoid hemorrhage (SAH) (6, 7). The suspected mechanisms include inadvertent microwire perforation, tearing of arterioles or venules, alterations in vascular permeability, or reperfusion injury. SAH can be seen on post-procedural imaging despite the absence of visualized vessel perforation or contrast extravasation on periprocedural digital subtraction angiography (6, 8). Based on the findings of several case series, the clinical course of isolated SAH is seemingly benign (6, 9–12). With technological advancements in endovascular devices, neurointerventionalists are pursuing more distal occlusions in medium-sized vessels achieving successful reperfusion (thrombolysis in cerebral infarction score (TICI) 2b/3) in 54–83% of cases (13–16). Accessing and retrieving devices from more distal and narrower vessels raises the concern for iatrogenic hemorrhage including SAH.

A limited number of studies have investigated clinical and procedural risk factors associated with SAH following mechanical thrombectomy (6–8, 12). They identified use of intracranial angioplasty, greater number of device passes, longer distal positioning of a stent retriever within a M2 segment branch, severe vasospasm in the involved vessel prior to thrombectomy, longer interval between stroke onset to recanalization, and longer procedural duration as significant predictors (6–8). These studies are limited by small sample sizes as well using techniques and devices that are becoming progressively dated. The objective of this study was to use the Systematic Evaluation of Patients Treated with Neurothrombectomy Devices for Acute Ischemic Stroke (STRATIS) registry, a prospective and multicenter cohort, to characterize the prevalence of thrombectomy-related SAH, its related risk factors, and impact on functional outcome. A systematic review of the literature was also performed for comparison.

METHODS

STRATIS Registry

Study Design and Participants

STRATIS was a prospectively-maintained registry of mechanical thrombectomies performed with the Solitaire and Mindframe Capture Low Profile Revascularization Devices (Medtronic, Minneapolis, MN, USA) at 55 US centers from August 2014 to June 2016. The Solitaire was predominantly used as the first thrombectomy device accounting for 96.9% of procedures. The objective of this initiative was to capture real-world outcomes treating acute ischemic stroke due to large vessel occlusion. Patients were included if they presented with a National Institutes of Health Stroke Scale (NIHSS) $\geq$8 and $\leq$30, pre-stroke modified Rankin Scale (mRS) score $\leq$1, and mechanical thrombectomy within 8 h of stroke onset. Those participating in any multicenter randomized controlled trial (RCT) were excluded. A total of 984 patients were enrolled with the maximum number that could be contributed from a single-center limited to 75. Complete details of the study design have been previously published (17).

Predictors and Outcomes

Data from the registry was collected on patient comorbidities, stroke presentation, as well as procedural details. Clinical characteristics included age, sex, existing diagnoses (hypertension, diabetes mellitus, coronary artery disease, and smoking status), presenting neurological status assessed by the NIHSS, and administration of intravenous tissue plasminogen activator (tPA). Radiological characteristics of interest were presenting infarct burden on CT based on the Alberta stroke program early CT score (ASPECTS) and location of vessel occlusion. The middle cerebral artery (MCA) was further divided into segments from M1 to M3. Occlusions at the MCA M2 segment or beyond were classified as distal and those involving the internal carotid artery terminus, MCA M1 segment, vertebral artery, and basilar artery considered proximal. Procedural characteristics included type of anesthesia (conscious or general), number of device passes, use of rescue devices, and total procedural time.

The primary outcome of interest was SAH following mechanical thrombectomy. This was diagnosed by non-contrast CT as hyperdensity within the subarachnoid space or MRI brain as decreased signal intensity in the subarachnoid space on gradient echo T2* sequences which were performed 24 ± 8 h post-procedure. All imaging, including mechanical thrombectomy angiography, was assessed in an independent, blinded fashion by an imaging core laboratory. The remainder of intracranial hemorrhages seen on follow up imaging were categorized according to the Hiedelberg Bleeding Classification (HBC) (18). Symptomatic hemorrhage was defined as having an associated decline in NIHSS scale by ≥4 points. Secondary outcome measures included NIHSS at discharge and functional outcome assessed by the mRS at 90 days.

Systematic Review and Meta-Analysis

Search Strategy and Study Selection

A comprehensive search of the literature was conducted through the Medline, EMBASE, and Cochrane Library databases using the OVID interface including publications up to April 2020. The concepts of subarachnoid hemorrhage, endovascular thrombectomy, stent retriever, direct aspiration, and stroke were identified in our search strategy employing controlled vocabulary (National Library of Medicine's medical subject headings—MeSH) and keywords. Only publications written in the English language were considered. The references of all included studies and relevant systematic reviews and meta-analyses were manually reviewed for other eligible articles.

Identified citations and their associated full text articles were reviewed by two independent investigators using pre-determined eligibility criteria after duplicates were removed using Endnote (Version X7, Thomson Reuters). The inclusion criteria applied to these studies were: (1). RCT or observational study including prospective or retrospective cohort and case control design, (2). acute ischemic stroke patients presenting with medium-to-large vessel occlusion treated with endovascular therapy, (3). adult patients (age ≥18 years). The intervention must have been performed using a stent retriever, direct aspiration catheter, or any combination of the two strategies with a post-procedural computerized tomography (CT) or magnetic resonance imaging (MRI) of the brain performed within 48 h. Systematic reviews, meta-analyses, editorials, case reports, and studies utilizing the mechanical embolus removal in cerebral ischemia (MERCI) retriever device as the initial primary treatment were excluded. The number of studies excluded from the initial screen and the number of full text articles excluded with an associated rationale are presented as a flow diagram in accordance with the preferred reporting items for systematic review and meta-analysis protocol (PRISMA) (see **Figure 1**).

Data Extraction and Risk of Bias Assessment

Data from the articles eligible for full review were extracted independently by two reviewers. The study details recorded included the first author's name, year of publication, study design, number of centers and countries involved, study duration, inclusion and exclusion criteria, and population size. Clinical, radiological, and interventional characteristics were also collected including age, sex, presenting NIHSS, presenting ASPECTS, tPA use, location of vessel occlusion, procedural anesthesia type, primary thrombectomy device used, number of device passes, procedural angiographic result (thrombolysis in cerebral infarction (TICI) score), incidence of post-procedural SAH, incidence of symptomatic SAH, and mRS at 90 days. All variables, except for study characteristics, were dichotomized based on post-procedural SAH status. The study quality of RCTs were assessed with the Cochrane risk-of-bias tool for randomized trials version 2 and the Newcastle-Ottawa Scale (NOS) or Joanna Briggs Institute (JBI) Case Series Critical Appraisal Tool used for observational studies (19, 20). Studies earning a NOS score of 6–9 were considered high quality whereas those scoring 5 or less were deemed low quality.

Statistical Analysis

For the STRATIS database, univariate analysis between collected clinico-radiologic variables and the presence of SAH following mechanical thrombectomy was performed using the Fisher exact or chi-squared tests for categorical variables and two-sided *t*-tests for continuous variables. Predictors achieving a $p < 0.10$ underwent multivariate stepwise logistic regression with bidirectional elimination retaining only those that reached significance set at a $p < 0.05$. The contribution of each predictor to the occurrence of SAH following mechanical thrombectomy was expressed as an odds ratio (OR) with a corresponding 95% confidence interval (CI).

For the systematic review and meta-analysis, a pooled prevalence of the included studies was calculated using R statistical software [version 4.0.2, (21)] with the metafor package (22). Random effects modeling was used with the restricted maximum-likelihood estimator to account for between-study variance and individual effect sizes underwent double arcsine transformation. Sensitivity analysis was performed to identify outlying effect sizes, which was defined as an externally studentized residual >3. The associations between clinical, radiographic, and procedural variables to post-thrombectomy SAH were summarized as OR with corresponding 95% CI using the Mantel-Haenszel method and random effects model. These were performed on Review Manager software (version 5.4, The Cochrane Collaboration, Oxford, UK). Only variables with data available from 3 or more studies were analyzed. Heterogeneity was assessed for both the pooled prevalence and pooled estimates with the Higgins I^2 statistic, where a value >50% was considered significant. Subgroup analysis was conducted to quantify the impact of important sources of heterogeneity with study design as the main suspected source.

RESULTS

STRATIS Registry

Prevalence of SAH and Associated Clinical Characteristics

Post-procedural neuroimaging performed at 24 h was available in 841 of the 984 enrolled patients. A total of 44 cases of SAH were detected, 7 were symptomatic, resulting in a prevalence of

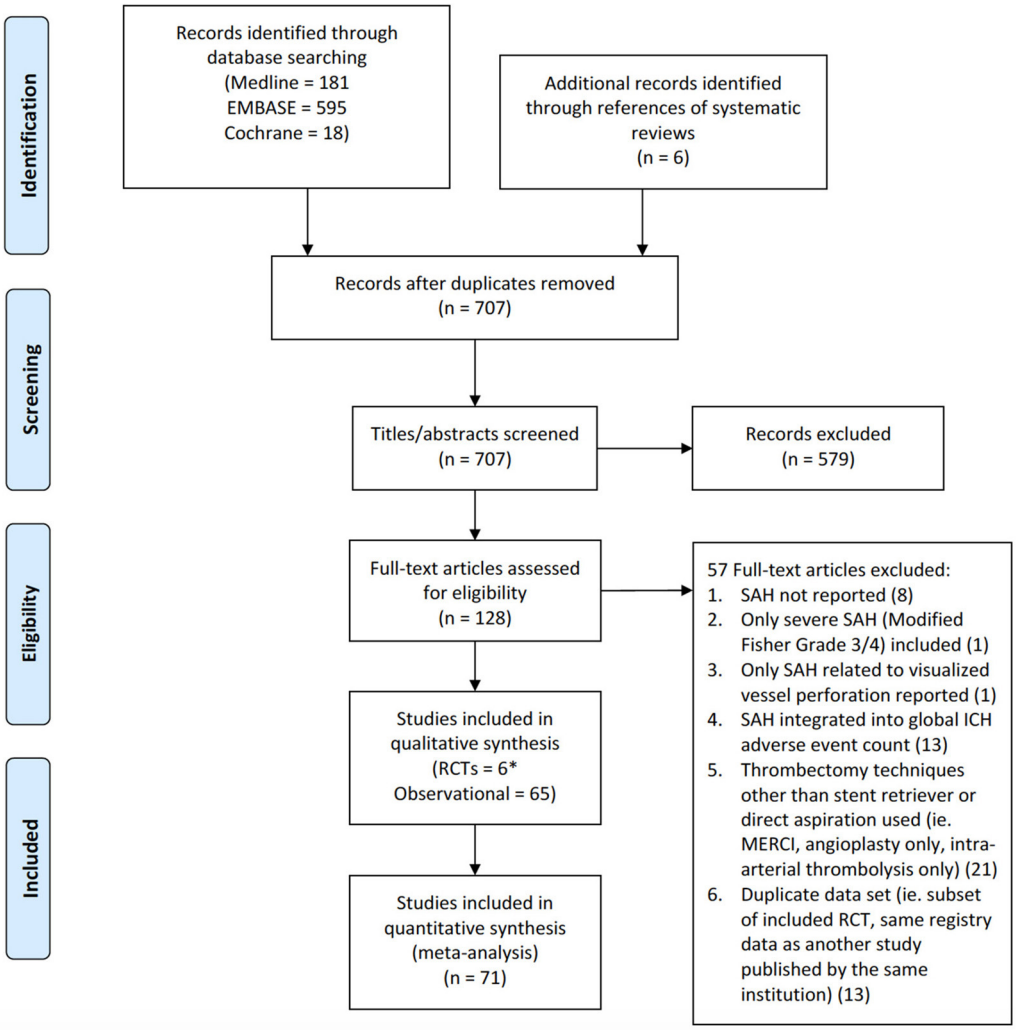


FIGURE 1 | PRISMA flow diagram of study identification and selection. *For the RCT by Saver et al.—SWIFT, only one treatment arm (Solitaire stent retriever) was used.

5.23% overall and 1.84% associated with neurological decline. Patients with and without SAH were similar with respect to demographics and their clinical presentation (see **Table 1**). Of the comorbidities investigated, diabetes mellitus was the only present in a greater percentage of patients with SAH post-mechanical thrombectomy (38.6 vs. 25.3%) however this difference only approached significance ($p = 0.05$). The location of vessel occlusion was significantly associated with occurrence of SAH with a higher frequency when the thrombus was located in the M2 segment of the MCA (36.4 vs. 15.8%) and a lower frequency at the carotid terminus (11.4 vs. 22.9%, $p = 0.02$). Several treatment factors were also related to post-mechanical thrombectomy SAH. A smaller proportion of patients with SAH received intravenous tPA (47.7 vs. 66.3%, $p = 0.01$). Clot retrieval resulting in SAH was performed with a higher percentage of device passes totaling more than 3 (20.5 vs. 9.6%, $p = 0.02$) in addition to longer mean procedural times (78.4 ± 39.3 min vs. 64.4 ± 36.9 min, $p = 0.02$).

Predictors of SAH

A total of 5 variables were highly associated with post-thrombectomy SAH based on a pre-determined $p < 0.10$. This included diabetes mellitus, administration of intravenous tPA, location of vessel occlusion, number of device passes, and total procedural time. The occluded vessel segment was further categorized as M2 and M3 vs. carotid terminus and M1 (excluding all posterior vessels) as well as M2 and M3 vs. all other sites except the posterior cerebral artery (PCA) to analyze the impact of distality in the anterior circulation and overall, respectively. This was necessary as the PCA data (overall few at 2/834) was not specific to the exact segment involved. The length of the procedure was analyzed in increments of 10 minutes of additional time.

Following multivariate logistic regression of these predictors, only intravenous tPA use, location of vessel occlusion, and number of device passes remained significant (see **Tables 2**, **3**). Mechanical thrombectomy of a distally-situated thrombus had a

TABLE 1 | Clinical, radiographic, and procedural characteristics in STRATIS ($N = 841$).

Mean ± SD [N], Median (IQR), or % (n/N)	No SAH	SAH	*P*-value
Age (years)	68.1 ± 15.0 [797] 69.6 (59.3–79.7)	70.3 ± 14.2 [44] 68.0 (61.4–81.7)	0.342
Female	46% (366/797)	55% (24/44)	0.264
Comorbidities			
Hypertension	73% (581/797)	70% (31/44)	0.723
Diabetes mellitus	25% (202/797)	39% (17/44)	0.050
Coronary artery disease	27% (216/797)	30% (13/44)	0.723
Smoking			0.650
Current	21% (167/797)	25% (11/44)	
Former	26% (210/797)	18% (8/44)	
Never	43% (342/797)	48% (21/44)	
Unknown	10% (78/797)	9% (4/44)	
Preprocedural			
Baseline NIHSS	17.3 ± 5.5 [797] 17.0 (13.0–22.0)	17.3 ± 5.8 [44] 16.0 (13.0–22.5)	0.983
ASPECTS	8.2 ± 1.6 [699] 8.0 (8.0–9.0)	8.1 ± 1.9 [40] 9.0 (7.5–9.0)	0.849
IV t-PA Use	66% (528/796)	48% (21/44)	0.012
Location of vessel occlusion			0.018
ICA terminus	23% (181/790)	11% (5/44)	
MCA (M1)	56% (443/790)	52% (23/44)	
MCA (M2)	16% (125/790)	36% (16/44)	
MCA (M3)	0% (2/790)	0% (0/44)	
Vertebral artery	0% (1/790)	0% (0/44)	
Basilar artery	5% (36/790)	0% (0/44)	
PCA	0% (2/790)	0% (0/44)	
Thrombectomy			
General anesthesia	33% (234/703)	40% (16/40)	0.382
Number of passes			0.021
≤3	90% (714/790)	80% (35/44)	
>3	10% (76/790)	20% (9/44)	
Rescue therapy	11% (85/790)	16% (7/44)	0.289
Procedure time (minutes)	64.4 ± 36.9 [777] 56.0 (38.0–81.0)	78.4 ± 39.3 [44] 75.0 (51.5–93.5)	0.015

NIHSS, National Institutes of Health Stroke Scale; ASPECTS, Alberta Stroke Program Early CT Score; IV-tPA, intravenous tissue plasminogen activator; ICA, internal carotid artery; MCA, middle cerebral artery; PCA, posterior cerebral artery.

TABLE 2 | Predictors of post-thrombectomy SAH—multivariate logistic regression for anterior circulation vessel occlusions.

	Univariate			Multivariate		
	OR	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
Diabetes mellitus	1.85	(0.99, 3.47)	0.054	–	–	–
Location of vessel occlusion: proximal vs. distal*	2.81	(1.48, 5.34)	0.002	3.18	(1.63, 6.18)	<0.001
IV t-PA Use	0.46	(0.25, 0.85)	0.013	0.46	(0.25, 0.85)	0.014
Number of passes	1.27	(1.04, 1.55)	0.017	1.34	(1.09, 1.64)	0.005
Procedure Time (10 min)	1.08	(1.02, 1.16)	0.016	–	–	–

Variables no longer significant following multivariate logistic regression are denoted by "–". IV-tPA, intravenous tissue plasminogen activator; OR, odds ratio; CI, confidence interval.
**Only anterior circulation vessel occlusions were included comparing internal carotid artery terminus and middle cerebral artery (MCA) M1 (proximal) to MCA M2 and M3 (distal).*

greater odds of periprocedural SAH in the anterior circulation (OR = 3.18 [95% CI: 1.63–1.68], $p < 0.001$) and when vertebrobasilar occlusions were included (OR = 3.41 [95% CI: 1.75–6.63], $p < 0.001$).

Outcomes Following SAH

The mean NIHSS at discharge was not statistically different between the two groups but trended toward a higher value in patients with SAH (8.3 ± 8.7 vs. 5.3 ± 6.6, $p = 0.07$). This is

TABLE 3 | Predictors of post-thrombectomy SAH—multivariate logistic regression for anterior and posterior circulation vessel occlusions.

	Univariate			Multivariate		
	OR	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
Diabetes mellitus	1.85	(0.99, 3.47)	0.054	–	–	–
Location of vessel occlusion: proximal vs. distal*	2.97	(1.56, 5.66)	<0.001	3.41	(1.75, 6.63)	<0.001
IV t-PA Use	0.46	(0.25, 0.85)	0.013	0.48	(0.26, 0.89)	0.020
Number of passes	1.27	(1.04, 1.55)	0.017	1.34	(1.09, 1.64)	0.005
Procedure Time (10 min)	1.08	(1.02, 1.16)	0.016	–	–	–

Variables no longer significant following multivariate logistic regression are denoted by "-". IV-tPA, intravenous tissue plasminogen activator; OR, odds ratio; CI, confidence interval.
**Comparison of proximal (internal carotid artery terminus, middle cerebral artery (MCA) M1, vertebral artery, basilar artery) to distal (MCA M2 and M3) vessel occlusions.*

TABLE 4 | Procedural and functional outcomes in STRATIS.

Mean ± SD [N], Median (IQR), or % (n/N)	**No SAH**	**SAH**	***P*-value**
Final reperfusion (TICI 2b/3)	93% (732/788)	91% (40/44)	0.620
NIHSS at discharge	5.3 ± 6.6 [620] 3.0 (1.0–7.0)	8.3 ± 8.7 [31] 4.0 (2.0–17.0)	0.070
mRS at 90 days			0.018
0	21% (157/737)	15% (6/40)	
1	23% (168/737)	8% (3/40)	
2	14% (101/737)	10% (4/40)	
3	13% (96/737)	20% (8/40)	
4	10% (76/737)	8% (3/40)	
5	4% (32/737)	10% (4/40)	
6	15% (107/737)	30% (12/40)	
mRS 0-1 at 90 days	44% (325/737)	23% (9/40)	0.007
mRS 0-2 at 90 days	58% (426/737)	33% (13/40)	0.002
Adverse events (within 24 h)			
Neurological deterioration*	8% (55/708)	18% (7/38)	0.020
Parenchymal hemorrhage (HBC 1c + 2)**	3% (22/797)	7% (3/44)	0.123
Remote intraparenchymal hemorrhage (HBC 3a)	0.1% (1/797)	0.0% (0/44)	0.814
Intraventricular hemorrhage (HBC 3b)	0.0% (0/797)	2.3% (1/44)	<0.001

TICI, thrombolysis in cerebral infarction; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; HBC, Heidelberg Bleeding Classification.
**≥ 4 point worsening in NIHSS. **Equivalent to European Cooperative Acute Stroke Study (ECASS) III PH1 and PH2.*

despite both cohorts having nearly identical presenting NIHSS prior to mechanical thrombectomy. Having a periprocedural SAH was associated with a reduced rate of functional independence (mRS 0–2: 32.5 vs. 57.8%, $p = 0.002$) and greater fatality rate (mRS 6 30 vs. 14.5%) at 90 days (see **Table 4**). Of the 12 fatal cases of SAH, 4 were symptomatic post-operatively with neurological decline. No patients with symptomatic SAH or SAH associated with parenchymal hemorrhage achieved functional independence.

Systematic Review and Meta-Analysis

Search Results and Risk of Bias of Included Studies

The initial search strategy identified 701 citations following removal of duplicates with an additional 6 studies included from the reference lists of relevant systematic reviews (see **Figure 1**). Of these, the full-text of 128 studies were reviewed in detail. Twenty-one studies were excluded as the frequency of SAH could not be determined as it was not reported at all in 8 or pooled with other intracranial hemorrhages in 13. Only SAH inferred by visualization of contrast extravasation on angiography from vessel perforation was reported in 1 article leading to removal. An additional study was discarded as it only included thick SAH. Thrombectomy techniques other than stent retrieval or direct aspiration was used in 21 citations resulting in exclusion. Lastly, 13 studies analyzed duplicated datasets as a subset of an included RCT or the same registry as an included observational study. This resulted in 71 studies consisting of 6 RCTs and 65 observational studies equating to a pooled population of 10,186 patients. For one RCT, only data from the stent retriever arm was used as the control group was treated with the MERCI retriever device (23).

The study quality was generally high across all design types. Only one RCT was considered high risk for compliance bias secondary to a 22% crossover rate of patients assigned to the standard medical therapy whose families could not accept the randomization result (24) (see **Supplementary Table 1**). Of the

included cohort studies, most rated poorly on the NOS for comparability as thrombectomy success and safety were typically reported with unadjusted univariate analysis. Selection bias was also inherent to the design of before-and-after cohort studies further impacting their NOS score (see **Supplementary Table 2**). Similar to poor comparability as judged by the NOS many case series used descriptive statistics resulting in "not applicable" for assessment of statistical analysis with the JBI appraisal tool. A total of 4 case series were deemed lower quality due to incomplete description of participant and institutional demographics, which is relevant to the external validity of individual studies but likely imparts minimal effect on pooled statistics (see **Supplementary Table 3**).

Prevalence of SAH

The pooled prevalence of SAH following thrombectomy by stent retriever, direct aspiration, or a combination of these techniques was 6.26% [95% CI: 4.75–7.93%] (655 cases in 10,186 patients). The heterogeneity was high with a Cochrane Q statistic of 407.06, p-value < 0.0001, and I^2 of 88%. Following removal of one outlying study based on pre-determined criteria (25), the prevalence reduced to 5.85% [95% CI: 4.51–7.34%] with a small improvement in the I^2 to 85.2% ($p < 0.0001$) (see **Figure 2**). Subgroup analysis based on study design revealed a lower pooled prevalence amongst RCTs, 4.3% [95% CI: 2.75–6.11%], as compared to the cohort studies and case series, 6.55% [95% CI: 4.87–8.42%]. This difference in summary estimates approaches, but does not reach significance ($p = 0.07$). Heterogeneity was low in the RCT subgroup with an I^2 of 13.57% ($p = 0.6$) but remained substantial in the observational study subgroup with an I^2 of 89% ($p < 0.0001$).

Effect of Vessel Occlusion Location and Procedural Technique on SAH

A total of 5 studies reported data separated by internal carotid artery or vertebrobasilar involvement demonstrating similar rates of SAH following mechanical thrombectomy of the anterior circulation, 5.44%, compared to the posterior circulation, 5.88%, with an OR of 1.12 [0.60, 2.11] ($p = 0.82$, $I^2 = 0\%$) (see **Figure 3**). When location of vessel occlusion was characterized by distance from the Circle of Willis, distal occlusions resulted in more cases of SAH, 9.09%, than proximal occlusions, 3.02%. This difference was significant with a corresponding OR of 2.89 [1.14, 7.35] ($p = 0.82$, $I^2 = 0\%$) (see **Figure 4**). Pre-operative treatment with tPA in 10 studies had no significant effect on SAH occurrence compared to no tPA administration (8.03 vs. 8.03%, OR 0.90 [0.58, 1.38], $p = 1.00$, $I^2 = 0\%$) (see **Figure 5**). When direct aspiration was used as the first-line mechanical thrombectomy device, there was a lower rate of SAH, 5.47%, in contrast to using a stent retriever, 8.87%. However, this difference did not reach significance with an OR of 0.70 [0.40, 1.25] ($p = 0.67$, $I^2 = 0\%$) (see **Figure 6**). Reporting of the number of device passes was variable ranging from summary statistics (e.g., median) to categories (e.g., 3 or less vs. more than 3) preventing a meaningful quantitative analysis despite adequate number of studies.

DISCUSSION

SAH following mechanical thrombectomy is a known complication whose rate of occurrence has been variably reported. With the initial use of the MERCI retriever device, SAH occurred in as many as 14.1–16.4% of patients (23, 26). This was greater in frequency compared to intra-arterial thrombolysis (14.1 vs. 6.5%) (26), and later, stent retrievers (16.4 vs. 3.4%) (26). Koh et al. performed a systematic review investigating the safety and efficacy of thrombectomy using the Solitaire stent identifying 5 cases of SAH in 262 patients (1.9%) (27). A more recent pooled analysis of the Solitaire With the Intention For Thrombectomy (SWIFT) trial, SWIFT as PRIMary Endovascular Treatment (SWIFT PRIME) trial, and Study of Mechanical Thrombectomy Using Solitaire FR in Acute Ischemic Stroke (STAR) data found 2.3% of 389 patients had SAH detected on post-operative imaging (12). At the time of recruitment for these studies, mainly proximal vessel occlusions were targeted evidenced by just under 90% of the thrombectomies performed at the internal carotid terminus or first segment of the MCA. In STRATIS, 81.5% of the target anterior circulation occlusion locations were proximal resulting in a SAH prevalence of 5.23%. This is comparable to the pooled prevalence of 5.85% from the current meta-analysis. Interestingly, the combined prevalence across 6 RCTs was lower at 4.3%, particularly when compared to observational studies, perhaps due to improved operator experience or access to more modern devices. These results contrast those of Qureshi et al. who analyzed a nationwide database comparing acute ischemic stroke patients undergoing thrombectomy within or outside clinical trials finding no significant difference in the rate of SAH and intracerebral hemorrhage after adjusting for age, gender, and admission to a teaching hospital although the type of hemorrhages were combined (28). SAH resulted in neurological decline in 1.84% of STRATIS patients, which falls within the reported range (0%-7.4%) of symptomatic SAH from mechanical thrombectomy (1, 6–9).

The location of vessel occlusion, which influences the technical approach to mechanical thrombectomy, has yet to be shown as a predictor of post-procedural SAH. Weber et al. investigated 139 posterior circulation large vessel occlusions treated endovascularly (84.9% involving the basilar artery) finding this cohort consisted of more men of younger age, lower NIHSS at presentation, but comparable SAH prevalence (2.9 vs. 3.3%) when compared to anterior circulation occlusions (29). Similar results were observed in a cohort of 345 patients (50 involving the basilar artery) who underwent mechanical thrombectomy with SAH occurring in 14 vs. 12.9% in posterior and anterior circulation occlusions, respectively (30). The pooled analysis comparing anterior to posterior circulation thrombectomies identified only 3 additional smaller observational studies in the literature, not surprisingly, concluding no relationship to SAH. When the occlusion site was dichotomized as proximal or distal, there was a strong association to SAH following thrombectomy, with a higher frequency in patients with distally-located thrombus both in the STRATIS registry and our meta-analysis that included 6 observational studies (8, 31–35). Of these studies, only one

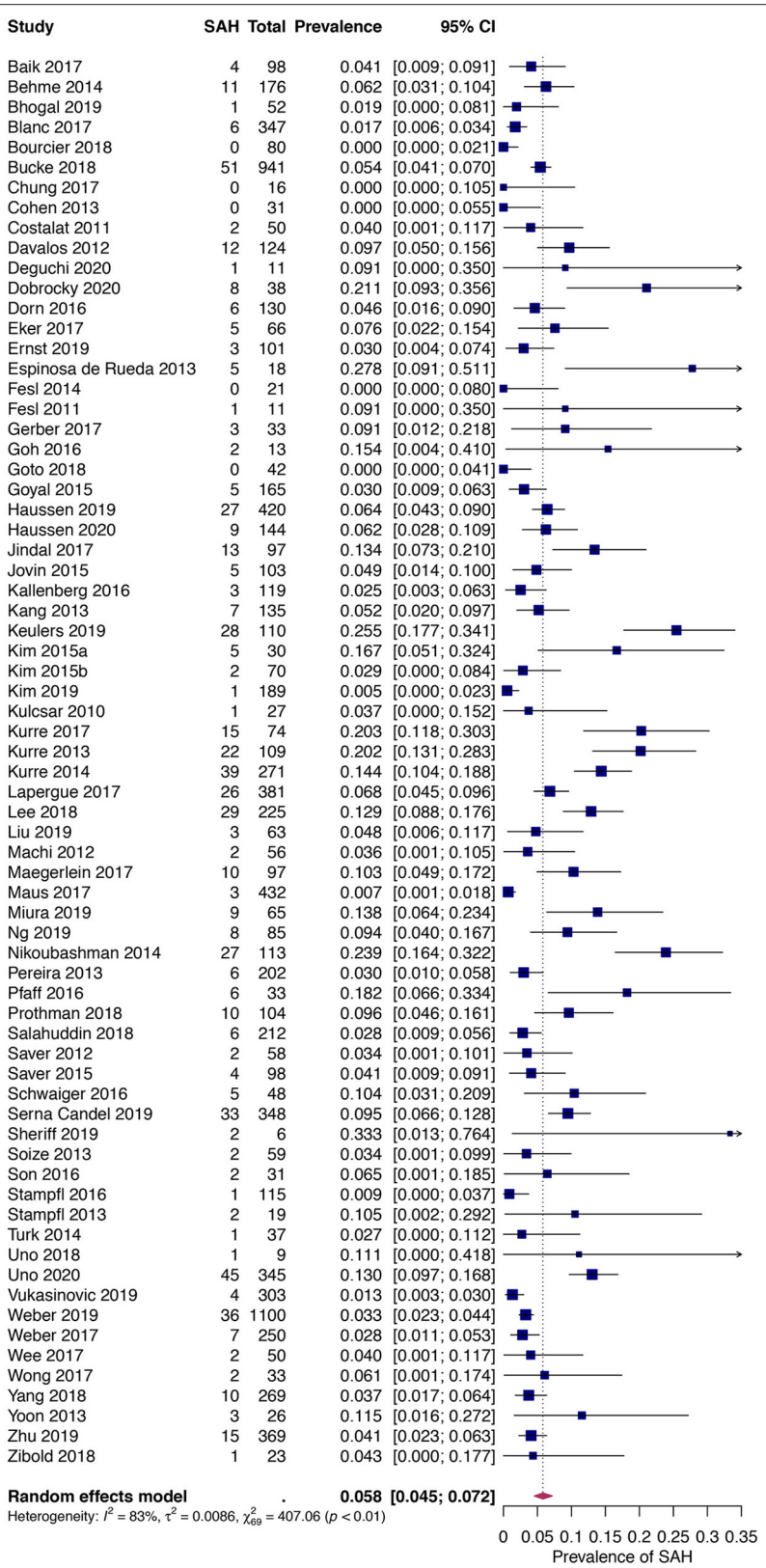


FIGURE 2 | Pooled prevalence of subarachnoid hemorrhage following mechanical thrombectomy.

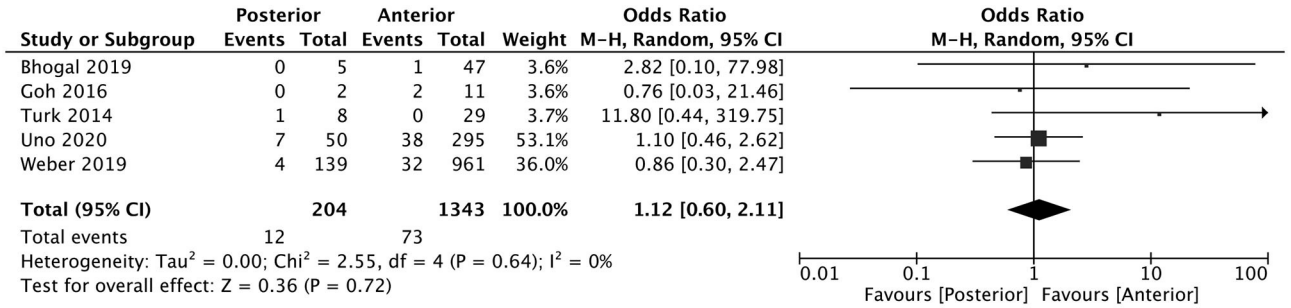


FIGURE 3 | Effect of anterior vs. posterior circulation vessel occlusion on subarachnoid hemorrhage following mechanical thrombectomy.

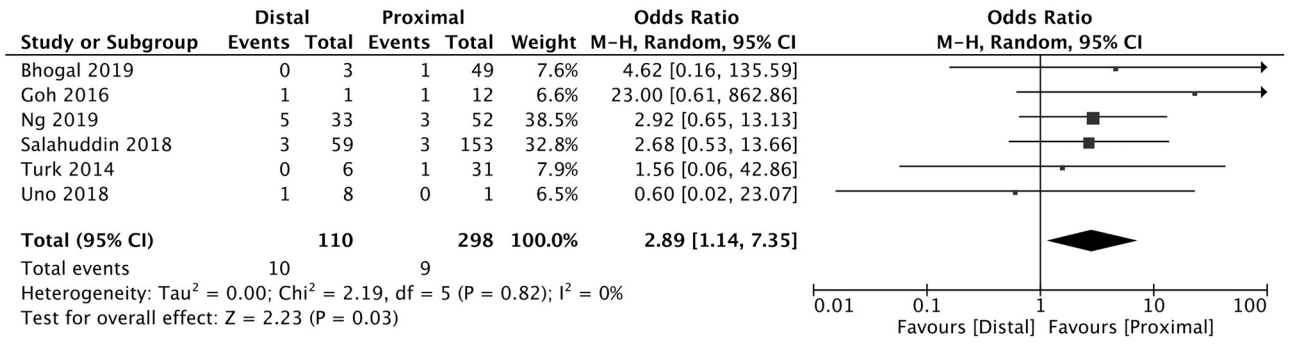


FIGURE 4 | Effect of proximal vs. distal vessel occlusion on subarachnoid hemorrhage following mechanical thrombectomy.

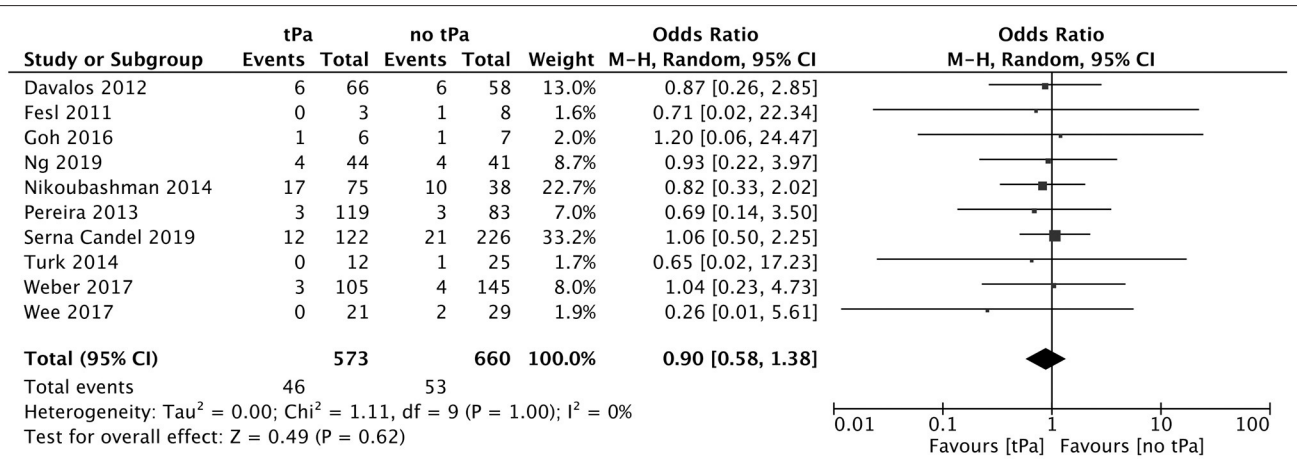


FIGURE 5 | Effect of IV tPA administration on subarachnoid hemorrhage following mechanical thrombectomy.

independently suggested distality to be a risk factor for SAH with more instances following thrombectomy of M2-situated clots (62.5 vs. 37.7%, $p = 0.26$) and when >2 cm of the stent was deployed within a M2 branch (100 vs. 30.2% $p = 0.002$) (8). The M2 segment of the MCA is on average 1 mm (25%) smaller than the parent vessel at its origin (36, 37). These smaller diameter vessels that closely-match the outer diameter of the smallest stent retrievers and distal access catheters coupled with the sharp curves the MCA takes as it courses over the insula and operculum predispose the vessel to endothelial injury as well as neighboring arterioles and venules to higher tensile forces and possible rupture (38). Distal positioning is also more challenging to achieve having to navigate more branching points and vessel tortuosity, often without roadmap guidance, contributing to a higher risk of vessel perforation and subsequent SAH.

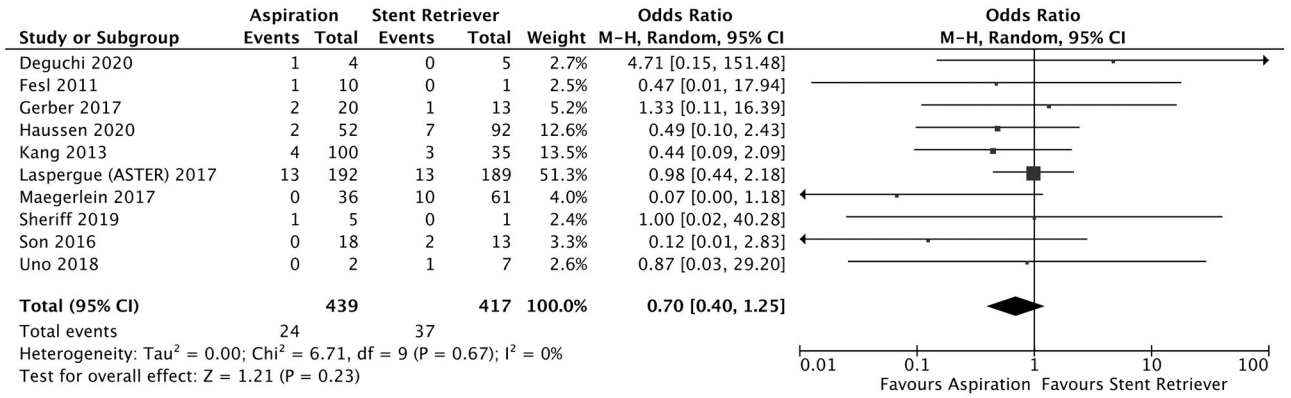


FIGURE 6 | Effect of direct aspiration vs. stent retriever use on subarachnoid hemorrhage following mechanical thrombectomy.

Greater number of passes of a thrombectomy device was found to significantly increase the prevalence of SAH in the current study. This correlates with previous studies that have included both stent retrieval and a direct aspiration first pass technique (ADAPT) in addition to other summary statistics such as the median or a differing cut-off for number of passes (7, 8, 39). The increased prevalence likely stems from compounding of the inherent risk of iatrogenic vessel injury from each revascularization attempt over repeated trials. Microperforation of the vessel wall or endothelial damage altering the blood brain barrier function may initially be minor but later exacerbated with recurrent passage of the device or subsequent reperfusion. The harm in performing multiple passes is further highlighted by a recent study showing a progressive decline in percentage of patients with good functional outcome (mRS 0–2) following mechanical thrombectomy as the number of device passes increased despite achieving a final result of good reperfusion (40).

Use of IV tPA as a bridging therapy to mechanical thrombectomy is the recommended treatment according to guidelines in the absence of contraindications however it is topic of controversy. Among the few published studies assessing its added utility, only Weber et al. reported rates of SAH finding they were similar between patients receiving IV tPA and those who did not (2.9 vs. 2.8%) (41). This absence of effect persisted when pooled with an additional 9 observational studies in our meta-analysis but contrasts the STRATIS observation of higher rates of SAH in the cohort that was not treated with IV tPA [7.9% (23/291) vs. 3.8% (21/549)] (7, 8, 32, 34, 42–46). It is difficult to rationalize this association between IV tPA administration and SAH to be causal given tPA's thrombolytic property. In fact, one of its theoretical advantages is to soften the thrombus to facilitate removal during mechanical thrombectomy promoting fewer required device passes however IV tPA use remained significant after adjustment for number of passes in the multivariate logistic regression. It could be mediated by unmeasured between-group differences in contraindications to receiving IV tPA including use of oral anticoagulants, inherent coagulopathy, or presentation outside the safe therapeutic window for intravenous thrombolysis. Of note, the mean stroke onset-to-arrival time at the recruiting center was no different when post-procedural SAH was present or absent (150.9 ± 94.9 min ($n = 40$) vs. 148.5 ± 102.6 min ($n = 728$), $p = 0.89$).

The type of anesthesia administered to patients undergoing mechanical thrombectomy has been of interest as it impacts airway protection, hemodynamic control, procedural timing, as well as patient comfort and cooperation. General anesthesia has the benefit of patient immobilization, particularly important in left-hemispheric stroke with receptive aphasia, reducing patient movements which otherwise can impair visualization and may precipitate accidental vessel injury. Randomized controlled trials have focused on determining if a difference in functional outcome exists between modalities with a meta-analysis by Schonenberger et al. including 368 patients demonstrating less disability amongst patients undergoing general anesthesia (mRS > 2: 50.8 vs. 64.9%, $p = 0.003$) with a lower frequency of intracerebral or subarachnoid hemorrhage (1 vs. 5, *not tested for significance due to low numbers*) (47). In a larger cohort of 4429 patients prospectively enrolled in the Italian Registry of Endovascular Treatment in Acute Stroke, no significant difference in SAH was seen between the general anesthesia and conscious sedation groups (3.8 vs. 2%, OR 2.230 [95% CI: 0.901–4.932]) or when compared to the local anesthesia group (3.8 vs. 2.6%, OR 1.158 [95% CI: 0.548–2.445]) after adjusting for age, sex, comorbidities, presenting NIHSS and ASPECTS, IV tPA, procedural time metrics, and thrombectomy techniques (48). This corresponds with the results of the current study and do not support a protective role of anesthesia modality against procedural SAH.

Contrary to the existing literature, SAH following thrombectomy in the STRATIS cohort had a significant deleterious effect on functional outcome. Fewer patients with SAH achieved functional independence, particularly if there was attributable neurological deterioration. This is not explained by a higher-than-normal rate of good clinical outcome in patients without SAH at 58%, which is comparable to previously published studies (2, 4, 5, 12). Raychev et al. found no significant difference in patients with and without post-thrombectomy SAH who achieved a mRS 0–2 at 90 days, however the proportion

was lower in the SAH group at 44.4 vs. 55.2% (12). Similarly, a relatively reduced frequency of functional independence in patients with SAH (33.3 vs. 43.5%) was observed in the study by Yoon et al. that also did not reach significance (6). These studies had few cases of SAH, 9 and 12, respectively, which may have contributed to an underpowered analysis. The relationship between post-thrombectomy SAH, neurological decline, and resulting functional outcome is likely more complex than the presence or absence of SAH but rather dependent on factors such as thickness and distribution similar to aneurysmal SAH. The co-occurence of SAH with intraparenchymal hemorrhage has also been shown to reduce the incidence of post-thrombectomy functional independence however this data is mainly derived from series where older thrombectomy devices were used as first-line (26, 49). We found that 7% of STRATIS SAH patients also had HBC 2 (PH2) hemorrhages and all experienced poor outcomes. For comparison, Enomoto et al. reported that 18.9% (14 of 74) of patients with SAH following endovascular thrombectomy also had intraparenchymal hemorrhage but unfortunately outcome data was not included (50).

Several limitations exist in this STRATIS cohort study. Independent evaluation of radiographic features and clinical outcomes by a core lab and clinical events committee aided standardization across recruitment sites. However, variability inherent to its observational design likely remained across patient treatments including pre-interventional care, thrombectomy technique (outside the use of a Medtronic-marketed stent retriever) and post-procedural management that may be a source of confounding. Secondly, only stent retrievers were used as the primary treatment device from a single manufacturer. It is uncertain whether our results extend to stroke patients treated with ADAPT particularly with the knowledge that distal aspiration catheters produce a different pattern of endothelial cell injury *in vitro* compared to stent retrievers (38). New stents designed specifically for thrombectomy, including an updated version of the Solitaire, have also since been introduced. Lastly, only patients undergoing mechanical thrombectomy within 8 h of stroke onset were included however the therapeutic window has since been extended to 24 h (51). Future analysis of cohorts eligible for mechanical thrombectomy beyond the 8 h timeframe including direct aspiration techniques are required to further characterize their SAH risk. Our meta-analysis results should also be interpreted knowing much of the data was pooled from observational studies. Without access to individual-level data, the possible influence of selection bias or confounding arising from choice of stroke patients for mechanical thrombectomy or the procedural technique could not be accounted for. An additional limitation lies in the non-standardized definition of subarachnoid hemorrhage across the included studies with few specifying criteria, such as Hounsfield units range on CT or use of dual energy CT to improve differentiation of contrast extravasation from hemorrhage (52). As both entities appear hyperdense on CT within the subarachnoid space, misclassification could occur and partly explain the high degree of heterogeneity between studies by contributing to variable prevalence rates. Dual energy CT was also not uniformly available for the STRATIS registry which may have influenced the observed SAH rate and the resulting outcomes if those associated with contrast vs. hemorrhage truly differ.

CONCLUSIONS

SAH following mechanical thrombectomy occurs in a small, but not insignificant, proportion of acute ischemic stroke patients. It is associated with distally-located vessel occlusions and a higher number of thrombectomy device passes required to achieve reperfusion. The influence of bridging IV tPA is uncertain with a reduced frequency of SAH when administered. Patients with post-thrombectomy SAH have poorer clinical outcomes particularly with concurrent parenchymal hemorrhage or neurological decline. Improved reporting of SAH is required in future randomized control trials.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

VP and HL contributed to the conception and design of the overall study. The STRATIS Registry was conceived, collected, and organized by NM-K, OZ, MF, and DL. The systematic review and meta-analysis was designed and conducted by VP, HL, and AQ. Statistical analysis was performed by HL. VP, HL, and AQ contributed to data analysis and interpretation with HL completing the first draft of this manuscript with further additions by AQ and VP. All authors contributed to subsequent revisions and approve of the final version.

FUNDING

Medtronic assisted with the analysis of the STRATIS registry. The concept, systematic review and meta-analysis, and manuscript preparation were performed independent of the industry sponsor.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.663058/full#supplementary-material

REFERENCES

1. Berkhemer OA, Fransen PSS, Beumer D, Van Den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med.* (2015) 372:11–20. doi: 10.1056/NEJMoa1411587
2. Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. *N Engl J Med.* (2015) 372:1019–30. doi: 10.1056/NEJMoa1414905
3. Jovin TG, Chamorro A, Cobo E, de Miquel MA, Molina CA, Rovira A, et al. Thrombectomy within 8 hours after symptom onset in ischemic stroke. *N Engl J Med.* (2015) 372:2296–306. doi: 10.1056/NEJMoa1503780
4. Campbell BCV, Mitchell PJ, Kleinig TJ, Dewey HM, Churilov L, Yassi N, et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N Engl J Med.* (2015) 372:1009–18. doi: 10.1056/NEJMoa1414792
5. Saver JL, Goyal M, Bonafe A, Diener H-C, Levy EI, Pereira VM, et al. Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. *N Engl J Med.* (2015) 372:2285–95. doi: 10.1056/NEJMoa1415061
6. Yoon W, Jung MY, Jung SH, Park MS, Kim JT, Kang HK. Subarachnoid hemorrhage in a multimodal approach heavily weighted toward mechanical thrombectomy with solitaire stent in acute stroke. *Stroke.* (2013) 44:414–9. doi: 10.1161/STROKEAHA.112.675546
7. Nikoubashman O, Reich A, Pjontek R, Jungbluth M, Wiesmann M. Postinterventional subarachnoid haemorrhage after endovascular stroke treatment with stent retrievers. *Neuroradiology.* (2014) 56:1087–96. doi: 10.1007/s00234-014-1424-1
8. Ng PP, Larson TC, Nichols CW, Murray MM, Salzman KL, Smith RH. Intraprocedural predictors of post-stent retriever thrombectomy subarachnoid hemorrhage in middle cerebral artery stroke. *J Neurointervent Surg.* (2019) 11:127–32. doi: 10.1136/neurintsurg-2018-013873
9. Machi P, Costalat V, Lobotesis K, Maldonado IL, Vendrell JF, Riquelme C, et al. Solitaire FR thrombectomy system: immediate results in 56 consecutive acute ischemic stroke patients. *J Neurointervent Surg.* (2012) 4:62–6. doi: 10.1136/jnis.2010.004051
10. Kurre W, Aguilar-Perez M, Schmid E, Sperber W, Bazner H, Henkes H. Clinical experience with the pREset stent retriever for the treatment of acute ischemic stroke–a review of 271 consecutive cases. *Neuroradiology.* (2014) 56:397–403. doi: 10.1007/s00234-014-1346-y
11. Schwaiger BJ, Kober F, Gersing AS, Kleine JF, Wunderlich S, Zimmer C, et al. The pREset stent retriever for endovascular treatment of stroke caused by mca occlusion: safety and clinical outcome. *Clin Neuroradiol.* (2016) 26:47–55. doi: 10.1007/s00062-014-0329-z
12. Raychev R, Saver JL, Jahan R, Nogueira RG, Goyal M, Pereira VM, et al. The impact of general anesthesia, baseline ASPECTS, time to treatment, and IV tPA on intracranial hemorrhage after neurothrombectomy: pooled analysis of the SWIFT PRIME. SWIFT, and STAR trials. *J Neurointervent Surg.* (2020) 12:2–6. doi: 10.1136/neurintsurg-2019-014898
13. Haussen DC, Lima A, Nogueira RG. The Trevo XP 3x20 mm retriever ('Baby Trevo') for the treatment of distal intracranial occlusions. *J Neurointervent Surg.* (2016) 8:295–9. doi: 10.1136/neurintsurg-2014-011613
14. Gory B, Lapergue B, Blanc R, Labreuche J, Machaa MB, Duhamel A, et al. Contact aspiration versus stent retriever in patients with acute ischemic stroke with M2 occlusion in the aster randomized trial (contact aspiration versus stent retriever for successful revascularization). *Stroke.* (2018) 49:461–64. doi: 10.1161/STROKEAHA.117.019598
15. Munich SA, Hall SL, Cress MC, Rangel-Castilla L, Snyder KV, Hopkins LN, et al. To treat or not to treat M2 occlusions? the question (and answer) from a single institution. *Clin Neurosurg.* (2016) 79:428–36. doi: 10.1227/NEU.0000000000001182
16. Grossberg JA, Rebello LC, Haussen DC, Bouslama M, Bowen M, Barreira CM, et al. Beyond large vessel occlusion strokes: distal occlusion thrombectomy. *Stroke.* (2018) 49:1662–8. doi: 10.1161/STROKEAHA.118.020567
17. Mueller-Kronast NH, Zaidat OO, Froehler MT, Jahan R, Aziz-Sultan MA, Klucznik RP, et al. Systematic evaluation of patients treated with neurothrombectomy devices for acute ischemic stroke: primary results of the STRATIS registry. *Stroke.* (2017) 48:2760–8. doi: 10.1161/STROKEAHA.117.016456
18. von Kummer R, Broderick JP, Campbell BCV, Demchuk A, Goyal M, Hill MD, et al. The heidelberg bleeding classification: classification of bleeding events after ischemic stroke and reperfusion therapy. *Stroke.* (2015) 46:2981–6. doi: 10.1161/STROKEAHA.115.010049
19. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ.* (2019) 366:l4898. doi: 10.1136/bmj.l4898
20. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrando- Mised Studies in Meta-Analyses.* Available online at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (accessed November 8, 2020).
21. R Core Team (2020). *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna. Available online at: http://www.R-project.org/
22. Viechtbauer W. Conducting meta-analyses in R with the meta for package. *J Statist Softw.* (2010) 36:1–48.
23. Saver JL, Jahan R, Levy EI, Jovin TG, Baxter B, Nogueira RG, et al. Solitaire flow restoration device versus the Merci Retriever in patients with acute ischaemic stroke (SWIFT): a randomised, parallel-group, non-inferiority trial. *Lancet.* (2012) 380:1241–9. doi: 10.1016/S0140-6736(12)61384-1
24. Liu X, Dai Q, Ye R, Zi W, Liu Y, Wang H, et al. Endovascular treatment versus standard medical treatment for vertebrobasilar artery occlusion (BEST): an open-label, randomised controlled trial. *Lancet Neurol.* (2020) 19:115–22. doi: 10.1016/S1474-4422(19)30395-3
25. Renu A, Laredo C, Lopez-Rueda A, Llull L, Tudela R, San-Roman L, et al. Vessel wall enhancement and blood-cerebrospinal fluid barrier disruption after mechanical thrombectomy in acute ischemic stroke. *Stroke.* (2017) 48:651–7. doi: 10.1161/STROKEAHA.116.015648
26. Shi Z-S, Liebeskind DS, Loh Y, Saver JL, Starkman S, Vespa PM, et al. Predictors of subarachnoid hemorrhage in acute ischemic stroke with endovascular therapy. *Stroke.* (2010) 41:2775–81. doi: 10.1161/STROKEAHA.110.587063
27. Koh JS, Lee SJ, Ryu C-W, Kim HS. Safety and efficacy of mechanical thrombectomy with solitaire stent retrieval for acute ischemic stroke: a systematic review. *Neurointervention.* (2012) 7:1. doi: 10.5469/neuroint.2012.7.1.1
28. Qureshi AI, Singh B, Huang W, Du Z, Lobanova I, Liaqat J, et al. Mechanical thrombectomy in acute ischemic stroke patients performed within and outside clinical trials in the United States. *Neurosurgery.* (2020) 86:E2–8. doi: 10.1093/neuros/nyz359
29. Weber R, Minnerup J, Nordmeyer H, Eyding J, Krogias C, Hadisurya J, et al. Thrombectomy in posterior circulation stroke: differences in procedures and outcome compared to anterior circulation stroke in the prospective multicentre REVASK registry. *Europ J Neurol.* (2019) 26:299–305. doi: 10.1111/ene.13809
30. Uno J, Kameda K, Otsuji R, Ren N, Nagaoka S, Maeda K, et al. Mechanical thrombectomy for basilar artery occlusion compared with anterior circulation stroke. *World Neurosurg.* (2020) 134:e469–475. doi: 10.1016/j.wneu.2019.10.097
31. Bhogal P, AlMatter M, Hellstern V, Perez MA, Ganslandt O, Bazner H, et al. Mechanical thrombectomy for recurrent large vessel occlusion. *J Clin Neurosci.* (2019) 66:107–12. doi: 10.1016/j.jocn.2019.05.010
32. Goh DH, Jin SC, Jeong HW, Ha SY. Mechanical solitaire thrombectomy with low-dose booster tirofiban injection. *Neurointervention.* (2016) 11:114–9. doi: 10.5469/neuroint.2016.11.2.114
33. Salahuddin H, Ramaiah G, Slawski DE, Shawver J, Buehler M, Zaidi SF, et al. Mechanical thrombectomy of M1 and M2 middle cerebral artery occlusions. *J Neurointervent Surg.* (2018) 10:330–34. doi: 10.1136/neurintsurg-2017-013159
34. Turk AS, Spiotta A, Frei D, Mocco J, Baxter B, Fiorella D, et al. Initial clinical experience with the ADAPT technique: a direct aspiration first pass technique for stroke thrombectomy. *J Neurointervent Surg.* (2014) 6:231–7. doi: 10.1136/neurintsurg-2013-010713
35. Uno J, Kameda K, Otsuji R, Ren N, Nagaoka S, Kazushi M, et al. Mechanical thrombectomy for acute anterior cerebral artery occlusion. *World Neurosurg.* (2018) 120:e957–61. doi: 10.1016/j.wneu.2018.08.196
36. Gibo H, Christopher C. Carver, Rhoton AL, Lenkey C, Mitchell RJ. Microsurgical anatomy of the middle cerebral artery. *J Neurosurg.* (1981) 54:151–69. doi: 10.3171/jns.1981.54.2.0151

37. Rai AT, Hogg JP, Cline B, Hobbs G. Cerebrovascular geometry in the anterior circulation: an analysis of diameter, length and the vessel taper. *J NeuroIntervent Surg.* (2013) 5:371–5. doi: 10.1136/neurintsurg-2012-010314
38. Teng D, Pannell JS, Rennert RC, Li J, Li Y-S, Wong VW, et al. Endothelial trauma from mechanical thrombectomy in acute stroke: *in vitro* live-cell platform with animal validation. *Stroke.* (2015) 46:1099–106. doi: 10.1161/STROKEAHA.114.007494
39. Lapergue B, Blanc R, Gory B, Labreuche J, Duhamel A, Marnat G, et al. Effect of endovascular contact aspiration vs stent retriever on revascularization in patients with acute ischemic stroke and large vessel occlusion: the ASTER randomized clinical trial. *JAMA.* (2017) 318:443–52. doi: 10.1001/jama.2017.9644
40. García-Tornel Á, Requena M, Rubiera M, Muchada M, Pagola J, Rodriguez-Luna D, et al. When to stop: detrimental effect of device passes in acute ischemic stroke secondary to large vessel occlusion. *Stroke.* (2019) 50:1781–8. doi: 10.1161/STROKEAHA.119.025088
41. Weber R, Nordmeyer H, Hadisurya J, Heddier M, Stauder M, Stracke P, et al. Comparison of outcome and interventional complication rate in patients with acute stroke treated with mechanical thrombectomy with and without bridging thrombolysis. *J Neurointervent Surg.* (2017) 9:229–33. doi: 10.1136/neurintsurg-2015-012236
42. Davalos A, Pereira VM, Chapot R, Bonafe A, Andersson T, Gralla J. Retrospective multicenter study of solitaire FR for revascularization in the treatment of acute ischemic stroke. *Stroke.* (2012) 43:2699–705. doi: 10.1161/STROKEAHA.112.663328
43. Fesl G, Wiesmann M, Patzig M, Holtmannspoetter M, Pfefferkorn T, Dichgans M, et al. Endovascular mechanical recanalisation of acute carotid-T occlusions: a single-center retrospective analysis. *Cardiovasc Intervent Radiol.* (2011) 34:280–6. doi: 10.1007/s00270-010-9980-1
44. Pereira VM, Gralla J, Davalos A, Bonafe A, Castano C, Chapot R, et al. Prospective, multicenter, single-arm study of mechanical thrombectomy using solitaire flow restoration in acute ischemic stroke. *Stroke.* (2013) 44:2802–7. doi: 10.1161/STROKEAHA.113.001232
45. Serna Candel C, Aguilar Perez M, Hellstern V, AlMatter M, Bazner H, Henkes H. Recanalization of emergent large intracranial vessel occlusion through intravenous thrombolysis: frequency, clinical outcome, and reperfusion pattern. *Cerebrovasc Dis.* (2019) 48:115–23. doi: 10.1159/000503850
46. Wee CK, McAuliffe W, Phatouros CC, Phillips TJ, Blacker D, Singh TP, et al. Outcomes of endovascular thrombectomy with and without thrombolysis for acute large artery ischaemic stroke at a tertiary stroke centre. *Cerebrovasc Dis Extra.* (2017) 7:95–102. doi: 10.1159/000470855
47. Schönenberger S, Hendén PL, Simonsen CZ, Uhlmann L, Klose C, Pfaff JAR, et al. Association of general anesthesia vs procedural sedation with functional outcome among patients with acute ischemic stroke undergoing thrombectomy: a systematic review and meta-analysis. *JAMA.* (2019) 322:1283. doi: 10.1001/jama.2019.11455
48. Cappellari M, Pracucci G, Forlivesi S, Saia V, Nappini S, Nencini P, et al. General anesthesia versus conscious sedation and local anesthesia during thrombectomy for acute ischemic stroke. *Stroke.* (2020) 51:2036–44. doi: 10.1161/STROKEAHA.120.032094
49. Qureshi AI, Saleem MA, Aytac E. Postprocedure subarachnoid hemorrhage after endovascular treatment for acute ischemic stroke: subarachnoid hemorrhage after endovascular ischemic stroke treatment. *J Neuroimaging.* (2017) 27:493–8. doi: 10.1111/jon.12430
50. Enomoto M, Shigeta K, Ota T, Amano T, Ueda M, Matsumaru Y, et al. Predictors of intracranial hemorrhage in acute ischemic stroke after endovascular thrombectomy. *Interv Neuroradiol.* (2020) 26:368–75. doi: 10.1177/1591019920926335
51. Nogueira RG, Jadhav AP, Haussen DC, Bonafe A, Budzik RF, Bhuva P, et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. *N Engl J Med.* (2018) 378:11–21. doi: 10.1056/NEJMoa1706442
52. Phan CM, Yoo AJ, Hirsch JA, Nogueira RG, Gupta R. Differentiation of hemorrhage from iodinated contrast in different intracranial compartments using dual-energy head CT. *AJNR Am J Neuroradiol.* (2012) 33:1088–94. doi: 10.3174/ajnr.A2909

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer KK declared a past co-authorship with one of the authors DL to the handling Editor.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership