



On the dark side of therapies with immunoglobulin concentrates: the adverse events

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Therapy by human immunoglobulin G (IgG) concentrates is a success story ongoing for decades with an ever increasing demand for this plasma product. The success of IgG concentrates on a clinical level is documented by the slowly increasing number of registered indication and the more rapid increase of the off-label uses, a topic dealt with in another contribution to this special issue of *Frontiers in Immunology*. A part of the success is the adverse event (AE) profile of IgG concentrates which is, even at life-long need for therapy, excellent. Transmission of pathogens in the last decade could be entirely controlled through the antecedent introduction by authorities of a regulatory network and installing quality standards by the plasma fractionation industry. The cornerstone of the regulatory network is current good manufacturing practice. Non-infectious AEs occur rarely and mainly are mild to moderate. However, in recent times, the increase in frequency of hemolytic and thrombotic AEs raised worrying questions on the possible background for these AEs. Below, we review elements of non-infectious AEs, and particularly focus on hemolysis and thrombosis. We discuss how the introduction of plasma fractionation by ion-exchange chromatography and polishing by immunoaffinity chromatographic steps might alter repertoire of specificities and influence AE profiles and efficacy of IgG concentrates.

Keywords: adverse events, hemolysis, thrombosis, complement, cytokines, SCIG, IVIG

INTRODUCTION – THE TINGE OF THE DARK SIDE OF THERAPIES WITH IMMUNOGLOBULIN CONCENTRATES

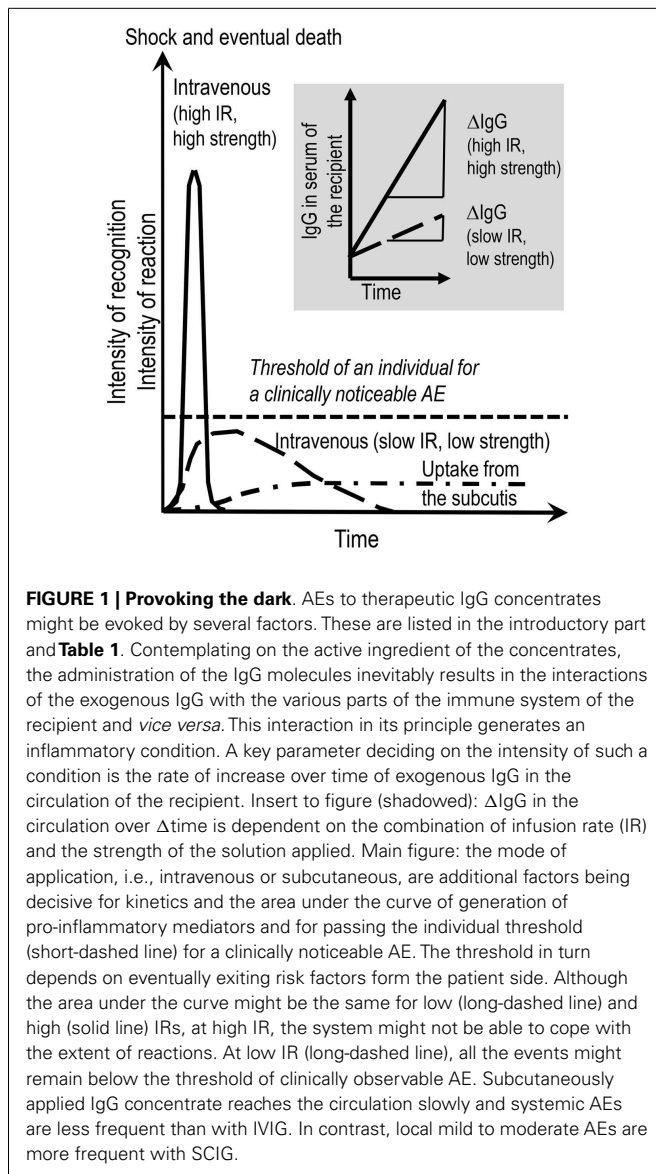
Since the initial clinical use of immunoglobulin G (IgG) concentrates of human origin, transmission of pathogens and non-infectious adverse events (AEs) were reported (1–7). Before the mid 90s, transmission of pathogens depended on the pool size and the fractionation methods used, particularly the polishing steps of an IgG concentrate (8). Mode of fractionation,

i.e., cold-ethanol or ion-exchange chromatography, contaminants, route of application, i.e., intra muscular (IMIG), intravenous (IVIG), or subcutaneous (SCIG), the rate of increase of the exogenous IgG in the circulation of the recipient over time and, last but not least an eventually existing risk factor from patients' side (**Figure 1**) as well as incorrect handling of the concentrate are factors having a role in inducing non-infectious AEs related to administration of IgG concentrates (**Table 1**). IgG concentrates represent a defined part of the adaptive immune system, are isolated from pooled human plasma of at least 1000 donors, which contribute to the repertoire diversity in the final product. Therapies with IgG concentrates manufactured according to regulators requirements are acknowledged to be safe in general. This does not exclude the occurrence of AEs which in their majority are rare and clinically mild to moderate. Below, we like to give a few insights into various aspects and possible mechanisms of AEs.

PATHOGEN SAFETY OF IgG CONCENTRATES – HOW TO EXCLUDE THE MENACES FROM A DARK AND FRIGHTENING ENVIRONMENT

Manufacturing of modern IgG concentrates has to occur in a regulatory framework and the quality standards implemented by the plasma fractionating industry (**Figure 2**). The cornerstone of the regulatory framework is current good manufacturing practice (cGMP). A pillar of pathogen safety is the validation of

Abbreviations: ACA, anti-complementary activity=unwanted spontaneous complement-activating capacity of an Ig concentrate; AE, adverse event; BCR, B cell receptor; BT, body temperature; C3a/C3a[desArg], anaphylatoxin C3a and its arginine deprived form which were detected together; CH50, total complement hemolytic activity 50%; cGMP, current good manufacturing practice; CVID, common variable immunodeficiency; DAT, direct antiglobulin test; DVT, deep venous thrombosis; HA, hemolytic anemia; Hb, hemoglobin; HR, heart rate; IC, immune complex; IgA, immunoglobulin A; IgA1, subclass 1 of IgA; IgA2, subclass 2 of IgA; IgAD, IgA deficiency; IgG, immunoglobulin G; IgM, immunoglobulin M; IL-1ra, interleukin 1 receptor antagonist; IL-6, interleukin 6; IL-8, interleukin 8; IMIG, immunoglobulin G concentrate for intramuscular application; IR, infusion rate; IVIG, immunoglobulin G concentrate for intravenous application; IEP, isoelectric point; ITP, immune thrombocytopenic purpura; LDH, lactate dehydrogenase; MERS, middle east respiratory syndrome; MW, molecular weight; NAb, natural antibody or autoantibody; PKA, prekallikrein activator; PE, pulmonary embolism; RBC, red blood cell, erythrocyte; SARS, severe acute respiratory syndrome; SCIG, immunoglobulin concentrate for subcutaneous application; sIgAD, selective IgAD; TCR, T cell receptor; TNF α , tumor necrosis factor alpha; V-region, variable region of antibodies, TCR and BCR; vCJD, variant Creutzfeldt-Jakob disease.



virus inactivation and virus elimination methods by validating an already performed step of the fractionation process or by introduction of dedicated steps (**Figure 3**). A hallmark of virus elimination introduced in the late 90s in Berne by the team of Christoph Kempf is the large-scale virus filtration technique (formerly also termed “nanofiltration”) (8). Meanwhile, virus filtration became a versatile tool to eliminate a variety of pathogens, the suspected agent of variant Creutzfeldt-Jakob disease included. Thanks to the tightly implemented regulatory framework, pathogen safety of plasma products is at a level never reached before. This is well supported by the fact of reports missing in the last decade of transmission by IgG concentrates of emerging viruses (SARS coronavirus, West Nile Virus, MERS coronavirus, and others), zoonotic pathogens, or the agent of variant Creutzfeldt-Jakob disease (vCJD). Furthermore, the development of specific mass screening techniques might help to eradicate in any blood product the transmission of vCJD in the future (9).

HOW THE SHADOW MIGHT GROW – SOME BASIC INSIGHTS INTO POSSIBLE MECHANISMS OF NON-INFECTIOUS ADVERSE EVENTS

The human immune system is in charge of controlling invading organisms and mediates homeostasis. The immunoglobulin pools in mammals (IgM, IgG, and IgA) to its smaller part provide defense and to the larger part homeostasis. Human IgG has a role in both. Efficient host defense is supported by “immune antibodies.” These have undergone somatic hypermutations and have in their vast majority narrow specificities and high affinities. Antibodies, generated in absence of external stimuli are termed “natural antibodies” (NAb). They occasionally recognize self structures. In general, the V(D)J genes of NAb are in germ-line configuration or have undergone a few somatic hypermutations only. Furthermore, they have broad specificity, are of low affinity (with exceptions) and high avidity (10). These NAb can participate in primary host defense, i.e., at a time point when an immunologic reaction has not provided the specific antibodies, react, e.g., with repetitive structures which can be found on bacteria or viruses (11). The self-reactive NAb, which we like to term physiologic autoantibodies, comprises various populations of antibodies such as (i) those able to interact through their complementary variable regions (V-regions) with the V-regions of circulating and membrane-bound (BCR) immunoglobulins and the T cell receptor (TCR) β -chain variable region, providing a peripheral immune network (V-connected network) (10); (ii) the NAb reacting in a non-idiotypic manner with the hinge region of immunoglobulins (12, 13); (iii) populations of NAb showing a wide variety of specificities toward growth factors, cytokines, or anaphylatoxin (10); (iii) and the population reacting with the soluble or membrane-bound forms of cell surface molecules having immunological importance, the last described being the Fc receptors CD16 and CD32 (14). Antibodies reacting with docking structures for viruses or bacteria can have additional first-line defense potential (15). These populations of NAb were described having a peripheral immune network homeostatic and anti-inflammatory function (16, 17). Although the primordial humoral proteins comprising the complement and lectin-like proteins in the plasma play a definite role, another population of self-reactive NAb reacting with, e.g., epitopes conserved over the evolution apparently has tissue homeostatic function and might support the efficient removal of roughly 10^{12} altered/senescent cells of the body per day (for references see below). The signal for research on NAb in IVIG was the description of IgG autoantibody-mediated immune thrombocytopenia (ITP) being corrected by infusion of a polyclonal, polyspecific IgG concentrate (18, 19). This research has expanded ever since.

The populations of immune antibodies and NAb in IgG concentrates upon infusion/injection inevitably react with occasional pathogens, toxins, or superantigens and concomitantly infusion/injection also results in recognition of a wide array of tissue antigens and V-regions of the recipient’s immune system. Reactions with tissue antigens and V-regions are conveyed by the self-reactive antibodies of the many donors in the IgG concentrate. *Vice versa*, the recipient’s immune system reacts with the infused IgG. A bewildering wide range of possible reactions can occur which primarily are dependent on the immune status of the recipient at the time of therapy and to a smaller part on the IgG concentrate(s).

Table 1 | The tinge of the dark.

Symptoms and signs	Frequency	IRR or total dose	PRR	System	Class, severity, and duration	Part of the product likely being involved in AEs
Fatigue	Common (SCIG as well)	No		Constitutional or systemic (generalized)	Immediate, mild, transient	
Malaise	Common	No	Yes	Constitutional or systemic (generalized)	Immediate, mild, transient	
Fever	Common	Yes	Yes	Constitutional or systemic (generalized)	Immediate, mild, transient	
Flushing	Common	Yes	Yes	Constitutional or systemic (generalized)	Immediate, mild, transient	
Chills	Common	Yes	Yes	Constitutional or systemic (generalized)	Immediate, mild, transient	
Anorexia	Common	No		Constitutional or systemic (generalized)	Immediate, mild, transient	
Myalgia	Common	Yes	Yes	Constitutional or systemic (generalized)	Immediate, mild, transient	
Arthralgia	Common	Yes	Yes	Constitutional or systemic (generalized)	Immediate, mild, transient	
Joint swelling	Common	Yes	Yes	Constitutional or systemic (generalized)	Mild, transient	
“Flu-like” symptoms	Common	Yes	Yes	Constitutional or systemic (generalized)	Immediate, mild, transient	Increase in A (dimers)
Anaphylactoid symptoms	Rare Complement activation Immune complexes (presence of acute infection)	No	Yes	Constitutional or systemic (generalized)	IgA: acute to immediate; other late, severe transient	I: IgA, very rare immune complexes
Full blown anaphylaxis	Rare Complement activation (in the presence of acute infection)	No	Yes	Constitutional or systemic (generalized)	Late, severe, hopefully transient (ICU)	I: IgA, very rare immune complexes
Headache	Common	Yes	Yes	Neurologic	Immediate, mild, transient	Increase in A
Migraine	Common	Yes	Yes	Neurologic	Transient	Increase in A
Dizziness	Common	Yes	Yes	Neurologic	Transient	Increase in A
Aseptic meningitis	Rare	No	No	Neurologic	Delayed, moderate, transient	Increase in A
Diffuse pain, muscle pain	Rare	Yes	Yes	Neurologic	Transient	Increase in A
Dysesthesia	Rare		Contributes	Neurologic		Increase in A
Weakness	Rare		Contributes	Neurologic		Increase in A

(Continued)

Table 1 | Continued

Symptoms and signs	Frequency	IRR or total dose	PRR	System	Class, severity, and duration	Part of the product likely being involved in AEs
Persistent headache	Rare		Yes	Neurologic	Delayed, moderate	Increase in A
Shortness of breath	Common	Dose	Yes	Respiratory		
Bronchospasm	Common	Yes	Yes	Respiratory		
Pleural effusion	Rare	Dose	Contributes	Respiratory	Severe, transient	
TRALI	Rare	Dose	Likely	Respiratory	Late, severe, transient (ICU)	
Hypotension	Common	Yes	Yes	Cardiovascular	Immediate, mild, transient	
Hypertension	Common	Yes	Contributes	Cardiovascular	Immediate, mild, transient	
Tachycardia	Common	Yes	Yes	Cardiovascular	Immediate, mild, transient	
Chest/back pain	Common	Yes	Yes	Cardiovascular	Immediate, mild, transient	
Arrhythmia	Rare	Dose	Contributes	Cardiovascular	Severe, hopefully transient	
Myocardial infarction	Rare	Dose	Contributes	Cardiovascular	Severe to fatal	Increase in A
Anorexia	Common			Gastrointestinal		
Nausea	Common	Yes	Yes	Gastrointestinal	Immediate, mild, transient	
Vomiting	Common	Yes	Yes	Gastrointestinal	Immediate, mild, transient	
Cramping	Common	Yes	Contributes	Gastrointestinal		
Diarrhea	Common		Contributes	Gastrointestinal		
Colitis	Rare		Contributes	Gastrointestinal	Late, severe	
Tubular swelling	Rare	Dose	Contributes	Renal	Severe, reversible; scars might remain	E: sucrose >> other sugars
Renal failure	Rare		Contributes	Renal	Delayed, severe, ICU	Increase in A (Complement deposition)
Infusion site pain, swelling, erythema	Common (SCIG more frequent)			Cutaneous	Immediate, mild, transient	SCIG: volume
Urticaria	Common		Yes	Cutaneous		Increase in A
Non-specific macular or maculopapular eruptions/eczema	Common		Yes	Cutaneous		Increase in A
Pruritus	Common		Contributes	Cutaneous		
Erythema multiforme	Rare		Contributes	Cutaneous		Increase in A

(Continued)

Table 1 | Continued

Symptoms and signs	Frequency	IRR or total dose	PRR	System	Class, severity, and duration	Part of the product likely being involved in AEs
Cutaneous vasculitis	Rare	Dose	Contributes	Cutaneous	Delayed, severe	
Hemolysis (clinically not significant)	Common	Yes	Contributes	Hematologic	Delayed, moderate, transient	
Acute hemolysis/hemolytic anemia	Rare	Yes	Yes	Hematologic	Delayed, severe	Increase in A
Thrombotic phenomena (DVT, stroke, cardiac infarction)	Rare	Yes	Yes	Hematologic	Severe, ICU	Increase in A
Hyperviscosity	Rare	Yes	Contributes	Hematologic	Immediate	Increase in A
Neutropenia	Rare	Yes		Hematologic	Delayed, mild, transient	Increase in A
Blood borne infectious disease	Rare	No	No	Microbiological	Late, severe	I: blood borne viruses, spongiform encephalopathy agent
Inappropriate handling before infusion			No		Immediate, mild to severe	A: incomplete dissolution of lyophilized product; denaturation and aggregate formation due to foam E: lyophilized product dissolved to result in too high concentrations; lyophilized product dissolved to result in too high osmolality; low temperature of concentrate at the time of infusion

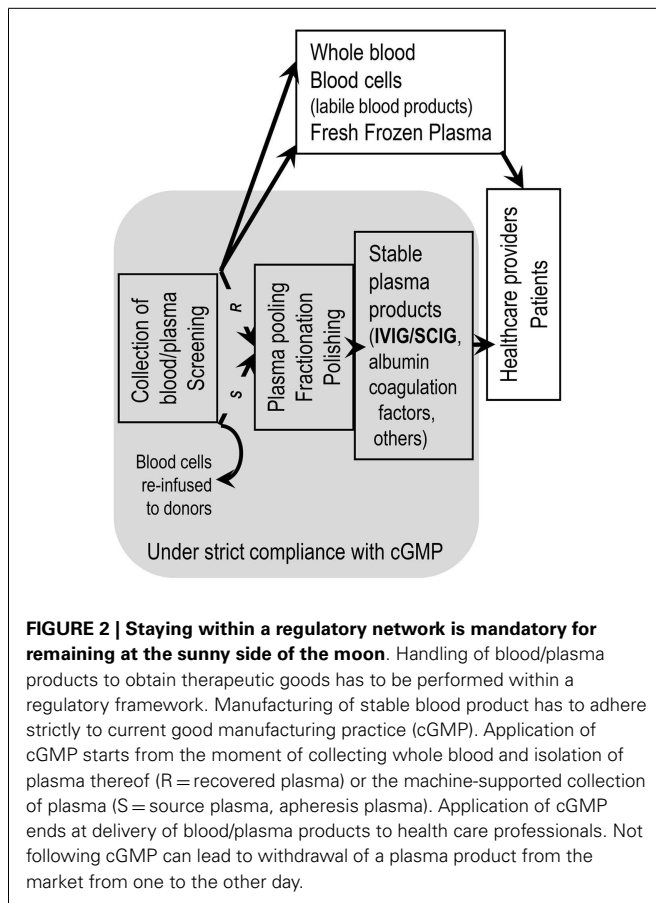
The various manifestations of AEs in recipients of polyvalent immunoglobulins. Frequency, severity, duration, timing, possible causes, risk factors.

A, active ingredient = IgG; I, impurities; E, excipients/stabilizers; "immediate," immediate reaction – within 6 h from the onset of infusion; "delayed," delayed reaction – 6 h to 1 week after infusion; "late," late reaction: weeks to months after infusion; IRR, infusion rate-related; PRR, patient-related risk factors (acute infection at the time of infusion); ICU, intensive care unit; SCIG, IgG concentrate for subcutaneous application.

The therapeutic effect achieved depends on the disease treated, and can depend on the concentration reached locally, i.e., can have agonistic or antagonistic effects (17, 20–22). In summary, it is our opinion that IgG concentrates always provide more or less the same "bouquet" of IgG specificities (similarity); however, it is the recipient's actual immune condition which decides from which IgG specificities the patient's derailed immune system is profiting (diversity).

Parameters of IgG-mediated AEs are: (i) the content in the product of biologically highly active likely beneficial ingredients that have to be kept under control (e.g., content of "dimers" devoid of remarkable complement activation *in vivo*; see below),

and the content in unwanted active ingredients that have to be discarded during manufacturing (alloantibodies); (ii) impurities such as IgA (anaphylactoid reaction); (iii) activated coagulation and contact activation factors (thromboembolic events) and; (iv) excipients such as sucrose (osmotic nephrosis). Below, we like to add and contemplate on how fully native IgG molecules not harmed by the manufacturing process might add to AEs. The above mentioned inevitable interaction of the exogenous IgG with the immune system of the recipient and *vice versa* in its principle might evoke an inflammatory condition. The sum of the potentially beneficial reactions might overshoot and lead to AEs (Figure 1). The principle of induction of mild inflammatory



conditions upon each infusion of a well-tolerated IVIG was confirmed when several dozen normogammaglobulinemic volunteers in all cases except one, showed a more or less moderate inflammatory reaction as indicated by the increase of tumor necrosis factor alpha (TNF α) at 2.5 h post initiation of infusion. The only person in the cohort not showing a measurable TNF α increase was a woman caring at home for her brother with full blown AIDS (22).

Subcutaneously applied IgG concentrate reaches the circulation slowly and systemic AEs are less frequent compared to IVIG but they are not absent (23–28) (a case of unintentional i.v. application of SCIG is not considered). In contrast, local mild to moderate AEs are more frequent with SCIG (29). In summary, the intensity of the resulting AEs is depending on the immune status of the recipient, the infusion rate (IR), e.g., how rapidly the active ingredients (the various IgG specificities), the impurities, and the excipients reach the circulation of the recipient. Thus, the i.v. application has the highest chance for the occurrence of AEs.

In the early days of IVIG therapy, complement-mediated “anaphylactoid” (i.e., immediate) and “phlogistic” (i.e., inflammatory) AEs were distinguished (30, 31). The complement-mediated AEs were considered to be caused by aggregates in the product (“spontaneous complement activation” or anti-complementary activity or ACA) or by *in vivo* formation of immune complexes (ICs, patient’s condition related; e.g., subclinical infections or the

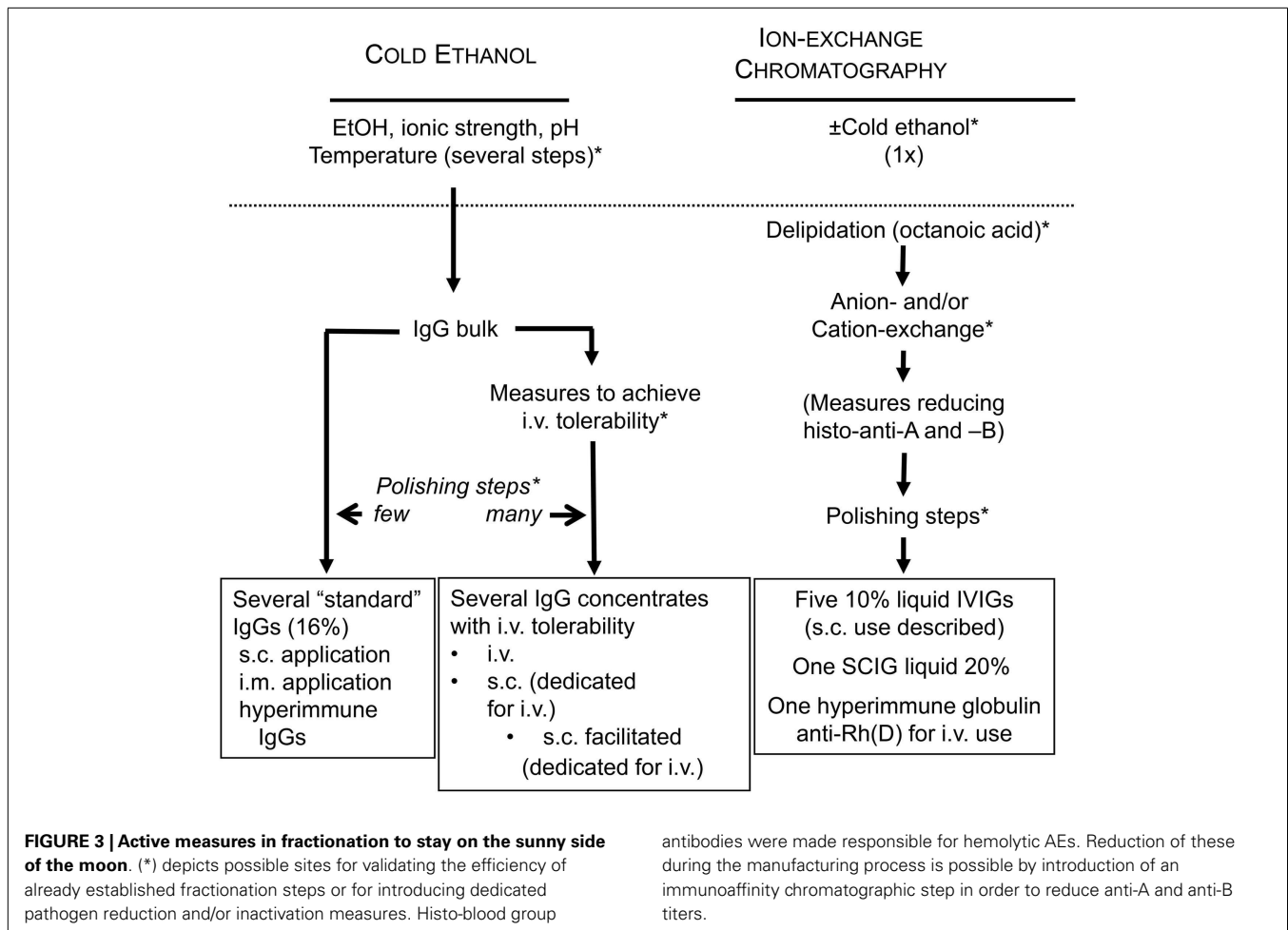
unnoticed presence of anti-IgA antibodies) and therefore only IgG concentrates with low or absent ACA is accepted by authorities for human use. Below, we present one instructive case of each type of reaction.

IMMEDIATE ADVERSE EVENTS – THE RAPID ONSET OF DARKNESS

The first reports of rapid onset AEs concerned either the application of complement-activating fractions in an IgG concentrate or the *in vivo* formation of complement-activating ICs (2–4). A very rare but potentially fatal condition is the formation of IgA/anti-IgA complexes in patients being initiated on replacement therapy and having serum IgG antibodies against infused IgA not recognized before the start of the IVIG infusion (32). Prerequisite for the presence of anti-IgA antibodies is the most common primary immunoglobulin defect, i.e., selective IgA deficiency (sIgAD) or IgAD associated with diminution of other immunoglobulin classes. IgAD is defined by serum levels of <0.05 or <0.07 g/L (depending on laboratories). A marked diminution of serum IgA consistent with IgAD in various ethnic groups is estimated being 1:155 to 1:18,550 (33). The mean frequency in Caucasians is approximately 1:700 (34). Up to 40% of patients with IgAD have been reported having anti-IgA antibodies in the serum with titers ranging between 1:4 and 1:262,144. In approximately 10% of patients with common variable immunodeficiency (CVID), and occasionally in patients with other primary immunodeficiency diseases, measurable anti-IgA can be detected (35, 36). These antibodies are predominantly of the IgG class, but anti-IgA antibodies of other immunoglobulin classes have been described as well (37, 38). The reason for their emergence remains unknown.

Taken the above numbers, the infusion of human-derived products containing IgA resulting in severe anaphylactoid type AEs should be considerable. This is not the case (39). Questions about the clinical relevance of above numbers emerge as soon as blood banks (i) estimate the theoretical risk of IgA anaphylactic reactions (32); (ii) assess the relation of severe IgAD with the presence or absence of anti-IgA antibodies (40); (iii) screen donors for very low IgA levels in order to become able to provide blood and plasma-derived products free of IgA and find a considerably lower frequency than expected (41). Alternatively, the test systems may not reliably detect anti-IgA antibodies being as yet insensitive and inaccurate or – at least – do not correspond to the clinically relevant fraction of antibodies. This comes to mind when a more close look to “anti-IgA” gives “unexpected” results, including “anti-IgA” in blood donors with normal serum IgA level or “anti-IgA” that cannot be neutralized with purified IgA (42); or when blood products containing proven anti-IgA do not elicit severe AEs (43).

Among patients on replacement therapy, those with CVID may rarely develop severe immediate AEs (32). The discrepancy between anti-IgA positive patients and frequency of AEs raises the question about the nature of the many reported anti-IgA antibodies and also raises the question about the immunologic condition which allows the formation of anaphylactoid anti-IgA antibodies. There might be some logic in supposing that anaphylactoid anti-IgA cannot evolve at IgA levels otherwise fulfilling the definition of IgAD. Such a condition would constantly generate ICs which in turn could activate complement, react with immune cells, and be deposited in lung and kidney. Indeed, Horn et al. found anti-IgA



antibodies in CVID patients missing IgA⁺ B cells and presenting with IgA levels <0.0009 g/L, a level which is more than 50- to 70-fold lower than the threshold for IgAD (44). However, a possibility for an IgA-mediated anaphylactoid reaction at measurable IgA serum levels might exist. Serum IgA contains approximately 85% subclass 1 of IgA (IgA1) and only 15% subclass 2 of IgA (IgA2). Selective deficiency of IgA2 and – although evidence is lacking – the presence of a highly specific anti-IgA2 antibody theoretically could elicit a severe AE.

The kinetics of anti-IgA after infusion of blood products have been studied in a few cases. In these patients, a fall in anti-IgA titers has been noticed followed by an increase during subsequent weeks or months. This suggests that at appropriate proportions, IgA of the infused material and anti-IgA present in the patients' serum combine with each other to form ICs. In turn, ICs activate complement that are bound and eliminated by macrophages most likely leading to cytokine release. The increase in anti-IgA titers over time indicates that the infused IgA-containing product has a booster effect (36, 37, 45). Such boosting effect together with the presence of anti-IgA before the application of an IgG concentrate can be taken as the ultimate confirmation of a supposed IgA/anti-IgA reaction. **Figure 4** depicts a well-documented case of IgA/anti-IgA reaction in a patient who progressed from sIgAD to

CVID. The events during the first 12 h at occasion of the first infusion of IVIG were as follows (shadowed area in **Figure 4**): 2 min after the start of the infusion, having received eight drops of an IgG solution (IgA < 1.2 g/L; 3% solution), she experienced a flush, back pain, rigors, difficulty in breathing, and hypotension. The infusion was immediately stopped. After approximately 1 h, the reaction has weaned, and 2 h later the patient felt well again, and the infusion of total 6 g IgG could be continued without further complications. Although the patient fairly assured having never received any blood or plasma product in the past, the follow-up of her anti-IgA titers from before infusion to 1 year later confirmed a true anaphylactoid reaction mediated by anti-IgA, as the anti-IgA became undetectable immediately after the infusion and showed a boosting phenomenon during the following months. True anaphylactoid reaction was further confirmed by follow-up of total complement hemolytic activity (CH50) on the day of infusion. Interestingly enough, the CH50 value reached its nadir at the end of the infusion when the patient had no complains. Although a single case only, the events during the first infusion call for the following remarks: (i) severe AEs most likely occur at concomitant complement and cell activation with cytokine release; (ii) infusion of minute to low amounts of IVIG hours before the main infusion can "anergize" cells and stop release of pro-inflammatory

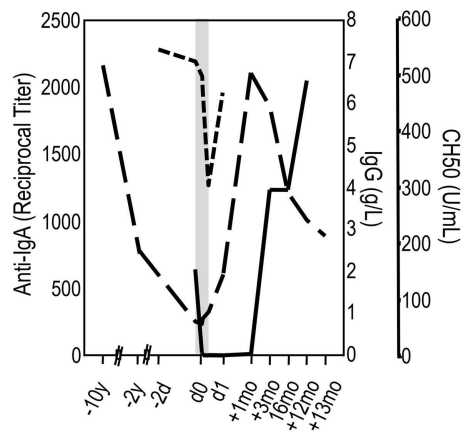


FIGURE 4 | An individual's slithering into the dark. A female patient has been suffering from recurrent airway infections since adolescence, occasionally complicated by pneumonia. At age 34, selective IgA deficiency was diagnosed. Ten years later, she was hospitalized with pneumonia. Within these 10 years, her serum IgG had dropped from 7 to 0.87 g/L (long-dashed line) and IgA was undetectable. The diagnosis was corrected into CVID, and IVIG replacement therapy was initiated (shadowed area). Two minutes after the start of the infusion of a 3% IgG solution (IgA < 1.2 g/L; 3% solution), she experienced a flush, back pain, rigors, difficulty in breathing, and hypotension. The infusion was immediately stopped and later continued without further complications (see text). The confirmation of a true anaphylactoid reaction due to anti-IgA in the serum of the patient was achieved by follow-up of anti-IgA (solid line) and CH50 (short-dashed line).

cytokines; (iii) “anergized” cells loose reactivity toward ongoing formation of ICs and complement activation products.

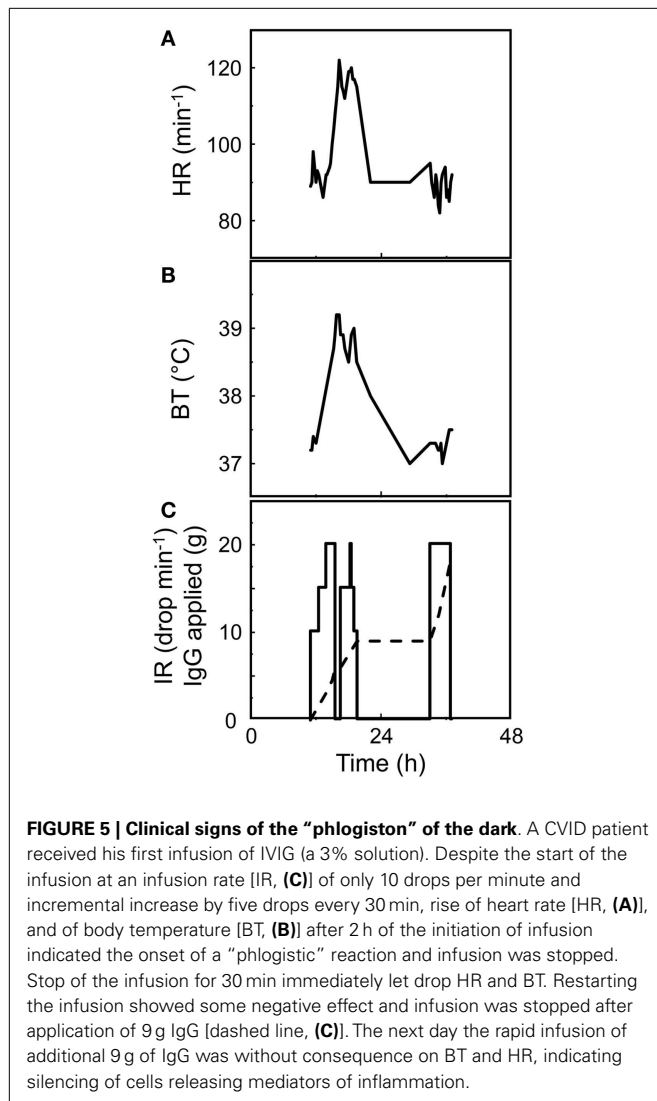
A non-complement-mediated anaphylactoid reaction was ascribed to the unforeseen release of elastase and other pro-inflammatory substances from neutrophils activated by the formation of *in vivo* IgA/anti-IgA complexes. Complement activation or mast cell-dependent release of vasoactive substances was excluded as pathogenic mechanisms. Although the IgA/anti-IgA complexes usually do not cause clinically relevant neutrophil degranulation within the circulation, the presence of a rare genotype encoding a novel gain-of-function IgG receptor on neutrophils may provoke premature degranulation by these complexes. This phenomenon was only relevant in hypogammaglobulinemic patients in the presence of *in vivo* IgA/anti-IgA complexes (46). The low prevalence of this genotype combined with an IgAD or CVID may add how to explain the rarity of serious anaphylactoid reactions in newly IVIG-treated patients. Authors share the opinion of Janne Björkander who at occasion of a discussion panel “Dilemmas in Diagnosis and Management of Antibody Deficiencies: Ask the Experts” held at occasion of the 58th Annual Meeting of the American Academy of Allergy, Asthma & Immunology (AAAAI), New York City, March 1–6, 2002 came to the following conclusion: a clinician has to be aware of the risk, particularly at occasion of first infusions, but otherwise IgA is not a major concern (from tape record).

PRO-INFLAMMATORY CYTOKINES – THE PHLOGISTON OF THE DARK

In the early days of Ig-therapy, the nature of the “phlogistic” AEs was obscure. However, it was already known that an AE can be

prevented or its evolution halted when the patient receives a low dose of IVIG first or the infusion is stopped early and is continued several hours later. Hours later the infusion can be (re)started at high rates without further problems (Figure 5). One of the authors had a particular opportunity to get an insight into what a “phlogistic reaction” might be. At the occasion of a voluntary infusion of an investigational liquid IVIG, he encountered a severe flu-like AE of more than 12 h duration. Before injection, the investigational liquid preparation had passed all release criteria for human use, including spontaneous complement activation assessed by ACA and was free of prekallikrein activator (PKA). In those days, assays for cytokines in biological samples just began to become available and were included into the parameters assessed in the study. Infusion was stopped after 1 h because of a drop of pulse rate and heavy discomfort provoking the laconic comment by the proband’s technician who was taking samples: “you look green.” The infusion was continued after another 90 min when the heart rate had almost normalized. The infusion could be completed within an additional 3.25 h (a total of 0.4 g/kg b.w.) without further aggravation of malaise. The leukocyte count transiently had dropped to a nadir of 40% at 2 h followed by a leukocytosis peak at 8 h. Complement activation, as assessed by generation of C3a/C3a[desArg] and the formation of the terminal complement complex C5b-9, apparently did not occur: the C3a/C3a[desArg] value reached a maximum of 260 ng/mL (norm: <200 ng/mL) at 7 h while the C5b-9 value never moved outside the normal range. Instead, a sequence of rapid transient massive increases of pro-inflammatory cytokines was observed: (i) TNF α started to increase 30 min post initiation of infusion from a value of 20 pg/mL to a peak value at 2 h which was above the calibration range of the test kit of 1500 pg/mL; (ii) interleukin 8 (IL-8) increase started after 1 h from 29 pg/mL and peaked at 2.5 h with 4400 pg/mL post initiation of infusion; (iii) interleukin 6 (IL-6) secretion started after 1 h with an undetectable level and peaked at 4 h with 345 pg/mL. All pro-inflammatory cytokines fell sharply while the second part of the infusion was still ongoing. The day after infusion, the pro-inflammatory cytokine profiles were back to normal and the flu-like syndrome was gone. In contrast, interleukin 1 receptor antagonist (IL-1ra) values started increasing at 1.5 h (300 pg/mL), peaked at 4 h (>32,000 pg/mL), and decreased slowly to reach a value of 10,500 pg/mL 24 h after initiation of the infusion. Soluble TNF receptor p75 level started at 2.25 ng/mL, reached a peak with 16.5 ng/mL at the same time as IL-1ra, and 24 h after initiation of infusion was still at 10.5 ng/mL. Thus, in this normogammaglobulinemic subject similar cytokine profiles and leukocyte number changes were observed as reported for hypogammaglobulinemia under replacement therapy (47, 48).

A series of further experiments with investigational and marketed IVIGs was performed. All IgG concentrates were analyzed for their molecular weight (MW) distribution. The most remarkable differences emerged in the MW range of dimers while the presence of minute amounts of higher oligomers could not be excluded with certainty. Below, we will use the term “dimers” for that fraction of IgG with higher MW. Subsequent findings indicated that levels of “dimers” >12% were responsible for complement-independent cell activation and cytokine release. The TNF α peaks assessed at 2.5 h post initiation of infusions correlated with “dimer” content of the IVIGs and mirrored a clinical score of AEs (49–51).



A few years before a complement-independent induction of a hypotensive factor by IgG di- and oligomers was reported in animal experiments (52). A key role for macrophages in the generation of the hypotensive lipid factor was identified as platelet-activating factor, being induced by dimers and polymers (53, 54). Several years later, the dimer-mediated AEs in animal experiments were confirmed (55, 56). Yet, at the same time, the dimer content of IVIG apparently correlated with the clinical efficacy in a murine ITP model (55, 57). Variables such as “IR,” “genetic background,” “endogenous immunoglobulin levels,” or “proportional fraction of polymers versus dimers” may impact on the balance between the phlogiston (being cytokines, active lipid substances, or a combination of factors) and the therapeutic efficacy (blocking IgG receptors on liver/spleen macrophages to prevent clearance of “opsonized” material such as platelets in ITP). As of today, reports on release of cytokines in humans in association with AEs or tolerability toward dimers remain scarce and to the best of our knowledge studies in humans of causative factors/fraction in an IgG concentrate has not been adequately addressed (47, 48, 58–61).

In IgG preparations, various forms of dimers might be present: formed through covalent binding (62) by denaturation, hydrophobic interactions of the Fc-parts, and by idiotype/anti-idiotype interactions, as part of the V-connected network of peripheral immune homeostasis (63). For a commercially viable fractionation process, pooling of donated plasma is mandatory in order to obtain a volume of starting material large enough to cover ever increasing costs for documentation, in-process, and batch-release testing as it is required by cGMP. Pooling also intends smoothing the batch-to-batch differences in antibody titers, a goal apparently difficult to achieve to levels as theory might imply (64). Consequences of pooling are on the one hand the enrichment of public/common immune antibodies while diluting out individual specificities; on the other hand, the antibodies of the immune network of an individual donor are exposed to those of many other donors. The more individuals contribute to the pool, the more complex the possible “immune-network” interactions among IgA, IgG, and IgM will become. The subsequent fractionation process has far-reaching effects on immunoglobulins from a given pool: only trace amounts of IgA and IgM are retained in the final product, i.e., IgG is deprived of its counterparts of the V-connected immune network. The IgG molecules of the homeostatic network “naked” at their V-regions can interact with each other at random combining site-interactions of single donor-derived monomeric IgG (65), otherwise not existing *in vivo*. This interaction is largely reversible. With increasing numbers of donors included into the pool, the immune network recognition among the “naked” IgG molecules of the V-connected network becomes more and more complex, and the dimer and lower oligomer content in the resulting IgG concentrate increases (66–68). In lyophilized IgG concentrates, the dimer formation is “frozen” at a low level while in liquid preparations an equilibrium between monomers and dimers is achieved over time reaching a dimer content of 12% or more if not hampered by stabilizers. Specificities, as far as they have been addressed, in the dimer fraction considerably differ from the monomeric fraction (69–73). In conclusion, the immunomodulatory efficacy of IgG concentrates in part depends on the capacity and extent to form “dimer” fractions devoid of remarkable complement activation *in vivo*. The “art” of manufacturing a liquid IgG concentrate is not to eliminate the monomeric IgG having potential for “dimer” formation but to inhibit extensive “dimerization.” In summary, AEs might be associated with the induction of pro-inflammatory cytokines in absence of measurable complement activation *in vivo* where all regulatory mechanisms and removal processes of a body are at disposition. At reasonable IRs in the open system of the human body, clinically relevant systemic complement activation apparently needs oligomers formed of three or more IgG molecules.

THE MISSING OXYGEN ON THE DARK SIDE – IMMUNOGLOBULIN-INDUCED HEMOLYSIS

There are multiple reports of Ig-induced hemolytic anemia (HA) in patients receiving high doses of IVIG (60, 74–110) (Table 2; Figure 6; www.adrreports.eu). By spontaneous reporting, risk factors recognized for Ig-induced hemolysis include beside high doses (more than 100 g IVIG over 2–4 days), female gender and histo-blood group type A, B, or AB of recipients.

Table 2 | The missing oxygen on the dark side – Ig-induced hemolysis in recipients of polyvalent immunoglobulins.

Publication	Number of patients	Blood group	Monthly Ig dosage (mg/kg)	DAT	Eluted antibody	Alloantibody passively administered	Hemoglobin drop (g/L)	Outcome
Quinti et al. (110)	8	A+ (5), A- (1), O+ (2)	Low	IgG (2), IgG and C3d (4)	anti-A (5), anti-C (1), anti-C and anti-D (1)	anti-A, anti-C, anti-D	6.4, 1.5, 5.1, 1.4, 6.9, 1.1, 1, 1	Recovery (7), death (1)
Desbourouh et al. (76)	1	AB+	High	IgG and C3	anti-A and anti-B	anti-A and anti-B and anti-D	6.5	Recovery
Mohamed et al. (77)	1	A+	High	IgG	anti-A	nd	4	Recovery
Rink et al. (78)	3	A+ (2)	High	IgG	nd	nd	1.2, 3.8, 4.4	Recovery
Berard et al. (79)	4	A+ (2), B+ (1), AB+ (1)	High	IgG	anti-A (1), anti-B (1), anti-A and anti-B (1)	nd	2.9, 5.8, 5.8, 3.7	Recovery
Michelis et al. (60)	1	A+	High	IgG	anti-A	nd	3.5	Recovery
Pintova et al. (80)	2	AB+, A+	Low	IgG	anti-A	nd	6.6, 7.2	Recovery
Morgan et al. (81)	3	AB+(1), A- (1), A- (1)	High	IgG	anti-A (2), anti-A and anti-B (1)	anti-A (2), anti-A and anti-B (1)	4.8, 5.0, 1.8	Recovery
Welles et al. (82)	1	nd	High	IgG	nd	nd	4.3	Death
Canadian Group (83)	20	A (14), AB (6)	High	IgG	nd	nd	3.2, 2.8, 5.1, 5, 5.6, 5, 3.5, 4.1, 7, 3.2, 5.6, 3.2, 6.6, 2.9, 3.1, 4, 3.9, 7.8, 4.9, 4.8	Recovery (10), death (1), unknown (8)
Gordon et al. (84)	4	A+ (3), AB+ (1)	High	IgG	nd	nd	5.3, 5.5, 4.8, 4.8	Recovery
Kahwaji et al. (85)	16	A+ (10), A- (2), B+ (3), AB+ (1)	High	IgG	nd	anti-A, anti-B	5.3, 4.7, 5.6, 4.9, 5.8, 5.7, 3.3, 2.4, 3.1, 4.0, 3.6, 2.1, 2.2, 2.8, 5.3, 1.9, 2.6, 3.0	Recovery
Daw et al. (86)	16	A+ (7), AB+ (1), AB- (1), B+ (6), O- (1)	High	IgG	anti-A (6), anti-B (4)	anti-A, anti-B	1.4, 3.6, 4.3, 3.6, 3.2, 3.4, 3, 4.7, 5.1, 5, 2.4, 8, 5.2, 1.3, 3	nd
Yin et al. (87)	1	AB+	High	Negative	nd	anti-A and anti-B and anti-D	nd	Recovery

(Continued)

Table 2 | Continued

Publication	Number of patients	Blood group	Monthly Ig dosage (mg/kg)	DAT	Eluted antibody	Alloantibody passively administered	Hemoglobin drop (g/L)	Outcome
Coghil et al. (88)	1	A+	High	IgG	anti-A	anti-A	4	Recovery
Chamouni et al. (90)	1	AB+	High	IgG	nd	nd	8	Recovery
Karaaslan et al. (91)	1	nd	High	IgG	nd	nd	3.9	Recovery
Trifa et al. (92)	1	AB+	High	IgG	anti-A and anti-B	anti-A and anti-B	7.8	Recovery
Nagakawa et al. (94)	1	A+	High	IgG	nd	nd	2	Recovery
Wilson et al. (95)	12	A+ (11), O+ (1)	High (10), low (2)	IgG	anti-A (9), anti-A and anti-D (2), anti D (1)	nd	3.7, 3.8, 1, 1.6, 1.9, 2.9, 1.4, 1.9, 3, 1.8, 1, 0.9	nd
Tamada et al. (97)	2	nd	High	IgG	anti-A, anti-B	nd	nd	nd
Thomas et al. (99)	1	A+	High	IgG	anti-A	anti-A	6.3	Recovery
Comenzo et al. (100)	1	nd	High	nd	nd	nd	nd	Recovery
Okubo et al. (102)	1	A+	High	IgG	anti-A	anti-A	nd	nd
Hillyer et al. (103)	1	AB+	High	IgG	anti-A, anti-B	nd	nd	Recovery
Nicholls et al. (104)	2	nd	High	IgG	anti-A, anti-A and anti-D	nd	nd	nd
Kim et al. (105)	2	B+	High	IgG	anti-B	nd	nd	nd
Brox et al. (106)	1	nd	High	IgG	anti-A	nd	nd	nd

Clinical and immunological characteristics of patients described in case reports. Numbers in parenthesis indicate the number of patients with the given condition.

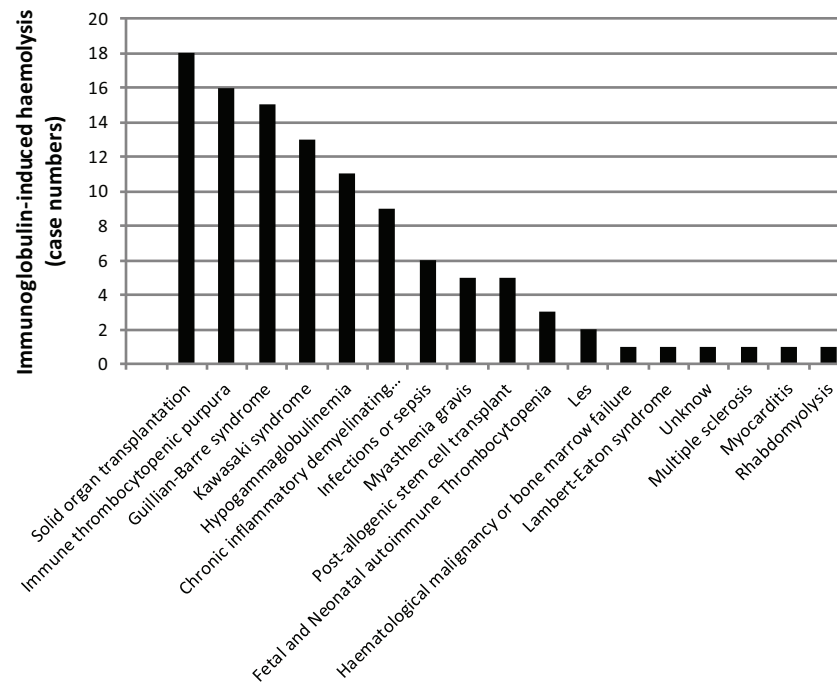


FIGURE 6 | The missing oxygen on the dark side: Ig-induced hemolysis in patients on Ig treatment. Black bars indicate the number of patients with a given clinical condition.

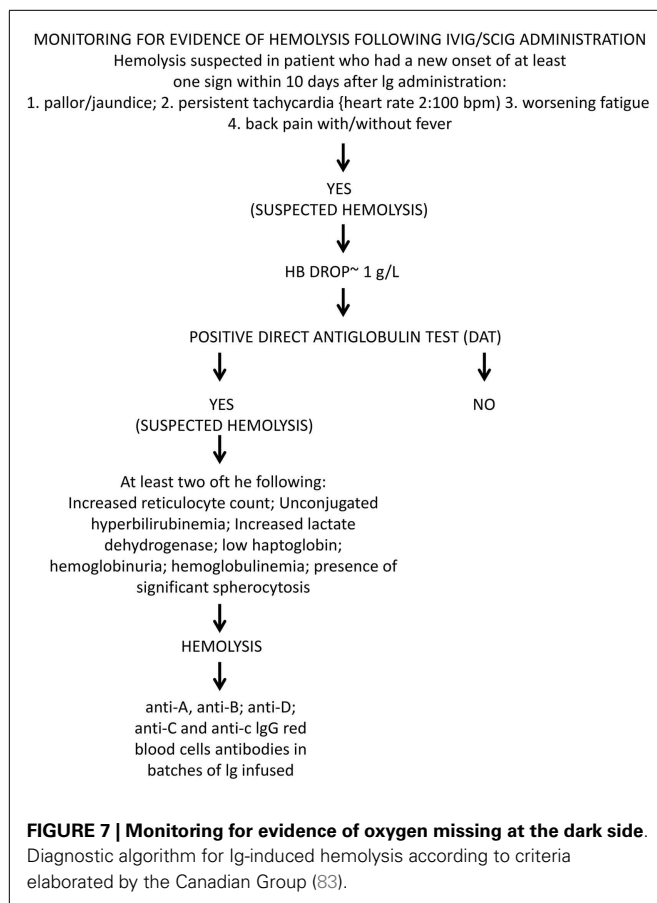
A significant proportion of patients receiving IVIG develop a positive direct antiglobulin test (DAT) detectable after 24 h for up to 10 days after the IVIG infusion (109, 110). However, it should be underlined that the DAT positivity due to the factors mentioned above (111, 112) is not sufficient *per se* to diagnose hemolysis and DAT positivity does not necessarily imply the presence of active hemolysis. DAT-positive mild hemolytic reactions can be easily missed and the true incidence of such reactions is difficult to document without careful clinical and laboratory follow-up.

In the majority of reports on HA, intravascular red blood cell (RBC) destruction via complement activation or extravascular RBC sequestration and removal by the reticulo-endothelial system was proposed to result from IgG alloantibodies with specificity for RBC antigens A, B, D, or C.

Hemolytic anemia induced by high-dose IVIG has an average incidence of 5.8% (85). Low-dose IgG replacement therapy is considered universally as safe, and only few cases of hemolysis following low-dose IVIG or SCIG administration have been described (80, 95, 110). A baseline WBC and RBC count prior to IVIG initiation and a close clinical and laboratory follow-up was suggested as a useful tool for early diagnosis and treatment. A possible work up might be to check hemoglobin (Hb) level prior and 48–78 h after Ig infusion. In case of a drop of Hb, the presence of DAT, an increase in unconjugated bilirubin, lactate dehydrogenase (LDH), and reduced haptoglobin level, followed by a rise in reticulocyte count should be assessed (Figure 7). We systematically reviewed case reports related to IVIG-induced hemolysis from 1987 to 2014 and identified 29 articles containing reports of 109 patients. Baseline characteristics of the patients are shown in

Table 2. When available, blood group, DAT, Hb drop, and outcome are indicated. All reports showed positive DAT, except for a case of Yin et al. (87); in this case, DAT was performed 10 days after IVIG administration and the DAT negativity might have been due to a rapid removal of sensitized RBCs.

In the majority of patients, the outcome was positive: 106 out of 109 patients recovered with or without packed RBC transfusions; three patients died after HA, with the hemolytic episode representing a precipitating factor of a severe underlying condition. Elution experiments were performed and the search for blood group antibodies revealed anti-A and anti-B specificity in the majority of cases; anti-D specificity was assessed in four reports, often associated with other specificities (95, 106, 110). A search for other specificities such as anti-band 3 or anti-Gal was not performed. Only one report detected anti-C specificity in three patients; in one of them associated with anti-D irregular antibodies (110). Although studies were restricted to blood group antibodies, this finding demonstrated that polyvalent IgG preparations might contain clinically significant non-blood group antibodies, which are not part of the lot-release criteria in that their titration is not yet required by the European Pharmacopeia. Antibodies in HA, such as anti-C, may have unexpected hemolytic consequences (113–117). Beside passive transfer of alloantibodies, IgG administration also has been demonstrated to lead to unspecific enhanced erythrocyte sequestration, in particular, in patients with underlying inflammatory disorders (109, 118). In 2009, the Canadian IVIG Hemolysis Pharmacovigilance Group elaborated criteria to define an “IVIG-induced hemolysis” (83). They included a reduction of Hb levels ≥ 1 g within 10 days after Ig administration, with



appearance of a positive DAT and, at least, two of the following criteria: increase in the reticulocyte count, elevation of LDH and unconjugated bilirubin serum levels, low haptoglobin, hemoglobinuria, hemoglobinemia, presence of significant spherocytosis, in the absence of alternative causes of anemia. The passive transfer of IgG alloantibodies through IgG concentrates is difficult to explain as polyvalent IgG is prepared from plasma of thousands of donors. Since immunization to RBC alloantigens can occur because of past transfusions or pregnancy, the hypothetical numbers of alloimmunized plasma donors should be rather low. Recently, other mechanisms underlying alloimmunization related to molecular mimicry have been demonstrated (119). The mechanism of high-dose IVIG-induced HA is complex and it might vary from patient to patient. IVIG cause hemolysis due to: (i) disease-associated pre-coating of RBCs; (ii) IgG with hemolysis triggered by passive transfer of IgG binding to blood group antigens; (iii) transfer of high levels of alloantibodies to RBC pre-coated at a low level only; or (iv) transfer of clinically tolerable levels of isoagglutinins plus transfer of additional RBC-reacting physiological autoantibodies. Indeed, hemolytic reactions could not be related exclusively to transfer of alloantibodies. Hence, antibodies other than histo-blood group alloantibodies (pre-)coated to RBCs might contribute to hemolysis in IgG recipients need to be identified. In addition, hemolytic episodes may possibly be precipitated by some sort of complexed/denatured IgG that co-purify with other IgG in the product (76, 109, 118, 120). Recently, a two hit mechanism for

IVIG-induced hemolysis has been proposed: the passive transfer of alloantibodies through IVIG representing the first hit and the underlying inflammatory state representing the second hit (121). Nowadays all commercial Ig products have to undergo anti-A and anti-B testing and regulatory requirement ask for respective IgG antibody titers of $\leq 1:64$ at 5% solution strength (w/v) (103, 104). Nevertheless, hemolysis might occur even in recipients of IgG products that meet these specifications (76). Consequently, it has been suggested that IgG recipients should be monitored for clinical signs and symptoms of hemolysis (122).

REDUCTION OF HISTO-BLOOD GROUP A AND B ALLOANTIBODIES IN IgG CONCENTRATES RAISES THE CHANCE FOR STAYING ON THE SUNNY SIDE OF THE MOON

With the detection of the immunomodulatory potential of IgG concentrates, their clinical use has continuously increased (123). To cover the need, at a first glance, an increase of the volume of plasma fractionated seems to be the most convenient option. However, this might economically not be viable because fractionation of plasma products is interconnected (124) and before increasing output of one product (e.g., IVIG), the market absorbance of the other products as well (e.g., albumin) must be ascertained. On a longer-run, a more viable option is to improve recovery. Considering recovery, the cold-ethanol fractionation apparently has reached its limits. As of today, four manufacturers have invested into a “modern” fractioning technique on the basis of ion-exchange chromatography. Ion-exchange chromatography allows elevated recovery at high purity. As of today, five IVIGs, one SCIG, and one anti-D concentrate are fractionated by ion-exchange chromatography. Pharmacovigilance has shown that all chromatographically fractionated IVIG and SCIG, more or less prominently, show a tendency for elevated frequencies of hemolytic AEs. Anti-A and anti-B alloantibody titers are now lot-release criteria (see above) as they constitute the major risk parameter for hemolytic reactions mediated by IgG concentrates. To overcome the threat of end up on the dark side of the moon, two manufacturers have taken measures to reduce anti-A and anti-B titers in their IgG products. One measure chosen was adsorption of the alloantibodies by affinity chromatography (125). Reported reduction in both alloantibodies was significant and levels were similar to those in cold-ethanol fractionated immunoglobulins (126). The other measure chosen was reduction in anti-A using an automated indirect agglutination test for donor screening and exclusion of high-titer donations (approximately 5.1%) from plasma pooling and fractionation (127). This measure reduced anti-A in the IgG concentrate by one titer step. To ensure staying on the safe and sunny side, the manufacturer has announced the introduction of an alloanti-A and alloanti-B immune-affinity chromatography step into the manufacturing process (128). Preliminary results indicate depletion in anti-A and anti-B by $>80\%$ in investigational lots. Subsequently, we want to discuss possible consequences of (extensive) removal of antibodies reacting with histo-blood group antigens A and B.

REASONING ABOUT ANTIBODIES REACTING WITH TERMINAL SUGARS OF THE MAJOR HISTO-BLOOD GROUP ANTIGENS A AND B

Three facts have initiated our thinking about possible consequences of removal of histo-blood group A and B reacting

antibodies from IgG concentrates. (I) In collaboration with Hans U. Lutz, formerly Biochemistry ETH Zurich, we have observed the non-intended removal of natural anti-C3 autoantibodies regulating complement activation by large-scale immune-affinity adsorption of IgA from an IgG concentrate (129). Anti-C3 antibodies belong to the family of “NABs” and have a particular role in homeostasis: they control activation of complement, among others, in the frame of NAb-mediated opsono-phagocytosis of altered or senescent cells, including RBCs (130–132). Thus, the intention to target one particular antibody by affinity chromatography might reduce that antibody specificity but at the same time affect other specificities as well. (II) It should be kept in mind that the blood groups A and B are in fact “histo-blood group” antigens, i.e., they are also found on white blood cells, T lymphocytes, and proteins and also can be found in soluble form (133). Alloantibodies reacting with histo-blood group antigens A and B thus have much broader tissue recognition than RBCs only. In addition, alloanti-A and alloanti-B belong to the population of NABs recognizing non-self and most likely participate in primary host defense (134). (III) In contrast to cold-ethanol fractionation, where low titers of alloanti-A and alloanti-B are achieved on basis of their isoelectric points (IEPs), the (extensive) immune-affinity removal might affect a much wider IEP range, thereby removing broadly reacting antibodies and impairing some desirable functions of the IgG concentrate. Thus, the struggle for staying on the sunny side of the moon might have consequences for the antibody repertoire in an IgG concentrate.

Antibodies reacting with terminal di-, tri-, and tetra-saccharides belong to the large family of human anti-glycan NABs. Histo-blood group A and B epitopes in terminal position are tetra-saccharides. Alloantibodies to these tetra-saccharides are found in the plasma of healthy individuals depending on the blood group they have. A considerable body of research into the nature of these NABs has been performed so far, all using for isolation the corresponding terminal di- or tri-saccharides (135–137). Recently, the repertoire and epitope specificity of such immunoglobulins was addressed in depth by including the tetra-saccharide as well (138, 139). It proved that serum of healthy individuals contain respectable amounts of di- or tri-saccharide-reacting NABs. These NABs proved to be pseudo-anti-A and pseudo-anti-B NABs as they are not reacting with the tetra-saccharide of histo-blood groups A and B. In contrast, alloanti-A and -B antibodies able to react with tetra-saccharides are reacting with the corresponding terminal di- and tri-saccharides. Reasoning about the biological role of these “high-titer and population conservative” anti-di- and anti-tri-saccharide NABs and the consequence of their potential removal by immunoaffinity is outlined below.

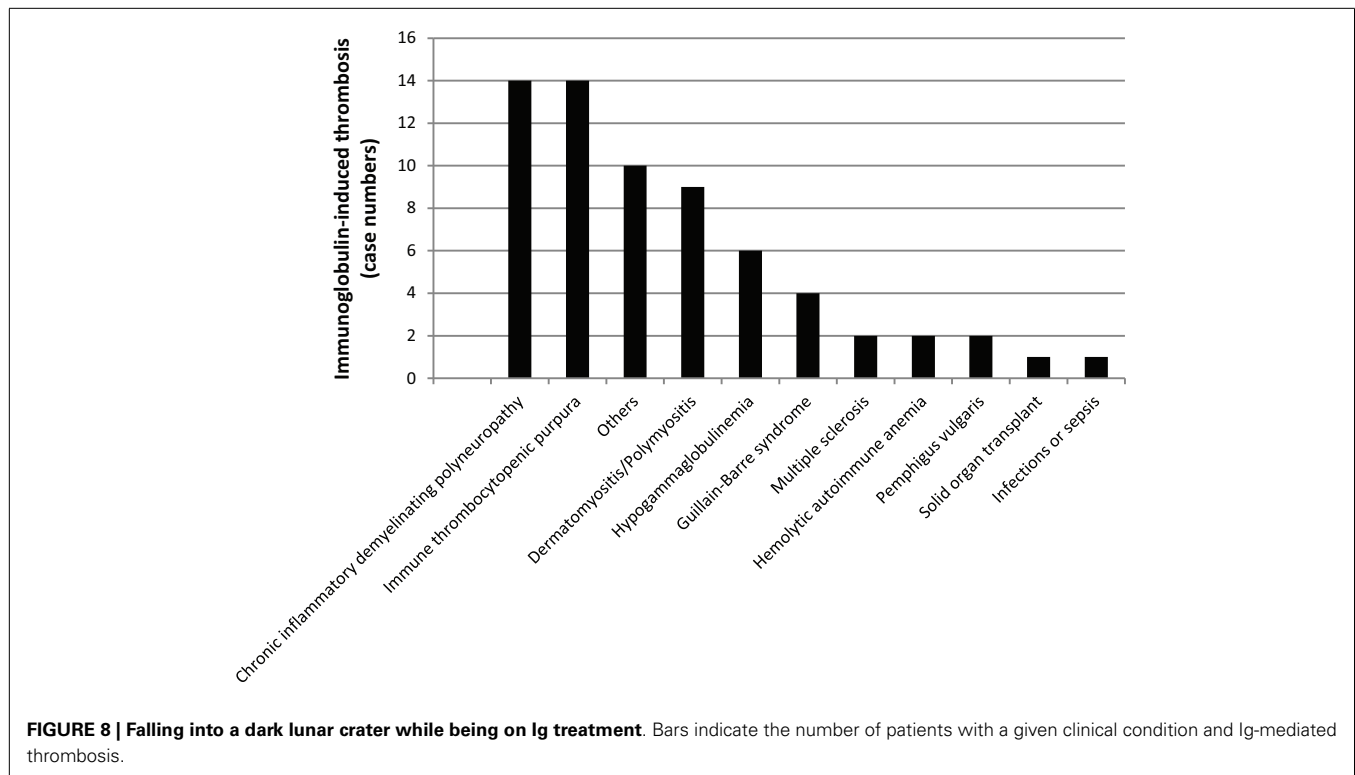
A population of the anti-glycan NABs are the anti- α Gal NABs which recognize Gal α 1-3Gal and Gal α 1-3(Fuc α 1-2)Gal epitopes. Anti- α Gal NABs have been described being xenoreactive, recognizing bacterial Gal α 1-3Gal (140) and having tissue homeostatic function. The daily removal of altered/senescent cells of the body is $\sim 10^{12}$. Removal is mainly mediated by apoptosis (no inflammation, no necrosis). RBCs, when they do not encounter a pathological condition, over their life span of 100–120 days remain intact although they shrink, do not undergo apoptosis but progressively become senescent, mainly due to cumulative oxidative stress.

Removal of intact RBCs with a daily turnover of $\sim 2 \times 10^{11}$, corresponding to ~ 20 g cell mass, is effectuated by increased exposure of otherwise cryptic structures such as spectrin, band 3, or α Gal epitopes. These exposed structures are recognized by low affinity, high avidity, C3-bearing NABs, which promote the efficient removal of intact senescent RBCs (130, 141, 142). Immunoaffinity adsorption by tri-saccharide columns of di- and tri-saccharide reacting NABs from IgG concentrates can eliminate anti-histo-blood A and B alloantibodies while it also eliminates α Gal and this might have a Janus effect. The face directed to the sun tells that adsorbing α Gal NABs reacting with altered and senescent self on RBC might prevent an increase in the IgG load of RBCs over the threshold level of relevant hemolysis in individuals at risk. The face directed to the dark indicate that adsorption of tissue homeostatic antibodies might deprive an IgG concentrate of potentially beneficial antibodies. Although they are NABs, tri-saccharide reacting antibodies can be induced by feeding bacteria bearing the corresponding carbohydrate epitopes (134). These inducible NABs are considered to participate in primary host defense. Other antibodies possibly involved in primary host defense are the anti- α Gal NABs. They show a broad specificity and can react with a number of related α Gal-terminated oligosaccharides, including those on bacteria (143). Thus, the immuno-adsorption of di- and tri-saccharide reacting NABs might diminish the potential of an IgG concentrate to mediate primary host defense. Therefore, when choosing affinity resins for immuno-adsorption, there might be some aspects worth to consider.

In summary, the principles of avoiding co-fractionation through cold-ethanol fractionation (144) versus immune-affinity removal of histo-blood group alloantibodies can have an impact on the presence of homeostatic and first-line defense antibodies. According to present knowledge, only resins coated with the corresponding tetra-saccharides can ascertain the selective removal of histo-blood group alloantibodies presumably involved in HA. Resins coated with the corresponding di- and tri-saccharides also remove blood group alloantibodies, however not selectively. Such resins in addition might remove a broad range of NABs present in IgG concentrates at relative high amounts. In the literature, the use of tri-saccharide-coated resin was reported (145–147). We have found no information available in the public domain indicating which type of resin is/will be used for reduction of the histo-blood group alloantibodies in large-scale fractionation of IgG. Furthermore, we suggest that the effect of reduction of anti-A and anti-B reacting antibodies by immune-affinity on the antibody repertoire of IgG concentrates can only be assessed by, e.g., using pathogens/commensals, which share the saccharide epitopes, that have been used to coat the affinity resins or alternatively by exposing senescent RBCs stripped off the IgGs coated *in vivo*. Finally, techniques are required, which allow detection of low affinity, high avidity NABs.

THROMBOSIS – FALLING INTO A DARK LUNAR CRATER

IVIG administration-related AEs, including thrombosis, have been extensively described (148). Thrombotic AEs are severe AEs and patients with risk factors require a special care. Reported average incidence of IVIG-induced thrombosis ranges from 3 to 13% (149). Recognized risk factors for IVIG-induced thrombosis



include male gender; age >60; diabetes; renal insufficiency, dyslipidemia; hypertension; immobility; coronary disease; pre-existing vascular disease, family history of early thromboembolic disease; atrial fibrillation, high-dose and high-speed IVIG infusions. IVIG-induced thrombosis is reported both as venous events such as thrombosis stroke, pulmonary embolism (PE), deep venous thrombosis (DVT), and arterial ischemia events such as myocardial infarct and stroke. The mechanisms leading to IVIG-associated thrombosis are still not completely clear; three main mechanisms have been proposed, emphasizing the role of an increased blood viscosity causing a hypercoagulable state (150), the role of anticardiolipin antibodies passively transferred through IVIG (151), and the role of factor XIa or other biologically highly active factors passively transferred via IgG concentrates, such as PKA. Avoiding activated coagulation factors in IgG concentrates starts with appropriate anticoagulation of donated blood/plasma, i.e., careful mixing of anticoagulant and sample over the whole donation process. Alterations in an established manufacturing process neglecting appropriate controls can also lead to increased risk of transmission of activated coagulation factors. High MW proteins passively transferred by IVIG are probably contributing to this phenomenon (152). In patients with other risk factors, such as vascular disease, the increase in blood viscosity can precipitate thromboembolic events. As elderly individuals are prone for such AEs, we like to point to the possibility of elevated altered/senescent self-reacting with infused homeostatic NABs being a possible factor facilitating thrombotic events as well. A relationship between IVIG administration and cerebral vasospasm has also been suggested by Sztajzel et al. (153); blood viscosity is a determinant for oxygen delivery to the tissues, and changes

in viscosity can lead to a reduction in cerebral or myocardial perfusion.

We systematically reviewed case reports related to IVIG-induced thrombosis from 1986 to 2014 (Figure 8). Literature search identified 35 articles containing reports concerning 65 patients (6, 24, 149, 154–183). When data were available, diagnosis, risk factors, the number of IVIG infusion prior to thrombosis event, and outcome have been indicated. Baseline characteristics of the patients are shown in Table 3. High-dose IVIG induced thromboembolic events in 59 patients at low to medium IVIG doses. Marie et al. (163) observed that the frequency and type of arterial events was inversely related to the time elapsed from IVIG infusion; almost 50% (23 versus 21 reports) of arterial ischemic events occurred within 12 h following IVIG, while about 75% of venous thrombosis occurred after more than 24 h. No correlation between number of infusions and occurrence of AE was observed. The main risk factors observed in this review were hypertension (19 cases, 33% of prevalence), previous vascular disease (18%), and dyslipidemia (17%). Average mortality for thrombotic events was 10% (arterial ischemia 9% versus venous thrombosis 11%, PE representing the main venous fatal event). Predicting IVIG-induced thrombosis is difficult. Risk factors should be assessed for each patient including instrumental exams when needed. Doppler ultrasound can be useful as early diagnostic tool for thrombosis or to detect the presence of abnormal blood flow especially after prolonged immobility. IVIG should be administered at low IR to reduce the risk. The administration of antiplatelet or anticoagulant prophylaxis was suggested in patients with several risk factors (162). However, thrombotic events have been reported even after several previous uncomplicated courses of treatment.

Table 3 | Falling into a deep lunar crater – Ig-induced thrombosis in recipients of polyvalent immunoglobulins.

Publication	Number of patients	Age	Diagnosis	Ig dosage	Predisposing factors	Number of IVIG infusion prior to thrombosis event	Thrombosis (arterial or venous)	Time from the last infusion	Outcome
Vinod et al. (154)	1	>65	Guillain-Barré	High		First	Arterial	72 h	Recovery
Sin et al. (157)	1	<65	Solid organ transplantation	High		First	Arterial	48 h	Recovery post-emergency renal transplant
Min et al. (24)	1	<65	CVID	Low (SCIG)	Hypercoagulability (oral contraceptive)	Several	Venous		nd
Rajabally et al. (149)	5	<65 (2), >65 (3)	CIDP	High	Diabetes (2), hypertension (2), immobility (4), coronary disease (2), arrhythmia (1)	First (3), several (2)	Arterial (3), venous (2)	14 days	Recovery (4), death (1)
Al-Riyami et al. (156)	1	11	ITP	High		Several	Venous	10 days	Recovery
Iroh et al. (155)	1	13	ITP	High	Estrogen treatment	First	Venous	12 h	Death
Barada et al. (158)	1	11	XLA	Low		Several	Venous	nd	nd
Lee et al. (159)	1	56	ITP	High		First	Venous	72 h	Recovery
White et al. (160)	1	43	Dermatomyositis	High		First	Arterial	2 h	Recovery
Feuillet et al. (161)	1	38	Multiple sclerosis	High	Oral contraceptives	Seventh	Venous	6 days	Recovery
Marie et al. (162)	2	51, 55	Polyarteritis nodosa (1), polymyositis (1)	High		Third, 15th	Venous (2)	2 h, 7 days	Recovery
Marie et al. (163)	6	76, 49, 63, 45, 64, 64	AHA (1), polymyositis (5)	High	Hypertension (3), hypercholesterolemia (3)	Second, sixth, several (4)	Venous (3), arterial (3)	2 days, 6 h (5)	Recovery
Geller et al. (165)	1	28	Streptococcal toxic shock syndrome	High		First	Venous	8 days	Recovery
Sheehan et al. (167)	1	43	<i>Pemphigus vulgaris</i>	High	Immobility, hypertension	First	Venous	16 days	Recovery
Hefer et al. (164)	1	85	ITP	High	Hypertension, chronic myelogenous leukemia	Second	Arterial	3 h	Recovery

(Continued)

Table 3 | Continued

Publication	Number of patients	Age	Diagnosis	Ig dosage	Predisposing factors	Number of IVIG infusion prior to thrombosis event	Thrombosis (arterial or venous)	Time from the last infusion	Outcome
Feuillet et al. (166)	1	38	Multiple sclerosis	High	Oral contraceptives	First	Venous	nd	Recovery
Vucic et al. (168)	7	57, 69, 75, 81, 79, 62, 80	CIPD (4), anti-MAG neuropathy (1), multifocal motor neuropathy (1)	High	Hypertension (3), hypercholesterolemia (3), previous stroke (2), arrhythmia (1)	Second (1), third (2), several (5)	Arterial (6), venous (1)	1 h (2), 2 days (3), 2 weeks (2)	Recovery (5)
Stamboulis et al. (169)	1	36	CIPD	High	Heavy smoker	First	Arterial	nd	Recovery
Katz et al. (170)	2	67, 65	<i>Pemphigus vulgaris</i> , dermatomyositis	High	Hypertension	Second, first	Arterial (1), venous (1)	6 h	Recovery
Zaidan et al. (171)	3	47, 65, 70	GBS (1), CIDP (2)	High	Hypercholesterolaemia (1), diabetes (2)	Third (1), several (2)	Arterial (3)	1 h (2), 1 day (1)	Recovery (2)
Brown et al. (172)	3	70, 91, 42	CVID	Low	Diabetes (1), myocardial infarction (2)		Arterial (3)	6 h	Recovery
Evangelou et al. (173)	1	54	CVID	Low	High platelets count	Several	Venous	24 h	nd
Emerson et al. (174)	2	54, 33	ITP, Evans Syndrome	High	Obesity (1)	Second	Arterial (2); venous (1)	2 h, 2 days	Death (1), recovery (1)
Alliot et al. (175)	1	63	ITP	High	Hypertension	Fifth	Venous	3 days	Death
Sherer et al. (176)	2			High			Venous		
Elkayam et al. (177)	4	60, 41, 67, 67	ITP, polymyositis, connective disease, CIPD	High	Hypercholesterolemia (2), hypertension (2)	several (3), first (1)	Arterial	4 days	Recovery
Go et al. (178)	1	52	ITP	High		First	Venous	1 day	Recovery
Turner et al. (179)	1	60	Miller-Fisher syndrome	High		Fifth	Arterial	5 days	Recovery
Harkness et al. (180)	1	40	CIDP	High	Hypercholesterolemia		Arterial and venous	7 days	Recovery

(Continued)

Table 3 | Continued

Publication	Number of patients	Age	Diagnosis	Ig dosage	Predisposing factors	Number of IVIG infusion prior to thrombosis event	Thrombosis (arterial or venous)	Time from the last infusion	Outcome
Paolini et al. (181)	1	78	ITP	High	Hypercholesterolemia	First	Arterial	7 days	Recovery
Rosenbaum et al. (182)	1	76	Miller-Fisher syndrome	High		First	Arterial	12 h	Recovery
Oh et al. (183)	1	17		High		Several	Venous	1 day	Recovery
Dalakas et al. (150)	2	62, 52		High		Second	Venous (1), arterial (2)	7 days, 2 days	Death (1), recovery (1)
Woodruff et al. (6)	4	72, 73, 62, 83	ITP	High	Hypertension (3), obesity (3), previous stroke (2), previous myocardial infarction (1)	Several (4)	Arterial (4)	2 h (3), 2 days (1)	Death (3)

Clinical and immunological characteristics of patients described in case reports. Numbers in parenthesis indicate the number of patients with the given condition.

In such cases, patients should be examined for signs and symptoms of thrombosis during each courses of IVIG.

Immunoglobulin G concentrates are widely acknowledged to offer a safe, high-dose, long-term therapy option for a variety of diseases. AEs occur rarely and mainly are mild to moderate. Deviations from this rule of thumb are addressed by authorities and the plasma fractionation industry to achieve corrections. Above, we have reviewed two types of AE which have shown elevated frequency in the near past. We tried to give some insights which might help in reducing frequencies of AEs bed side.

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