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Real-world data and real-world evidence in hematologic malignancies

Edited by
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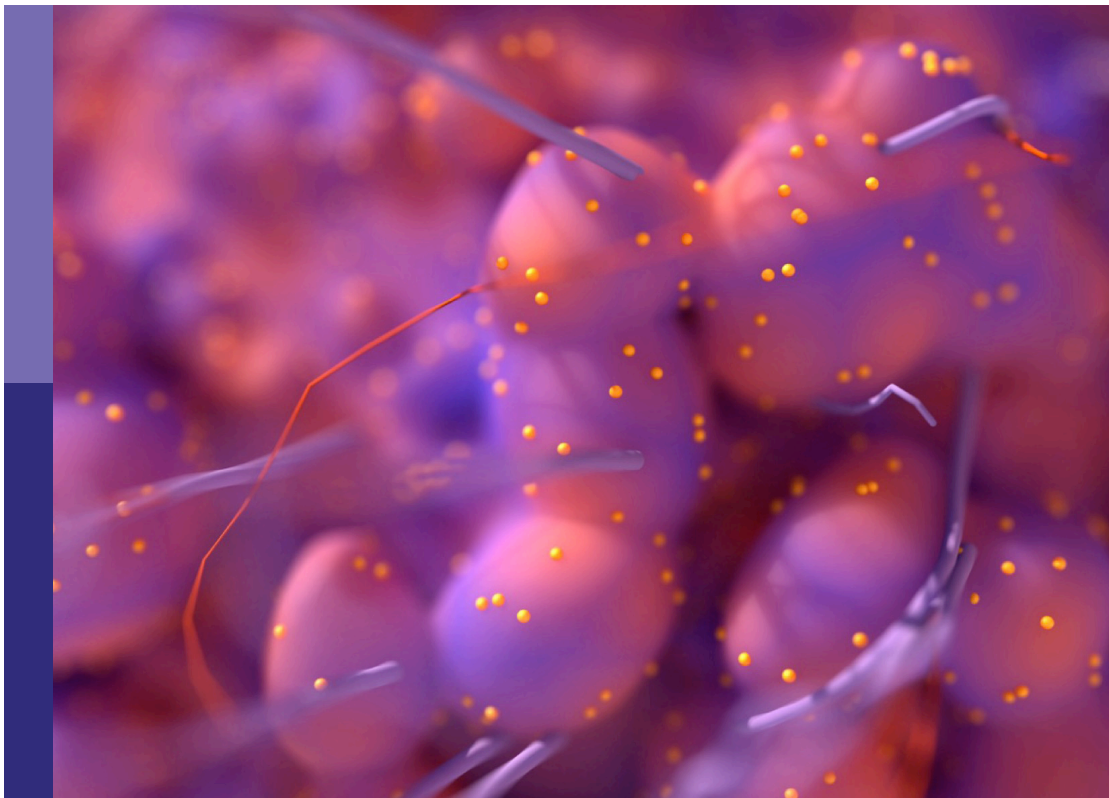

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Real-world data and real-world evidence in hematologic malignancies

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Table of contents

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Editorial: Real-world data and real-world evidence in hematologic malignancies

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KEYWORDS

real-world data, real-world evidence, hematological malignancies, clinical trials, real-life

Editorial on the Research Topic
Real-world data and real-world evidence in hematologic malignancies

Advances in the availability and analysis of real-world data (RWD) have substantially contributed to generate robust real-world evidence (RWE), thus supporting the development of recommendations/guidelines and regulatory decisions closer to real-life experience.

RWD and RWE are closely related but not interchangeable. RWD are data related to patient health status and/or the delivery of health care routinely collected from a variety of sources, processed and analyzed through advanced analytical methods such as data mining, machine learning, and artificial intelligence (1). RWE refers to the meaningful insights and conclusions extracted from RWD (2).

In 2023, clinical care guidelines and available treatments are changing so rapidly that making decisions based only on clinical trial data is becoming outdated in many areas, including hematology. Moreover, in the transplant and cellular therapy settings, clinical patient care is generally localized, practices may differ across countries and centers, generating interest towards harmonization (3). RWD is paving the way towards generating insights that can drive decisions in life sciences and healthcare research. Indeed, RWD can help validate findings from clinical trials, evaluating the effectiveness and safety of treatments, strategies, and programs in a real-world setting. This may be useful to identify patient subgroups that may benefit more from specific treatments. RWD can also support research and development, including the design of clinical trials and the identification of unmet healthcare needs. In this context, RWE generated from RWD may facilitate appropriate clinical decisions, recommendations, and healthcare planning. These outcomes can then be used for a variety of decisions and drive improvements in patient care, including more accurate diagnoses, better algorithms, and personalized treatments.

Indeed, RWD are expected to play an increasingly important role in healthcare research and decision-making in the years to come, as witnessed by this Frontiers in Immunology Research Topic on "*RWD and RWE in hematological malignancies*".

Twenty articles have been accepted and included in this Research Topic. Twelve are published in the form of Original Research Articles, 5 are Case Reports, 2 Brief Research Reports and 1 is a Systematic Review.

Among the Research Articles, 6 focus on Acute Leukemias, 3 on Lymphomas, and 3 on Multiple Myeloma. Four out of the 6 articles on Leukemias are focused mainly on the biology of the disease: this is a clear evidence that the biology study of these diseases is still of major interest in the community of Hematologists. In particular, these studies focus on relatively rare entities and/or peculiar biological findings: early T-precursor Acute Lymphoblastic Leukemias (ETP-ALL; Chen et al.), Myeloid Sarcoma (MS; Xing et al.), bone marrow fibrosis in acute myeloid leukemia (AML; Zhang et al.), and lipid profile in AML (Bai et al.). In particular, these RWD highlight some crucial aspects: the prognosis of ETP-ALL following allogeneic stem cell transplantation seems similar to that of non-ETP ALL (Chen et al.), the clinical features and the prognosis of patients with MS involving hematopoietic vs non-hematopoietic sites is different (Xing et al.), bone marrow fibrosis is an independent adverse prognostic factor in AML patients (Zhang et al.), and lipid profile together with clinical characteristics of AML patients may improve patients' prognostication (Bai et al.). Although these findings should be confirmed in larger and possibly prospective studies, they represent interesting aspects to be considered in the clinical management of our patients. Two other Research Papers on AML are clinically oriented and covers peculiar aspects. Zhang et al. explore the effects of intensive chemotherapy on megakaryoblast AML nonrelated to Down's syndrome: although the prognosis of this peculiar entity remains poor, intensive chemotherapy may have some advantages in terms of long term survival. The other research article on AML focuses on a classical dilemma in the field of AML: is standard dose cytarabine-based consolidation chemotherapy superior to high dose? To address this issue, Wang et al. analyze a series of 183 patients younger than 60 years, suggesting that consolidation with high-dose cytarabine leads to superior outcomes, particularly in intermediate-risk group according to the 2022 ELN classification.

Moving to the three Research Articles on Lymphomas, it is notable that they are all dedicated to Central Nervous System Lymphomas (CNSL), suggesting that this group of lymphomas represents a clear hot topic of research and study. Wu et al. propose a prognostic scoring model, including lesion number, beta-2 microglobulin, systemic inflammation response index and Karnofsky performance status. This score has been tested on a cohort of 122 patients with PCNSL, 72 of whom were used to develop the model and 50 of whom were used as a validation set. Three groups of patients with different long-term outcome are identified, and this is reproducible across different treatments (chemotherapy vs Bruton's tyrosine kinase inhibitors) and in elderly patients. The topic of the best treatment for PCNSL (chemotherapy vs. radiotherapy) is covered in the manuscript by Yang et al., in which 105 relapsed/refractory PCNSL are addressed to salvage treatment with chemo or radiotherapy. Interestingly, the overall response rate is higher in patients treated with radiotherapy both in the relapsed and in the refractory group. Moreover, age, cerebral spinal fluid protein level and ocular involvement are factors associated with impaired outcome. Overall, these data clearly suggest that the prognosis of PCNSL is influenced by several different biological and clinical factors and that conventional therapy (chemo and radiotherapy) still play a major role in the management of the advanced phase of the disease. The topic of central nervous system (CNS) involvement has been explored also in the manuscript by Jeong et al. In particular they focus on CNS localization in diffuse large B cell lymphomas and explore the feasibility and efficacy of autologous stem cell transplantation (ASCT) following high-dose methotrexate reinduction. This treatment algorithm was safely performed on 43 patients. After ASCT, 17 patients (39%) maintain the complete remission (median follow up 14.7 months) suggesting that this treatment option is feasible. This result is of interest, because the salvage treatment of patients with CNS involvement is an unmet clinical need, as CAR-T cell therapy, at present, is still a matter of debate.

The three Research articles on Multiple Myeloma covers distinct areas. The adverse prognostic impact of chromosome 1q21 gain in patients treated with bortezomib-based therapy is underlined in the manuscript by Liu et al. Xu et al. cover a very important aspect of multiple myeloma treatment in the era of new molecular target drugs: the socioeconomic status strongly influences survival disparities, suggesting that non-Hispanic, white, married, insured and urban patients have an increasing linear trend in survival benefits. Finally, Bao et al. suggest the usefulness of a machine learning tool to predict survival in elderly patients with multiple myeloma without genomic data and showed that patients who received an immunomodulator agent as maintenance had the best survival.

The two brief research reports are highly interesting. Morin et al. cover the topic of post-allogeneic stem cell maintenance (allo-SCT) with Sorafenib in FLT3-ITD positive AML. 30 patients receive post-transplant maintenance and data on long-term survival are intriguing: after 12 months of median follow up, median overall survival is not reached. The topic of post-transplant maintenance is of high interest now that we have molecular target drugs, such as FLT3 inhibitors, but also azacitidine and venetoclax. It is highly probable that the scenario of the next future will change, and that the great majority of AML patients will receive an individualized maintenance following allo-SCT. The other brief Research Report covers the topic of defibrotide prophylaxis of sinusoidal obstruction syndrome (SOS) in adults submitted to allo-SCT following Inotuzumab Ozogamicin-based treatment (Giglio et al.). Seven patients were treated, four of whom received a double-alkylator based conditioning regimen. Three patients developed fatal SOS and all the three patients received the double-alkylator conditioning regimen. Several data suggest that defibrotide plays a crucial role in the treatment of SOS, but further data are warranted to better define its role in prophylaxis.

This Research Topic also includes an interesting review, focusing on agents contributing to secondary immunodeficiency development in patients with chronic lymphoproliferative disorders (Jolles et al.). As expected, multiple myeloma patients treated with monoclonal antibodies, as well as patients with chronic lymphocytic leukemia and non-Hodgkin's lymphomas treated with a tyrosine kinase inhibitors are those at major risk of developing infectious complications. Moreover, the Authors reported a global under-

reporting of hypogammaglobulinemia and lymphocytopenia before and during therapies: this suggests that a higher attention should be addressed to this aspect, as infectious complications represent a major cause of morbidity and mortality, as well as healthcare costs.

Finally, 5 case reports are included in the Research Topic (Giglio et al., Pederzolli et al., Wang et al., Wang et al., and Ji et al.). Without going into details (please refers to the electronic links), all these reports cover interesting and peculiar clinical situations: ponatinib as bridge to CAR-T (Giglio et al.), intravitreal methotrexate in ocular acute lymphoblastic leukemia (Pederzolli et al.), Zanubrutinib-induced dermatological toxicity (Wang et al.), association of acute promyelocytic leukemia and metachronous multiple primary carcinoma (Wang et al.) and Langerhans cell histiocytosis of the thymus and heart (Ji et al.). Although rare entities, these case reports represent valid tools for clinicians who may be involved in the management of patients with similar conditions.

In conclusion, we think that this Research Topic helped us to collect several interesting articles on real-life studies, covering different aspects of different hematological diseases. Taken together these data suggest that real life is still an important way to collect informative data useful for the design of prospective, controlled trials, that are fundamental to confirm any preliminary result.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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

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CASE REPORT
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Langerhans Cell Histiocytosis Involving the Thymus and Heart With Simultaneous Thymoma: A Case Report

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Langerhans cell histiocytosis (LCH) is a rare disease characterized by clonal expansion of CD1a+/CD207+ cells in lesions. The most frequent sites involved are bone and, less commonly, lymph nodes, lungs, and skin. The thymus or heart is rarely involved with LCH. In this case, we present a 73-year-old woman with a mediastinal mass. Histopathology after thymectomy identified this mass as type AB thymoma; notably, subsequent immunohistochemical tests showed lesions of LCH scattered in the region of thymoma. 18-Fluorodeoxyglucose PET/CT (18-FDG-PET/CT) was performed to make an overall assessment of the extent of this disease, which demonstrated suspicious cardiac involvement of LCH. This report highlights the importance of differentiating abnormalities of the thymus or mediastinal mass from LCH and the necessity of comprehensive evaluation for patients with LCH.

Keywords: Langerhans cell histiocytosis, thymoma, thymus, heart, ^{18}FDG-PET/CT

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INTRODUCTION

Langerhans cell histiocytosis (LCH) is a rare disease characterized by clonal expansion of CD1a+/CD207+ cells, which often affects children and young adults (1). Thymoma, as the most common neoplasm in the anterior mediastinum, originates from epithelial cells in the thymus regardless of the presence or abundance of lymphoid component (2). It is extremely rare for two lesions of LCH and thymoma to occur in the same organ. Herein, we present a 73-year-old woman diagnosed with thymic LCH concurrent with type AB thymoma after thymectomy, with suspicious cardiac involvement of LCH demonstrated by 18-fluorodeoxyglucose PET/CT (^{18}FDG-PET/CT). We believe that LCH should be included in the differential diagnosis of thymic abnormalities or mediastinal masses.

CASE PRESENTATION

A 73-year-old woman presented with chest tightness without evidence of myasthenia gravis and other complaints, such as fever, weight loss, pain, mass, skin lesion, and cough. She had no previous history of carcinoma or other comorbidities. Physical examination showed no abnormal signs. Her blood tests revealed no apparent abnormalities, including blood cell count, renal and liver function, and tumor markers (NSE, CYFRA21-1, CA15-3, CA19-9, and CA12-5). Chest CT showed an

irregular mass (51 mm × 35 mm) in the anterior mediastinum, with inhomogeneous enhancement on contrast-enhanced CT (**Figure 1**). A mediastinal tumor was highly suspected, and a biopsy was considered. After the physician communicated with this patient and her family, the patient had chosen direct thymectomy, not biopsy. Histopathology identified this mass as a type AB thymoma composed of lymphocyte-poor areas and lymphocyte-rich areas at low magnification. Small nodules of mononuclear cells and eosinophils were surrounded by lesions of thymoma, and immunohistochemical tests showed that these nodules were positive for S100, CD1a, and Langerin (**Figure 2**). The genomic analysis revealed no $BRAF^{V600E}$ mutation. Therefore, thymic LCH co-occurrence with type AB thymoma was considered. This patient also received radiotherapy after thymectomy (GTV5500cGy/25f, CTV5000cGy/25f). ^{18}F-FDG-PET/CT was performed (**Figure 3**) and showed abnormal hypermetabolic regions in the chest, left femur, and right thigh, with the following maximum standardized uptake value (SUVmax): surgery-related changes of the sternum, 4.08; right atrial appendage, 7.08; a round mass with a size of 37 mm × 31 mm at the right upper thigh, 3.20; and left upper femur, 3.98. The patient underwent a biopsy for the mass of the right upper thigh in another hospital, of which immunohistochemical tests also proved the involvement of LCH. We could not be able to

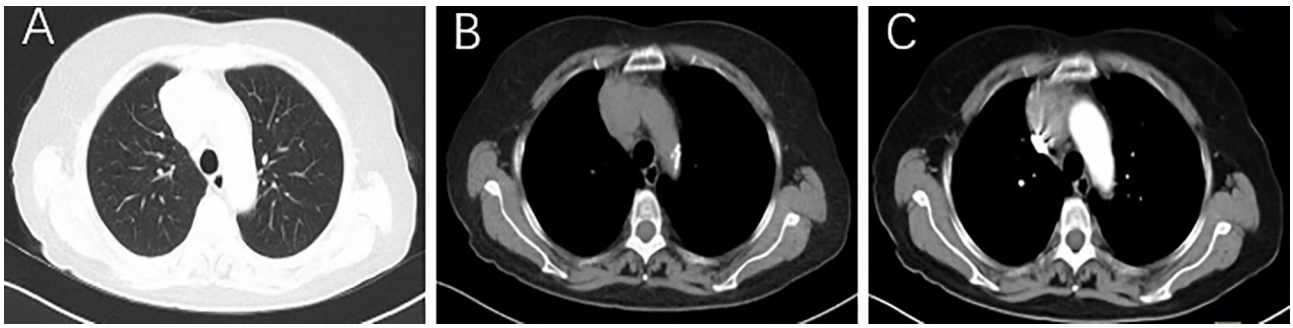

FIGURE 1 | Lung **(A)** and mediastinal window **(B)** of axial CT images show an irregular mass (about 5.1 × 3.5 cm) in the right anterior upper mediastinum, with inhomogeneous enhancement on contrast-enhanced CT **(C)**.

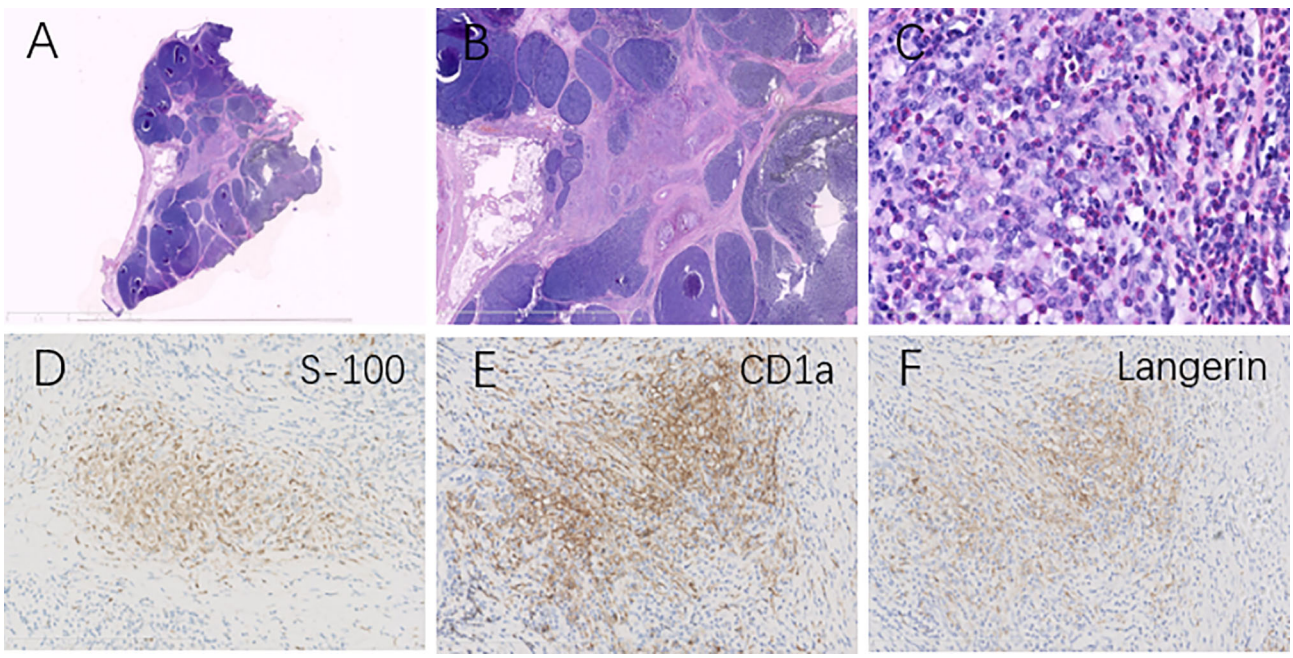

FIGURE 2 | Histopathological analysis revealed type AB thymoma composed of a lymphocyte-poor area and lymphocyte-rich area at low magnification **(A, B)**. Clusters of mononuclear cells and eosinophils **(C)** were surrounded by lesions of thymoma (H&E, magnification ×400), and immunohistochemical tests showed these clusters were positive for S100 **(D)**, CD1a **(E)**, and Langerin **(F)**, identifying the diagnosis of Langerhans cell histiocytosis in the thymus.

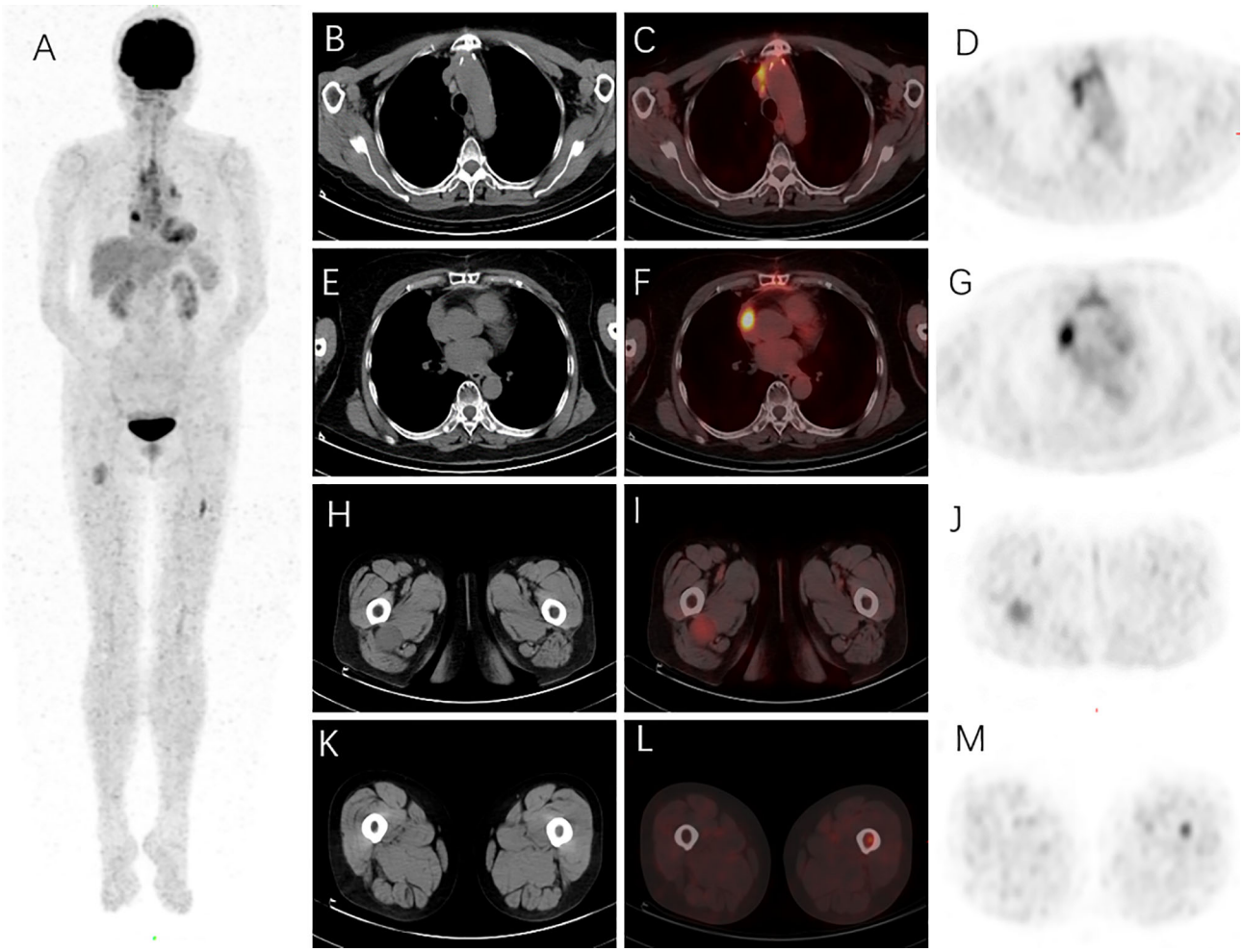

FIGURE 3 | ^{18}F-FDG PET/CT was performed on this patient to evaluate the extent of the Langerhans cell histiocytosis (LCH). The maximum intensity projection (MIP) revealed increased ^{18}F-FDG uptake in the chest, left femur, and right thigh regions **(A)**. Surgery-related changes of the sternum on the mediastinal window of axial CT **(B)** were shown with diffusely high ^{18}F-FDG uptake and SUVmax as 4.08 on axial PET/CT fusion **(C)** and PET images **(D)**. An ^{18}F-FDG-avid lesion in the right atrial appendage was found with SUVmax as 7.08 on axial PET/CT fusion **(F)** and PET images **(G)**, and no specific abnormal density was found in the corresponding region on the mediastinal window of axial CT image **(E)**. A round mass (about 3.7 × 3.1 cm) in the right upper thigh on the soft tissue window of axial CT **(H)** was revealed with SUVmax as 3.20 on axial PET/CT fusion **(I)** and PET images **(J)**. A hypermetabolic lesion in the left upper femur was found with SUVmax as 3.98 on axial PET/CT fusion **(L)** and PET images **(M)**; bone marrow density was slightly increased **(K)**.

define the nature of lesions in the left upper femur and right atrial appendage because of difficulty in the biopsy. Eighteen months after thymectomy, there were no signs of recurrence on the chest contrast-enhanced CT, and the mass of the right upper thigh remains the same size. She also did not complain of symptoms of heart failure and bony pain.

DISCUSSION

The annual incidence of LCH is 5–9 cases per million in children older than 15 years and 1 case per million in patients older than 15 years (3). Thymoma is also a rare tumor of the mediastinum with an annual age-adjusted incidence of 0.9–2.3 cases per million (2). Therefore, thymoma and LCH rarely occur in the same organ.

LCH commonly affects the bone (80%), skin (33%), pituitary gland (25%), and lungs (15%) (3). The thymus is rarely involved (4). A retrospective study reported that 1.4% of pediatric LCH cases were found to have thymic involvement (5). However, data about thymic LCH in adults are lacking. Patients with thymic LCH could be asymptomatic; some patients are accidentally diagnosed by imaging examination, and others have undergone thymectomy during cardiothoracic surgery and were identified as thymic LCH by pathology (4, 6, 7). In addition, adults suffering from thymic LCH can also present with myasthenia gravis (4), which did not occur in our case. Previous literature had demonstrated the status of thymic LCH combined with lymphoid hyperplasia but no features of thymoma (8).

Researchers previously suggested routine imaging screening of the thymus in patients with LCH, especially in young children (5). To some degree, this abnormality of the thymus promotes early diagnosis in our case. We recommend routine screening of the biomarkers of LCH in thymic samples to investigate the presence of LCH.

Cardiac lesions are exceedingly rare in patients with LCH (9). There are no available data for the prevalence of cardiac involvement of LCH. Four case reports have shown the infiltration of the septum (10) and pericardium (11–13) by LCH. In our case, the hypermetabolic lesions in the right atrial appendage and left proximal femur demonstrated by ^{18}F-FDG PET/CT were considered neoplastic changes. Due to the high risk of cardiac biopsy, we did not perform it on this patient. Based on the pathological identification of LCH in two regions for this patient, we highly suspect that the hypermetabolic lesions of the heart and femur were caused by LCH. It is true that we must consider the possibility of mixed LCH and Erdheim-Chester disease (ECD) when a patient with LCH has suspicious cardiac lesions (9). However, pericardial infiltration and effusion, sometimes complicated by cardiac tamponade, and pseudotumor of the right atrioventricular groove are the most common regions of cardiac involvement with ECD (9), which is not in line with our case. Due to the steel wire retention sutures in the chest during thoracotomy, cardiac magnetic resonance to help us define the nature of the cardiac lesion in this case cannot be performed on this patient. In most cases, the diagnosis and evaluation of LCH are challenging and delayed because clinical findings are non-specific and complicated. Late diagnosis and assessment could exacerbate LCH and lead to sequelae, such as disability and malformation associated with pathological fracture and growth retardation caused by the progression of LCH in the pituitary and even death (1). Besides, initial evaluation and diagnosis of LCH depend on full-body screening, and all lesions are FDG-avid (9, 14). Therefore ^{18}F-FDG PET/CT could be a very useful tool to make a comprehensive evaluation in patients with LCH, guiding therapy for this disease (14, 15).

Currently, although treatment of LCH is not well established, which depends on lesions' location and number, therapy strategies have been generally agreed upon (1, 16). Single-system lesion confined to only one single site needs local medication, curettage, or observation, such as skin and bone lesion (17). Multiple lesions in single-system LCH (SS-LCH) or multisystem LCH (MS-LCH) require systematic chemotherapy (1, 16). Along with the discovery of the mutated MAPK pathway in the pathogenesis of LCH, targeted therapy by BRAF or MEK inhibitors has become a novel therapeutic strategy for patients with LCH (18). Timely and proper treatment, which prevents the invasion of disease into risky organs (bone marrow, liver, and spleen), could promote a good prognosis (1, 16).

In conclusion, we report an "incidental" thymic LCH combined with thymoma in an old woman with highly suspicious cardiac involvement of LCH and emphasize the necessity of differentiating abnormalities of the thymus from LCH and comprehensive evaluation for patients with LCH, if possible, by PET/CT.

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.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the West China Hospital of Sichuan University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors have made a significant contribution to this paper. TJ and YZ drafted the manuscript. DC revised the manuscript. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Metachronous Multiple Primary Carcinoma With Acute Promyelocytic Leukemia: 2 Cases Report and Literature Review

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The co-occurrence of multiple primary cancers with hematological malignancies is uncommon, and acute promyelocytic leukemia (APL) with MPC is even rarer, with only a few cases reported in the literature. Herein, we introduce the diagnosis and treatment of 2 cases of MPC complicated with APL in our hospital and review the relevant literature. Both patients were primary solid tumor patients and were treated with surgery and chemotherapy, and had stable disease (SD). However, more than 1 year after the primary tumor was diagnosed, clinical symptoms were found and APL was diagnosed. Both patients received standard remission-induction therapy, but unfortunately died in the short term due to hemorrhagic complications. In conclusion, treatment of hematological neoplasms, especially acute leukemia combined with multiple primary cancers, is challenging. The prognostic factors and survival analysis of MPC patients with combined APL still need further clinical research and analysis.

Keywords: leukemia, promyelocytic, acute, solid tumor, multiple primary carcinomas, diagnosis, prognosis

INTRODUCTION

Multiple primary cancer (MPC) refers to the occurrence of two or more independent primary malignancies in one or more organs of the same patient, either simultaneously or sequentially. Multiple primary cancers occurring within 6 months of each other are called synchronous carcinoma (SC), while multiple primary cancers occurring more than 6 months apart are called metachronous carcinoma (MC). The diagnostic criteria are: each tumor is histologically malignant; each tumor has its pathological pattern; and there are ≥2 lesions, clearly excluding metastases or recurrence. The combination of multiple primary cancers with hematological tumors is rare, with acute promyelocytic leukemia (APL) combined with multiple primary cancer (MPC) being even more rare, with only a few cases reported in the literature. To discuss the diagnosis, treatment and prognosis of MPC in combination with APL, the data of two patients with multiple primary cancer in combination with APL admitted to our hospital are summarized and analyzed.

CASE DESCRIPTION

Case 1 Male, 51 years old, presented to Gansu Cancer Hospital in December 2017 with a clear diagnosis of lung adenocarcinoma due to cough and shortness of breath, underwent right upper lobe lung resection and was given pemetrexed + cisplatin chemotherapy for 6 cycles after surgery, followed by continuous oral gefitinib treatment until this admission. The patient was reviewed several times during this period and the clinical outcome was evaluated as SD. In mid-June 2019 thepatient had frequent gingival bleeding and blood blisters in the buccal mucosa on both sides of the mouth and visited our department on 20 June 2019. Routine blood tests were performed: white blood cells 26.98×109/L, neutrophil count 1.66×109/L, red blood cells 4.84 1012/L, hemoglobin 148 g/L, and platelets 10 x 109/L. Bone marrow aspiration smear: the bone marrow proliferation is obviously active; the granulocyte lineage is abnormally proliferated, 87.5% of the nucleated cells, of which 76.5% are early granulocytes with increased granules, the cells are of different sizes, the nuclei are of various shapes, the nuclei are twisted and folded, small and dense anilinophilic blue granules are seen in the pulp, Auer vesicles in the shape of firewood bundles are easily seen, meganuclei are occasionally seen, platelets are single and rare; blood picture: leukocytes are increased, the early The diagnosis is acute promyelocytic leukemia (APL). Karyotype: 46,XY,t(15;17)(q24;q21),add(18)(q23) [10]. Quantitative PML-RARα fusion gene test: positive, the copy number of PML-RARα fusion gene: 65132 copies. The diagnosis was "acute promyelocytic leukemia (APL)" (**Figure 1**). Treatment regimens for patient was developed according to the 2019 European LeukaemiaNet (ELN)guidelines (1). Retinoic acid 20 mg orally 2 times/day was given from 22 June 2019, and 5 tablets (1.35 g) of compound Huang Dai were added 3 times/day orally from 25 June to induce remission, while intermittent transfusions of plasma, cold precipitation, fibrinogen, platelet supplementation coagulation factors and platelets, and anti-infective, nutritional support, hydration and alkalization, and correction of electrolyte disturbances. On 26 June 2019, the patient developed nausea and vomiting, with 300 ml of stomach contents, followed by confusion and unconsciousness. Emergency cranial CT showed that the right frontoparietal and left frontal lobes had a cerebral hemorrhage, resulting in a local left shift of the midline structures and a small amount of hemorrhage in the subarachnoid space (**Figure 1**). The patient did not recover consciousness and was in a coma. The patient's family gave up treatment and discharged him from the hospital.

Case 2, male, 52 years old, the patient visited our hospital in May 2019 with gastric discomfort and was diagnosed with "gastric cancer" by electronic gastroscopy. The postoperative pathological examination (170259) showed: ulcerated hypofractionated adenocarcinoma of the gastric body, Lauren's staging: diffuse; tumor size 4.5×3 cm; cancerous tissue infiltrated the plasma layer to the extra-plasma fatty tissue, nerve invasion; cancerous thrombus formation in the lymphatic vessels, no clear cancerous thrombus in the vessels. Definite cancer thrombus, no cancerous tissue was observed in the upper and lower cut margins of the specimen and in the other cut margin sent for examination, large omentum (-), cancer metastases were noticed in the regional lymph nodes (0/44), of which (group 1) lymph nodes (0/7), (group 2) lymph nodes (0/4), (group 3) lymph nodes (0/3), (group 4) lymph nodes (0/4), (group 5) lymph nodes (0/0), (group 6) lymph nodes (0/3), (group 7) lymph nodes (0/5), (group 8a) lymph nodes (0/5), (group 9) lymph nodes (0/3), (group 11) lymph nodes (0/0), (group 12) lymph nodes (0/0); immunohistochemistry: P53 (40% positive), P-GP (-), GSTπ (++), TopoII (++), Ki-67 (80% positive), TS (-, C erbB-2 (-) (**Figure 2**). The patient was treated with SOX regimen chemotherapy (oxaliplatin 200mg IV d1, tegafur 60mg oral bid d1-14) for 3 cycles and XELOX regimen (oxaliplatin 200mg IV d1, capecitabine 1.5g oral bid d1-14) for 3 cycles. The patient's blood count showed 13.34×109/L white blood cells, 3.84×109/L

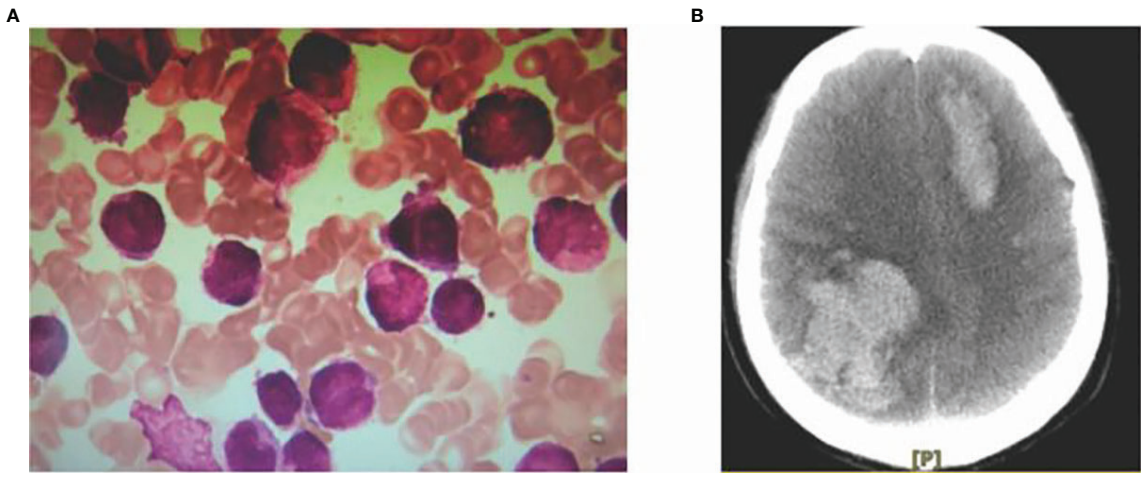

FIGURE 1 | Myeloid morphology **(A)** and CT signs of intracranial hemorrhage in acute promyelocytic leukemia **(B)**.

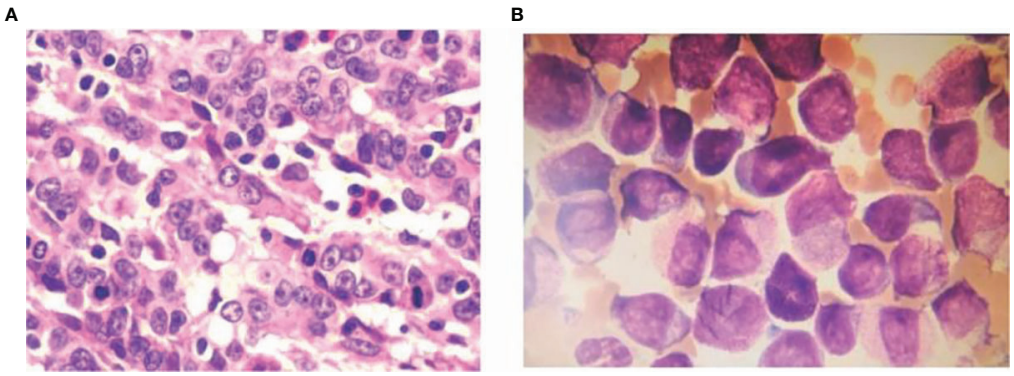

FIGURE 2 | Ulcerated hypofractionated adenocarcinoma of the lesser curvature of the gastric body HE staining 10×40 **(A)** Examination (170259) shows: ulcerated hypofractionated adenocarcinoma of the lesser curvature of the gastric body, Lauren's staging: diffuse; tumor size 4.5×3 cm. Cancerous tissue infiltrated the plasma layer to the extra-plasma fatty tissue, nerve invasion; cancerous thrombus formation in the lymphatic vessels, no clear cancerous thrombus in the blood vessels; no cancerous tissue was seen in the upper and lower cut edges of the specimen and another sent for examination. No cancerous tissue was seen in the cut margins, the greater omentum (-), regional lymph nodes (0/44) were seen to be metastatic, including (group 1) lymph nodes (0/7), (group 2) lymph nodes (0/4), (group 3) lymph nodes (0/3), (group 4) lymph nodes (0/4), (group 5) lymph nodes (0/0), (group 6) lymph nodes (0/3), (group 7) lymph nodes (0/5), (group 8a) lymph nodes (0/5), (group 9) lymph nodes (0/3), (group 11) lymph nodes (0/0), (group 12) lymph nodes (0/0); immunohistochemistry: P53 (40% positive), P-gp(-), GSTπ (++), TopoII (++), Ki-67 (80% positive), TS (-, C-erbB-2 (-). and bone marrow smear **(B)**.

neutrophils, 3.87×1012/L red blood cells, 121 g/L hemoglobin and 34×109/L platelets. bone marrow aspiration results suggested acute promyelocytic leukemia (APL). The flow results were consistent with an acute myeloid leukemia immunophenotype with a high probability of APL. The fusion gene was positive for PML-RARαS subtype (bcr-3) (+) with positive WT1 expression. The diagnosis of "acute promyelocytic leukemia (APL)" was confirmed and the patient was given vincristine 20 mg orally twice/day from 4 December 2020 and augmented with cytarabine 100 mg IV once/day from 7 December to induce remission. The above treatment regimens were determined according to the European LeukemiaNet (ELN) 2019 (1). At 20:30 on 8 December 2020, the patient vomited about 30 ml blood and did not respond to calls. The patient was checked for bilateral pupils about 2 mm, blunted reflex to light and cyanotic petechiae in the left eye sockets. Resuscitation treatment such as hemostasis and dehydration were given, the patient vomited blood again in an amount of about 100 ml. The patient was comatose, sigh-like breathing, bilateral pupils of 2 mm, blunted reflex to light, heart rate of 65 beats per heart rate of 65 beats/min, blood pressure of 95/50mmHg, oxygen saturation between 65% and 88%. After explaining his condition to his family, the patient was discharged after his family refused further resuscitation and the patient died the night after discharge. Summary of 2 cases of MPC combined with APL see **Table 1**.

DISCUSSION

Multiple primary carcinomas mainly occur in organs with similar tissue types, such as the upper respiratory tract, upper gastrointestinal tract and genitourinary system. The incidence of multiple primary carcinomas in combination with solid tumors in the hematologic system is rare, with only 0.1% reported by Xu Hao et al. (2) in China and 0.5% reported by Cuit et al. (3) abroad. Moertel et al. (4) reported only 9 cases of acute myeloid leukemia (AML) among 194 patients with multiple primary cancers with haematological malignancies, all of which were non-APL subtypes. Xie Xiaoyan et al. (5) reported 6 cases of solid tumors combined with acute leukemia, including 2 cases with M2, 1 case each with M5, M3 and M4, and 1 case with AML that could not be classified (**Table 2**).

TABLE 1 | Summary of MPC in 2 cases with combined APL.

Case		1	2
Solid tumor	Type	Adenocarcinoma of the lung	Hypofractionated adenocarcinoma of the stomach
	Treatment modality	Surgery + chemotherapy	Surgery + chemotherapy
	Chemotherapy drug	Pemetrexed + cisplatin, gefitinib	Oxaliplatin + Tegeo, Oxaliplatin + Capecitabine
Diagnosis of APL		18 months after treatment	17 months after treatment
Diagnostic basis		MICM	MICM
Cytogenetic characteristics		t (15;17) (q24;q21),add (18) (q23)	t (15;17) (q24;q21)
Molecular characteristics		PML-RARα fusion gene: positive	PML-RARαS subtype (bcr-3) (+) with positive WT1 expression.
Therapeutic regimen		Retinoic acid, compound Huang Dai	Retinoic acid, cytarabine
APL survival time		15days	15days

TABLE 2 | Clinical profile of patients with MPC combined with APL reported in the literature, 2000-2020.

Author	Year	NO.	Age	Gender	Solid tumours			APLTreatment		Prognosis
					Part	Pathology	Staging	Treatment		
Mi Rui Hua et al. (6)	2020	2	58	Female	Breast	Invasive ductal carcinoma	Stage II	Surgery + chemotherapy	Tretinoin 、As2O3、IA、HA、MA、DA	Stable follow-up visits
			52	Female	Esophagus	Squamous cell carcinoma	Stage I	Surgery	Tretinoin、As2O3、IA、HA、ID-Ara-cx2	Stable follow-up visits
ShaLiu, et al. (7)	2019	1	56	Male	Esophagus	Squamous cell carcinoma	Stage IIIB	Surgery + chemotherapy + radiotherapy	Tretinoin、As2O3	Died
Wu Zhijun et al. (8)	2009	1	54	Female	Ovarian	Mucinous cystic adenocarcinoma	Stage III C	Surgery + chemotherapy	Tretinoin	Died
Li Weibin et al. (9)	2002	1	55	Male	Gastric	–	–	Surgery + chemotherapy	–	Died

HA high trichostatin (HHT) in combination with cytarabine (Ara-C); ID-Ara-C is medium-dose cytarabine; MA is mitoxantrone in combination with cytarabine; As2O3 is arsenic trioxide; IA is idarubicin (IDA) in combination with cytarabine; DA is erythromycin (DNR) in combination with cytarabine.

It is well documented that the pathogenesis of MPC is multifaceted with genetic abnormalities, regional theories of carcinogenesis, infections, therapeutic factors, tumor immunity and *in vivo* hormones. It is well established that radiation therapy can lead to secondary tumourigenesis, especially exposure to brain, thyroid, breast, skin, bone and soft tissue. Systemic anti-tumor treatments such as chemotherapy, hormonal therapy and immunotherapy may increase the occurrence of multiple primary tumors. The treatment of solid tumors, in addition to surgery, mostly adopts integrated treatment modes such as chemotherapy and radiotherapy. With the use of cytotoxic drugs such as alkylating agents or the prolongation of radiotherapy, resulting in damage to normal cells of the body and affecting DNA repair, all may increase the prevalence of hematological tumors, especially leukemia. A report by Wang Xiaojiao et al. (10) in 2019 indicated that the incidence of treatment-related leukemia in patients with breast cancer using alkylating agents was on the rise. Literature reported by Yam et al. (11) abroad in 2018 indicated that the use of alkylating agents and anthracyclines for a longer time in the treatment of malignancies may have an increased risk for AML. The combined use of alkylating agents and anthracyclines further increases the proportion of patients with solid tumors secondary to myelodysplastic syndrome (MDS) or AML if local radiotherapy is also used (12). The alkyl group of the alkylating agent is capable of forming covalent bonds with biomolecules. When the alkylating agent binds DNA, it causes strand breaks and cross-linking. Topoisomerase II inhibitors prevent DNA forming double strands, leading to the accumulation of damaged DNA and inducing the formation of free radicals that further break DNA strands. This damage may also lead to genetic changes that predispose patients to MDS and AML. The risk of multiple primary solid tumors is associated with radiotherapy (RT) and/or alkylating agent exposure, and the incidence increases over time without a plateau (13). Alkylating agents are known to have leukemogenic effects and alkylating agents or radiotherapy-associated AML are now included as a separate subtype in the World Health Organization (WHO) staging criteria for hematological neoplasms (14). MORTON et al. reported that the use of certain alkylating agents, topoisomerase II inhibitors, and platinum-based drugs, often cause fatal chemotherapy complications such as AML or MDS. Alkylating agent-associated AML is usually diagnosed 3 to 7 years after treatment of the etiology. Chromosome 5 and/or 7 abnormalities were more common in cytogenetic analysis of AML patients (15). In contrast, AML associated with topoisomerase II inhibitors has a short incubation period and is typically presented as a translocation abnormality of 11q23, 21q22, or other chromosomes (16). These mutations appear to regulate transcription of genes critical to myeloid cell differentiation, leading to abnormal fusion of chromosomes (17). It has been reported that germline mutations with BRCA 1 or BRCA 2 may be a predisposition factor for AML secondary to solid tumors, as these mutations will result in dysfunctional proteins involved in error-free repair of DNA double-strand breaks (18–20). In the SEER-Medicare database, the use of known leukemogenic drugs in initial chemotherapy, especially platinum compounds, has increased substantially since 2000, especially in gastrointestinal cancers (oesophageal, gastric, colon, and rectal cancers) (21). In this paper, platinum-based agents were used in two patients with solid tumors, one with 6 cycles of pemetrexed + cisplatin and the other with 3 cycles of oxaliplatin combined with capecitabine and 3 cycles of oxaliplatin combined with tegafur. Due to the short survival time of the two patients after APL diagnosis, no further gene mutation test was conducted to confirm whether the patients' APL and solid tumor had the genetic susceptibility as described above. In addition to treatment-related factors such as chemotherapy and radiotherapy, patients' own lifestyle habits such as smoking and alcohol consumption, viral infections and immune deficiencies are common factors contributing to the development of multiple primary cancers, and whether AML or APL correlates with the development of multiple primary cancers needs to be further investigated in large clinical trials.

According to the literature (22), the treatment of MPC generally adheres to the following principles: surgical resection is preferred, with every tumor removed if possible, and staged

surgery if necessary; the tumors that are more malignant and more threatening to the patient are treated first, and multidisciplinary treatment is combined to optimize the treatment plan. There is no uniform standard for the treatment of MPC patients with combined APL. As APL is characterized by rapid onset, many bleedingsites and easy combination with DIC, the early use of retinoic acid is still advocated to improve the survival rate of patients when the combined multiple primary solid tumors are in a stable stage, but attention should be paid to the prevention of DIC, tumor lysis syndrome and retinoic acid syndrome. In this paper, two MPC patients with combined APL were given standard doses of retinoic acid combined with arsenic or cytarabine at the diagnosis of APL. Unfortunately, both patients developed bleeding at different sites during treatment, and their families abandoned further treatment and the two patients eventually died. Due to the low incidence and poor efficacy of AML combined with multiple primary cancers, there is no uniform treatment protocol and treatment strategies including chemotherapy, hematopoietic stem cell transplantation, immunomodulation and symptomatic support (23). Among the 12 cases of AML combined with multiple primary solid tumors reported by Mi Ruihua (6), the AML induction treatment regimen included induction remission, consolidation chemotherapy and other chemotherapy regimens commonly used in the treatment of myeloid leukemia. The overall survival [M (range)] of the 12 patients was 12.5 (3.8-48.0) months, depending on the stage of the tumor, the patient's blood picture, coagulation function and physical condition. Because of the small sample size and the fact that all the cases were AML patients without APL, the treatment options for patients with APL combined with MPC needs to be further investigated.

The two patients with MPC in combination with APL in this paper both died eventually. The prognosis of multiple primary cancers is influenced by a number of factors, including the chronological nature of tumorigenesis (24). In a study by Ventura (25) on patients with lung cancer combined with other solid tumors, it was shown that patients with heterochronic multiple primary cancers had a higher risk of death than those with simultaneous multiple primary cancers. This may be due to the weakening of the body's immune function as a result of receiving multiple anti-tumor treatments within a short period of time or at the same time. However, the two cases in this paper were both patients with heterochronic multiple primary solid tumors combined with APL, and the average survival time after diagnosis of APL was 14 days, which may be related to the aggressive early pathogenesis of APL disease. Therefore, the prognostic factors and survival analysis of MPC patients with combined APL still need further clinical research and analysis.

In summary, the presence of combined hematological malignancies should be considered in patients with solid tumors combined with unexplained blood changes, and prompt bone marrow aspiration and bone marrow biopsy should be performed to confirm the diagnosis. Treatment of hematological neoplasms, especially acute leukemia combined with multiple primary cancers, is challenging, and the difficulty lies in balancing different treatment modalities and risk assessment. Given the complexity of the etiology and pathogenesis of APL combined with heterochronous multiprogenitor carcinoma and the variability of clinical characteristics of patients with APL, clinical knowledge and experience is relatively limited. The treatment and survival of MPC patients with combined APL needs to be improved and enhanced.

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.

ETHICS STATEMENT

This study was reviewed and approved by the Ethics Committee of Drug Clinical Trials of Wuwei Tumor Hospital of Gansu Province, which affiliated to Wuwei Tumor Hospital of Gansu Province, China. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Conception and design: CW. Acquisition of data: YS, QL, and XH. Drafting of the manuscript: YS. Critical revision of the manuscript for important intellectual content: CW. Administrative, technical, or material support: HHZ, YZ, HZ, ZY, and FG. Supervision: CW. All authors contributed to the article and approved the submitted version.

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Defibrotide Prophylaxis of Sinusoidal Obstruction Syndrome in Adults Treated With Inotuzumab Ozogamicin Prior to Hematopoietic Stem Cell Transplantation

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Sinusoidal Obstruction Syndrome (SOS) is a life threatening HSCT complication and it can rapidly evolve in Multiple Organ Dysfunction Syndrome, with a mortality exceeding 80%. Early treatment with defibrotide is the leading factor for efficacy. Its prophylactic use is recommended in the pediatric setting, but its value isn't validated for adults, although factors for individual risk assessment are debated. We here present a real-world experience of Defibrotide prophylaxis in adults at very high risk of SOS. We treated with prophylactic Defibrotide and Ursodeoxycholic Acid seven patients receiving allogeneic HSCT for high risk B-ALL, previously treated with single agent Inotuzomab-Ozogamicin. They all had other high risk factors for SOS such as previous hepatotoxicity, previous allo-HSCT, double alkylating conditioning. All patients received Treosulfan-Fludarabine conditioning, Thiotepa was added in 4 patients and 4GyTBI in 2 patients. GvHD prophylaxis included post-transplant cyclophosphamide, rapamycin and mycophenolate. Donor source was PBSC. Five patients received family MMRD transplant, 1 patient a MRD transplant and 1 patient a MUD transplant. Non-severe gastrointestinal bleeding occurred in two patients requiring defibrotide temporarily discontinuation. SOS occurred in 3/7 cases within 21 days after HSCT and no late-onset SOS were diagnosed. SOS caused death in all cases. All three patients were characterized by a common pattern of very high risk factors by prior HSCT, they all received a myeloablative conditioning with Treosulfan-Thiotepa and a MMRD transplant. Defibrotide prophylaxis apparently failed to protect against the development of SOS in those patients treated with a double alkylator-based conditioning regimen, while a possible efficacy for the other high-risk patients is debatable.

Keywords: Inotuzumab ozogamicin, defibrotide, prophylaxis, SOS, VOD, Allo-HCT, allogeneic hematopoietic stem cell transplantation

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INTRODUCTION

Hepatic Sinusoidal Obstruction Syndrome (SOS) is a potentially life-threatening complication of Hematopoietic Stem Cell Transplantation (HSCT). SOS pathogenesis is related to pro-inflammatory signals that determine the activation of hepatic stellate cells with subsequent collagen deposit, progressive sinusoidal obstruction, and portal hypertension. While mild SOS are underdiagnosed and spontaneously resolve, severe cases might evolve in liver dysfunction and multiple organ failure (MOF). Given the lack of pathognomonic features, several groups tried to identify clinical and laboratory criteria that define SOS (1). Several transplant, patient, and liver-related risk factors have been suggested (2) and, among them, the administration of Inotuzumab ozogamicin (IO), an anti-CD22 calicheamicin-linked monoclonal antibody approved for B-cell acute lymphoblastic leukemia (B-ALL), has been recognized as a major risk factor for both drug-induced liver injury and SOS (3). Given the lack of CD22 expression on liver cells, it has been hypothesized that drug-induced hepatic damage is secondary to the disposition of the calicheamicin metabolites by hepatocytes and the subsequent biliary excretion, which might expose liver cells to toxic injury; moreover calicheamicin uptake by endothelial cells might determine cell toxicity associated with platelet sequestration in liver sinusoids causing their obstruction (4, 5). Anti-inflammatory and anti-thrombotic properties of defibrotide, a mixture of oligonucleotides purified from porcine gut mucosa, reduce endothelial damage and potentially revert the cascade of organ dysfunction; therefore, defibrotide has been approved for severe SOS treatment (6). Whereas multiple dosages of defibrotide have been tested (7), the actual recommendations for the treatment of SOS indicate 25 mg/kg/day, administered for a minimum of 21 days, as the standard dose. Regarding prophylactic therapies, there is only low-quality evidence on the efficacy of defibrotide and ursodeoxycholic acid (UDCA) in reducing the incidence of SOS (8–10). The seminal paper by Corbacioglu et al. showed a borderline benefit in SOS incidence in children receiving prophylactic defibrotide that did not translate in overall survival improvement (10), and no clear data support its use in adults. Furthermore, the role of defibrotide in patients previously treated with IO is unknown. Herein, we present a real-world experience of peri-HSCT defibrotide prophylaxis in adult patients at risk of SOS because of a history of IO administration.

METHODS AND RESULTS

From May 2016 to April 2018, seven patients diagnosed with relapsed/refractory B-ALL with a history of IO administration underwent peripheral blood allogeneic HSCT at our Institution. Patient and transplant characteristics are shown in **Table 1**. IO was given as single agent for either one (n=1) or two cycles (n=6) at standard dose, with the last dose being administered at a median time of 41.5 days (range 34-61) prior to HSCT. Conditioning regimens were based on treosulfan (42 g/m^2) and fludarabine (150 mg/m^2) in all cases; thiotepa was added in four patients, whereas two cases received also 4Gy total body irradiation. As per institutional policy, graft versus host disease (GvHD) prophylaxis consisted in post-transplant cyclophosphamide (PT-

TABLE 1 | Patient, disease, and treatment characteristics.

Patient	#1	#2	#3	#4	#5	#6	#7
Sex and age	M, 25 y	M, 36 y	F, 22 y	M, 30 y	M, 21 y	M, 38 y	M, 26 y
Disease - status	B-ALL, active disease	B-ALL active disease	B-ALL, active disease	B-ALL, complete remission	B-ALL, complete remission	B-ALL, complete remission	B-ALL, complete remission
Previous lines of therapy	8	4	6	4	5	2	6
Previous allo-HSCT	yes	no	yes	yes	yes	yes	no
Pre-existing liver abnormalities	G2* Bilirubin and ALT increase	G2* ALT increase; mild steatosis	no	no	no	no	no
IO cycles numbers (cumulative dose)	2 cycles (3.3 mg/mq)	2 cycles (3.3 mg/mq)	2 cycles (3.3 mg/mq)	2 cycles (3.3 mg/mq)	2 cycles (3.3 mg/mq)	2 cycles (3.3 mg/mq)	1 cycle (1.8 mg/mq)
Days from IO to HSCT	25	61	49	25	34	60	49
Conditioning	Treo/Flu TBI4Gy	Treo/Flu TBI4Gy	Thio/Treo/Flu	Thio/Treo/Flu	Thio/Treo/Flu	Thio/Treo/Flu	Treo/Flu
Type of transplant	Mismatched related	Matched related	Mismatched related	Mismatched related	Mismatched related	Mismatched related	Matched unrelated
Days to engraftment	29	30	Died in aplasia	19	15	18	35
Days of defibrotide	27	32	11	35	33	35	64
SOS diagnosis	no	no	no	**YES**	**YES**	**YES**	no
Days from HSCT to SOS	na	na	na	10	13	9	na
Days of follow up	116	1092	11	28	26	28	229
Cause of death	Disease relapse	Disease relapse	Septic shock	SOS	SOS	SOS	Disease relapse.

B-ALL B-cell acute lymphoblastic leukemia; HSCT allogeneic Hematopoietic Stem Cell Transplantation; IO Inotuzumab ozogamicin; Treo/Flu Treosulfan/Fludarabine; Thio/Treo/Flu Thiotepa/Treosulfan/Fludarabine; SOS sinusoidal obstruction syndrome.

**According to CTCAE 5.0.*

bold values means to better identify case with SOS..

na, not applicable.

Cy) on day +3 and day +4, rapamycin and mycophenolate. All patients received peri-transplant UDCA 300 mg twice daily. Defibrotide was administered intravenously in four daily doses for a total dose of 25 mg/kg/day, starting the first day of conditioning and throughout engraftment. Informed consent for off-label use of prophylactic defibrotide was signed by all patients.

The median duration of defibrotide administration was 33 days (range, 11-64); the drug was overall well tolerated as it was temporarily discontinued only in two cases, respectively for two and four days, due to transient gastrointestinal bleeding (CTCAE v5.0 grade 3); no other adverse reactions were documented. Six patients engrafted after 18 days (range, 15-35), whereas one deceased in aplasia. One patient developed acute GvHD and required multiple lines of immunosuppressants; we documented one case of post-transplant microangiopathy shortly after SOS diagnosis.

Three out of seven patients (#4, #5, #6) developed all classical signs of SOS (hyperbilirubinemia, ascites, painful hepatomegaly, and weight gain) (1) a few days before engraftment, with a rapid evolution into a very severe form (2). They all received a double alkylator chemoconditioning; patient #3 also received the same chemoconditioning but died in aplasia at +11 post HSCT. No diagnostic liver biopsy was performed given the high risk of procedural hemorrhagic complications. SOS determined MOF and death in all three cases.

DISCUSSION

We here describe a real-world case series of patients at very high risk of SOS and 3 out of 7 patients developed this severe complication. These three patients showed common features: all were young adult males transplanted from a haploidentical donor for a B-ALL in complete remission after 2 cycles of IO; moreover, all had a history of a previous allogeneic HSCT and received a myeloablative conditioning with double alkylating agents prior to HSCT.

It is noteworthy that all patients in our series, including those not diagnosed with this complication, displayed at least one additional risk factor of SOS such as pre-existing hepatic abnormalities, active disease, rapamycin use, conditioning with TBI, use of HLA-mismatched donors, and previous allogeneic HSCT (1, 2, 6). Double alkylators and pre-HSCT hepatic abnormalities have been specifically associated with SOS in patients pre-treated with IO (3) and these risk factors were present in two and four patients, respectively (**Table 1**).

All these factors were scattered among patients whit and without SOS and, in this very small cohort, it is not possible to discriminate the role of each risk factor. However, it appears evident that those who were treated with double alkylator conditioning developed SOS despite prophylaxis with defibrotide plus UDCA; the other patient who also received thiotepa inside the conditioning died at day +11 after HSCT, preventing us from assessing the effectiveness of prophylaxis (**Table 1**). It is also worth mentioning the use of PT-Cy as GVHD prophylaxis in this case series, so that the three patients developing SOS effectively received three alkylators. It is not clear at this point if PT-Cy should be regarded as a risk factor for SOS, but it surely warrants further investigations.

All diagnoses occurred right before engraftment and the clinical picture evolved dramatically within few days, making SOS diagnosis striking. No differences in the number of IO cycles (never more than 2) and interval from last IO to HSCT were evident between patients diagnosed with SOS and those who did not develop this complication.

Whereas the approval of IO has greatly improved the treatment armamentarium for relapsed/refractory B-ALL, emergence of hepatic toxicity, namely SOS, has questioned the safety of this new drug. Recently, a learning curve has allowed a better use of IO, specifically as a bridge to allogeneic HSCT (11). In the setting of prophylaxis, Corbacioglu and colleagues reported a decrease of SOS incidence from 20% to 12% in a phase 3 trial conducted in a high-risk pediatric population, including patients who received gentuzumab ozogamicin but not IO (10). On the contrary, the role of prophylaxis is particularly debated in adult patients, where no evidence-based data were able to demonstrate its efficacy (9). Our experience suggests that defibrotide prophylaxis is safe in the adult population, with only two documented bleeding events promptly resolved with supportive care and temporary drug interruption. However, the drug, in association with UDCA, was only partially effective in preventing SOS. Our data confirm the detrimental effect of double alkylator conditioning for patients undergoing HSCT previously treated with IO, a feature that we now recognize as one of the most important risk factors for SOS in this treatment setting (3, 11). Furthermore, recent data confirmed the benefit of a TBI-based conditioning in children with ALL compared to double alkylator chemoconditioning in terms of both relapse and treatment related mortality (12). This approach should be considered as reasonable also in young adults and warrants investigations in the setting of IO pre-treated patients at risk for SOS.

Indeed, in our study, defibrotide prophylaxis apparently failed to protect against the development of SOS only in those patients treated with a double alkylator-based conditioning regimen, while a possible efficacy could be at least presumed for the other high-risk patients.

In conclusion, with the caution derived from analyzing the results of a small single-center case series, the use of peri-HSCT defibrotide prophylaxis in adult patients at very high-risk of SOS due to a previous history of IO exposure demonstrated to be safe and partially effective. Minimization of other known cumulative risk factors should be pursued to further reduce the incidence of SOS in this population, and, particularly, the addition of a second alkylating agent into the conditioning scheme must be avoided. Other tips, such as further reducing the number of IO administrations, longitudinal monitoring with liver ultrasound elastography to allow early diagnosis and avoiding other hepatotoxic drugs should be considered (13). Only well-designed prospective clinical trials could demonstrate the usefulness of defibrotide in this setting.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This study was reviewed and approved by San Raffaele Institutional Ethical Committee. Written informed consent was obtained from all participants for their participation in this study.

AUTHOR CONTRIBUTIONS

FG and FC designed the study. FG and EX wrote the manuscript. All authors were involved in patients management. All authors interpreted results and validated the manuscript's content. All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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Machine Learning–Based Overall Survival Prediction of Elderly Patients With Multiple Myeloma From Multicentre Real-Life Data

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Objective: To use machine learning methods to explore overall survival (OS)-related prognostic factors in elderly multiple myeloma (MM) patients.

Methods: Data were cleaned and imputed using simple imputation methods. Two data resampling methods were implemented to facilitate model building and cross validation. Four algorithms including the cox proportional hazards model (CPH); DeepSurv; DeepHit; and the random survival forest (RSF) were applied to incorporate 30 parameters, such as baseline data, genetic abnormalities and treatment options, to construct a prognostic model for OS prediction in 338 elderly MM patients (>65 years old) from four hospitals in Beijing. The C-index and the integrated Brier score (IBwere used to evaluate model performances.

Results: The 30 variables incorporated in the models comprised MM baseline data, induction treatment data and maintenance therapy data. The variable importance test showed that the OS predictions were largely affected by the maintenance schema variable. Visualizing the survival curves by maintenance schema, we realized that the immunomodulator group had the best survival rate. C-indexes of 0.769, 0.780, 0.785, 0.798 and IBS score of 0.142, 0.112, 0.108, 0.099 were obtained from the CPH model, DeepSurv, DeepHit, and the RSF model respectively. The RSF model yield best scores from the fivefold cross-validation, and the results showed that different data resampling methods did affect our model results.

Conclusion: We established an OS model for elderly MM patients without genomic data based on 30 characteristics and treatment data by machine learning.

Keywords: multiple myeloma, survival model, elderly patients, random survival forest (RSF), deep hit algorithms, cox proportinal hazards model (CPH), deep survival algorithms

1 INTRODUCTION

Multiple myeloma (MM) is an incurable neoplastic disease derived from abnormal plasma cells that predominantly affects elderly patients (1), and more than 60% of patients are over 65 years of age (2). Age has been deemed a prognostic factor and criterion of treatment regimen selection. MM patients ≥ 65 years are not candidates for autologous haematopoietic stem cell transplantation and show poor progression-free survival (PFS) and overall survival (OS) compared with patients younger than 65 years (3). The other limitation is that elderly patients usually do not meet the eligibility criteria of clinical studies, probably due to more comorbidities that cause higher Eastern Cooperative Oncology Group (ECOG) scores and lower estimated glomerular filtration rates (eGFRs). The application of novel agents, including bortezomib, lenalidomide and daratumumab, for nontransplant candidates recommended by the American National Comprehensive Cancer Network (NCCN) (4) is insufficient in the real world (5). Moreover, the elderly group was highly heterogeneous compared with the young group, with poor biological characteristics and more adverse reactions, resulting in a poor treatment response and no subsequent treatment after frontline therapy. Improving outcomes in the elderly MM population is dependent on selecting an appropriate therapy strategy according to elderly patients' specific prognostic stratification. Although there are many stratification systems, few survival prognostic models have been generated for elderly patients, especially using real-world data.

The widely used prognostic indexes are the International Staging System (ISS), the more recent revised ISS (R-ISS), and the International Myeloma Working Group (IMWG) recommendations for risk stratification derived from clinical trials (6–8). The concordance indexes (c-indexes) of the above stratification systems validated by real-world data range from 57% to 65% (9), revealing substantial room for improvement. In recent reports, machine learning, including deep learning and random forests, has been implemented in cancer prognosis prediction (10). Maria Victoria et al. created a 50-variable random forest model including 4 biochemical variables (age, ISS stage, β2-microglobulin and frontline regimen) and 46 gene expression variables (c-index 78%) (11). This model was also based on clinical trials and is not suitable for patients without genetic features. In addition, the treatment response and maintenance therapy also affect the OS of MM patients. In this study, we enrolled 338 elderly MM patients (age ≥65 years) from 4 centres in Beijing, China, and used machine learning methods to incorporate 30 parameters, such as baseline data, genetic abnormalities and treatment options, to construct a prognostic model for survival prediction.

Survival analysis includes a set of methods that analyses the expected duration and factors affecting the expected duration until one event occurs. Most commonly used statistical methods assume that this potential relationship follows certain distributions. For example, the Cox proportional hazards model assumes that the logarithm of the sample hazard rate is linearly related to the covariate, but in fact, it is difficult to determine the actual underlying relationships.

On the other hand, random survival forest (RSF) avoids making restrictive assumptions and is able to provide an unbiased estimate of the error rate even when there is missing data (12). Recently, researchers in the health care field have started to use RSF tools to analyse patient data (13, 14). There are also survival analysis studies being done in the deep neural network field. Farragi et al. first proposed the use of feedforward neural networks to study the relationship between variables and risk factors, and many subsequent studies extended their idea (15, 16). The DeepHit model emerged from this idea and learns the joint distribution of survival time and events directly, avoiding restrictive assumptions and time invariance (17).

In this study, we enrolled 338 elderly MM patients (age ≥65 years) from 4 centres in Beijing, China, and used the cox proportional hazards model (CPH); DeepSurv; DeepHit; and the random survival forest (RSF) model to incorporate 30 parameters, such as baseline data, genetic abnormalities and treatment options, to construct a prognostic model for OS prediction.

2 METHODS

2.1 Patient Selection and Variable Acquisition

All geriatric newly diagnosed multiple myeloma (NDMM) patients aged 65 years and older were reviewed at the Department of Hematology of four hospitals from January 2016 to September 2020. Patients who received no treatment or lost to follow-up were excluded. We selected 338 data which had >80% full annotation for 30 variables including baseline characteristics (sex, age, GA score, and ECOG score), myeloma-specific factors [haemoglobin, calcium, albumin, eGFR, M-spike, β2-microglobulin, LDH, ISS stage, RISS stage, and FISH detection including gain 1q21 and del 17p, t (11, 14), t (4, 14); t (14, 16), and t (14, 18)] and treatment condition (induction regimen, induction response, maintenance regimen, times for maintenance, and different treatment lines) (**Supplementary Table 1**). We defined 65 years old was the cut-off for elderly MM as patients ≥ 65 years old were not candidates for autologous hematopoietic stem cell transplantation and showed poor PFS and OS compared with patients younger than 65 (19). The OS was estimated from first treatment and censored at the last date at which they were known to be alive until September 30, 2020. The median follow-up was 27 months (1–60).

All of these study procedures were performed in accordance with the Declaration of Helsinki and were approved by the ethics committee of Beijing Jishuitan Hospital (201907–04). Written informed consent was obtained from each patient prior to data collection and analysis.

2.2 Data Preprocessing

2.2.1 Data Cleaning and Standardization

The 338 data entries suffer from missing data problems. Different imputation methods were implemented for the variables based

on their characteristics and our clinical knowledge. Detailed imputation methods for each variable were shown in **Supplementary Table 3**.

Simple imputation methods are widely used methods when dealing with missing data in health care studies (20). In this study, the observations were grouped according to the maintenance schema first, and continuous variables were imputed using the mean of its group. Group mean imputation can ensure that the mean of the variable in each group does not change after imputation. For variables with discrete values, we used hot-deck imputation and assumed that the data entries with similar survival time would have similar variable characteristics. Therefore, we sorted the data based on survival time, and discrete missing myeloma-specific factors were imputed using the corresponding value of the previous observation.

After data imputation, we addressed the problem that a large value range appeared between variables. To facilitate the training and convergence of the model, we first compressed the value space of each continuous variable to [0, 1] and then normalized it to form a dataset denoted as D.

2.2.2 Data Resampling

Two data resampling methods were implemented before performing the tests.

The first method resamples the dataset D by a ratio of 7:1.5:1.5, giving us the dataset $D^{\star} = (D_{train}, D_{valid}, D_{test})$. D_{train} contains 236 (70%) of the data points, while D_{valid} and D_{test} contain 51 (15%) each. Each data entry is a 31-dimensional vector. Based on this dataset, we conducted model building using four different methods. There was no difference between the three datasets by Mann–Whitney U nonparametric tests using SPSS 20.0 (SPSS, Inc., Chicago, IL) (**Supplementary Table 2**).

The result of the models might be affected by how the dataset was resampled because of our limited data size. Therefore, to further illustrate the effectiveness of the models and compare the pros and cons of the four models, we resampled the data with a second method and used fivefold cross-validation for model evaluation. The original data were divided into five equal subsets, and each subset was stratified and sampled while ensuring that the value range of the OS between the training set and the test set was roughly the same. After that, we took four of the subsets as the training set and the remaining one as the test set to train and test the four models. The dataset created here was denoted as $D^{\star\star}$.

A detailed data analysis flowchart is presented in **Figure 1**. R programming (R Core Team, Vienna, Austria) and Python v 3.6.7 (Python Software Foundation, Scotts Valley, USA) were used for the analysis in this paper.

2.3 Data Analysis

Four algorithms were selected to build models and analyse the influencing factors of the survival time: the cox proportional hazards model (CPH); DeepSurv; DeepHit; and the random survival forest (RSF).

2.3.1 CPH Model

The Cox proportional hazards model is one of the most widely used models in survival analysis. It can be used to assess the influence of quantitative and categorical risk factors on survival time and make forecasts (19). In this study, we used the Python lifelines library to build a CPH model and forecasted survival times.

2.3.2 DeepSurv Model

DeepSurv is a feed-forward neural network method based on the Cox proportional hazards model. The structure of DeepSurv is similar to the Faraggi-Simon network, and it can be used to model non-linear relationships between risk factors and survival time. DeepSurv has been proved to perform well on clinical data with missing datapoints and without prior assumptions on the risk function (20). We performed a grid search with the Pytorch framework to find the optimized hyper-parameter for the DeepSurv model in this study.

2.3.3 DeepHit Model

The DeepHit model was originally designed for analysing the competing risk of multiple events (18). In this study, we only considered a single event, which was patient survival. Therefore, we can use a simplified DeepHit model to analyse our data. Through the softmax layer of the model, we can obtain an estimated probability sequence $\{ y_1, y_2 \cdots, y_{T_{max}} \}$, where y_t represents the probability estimate of the patient's death at time t. While ensuring that $\sum_{i=1}^{T_{max}} y_1 = 1$, the estimated survival rate of the patient at each time point can be obtained according to $\hat{P}(t = t^*) = 1 - \sum_{i=1}^{t^*} y_i, t^* = 1, 2, 3, \cdots, T_{max}$, and the survival curve can then be drawn.

Because the DeepHit model is designed to deal with discrete survival time, the event time is discretized using an isometric grid between the minimum duration and the maximum duration in the dataset. The isometric grid was set to one day in this study. The loss function of DeepHit contains two parts as shown in equation 1:

$$L_{total} = \alpha * L_1 + (1 - a) * L_2 \tag{1}$$

The hyper-parameter α is used to set the proportion of each loss. L_1 is the negative log likelihood of the model, as shown in equation 2:

$$L_1 = \sum_i^N \left[\mathbb{I}\left(k^i = 1\right) * \log\left(y_{t^i}^i\right) + \mathbb{I}\left(k^i = 0\right) * log\left(\hat{P}_{k=1}\left(t^i \mid x^i\right)\right) \right] \tag{2}$$

where $I(\cdot)$ denotes the indicator function, and N denotes the sample size.

The idea of L_2 came from the concordance index, and the calculation method is shown in equation 3:

$$L_2 = \sum_{i \neq j} A_{i,j} * \eta\left(\hat{P}_{k=1}\left(t^i \mid x^i\right), \hat{P}_{k=1}\left(t^i \mid x^j\right)\right) \tag{3}$$

Where $A_{i,j} \triangleq \mathbb{I}(k^i = 1, t^i < t^j), \eta(x, y) = exp(\frac{y-x}{\sigma}), \sigma$ denotes the hyper-parameter.

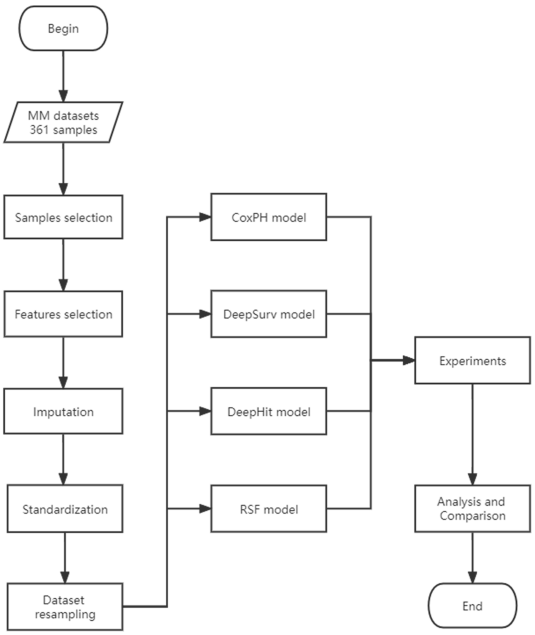

FIGURE 1 | Data Analysis Flowchart.

We performed grid search with the Pytorch framework to find the optimized hyper-parameter α, σ, and trained the DeepHit model.

2.3.4 RSF Model

The RSF model is similar to the general random forest model, while the main difference is that the basic unit of RSF is a binary survival tree (18). Unlike traditional decision trees, survival trees usually use log-rank scores to maximize survival differences and use it as a criterion for splitting tree nodes. The final evaluation standard is the consistency index (18). Due to the limited data size and feature dimension, pruning and feature selection were not performed in this study.

The randomForestSRC package was used to build an RSF model for data training. The number of trees in the forest was set to 1000, the feature importance ranking was obtained, and the c-index indicator was used to evaluate the model performance.

2.4 Model Performance Evaluation

In order to compare the performance of the four models, we measured the Harrell concordance index (C-index) and the integrated Brier score (IBS).

2.4.1 Concordance Index

The C-index is one of the most common indicators used in survival analysis. It is a generalization of the area under the ROC curve (AUC) (18), and represents the percentage of accurately-predicted patient pairs. The calculation method of C-index of patient i and patient j is shown in equation 4:

$$C_{index} = P\left\{\hat{S}\left(t_i \mid x_i\right) < \hat{S}\left(t_j \mid x_j\right) \mid t_i < t_j\right\} \tag{4}$$

Where $\hat{S}(t_i \mid x_i)$ represents the predicted survival time of patient i. C_{index} has a value between 0 and 1, $C_{index} = 1$ indicates that the model makes a perfect prediction.

2.4.2 Integrated Brier Score

In multi-classification problems, the Brier score is defined as the average variance between predicted value and true value as shown in equation 5 (21):

$$BS = \frac{1}{N}\sum_{i=1}^{N}\sum_{j=1}^{L}\left(\hat{y}_{ij} - y_{ij}\right)^2 \tag{5}$$

Where N denotes the sample size, L the number of classes, $\hat{y}_{ij}$ the model predicted value, and y_{ij} the real value.

When dealing with survival analysis that has censoring problems, the Inverse Probablity of Censoring Weighted (IPCW) (18) needs to be considered in calculating the Brier score. The calculation method is shown in equation 6:

$$BS(t) = \frac{1}{N}\sum_{i=1}^{N}\frac{\left(0 - \hat{S}(t \mid x)\right)^2}{\hat{G}(t_i \mid x)} I(t_i \le t, \delta_i = 1) + \frac{\left(1 - \hat{S}(t \mid x)\right)^2}{\hat{G}(t \mid x)} I(t_i > t) \tag{6}$$

Where $\hat{G}(t \mid x)$ is the Kaplan-Meier estimator, δ is the censoring indicator, and $\hat{S}(t \mid x)$ is the estimate of the survival function. Then we integrate the Brier score to get the integrated Brier score (IBS) as shown in equation 7:

$$IBS = \int_{\min(t)}^{\max(t)} BS(t)dt \tag{7}$$

3 RESULTS

3.1 Clinical Characteristics of the Cohort

A summary of the baseline characteristics and treatment conditions of the patients in the cohort is presented in **Supplementary Table**. The median age was 70 years (65–86). Proteasome inhibitors (PIs), including bortezomib and ixazomib, were the most common induction regimen (64.5%), and PIs in combination with immunomodulatory drugs (IMiDs) were the second most common first line of therapy (18.4%). Few patients received IMiD-based (14.2%) and traditional regimens (2.9%). Of note, 20.4% of patients had a lower eGFR (<30 ml/min per 1.73 m), and 57.7% had an ECOG score higher than 2 at baseline.

3.2 Model Analysis

As mentioned above, we used the training set and validation set of $D^{\star}$ to find the value of hyper-parameters in DeepSurv and DeepHit models. In order to minimize the influence on model performance by data resampling, we further tested model performances using the five-fold cross-validation with $D^{\star\star}$.

3.2.1 Model Parameter Tuning and Visualization

The best parameters we obtained from the training and the validation set of $D^{\star}$ are: DeepSurv: Layers=3, Nodes per layer=32, dropout=0.4, learning rate = 0.003; and DeepHit: Layers=3, Nodes per layer=[32,32,60], dropout=0.4, learning rate = 0.0002, $\alpha = 0.1$, $\sigma = 0.3$.

Based on these parameters, the training set D_{train} from $D^{\star}$ was used to build four models. CPH and DeepSurv models did not yield results that are as good as RSF or DeepHit, so we further analysed the RSF and the DeepHit models.

Figure 2 shows one RSF model that had a result near to average. The graph presents the predicted survival curve of the patients in the test set, where each dotted line represents the predicted survival curve for one patient, and the thick red line represents the average survival curve.

Additionally, we obtained the variable importance ranking based on their influences on the OS rate. **Figure 3** shows that the three variables related to the maintenance schema had the strongest influences on survival. This indicates that in the actual treatment of MM, maintenance therapy is very important.

The DeepHit neural network model of a single event was built to discover information from more feature variables. **Figure 4** shows one model that had a result near the average under this case. The predicted survival curves of the patients in the test set are displayed.

From **Figure 3**, we observed that the results are largely affected by the maintenance schema variables. Therefore, we generated a groupwise survival curve graph based on these variables. The results are shown in **Figure 5**, with the left subgraph presenting the RSF model and the right subgraph presenting the DeepHit model. It is obvious that the classification results of both models are acceptable. For the data entries with maintenance schema groups 1, 5, and 7, which represent maintenance treatment with IMiDs, no maintenance treatment due to relapse and refractory disease, and still in induction treatment, respectively, the model can effectively distinguish them; however, the classification effect of groups 2, 3, 4, 5, and 6 is not strong. The reason might be that the sample size of these indistinguishable groups is too small; for example, there is only one sample in group 6 in the test set. Another reason might be that when the missing values of the data were filled, a strong correlation between samples was introduced. Hence, it is difficult for the model to distinguish some of the groups.

We then calculated the variance of each group to characterize the degree of aggregation of the survival curves. The formula we used to calculate the variance of the curve is shown in equation (8), where T_{max} denotes the maximum survival time, $count_k$ denotes the

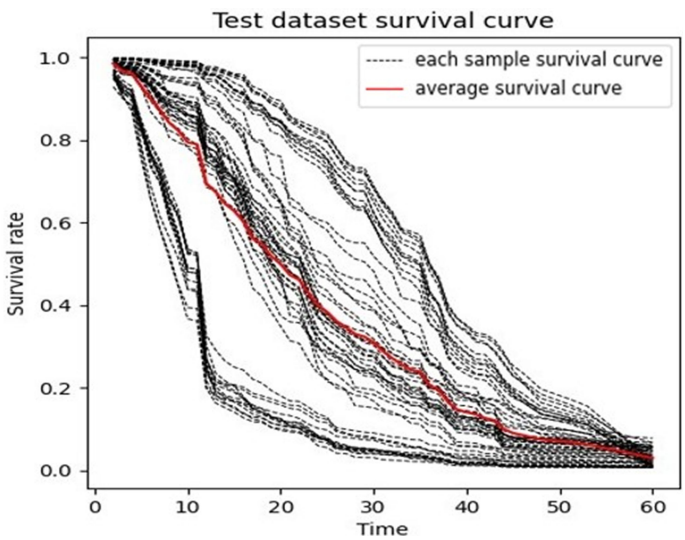

FIGURE 2 | Predicted Survival Curve (Random Survival Forest).

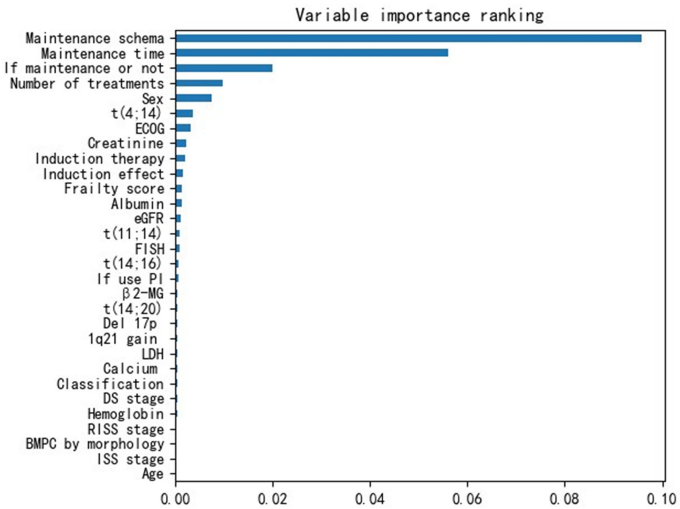

FIGURE 3 | Variable Importance Ranking.

number of curves in the k^{th} group, and P_i^k denotes a probability sequence $\{P_{i,2}^k, P_{i,2}^k, \cdots, P_{i,count_k}^k\}$; $std(\cdot)$ represents the variance function.

$$\sigma_k = \left(\sum_{i=1}^{T_{max}} std\left(P_i^k\right)\right) / \ T_{max} \tag{8}$$

The calculation results are shown in **Table 1**. All the variances are less than 0.09, indicating that the aggregation of both models is acceptable. Because of the limited data size and the unequal data entry distribution in each group, it is difficult for us to compare the aggregation effect between the DeepHit model and the RSF model. However, the results showed that the two models have convincing performance on OS curve prediction for different groups of patients.

3.2.2 Cross-Validation

The actual data resampling result might affect the performance of the models due to our limited data size. Therefore, to further evaluate the performance of the four models, fivefold cross-validation was performed for each model. That is, four subsets of the dataset D^{**} were used as the training set, and the remaining subset was used as the test set. Five tests were carried out for each model. The average of the five test results was used as the final result of a model. This method can alleviate the impact of data resampling on model results and better demonstrate the model

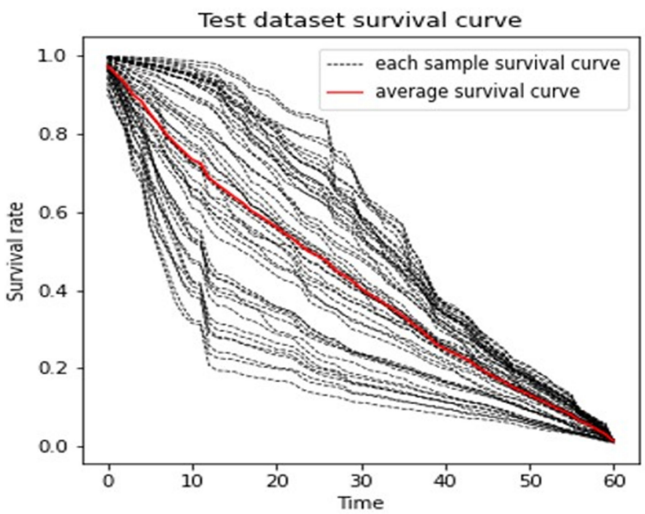

FIGURE 4 | Predicted Survival Curve (DeepHit).

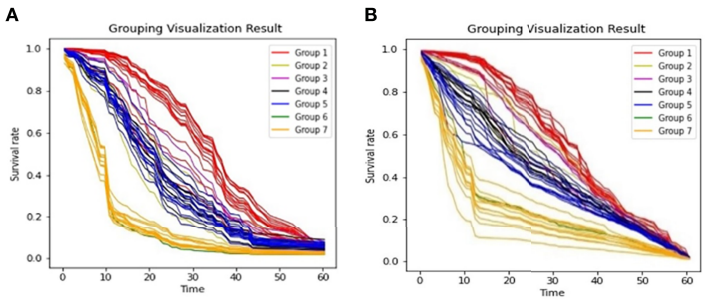

FIGURE 5 | Survival Curves by Maintenance Schema **(A)**. Grouping Visualization Result of RSF; **(B)**. Grouping Visualization Result of DeepHit.

performance. **Table 2** shows the C-index results, averaged over the fivefold cross-validation folds.

CPH and DeepSurv models did not perform well under this data resampling method as well. The reason that RSF and DeepHit models performed better might be that they have less requirements on the dataset, so that they can deal better with more complex survival data. Another reason that DeepHit performed better than DeepSurv might be that the loss function of DeepHit took concordance index into consideration.

In general, the results show that different data resampling methods have noticeable effects on the model results. One possible reason is the size of our dataset is limited, and there are missing values in our dataset. We can also conclude that RSF models are more susceptible to data quality because it is shown in the results that the standard deviation of the RSF model is larger than that of the DeepHit model. Cross-validation presented a C-index result different from our result in the model training section. The average RSF C-index result (0.798) was slightly better than the average DeepHit result (0.785), because RSF is more suitable for small sample size data analysis. At the same time, DeepHit yielded a smaller standard deviation of C-index (SD = 0.016) comparing to RSF (SD = 0.026), because part of its loss function was designed based on the concordance index.

Moreover, **Table 3** presents the IBS for the four models under cross-validation. RSF presented a mean IBS of 0.099 and a standard deviation of 0.002, while DeepHit presented a mean of 0.108 and a standard deviation of 0.002. RSF has a better IBS (a value closer to 0) with a lower standard deviation, so the accuracy and stability of the RSF model are better based on IBS.

TABLE 1 | Variance of each Maintenance Schema group.

Groups	Maintenance Schema	$count_i$	RSF	DeepHit
Group 1	Immunomodulator	**14**	0.0667	**0.0369**
Group 2	Proteasome Inhibitor	**3**	0.0859	**0.0515**
Group 3	PI+iMiDs	**2**	0.0249	**0.0037**
Group 4	No	**8**	0.0333	**0.0190**
Group 5	Disease Progression	**11**	0.0434	**0.0408**
Group 6	Death	**1**	0.0000	0.0000
Group 7	Inducing	**12**	**0.0217**	0.0417

The bold value was the best results compared among groups.

Overall, the RSF model presented better discriminatory accuracy and provided the best model results on the elderly MM patient dataset.

4 DISCUSSION

Due to the strong heterogeneity of MM, although there are many traditional assessment methods, such as ISS, RISS, chromosomal abnormalities and CIC, they still cannot meet clinical needs. There are also previous studies on machine learning methods in the MM field. Terebelo et al. reported a tool based on 3011 patients with NDMM from multiple centres in the US by multivariable Cox regression using weighted observations, achieving c-indexes of 64.7%-69.8% (21). In another recent report, Maria Victoria et al. developed a random forest model including the characteristic and GEP data of 730 patients for OS prediction with good discrimination (c-indexes of 0.818 and 0.780 in training and validation sets) (11). Groups from India proposed k-adaptive partitioning derived simple stage system using five baseline parameters and validated higher values of C-index on both MMIn and MMRF datasets, which outperformed ISS for OS calculation but was equivalent in the prognosis of PFS (22). However, most of these data came from clinical trials, and their role in real-world MM predictions, especially in older patients, is unclear (23).Although there have also been researches that applied machine learning and deep learning algorithms to build models and make survival predictions using real-world oral cancer (24, 25), breast cancer (26) and glioblastoma (27) patient data, the implementation of these methods on elderly MM patient data have not been fully discussed.

In this study, we presented feasible machine learning models for predicting the OS of elderly MM patients based on baseline clinical, biochemical, and treatment data. Our deep learning and random forest model involved 30 parameters, which combined frontline and maintenance treatment information, and achieved a high c-index of 80%. The RSF model presented the best model results on our dataset. Although the number of people in this study is small, all of them are elderly and represent multicentre data in the real world. Therefore, this model may provide dynamic prediction during the whole process of MM.

TABLE 2 | C-index for Cross-validation.

	Fold 1	Fold 2	Fold 3	Fold 4	Fold 5	Mean	SD
CPH	0.760	0.802	0.776	0.772	0.734	0.769	0.022
DeepSurv	0.760	0.821	0.754	0.773	0.792	0.780	0.024
DeepHit	0.785	0.810	0.770	0.767	0.795	0.785	**0.016**
RSF	0.816	0.811	0.753	0.784	0.824	**0.798**	0.026

The bold value was the best results compared among groups.

TABLE 3 | IBS for Cross-validation.

	Fold 1	Fold 2	Fold 3	Fold 4	Fold 5	Mean	SD
CPH	0.1409	0.1341	0.1160	0.1644	0.1522	0.1415	0.0164
DeepSurv	0.1154	0.1070	0.1032	0.1174	0.1147	0.1115	0.0055
DeepHit	0.1092	0.1085	0.1086	0.1100	0.1041	0.1081	0.0021
RSF	0.0974	0.0964	0.0991	0.1001	0.1009	**0.0988**	**0.0017**

The bold value was the best results compared among groups.

The visualization results showed that the use and prolonged use of maintenance therapy are critical for OS in MM, and the most commonly used maintenance therapy is lenalidomide. Real-life data from the US showed that approximately 50% of nontransplant patients do not receive follow-up therapy after first-line therapy (28). Similarly, many elderly Chinese patients do not receive maintenance therapy for various reasons, such as poor compliance, multiple comorbidities, poor physical fitness, and economic conditions.

Both ECOG and frailty scores had higher contribution rates, indicating the importance of applying performance status scores in elderly patients. The high attrition rate also suggests that choosing the optimal frontline treatment is crucial for prolonging OS in elderly MM patients. The recommended treatment regimens for MM patients ineligible for autologous stem cell transplantation (ASCT) include VRD, DaraRD, Rd and PCD (4). Over 80% of elderly MM patients in our group received PI and PI+IMiD-based first-line regimens in accordance with their treatment status in first-tier cities of China. Although DaraRD has been reported to improve PFS in patients ineligible for ASCT compared with RD (34.4 m vs. not reached) (29), the application of Dara was not extensive. Compared with survival model reported by previous studies (11, 21), the model derived from our real-life data is suitable for elderly MM patients without genomics data who received first-line therapy without daratumumab, so it will be easy applicable in real world.

One limitation of our study arises at the data imputation step. Although the current imputation methods we use are commonly used in health care studies, we are aware that more advanced imputation methods exist and might be able to lead to better results. We are planning to further discuss the influence of imputation methods on model results in our future studies.

What is more, due to the limited sample size, the results from our models are not stable enough. We will continue to enlarge the data size and improve the data quality. We believe that our model has a very good function and can reveal the relationship between different variables more clearly. We aim to provide credible and accurate reference guidance for medical clinical treatment with our models.

In conclusion, this work utilized all process variables, including baseline characteristics and treatment parameters, to provide a reliable OS prediction model for elderly MM patients. It is also suitable for patients without genomic testing and monoclonal antibody therapy due to economic and/or geographic constraints. The model is applicable to any disease stage, can be optimized on larger datasets, and can be used to select the appropriate intensity of treatment.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

LB designed the study. Y-TW and LB analysed the data and wrote the manuscript, and all authors contributed to the interpretation of the data, prepared the manuscript, and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.922039/full#supplementary-material

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Case report: Zanubrutinib-induced dermatological toxicities: A single-center experience and review

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Zanubrutinib, a next-generation non-covalent Bruton's tyrosine kinase (BTK) inhibitor, shows great efficacy in the treatment of B cell malignancies. Some patients may experience a series of side effects after the treatment of zanubrutinib. Grade 4 dermatological toxicities are rare, which present as severe rash and skin infection. Herein, we retrospectively reported the grade 4 dermatological toxicities of zanubrutinib in three consecutive patients. They were treated with zanubrutinib 160 mg twice daily orally. One patient was diagnosed with Primary Breast Diffuse Large B-cell Lymphoma(PB-DLBCL) and two patients were diagnosed with Chronic Lymphocytic Leukemia(CLL). Within one month after zanubrutinib treatment, all three patients developed grade 4 dermatological toxicities, including bruising, maculopapular rash, petechiae, ecchymosis, hemorrhagic blister, acne-Like rash, papulopustular rash, and skin infections. Zanubrutinib was discontinued in two patients due to unacceptable dermatological toxicities. Safety data from pre-licensing clinical trials showed that zanubrutinib-related side effects were frequent but well tolerated. To date, no severe dermatological toxicities were reported. The majority of patients can be relieved with symptomatic treatment, but a very small percentage of patients may face discontinuation of the drug.

KEYWORDS

zanubrutinib, dermatological toxicity, maculopapule rash, papulopustular rash, skin infection, epithelial growth factor receptor

Introduction

BTK is a non-receptor tyrosine kinase in the Tec(transient erythroblastopenia of childhood) family that is mainly expressed in hematopoietic stem cells such as B cells, monocytes, macrophages, and basophils. Since BTK plays an important physiological function in the B-cell receptor and FcγR-mediated signaling pathway, BTK has become

an important therapeutic target for the treatment of B cell lymphoma. There are currently five BTK inhibitors approved for marketing. Zanubrutinib is a highly selective BTK inhibitor that forms covalent bonds with cysteine residues at the BTK active site, thus inhibiting BTK activity. Zanubrutinib, a representative drug of the second-generation BTK inhibitors, still inhibits EGFR, HER2, ITK, JAK3, TEC, BMX, and BLK, although its off-target effect is significantly reduced compared to first-generation BTK inhibitors (1). Dermatological toxicities are off-target effects and are supposed to relate to EGFR inhibition, which frequently appears during the first year of treatment. The overwhelming majority can be improved over time, so reports about dermatological toxicities related to zanubrutinib are diminished as the duration of treatment increases (2). Herein, we reported our experience with zanubrutinib-induced dermatological toxicities in three consecutive patients with B cell malignancies. Once grade 4 dermatological toxicities occur, topical hormonal medication and anti-allergic treatment are recommended. Some patients require empirical anti-inflammatory treatment. In severe cases, consultation with a dermatology specialist is recommended.

Methods

Three consecutive patients were diagnosed with B cell malignancies in the Department of Hematology of The First people's Hospital of Yancheng. Bone marrow aspiration and image logical examination were done in all patients to confirm the diagnosis. One patient diagnosed with PB-DLBCL, received a regimen containing rituximab, lenalidomide, and zanubrutinib. The other two patients were diagnosed with relapsed CLL and treated with oral zanubrutinib. All patients were treated with zanubrutinib 160 mg twice daily continuously, until disease progression or unaccepted toxicities.

Results

Case 1

A 71-year-old female patient was admitted to our hospital because of her inadvertent discovery of a right breast mass in June 2021. The patient denied any relevant personal or family history. Palpation examination revealed an irregular mass with a medium texture. Mastectomy was performed in the Department of Surgery of our hospital. The pathological report revealed the lesion diffuse large B-cell lymphoma(DLBCL). The pathological immunohistochemical staining were as follows: CD10+, CD138-, CD20+, CD21-, CD23-, CD3+, CD38+, CD45RO+, CD5-, CD79α+, MUM1+, c-Myc+, CyclinD1-, Bcl2+, Bcl6+, Ki67 (+:80%), SOX-11-, PAX5+, Kappa-, Lambda+, CK-P-, Vim+. The whole-body positron emission tomography-computed tomography (PET-CT) scan and bone marrow aspiration were performed to evaluate the condition. She was finally diagnosed as PB-DLBCL, germinal center B-cell type (GCB), Ann Arbor stage IVA. The Patients received three cycles of targeted therapy including zanubrutinib, rituximab, and lenalidomide in August and September of 2021. Her blood profile was monitored three times a week. After 26 days of treatment, she developed bruising and skin ecchymoses on the right upper extremity and the right chest wall, but she continued to take the drug. After three cycles of chemotherapy, there was no significant improvement in bruising and skin ecchymoses. More severely, swelling of the right upper extremity was much more obvious. Zanubrutinib was withheld as a suspected cause of dermatological toxicity, and third-generation cephalosporin therapy was undertaken. Furthermore, the patient developed three severe skin necrosis with abscesses in the right chest wall, which evolved into cheese-like changes over time. The final decision was made to perform surgical resection of the local masses after multidisciplinary discussion, and the pathological findings were consistent with lymphoma. The pathological immunohistochemical staining were as follows: CD3-, CD5-, CD20+, CD79a+, CD21-, CD23-, CD10+, CD15-, CD30-, CyclinD1-, MUM1+, Bcl-2+, Bcl-6+, Ki67+, PAX5+. This suggested that necrosis of the right chest wall skin was associated with disease progression. Reexamination of CT indicated the patient's progress, then we switched the regimen to lenalidomide, along with rituximab-cyclophosphamide, hydroxydaunomycin, oncovin, and prednisone(R-CHOP) chemotherapy. The bruising and skin ecchymoses on the right upper extremity subsided gradually in the following months. After four cycles of R-CHOP chemotherapy, PET-CT showed that the patient achieved complete remission. The patient was given orelabrutinib plus lenalidomide as a maintenance regimen. No similar dermatological toxicities occurred again during any of these periods (Figure 1).

Case 2

A 56-year-old man was diagnosed with CLL at the age of 45 and was previously treated with rituximab, fludarabine, and cyclophosphomide chemotherapy. In April 2021, the patient presented with progressive splenomegaly, lymphadenopathy, peripheral blood thrombocytopenia, and lymphocytosis. Fludarabine and cyclophosphamide treatment was restarted. The patient achieved partial response quickly and received zanubrutinib as maintenance treatment. After 30 days of treatment of zanubrutinib, acne-Like rash and ecchymosis ran over the whole body, and diffused maculopapular rash with purpuric lesions gradually appeared. The patient developed a papulopustular rash involving the nape of the neck, trunk, axilla, limbs, and groin area after two months of treatment. He did not show any symptoms of fever or systemic allergy. Bacterial

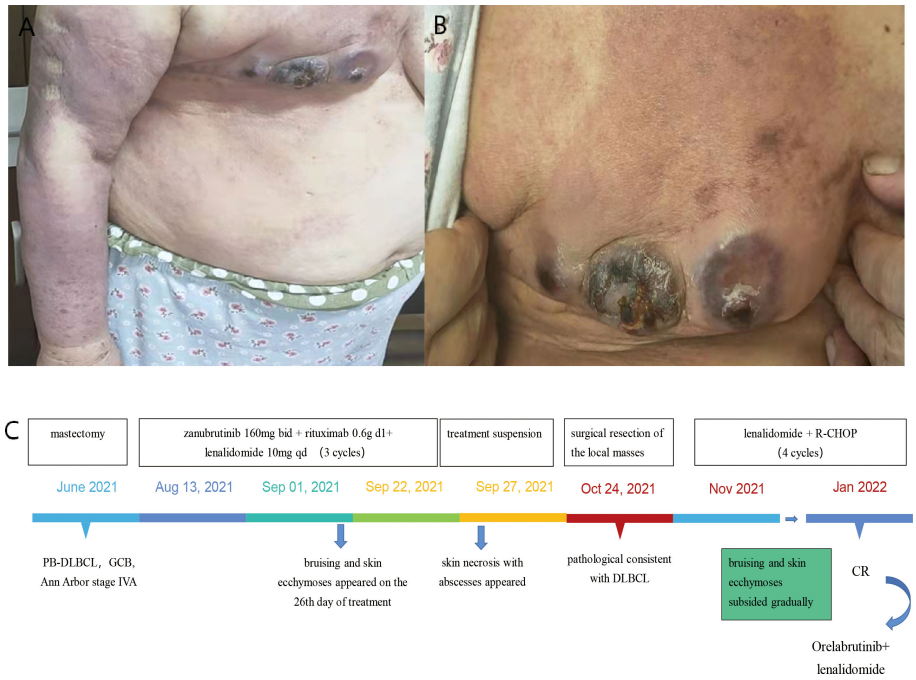

FIGURE 1
Case 1 : A 71-year-old woman with PB-DLBCL. **(A)** swelling of the right upper extremity. **(B)** bruising and skin ecchymoses on the right upper extremity and the right anterior chest wall. **(C)** The time line of this patient.

cultures were taken several times, but the results were all negative. We had suggested skin biopsy several times, but the patient refused skin biopsy. Due to our experience, the special skin rash was related to the side effect of zanubrutinib. After careful consideration, zanubrutinib was stopped. Treatment with topical corticosteroids was proposed and the effect was not obvious. We empirically treated the patient with vancomycin according to the local epidemiological characteristics, and the patient's skin erythema and rupture did improve significantly after vancomycin treatment. On our advice, the patient continued oral linezolid tablets for maintenance treatment. The patient is currently in the follow-up phase and the generalized rash has significantly subsided and improved, but the patient refused to continue the treatment of zanubrutinib (Figure 2).

Case 3

In July 2020, a 69-year-old male patient was admitted with a relapse of CLL, diagnosed 8 years ago, initially treated with FC (i.e., fludarabine and cyclophosphamide) regimen chemotherapy and discontinued in March 2013. He had severe anemia, lymphadenopathy, hyperleukocytosis with lymphocytosis, and mild thrombocytopenia. Peripheral blood flow cytometry analysis was consistent with CLL, bone marrow studies showed massive and diffuse infiltration with clonal small B lymphocytes. He was treated with BR(i.e., rituximab and Bendamustine) regimen chemotherapy and received a therapeutic response. The patient achieved partial remission after two cycles of treatment, then switched to zanubrutinib as maintenance therapy. One month after treatment, multiple lesions began to appear on his left lower extremity, initially presenting as papules, which later developed into pustules and cellulitis. The patient was followed by Dermatology Department for clearing the wound. Topical fusidic acid cream was used as an anti-inflammatory agent, and compound calamine lotion was used to reduce itching. However, the skin lesions were persistent over his left lower limb for about six months. The patient had necrotic tissue removed from the left lower extremity wound in the Department of Dermatology, and the skin biopsy results suggested that the tissue was infiltrated by acute and chronic inflammatory cells with necrosis. We also retained pus from this area for flow cytometry analysis and the results were as follows: CD19+, CD5+, CD20+, CD25+, CD23, cBcl-2+, cKappa-, sKappa-, sLambda-, CD138-, CD10-, CD38-, sIgM-, CD102-, CD11c-, FMC-7-, CD22-. Treatment and follow-up in the dermatology department were carried out for more than 6 months and the skin eventually healed well. On our advice, the patient was reintroduced to oral zanubrutinib in October 2021, after which the patient did not experience similar dermatological toxicities (Figure 3).

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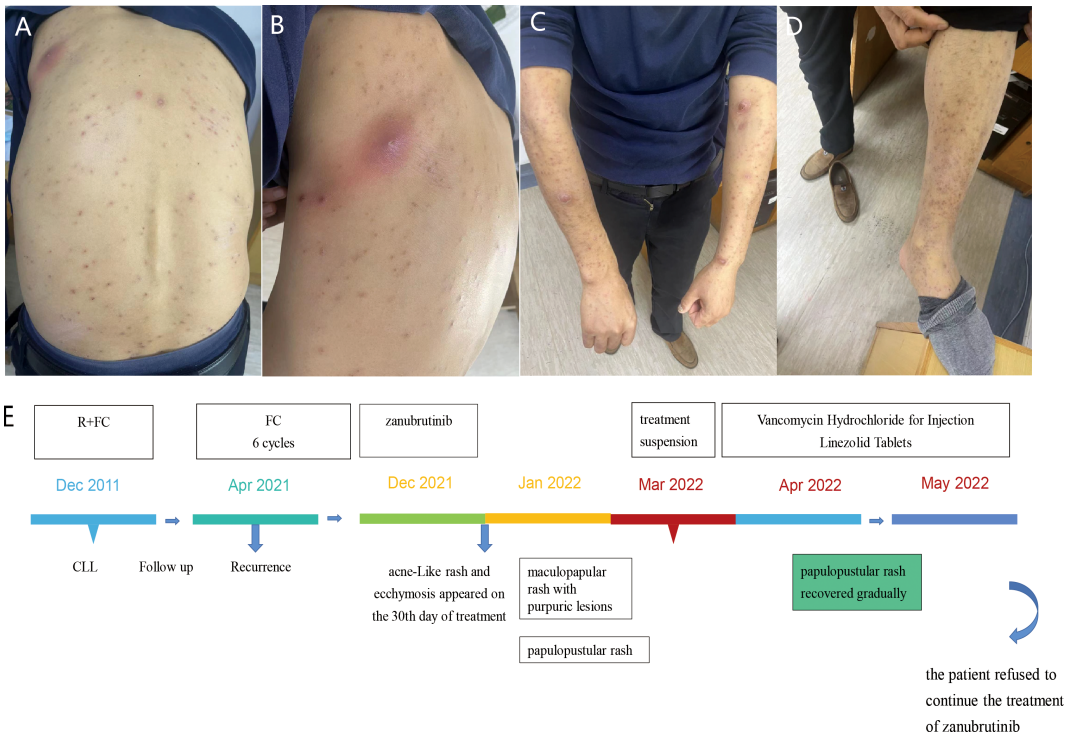

FIGURE 2
Case 2 : A 56-year-old man with CLL. **(A, C, D)** difused and severe maculopapular rash with purpuric lesions on the trunk, upper extremity and lower extremity. **(B)** necrotizing folliculitis with suspected Staphylococcus aureus superinfection on the back. **(E)** The time line of this patient.

Discussion

Dermatological toxicities are among the most common toxicities of BTK inhibitors (3). Bruising, skin ecchymoses, eczema-like rash, acne-like rash (folliculitis), pityriasis rosea-like rash, and panniculitis are the typical dermatological adverse events. Nail changes include brittleness onycholysis, onychorrhexis, onychoschizia, koilonychia, or trachyonychia paronychia, and subungual splinter hemorrhages. Hair changes are also common, including alopecia, and the hair follicle changes from curly to straight. Stomatitis is not rare. Other dermatological toxicities include neutrophilic dermatosis, skin carcinomas, autoimmune skin disorders, xerosis, peripheral edema, and eosinophilic dermatosis of hematologic malignancy (EDHM).

Here, we retrospectively reported three cases of dermatological toxicities induced by zanubrutinib. All three patients developed grade 4 skin adverse effects, which lead to a suspension of treatment in two patients. The first patient was diagnosed with PB-DLBCL, received combination chemotherapy containing zanubrutinib, lenalidomide, and rituximab, and developed widespread bruising and skin ecchymoses on the right chest wall and right upper extremity on day 26 of treatment. All three medicines have the potential to produce dermatological toxicity, and we took into account this patient's zanubrutinib-related dermatological toxicity for the following reasons. When given rituximab for the first time, some people may develop a rash allergy. This is usually a transitory reaction that can be alleviated by using anti-allergic medications and slowing down the titration rate. On the day of rituximab treatment, the patient did not experience any usual adverse symptoms such as rash, pruritus, chest tightness, or dyspnea. The dermatological toxicities did not occur when the patient had chemotherapy with the R-CHOP regimen and lenalidomide, and the timing of the skin reaction in this patient does not match the time point of rituximab treatment. Therefore, we concluded that the rash in this patient was not related to rituximab or lenalidomide. In contrast, skin necrosis at the right breast incision was confirmed by biopsy to be associated with lymphoma progression. PET-CT corroborated the recurrence of the disease. Both the second and third patients were diagnosed with relapsed CLL, received FC regimen chemotherapy and achieved remission, and were maintained on zanubrutinib without taking other drugs that could cause dermatological toxicity during maintenance therapy. Therefore, the dermatological toxicities in these two patients were related to zanubrutinib.

To date, there are no reported cases of dermatological toxicities secondary to zanubrutinib in China. We report the first three cases of dermatological toxicities due to zanubrutinib.

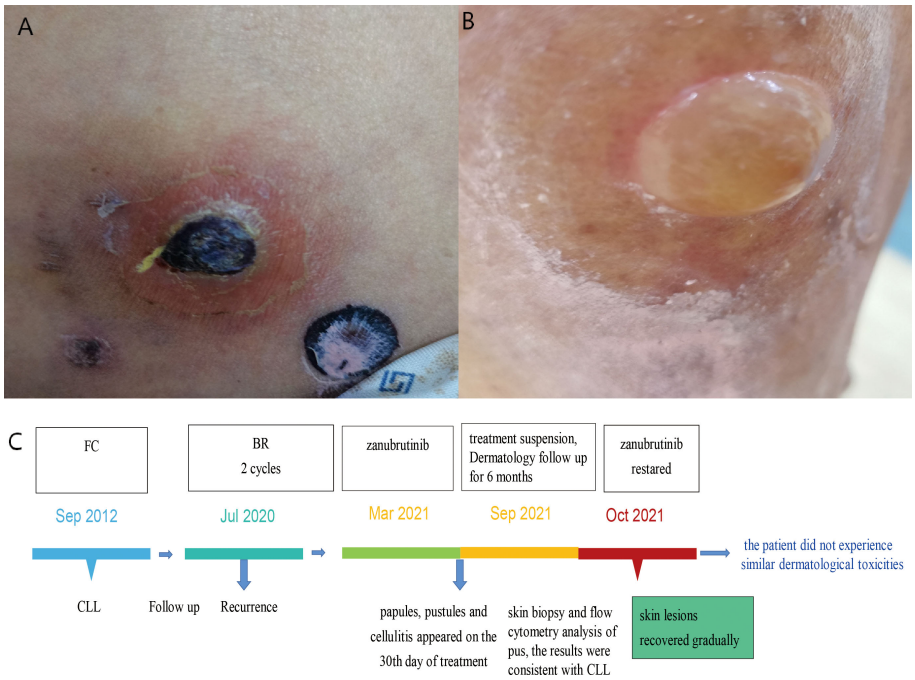

FIGURE 3
Case 3 : A 69-year-old man with CLL. **(A)** cutaneous ecchymosis with central necrosis. **(B)** pustules on the left lower extremity. **(C)** The time line of this patient.

Our team hopes these cases could raise clinical awareness regarding zanubrutinib-induced dermatological toxicities and the importance of drug withdrawal in the event of dermatological toxicities. We review the underlying mechanisms of dermatological toxicities, and the incidence of zanubrutinib-associated dermatological toxicities, and propose our experience in managing them.

Underlying mechanisms of dermatological toxicities

It has been postulated that the direct binding to both BTK and other 'off-target' kinases leads to BTK inhibitors-related dermatological adverse events. Zanubrutinib has overwhelming advantages in optimizing BTK inhibition and minimizing off-target inhibition of alternative kinases (Tec, ITK, EGFR, SRC-family kinases) (4). EGFR and downstream signaling pathways are involved in numerous key biological processes such as cell proliferation, differentiation, migration, and apoptosis. In skin tissues, EGFR receptors are expressed on keratinocytes, which are distributed in the basal and suprabasal layers of the epidermis as well as in the outer layer of the hair follicle. The EGFR pathway regulates the normal growth and differentiation process of the epidermis, stimulating epidermal growth, inhibiting differentiation, and accelerating wound healing. Blocking the EGFR pathway in the skin can lead to a series of inflammatory reactions, and thus manifest the corresponding skin adverse effects (5). EGFR-TKI could evoke dermatological and gastrointestinal toxicities through block down epidermal growth factor signals. Dermatological toxicities involved rash acneiform, skin fissure, and xerosis, which are related to pruritus (6). Acne-like rash with erythematous papules or pustules centered on hair follicles is the most common clinical manifestation of adverse skin reactions. With few subjective symptoms, no effect on daily life, and no secondary infection, grade 1 adverse reactions are limited to the head, face, and upper trunk. Grade 2 side effects along with minor subjective symptoms, little impact on daily life, and no signs of secondary infection. To adverse reactions of grade 3/4, the subjective symptoms are severe, causing significant disruption in daily life and the risk of secondary infection. Premature differentiation, inflammation, apoptosis, skin atrophy, telangiectasia, and photosensitivity are all side effects of EGFR inhibition (7). Although zanubrutinib is a highly selective BTK inhibitor, it also inhibits cell cycle progression and increases apoptosis by acting on other kinases such as EGFR. The most likely mechanism of zanubrutinib-induced skin rash appears to be off-target inhibition of EGFR. Inhibition of c-kit and platelet-derived growth factor receptors is another mechanism proposed

for zanubrutinib-induced drug eruption. Iberri hypothesized that some of these rash types, particularly those that appeared within the first month of treatment, could be related to the transient hyperlymphocytosis associated with BTK, which is caused by CLL cells egressing from lymph nodes and spleen (8).

We believe that the dermatological toxicities seen in these three patients were not the same phenomenon as CLL-associated insect bite-like reactions. Insect bite-like reaction, also known as eosinophilic dermatitis associated with hematologic malignancies, is a nonspecific skin reaction to a hematologic disease, which results in an altered immune response and increased secretion of TH2 cytokines (IL4 and IL5) that stimulate the development of eosinophilic skin infiltrates (9, 10). All three patients denied a history of any insect stings, food allergies, and drug allergies. The first patient was newly diagnosed with DLBCL and did not undergo any treatment that could have induced dermatological toxicities before zanubrutinib-based targeted therapy. The second and third cases were diagnosed with relapsed CLL and did not experience any degree of dermatological toxicities during previous chemotherapy and follow-up. All three patients developed varying forms of dermatological toxicities within one month of zanubrutinib treatment, and we do not believe that these were coincidences. Dermatological toxicities associated with ibrutinib have been reported, and most of these dermatological toxicities occur within 1 year after ibrutinib, and the incidence decreases gradually with time. The three patients observed in our center had common features with previous cases reported in the literature. Compared to ibrutinib, zanubrutinib is more precisely targeted and has fewer toxic side effects. As a result, the incidence of dermatological toxicities associated with zanubrutinib is much lower, and there are very few reports in the literature. There are three kinds of BTK inhibitors currently in use in our center, namely ibrutinib, zanubrutinib, and orelabrutinib. Based on the results of the follow-up, only these 3 patients have experienced grade 4 dermatological toxicities so far. This is the purpose of our study as a way to draw the attention of investigators and to present our treatment experience to better manage the adverse reactions of BTK inhibitors.

Incidence of zanubrutinib-associated dermatological toxicities

The efficacy and safety of zanubrutinib were evaluated in the following clinical trials (Table 1). Safety data were obtained from five multicenter studies that enrolled a total of 394 patients who received zanubrutinib. No grade ≥3 dermatological toxicities were reported. No deaths (all R/R patients) were attributed to dermatological toxicities. Almost all patients (98%) reported ≥1 Treatment-Emergent Adverse Events(TEAE). Dermatological toxicities reported in ≥10% of the study population were rash, bruising, petechiae, purpura, contusion, and rash maculo-papular (11).

Management of dermatological toxicities

Multiple treatment regimens could alleviate zanbrutinib-related dermatological toxicities. Moisturizers are typically used to manage dermatological toxicities in patients with grade 1/2 skin reactions. Patients should avoid alcohol and perfume-containing products because they can dry out the skin and limit its ability to heal under stress. In patients with moderate to severe dermatological toxicities, topical steroid ointments such as hydrocortisone can be used alone or in combination with topical emollients. In cases where skin toxicity leads to infection, antibiotic ointments or systemic antibiotics must be used. In addition, multidisciplinary cooperation is recommended, and the patient is advised to visit the dermatology department for further consultation. Due to the long-term use of immunosuppressive drugs in patients with hematologic diseases, immune function is deficient and they are prone to a variety of opportunistic infections in combination. Our recommendation is to temporarily discontinue the drug if grade 4 dermatological toxicities occur to give the patient time to deal with the severe events while still allowing the patient to continue using the drug, thereby avoiding disease progression due to discontinuation.

In conclusion, physicians should be aware of the potential dermatological toxicities of zanubrutinib, which requires

TABLE 1 Incidence Of Zanubrutinib-Associated Dermatological Toxicities.

Clinical Trial	Phase	B-Cell Malignancies	Patients, No.	Rash	Purpura	Rash Maculo-Papular	Ecchymosis
Nct03189524	1	Cll/Sll, Mcl, Wm/Lpl, Fl, Mzl, Hcl, Or Non-Gcb Dlbcl	44	10/44 (22.72%)	5/44 (11.36%)	5/44 (11.36%)	–
Nct03206918	2	Cll/Sll	91	17/91 (18.68%)	31/91 (34.07%)	1/91 (1.10%)	–
Nct03206970	2	Mcl	86	31/86 (36.05%)	–	–	–
Nct03332173	2	Wm	44	8/44 (18.18%)	8/44 (18.18%)	–	4/44 (9.09%)
Nct03053440	3	Wm	129	No Study Results Posted			

vigilance. With appropriate treatment, they can be managed, minimizing patient discomfort and reducing the need for therapy interruption or discontinuation.

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.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of The First people's Hospital of Yancheng. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

LW and JT conceived and designed the study. JF, YH, YC, HX collected the clinical data. LW and YM wrote the paper. All authors contributed to the article and approved the submitted version.

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.

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Incidence, clinical characteristics, and prognostic nomograms for patients with myeloid sarcoma: A SEER-based study

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Background: Myeloid sarcoma (MS) is a rare hematological tumor that presents with extramedullary tumor masses comprising myeloid blasts. A controversial issue is whether MS involving normal hematopoietic sites (liver, spleen, and lymph nodes) should be excluded in future studies. We aimed to compare MS characteristics and outcomes involving hematopoietic and non-hematopoietic sites and construct a prognostic nomogram exclusively for the latter.

Methods: Data from patients diagnosed with MS between 2000 and 2018 were collected from the Surveillance, Epidemiology, and End Results (SEER) database. According to the primary site, patients were classified as having MS involving hematopoietic sites (hMS) or non-hematopoietic sites (eMS). Clinical characteristics and survival outcomes were compared between the two groups using Wilcoxon, chi-square, and log-rank tests. Cox regression analysis was used to identify eMS prognostic factors to establish prognostic nomograms. The models' efficiency and value were assessed using receiver operating characteristic (ROC) curves, calibration curves, and decision curve analysis (DCA).

Results: In total, 694 patients were enrolled, including 86 with hMS and 608 with eMS. There were no sex, race or marital status distribution differences between the two groups. Patients with eMS had better overall and cancer-specific survival rates than those with hMS. Additionally, prognostic factor effects differed between the two groups. Patients with eMS were randomly divided into the training (number of patiens, n=425) and validation cohorts (n=183). Age, first primary tumor, primary site, and chemotherapy were used to establish nomograms. The C-index values of overall survival (OS) and cancer-specific survival (CSS) nomograms were 0.733 (validation: 0.728) and 0.722 (validation: 0.717), respectively. Moreover, ROC, calibration curves, and DCA confirmed our models' good discrimination and calibration ability and potential clinical utility value.

Conclusion: Our study described the differences between patients with eMS and those with hMS. Moreover, we developed novel nomograms based on clinical and therapeutic factors to predict patients with eMS' 1-, 3- and 5-year survival rates.

KEYWORDS

SEER, myeloid sarcoma, nomogram, prognosis, hematopoietic site

Introduction

Myeloid sarcoma (MS), a term that accurately summarizes the two features of this disease, is a rare hematologic tumor composed of myeloid cells in bone, soft tissues and other anatomical sites (1). Due to this entity's rarity, much of our current limited MS clinical and prognostic characteristics understanding is derived from case reports or single-center studies. In addition, several terms are used in clinical diagnoses and academic reports to describe MS, including chloroma, granulocytic sarcoma, and extramedullary acute myeloid leukemia (eAML) (1–3). The confusion over MS terminologies has further impeded this disease's comprehensive study, especially its epidemiological features.

Other important MS features are that it can occur at any site of the body, except the bone marrow, and present synchronously or subsequently with various myeloid malignancies, including acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), myeloproliferative neoplasm (MPN), or chronic myelogenous leukemia (CML) (4–6). Because infiltration of leukemic cells in the liver, spleen or lymph nodes is generally considered to be an indication of the natural spread of tumor cells from the bone marrow, several scholars have argued that myeloid neoplasms originating from normal hematopoietic sites should not be included in MS (5, 7). The term "extramedullary acute myeloid leukemia (eAML)" was proposed to describe MS involving non-hematopoietic sites in their studies (8). However, excluding myeloid masses involving normal hematopoietic sites from MS is based only on theoretical derivation. The differences between patients with MS involving normal hematopoietic and non-hematopoietic sites have yet to be illustrated in the literature.

The Surveillance, Epidemiology, and End Results (SEER) database, a population-based oncology clinical database in the United States, provides a wealth of information for research on rare tumors. However, the latest published literature on MS, based on the SEER database, only included patients (≥15 years old) from 1973 to 2010 (9). We enrolled patients diagnosed with MS between 2000 and 2018 from the SEER database and divided them into two categories: MS involving hematopoietic sites (hMS) and those involving non-hematopoietic sites (eMS). We aimed to update our understanding of MS regarding its epidemiological, clinical, and prognostic characteristics, describe the differences between patients with eMS and hMS, and further develop MS prognostic nomograms.

Abbreviations: MS, Myeloid sarcoma; DCA, decision curve analysis; OS, overall survival; CSS, cancer-specific survival; hMS, myeloid sarcoma involving hematopoietic site; eMS, myeloid sarcoma excluding those involving hematopoietic site; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; ML, chronic myelogenous leukemia; eAML, extramedullary acute myeloid leukemia; AIC, Akaike information criterion; AUC, area under the receiver operating curve; APC, annual percentage change; DCA, Decision curve analysis; RT, radiotherapy; HSCT, hematopoietic stem cell transplantation.

Materials and methods

Patient selection and data collection

All data involved in this study were obtained from the SEER database software (SEER*Stat version 8.4.0). Age-adjusted rates and trends in rates of MS from 2000 to 2018 were calculated in the rate session. MS patient selection and clinical data collection were carried out in the case listing session based on the SEER reseach dataset (18 registries, [2000-2018]). As shown in the flow chart (Figure S1), the inclusion criteria of MS patients were as follows: (1) the International Classification of Disease for Oncology, Third Edition (ICD-O-3) histology code 9930/3; (2) positive exfoliative cytology or positive histology diagnosis. The exculsion criteria were as follows: (1) clinical diagnosis, image diagnosis, or unknown diagnosis; (2) primary site labels C42.0 Blood, C42.1 Bone marrow, or unknown site. To describe the clinical characteristics of MS patient, the following clinical information was extracted: age and marital status at diagnosis, sex, race, year of diagnosis, total number of tumors per patient, first malignant primary indicator, primary site, treatment (surgery, radiotherapy, and chemotherapy), survival time, survival status, and cause of death. SEER is a free and publicly available database and has anonymized the patient's identifying

information. Therefore, there are no ethical issues, and approval from the ethics committee was not required.

According to anatomic sites, patients were classified into two groups: MS involving hematopoietic sites (hMS) and those involving non-hematopoietic sites (eMS). Hematopoietic sites include the spleen, liver and lymph nodes. According to the classification principles proposed by Goyal et al., non-hematopoietic sites can be further divided into 9 major categories as follows: soft tissue(st), skin/breast (s/b), bone (b), nervous system (ns), head/neck (h/n), digestive system(ds), cardiopulmonary/mediastinum (c/m), reproductive system (rs), and kidney/bladder/retroperitoneum (k/b/r) (Table S1) (10). Furthermore, patients with eMS were randomly divided into a training and a validation cohort by a ratio of 7:3 to develop prognostic prediction models. This study's two endpoints, overall survival (OS) and cancer-specific survival (CSS), were defined as the time from the initial diagnosis to death related to any cause and MS, respectively.

Construction, validation and evaluation of nomograms

Univariate and multivariate COX regression analyses were performed to identify the independent risk factors for OS and CSS of patients with eMS in the training cohort. Moreover, the C-index and Akaike information criterion (AIC) were calculated to determine the final independent prognostic factors for inclusion in the prognostic nomograms. Regarding the performance of the prognostic nomograms, the area under the receiver operating curve (AUC) was calculated to examine the discrimination power; calibration curves were plotted to test the predictive accuracy; and decision curve analysis (DCA) was used to evaluate the clinical utility. Based on the nomograms, each patient was assigned a total point to predict survival rates. As the scores rise, the survival rates fall. Then, the best cutoff values for the total points were generated using X-tile software and used for risk stratification (low and high). To evaluate the significance of the OS and CSS differences between the low- and high-risk groups, we also conducted Kaplan-Meier survival analysis and log-rank tests.

Statistical analysis

Continuous variables were compared between groups using the Mann-Whitney U-test, whereas categorical variables were compared using the chi-squared test. Survival outcomes between the groups were visualized using Kaplan–Meier curves and compared using a log-rank test. Overall, statistical analyses in this study were conducted using R software (version 4.0.5) with the "survival," "survminer," "rms," and "ggDCA" packages. P-value <0.05 was considered statistically significant.

Results

Incidence trends of MS

We used the SEER database to calculate age-adjusted MS incidence rates by year of diagnosis and gender. A rising pattern in MS incidence from 2000 to 2018 was observed, with an annual percentage change (APC) of 3.20% (95% confidence interval [CI]: 1.21–5.23%, P=0.003) (Figure 1A). After peaking at 0.077 per 100,000 persons in 2015, the age-adjusted MS incidence plummeted to 0.035 per 100,000 persons in the following two years. Grouped by sex, we found the waveringly increasing trend was more noticeable in male patients, with an APC of 3.44% (95% CI 0.99-5.96%, P=0.008) and 2.65% (95%CI 0.14-5.23%, P=0.039) in male and female patients, respectively (Figure 1B). In general, male patients had a substantially higher incidence than females.

Clinical characteristics of patients

We identified 694 patients with MS from the SEER database between 2000 and 2018 and summarized their demographic and

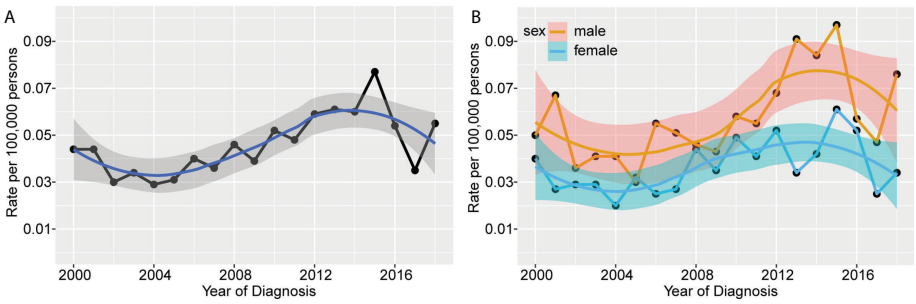

FIGURE 1
Incidence rates of MS according to year of diagnosis. The waveringly increasing trends were observed in incidence rates of all patients with MS from 2000 to 2018 **(A)**, and patients grouped by sex **(B)**.

clinical characteristics in Table 1. The median age of patients with MS in this study was 62 years, ranging from 0 to 96 years. Most patients were: aged above 60 years (53.2%), male (57.5%), white (80.1%), married (49.7%), and diagnosed between 2010 and 2018 (63.7%). In addition, most had MS as the first primary tumor (56.8%). Furthermore, half of the patients (49%) underwent chemotherapy, whereas only a small proportion underwent radiotherapy (24.9%) or surgery (13.1%).

TABLE 1 Baseline characteristics of patients diagnosed with MS from the SEER database.

Characteristic	eMS N(%)	hMS N(%)	Total N(%)	P.Value
Sum	608(87.6)	86(12.4)	694(100)	
Age at diagnosis (years)				0.011
Mean (SD)	55.5(22.5)	61.7 (19.7)	56.2(22.3)	
Median [Min, Max]	61.0 [0, 96.0]	67.5 [3,91.0]	62.0 [0, 96.0]	
Age group (years)				0.149
<40	140 (23.0)	14 (16.3)	154 (22.2)	
40-59	153 (25.2)	18 (20.9)	171 (24.6)	
≥60	315 (51.8)	54 (62.8)	369 (53.2)	
Year of diagnosis				0.513
2000-2009	224 (36.8)	28 (32.6)	252 (36.3)	
2010-2018	384 (63.2)	58 (67.4)	442 (63.7)	
Sex				0.826
Female	257 (42.3)	38 (44.2)	295 (42.5)	
Male	351 (57.7)	48 (55.8)	399 (57.5)	
Race				0.282
Asian	47 (7.7)	5 (5.8)	52 (7.5)	
White	490 (80.6)	66 (76.7)	556 (80.1)	
Others	71 (11.7)	15 (17.4)	86 (12.4)	
Marital.status				0.096
Single	151 (24.8)	18 (20.9)	169 (24.4)	
Married	304 (50.0)	41 (47.7)	345 (49.7)	
Widowed	49 (8.1)	14 (16.3)	63 (9.1)	
Others	104 (17.1)	13 (15.1)	117 (16.9)	
Number				0.074
≥3	94 (15.5)	19 (22.1)	113 (16.3)	
1	265 (43.6)	42 (48.8)	307 (44.2)	
2	249 (41.0)	25 (29.1)	274 (39.5)	
1st primary tumor				0.875
No	264 (43.4)	36 (41.9)	300 (43.2)	
Yes	344 (56.6)	50 (58.1)	394 (56.8)	
Surgery				0.103
No/Unknown	523 (86.0)	80 (93.0)	603 (86.9)	
Yes	85 (14.0)	6 (7.0)	91 (13.1)	
Radiation				0.008
No/Unknown	446 (73.4)	75 (87.2)	521 (75.1)	
Yes	162 (26.6)	11 (12.8)	173 (24.9)	
Chemotherapy				0.205
No/Unknown	310 (51.0)	37 (43.0)	347 (50.0)	
Yes	298 (49.0)	49 (57.0)	347 (50.0)	

As previously stated, patients were divided into two groups depending on the site involved: hMS (n=86) and eMS (n=608). A comparison of the two groups' demographic and clinical features is shown in Table 1. Except for age and radiotherapy proportion received, there were no statistical differences in the other variables between the eMS and hMS groups. Specifically, the hMS group had a higher patient proportion aged >60 years, with a median age of 67.5 years. Regarding treatment, patients with hMS were more likely to undergo chemotherapy (57% vs. 49%, P=0.205) and were less likely to undergo surgery (7% vs.14%, P=0.103) and radiotherapy (12.8%vs. 26.6%, P=0.008) than patients with eMS. In addition, the three most common involvement sites were soft tissues (35.6%), skin/breast (13.0%), and the digestive system (9.4%) (Table S1). The mean age of patients with eMS involving the digestive system, reproductive system, and head/neck was lower (<50 years) than that of the patients with eMS involving other sites (>50 years).

In consideration that pediatric patients were not included in the previous SEER-based study, we analyzed MS clinical characteristics in pediatric patients aged <15 years old (9). Among the 694 patients with MS, only 47 pediatric patients were identified. As shown in Table S2, pediatric patients' mean age was 5 years. Furthermore, 87.2% of children developed MS as the first primary tumor, which is far more common than in adults. In addition, the three most common sites in pediatric patients differed slightly from those in adults, with the head/neck rather than the digestive system being one of our study's three most common sites. Nearly three-quarters of pediatric patients receive chemotherapy, higher than the 50% in adults. Overall, pediatric patients' prognosis was also good, with a 3-year OS rate of 67.4%.

Survival analysis

Of the 694 patients with MS in this study, 498 died (71.76%); 410 died of MS (59.08%). We performed a Kaplan-Meier survival analysis to quantify and visualize OS and CSS in patients and used the log-rank test to compare survival outcomes between patients grouped by year of diagnosis and primary sites. As shown in Figure 2, the median OS time for patients with MS was 9 months, with a 31.3% 3-year OS rate. The median CSS time was 11 months, with a 37.7% 3-year CSS rate. Despite the growing number of patients, the OS and CSS of patients diagnosed in the last decade (2010-2018) did not differ from those in the previous decade (2000-2009), showing no significant improvement in survival outcomes over the last two decades. Additionally, patients with hMS had significantly lower OS and CSS rates than those with eMS, with a median OS of 5 and 10 months and median CSS of 5 and 13 months, respectively (Table S1).

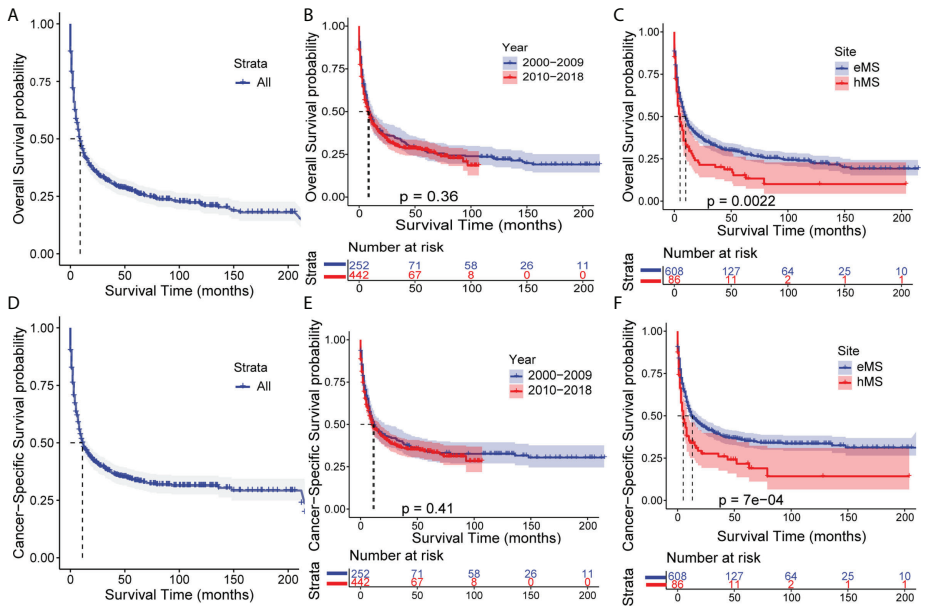

FIGURE 2
Kaplan–Meier analysis of overall survival (OS) and cancer-specific survival (CSS) in MS. Kaplan–Meier survival curves of OS for all patients **(A)**, patients stratified by year of diagnosis **(B)** and primary sites **(C)**. Kaplan–Meier survival curves of CSS for all patients **(D)**, patients stratified by year of diagnosis **(E)** and primary sites **(F)**. eMS, MS excluding those involving hematopoetic sites; hMS, MS involving hematopoetic sites.

We performed a subgroup survival analysis to determine whether different variable prognostic effects were consistent between hMS and eMS. As shown in Figure 3 and Figure S2, age, race, and tumor number had different effects on eMS and hMS prognosis. Given that the number of patients undergoing surgery and radiotherapy in the hMS group was too small, we should be cautious when concluding how treatment affects prognosis. The fact that the variables had different prognostic effects between the two groups suggested that hMS should be distinguished from eMS in future studies.

According to the 3-year OS rate shown in Table S1, the nine primary site categories in patients with eMS were further divided into four sets for the prognosis analysis as follows: setA (3-year OS <25%; nervous system and bone), setB (3-year OS: 25%–35%; cardiopulmonary/mediastinum, kidney/bladder/retroperitoneum, and soft tissue), setC (3-year OS: 35%–50%; skin/breast, head/neck, digestive system), and setD (3-year OS >50%; reproductive system). Patients with setA had the poorest prognosis among the four sets, and those with setD had the best prognosis.

Prognostic factors selection and nomograms construction

Patients with eMS were randomly divided into the training (n=425) and validation cohorts (n=183). Patients in the training cohort were screened for independent prognostic markers. None of the eleven variables included in the univariate COX analysis was statistically different between the training and validation cohorts (Table S3). The univariate Cox analysis showed that the year of diagnosis, sex, race, and radiotherapy were not significantly associated with the OS and CSS of patients with eMS (Table S4). As shown in Table 2, we included the remaining seven variables in the multivariate Cox analysis and demonstrated that marital status, tumor number, and surgery were not significant risk factors for OS and CSS (P >0.05). Patients aged >40 years, diagnosed with MS involving the nervous system or bone and not as 1st primary tumor, and who did not undergo chemotherapy had worse survival outcomes (HR>1, P <0.05). Combining the multivariate Cox regression analysis results and AIC, we eventually identified age (<40, 40-59, ≥60), first primary tumor (yes, no), site (setA, B, C, D), and chemotherapy (no/unknown, yes) as significant prognostic factors to construct OS and CSS nomograms (Figure 4). The OS and CSS nomograms' C-index values were 0.733 (95%CI: 0.703-0.762) and 0.722 (95%CI: 0.698-0.755) in the training cohort, 0.728 (95%CI: 0.679-0.757) and 0.717 (95% CI: 0.664-0.770) in the validation cohort, respectively.

Nomogram validation and evaluation

Figure 5 and Figure S3 show that time-dependent receiver operating characteristic (ROC) and calibration curves were

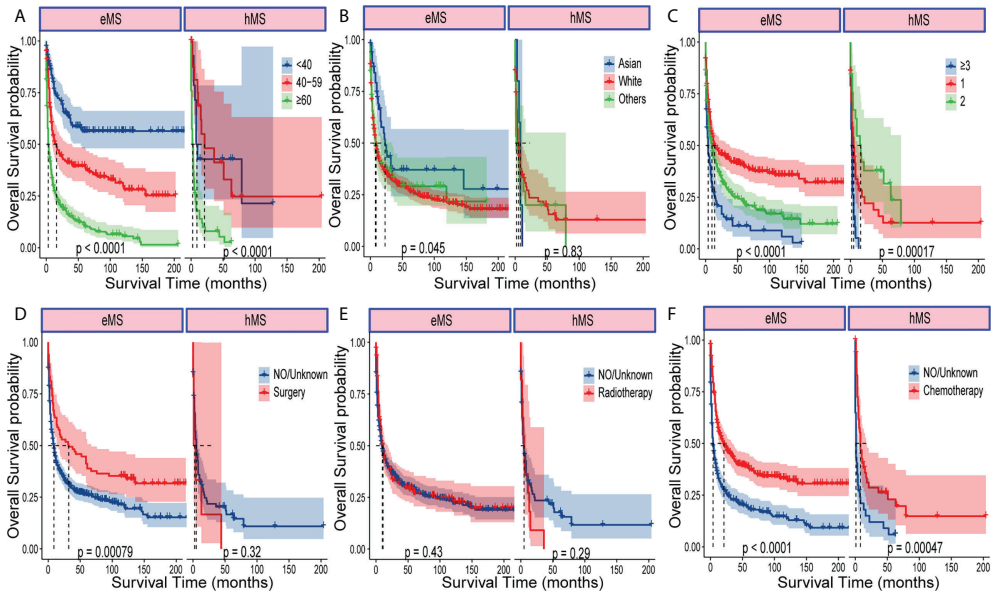

FIGURE 3
Kaplan–Meier analysis of OS in eMS and hMS, respectively. Kaplan–Meier survival curves of OS for patients with eMS or hMS stratified by age **(A)**, race **(B)**, number of tumors **(C)**, surgery **(D)**, radiotherapy **(E)** and chemotherapy **(F)**.

drawn to evaluate OS and CSS nomograms' discrimination and calibration ability, respectively. The AUC was calculated to assess the performance. The OS and CSS nomograms presented an AUC value of 0.774-0.823-0.829 and 0.758-0.812-0.822 for the training cohort's 1-, 3-, and 5-year survival rates, and 0.768-0.754-0.801 and 0.755-0.756-0.811 for the validation cohort's 1-, 3-, and 5-year survival rates. In addition, the calibration curves revealed outstanding consistency between actual survival rates and nomogram-predicted survival rates at 1, 3, and 5 years in both the training and validation cohorts. DCA was used to assess the nomograms' clinical utility. Figure 6 and Figure S4 showed that the OS and CSS nomograms had a major positive net benefit, indicating good clinical utility and favorable efficiency in predicting 1-, 3-, and 5-year survival rates.

To extend the nomograms' clinical application, we stratified patients into two groups based on their nomogram points: high-risk with higher points and low-risk with lower points. The best cutoff values for OS and CSS nomogram points were 139.3 and 130.7, respectively (Figure S5). In general, Kaplan-Meier survival analysis revealed that patients with eMS could be classified into low-risk patients with a better prognosis and high-risk patients with a worse prognosis (P<0.0001, Figure 7).

Discussion

Given MS's rarity and terminology confusion, most of its knowledge is based on a case series of single-center studies, wherein the retrospective analysis of a limited number of patients may lead to conflicting findings (7, 11). Although a previous MS study using the SEER database was published, it only covered adult patients aged >15 years from 1973 to 2010 (9). MS's epidemiological, clinical, and prognostic characteristics are poorly understood. Our study updates MS understanding based on the SEER database by including patients from 2000 to 2018 and provides MS epidemiological features and prognostic nomograms.

As previously stated, MS was listed as an AML subtype by the World Health Organization (WHO) and considered as a specific presentation of many other myeloid neoplasms, including MDS, MPN, and CML (1, 12, 13). Recently, two cases were reported in which MS and acute lymphoblastic leukemia occurred simultaneously (14, 15). MS's complex association with other hematologic neoplasms has resulted in a lack of separate epidemiologic data. Previous studies have only focused on the patient proportion who presented with MS in AML and showed that the MS occurrence rate in AML was 2-9% in adults and 6.8-23.3% in children (2, 9, 10, 16, 17). Our study is the first to report the MS age-adjusted incidence among the US population from 2000 to 2018. We showed a fluctuating increasing trend in the incidence rate and observed a peak in incidence in 2015, one year before the WHO re-adopted the term MS (4). Consistent with the higher male patient proportion, their incidence rate was notably higher than that of female patients. However, since MS is easily ignored or misdiagnosed in clinical practice, with up to 50% of cases

TABLE 2 Multivariate COX regression analyses of OS and CSS in the training cohort.

Characteristics	OS			CSS		
	HR	95%CI	P.Value	HR	95%CI	P.Value
Age*						
<40	Ref			Ref		
40-59	2.17	1.39-3.40	<0.001	1.82	1.12-2.96	0.015
≥60	4.21	2.69-6.58	<0.001	3.57	2.21-5.76	<0.001
Marital.status						
Single	Ref			Ref		
Married	0.80	0.55-1.17	0.251	0.76	0.50-2.25	0.199
Widowed	1.34	0.80-2.24	0.271	1.20	0.68-2.13	0.529
Others	0.91	0.59-1.39	0.654	0.84	0.53-1.34	0.461
Number						
≥3	Ref			Ref		
1	0.98	0.60-1.58	0.929	0.86	0.51-1.47	0.589
2	0.84	0.61-1.17	0.311	0.74	0.52-2.06	0.099
1st Primary Tumor*						
No	Ref			Ref		
Yes	0.67	0.46-0.98	0.038	0.69	0.45-1.05	0.086
Site*						
SetA	Ref			Ref		
SetB	0.62	0.42-0.90	0.013	0.58	0.38-0.87	0.009
SetC	0.48	0.32-0.72	<0.001	0.45	0.29-0.71	<0.001
SetD	0.43	0.23-0.80	0.008	0.38	0.18-0.77	0.007
Surgery						
No/Unknown	Ref			Ref		
Yes	0.73	0.51-1.05	0.091	0.75	0.49-1.12	0.161
Chemotherapy*						
No/Unknown	Ref			Ref		
Yes	0.65	0.51-0.84	<0.001	0.70	0.53-0.93	0.012

OS, overall survival; CSS, cancer-specific survival; HR, hazard ratio; CI, confidence interval; setA, nervous system and bone; setB, cardiopulmonary/mediastinum, kidney/bladder/retroperitoneum and soft tissue; setC, skin/breast, head/neck, and digestive system; setD, reproductive system. The symbol *indicates that the variable is statistically significantly in the COX regression analysis with P. value < 0.05.

being misdiagnosed as lymphoma or Ewing sarcoma, the actual prevalence may be underestimated (18, 19).

In this study, we comprehensively analyzed MS's demographic and clinical characteristics. The patients were predominantly older Caucasian men, consistent with previous studies based on the SEER and NCDB databases (9, 10). Our study analyzed tumor numbers in patients with MS and whether MS was the first primary tumor. As reported in previous studies, nearly 80-90% of newly diagnosed patients with MS may have an AML or other hematological neoplasm history, either at diagnosis or later in the disease course (7, 20). However, patients with MS as the initial and only tumor, also known as isolated MS, accounted for nearly half of the patients in our study, suggesting that isolated MS was more prevalent than MS combined with other hematologic malignancies. Understanding of MS in association with other hematologic cancers is improving. However, there is still more to learn about isolated MS (21).

MS can occur at any extramedullary anatomical site; however, the primary site classification principles have not been standardized. The orbit and gonads, considered privileged sanctuary sites with a better prognosis, were highlighted as separate categories in previous SEER-based studies (9). The primary site classification in the NCDB-based study, which integrates anatomical locations, organ systems, and prognosis, was adopted in this study (10). As previously described, we grouped the lymph nodes, spleen, and liver as normal hematopoietic sites, whereas the remaining non-hematopoietic sites were divided into nine categories and then grouped into four sets based on the 3-year OS. We found that the most common sites were soft tissue, skin/breast, and digestive system, consistent with NCDB- and SEER-based study results. In addition, numerous studies have identified the primary site as an independent prognostic factor (7, 22). In our study, patients with MS involving the head/neck, reproductive system, or digestive system had a better prognosis. However, the better prognosis could be partly due to the younger age of patients with MS involving these sites.

According to Shallis et al., several scholars recommend distinguishing patients with hMS from those with eMS, stating that myeloid blasts involving normal hematopoietic sites should be diagnosed as extramedullary leukemia infiltrates, rather than MS (8, 22, 23). However, apart from the distinct onset sites, the differences between these two concerning critical elements, including clinical and molecular characteristics, have not been published in the literature. In this study, we demonstrated demographic and clinical characteristic differences between patients with hMS and those with eMS. On average, patients with hMS were older than those with eMS, with a higher proportion of patients receiving local therapy. We also observed that the effects of age, race, and tumor number on prognosis differed between the two groups. These findings emphasize the importance of identifying eMS as a separate subtype in future prognostic studies.

Regarding treatment options, surgery, chemotherapy, radiotherapy (RT), and hematopoietic stem cell transplantation (HSCT) are all available for patients with MS (23). However, there are no agreed guidelines for MS due to its rarity, neither criteria for local therapy and chemotherapy nor indications for HSCT. For either isolated MS or MS that is synchronous with AML, intensive anti-AML chemotherapeutic protocols are currently recommended as systemic therapy, and HSCT is recommended as consolidation therapy (5, 19). In our study, half of the patients underwent chemotherapy, nearly a quarter received RT, more than one in eight patients underwent surgery, and the number of those who received HSCT was unavailable. Although the NCDB-based study included information on individuals who received HSCT, our SEER-based study included more patients who received the other

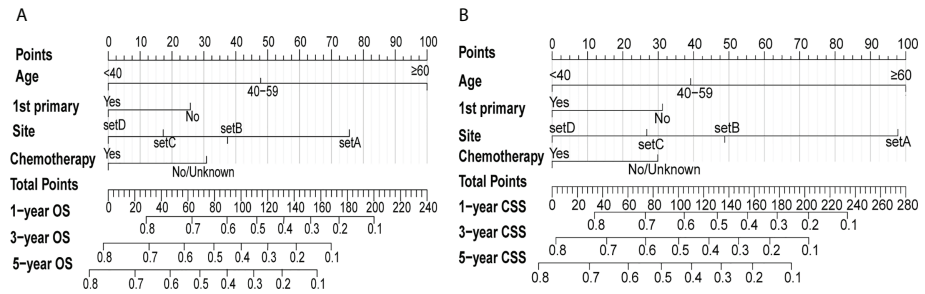

FIGURE 4
Nomograms of predicting the 1-, 3- and 5-year OS rates **(A)** and CSS rates **(B)** for eMS patients. The first line called "Points" is the score reference of the 4 variables below. For any given patient, the score of each variable can be obtained by drawing a vertical line from the corresponding scale axis to the first line "Points". Then, sum up the 4 scores to obtain the total points, which can be mapped to predict 1-, 3-, and 5-year survival rates by drawing a line descending from the axis labeled "Total points" to the 3 survival axes.

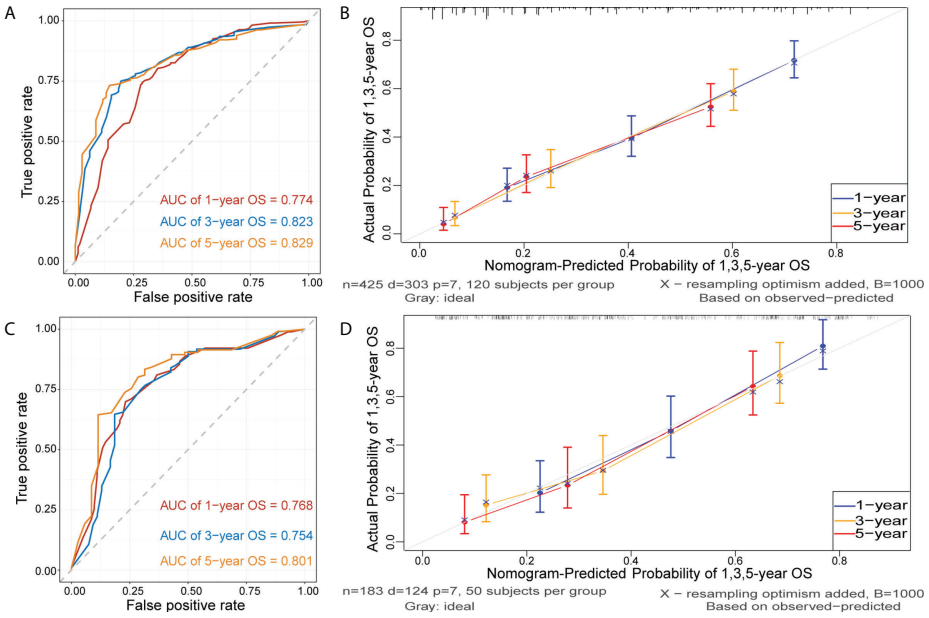

FIGURE 5
Receiver operating characteristic (ROC) and calibration curves of the nomogram for OS. ROC curves were plotted to evaluate the performance of the model to discriminate between patients with different survival outcomes (alive or dead), quantified by calculating the area under the ROC curve (AUC). The AUC of nomogram for predicting 1-, 3-, and 5-year OS were 0.774, 0,823 and 0.829 in the training cohort **(A)** and 0.768, 0.754, and 0.801 in the validation cohort **(C)**. Calibration curves were plotted to evaluate the accuracy of the nomogram model. The horizontal axis represents the survival rate predicted by the model, and the vertical axis represents the actual survival rate. The diagonal line represents the ideal situation where the predicted and actual survival rates consist, and the blue, orange, and red lines represent the model's predicted and actual survival rates for 1-year, 3-year, and 5-year OS, respectively. Calibration curves of the nomogram for predicting 1-, 3-, and 5-year OS in the training cohort **(B)** and in the validation cohort **(D)**.

three treatment types (10). Regarding local treatment, several studies have revealed that surgery or RT can shrink tumors, relieve local symptoms, and sometimes aid in diagnosis; however, they do not affect survival (24–26). In addition, as MS molecular mechanism's understanding improves, targeted therapy is also on the horizon (27, 28).

Although extensive studies have been conducted on MS prognosis, the published findings are controversial, and various prognostic factors have been identified in different studies (17, 29, 30). Patients with MS have a dismal prognosis, with a reported median OS time of <12 months (10, 21). This study found that the median OS times for MS and eMS were 9

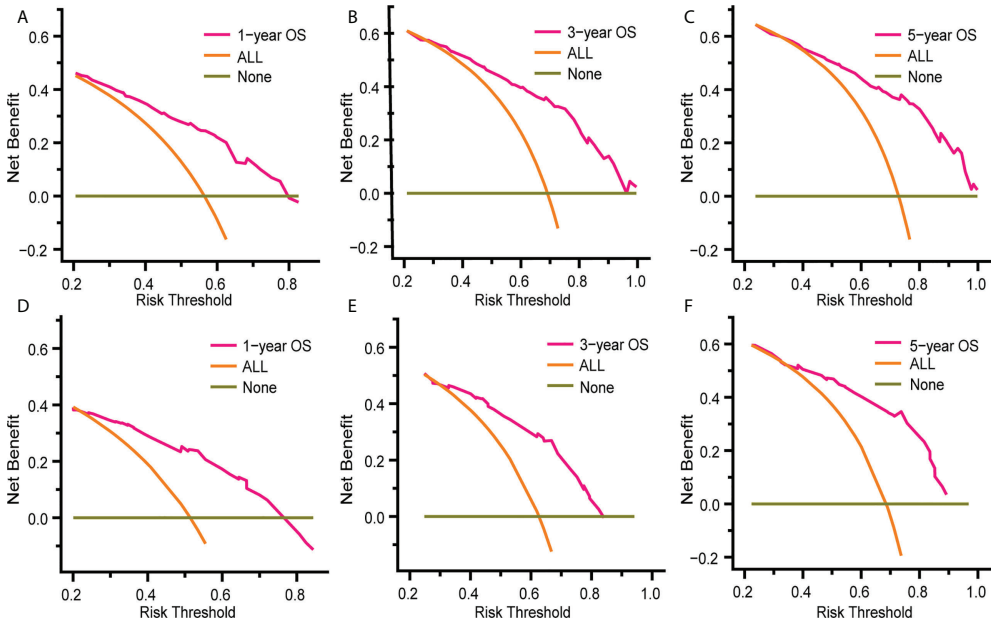

FIGURE 6
Decision curve analysis (DCA) of the nomogram for predicting 1-, 3- and 5- year OS rates in patients with eMS in the training cohort **(A–C)** and the validation cohort **(D–F)**. DCA was used to evaluate the clinical utility of the nomogram by calculating the net benefits of the model under different thresholds. The x-axis represents threshold probability, and the y-axis represents net benefit. The horizontal dark green line represents no deaths occurring, and the orange line represents all patients died. The pink line represents our nomogram model and when it is maintained above the dark green and orange line mentioned above, the net benefit value of the model is positive, which implies that our model has good clinical utility.

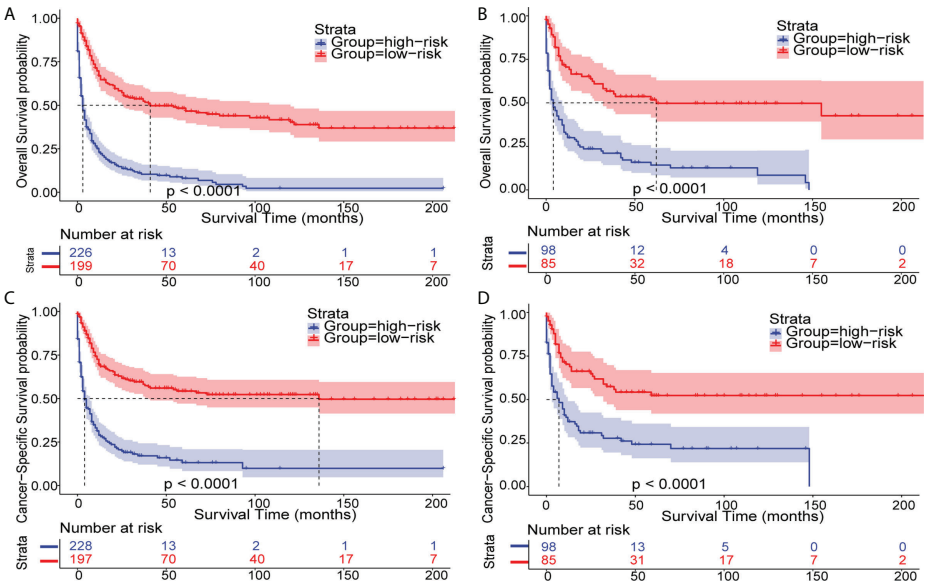

FIGURE 7
Kaplan–Meier analysis of OS and CSS in patients with eMS. Kaplan–Meier survival curves of OS and CSS in eMS patients stratified by risk levels in the training cohort **(A, C)** and validation cohort **(B, D)**.

and 10 months, respectively. We also revealed that the hMS prognosis was significantly worse, with a median OS time of 5 months. Our study demonstrated that eMS survival outcomes varied significantly with age, primary site, first primary tumor, and chemotherapy. A disparity in OS according to sex and race was observed in another NCDB-based study but not in this study (31). In line with the finding that patients with AML secondary to MS have a better prognosis than those with non-MS AML, patients with eMS as the first primary malignancy have a higher survival rate. This may have resulted from a lead-time advantage in patients with MS, indicating delayed AML development (5, 9). Chemotherapy has long been recognized as an independent prognostic factor, and patients who received chemotherapy had a significantly higher survival rate than those who did not (31). Two NCDB-based studies provided a more thorough analysis of chemotherapy's effect on the prognosis of patients with MS. Lontos et al. argued that combining chemotherapy with surgery and RT did not improve survival in isolated MS. Goyal et al. found that early chemotherapy was associated with a higher mortality rate among the elderly but had no effect on survival in younger patients (10, 31).

The nomogram can assist clinicians in assessing patient prognosis by converting the miscellaneous COX regression analysis results into a visual predictive model. Although numerous cancer nomograms have been constructed using the SEER database, no nomogram for MS has been reported (32). Our study constructed eMS nomograms for OS and CSS with four independent prognostic factors (age, primary site, first primary tumor, and chemotherapy). Our models had excellent discriminating and calibration abilities and potential clinical utility in the training and validation cohorts. To our knowledge, there is no risk stratification model for patients with MS. Clinicians can predict the 1-, 3-, and 5-year survival rates of patients with eMS and categorize them into low- or high-risk groups using the total points of the nomograms in this study.

Limitations

This study has numerous limitations, similar to those found in other studies based on retrospective datasets, including NCDB and SEER (9, 10). First, the SEER database was based on the US population, which may have limited our findings' generalizability. Second, although HSCT has been shown to affect MS prognosis, it was not incorporated into our clinical characteristics-based model because the information was unavailable in the SEER database (33). A further limitation on the predictive significance of chemotherapy is that the SEER database only provides information on whether patients underwent chemotherapy, not detailed individual chemotherapy regimens. As MS receives more attention, we will have additional information from single-center institutions or multi-center collaborative groups to help address this issue. Recently, oncology researchers have focused on imaging and genetic characteristics' impact on prognosis (34). The PET/CT potential utility in monitoring and assessing therapeutic response in MS has been emphasized by Lee et al. (35). Although we have gained further insight into the cytogenetic and molecular abnormalities of MS, such as chromosomal abnormalities like inv (16) and t (8, 21), and mutations in NPM1 and FLT3, the prognostic impact of genetic features remains poorly understood due to the small sample size (16, 36, 37). Therefore, another limitation is that it lacks information on the patients' imaging, cytogenetic, and molecular features. As a result, we could not investigate these features' predictive significance even with the sufficiently large sample size.

Conclusion

Using the SEER database, we updated the information on patients with MS and compared the clinical features and prognostic markers between the eMS and hMS groups, supporting the recommendation of distinguishing patients with eMS from those with hMS in future studies. Furthermore, our research developed and validated novel nomograms exclusively for patients with eMS. These models may assist clinicians in predicting overall and cancer-specific survival rates. In future studies, patients will benefit from new prognostic models that combine the clinical features with MS genomic, transcriptomic, and metabolomic features.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://seer.cancer.gov/data-software/.

Author contributions

XWZ, ZX, and XHZ made major contributions to the design and data collection of this study. ZX, XHZ, ZL, HW, and MQ participated in the data analysis, graphing, and compilation of the results. XWZ, ZX, and XHZ wrote the manuscript. ZL, HW, and MQ revised the manuscript. All authors contributed to the article and approved the submitted version.

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.989366/full#supplementary-material

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Socioeconomic status-based survival disparities and nomogram prediction for patients with multiple myeloma: Results from American and Chinese populations

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Objective: This study aimed to comprehensively investigate the relationship between the survival differences and socioeconomic status (SES) in patients with multiple myeloma (MM) and construct a predictive nomogram to assess clinical outcomes of MM patients.

Methods: The Surveillance, Epidemiology, and End Results (SEER) census tract-level SES database provides two specialized attributes: SES index and rurality. Using this database, 37,819 patients diagnosed with MM between January 2007 and December 2016 were enrolled. We evaluated the effects of SES index on overall survival (OS) and myeloma-specific survival (MSS) using Kaplan-Meier curves and Cox regression analyses. Thereafter, we included 126 patients with MM from two independent medical centers in China and divided them into training (Center 1) and validation (Center 2) cohorts. Univariate and multivariate Cox analyses were used in the training cohort to construct a nomogram for predicting clinical outcomes. Nomogram performance was assessed using the area under the curve (AUC) and calibration curves.

Results: In the SEER cohort, lower SES was significantly associated with worse OS rates and MSS rates (both $P < 0.001$). Multivariate analysis confirmed SES as an independent predictor of survival. Subgroup analysis indicated an increasing linear trend in survival benefits in non-Hispanic White, married, insured, and urban populations with increasing SES (all $P < 0.001$). In the training cohort, albumin, creatinine, rurality, and SES were confirmed as independent prognostic indicators. A nomogram for OS prediction was developed using these four factors, and it showed satisfactory discrimination and calibration. The 18- and 36-month AUC values of the nomogram were 0.79 and 0.82, respectively. Based on the total nomogram points, patients were categorized into two risk levels with good separation.

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Conclusion: SES strongly influences survival disparities in patients with MM. Our nomogram consisting of clinical and sociodemographic characteristics can potentially predict survival outcomes.

KEYWORDS

SES, multiple myeloma, nomogram, risk stratification, myeloma-specific survival

Introduction

Multiple myeloma (MM) is an incurable plasma cell dyscrasia that is characterized by the proliferation of clonal plasma cells, and it is the second most common hematologic malignancy (1, 2). The incidence of MM is notably high in developed and high-income countries such as Australia, the United States, and those in Western Europe (3, 4). The survival rates of patients with MM have continuously increased since 2000, with a 5-year relative survival rate of 55.6% between 2011 and 2017 (5). Improved survival in patients with MM is mainly attributed to the availability of novel therapies, including stem cell transplantation (SCT), advanced immune-modifying drugs, and proteasome inhibitors, but these are accompanied by increased treatment costs (6). Currently, survival outcomes vary substantially between individuals, which may largely depend on the recognized MM prognostic factors: age, sex, comorbidities, cytogenetics, the International Staging System (ISS) stage, response to chemotherapy, and social determinants (7).

Disparities in race, income, marital status, and insurance coverage are associated with survival in MM (8–10). Socioeconomic status (SES) and rurality are also imperative sociodemographic factors that potentially affect prognosis. A report showed that lower SES is independently associated with worse overall survival (OS) in patients with MM, when SES is estimated by household income alone (11). In the era of precision therapy, real-world data show that the impact of low SES on OS is more discernable in elderly patients (12). Additionally, survival in MM patients improved with a widening SES-level poverty gap over the last three decades (13). Nevertheless, these studies evaluated OS rather than myeloma-specific survival (MSS), which is more specific for predicting MM outcomes with less interference from other causes. In particular, the measures of SES varied considerably in previous studies, with lack of unified, professional, and standardized approaches for taking measurements. Given these limitations, we aimed to employ the Surveillance, Epidemiology, and End Results (SEER) census tract-level SES database for further demonstration.

The SEER census tract-level SES database is specifically designed to allow for improved investigation of the effects of SES on cancer survival. The census tract-level SES index is a composite of seven variables measuring different SES aspects, including median household income, median house value, median rent, percentage below 150% of the poverty line, working class percentage, unemployed percentage, and education level. These data are more reliable than the isolated methods of measuring SES (14, 15). In addition, the database provides another census tract-level attribute: rurality as measured by rural-urban commuting area codes. Researchers have examined the urban/rural differences in the survival of lung and breast cancer (16). Since few studies have focused on the impact of rurality on myeloma, to understand the relationship between rurality levels and prognosis of patients with MM, we hypothesized that rurality would serve as a prognostic factor for clinical outcomes.

Therefore, this study investigated the prognostic effects of SES and rurality on the survival of MM patients using the census tract-level SES database. Further, we developed and validated a novel nomogram using the data from patients at two independent medical centers in China. This nomogram will provide quick assessment of risk levels and individualized prediction of clinical outcomes.

Abbreviations: MM, multiple myeloma; SES, socioeconomic status; SEER, Surveillance, Epidemiology, and End Results; SCT, stem cell transplantation; OS, overall survival; MSS, myeloma-specific survival; HR, hazard ratio; CI, confidence interval; NHW, non-Hispanic White; NHB, non-Hispanic Black; NHAPI, non-Hispanic Asian or Pacific Islander; ROC, receiver operating characteristic; AUC, area under the curve; ISS, International Staging System; BMPC, bone marrow plasma cells; ALB, albumin; β2-MG, β2-microglobulin; HGB, hemoglobin; CREAT, creatinine; DM, diabetes mellitus; HTN, hypertension; PIs, proteasome inhibitors.

Materials and methods

Patients and variables in the SEER cohort

In the SEER-based analysis, patient data were obtained from the specialized Census Tract-level SES and Rurality Database covering 18 cancer registry areas (excluding Alaska) using

SEER*Stat software (version 8.3.9). Census tracts were categorized into SES quintiles with equal populations in each quintile within the overall area or in each registry. For instance, the first quintile (Q1, the group with the lowest SES) refers to the 20th percentile or lower, and the fifth quintile (Q5, the group with the highest SES) refers to the 80th percentile or higher. MM cases were identified using the International Classification of Disease for Oncology, Third Edition (ICD-O-3) histologic code 9732, and primary site code C42.1. We initially screened 42,210 patients diagnosed with MM between January 2007 and December 2016 according to the following inclusion criteria: (a) non-autopsy/death certificate-only cases, (b) unambiguous insurance information, and (c) first primary tumor. Subsequently, 37,819 patients were enrolled in the cohort for further research, grouped with SES quintiles by the baseline characteristics, after excluding the following cases: (a) unknown race (n=236); (b) non-Hispanic American Indian/Alaska Native (n=208); (c) unknown marital status (n=2,027); and (d) missing or no match for SES quintile (n=1,920).

We extracted the following sociodemographic variables from the cohort: age at diagnosis, year of diagnosis, sex, race, insurance status, marital status, rurality, and SES index. Race included four categories: non-Hispanic white (NHW), non-Hispanic black (NHB), non-Hispanic Asian or Pacific Islander (NHAPI), and Hispanic. Insurance status was categorized as insured, uninsured, or Medicaid. Marital status was defined as married (including separated), divorced, single (including unmarried or domestic partner), or widowed. According to the rural-urban commuting area codes, rurality was classified as rural or urban. Regarding the survival variables, OS was defined as the interval between diagnosis and death from any cause. MSS was defined as the interval between diagnosis and death due to myeloma.

Patients and variables in the real-world Chinese cohorts

We retrospectively included 126 patients with newly diagnosed MM from two cancer centers in China (Jingjiang People's Hospital, from January 2012 to November 2021; Nanjing Drum Tower Hospital, from May 2016 to June 2019). All patients were diagnosed according to the current International Myeloma Working Group consensus recommendations (17). We collected and analyzed the following patient-specific information: age, sex, bone marrow plasma cells (BMPC), albumin (ALB), β2-microglobulin (β2-MG), hemoglobin (HGB), creatinine (CREAT), history of hypertension, diabetes, smoking, insurance status, employment status, rurality, and SES. Here, SES was evaluated based on each patient's occupation, place of residence, and the ability to pay for treatment. Patients were divided equally into two groups.

Nomogram development and validation

We divided the enrolled patients into training and validation sets according to the medical centers. Patients registered at Jingjiang People's Hospital (Center 1) were selected as the training cohort (n=85), and patients registered at Nanjing Drum Tower Hospital (Center 2) were selected as the validation cohort (n=41). Variables with statistical significance in the multivariate analysis were used to create the nomogram of the training cohort. The receiver operating characteristic (ROC) curves with the area under the curve (AUC) values were employed in both the training and validation cohorts for validity and sensitivity. To measure accuracy, we constructed calibration plots with 1,000 bootstrap resamples to observe errors between the actual and predicted survival rates. Moreover, the stratification of risk levels was constructed based on the nomogram total scores.

Statistical analysis

Baseline characteristics of patients were presented as a proportion for categorical variables. The chi-squared test was used to compare the distribution of patient characteristics between the training and validation cohorts. Survival analysis was conducted using the Kaplan-Meier method and assessed using a log-rank test. Univariate and multivariate Cox proportional-hazards models were applied to evaluate the hazard ratio (HR) with corresponding 95% confidence interval (CI). The above analyses were performed using GraphPad Prism 8 and R software (Version 4.0.2). Results were considered statistically significant when the two-tailed *P*-value was less than 0.05.

Results

Sociodemographic characteristics of SEER patients

Baseline characteristics of the 37,819 patients in the SEER cohort are summarized in Table 1. The patients were divided into five groups according to SES quintiles: 7,365 in quintile 1 (Q1, lowest), 7,236 in quintile 2 (Q2, lower), 7,382 in quintile 3 (Q3, medium), 7,805 in quintile 4 (Q4, higher), and 8,031 in quintile 5 (Q5, highest). Patients in high SES groups (Q4 and Q5) were more likely to be male, NHW, insured, and married, and tended to reside in urban tracts. In the lowest SES group (Q1), the relative proportions of those designated as NHB, Medicaid, or single were the largest.

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TABLE 1 Baseline sociodemographic characteristics of MM patients in the SEER cohort, grouped by SES index.

Variables	Overall N=37819 (%)	Q1 (lowest) N=7365 (%)	Q2 (lower) N=7236 (%)	Q3 (medium) N=7382 (%)	Q4 (higher) N=7805 (%)	Q5 (highest) N=8031 (%)
Age (years)						
<60	10417 (27.5%)	2131 (28.9%)	2006 (27.7%)	2001 (27.1%)	2051 (26.3%)	2228 (27.7%)
60-69	11150 (29.5%)	2188 (29.7%)	2046 (28.3%)	2172 (29.4%)	2372 (30.4%)	2372 (29.5%)
70-79	9852 (26.1%)	1949 (26.5%)	1924 (26.6%)	1916 (26.0%)	2037 (26.1%)	2026 (25.2%)
≥80	6400 (16.9%)	1097 (14.9%)	1260 (17.4%)	1293 (17.5%)	1345 (17.2%)	1405 (17.5%)
Year of diagnosis						
2007-2011	17349 (45.9%)	3332 (45.2%)	3352 (46.3%)	3307 (44.8%)	3618 (46.4%)	3740 (46.6%)
2012-2016	20470 (54.1%)	4033 (54.8%)	3884 (53.7%)	4075 (55.2%)	4187 (53.6%)	4291 (53.4%)
Sex						
Male	20762 (54.9%)	3806 (51.7%)	3883 (53.7%)	4114 (55.7%)	4401 (56.4%)	4558 (56.8%)
Female	17057 (45.1%)	3559 (48.3%)	3353 (46.3%)	3268 (44.3%)	3404 (43.6%)	3473 (43.2%)
Race						
NHW	23116 (61.1%)	2581 (35.0%)	4135 (57.1%)	4735 (64.1%)	5518 (70.7%)	6147 (76.5%)
NHB	7893 (20.9%)	3347 (45.4%)	1709 (23.6%)	1320 (17.9%)	909 (11.6%)	608 (7.6%)
NHAPI	2285 (6.0%)	206 (2.8%)	301 (4.2%)	458 (6.2%)	587 (7.5%)	733 (9.1%)
Hispanic	4525 (12.0%)	1231 (16.7%)	1091 (15.1%)	869 (11.8%)	791 (10.1%)	543 (6.8%)
Insurance status						
Insured	32166 (85.1%)	5352 (72.7%)	5914 (81.7%)	6406 (86.8%)	7014 (89.9%)	7480 (93.1%)
Uninsured	996 (2.6%)	341 (4.6%)	231 (3.2%)	167 (2.3%)	138 (1.8%)	119 (1.5%)
Medicaid	4657 (12.3%)	1672 (22.7%)	1091 (15.1%)	809 (11.0%)	653 (8.4%)	432 (5.4%)
Marital status						
Married	23144 (61.2%)	3615 (49.1%)	4179 (57.8%)	4599 (62.3%)	5094 (65.3%)	5657 (70.4%)
Divorced	3577 (9.5%)	860 (11.7%)	746 (10.3%)	704 (9.5%)	708 (9.1%)	559 (7.0%)
Single	5639 (14.9%)	1636 (22.2%)	1173 (16.2%)	1013 (13.7%)	953 (12.2%)	864 (10.8%)
Widowed	5459 (14.4%)	1254 (17.0%)	1138 (15.7%)	1066 (14.4%)	1050 (13.5%)	951 (11.8%)
Rurality						
Urban	34978 (92.5%)	6291 (85.4%)	6234 (86.2%)	6792 (92.0%)	7655 (98.1%)	8006 (99.7%)
Rural	2841 (7.5%)	1074 (14.6%)	1002 (13.8%)	590 (8.0%)	150 (1.9%)	25 (0.3%)

Q1, quintile 1; Q2, quintile 2; Q3, quintile 3; Q4, quintile 4; Q5, quintile 5; NHW, non-Hispanic White; NHB, non-Hispanic Black; NHAPI, Non-Hispanic Asian or Pacific Islander.

Survival analysis of the SEER cohort

To assess the effects of SES and rurality on OS and MSS, Kaplan-Meier survival curves stratified by SES quintiles and rurality were analyzed. Patients in the highest SES group (Q5) had a median OS of 63 months, which was much higher than that of the other four groups (53, 48, 43, and 39 months, for Q4 to Q1, respectively, $P < 0.001$) (Figure 1A). Patients in urban tracts exhibited higher median OS than those in rural tracts (50 vs. 41 months, $P < 0.001$) (Figure 1B). Similarly, patients with highest SES had a median MSS of 91 months, which was quite higher than that of the other four groups (77, 69, 68, and 63 months, for Q4 to Q1, respectively, $P < 0.001$) (Figure 1C). Patients in urban tracts had a higher median MSS time than those in rural tracts (75 vs. 59 months, $P < 0.001$) (Figure 1D).

Cox regression analysis identified the prognostic values of these sociodemographic factors for OS (Table 2) and MSS (Table 3). All variables except sex proved to be significantly associated with survival outcomes in the univariate analysis. Furthermore, age, year of diagnosis, sex, race, insurance status, marital status, and SES index were independent prognostic indicators of both OS and MSS in the multivariate Cox proportional-hazards models. Notably, compared with the Q1 group, the risk of a poor MSS gradually decreased from Q2 to Q5 (adjusted HR, Q2: 0.93, $P = 0.006$; Q3: 0.90, $P < 0.001$; Q4: 0.81 $P < 0.001$; Q5: 0.69, $P < 0.001$). To further visualize the effect of SES on MSS in subgroups, forest plots displayed the HRs by SES quintiles within the race, marital status, insurance status, and rurality groups (Figures 2A–D). In the NHW, married, insured, and urban groups, SES had the most significant effect on prognosis (all $P < 0.001$).

Baseline characteristics and survival of Chinese patients

The clinical and demographic characteristics of the training and validation cohorts are described in Table 4. A total of 126

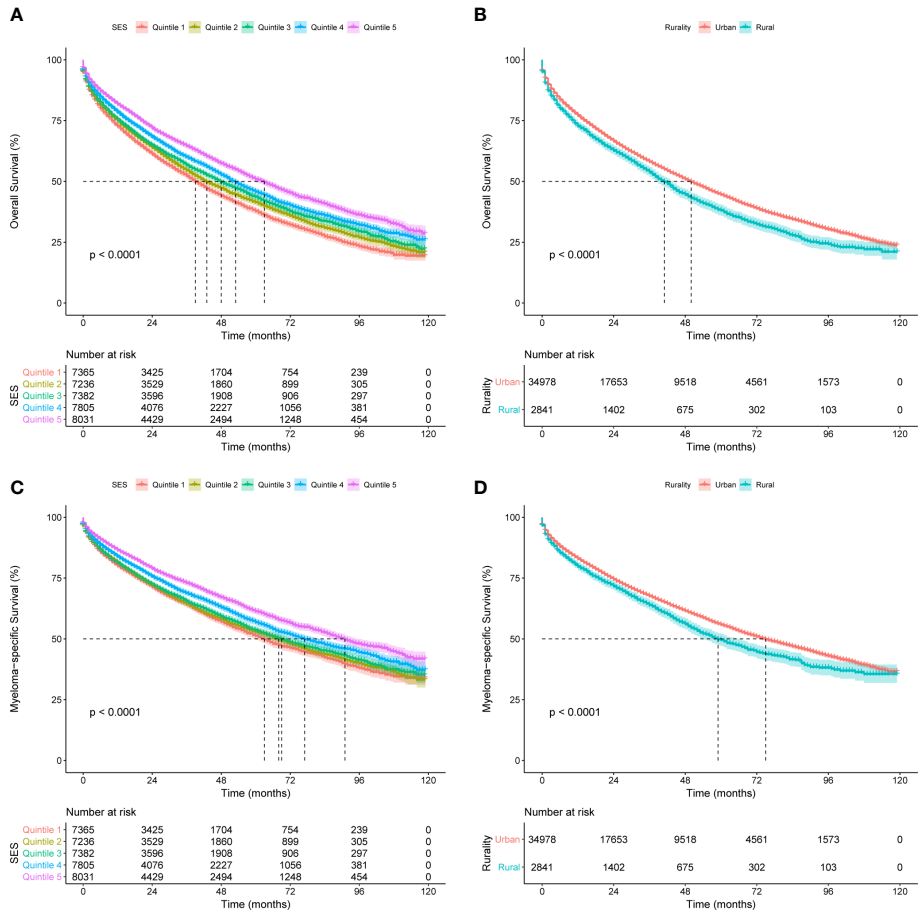

FIGURE 1
Kaplan-Meier survival curves of the SEER cohort for **(A)** OS stratified by SES quintiles, **(B)** OS stratified by rurality, **(C)** MSS stratified by SES quintiles, and **(D)** MSS stratified by rurality.

eligible patients with MM were included in this study, with 66 (52.4%) patients aged 65 years or older and 48 (38.1%) female patients. Overall, 105 (83.3%) patients received proteasome inhibitor (PI)-based treatment regimens (bortezomib or carfilzomib), while other patients were treated with traditional medication. There were 50 (39.7%) patients with high insurance coverage, 40 (31.7%) employed patients, and 48 (38.1%) urban residents. Cases were separated into two cohorts with 85 cases from Center 1 assigned to the training cohort, and 41 patients from Center 2 assigned to the validation cohort. No significant differences were observed between the two cohorts by any of the included variables.

Kaplan–Meier curves were generated to evaluate the prognostic value of the socioeconomic factors. Insurance status ($P = 0.05$, Figure 3A), employment status ($P = 0.03$, Figure 3B), rurality ($P = 0.004$, Figure 3C), and SES ($P = 0.002$, Figure 3D) were linked to survival disparities in OS. Univariate and multivariate analyses were used to identify the prognostic effect of each factor in the training cohort (Table 5). ALB, CREAT, ISS stage, employment, rurality, and SES were correlated with OS in the univariate Cox analysis. Then, multivariate analysis confirmed that ALB, CREAT, rurality, and SES could serve as independent prognostic indictors of OS in patients with MM. ALB <3 g/dL ($P = 0.027$) or CREAT ≥2 mg/dL ($P = 0.019$) indicated worse outcomes in OS. Moreover, patients with low SES ($P = 0.005$) and those living in rural areas ($P = 0.023$) had a worse prognosis.

Construction and validation of a nomogram

We established a predictive nomogram in the training cohort to estimate the 18- and 36-month OS probabilities (Figure 4A). ALB, CREAT, rurality, and SES were included in the nomogram. Different categories of these risk factors could be projected onto matching scores, which were added up to correspond to specific survival probabilities. The 18- and 36-

TABLE 2 Univariate and multivariate Cox regression analysis for OS in the SEER cohort.

Characteristics	Levels	Univariate analysis			Multivariate analysis		
		HR	95% CI	*P* value	Adjusted HR	95% CI	*P* value
Age	<60 years	Ref			Ref		
	60-69 years	1.39	1.33-1.45	<0.001	1.44	1.38-1.51	<0.001
	70-79 years	2.22	2.12-2.31	<0.001	2.30	2.20-2.40	<0.001
	≥80 years	4.13	3.95-4.32	<0.001	4.18	3.99-4.39	<0.001
Year of diagnosis	2007-2011	Ref			Ref		
	2012-2016	0.87	0.84-0.90	<0.001	0.87	0.84-0.90	<0.001
Sex	Male	Ref			Ref		
	Female	0.96	0.93-0.99	0.003	0.83	0.81-0.86	<0.001
Race	NHW	Ref			Ref		
	NHB	0.92	0.88-0.95	<0.001	0.88	0.85-0.92	<0.001
	NHAPI	0.90	0.84-0.95	<0.001	0.91	0.85-0.97	0.006
	Hispanic	0.95	0.91-0.99	0.027	0.93	0.88-0.98	0.003
Insurance status	Insured	Ref			Ref		
	Uninsured	0.87	0.79-0.95	0.003	1.18	1.07-1.30	0.001
	Medicaid	1.30	1.25-1.36	<0.001	1.37	1.31-1.43	<0.001
Marital status	Married	Ref			Ref		
	Divorced	1.16	1.10-1.22	<0.001	1.23	1.17-1.29	<0.001
	Single	1.16	1.11-1.21	<0.001	1.28	1.23-1.34	<0.001
	Widowed	1.95	1.88-2.03	<0.001	1.26	1.21-1.32	<0.001
Rurality	Urban	Ref			Ref		
	Rural	1.18	1.12-1.24	<0.001	0.99	0.94-1.05	0.767
SES	Quintile 1	Ref			Ref		
	Quintile 2	0.91	0.87-0.95	<0.001	0.89	0.85-0.94	<0.001
	Quintile 3	0.86	0.82-0.90	<0.001	0.85	0.81-0.89	<0.001
	Quintile 4	0.78	0.75-0.82	<0.001	0.78	0.74-0.82	<0.001
	Quintile 5	0.68	0.65-0.71	<0.001	0.68	0.64-0.71	<0.001

OS, overall survival; NHW, non-Hispanic White; NHB, non-Hispanic Black; NHAPI, Non-Hispanic Asian or Pacific Islander.

month AUC values of the nomogram were 0.79 and 0.82, respectively, in the training cohort (Figure 4B) and 0.90 and 0.76, respectively, in the validation cohort (Figure S1), indicating adequate sensitivity and specificity. The calibration plots of both cohorts for 18- and 36-month OS showed close proximity of the predicted lines to the actual reference lines (Figures 4C, D; Figure S2), which confirmed the accuracy and reliability of our model.

To better assist patients with MM in predicting their survival, we created a risk stratification based on the total points (TP) of the nomogram. Using the median risk score (TP: 153) of the nomogram model, all patients were divided into high- and low-risk groups. Kaplan-Meier curves were used to assess the discriminatory ability of the nomogram stratification. Compared to those with low-risk level, patients of the high-risk group showed a significantly worse OS in the entire cohort ($P < 0.001$, Figure 5A), training cohort ($P < 0.001$, Figure 5B), and validation cohort ($P < 0.001$, Figure 5C). These results revealed the effective discriminatory ability of the nomogram's risk stratification.

Discussion

Determining the role of socioeconomic factors in the survival of patients with MM is important (18), and we completed a large-scale retrospective cohort study to obtain more evidence regarding the role of SES in MM patient prognosis. Using the SEER census tract-level data, we found that lower SES and rural tracts were significantly associated with poorer OS and MSS. Subgroup analyses of the other demographic factors indicated that the impact of SES was more notable in the NHW, married, insured, and urban groups, with clear linear trends. In addition, this study enrolled two independent cohorts of Chinese patients with MM to confirm the effects of SES on survival. The nomogram and risk stratification showed satisfactory results for survival prediction and risk assessment.

In several studies, SES was an independent predictor of MM patient survival in multiple cohorts, which is in accordance with our results (11, 12, 19, 20). We provide clear evidence that SES inequalities are associated with survival differences among

TABLE 3 Univariate and multivariate Cox regression analysis for MSS in the SEER cohort.

Characteristics	Levels	Univariate analysis			Multivariate analysis		
		HR	95% CI	*P* value	Adjusted HR	95% CI	*P* value
Age	<60 years	Ref			Ref		
	60-69 years	1.31	1.25-1.38	<0.001	1.35	1.28-1.42	<0.001
	70-79 years	1.99	1.89-2.09	<0.001	2.03	1.93-2.14	<0.001
	≥80 years	3.52	3.35-3.71	<0.001	3.52	3.32-3.72	<0.001
Year of diagnosis	2007-2011	Ref			Ref		
	2012-2016	0.86	0.82-0.89	<0.001	0.85	0.82-0.89	<0.001
Sex	Male	Ref			Ref		
	Female	1.00	0.97-1.04	0.886	0.9	0.87-0.93	<0.001
Race	NHW	Ref			Ref		
	NHB	0.86	0.82-0.90	<0.001	0.82	0.79-0.87	<0.001
	NHAPI	0.90	0.84-0.97	0.008	0.92	0.85-0.99	0.026
	Hispanic	0.95	0.90-1.00	0.053	0.92	0.87-0.98	0.006
Insurance status	Insured	Ref			Ref		
	Uninsured	0.91	0.81-1.01	0.088	1.20	1.07-1.34	0.001
	Medicaid	1.27	1.20-1.33	<0.001	1.34	1.27-1.41	<0.001
Marital status	Married	Ref			Ref		
	Divorced	1.13	1.07-1.20	<0.001	1.19	1.12-1.26	<0.001
	Single	1.12	1.06-1.17	<0.001	1.22	1.16-1.29	<0.001
	Widowed	1.82	1.74-1.91	<0.001	1.21	1.15-1.27	<0.001
Rurality	Urban	Ref			Ref		
	Rural	1.17	1.10-1.25	<0.001	0.99	0.93-1.06	0.741
SES	Quintile 1	Ref			Ref		
	Quintile 2	0.96	0.91-1.01	0.136	0.93	0.88-0.98	0.006
	Quintile 3	0.93	0.88-0.98	0.010	0.90	0.85-0.96	<0.001
	Quintile 4	0.83	0.79-0.88	<0.001	0.81	0.76-0.85	<0.001
	Quintile 5	0.72	0.68-0.76	<0.001	0.69	0.65-0.74	<0.001

MSS, myeloma-specific survival; NHW, non-Hispanic White; NHB, non-Hispanic Black; NHAPI, Non-Hispanic Asian or Pacific Islander.

patients with MM, regardless of OS or MSS. A summary of the main papers regarding the association between SES and MM survival, is presented in Table 6. In contrast, the advantages of our SEER study include a notably large sample size, more classifications of the SES index, and specialized measures for SES. We also conducted a dual-center, real-world, cohort study of Chinese patients. Detailed clinical variables were included, and the importance of SES was confirmed after adjusting for covariates. With respect to rurality, rural patients experienced worse survival than patients in urban areas, which is consistent with current research related to residence. In both China and Queensland, Australia, rural patients were found to have worse survival across all age groups (19, 23).

Apart from SES and rurality, we also identified other covariates that could directly or indirectly affect survival and explain differences in clinical prognosis. Low SES in elderly cancer patients was linked to poor survival (24). Since MM is primarily a malignancy of the elderly, increased age at diagnosis accounts for a higher risk of MM, as older patients usually have more comorbidities, less social care, and worse response to therapies (25). Interestingly, although the proportion of NHB patients increased as the SES index decreased, NHB patients had a better prognosis than NHW patients. This finding is supported by a few clinical trials in which African Americans who underwent SCT or novel therapies had higher MSS and OS than White patients, with equal access to healthcare and treatment patterns in both groups (26–29). Race-related heterogeneity in biology and genomics may play an important role in the therapeutic effects and survival time of patients with MM. Previous research suggested that discrepancies in survival are mainly attributed to socioeconomic factors, especially SES, rather than race (8, 11, 26). Unmarried individuals, including divorced, single, and widowed, occupied a larger proportion within the lower SES groups and were proven to have worse survival outcomes. This phenomenon could be possibly explained by chronic psychological stress due to an unmarried status. Stress caused by anxiety, severe life events, and insufficient coping strategies accelerate the cellular aging

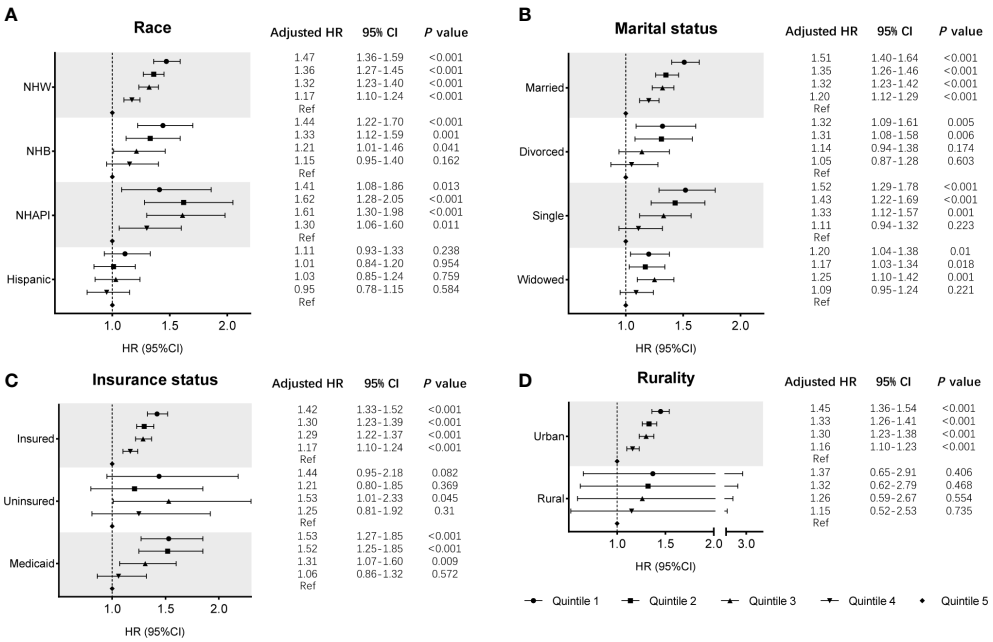

FIGURE 2
Subgroup analyses of the effects of SES on MSS in the SEER cohort, according to the subgroups of **(A)** race, **(B)** marital status, **(C)** insurance status, and **(D)** rurality, using multivariate Cox regression analysis with adjusted covariables.

process and tumor progression, which leads to increased cancer risk and mortality (30).

Despite the rapid emergence of new drugs and therapies in the field of MM, patients with different sociodemographic attributes are provided different therapies and experience different outcomes. Disparities in myeloma care are due to the limited access to health services for the more deprived patients. As a crucial part of the initial treatment, SCT was less likely to be given to patients with older age, low levels of education or income, or no medical insurance (31, 32). Further, patients with an unmarried status and lower household income had a higher burden of treatment costs, which may result in treatment interruptions (33). The accessibility and persistence of treatment modalities also depend on insurance status. The percentage of the insured population was larger in the higher SES groups. Those who were insured had more substantial survival gains in OS and MSS than those with uninsured or Medicaid status. With less insurance support, patients will have more obstacles in accessing qualified healthcare, social support, and advanced therapies (9). Moreover, patients with higher SES are more likely to live in urban tracts, where there is easier access to higher-volume facilities and better management (34).

In the context of sociology, patients with higher SES tend to obtain more social utility, leading to a lower risk of cancer mortality. The underlying mechanism is that inflammatory processes are involved in regulating the relationship between social support and cancer mortality, with patients at higher levels of social support and satisfaction having lower levels of inflammatory factors, including IL-6, TNF-α, CRP, and VEGF (35). From a psychiatric perspective, patients with low SES have a higher prevalence of depression (36). The interaction between SES and depressive symptoms is potentially mediated by interpersonal trust and reciprocity, or education level (37, 38). As a psychosocial stressor in cancers, depression promotes inflammatory reactions and oxidative stress, represses immune surveillance, and abnormally activates the hypothalamic-pituitary-adrenal axis, thus promoting tumor progression and a worse prognosis (39, 40).

Hence, we emphasized the importance of SES on survival and provided a pragmatic nomogram for clinicians and patients to better understand MM prognosis. Our nomogram contained both clinical and sociodemographic features, with good accuracy in both the training and validation cohorts. Once a diagnosis of MM is made, patients could easily predict survival prognosis according to their individual characteristics. Additionally, the risk stratification distinctly identifies two risk levels and displays marked differences in survival outcomes between the two populations. The nomogram along with the risk system may become a complementary tool in clinical practice, and more potential risk factors are expected to be identified and included in future research.

There were several limitations to our study. First, the SEER database does not include clinicopathological or molecular

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TABLE 4 Baseline clinical and sociodemographic characteristics of MM patients in the two-center cohorts.

Characteristics	Entire cohort N=126 (%)	Training cohort (Center 1) N=85 (%)	Validation cohort (Center 2) N=41 (%)	*P* value
Age (years)				0.058
<65	60 (47.6%)	35 (41.2%)	25 (61.0%)	
≥65	66 (52.4%)	50 (58.8%)	16 (39.0%)	
Sex				1.000
Male	78 (61.9%)	53 (62.4%)	25 (61.0%)	
Female	48 (38.1%)	32 (37.6%)	16 (39.0%)	
M-protein subtype				0.659
IgG	52 (41.3%)	32 (37.6%)	20 (48.8%)	
IgA	41 (32.5%)	30 (35.3%)	11 (26.8%)	
FLC	27 (21.4%)	19 (22.4%)	8 (19.5%)	
Other	6 (4.76%)	4 (4.71%)	2 (4.88%)	
ISS stage				0.226
I	27 (21.4%)	17 (20.0%)	10 (24.4%)	
II	55 (43.7%)	34 (40.0%)	21 (51.2%)	
III	44 (34.9%)	34 (40.0%)	10 (24.4%)	
BMPC (%)				0.058
<25	72 (57.1%)	54 (63.5%)	18 (43.9%)	
≥25	54 (42.9%)	31 (36.5%)	23 (56.1%)	
ALB (g/dL)				0.848
<3	37 (29.4%)	24 (28.2%)	13 (31.7%)	
≥3	89 (70.6%)	61 (71.8%)	28 (68.3%)	
β2-MG (mg/L)				0.128
<5.5	82 (65.1%)	51 (60.0%)	31 (75.6%)	
≥5.5	44 (34.9%)	34 (40.0%)	10 (24.4%)	
HGB (g/dL)				0.542
<10	89 (70.6%)	62 (72.9%)	27 (65.9%)	
≥10	37 (29.4%)	23 (27.1%)	14 (34.1%)	
CREAT (mg/dL)				0.067
<2	104 (82.5%)	66 (77.6%)	38 (92.7%)	
≥2	22 (17.5%)	19 (22.4%)	3 (7.32%)	
DM				0.762
Yes	25 (19.8%)	18 (21.2%)	7 (17.1%)	
No	101 (80.2%)	67 (78.8%)	34 (82.9%)	
HTN				0.844
Yes	43 (34.1%)	30 (35.3%)	13 (31.7%)	
No	83 (65.9%)	55 (64.7%)	28 (68.3%)	
Smoking				0.050
Yes	23 (18.3%)	20 (23.5%)	3 (7.32%)	
No	103 (81.7%)	65 (76.5%)	38 (92.7%)	
Therapy regimens				0.734
PIs-based	105 (83.3%)	72 (84.7%)	33 (80.5%)	
Traditional drugs-based	21 (16.7%)	13 (15.3%)	8 (19.5%)	
Insurance status				0.929
High	50 (39.7%)	33 (38.8%)	17 (41.5%)	
Low	76 (60.3%)	52 (61.2%)	24 (58.5%)	
Employment				0.843
Employed	40 (31.7%)	26 (30.6%)	14 (34.1%)	
Unemployed	86 (68.3%)	59 (69.4%)	27 (65.9%)	

(Continued)

TABLE 4 Continued

Characteristics	Entire cohort N=126 (%)	Training cohort (Center 1) N=85 (%)	Validation cohort (Center 2) N=41 (%)	*P* value
Rurality				0.259
Urban	48 (38.1%)	29 (34.1%)	19 (46.3%)	
Rural	78 (61.9%)	56 (65.9%)	22 (53.7%)	
SES				1.000
High	64 (50.8%)	43 (50.6%)	21 (51.2%)	
Low	62 (49.2%)	42 (49.4%)	20 (48.8%)	

ISS, International Staging System; BMPC, bone marrow plasma cells; ALB, albumin; β2-MG, β2-microglobulin; HGB, hemoglobin; CREAT, creatinine; DM, diabetes mellitus; HTN, hypertension; PIs, proteasome inhibitors. P value is for comparison between the training cohort and validation cohort.

variables for MM, and we were therefore unable to assess disease-specific factors in the multivariate analysis. Second, the SES index provided by SEER was at the census-tract level instead of the individual level. Detailed individual information may afford patients with MM a more personalized prediction of their survival. Third, controversy persists in real-life when assigning causes of death to underlying diseases. For instance, it is not mentioned in SEER whether infections are considered related to death caused by myeloma, which may affect the accuracy of MSS.

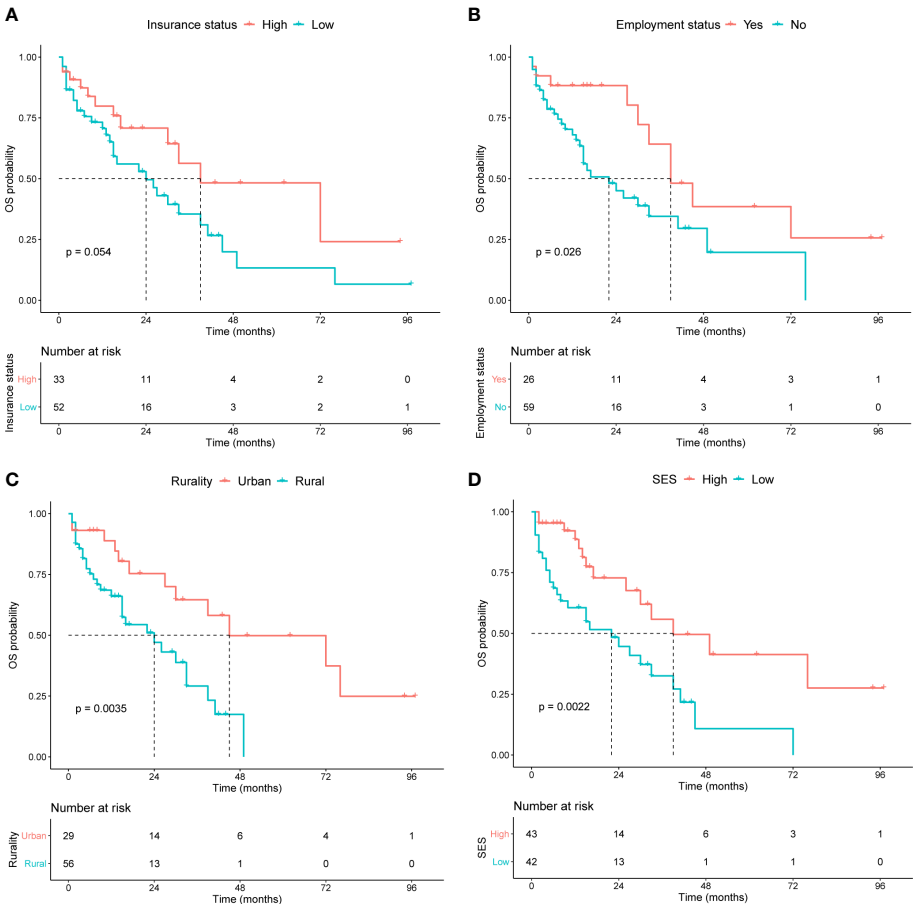

FIGURE 3
Kaplan-Meier survival curves in the training cohort for OS stratified by **(A)** insurance status, **(B)** employment status, **(C)** rurality, and **(D)** SES.

TABLE 5 Univariate and multivariate Cox regression analysis for OS in the training cohort.

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P* value	Adjusted HR	95% CI	*P* value
Age (≥65 years vs. <65 years)	1.48	0.78-2.81	0.228			
Sex (female vs. male)	0.84	0.44-1.57	0.579			
BMPC (≥25% vs. <25%)	1.39	0.76-2.56	0.288			
HGB (≥10 vs. <10 g/dL)	0.76	0.37-1.57	0.461			
β2-MG (≥5.5 vs. <5.5 mg/L)	1.97	1.00-3.87	0.051			
ALB (≥3 vs. <3 g/dL)	0.49	0.26-0.93	0.028	0.39	0.17-0.90	0.027
CREAT (≥2 vs. <2 mg/dL)	2.33	1.11-4.89	0.025	3.10	1.20-7.98	0.019
ISS stage (II vs. I)	2.03	0.85-4.86	0.110	1.16	0.40-3.34	0.788
ISS stage (III vs. I)	2.94	1.13-7.66	0.027	1.53	0.51-4.53	0.447
DM (yes vs. no)	0.99	0.48-2.02	0.972			
HTN (yes vs. no)	0.74	0.38-1.45	0.379			
Smoking (yes vs. no)	1.12	0.56-2.23	0.757			
Insurance status (low vs. high)	1.91	0.98-3.74	0.059			
Employment (unemployed vs. employed)	2.22	1.08-4.59	0.031	1.56	0.67-3.63	0.299
Rurality (rural vs. urban)	2.93	1.39-6.19	0.005	2.64	1.14-6.09	0.023
SES (low vs. high)	2.73	1.40-5.31	0.003	2.72	1.35-5.46	0.005

BMPC, bone marrow plasma cells; ALB, albumin; β2-MG, β2-microglobulin; CREAT, creatinine; HGB, hemoglobin; ISS, International Staging System; DM, diabetes mellitus; HTN, hypertension.

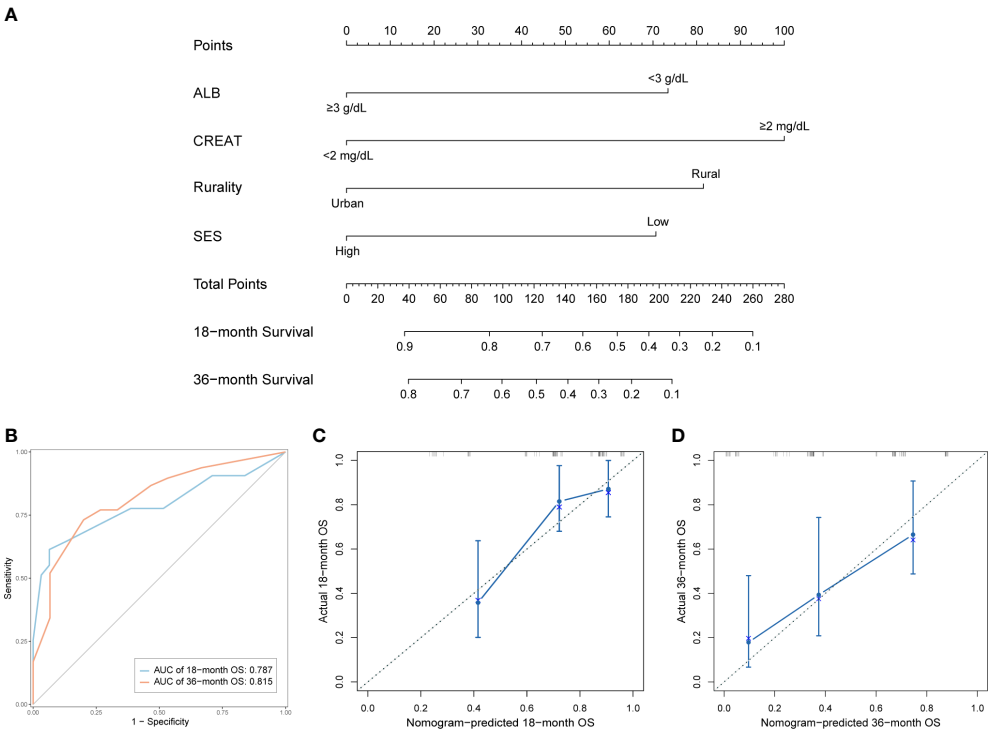

FIGURE 4
Development and validation of the prognostic nomogram in the training cohort. **(A)** Nomogram for predicting 18- and 36-month OS. **(B)** Time-dependent ROC curves and AUC values of the nomogram. **(C)** Calibration plot for predicting 18-month OS. **(D)** Calibration plot for predicting 36-month OS.

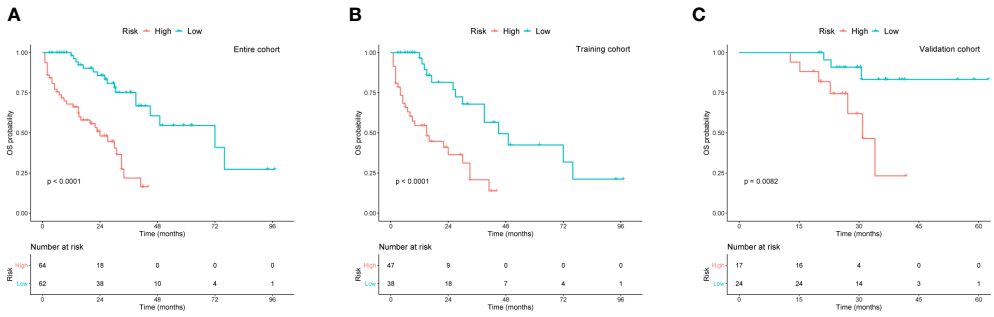

FIGURE 5
Kaplan-Meier survival curves for OS based on risk levels in **(A)** the entire cohort, **(B)** training cohort, and **(C)** validation cohort.

Although SES is difficult to change in a short period of time by patients, more equitable access to healthcare resources could diminish its impact on disease processes. Medical institutions and clinicians should focus on addressing these discrepancies, providing effective interventions, and seeking optimal practices. The underlying pathogenesis of socioeconomic causes also requires further elucidation. Monitoring future trends in incidence and mortality within different socioeconomic groups is recommended. Considering the pronounced relationship between SES and patient survival, it would be meaningful to track and appraise the quality of life with SES changes during long-term treatment.

To summarize, our study identified SES as an independent predictor of survival in MM. Patients with a higher SES tend to have more favorable survival outcomes. The prognostic nomogram and risk stratification model are reliable and convenient, which improves risk assessment for each patient. More effort is needed to improve survival for patients with adverse socioeconomic factors.

TABLE 6 A summary of other major studies regarding the association of SES with OS in MM patients.

Authors	Countries	Sample size	Year of diagnosis	SES assessment	Results (HR, 95% CI, *P* value)
Fiala et al. (11)	US (SEER 18 registries)	45,505	2000-2009	Median household income based on the 2000 US Census data	Low-SES vs. high-SES: 1.18 (1.15-1.22), *P* <0.001; middle-SES vs. high-SES: 1.10 (1.07-1.13), *P* <0.001
Fiala et al. (11)	US	562	2000-2009	Median household income based on the American Community Survey	Low-SES vs. high-SES: 1.54 (1.13-2.09), *P* =0.006; middle-SES vs. high-SES: 1.25 (0.95-1.65), *P* =0.114
Hong et al. (21)	US	346	2003-2013	Median household income based on the 2010 US Census data	High-SES vs. low-SES: 1.08 (0.71-1.64), *P*=0.72; middle-SES vs. low-SES: 1.40 (0.93-2.10), *P* =0.11
Sun et al. (13)	US (SEER 9 registries)	12,969	2001-2010	County poverty rate	High-SES vs. low-middle-SES: 0.88 (0.84-0.92), *P* <0.001
Chan et al. (22)	New Zealand	1,864	2012-2016	The New Zealand Deprivation Index (NZDep2013) including income, home ownership, employment, qualifications, family structure, housing, access to transport, and access to communication	Low-SES vs. high-SES: 1.10 (1.04-1.16), *P*<0.05
Harwood et al. (19)	Australia	6,025	1982-2014	The Socio-Economic Indexes for Areas (SEIFA) index including income, education, employment, occupation, and housing	Low-SES vs. high-SES: 1.23 (1.07-1.40), *P*=0.004; middle-SES vs. high-SES: 1.04 (0.93-1.17), *P*=0.476
Intzes et al. (12)	Greece	223	2005-2019	The modified Kuppuswamy scale evaluated by marital status and median annual income	Low-SES vs. high-SES: 2.09 (1.36-3.20), *P* <0.001
Xu et al. (23)	China	773	2006-2019	Individual education level	High-SES vs. low-SES: 0.32 (0.19-0.56), *P*<0.001
Evans et al. (20)	US	2,543	2005-2015	Median household income, education level, and marital status	Low-SES vs. high-SES: 1.36 (1.04-1.77), *P*=0.025

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.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas. Additionally, all authors participated in drafting, revising, and reviewing the article; giving final approval of the version to be published; agreeing on the journal to which the article has been submitted; and remaining accountable for all aspects of the work.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.941714/full#supplementary-material

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Case report: Intravitreal methotrexate in intraocular acute lymphoblastic leukemia

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Direct leukemic infiltration of the eye is most frequently associated with acute lymphoblastic leukemia (ALL), probably due to its well-known central nervous system (CNS) tropism. Systemic treatment alone may not be sufficient for intraocular leukemia. Data on local treatment are scarce. Here, we present two cases of intraocular ALL treated with intravitreal methotrexate (MTX). Initially, anatomical improvement and visual stability were observed. The first patient experienced anatomical and visual worsening after a year of treatment. Treatment was withheld after 2 months for the second patient due to poor systemic conditions. Corneal toxicity and intraocular pressure elevation were observed in the first case. In both cases, eye involvement was associated with CNS or systemic relapse. This highlights the importance of incorporating ocular disease management in a comprehensive approach to therapy. Our experience corroborates previous findings on MTX injections as an effective and safe therapeutic option for intraocular leukemia. Further evidence is needed to consolidate the use of intravitreal MTX to treat such a debilitating localization of leukemia.

KEYWORDS

intraocular leukemia, methotrexate, acute lymphoblastic leukemia, intravitreal injections, case report, intravitreal methotrexate

Introduction

Ocular involvement in acute and chronic leukemia is common, and ocular symptoms may manifest at presentation or appear in later stages (1). Ocular involvement may occur as direct leukemic infiltration or secondary hematological abnormalities. Intraocular infiltration may follow or precede central nervous system (CNS) involvement. CNS localization is particularly frequent in acute lymphoblastic leukemia (ALL) (2).

The mainstay of treatment for ocular leukemia is systemic chemotherapy. Nonetheless, systemic chemotherapy drugs have scarce penetration in ocular tissues, and adjunctive local treatment is often required (3, 4). Due to the scarcity of data on intraocular treatment of leukemic infiltration, there is a high interest in reporting the outcomes of these therapies (3, 5–7). We hereby present two cases of retinal ALL infiltration treated with intravitreal methotrexate (MTX) injections.

Case descriptions

Case 1

The first patient was a 51-year-old woman with a history of T-ALL. At diagnosis, her white blood cell count was 300 × 10 (8)/L, and she had no CNS involvement (failed karyotype, no molecular data available). She received one cycle of induction and two consolidation courses according to the pediatric-inspired polychemotherapy scheme NILG ALL 10/07 (9); she received adequate CNS prophylaxis with intrathecal chemotherapy and 7 high-dose MTX and cytarabine as per protocol. Considering the high risk of relapse, she then received an allogeneic hematopoietic stem cell transplant (HSCT) from an HLA-identical sibling after myeloablative conditioning with busulfan and cyclophosphamide (as total body irradiation was not available). No CNS-directed therapy was given after HSCT.

Five months after the transplant, while on complete hematologic remission with full donor chimerism, the patient lamented worsening bilateral visual loss. Best-corrected visual acuity (BCVA) was counting fingers in the right eye and 20/25 in the left eye. A combined evaluation of dilated fundus examination, ultra-widefield (UWF) retinography, and optic coherence tomography (OCT) unveiled vitritis, retinal vascular sheathing with frosted branch angiitis, and diffuse yellowish posterior-pole retinal infiltration in both eyes (Figures 1A, C). Fluorescein angiography confirmed bilateral retinal vasculitis of the large and small vessels (Figure 1E). Diagnostic vitrectomy with silicone oil was performed in the right eye: vitreous and retinal biopsies unveiled the presence of leukemic cells positive for CD34 and CD3 at immunohistochemistry. Vitreous polymerase chain reaction (PCR) analysis and culture resulted negative for viruses, bacteria, or fungi. Concurrent neurological evaluation, brain magnetic resonance imaging, and lumbar puncture were initially negative for the presence of CNS leukemic disease and graft-versus-host disease (GVHD). Since leukemia was confined to the eye, intravitreal MTX (400 µg/0.1 ml) was started in both eyes, biweekly in the first month, weekly for 2 months, and monthly thereafter [a protocol previously employed against intraocular lymphomas and leukemic infiltrates (3, 8)]. At this time, no systemic therapy was given.

During the first months of intravitreal treatment, vitreous and retinal infiltrates reduced in both eyes (Figures 1B, D), and visual acuity remained stable (improving to 20/20 in the left eye on one occasion). A few months later, lumbar puncture demonstrated isolated CNS recurrence of leukemia. She was treated with intrathecal methotrexate and whole-brain radiotherapy (RT) obtaining complete remission of CNS involvement. No systemic therapy was given.

Corneal toxicity presenting as superficial punctate epitheliopathy was found during treatment and was controlled with topical artificial tears and a short course of topical corticosteroids. Transient intraocular pressure (IOP) elevation (up to 40 mmHg) was noticed in the right eye and successfully managed with topical anti-glaucoma therapy. Injections were temporarily withheld.

Unfortunately, approximately 1 year after the beginning of treatment, an elevated chorioretinal mass projecting into the vitreous was observed in the right eye. CyberKnife stereotactic radiosurgery treatment was then undertaken in the right eye [anatomical outcomes are shown elsewhere (10)].

A second overt CNS relapse was documented 5 months later. The patient was treated with systemic chemotherapy with fludarabine, cytarabine, and idarubicin chemotherapy, followed by escalated dose donor lymphocyte infusion. She obtained

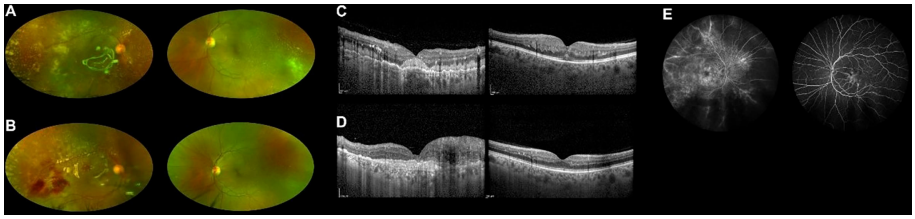

FIGURE 1
Ultra-widefield retinography of both eyes showing leukemic retinal infiltration before **(A)** and during **(B)** methotrexate treatment. OCT scans of both eyes before **(C)** and during **(D)** treatment, displaying reduced infiltration in the left eye. Fluorescein angiography at presentation showing signs of vasculitis **(E)**.

complete CNS remission, but she suffered from progressive ocular disease. Her vision decreased to no light perception in the right eye and counting fingers in the left eye. Eventually, she developed severe chronic GVHD with skin, eye, and lung involvement, and she required immunosuppressant therapy. Ocular treatment was withheld due to poor general conditions. A timeline for patient 1 is presented in Figure 2.

Case 2

The second patient was a 31-year-old man with a history of B-ALL with t(4;11)(q21;q23); *KMT2A-AFF1* rearranged. At the time of diagnosis, his white blood cell count was 175 × 10 (8)/L, and he had CNS involvement (failed karyotype, no other molecular data available). The patient obtained complete hematologic remission after pediatric inspired polychemotherapy induction (11) and four intrathecal injections of dexamethasone, MTX, and cytarabine. Persistent CNS disease was treated with high-dose cytarabine and MTX.

Four months after diagnosis, he experienced a hematologic relapse. CNS was negative for the disease. He was treated with bispecific monoclonal antibody blinatumomab, and a transient remission was obtained. During hospitalization, the patient reported visual impairment in both eyes. BCVA at presentation was counting fingers in the right eye and 20/50 in the left eye. Dilated fundus examination, UWF retinography, and OCT collectively showed yellowish retinal infiltration with retinal hemorrhages in the posterior pole in both eyes and prominent optic disc infiltration in the right eye (Figures 3A, C). Aqueous humor biopsy confirmed the presence of leukemic cells, and PCR analysis excluded infectious etiologies. The patient was diagnosed with leukemic retinal infiltration. Intravitreal rituximab was not an option considering CD20 negativity on leukemic blast. We planned to put the patient on the same intravitreal MTX scheme as our first case, but his general conditions did not allow frequent injections, and we privileged the treatment of the more compromised right eye.

Despite a rarefied regimen of treatment, fundus examination, UWF retinography, and OCT showed regression of retinal infiltration in both eyes, and the patient experienced mild bilateral visual acuity improvement (Figures 3B, D).

Two months after intravitreal treatment initiation, he suffered from systemic ALL relapse with CNS involvement. At this stage, the disease was unresponsive to further treatment. The patient eventually succumbed due to ALL progression and systemic complications. A timeline for patient 2 is presented in Figure 4.

Discussion

CNS involvement is frequent in patients with ALL and notoriously confers a poor prognosis, while data about ocular disease are limited (2). Here, we describe two cases of ALL intraocular infiltration that occurred despite adequate CNS treatment. Both patients had high-risk leukemia. The first patient had hyperleukocytosis and had a relapse after HSCT. The second patient had hyperleukocytosis, CNS involvement, and *KMT2A-AFF1* rearrangement.

Our experience corroborates existing evidence on the local treatment of intraocular leukemia with MTX (a drug that is particularly active against ALL). Both patients benefited from MTX injections, showing reduced retinal infiltration. In our first case, MTX treatment improved funduscopic features in both eyes and, at least initially, visual function in the left eye. The second patient had a good morphologic regression of disease, even though BCVA improvement was not substantial.

Systemic chemotherapy at normal dosage has scarce efficacy on intraocular leukemic infiltration because the blood–aqueous barrier and the inner and outer blood–retinal barriers hinder the penetration of macromolecules into the ocular chambers (4).

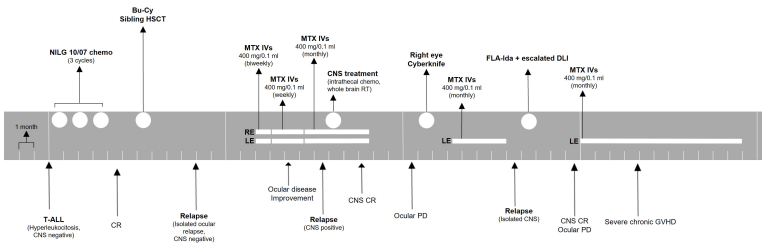

FIGURE 2
Treatment timeline for patient 1. The patient was monitored with ophthalmologic evaluations before every injection or monthly if no injection was administered. ALL, acute lymphoblastic leukemia; CNS, central nervous system; CR, complete remission; Bu-Cy, busulfan + cyclophosphamide; HSCT, hematopoietic stem cell transplant; MTX, methotrexate; IVs, intravitreal injections; RE, right eye; LE, left eye; PD, progressive disease; FLA-Ida, fludarabine, cytarabine, idarubicin; DLI, donor lymphocyte infusion; GVHD, graft-versus-host disease.

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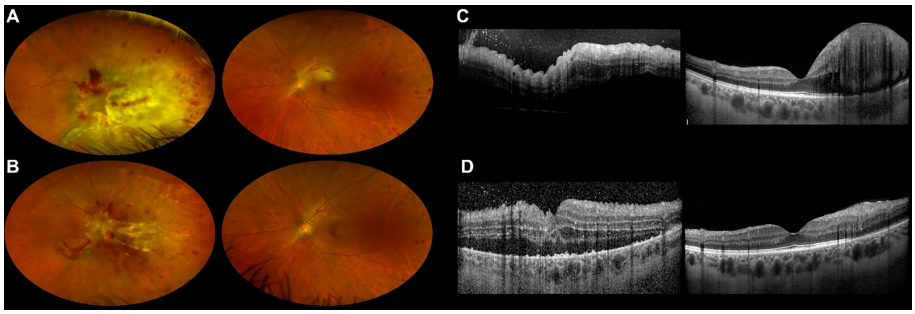

FIGURE 3
Ultra-widefield retinography of both eyes showing leukemic retinal infiltration before **(A)** and at the end **(B)** of our second patient's methotrexate course. OCT scans of both eyes before **(C)** and at the end **(D)** of the treatment confirm morphological improvement.

Leukemic infiltration has been treated locally with ocular radiation and surgical vitrectomy. Dexamethasone injections have been successfully used in conjunction with pars plana vitrectomy to treat leukemic retinal and vitreous infiltrates in a 4-year-old patient with ALL: the rationale for the corticosteroid treatment was controlling inflammation and promoting apoptosis of neoplastic cells (7).

Intravitreal rituximab has been employed against intraocular lymphomas, and it would be reasonable to use it in ocular infiltration from CD20-positive leukemia (12, 13).

MTX is also employed against intraocular lymphomas (13). MTX treatment for primary leukemic invasion was first reported by Ong and White, who described a case of biopsy-proven intraocular localization of lymphocytic leukemia refractory to intravitreal triamcinolone and intrathecal MTX. Mello et al. employed intravitreal MTX for ciliary body infiltration in a case of ALL (6). Lastly, a case series by Vishnevskia-Dai et al. described the effects of intravitreal MTX treatment in 11 eyes of six patients with intraocular leukemia: signs of neoplastic infiltration and related inflammation improved with treatment, but no patient reported visual improvements (3). Previous findings on intravitreal MTX treatment for intraocular leukemic infiltration are summarized in Table 1.

It is also interesting to note that intravitreal MTX (together with oral valganciclovir) reduced macular edema and disc swelling in a case of leukemia-related cytomegalovirus retinitis (14). It should be noted that in both cases of our series, anatomic improvement was not strictly associated with BCVA gains, consistent with previous findings (3). Nevertheless, maintaining an acceptable visual function is a desirable outcome in the context of a comprehensive care for leukemic patients. We believe that withholding treatment would have

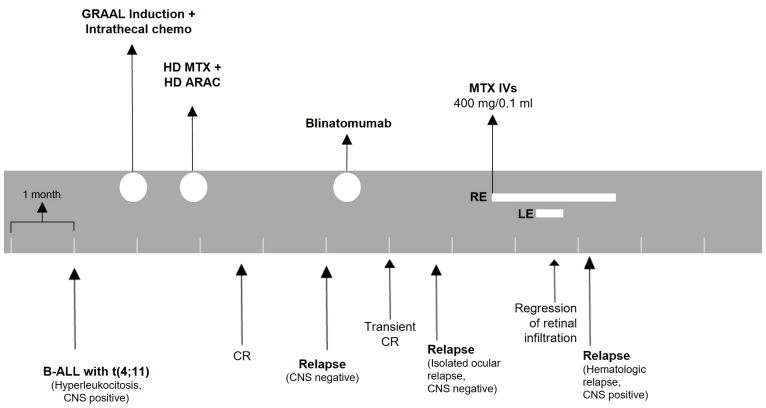

FIGURE 4
Treatment timeline for patient 2. The patient was monitored with ophthalmologic evaluations before every injection. ALL, acute lymphoblastic leukemia; CNS, central nervous system; HD, high dose; MTX, methotrexate; ARAC, cytarabine; CR, complete remission; IVs, intravitreal injections; RE, right eye; LE, left eye.

TABLE 1 Intravitreal treatment of ocular leukemic infiltration.

Paper	Systemic disease	Number of eyes (number of patients)	Localization of infiltration	Outcomes	Side effects
Ong and White	CLL	2 (1)	Vitreous, anterior chamber, eyelids	Resolution of orbital pain and signs of infiltration and rapid VA improvement after 2 injections	None
Mello et al.	ALL	1 (1)	Iris and ciliary body	Resolution of infiltration after 8 injections, VA improvement	Keratopathy (1)
Vishnevskia-Dai et al.	ALL (7), acute promyelocytic leukemia (3), AML (1), HCL (1)[a]	11 (6)	Anterior chamber (4), vitreoretinal (8)	Regression of infiltrates, resolution of inflammation (4 patients), demise without improvement (2 patients). No VA improvement observed	Keratopathy (1)
Current series	ALL	4 (2)	Vitreoretinal	Regression of infiltrates, initial VA stabilization	Keratopathy (1), transient IOP elevation (1)

CLL, chronic lymphatic leukemia; ALL, acute lymphoblastic leukemia; VA, visual acuity; IOP, intraocular pressure.
[a]Data regarding treated and untreated patients.

allowed faster progression of the intraocular disease, with early irreversible visual loss.

Treatment plans need to be personalized according to individual response and the severity of ocular involvement. As our series shows, ocular disease is often associated with and may precede overt systemic and CNS disease. A close collaboration between ophthalmologists and hematologists is central to providing the holistic approach these patients need.

Injections were well tolerated by patients. Compliance was facilitated by the strong impact of visual loss on the perceived quality of life of our patients. To reduce the burden of treatment, we scheduled injections on the same day of other hospital visits whenever possible. Injections were also feasible in a severe thrombocytopenic patient like our second case (CTCAE v.5 grade 4 thrombocytopenia). The only side effects were isolated episodes of corneal epitheliopathy and IOP elevation occurring in our first patient.

We acknowledge that our study has limitations. Our series only includes two patients. However, few cases have been reported previously. The design of this paper is retrospective.

In summary, intravitreal MTX injections have proven to be an effective and safe therapeutic option in two patients with intraocular leukemic involvement, leading to reduction of retinal infiltration and stabilization of visual acuity. Further evidence is needed to evaluate the effectiveness and safety profile of intravitreal MTX in the treatment of such a debilitating localization of leukemia.

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 authors.

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. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

All authors made substantial contributions in the clinical management of patients, study design, drafting or critical revision of the paper, and approved the final version of the paper.

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

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Chromosome 1q21 gain is an adverse prognostic factor for newly diagnosed multiple myeloma patients treated with bortezomib-based regimens

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Chromosome 1q21 aberration is one of the most common cytogenetic abnormalities in multiple myeloma, and is considered an important prognostic factor. The present study analyzed the clinical relevance and prognostic impact of 1q21 gain in 194 patients with newly diagnosed multiple myeloma treated with bortezomib-based regimens. 1q21 gain was detected in 45.9% (89/194) of patients, and those with 1q21 gain had a worse prognosis. Strikingly, our results showed that excluding the effects of other coinciding genetic anomalies, patients carrying at least four copies of 1q21 had worse survival outcome. Moreover, del(13q) strongly correlates with 1q21 gain, and the coexistence of del(13q) and 1q21 gain plays an important role in reducing PFS and OS times. Therefore, 1q21 gain should be considered a high-risk feature in multiple myeloma patients treated with a bortezomib-based regimen.

KEYWORDS

multiple myeloma, 1q21, bortezomib, cytogenetics introduction, prognostic factor and survival

Introduction

Multiple myeloma (MM) is a neoplastic plasma cell disorder characterized by the clonal proliferation of malignant plasma cells in the bone marrow microenvironment. MM accounts for approximately 1% of neoplastic diseases and 13% of hematologic cancers (1). MM is thought to develop through a multistep process, involving genomic

instability, epigenetic dysregulation, and interactions within the bone marrow niche during clonal evolution (2). IGH translocations, such as t (4;14), t (6;14), t (11;14), t (14;16), t (14;20), and hyperdiploidy, have been identified as initiation events in this process. Secondary genomic events, such as chromosomal copy number abnormalities, secondary chromosomal translocations, and gene mutations, occur in subclones of MM cells.

Previous studies have confirmed that the clinical heterogeneity of MM depends largely on cytogenetic abnormalities. Based on consensus, trisomies, t (11;14), t (6;14), and a normal karyotype are standard-risk factors associated with relatively good prognosis; t (4;14) is an intermediate-risk factor, while t (14;20), t (14;16), and del(17p) are high-risk factors associated with relatively adverse prognosis (3). 1q21 gain is one of the most common cytogenetic abnormalities in multiple myeloma (4–6). Chromosome 1q21 abnormalities involving the 1q12-23 region are usually complex and tend to become unstable during tumor progression. High-risk copy number gains of 1q21 partly originate from the hypomethylation of 1q12 pericentromeric heterochromatin (7). Nevertheless, the prognostic value of chromosome 1q21 aberrations is still controversial among cytogenetic abnormality studies (8–12).

In this present study, we aimed to explore the prognostic significance of 1q21 gain in patients with newly diagnosed multiple myeloma treated with bortezomib-based regimens to better understand the genetic basis of MM and guide treatment strategies.

Patients, materials, and methods

Study design and patients

This was a single-center, retrospective cohort study at the Xijing Hospital of the Air Force Military Medical University. Consecutive patients with *de novo* multiple myeloma receiving bortezomib-based regimens from 2014 to 2021 were included. The median follow-up time was 29 months. The bortezomib-based regimens in our cohort were mainly divided into two categories, one is that the regimen only contains one novel agent bortezomib including VD (bortezomib and dexamethasone) and VCD (bortezomib, cyclophosphamide, and dexamethasone), and the other is that it contains two novel agents bortezomib and immunomodulatory drugs (IMids) including VTD (bortezomib, thalidomide, and dexamethasone) and VRD (bortezomib, lenalidomide, and dexamethasone). All patients were aged at least 18 years and diagnosed according to International Myeloma Working Group (IMWG) criteria.

FISH analysis

Bone marrow samples were obtained before treatment. All 194 specimens were purified using magnetic-activated cell sorting (MACS) as CD138-positive cells. Post-sorting purity was checked as previously described, and only samples with ≥70% plasma cells after sorting were analyzed. The plasma cell purity was greater than 90% per sample. Then, these specimens were analyzed to detect the following cytogenetic aberrations: 1q21 gain, del(13q), del(17p), t (11;14), t (4;14), t (14;16), and complex karyotype. Complex karyotype was defined as the occurrence of more than two types of chromosome aberration within an abnormal clone by conventional karyotype analysis. The amplification and deletion cutoff values were set at 20%, and samples with translocation present in over 10% of plasma cells were taken into account.

Outcomes and statistical analyses

The primary objective was to determine the prognostic value of 1q21 gain in MM patients treated with bortezomib-based regimens. Progression-free survival (PFS) and overall survival (OS) times were analyzed as exploratory objectives. The objective response rate (ORR) according to IMWG criteria was assessed as a secondary objective (13). PFS was defined as the time from treatment initiation to the date of documented progression, death, or the last follow-up. OS was defined as the time from the date of treatment initiation to the date of death from any cause or the last follow-up. The Kaplan–Meier method was employed to plot the survival curves, and the log-rank test was used to assess the differences. Logistic regression analysis was used for univariate and multivariate analyses. SPSS version 25.0 (SPSS, Inc) was used for all statistical analyses. Statistical significance was reached if the p-value was less than 0.05.

Results

Clinical characteristics of 1q21 gain

A total of 194 patients diagnosed with multiple myeloma were enrolled in this study from March 2014 to January 2021, and baseline data are shown in Table 1. The median age was 59 years (35–88 years); 45.9% (89) of the patients had advanced ISS III stage, and 17.5% (34) had R-ISS III stage. Three patients were over 80 years of age (1.5%). The median follow-up time was 29 months. The median PFS was 29 months, and the median OS was not reached.

TABLE 1 Patient characteristics.

Characteristics	Overall (n = 194)	1q21 gain positive (n = 89)	1q21 gain negative (n = 105)	p
[a] Median age (range) years	59 (35–88)	59 (35–88)	58 (38–80)	0.429
[b] Gender (male)	116 (59.8%)	50 (56.2%)	66 (62.9%)	0.345
[c] Durie–Salmon stage				0.218
I	21 (10.8%)	6 (6.7%)	15 (14.3%)	
II	33 (17.0%)	17 (19.1%)	16 (15.2%)	
III	140 (72.2%)	66 (74.2%)	74 (70.5%)	
[c] ISS stage				0.003
I	35 (18.0%)	7 (7.9%)	28 (26.7%)	
II	70 (36.1%)	34 (38.2%)	36 (34.3%)	
III	89 (45.9%)	48 (53.9%)	41 (39.0%)	
[c] R-ISS stage				<0.001
I	23 (11.9%)	5 (5.6%)	18 (17.1%)	
II	137 (70.6%)	59 (66.3%)	78 (74.3%)	
III	34 (17.5%)	25 (28.1%)	9 (8.6%)	
[b] M component				0.175
IgG	97 (50.0%)	42 (47.2%)	55 (52.4%)	
IgA	47 (24.2%)	27 (30.3%)	20 (19.0%)	
Others	50 (25.8%)	20 (22.5%)	30 (28.6%)	
[d] Light chain				0.143
λ	101 (52.1%)	53 (59.6%)	48 (45.7%)	
κ	89 (45.9%)	35 (39.3%)	54 (51.4%)	
Others	4 (2.1%)	1 (1.1%)	3 (2.9%)	
[a] Marrow plasma cell (%) (range)	36.8 (2.0–94.8)	40.00 (10.4–94.8)	31.60 (2.0–89.6)	0.062
[b] Peripheral plasma cells	35 (18.0%)	20 (22.5%)	15 (14.3%)	0.140
[a] Hemoglobin (g/L) (range)	93.0 (42.0–165.0)	93.0 (42.0–143.0)	94.0 (46.0–165.0)	0.091
[a] Albumin (g/L) (range)	34.75 (13.40–51.40)	33.60 (13.40–45.00)	36.30 (18.60–51.40)	0.004
[a] Calcium (mmol/L) (range)	2.24 (1.74–3.47)	2.27 (1.74–3.47)	2.21 (1.77–3.37)	0.707
[a] β_2-MG (mg/L) (range)	5.01 (1.37–87.4)	6.09 (2.04–44.3)	3.90 (1.37–87.4)	0.266
[b] LDH high level	31 (16.0%)	20 (22.5%)	11 (10.5%)	0.023
[b] Extramedullary lesions	93 (47.9%)	46 (51.7%)	47 (44.8%)	0.336
[b] Novel therapy				0.413
BTZ + IMids-based	89 (45.9%)	38 (42.7%)	51 (48.6%)	
BTZ-based	105 (54.1%)	51 (57.3%)	54 (51.4%)	
[b] ASCT accepted	21 (10.8%)	11(12.4%)	10 (9.5%)	0.526

Ig, immunoglobulin; ISS, International Staging System; R-ISS: Revised International Staging System; β_2-MG, β_2-microglobulin; LDH, lactate dehydrogenase; BTZ: Bortezomib.
All 180 patients. (B) Patients with/without the gain of 1q21.
Statistical tests used:
[a]t-test.
[b]M-L χ^2 test.
[c]Kruskal–Wallis test.
[d]Fisher test.

Association between 1q21 gain and clinical and biologic parameters

In the present study, 1q21 gain was detected in 45.9% (89/194) of MM patients. Compared to patients without 1q21 gain, 1q21 gain patients demonstrated the following clinical characteristics (Table 1): (1) More advanced ISS (p = 0.003) and R-ISS (p<0.001) stages, (2) lower serum albumin levels (ALB: p = 0.004), and (3) higher lactic dehydrogenase levels (p = 0.023). There were no significant differences between the 1q21 gain and non-1q21 gain groups in other clinical baselines, including sex, DS stage, heavy and light chain, myeloid and serum plasma cell, calcium, β2-microglobulin, extramedullary disease, and receipt of ASCT (p > 0.05), and there were also no significant differences in treatment regimens (p = 0.413) (Table 1).

1q21 gain is an independent risk factor for newly diagnosed myeloma patients

In our cohort, we used univariable logistic regression to evaluate the risk factors of survival outcome in newly diagnosed multiple myeloma patients. The results showed that among all variables, only 1q21 copy number, β2-microglobulin, t (4;14), and presence of peripheral plasma cells were the risk factors (p<0.05) affecting the survival outcome. Finally, we picked up these four variables to perform multivariable logistic regression analysis. The results showed that 1q21 copy number was an independent risk factor for newly diagnosed multiple myeloma (Table 2).

Association between 1q21 gain and other cytogenetic abnormalities

In our assessment of concurrent chromosome aberrations, del(13q) and del(17p) were detected in 59.6% (p = 0.014) and 10.1% (p = 0.891) of patients with 1q21 gain, respectively; in addition, t (4;14), t (11;14), and t (14;16) were detected in 18.0% (p = 0.029), 15.7% (p = 0.381), and 3.4% (p = 0.662) of these patients, respectively. The incidence of other cytogenetic abnormalities, including del(17p), del(13q), and all types of IgH translocation, was 73.0% in 1q21 gain cases and 54.3% in non-1q21 gain cases (p = 0.007). Importantly, a complex karyotype was more common in patients with 1q21 gain than in those without (p<0.001). In conclusion, a significant correlation between 1q21 gain and del(13q), t (4;14), and other cytogenetic aberrations or complex karyotypes was observed (Table 3).

Survival analysis of patients with 1q21 gain

A survival analysis was performed to assess the impact of 1q21 gain on PFS and OS in MM patients. Patients with 1q21 gain had significantly shorter PFS and OS times than those without 1q21 gain (median PFS: 21 months *vs*. 35 months, p<0.001; median OS: 43 months *vs*. NR, p<0.001). Given that 1q21 gain is more likely to coexist with other cytogenetic abnormalities, especially high-risk cytogenetics, we further compared patients with isolated 1q21 gain and those who were FISH-negative. The analysis showed that isolated 1q21 gain was an adverse prognostic factor of OS (p = 0.034), and there were no significant differences in PFS between patients with isolated 1q21 gain and those who were FISH-negative (Figure 1).

Prognostic value of 1q21 gain at different copy numbers

To further analyze the effect of 1q21 gain on the survival and prognosis of patients with MM, we grouped patients according to different 1q21 copy numbers. Survival analysis results showed that patients with a normal copy number of 1q, Gain1q, and Amp1q had a median PFS of 35, 22, and 17 months, respectively (p<0.001), and the median OS of the three groups was NR, 38 months, and 41 months, respectively (p<0.001) (Figures 2A, B). Obviously, over three copies of 1q led to poor prognosis; however, there was no significant difference between Gain1q and Amp1q patients.

As mentioned above, we also considered the possible effects of coexisting cytogenetic abnormalities; thus, we further explored the impact of 1q21 copy number on prognostic data in isolated 1q21 gain and FISH-negative patients. Patients were grouped according to the protocol described above. The median PFS times of normal copy number of 1q, Gain1q, and Amp1q were 49, 50, and 26 months, respectively (p = 0.268), and the median OS times were NR, NR, and 41 months, respectively (p = 0.001) (Figures 2C, D).

Gain of 1q21 and response rate

Only 186 enrolled patients had evaluable results for the best treatment response after bortezomib-based chemotherapy.

TABLE 2 Univariable and multivariable logistic regression of survival outcome.

	Univariable analysis			Multivariable analysis		
	OR	**95% CI**	***p***	**OR**	**95% CI**	***p***
1q21 copy number						
Normal	–	–	–	–	–	–
Gain1q (3 copies of 1q21)	3.598	1.277–10.135	0.015	3.482	1.148–10.560	0.028
Amp1q (≥4 copies of 1q21)	5.009	2.109–11.899	<0.001	3.876	1.549–9.699	0.004
β_2-MG	1.045	1.010–1.081	0.012			
t (4;14)	6.259	2.539–15.428	<0.001	5.314	1.858–15.198	0.002
Peripheral plasma cell	2.889	1.297–6.435	0.009	3.177	1.210–8.345	0.019

TABLE 3 Associations between 1q21 gain subgroup and cytogenetic abnormalities.

	Overall (n = 194)	1q21 gain positive (n = 89)	1q21 gain negative (n = 105)	p
[a] Del(13q)	97 (50.0%)	53 (59.6%)	43 (41.9%)	0.014
[a] Del(17p)	19 (9.8%)	9 (10.1%)	10 (9.5%)	0.891
[a] t (11;14)	26 (13.4%)	14 (15.7%)	12 (11.4%)	0.381
[a] t (4;14)	24 (12.4%)	16 (18.0%)	8 (7.6%)	0.029
[b] t (14;16)	5 (2.6%)	3 (3.4%)	2 (1.9%)	0.662
[a] Other cytogenetic aberration	122 (62.9%)	65 (73.0%)	57 (54.3%)	0.007
[a] Complex karyotype	84 (43.3%)	65 (73.0%)	19 (18.1%)	<0.001

Other cytogenetic aberration was defined as del(17p), del(13q), and all type of IgH translocation by FISH.
Complex karyotype was defined as the occurrence of more than two types of chromosome aberrations within an abnormal clone by conventional karyotype analysis.
Statistical tests used:
[a]M-L χ^2 test.
[b]Fisher test.

Among patients with 1q21 gain (n = 89), the ORR was 74.2%, with the following distribution: CR 30.3%, VGPR 29.3%, and PR 14.6%. Among patients without 1q21 gain (n = 105), 87.6% achieved ORR, including 29.5% CR, 36.2% VGPR, and 21.9% PR. Patients without 1q21 gain had a higher ORR rate than patients with 1q21 gain (p = 0.027). Similarly, the patients with isolated 1q21 gain had a lower ORR rate than FISH-negative patients (63.6% *vs*. 89.1%, p = 0.020) (Table 4).

Prognostic value of 1q21 gain combined with other cytogenetic abnormalities

As mentioned above, 1q21 gain and other cytogenetic abnormalities significantly correlated; thus, we further explored the combined effects of 1q21 gain and other cytogenetic abnormalities on patient outcomes. Del(17p), t (4;14), and t (14;16) were considered high-risk cytogenetic

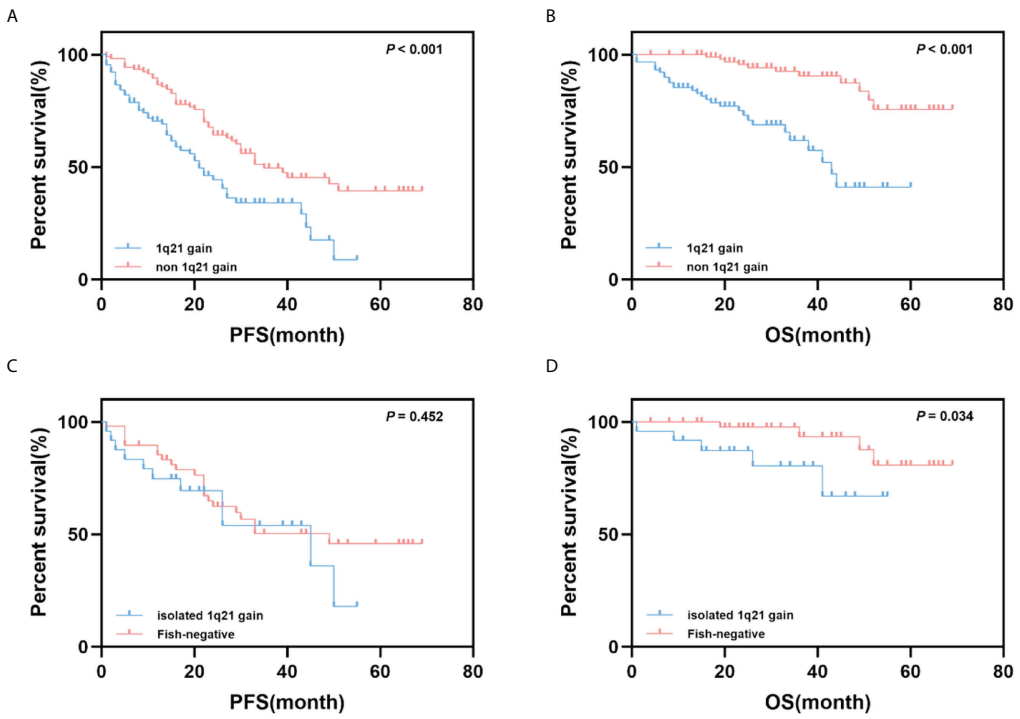

FIGURE 1
Impact of 1q21 gain on PFS and OS. **(A, B)** Kaplan–Meier curves are shown for PFS and OS for patients with 1q21 gain and without 1q21 gain. **(C, D)** Kaplan–Meier curves are shown for PFS and OS for patients with 1q21 gain only and with no cytogenetic abnormalities.

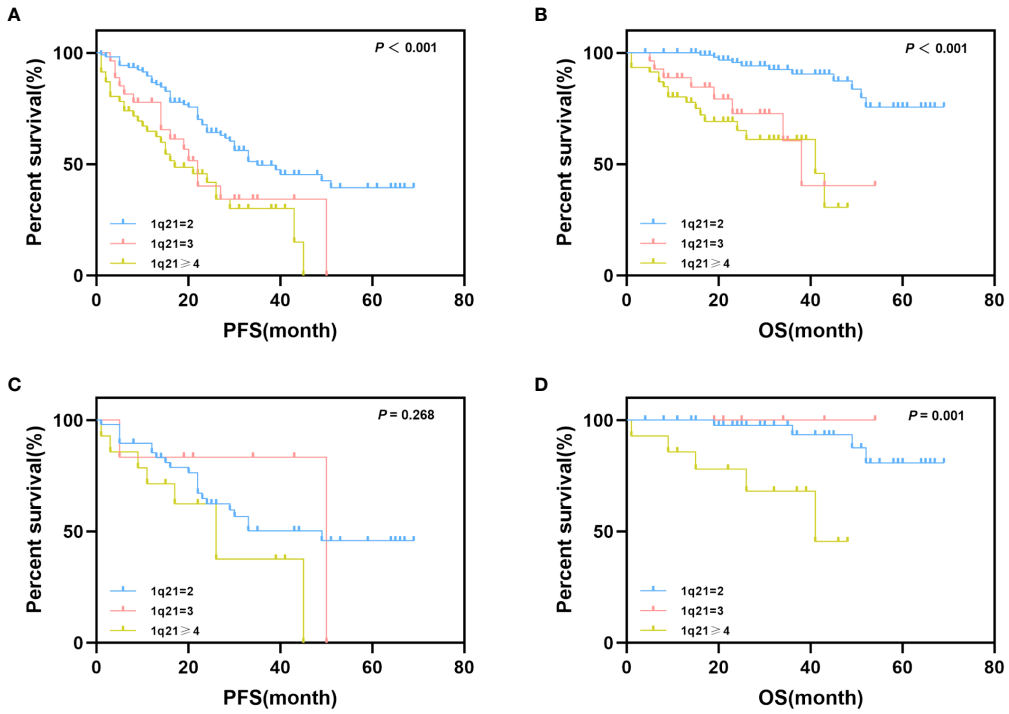

FIGURE 2
Impact of 1q21 gain copy numbers on PFS and OS. **(A, B)** Kaplan–Meier curves are shown for PFS and OS for patients with different copy numbers of 1q21 gain. **(C, D)** Kaplan–Meier curves are shown for PFS and OS for isolated 1q21 gain patients and FISH-negative patients with different copy numbers of 1q21 gain.

abnormalities (HRCAs) according to the IMWG standard (14). Patients were divided into four groups according to HRCAs and 1q21 gain in the subsequent analysis. No significant differences in PFS were observed in patients with HRCAs versus FISH-negative patients; however, patients with 1q21 gain showed shorter PFS times than HRCA patients (median PFS: 20 *vs.* 45 months, $p = 0.044$). Regarding OS, the adverse impact of 1q21 gain was enhanced when it coexisted with HRCAs (median OS: NR *vs.* 38 months, $p = 0.045$).

In addition, we analyzed the synergistic effect of 1q21 gain and del(13q), which was not an independent predictor of poor prognosis mentioned above (15). The results showed that PFS and OS among the four groups were significantly different ($p < 0.001$). Importantly, patients with del(13q) only [1q21- del (13q)+] had better OS times than those in the other three groups (NR *vs.* 44 months *vs.* NR *vs.* 38 months). The median OS of patients with 1q21 gain and del(13q) [1q21+ del(13q)+] was shorter than that of the OS of those with 1q21 gain only [1q21+ del(13q)-] (38 *vs.* 44 months, $p = 0.138$); however, the statistical analysis revealed that the difference was not significant (Figure 3).

Discussion

We report a real-world retrospective study of the prognosis and efficacy of 1q21 gain in patients with newly diagnosed multiple myeloma who received bortezomib-based chemotherapy. We found that 1q21 gain is common (45.9%)

TABLE 4 Treatment response rate.

	1q21 gain Negative ($n = 105$)	1q21 gain Positive ($n = 89$)	*P*	FISH-negative ($n = 46$)	1q21 gain only ($n = 22$)	*P*
ORR	92 (87.6%)	66 (74.2%)	0.027	41 (89.1%)	14 (63.6%)	0.020
CR	31 (29.5%)	27 (30.3%)	0.798	15 (32.6%)	10 (45.5%)	0.304
≥VGPR	69 (65.7%)	53 (59.6%)	0.516	30 (65.2%)	11 (50.0%)	0.230

ORR, overall response rate; CR, complete response; VGPR, very good partial response; PR, partial response.

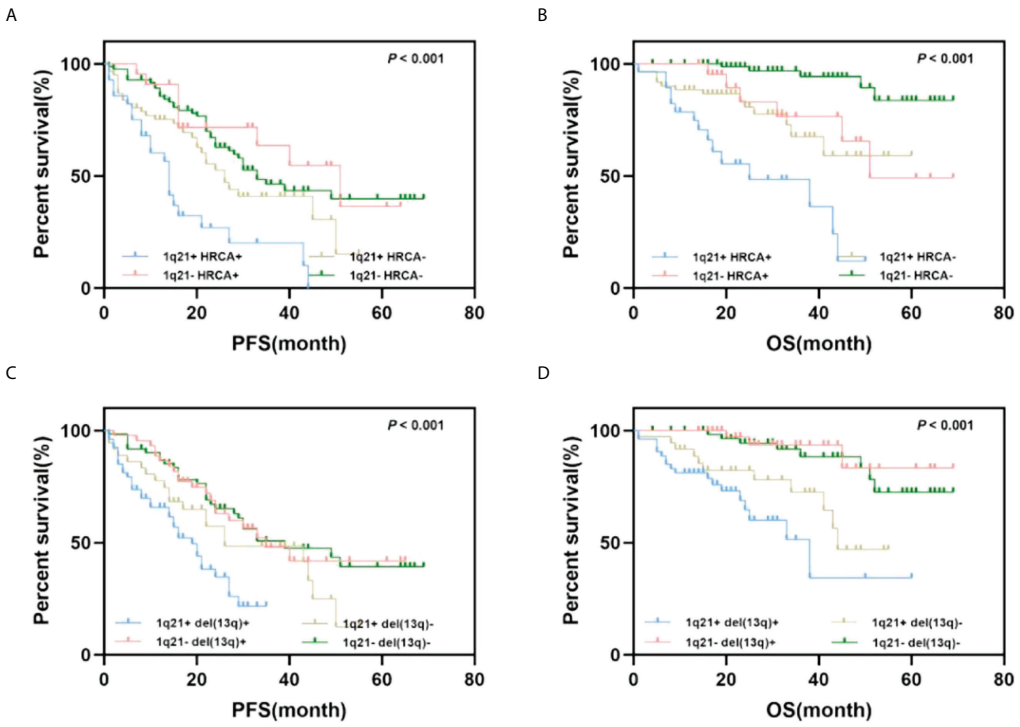

FIGURE 3
Impact of 1q21 gain coexisting with other cytogenetics on PFS and OS. **(A, B)** Kaplan–Meier curves are shown for PFS and OS for patients with or without 1q21 gain and high-risk cytogenetics abnormalities. **(C, D)** Kaplan–Meier curves are shown for PFS and OS for patients with or without 1q21 gain and del(13q).

at diagnosis in multiple myeloma, and this aberration is associated with later ISS stage levels, lower serum albumin levels, and elevated LDH concentrations, consistent with previous research results (3, 8, 16). Moreover, we further demonstrated the prognostic risk value of 1q21 gain in PFS and OS in MM patients. 1q21 gain could significantly result in an adverse outcome when the effects of other cytogenetic abnormalities were excluded.

In recent years, 1q21 gain has been identified as a potential poor prognostic factor. Nahi et al. (17) and Saxe et al. (4) found that even in the era of new drugs, 1q21 gain still led to poor PFS and OS. Shah et al. (18) and Mohan et al. revealed that CD38 monoclonal antibody and ASCT could not reverse the poor prognosis associated with 1q21 gain. According to the latest Mayo guide, MM patients with gain (1q21) are at a higher risk for progression, including those with MGUS, SMM, and multiple myeloma (3). A multivariate analysis conducted by Abdallah et al. revealed that 1q21 gain was an independent risk factor for OS in MM patients (16). However, 1q21 gain is not included in the stratification of the European myeloma consensus (19), and some studies failed to demonstrate the relationship between 1q21 gain and adverse prognosis, although some of the patients received conventional chemotherapies (12, 20, 21). Our results demonstrated that 1q21 gain is a poor prognostic factor in MM patients and is associated with poor clinical features, such as a high concentration of LDH.

In addition, the importance of 1q21 copy number in MM patient prognosis is contradictory. Neben et al. showed that compared with a normal copy number of 1q21, a copy number of three has a marginal negative effect, and having more than three copies significantly reduces PFS and OS times (22). Schmidt et al. also demonstrated that only copy numbers greater than or equal to 4 led to a poor prognosis (10). In contrast, Abdallah et al. observed similar prognostic effects between three and more than four 1q21 copies (16). Consistently, a Chinese study also failed to prove the relationship between different 1q21 copy numbers and prognosis (5). Moreover, Locher demonstrated that three or more than three copies of 1q21 are both adverse prognostic factors, and the effect of more than three copies is more obvious (23). We set the cutoff value of 1q21 gain to 20% and found that the PFS and OS of Gain1q and Amp1q were significantly shorter than those with normal copies of 1q21, but the effect of Gain1q and Amp1q on prognosis was similar. However, excluding the coexistence of other genetic abnormalities, we found that the prognosis of Amp1q patients was significantly worse than that of patients with a normal copy number or Gain1q.

Multiple myeloma patients often carry more than one cytogenetic abnormality, and recent studies have indicated that the coexistence of many abnormalities is a crucial prognostic indicator. For instance, Boyd et al. demonstrated that 1q21 gain, del(17p), and IgH translocation often coexist, and the accumulation of these adverse abnormalities is associated with gradually worsening survival outcomes (24). Pawlyn also found similar results (25). In contrast, Kumar et al. found that 1q21 chromosomal trisomy ameliorated the adverse effects of t (4;14), t (14;16), and t (14;20) (11). We found that 1q21 gain was associated with del(13q) and t (4;14), and patients with 1q21 gain often had complex karyotypes. Further analysis showed that the coexistence of 1q21 with other genetic abnormalities leads to a worse prognosis. Moreover, we found that del(13q) strongly correlates with 1q21 gain and that the coexistence of del(13q) and 1q21 gain plays an important role in reducing PFS and OS times.

Previous studies have shown that bortezomib-based regimens cannot overcome the adverse effects of 1q21 (10, 17). We further analyzed the response of enrolled patients to this treatment protocol. Patients with only 1q21 gain had a lower ORR. However, if the effects of coexisting genetic abnormalities are not excluded, it cannot be confirmed that patients with 1q21 gain have worse treatment responses. Coexisting genetic abnormalities may reverse the role of 1q21 in bortezomib resistance.

Our study has the standard limitations of retrospective studies, including inadequate number of cases and selection bias. Moreover, only a portion of the enrolled patients had 1q21 copy number aberrations, and the sample size used for the statistical analysis after excluding those with coexisting genetic abnormalities was small. In addition, the heterogeneity of the treatment regimens also needs to be considered; thus, our results need to be verified with further follow-up and prospective studies.

In conclusion, our study demonstrated the importance of 1q21 gain in myeloma patients treated with bortezomib-based regimens. 1q21 gain was an independent prognostic risk factor for PFS and OS and led to worse treatment responses. Routine testing should include FISH for 1q21 gain, and patients with this abnormality should be considered for alternative treatments and new drugs might improve their prognosis.

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 authors.

Ethics statement

This study was reviewed and approved by Ethics Committee of Xijing Hospital of Air Force Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

XL, SJ and YC analyzed the majority of data and prepared figures. XL wrote the manuscript. BT, YG, CZ, YZ, WJ and XXL collected the data. RY, NZ, JF and HD collated the data. XX, ZWC and ZCC assisted in data sorting and analysis. GG and HT conceived the study, designed the study, evaluated data, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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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.

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Pediatric non–Down's syndrome acute megakaryoblastic leukemia patients in China: A single center's real-world analysis

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Non-Down's syndrome acute megakaryocytic leukemia (non-DS-AMKL) is a subtype of childhood acute myeloid leukemia (AML), whose prognosis, prognostic factors and treatment recommendations have not yet to be defined in children. We conducted a retrospective study with 65 newly diagnosed non-DS-AMKL children from August 2003 to June 2020 to investigate the clinical impact of factors and clinical outcome. Among all 65 patients, 47 of them were treated at our center who received three different regimens due to time point of admission (CAMS-another, CAMS-2009 and CAMS-2016 protocol), and the efficacy were compared. Patients with newly diagnosed non-DS-AMKL accounted for 7.4% of pediatric AML cases. The median age of the patients was 18 months at diagnosis, and over 90% of them were under three-years-old. The overall survival (OS) rates were 33.3% ± 1.7%, 66.7% ± 24.4% and 74.2% ± 4.0% for three groups (CAMS-another, CAMS-2009 and CAMS-2016 regimen), respectively. In CAMS-2016 group, the complete remission (CR) rate after induction was 67.7% (21/31), while the total CR rate after all phases of chemotherapy was 80.6% (25/31). The 2-year survival probability did not significantly improve in patients underwent HSCT when compared with non-HSCT group (75.0% ± 4.7% vs. 73.9% ± 4.6%, p=0.680). Those who had a "dry tap" during BM aspiration at admission had significantly worse OS than those without "dry tap" (33.3% ± 8.6% vs. 84.0% ± 3.6%, p=0.006). Moreover, the results also revealed that patients with CD34+ had significantly lower OS (50.0% ± 6.7% vs. 89.5% ± 3.5%, p=0.021), whereas patients with CD36+ had significantly higher OS than those who were negative (85.0% ± 4.0% vs. 54.5% ± 6.6%, p=0.048). In conclusion, intensive chemotherapy resulted in improved prognosis of non-DS-

AMKL children and subclassification may base on "dry tap" and immunophenotypic. Although some progress has been made, outcomes of non-DS-AMKL children remain unsatisfactory, especially in HSCT group, when compared with other AML types.

KEYWORDS

non-DS-AMKL, pediatric, treatment, outcomes, prognostic factors

Introduction

Acute megakaryoblastic leukemia (AMKL) was first described in 1931 by Von Boros (1). In 1978, Breton-Gorius et al utilized immunoelectron microscopy (IEM) to show that the blast cells of AMKL patients were with positive reactions for platelet peroxidase (PPO) (2). AMKL is a subtype of acute myeloid leukemia (AML) classified as megakaryocyte lineage (M7) by the French-American-British (FAB) cooperative group classification system of hematological neoplasias in 1985 (3). According to recent studies, the proportion of AMKL is around 1% in all adult AML patients (4, 5), while the incidence of AMKL in AML children ranges from 4% to 15% (6–12). Identification of markedly decreased CD41 (GPIIb) and CD61 (GPIIIa) expression levels, which are diagnostic for AMKL patients (13). There are also likely to be many false positives in the result of flow cytometry due to bone marrow (BM) aspiration may be difficult allowing for extensive myelofibrosis caused by megakaryocytes, known as "dry tap". These increase the difficulty of diagnosis and lead to misdiagnosis. In view of the documented diagnostic bias for the reason above, elimination of misdiagnosis and improve of prognosis may be warranted.

AMKL can be divided into two subgroups in pediatrics: AMKL with Down syndrome (DS-AMKL) and without Down syndrome (non–DS-AMKL). AMKL is the most common kind of AML in children with Down syndrome, and the prognosis of DS-AMKL is better than non–DS-AMKL (14, 15). When comapred with DS-AMKL, non–DS-AMKL may be with biologically heterogeneity, and the prognosis of non–DS-AMKL was thought to be poor (6, 15, 16). However, in view of the low incidence of this type of childhood AML, the prognosis and potential risk factors for Non-DS-AMKL remains debatable (7, 11, 17, 18). The purpose of this study was to determine the prevalence, clinical symptoms at presentation, hematologic, immunophenotypic, cytogenetic, and molecular characteristics of childhood non–DS-AMKL. Furthermore, we analyzed the prognosis and evaluated the potential risk factors of these patients.

Patients and methods

Patients

We reviewed data from 65 patients with newly diagnosed non-DS-AMKL at the Institute of Hematology & Blood Diseases Hospital between August 2003 and June 2020. Patients with non-DS-AMKL were ≤16 years old. The diagnosis of AMKL was established based on the 2016 WHO categorization criteria (19). Diagnostic criteria of AMKL met one or more following criteria: 1) The BM aspirate exhibited a blast cell infiltrate that comprised ≥20% of all cells, and with >50% of the blast cells being identified as megakaryoblasts; 2) the expression of CD41, CD42b and/or CD61 was positive, as demonstrated by flow cytometry with monoclonal or polyclonal platelet-specific antibodies; 3) In cases with BM "dry tap" or myelofibrosis, a BM clot or biopsy was necessary, and the cell of origin was required to be identified as part of the megakaryocyte lineage. Positive immunocytochemical staining for platelet-specific antigens such as factor VIII, CD41, CD42b and CD61 revealed this; 4) In the absence of immunophenotyping or biopsy, the diagnosis was confirmed by electron microscopic identification of PPO activity or immunocytochemical staining for platelet-specific antigen CD41 positive in BM or peripheral blood samples, or both in blasts cells (3, 20). Immunophenotyping or immunohistochemistry should always be used to confirm the diagnosis (6). Exclusion criteria included DS-AMKL and AMKL as a secondary malignancy. Cytogenetic studies and Next-generation sequencing (NGS) were performed in some cases. This study was approved by our institution's ethical committee. Consent was obtained from all patients' parents or guardians.

Treatment protocols

During the study, three different treatment protocols were used. Six patients were treated according to the Chinese

Academy of Medical Science (CAMS)-another protocol, nine patients received CAMS-2009 protocol (21), and thirty-one patients received CAMS-2016 protocol. The CAMS-2016 of non-DS-AMKL regimen consists of induction and consolidation treatment. If the white blood cell (WBC) count was ≥4×10^9/L or associated with BM hyperactivity, the standard induction treatment regimen was used, included: etoposide, 150 mg/m^2 with a 2-hour infusion on days 1-5, idarubicin, 8 mg/m^2 with a 1-hour infusion on days 6-8 (mitoxantrone 5 mg/m^2 days 6-10, early availability), and cytarabine, 200 mg/m^2 with a 12-hour infusion on days 6-12. If the WBC count is less than 4×10^9/L and the degree of BM hyperplasia is less than active, the standard induction treatment regimen consisted of homoharringtonine 1 mg/m^2/d, cytarabine 10 mg/m^2/d, q12h, and granulocyte colony stimulating factor (G-CSF) 200ug/m^2/d (mix 200ug/d, if WBC ≥20×10^9/L, stop it) on days 1-14. In patients with severe infections, it can be reduced to 10 days. If CR was not achieved, a second course of induction therapy was administered. High-dose cytarabine combined with etoposide or idarubicin/mitoxantrone was used in the five courses of consolidation treatment. In the consolidation treatment, the course and dosage of medium and large doses of cytarabine have been increased. HSCT is recommended for high-risk patients with relapsed or refractory disease or high minimal residual disease (MRD). If HSCT was not feasible, consolidation and strengthening treatment should be continued. Intrathecal multi-drug chemotherapy was used once per course of treatment to provide prophylactic treatment for the central nervous system.

Definition and statistical analysis

CR was defined as BM with <5% blasts and evidence of normal hematopoietic cell regeneration. Early death was defined as an event that occurred within 30 days of a diagnosis. The study's primary endpoints were event-free survival (EFS) and overall survival (OS). EFS was defined as the time from diagnosis to the first event, which included failure to achieve remission, relapse, secondary malignancy, being lost to follow-up, or death from any cause. OS was defined as the time of death from any cause. Categorical variables are expressed as sums and percentages of total numbers. Since continuous variables are not normally distributed, median, minimum, and maximum values were utilized as descriptive statistics. To analyze the differences in continuous variables, a non-parametric test (Mann-Whitney U test) was used, and frequencies were analyzed using Fisher's exact test. The Kaplan-Meier survival analysis was used to estimate the 2-year probabilities of EFS and OS, and the log-rank test was used to compare survival. Bonferroni-adjusted log-rank tests were conducted to assess differences in separated groups, and the significance level was 0.017 after Bonferroni correction for multiple analysis. A multiple Cox regression model was used to perform multiple regression analysis on EFS and OS. All variables with a $P<0.10$ in univariate analysis were included in the multivariate analysis in logistic regression model. A two-sided P-value of <0.05 was deemed to be statistically significant. All clinical statistical analyses were performed using SPSS 25.

Next-generation sequencing

The DNA from the BM of the patients was extracted using the QIAamp DNA Mini kit (QIAGEN) and purified with the Twist Binding and Purification Beads Kit (Twist Bioscience) following the manufacturer's instructions. Then, using the Twist Fast Hybridization Target Enrichment protocol, target genes were enriched, amplified, and purified. The Illumina NovaSeq 6000 platform was used to sequence the target-enriched DNA libraries, with an average sequencing depth of 1000×. After quality control of the FASTQ files by FastQC (V 0.11.5), the reads were aligned to the reference genome (hg19) using BWA (V 0.7.10), sorted with SAMtools (V 0.1.19), and deduplicated with Picard (V 1.123). Somatic mutations were then detected with Pisces (V 5.1.6.54) and annotated with ANNOVAR.

Results

In this study, we included 65 non-DS-AMKL patients between January 2003 and June 2020, accounting for 2.1% (65/3034) of newly diagnosed acute leukemia and 7.4% (65/876) of AML (including AML-M3 patients) in our center. Table 1 showed the baseline characteristics of all 65 included non-DS-AMKL patients. The median age at diagnosis was 18 months (ranging from 5 to 89 months), and 59 cases (90.8%) were ≤3-years-old.

Baseline characteristics was showed in Table 1. Anemia, bleeding or fever were initial symptoms in 65 non-DS-AMKL cases in this study. There were 21 cases (32.3%) with pale skin, 38 cases (58.5%) with fever, 31 cases (47.7%) with skin ecchymosis or epistaxis, lymphadenopathy in 2 cases, and bone pain in 4 cases. Physical examination revealed palpable hepatosplenomegaly in 24 cases (36.9%). The morphology of BM varies (Figure 1A). The proportion of megakaryocytes stained with CD41 was 39.5% (7%–91%) in BM smears (Figure 1B) and 22.5% (2%–59%) in peripheral blood smears. BM biopsy was performed on 12 non-DS-AMKL children, four of whom were CD42b positive. There were six cases of MF-2 and three cases of MF-3. BM clot was performed on 6 non-DS-AMKL children, four of whom were CD42b and/or CD61 positive. Cytogenetic analysis was performed in 60 patients and 21 cases among them was with complex karyotypes.

TABLE 1 Baseline characteristics of included non-DS-AMKL patients (n = 65).

Characteristics	Patients
Gender ratio	43M/22F
Median age at diagnosis, months(range)	18 (5-89)
Median time from onset to diagnosis(range)	2 (0.2-7)
Median WBC count, $\times10^9$/L (range)	11.58 (2.44-55.35)
Median Hb count, g/L (range)	82.4 (27-129)
Median PLT count, $\times10^9$/L (range)	32 (6-222)
Hepatosplenomegaly, no. (%)	24 (36.9%)
Median BM blasts, % (range)	42.5 (4.0 -97.0)
Median PB blasts, % (range)	16 (0 - 81.0)
"Dry tap", no. (%)	19/65(29.2%)
PPO (n=47), no. (%)	
PPO positive	39 (83.0%)
Immunophenotype features (n=58), no. (%)	
CD61	28 (48.3%)
CD41	36 (62.1%)
CD42b	25 (43.1%)
CD34	19 (32.8%)
CD36	33 (56.9%)
Cytogenetic features (n=60), no. (%)	
complex karyotypes	21 (35.0%)
+21	18 (30.0%)
+19	19 (31.7%)
+8	20 (33.3%)
-7	2 (3.3%)
-13	3 (5.0%)
-15	4 (6.6%)

M, male; F, female; Hb, hemoglobin; PLT, platelet; BM, bone marrow; PB, peripheral blood; PPO,platelet peroxidase.

NGS were performed in 29 patients. 20 of all 29 cases were without disease-related mutations. Three cases carried MPL S505N mutation, as well as one case was with JAK2 V617F and R867Q mutations. The other five cases were with JAK2 M511I, JAK2 V617F, JAK2 R867Q, SUZ12 R286X, and RB1 R255X mutations respectively, and frequency of mutations ranging from 0.85% to 27.8%.

Outcomes

Prognosis of patients received three protocols

The treatment regimens were classified into three groups: previous treatment (from August 2003 to August 2009), the CAMS-2009 regimen (from September 2009 to December 2015), and the CAMS-2016 regimen (from January 2016 to June 2020). Among all 65 patients, 47 of them were treated who received three different regimens due to time point of admission (CAMS-another, CAMS-2009 and CAMS-2016 protocol). The baseline characteristics between patients who underwent treatment and dropout were compared, and there exist no difference between two groups (Supplementary Table 1). The percentage of non-DS-AMKL children who dropped out of treatment gradually decreased from 50.0% (6/12) to 20.5% (8/39) (Figure 2).

The estimated 2-year probability of OS rates in three different subgroups (CAMS-another, CAMS-2009, and CAMS-2016 regimen) were 33.3% ± 1.7%, 66.7% ± 24.4%, 74.2% ± 4.0%, respectively (p=0.023). The difference between CAMS-another and CAMS-2016 protocol was statistically significant (p=0.007). However, there was without statistical significance between

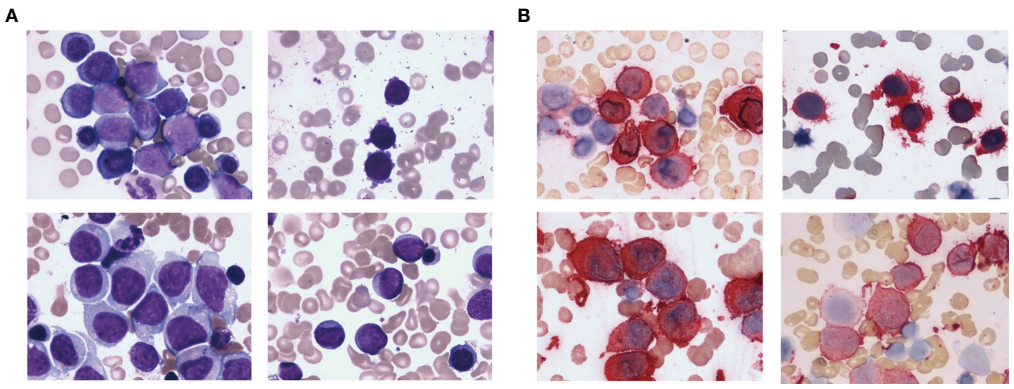

FIGURE 1
HE staining **(A)** and CD41 immunohistochemical staining **(B)** of the bone marrow from non-DS-AMKL children.

CAMS-another and CAMS-2009 regimen (p=0.101), nor CAMS-2009 and CAMS-2016 regimen (p=0.543) (Figure 3A).

The estimated 2-year probability of EFS rates in three different subgroups (CAMS-another, CAMS-2009, and CAMS-2016 regimen) were estimated to be 0.0% ± 0.9%, 33.3% ± 19.2%, 67.7% ± 4.1%, respectively (p<0.001). The difference between CAMS-another and CAMS-2009 regimen, as well as CAMS-another and CAMS-2016 regimen, was statistically significant (p=0.011, p<0.001, respectively). There was no significant difference between CAMS-2009 and CAMS-2016 regimen (p=0.113) (Figure 3B).

The prognosis and risk factors in CAMS-2016 protocol

For the credibility of the analysis (22, 23), only 31 patients received CAMS-2016 protocol was considered for the further analysis of prognostic factors (Supplementary Table 2). The median time of follow-up was 16.1 months (range, 4.6-71.8 months). Three children died as a result of a severe infection, gastrointestinal bleeding, and multiple organ failure during early induction chemotherapy. Three cases remained not remission (NR). In CAMS-2016 group, the complete remission (CR) rate after induction was 67.7% (21/31), while the total CR rate after all phases of chemotherapy was 80.6% (25/31). During the induction, 85.7% of patients with CR survived, and eight patients experienced MRD-negative remission while seven children were still alive in the first CR after induction.

The estimated 2-year probability of OS and EFS was 74.2% ± 4.0% and 67.7% ± 4.1%. 23 patients received intensive chemotherapy and eight patients received HSCT. In chemotherapy cohort, the 2-year OS and EFS was 73.9% ± 4.6% and 65.2% ± 4.9%, respectively, and in transplantation cohort, they were 75.0% ± 4.7% and 75.0% ± 5.4% (Figures 4A, B). The OS and EFS rates were similar in both cohorts.

Patients who had "dry tap", which indicated the possibility of myelofibrosis, had significantly worse OS and EFS than those who without (33.3% ± 8.6% vs. 84.0% ± 3.6%, p=0.006; 33.3% ± 8.8% vs. 76.0% ± 4.1%, p=0.030, respectively) (Figures 4C, D). CD34+ patients have lower 2-year OS and EFS rates than CD34- patients (50.0% ± 6.7% vs. 89.5% ± 3.5%, p=0.021; 41.7% ± 6.7% vs. 84.2% ± 4.0%, p=0.021, respectively) (Figures 4E, F). Patients who are CD36+ have superior 2-year OS and EFS rates than CD36- patients (85.0% ± 4.0% vs. 54.5% ± 6.6%, p=0.048; 85.0% ± 3.9% vs. 36.4% ± 6.2%, p=0.007, respectively) (Figures 4G, H) (Table 2).

Multivariate analysis demonstrated a trend toward a poor prognosis for patients with "dry tap" (p=0.064) and CD34+ (p=0.096). Patients with CD36+ demonstrated a trend toward a favorable prognosis in EFS (p=0.054). In the univariate analysis, they were statistically significant (Table 3).

There were insignificant differences in the outcomes of megakaryocytic differentiation-related antibodies (CD41, CD42b, and CD61). The clinical characteristics of the patients, such as gender and age, had no effect on survival as well as complex karyotypes (Table 2).

Discussion

This is a single-center retrospective study to report on the clinical characteristics, outcomes, and potential prognostic factors of newly diagnosed non-DS-AMKL in children. In our study, only 7.4% of pediatric AML cases were diagnosed as non-DS-AMKL. However, the diagnosis of AMKL is frequently challenging due to a high incidence of myelofibrosis, resulting

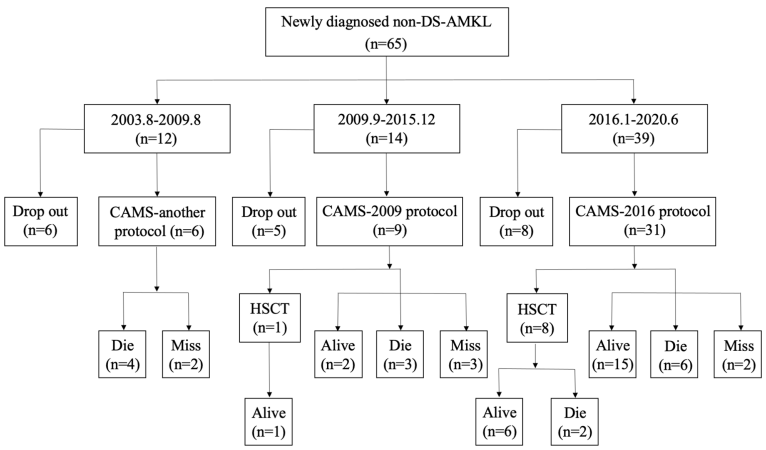

FIGURE 2
A flowchart depicting newly diagnosed cases of non-DS-AML at our center from August 2003 to June 2020.

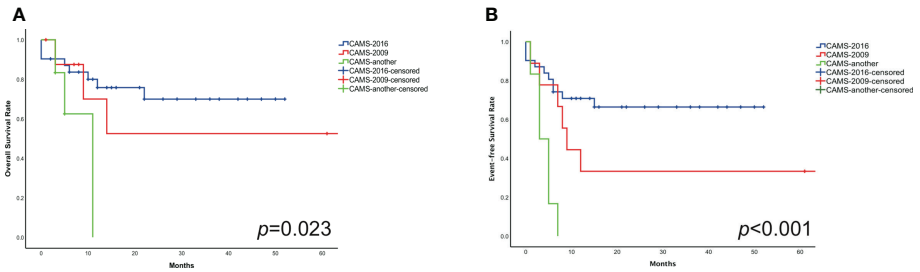

FIGURE 3
Compared the OS **(A)** and EFS **(B)** in three distinct subgroups of pediatric non-DS-AMKL.

in the failure of BM aspiration. This complicates the diagnosis of AMKL. In our clinical practice, PPO activity on electron microscopy (2, 20), or immunocytochemical staining for platelet-specific antigen CD41 positive in BM or peripheral blood samples, or both in blast cells (24, 25), was recommended for diagnosis when blasts in BM were <20% or absence of immunophenotyping or biopsy in non-DS-AMKL. What's more, immunophenotyping or immunohistochemistry may be also warranted to aid in diagnosis, which contributed to increased diagnostic capability in recent years (6).

Due to lack of consensus on treatment recommendations for non-DS-AMKL, children with non-DS-AMKL still experienced a poor prognosis and the survival rates vary substantially between studies (10%–70%) (6, 11, 26). This was one of the reasons for the high dropout rate at our center previously. Hence, more intensive induction and consolidation regimens were adopted in our center. By incorporating idarubicin, high-dose cytarabine and mitoxantrone into the protocol, 5-year OS in German AML-BFM (Berlin-Frankfurt-Münster)-04 of pediatric non–DS-AMKL improved to 70% ± 6% (11). Another Japanese study estimated the 10-year OS rate for patients with non-DS-AMKL to be 76% (7). Based on the findings of the preceding investigation, we added mitoxantrone, idarubicin, etoposide and high-dose cytarabine into our CAMS-2009 regimen. On the top of CAMS-2009, CAMS-2016 incorporated homoharringtonine into the protocol which results in a better prognosis of these patients during the past two decades, which was similar to the earlier studies (7, 11). Moreover, several steps have been taken to facilitate the diagnosis and optimize the treatment of childhood AML in recent years, which may also benefit these patients.

According to the discriminating educated degree and economic status of different family, some of the parents were reluctant to let their children to receive chemotherapy because of financial constraints. The dropout rate was higher during the CAMS-2009 or CAMS-another treatment. In recent years, with a number of measures were introduced, increasing number of children suspected of non-DS-AMKL were diagnosed and the clinical outcome of these patients were also improved. With the optimization of the chemotherapy regimen in our center during the past two decades, the prognosis has increased gradually and very few patients abandoned the treatment in recent years.

In view of the poor prognosis of non-DS-AMKL patients, Garderet et al. recommended allogeneic HSCT in the first CR in this cohort (17). However, the benefits of allo-HSCT continue to be inconclusive, for the small number of AMKL patients who received allogeneic HSCT in CR1 (18, 27). Several studies achieved superior survival rates with intensive chemotherapy alone, with no benefit observed when HSCT was used during post-remission treatment (7, 11). In our study, the estimated 2-year OS for patients with non-DS-AMKL was 73.9% ± 4.6% in chemotherapy group and 75.0% ± 4.7% in transplantation group (p=0.680), and the OS were comparable in comparison to two previous studies (7, 11). In this study, the benefit of HSCT was still not obvious. Additional research is required to develop new and more effective treatment options for these children.

AMKL is frequently associated with myelofibrosis (28), which frequently results in a "dry tap" in the BM aspiration. The cause of BM fibrosis is unknown. Previous research suggested that fibroblast growth may be correlated with the production of growth factors by malignant megakaryocytes and their dissemination into the BM microenvironment (29–31). However, very few study found that "dry tap" is related to the prognosis of non-DS-AMKL children. In our study, patients with non-DS-AMKL who had "dry tap" had significantly worse prognosis than those who did not. The multivariate analysis indicates that "dry tap" may be associated with a poor prognosis, but the difference is not statistically significant due to limited sample size.

In our study, non-DS-AMKL patients who are CD34+ have inferior 2-year OS and EFS rates (p=0.021, p=0.021, respectively). According to some studies, CD34-positive cells may be early lineage specific progenitors in AML-M7 (32). It explains that high CD34 expression on AMKL blasts indicates that megakaryocytes are more primitive and may be associated with a poor outcome. CD36 (thrombospondin receptor) is generally used as a marker for late differentiation in CD34- megakaryocytes.

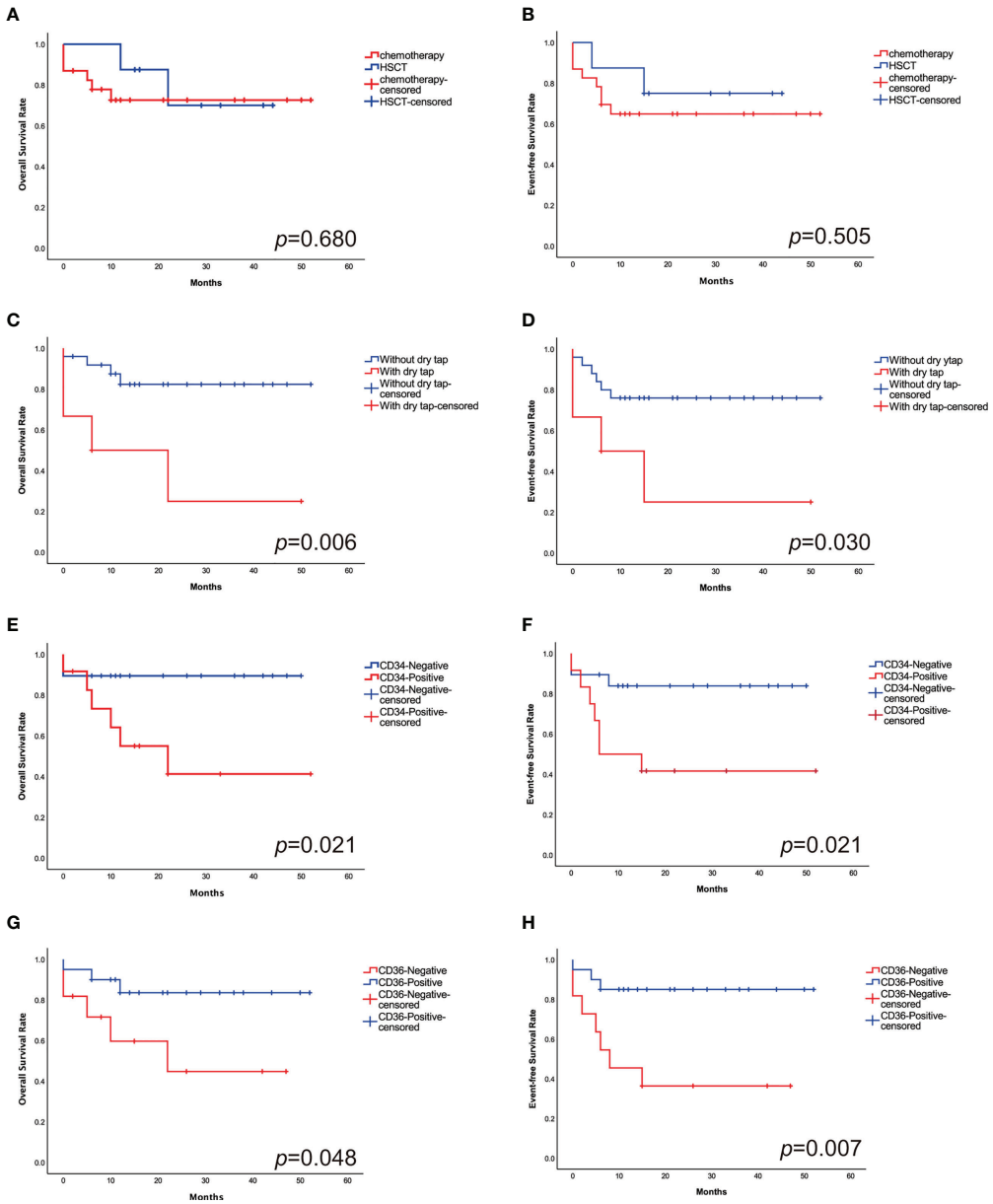

FIGURE 4
The 2-year probabilities of OS **(A)** and EFS **(B)** comparing the outcomes in HSCT cohort with chemotherapy cohort. There is no significant difference in outcomes between the study groups. The 2-year probabilities of OS **(C)** and EFS **(D)** comparing the outcomes of patients with and without "dry tap". The 2-year probabilities of OS **(E)** and EFS **(F)** comparing the outcomes of CD34+ and CD34- patients. "Dry tap" and CD34+ confer a poor outcome. The 2-year probabilities of OS **(G)** and EFS **(H)** comparing the outcomes of CD36+ and CD36- patients. CD36+ have a favorable outcome compared with CD36-.

In our study, non-DS-AMKL patients with CD36+ had significantly higher 2-year OS and EFS rates than patients without (p=0.048, p=0.007, respectively), which is consistent with previous literature reports (12, 33). CD34+ may be a poor prognostic factor and CD36+ may be a good prognostic factor in univariate analysis. Due to the small number of cases, there is no statistically significant difference in multifactorial analysis. However, in our study, non-DS-AMKL patients who are CD41, CD42b, or CD61 positive had no effect on prognosis.

Non-DS-AMKL cases are characterized by the presence of recurrent translocations (which are absent in DS-AMKL), such as complex karyotype or copy-number abnormalities. In non-DS-AMKL children, abnormal chromosome numbers, particularly +8, +19, +21, were more prevalent than in

TABLE 2 Effects of potential factors on clinical outcomes in CAMS-2016 protocol (n=31).

	Cases	OS, %	*P* value	EFS, %	*P* value
Gender					
male	22	77.3% ± 4.4%	0.426	72.7% ± 4.7%	0.302
female	9	66.7% ± 7.9%		55.6% ± 8.0%	
Age					
≤12month	8	87.5% ± 5.5%	0.401	62.5% ± 7.5%	0.699
>12month	23	69.6% ± 4.7%		69.6% ± 4.7%	
Dry tap					
Y	6	33.3% ± 8.6%	0.006	33.3% ± 8.8%	0.030
N	25	84.0% ± 3.6%		76.0% ± 4.1%	
Immunophenotype					
CD34					
positive	12	50.0% ± 6.7%	0.021	41.7% ± 6.7%	0.021
negative	19	89.5% ± 3.5%		84.2% ± 4.0%	
CD36					
positive	20	85.0% ± 4.0%	0.048	85.0% ± 3.9%	0.007
negative	11	54.5% ± 6.6%		36.4% ± 6.2%	
CD41					
positive	18	77.8% ± 4.6%	0.779	77.8% ± 4.6%	0.238
negative	13	69.2% ± 5.9%		53.8% ± 6.4%	
CD61					
positive	15	80.0% ± 2.6%	0.661	80.0% ± 2.6%	0.215
negative	16	68.8% ± 5.4%		56.3% ± 5.9%	
CD42b					
positive	16	81.3% ± 4.7%	0.529	81.3% ± 4.7%	0.171
negative	15	66.7% ± 5.6%		53.3% ± 6.0%	
Cytogenetic					
complex karyotypes					
Y	11	63.6% ± 3.9%	0.222	63.6% ± 3.7%	0.599
N	20	80.0% ± 4.4%		70.0% ± 4.9%	
Trisomy 8 and/or Trisomy 19 and/or Trisomy 21					
Y	14	57.1% ± 6.7%	0.057	57.1% ± 6.8%	0.233
N	17	88.2% ± 3.9%		76.5% ± 4.8%	

N, no; Y, yes.

children with DS-AMKL (7, 34). Furthermore, +8 and/or +19 can be found in MDS and other diseases (35, 36). Due to a few circulating leukemic cells, a "dry-tap" BM aspiration, and BM fibrosis, some newly diagnosed AMKL patients have both BM and peripheral blood blasts ≤19%. AMKL can be distinguished from MDS based on the age of onset, the course of the disease, and immunophenotyping or immunohistochemistry of peripheral blood or BM megakaryoblastic cells.

TABLE 3 Multivariable Cox Regression Analysis for OS and EFS in CAMS-2016 protocol n = 31.

	OS			EFS		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Dry tap	3.970	(0.925,17.051)	0.064	2.646	(0.723,9.683)	0.142
CD34-positive	4.038	(0.781,20.865)	0.096	3.006	(0.741,12.194)	0.124
CD36-positive	0.422	(0.093,1.919)	0.264	0.256	(0.064,1.026)	0.054

Hussein et al. reported that MPL W515L mutation occurs in a considerable proportion of AMKL with myelofibrosis that was unrelated to primary myelofibrosis (37). Malinge et al. also described a new gain-of-function MPL T487A mutation in non-DS-AMKL with features comparable to MPL W515 mutation (38). In this study, we did not find MPL T487A or MPL W515 mutation, but three cases of MPL S505N mutation were detected. The role of the MPL S505N mutation in the pathogenesis of AMKL is still unknown. JAK2 V617F mutation is rare in acute leukemias but occur in 2 of 11(18%) patients with AMKL (39). In our study, two individuals with non-DS-AMKL had JAK2 V617F mutation; one of them also had JAK2 R867Q mutation. JAK2 R867Q mutation promoted the expression of proliferation marker and inhibited the differentiation marker in AML cell-line (40). More research is needed to determine whether the other JAK2 M511I, SUZ12 R286X, and RB1 R255X mutations have functions in non-DS-AMKL.

There still exist limitations in this study. Despite our study has been conducted for nearly 20 years, owing to non-DS-AMKL (AML-M7) was a rare subtype of childhood AML, the number of participants engaged in this study is still limited and the results may not be fully elucidated.

Conclusion

In conclusion, intensive chemotherapy resulted in improved prognosis of non-DS-AMKL children and subclassification may base on “dry tap” and immunophenotypic. Although some progress has been made, outcomes of non-DS-AMKL children remain unsatisfactory, especially in HSCT group, when compared with other AML types.

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 authors.

Ethics statement

The studies involving human participants were reviewed and approved by The Ethics committee of the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

Author contributions

AZ and LL conceived and designed the study. AZ and SZ drafted the initial manuscript and analyzed the data. MR, XYC, and CL reviewed the initial manuscript. XZ, MR, and YiZ supervised the work. LC, XJC, WY, YG, LZ, YaZ, and YC collected and provided patient clinical data. XZ and MR assigned the protocol, and critically revised the manuscript for relevant intellectual content. All authors contributed to the article and approved the submitted version.

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.940725/full#supplementary-material

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Significance of bone marrow fibrosis in acute myeloid leukemia for survival in the real-world

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Acute myeloid leukemia (AML) is a highly heterogeneous hematologic malignancy characterized by the proliferation of myeloid blasts. Bone marrow fibrosis (BMF), characterized by increased deposition of reticulin or collagen fibers, can occur in AML. International authoritative guidelines do not mention AML patients with BMF and the reported studies are inconsistent. Therefore, we retrospectively analyzed the clinical data of newly diagnosed AML patients in our hospital and compared the clinical characteristics, gene mutations and prognosis of AML patients with or without BMF. We found AML patients with BMF tended to be older, were more prone to hepatosplenomegaly, their level of β2-MG was higher and they often had karyotypes associated with a poor prognosis. The proportion of AML patients without BMF was high in the intermediate-risk group and low in the high-risk group. The mutation rates of ASXL1 and TET2 genes were higher and that of CEBPA was lower in the BMF group. Multivariate analysis showed BMF had independent prognostic significance. AML patients without BMF had higher CR/CRi rate, and the time of hematopoietic recovery in patients achieving CR/CRi was longer in BMF group. The degree of BMF, prognostic level and blasts in peripheral blood were independent risk factors for CR/CRi in newly diagnosed AML. AML patients in the BMF group, especially those with BMF ≥ 2, had a lower OS rate. In age<60 years old group, the higher the degree of BMF was, the shorter the median survival time and the lower the OS rate. In age ≥ 60 years old group, the median survival time in the BMF-1 and the BMF-2/3 groups was shorter. For AML with low, intermediate and high risk, there was always a lower OS rate in patients with BMF. The median survival of AML patients decreased with an increasing degree of BMF in different risk stratifications. BMF had no effect on OS of AML patients with HSCT. In conclusion, AML patients with BMF have a poor prognosis, and BMF was an independent prognostic factor for OS. The assessment of BMF was of great significance for the treatment efficacy and prognosis of newly diagnosed AML.

KEYWORDS

acute myeloid leukemia, bone marrow fibrosis, overall survival, prognosis, complete remission

Introduction

Acute myeloid leukemia (AML) is a highly heterogeneous hematologic malignancy characterized by the proliferation of myeloid blasts or progranulocytes that fail to undergo normal differentiation. The pathogenesis of AML is mainly attributed to chromosomal translocations and mutations of the genes involved in hematopoietic proliferation and differentiation, which results in the accumulation of poorly differentiated myeloid cells (1). The bone marrow microenvironment (BMM) is a complex network composed of blood vessels, nervous systems, hematopoietic cell populations, stromal cell populations, bone marrow adipocytes, cytokines and adhesion molecules and extracellular matrix (ECM) (2). Damage to stromal cell populations and the ECM may lead to bone marrow fibrosis (3). However, many recent studies found that genetic lesions and BMMs that could not regulate hematopoietic stem cells (HSCs) were responsible for the transition to leukemia stem cells (LSCs) (4). In turn, the transformed LSCs promoted the remodeling of the BMM (5). Consequently, the BMM is considered to play a crucial role in both hematopoiesis and leukemogenesis.

Bone marrow fibrosis (BMF) is characterized by increased deposition of reticulin fibers or collagen fibers (6). However, BMF, observed in any type of AML, is more frequent in acute megakaryocytic leukemia (AML-M7) (7, 8). In recent years, there have also been some reports about chronic myeloid leukemia (CML) (9) and myelodysplastic syndrome (MDS) (10) combined with BMF. Tumor aggression and poor prognosis were found to be correlated with the degree of tissue fibrosis and level of stromal stiffness in solid tumors (11). However, the study of BMF in hematological malignancies is relatively rare. Research reports marrow fibrosis is a factor predictive of a poor prognosis in patients with MDS (12). International authoritative guidelines, such as the NCCN clinical practice guidelines (13), ESMO clinical practice guidelines (14), World Health Organization (WHO) (15) and ELN (16) guidelines, do not mention the gene mutation and prognosis analysis of AML patients with BMF. Studies have reported on this issue, but the results are inconsistent. One study by Manoharan A et al. showed that BMF did not affect the overall survival (OS) of patients with AML and that effective anti-leukemia treatment could reverse BMF (17). However, another study found a poor prognosis in AML patients with BMF (18). Therefore, we retrospectively analyzed the clinical data of newly diagnosed AML patients in our hospital and compared the clinical characteristics, gene mutations and prognosis of newly diagnosed AML patients with or without BMF. In order to clarify the influence of BMF on the efficacy and prognosis of newly diagnosed AML, further explore whether AML with BMF can be regarded as an independent clinicopathological feature or be included in prognosis stratification and guide such patients to make more reasonable treatment plans.

Materials and methods

Patients and clinical procedures

This retrospective study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University, and written informed consent was obtained from all subjects or their guardians. Clinical samples of hospitalized patients were collected from December 2014 to September 2021. A total of 605 newly diagnosed AML patients were enrolled in our study. All patients underwent examinations of morphology, immunology, cytogenetics, molecular biology and bone marrow biopsy. The diagnosis and prognosis of AML were made according to the guidelines and the WHO classification systems (15). A bone marrow biopsy was performed in 190 AML (non-acute promyelocytic leukemia, non-APL) patients. The CAG regimen (low-dose cytarabine 10 mg/m^2 every 12 hours on days 1-14, aclarubicin (14 mg/m^2 every day on days 1-4 and granulocyte colony-stimulating factor 200 μg/m^2 every day on days 1-14) as induction therapy for the treatment of poor-prognosis AML (19). Other AML patients received conventional "7 + 3" regimens: DA (cytarabine 200 mg/m^2 every day on days 1-7, daunorubicin 60 mg/m^2 every day on days 1-3), IA (cytarabine 200 mg/m^2 every day on days 1-7, idarubicin 12 mg/m^2 every day on days 1-3), MA (cytarabine 200 mg/m^2 every day on days 1-7, mitoxantrone 12 mg/m^2 every day on days 1-3), which was used for induction chemotherapy (13). Consolidation chemotherapy was conducted after complete remission (CR), which included the original induction chemotherapy plus intermediate- or high-dose cytarabine. According to the NCCN guidelines, lumbar puncture (LP) and intrathecal injection were performed to prevent or treat central nervous system involvement in AML patients. LP is not recommended in asymptomatic patients at diagnosis. Patients with headache, confusion, and paresthesia should be examined first by radiology (CT/MRI) to rule out neurological bleeding or mass. If there is no evidence of intracranial hemorrhage, LP can be performed after correcting the coagulation disorder and platelet transfusion. If leukemic cells are found in cerebrospinal fluid, LP and intrathecal injection should be performed with systemic chemotherapy. If symptoms persist but cerebrospinal fluid is normal, LP should be performed (13). The hematopoietic stem cell transplantation (HSCT) was performed in accordance with clinical guidelines based on the classification and AML risk stratification (13). Complete remission and progression of the disease were defined according to references (20–22).

Bone marrow biopsy, pathological film and reticular fiber staining

Qualified bone marrow tissue (1-2 cm in length and more than 0.2 cm in diameter) was removed. Then the tissues were fixed in 4% paraformaldehyde. After decalcification, dehydration and paraffin embedding, the wax blocks were thinly sliced. The paraffin sections were stained with hematoxylin-eosin and immunohistochemistry. The reticular fibers were stained by the Gomori method. The radiographs were reviewed by professional physicians of the Institute of Hematology in our hospital. The grading criteria of bone marrow fibrosis were in accordance with the 2005 European consensus on grading bone marrow fibrosis (23).

Other indicators of detection

Bone marrow aspiration was subjected to chromosome karyotype analysis, second-generation sequencing, preliminary screening and prognostic gene detection to assess the AML patient's prognosis and guide the treatment. General information about the patients (gender, age) was also recorded. Routine blood tests, the percentage of peripheral blood/bone marrow primitive cells, lactate dehydrogenase (LDH), β2 microglobulin (β2-MG), blood type and hepatosplenomegaly were also assessed.

Follow up

All cases were followed up to May 30, 2022. Follow-up data were obtained from inpatient and outpatient medical records. Patients who died during the follow-up period were confirmed according to the course of the disease records or by telephone contact with the patient's family members. Survival time was calculated from diagnosis to death or from diagnosis to May 30, 2022.

Statistical analysis

Data were analyzed using SPSS 26.0 software (SPSS, Chicago, IL, US). Quantitative data were compared using a t-test (for a normal distribution) or a nonparametric test (Mann–Whitney Test, not a normal distribution). The chi-squared ($\chi 2$) test was used for comparison of the categorical data. First, a t-test was used for univariate analysis. Second, a nonconditional logistics regression model was used for multivariate analysis. The Kaplan–Meier method was used to plot cumulative survival curves. A Cox regression model was used for multivariate analysis of overall survival. Only the independent variables that $P < 0.05$ in the univariate analysis in the previous step, were subjected to multivariate regression analysis. Data analysis was performed using GraphPad Prism 9 software (GraphPad Software, La Jolla, California). $P<0.05$ was considered statistically significant.

Results

Patient characteristics

In this retrospective study, there were a total of 190 newly diagnosed patients with AML (non-APL). Among them, there were 130 AML patients with BMF and 60 without BMF. There were more men than women in both groups (AML with BMF vs. without BMF, 72/58 vs. 31/29). The median age was 51.9 and 42.5 years old among AML patients with and without BMF, and there was a significant difference between the two groups ($P = 0.000$). The level of serum β2-MG in AML patients with BMF was higher than that in those without BMF, and the difference was statistically significant ($P = 0.000$). Hepatosplenomegaly was more common in AML patients with BMF than in those without BMF (39.50% vs. 22.00%) ($P = 0.045$). In the high-risk cytogenetics group, AML patients with BMF accounted for a higher proportion (13.59% vs. 2.22%), and the difference was statistically significant ($P = 0.006$). Prognostic risk stratification was significantly different in AML patients with or without BMF ($P =0.036$). In the intermediate group, the proportion of AML without BMF was higher than AML with BMF (58.33% vs. 21.54%), and the P value was 0.000. Conversely, the proportion of AML without BMF was lower than AML with BMF in the poor prognosis group (18.33% vs. 61.54%), and the P value was 0.000. However, there was no significant difference in the number of AML patients with or without BMF in the good prognosis group. At the same time, there was no significant difference between blasts in the peripheral blood and bone marrow between the two groups. There was no significant difference in white blood cell count, hemoglobin and platelet count in the peripheral blood, LDH, AST, ALT, α1-MG, D dimer, ferritin and blood type between the two groups ($P > 0.05$). There was no therapy related AML in either group, but there was secondary AML in both groups (AML with BMF vs. without BMF, 13/130 vs. 5/60), and there was no significant difference between the two groups ($P = 0.796$).

Details of the clinical features are listed in Table 1.

Gene mutation analysis in newly diagnosed AML patients with and without BMF

The results of gene mutation analysis in the two groups are shown in Table 2. The mutation rates of the ASXL1 and TET2 genes were higher in the AML with BMF group than in the AML

TABLE 1 Comparison of clinical features in newly diagnosed AML patients with and without BMF.

Clinical characteristics	AML with BMF (130)	AML without BMF(60)	P value
Gender (male/female)	72/58	31/29	0.639
Age, year, median (range)	51.9 (15~82)	42.5 (14~73)	0.000
WBC ($\times10^9$/L), median (range)	35.1 (0.5~332)	22.2 (0.85~153.6)	0.051
HB (g/L), median (range)	74.9 (11~142)	80.6 (42~152)	0.099
PLT ($\times10^9$/L), median (range)	95.3 (2~1262)	76.6 (2~1510)	0.513
Blasts in PB (%), median (range)	37.3 (0~98)	40.5 (0~96)	0.514
Blasts in BM (%), median (range)	51.7 (20~95.6)	57.3 (20~93.2)	0.101
LDH(IU/L), median (range)	611.0 (109~2518)	478.6 (110~2111)	0.131
ALT(U/L), median (range)	26.7 (5~140)	26.0 (4~119)	0.875
AST(U/L), median (range)	26.9 (5~161)	21.5 (8~89)	0.098
β2-MG (mg/L), median (range)	2.5 (0.61~8.2)	1.58 (0.62~3.33)	0.000
α1-MG (mg/L), median (range)	21.1 (7.81~49)	20.3 (10~29)	0.605
D dimer (mg/L), median (range)	5.9 (0.06~430.6)	1.59 (0.09~12.83)	0.523
Ferritin (ng/mL), median (range)	1375.1 (37~10738)	826.3 (10.8~2384.1)	0.215
Blood type (%)			0.238
A+	19.83	32.14	0.073
B+	34.71	32.14	0.737
AB+	11.57	14.29	0.611
O+	33.88	21.43	0.092
Hepatomegaly/Splenomegaly (%)	39.50	22.00	0.045
Cytogenetics (%)			0.140
Low risk	9.71	22.22	0.597
Intermediate risk	76.70	75.56	0.881
High risk	13.59	2.22	0.006
Prognostic level, n (%)			0.036
Low risk	22 (16.92%)	14 (23.33%)	0.320
Intermediate risk	28 (21.54%)	35 (58.33%)	0.000
High risk	80 (61.54%)	11 (18.33%)	0.000
AML diagnosis			
De novo	117 (90.00%)	55 (91.67%)	0.796
Secondary AML	13 (10.00%)	5 (8.33%)	0.796

WBC, white blood cells; HB, hemoglobin; PLT, platelet; PB, peripheral blood; BM, bone marrow; BMF, bone marrow fibrosis; AML, acute myeloid leukemia; BMF, bone marrow fibrosis.

without BMF group (P = 0.004 and 0.048, respectively). However, the mutation frequency of CEBPA was significantly lower than that of patients without BMF (P = 0.000). Other mutated genes, such as FLT3-ITD, FLT3-TKD, TP53, DNMT3A, and NPM1, showed no significant difference between the two groups (P > 0.05).

The impact of different induction therapies on CR/CRi rate and overall survival

In our retrospective study, the standard DA, IA, MA or CAG formula was used for induction chemotherapy in our retrospective study. There were only two AML patients with BMF receiving MA formula, but none in AML without BMF. Consequently, we analyzed the impact of the remaining three induction therapies on complete response (CR)/morphologic complete remission with an incomplete blood count recovery (CRi) rate and overall survival (OS). The CR/CRi rate of the IA, DA and CAG groups in AML with the BMF group was 57.78%, 53.85% and 41.67% (P = 0.446), respectively, and there were no statistically significant differences between any two groups of the three induction therapies (P > 0.05). In AML without the BMF group, the CR/CRi rate in the IA, DA and CAG groups was 85.71%, 62.50% and 75.00% (P = 0.359), respectively. Similarly, there was no statistical difference between any two groups of the three induction therapies in this group (P > 0.05) (Table 3).

The median survival time of the IA, DA and CAG groups in AML with BMF receiving induction therapies was 7.800 months, 4.733 months and 5.167 months, respectively. There was no significant difference among the three groups (χ^2 = 5.061, P = 0.080)

TABLE 2 Gene mutation analysis in newly diagnosed AML patients with and without BMF.

Gene name	AML with BMF, n (%)	AML without MF, n (%)	P value
FLT3-ITD	21 (16.15%)	8 (13.33%)	0.618
FLT3-TKD	5 (3.85%)	7 (11.67%)	0.054
CEBPA	10 (7.69%)	21 (35%)	0.000
NPM1	20 (15.38%)	6 (10%)	0.287
C-kit	8 (6.15%)	5 (8.33%)	0.583
TP53	5 (3.85%)	2 (3.33%)	0.862
RUNX1	10 (7.69%)	2 (3.33%)	0.190
ASXL1	40 (30.77%)	8 (13.33%)	0.004
DNMT3A	18 (13.85%)	5 (8.33%)	0.244
IDH1	1 (0.77%)	3 (5%)	0.155
IDH2	6 (4.62%)	2 (3.33%)	0.684
SF3B1	3 (2.31%)	2 (3.33%)	0.683
U2AF1	9 (6.92%)	3 (5%)	0.615
SRSF2	7 (5.38%)	3 (5%)	0.913
ZRSR2	1 (0.77%)	2 (3.33%)	0.301
EZH2	2 (1.54%)	0 (0)	0.337
TET2	83 (63.85%)	29 (48.33%)	0.048
CBL	8 (6.15%)	2 (3.33%)	0.421
JAK2/V617F	8 (6.15%)	1 (1.67%)	0.097
NRAS	30 (23.08%)	9 (15.00%)	0.177
KRAS	1 (0.77%)	0 (0)	0.498
ETV6	4 (3.08%)	4 (6.67%)	0.320
SETBP1	6 (4.62%)	1 (1.67%)	0.318
GATA2	1 (0.77%)	1 (1.67%)	0.576
IKZF1	0 (0)	1 (1.67%)	0.321

AML, acute myeloid leukemia; BMF, bone marrow fibrosis.

(Figure 1A). In AML without BMF group, the median survival time of IA, DA and CAG groups was 21.500 months, 32.533 months and 21.667 months. There was no significant difference among the three groups (χ^2 = 1.060, P = 0.588) (Figure 1B).

Effect of BMF on the induction remission rate in primary diagnosed AML patients

The CR/CRi rate was 54.25% in AML with BMF and 77.19% in AML without BMF, and there was a significant difference between the two groups (P = 0.004). AML patients without BMF had higher CR rate (AML with the BMF vs. without the BMF, 39.36% vs. 61.40%, P = 0.008). The proportion of induction failure in AML with the BMF group was higher than that without the BMF group (23.40% vs. 7.02%) (P = 0.010). The recovery time of bone marrow hematopoietic function in patients achieving CR/CRi was longer in the BMF group (P = 0.034) (Table 4). Multivariate analysis using a non-conditional logistic regression model showed that BMF had independent prognostic significance (P = 0.001). The degree of fibrosis was an independent risk factor for CR/CRi in newly diagnosed AML patients [BMF-2/3 vs. BMF-0, HR, 95% CI, 0.351 (0.194-0.634), P = 0.001; BMF-2/3 vs. BMF-1, HR, 95% CI, 0.189 (0.068-0.521), P = 0.001]. The prognostic level was an independent risk factor for CR/CRi in newly diagnosed AML patients [high risk vs. low risk and intermediate, 0.369 (0.163-0.834), P = 0.017]. Blast in peripheral blood (PB) was a risk factor [blast vs. without blast in PB, 0.098 (0.012-0.800), P = 0.030] (Table 5).

TABLE 3 Impact of different induction therapies on CR/CRi rate.

Group	IA	DA	CAG	P value
AML with BMF, n (%)	26 (57.78%)	14 (53.85%)	10 (41.67%)	0.446
AML without BMF, n (%)	12 (85.71%)	10 (62.50%)	21 (75.00%)	0.359

AML, acute myeloid leukemia; BMF, bone marrow fibrosis; CR, complete remission; CRi, morphologic complete remission with incomplete blood count recovery.

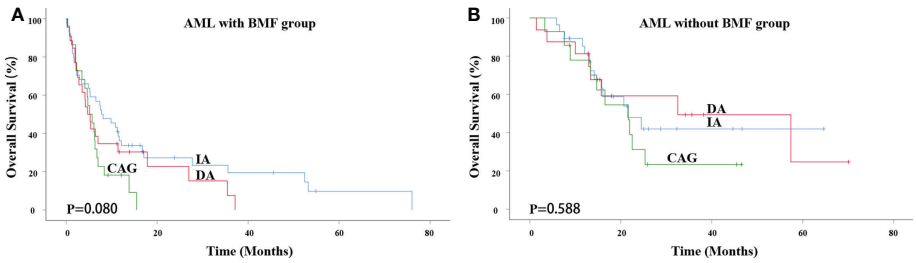

FIGURE 1
Impact of different induction therapies on OS in primary AML patients. **(A)** Kaplan-Meier curves comparing the OS of patients with BMF receiving induction therapies, such as IA (blue), DA (red) and CAG (green) (7.800 months vs. 4.733 months vs. 5.167 months, P = 0.080). **(B)** Kaplan-Meier curves comparing the OS of patients with BMF receiving induction therapies, such as IA (blue), DA (red) and CAG (green) (21.500 months vs. 32.533 months vs. 21.667 months, P = 0.588). AML, acute myeloid leukemia; BMF, bone marrow fibrosis; OS, overall survival.

TABLE 4 Induced chemotherapy response and recovery time of BMHF in newly diagnosed AML patients with and without BMF.

Treatment response	AML with BMF	AML without BMF	P value
CR/CRi, n (%)	51 (54.25%)	44 (77.19%)	0.004
CR, n (%)	37 (39.36%)	35 (61.40%)	0.008
CRi, n (%)	14 (14.89%)	9 (15.79%)	0.883
PR, n (%)	21 (22.34%)	9 (15.78%)	0.331
Induction failure, n (%)	22 (23.40%)	4 (7.02%)	0.010
Abandoning therapy, (n)	36	3	
Recovery time of BMHF			
CR/CRi [a], day, median (range)	26 (13~73)	22 (7~61)	0.034
CR [b], day, median (range)	24 (13~73)	22 (7~61)	0.067

AML, acute myeloid leukemia; BMF, bone marrow fibrosis; CR, complete remission; CRi, morphologic complete remission with incomplete blood count recovery; PR, partial remission. [a]the recovery time of bone marrow hematopoietic function in patients achieving CR/CRi; [b]the recovery time of bone marrow hematopoietic function in patients achieving CR.

Overall survival

The median survival time of AML without BMF was 21.667 months and that of AML with BMF was 4.200 months. The 3-year overall survival (OS) rate of AML patients without BMF was 35.4% and that of AML patients with BMF was 9.6%. There was a significant difference between the two groups (χ^2 = 35.200, P=0.000) (Figure 2A). AML with BMF was divided into two subgroups according to the degree of fibrosis: the BMF-1 group and the BMF-2/3 group. The OS of the BMF-1 group and the BMF-2/3 group was compared with that of the AML without BMF group, and there were statistically significant differences among the three groups (χ^2 = 41.140, P=0.000). The median survival of the AML without BMF group was 21.667 months, 5.400 months in the BMF-1 group, and 2.533 months in the BMF-2/3 group. The 3-year OS rate of AML patients without BMF was 35.4%, AML patients with BMF-1 was 16.6%, and BMF-2/3 was 2.3% (Figure 2B).

Cox multivariate analysis showed that BMF had independent prognostic significance for the OS of primary AML patients (P = 0.000), especially AML patients in the BMF-2/3 group, who had worse OS [HR, 95% CI, 2.203 (1.661-2.924), P = 0.000]. Meanwhile, Cox multivariate analysis showed that age had independent prognostic significance for the OS of primary AML patients (P=0.000), especially age ≥60 years had a worse OS [HR, 95% CI, 2.495 (1.708-3.644), P = 0.000] (Table 6).

Survival by age

For AML patients younger than 60 years old, the AML with BMF group had a lower OS rate (3-year OS rate: 42.8% vs. 12.9%, χ^2 = 24.276, P = 0.000). The median survival time of AML without BMF was 21.667 months while that of AML with BMF was 6.133 months (Figure 3A). The OS of the BMF-1 group and the BMF-2/3 group was compared with that of the AML without BMF group for AML patients < 60 years old, and there were statistically significant differences among the three groups (χ^2 = 31.205, P = 0.000). The median survival of the AML without

TABLE 5 Univariate and multivariate analyses of CR/CRi for patients with newly diagnosed AML.

Covariates	Univariate		Multivariate	
	HR (95% CI)	P	HR (95% CI)	P
Age[a]	0.433 (0.179-1.047)	0.063		
Gender (male vs. female)	1.266 (0.653-2.457)	0.485		
WBC[b]	0.863 (0.445-1.671)	0.662		
Blasts in PB[c]	0.118 (0.015-0.927)	0.042	0.098 (0.012-0.800)	0.030
Blasts in BM[d]	0.552 (0.056 -5.434)	0.610		
Cytogenetics[e]	0.306 (0.084-1.120)	0.074		
Prognostic level[f]	0.334 (0.169-0.663)	0.002	2.710 (1.199-6.123)	0.017
Bone marrow fibrosis		0.008		0.001
BMF-0 VS BMF-1	0.802 (0.336-1.916)	0.619	0.906 (0.323-2.543)	0.851
BMF-0 VS BMF-2/3	0.344 (0.213-0.555)	0.000	0.351 (0.194-0.634)	0.001
BMF-1 VS BMF-2/3	0.147 (0.057-0.383)	0.000	0.189 (0.068-0.521)	0.001

HR, hazard ratio; CI, confidence interval; [a]Age < 60 years old versus ≥ 60 years old; [b]WBC ≤10×10^9/L versus WBC >10×10^9/L; [c]Blasts in peripheral blood versus without blasts; [d]Blasts in bone marrow >20% versus = 20%; [e]risk versus intermediate and high risk; [f]Low and intermediate risk versus high risk. CR, complete remission; CRi, Morphologic complete remission with incomplete blood count recovery; AML, acute myeloid leukemia.

BMF group was 21.667 months, 7.800 months in the BMF-1 group, and 3.133 months in the BMF-2/3 group. The 3-year OS rate of AML patients without BMF was 42.8% and that of AML patients with BMF-1 was 22.4%, with BMF-2/3 being 3.3% (Figure 3B).

For AML patients older than 60 years old, the AML with BMF group also had a lower OS rate (3-year OS rate: 0 vs. 0, χ^2 = 8.215, P = 0.004). The median survival time of AML without BMF was 14.700 months and that of AML with BMF was 0.767 months (Figure 3C). The OS of the BMF-1 group and the **B**MF-2/3 group was compared with that of the AML without BMF group for AML patients ≥ 60 years old, and there were statistically significant differences among the three groups (χ^2 = 8.697, P = 0.013). The median survival of the AML without BMF group was 14.700 months, 0.733 months in the BMF-1 group, and 2.167 months in the BMF-2/3 group. All of the 3-year OS rate in BMF subgroups was 0 (Figure 3D).

Survival by different prognostic levels

Next, we performed survival analysis for AML patients with or without BMF at different risk stratifications. For primary AML patients with low risk, the AML with BMF group also had a lower OS rate (3-year OS rate: 39.2% vs. 9.5%, χ^2 = 16.533, P = 0.000). The median survival time of AML without BMF was 24.500 months while that of AML with BMF was 1.933 months (Figure 4A). The OS of the BMF-1 group and the BMF-2/3 group was compared with that of the AML without BMF group for AML patients, and the results showed statistically significant differences among the three groups (χ^2 = 19.709, P = 0.000). The median survival of the AML without BMF group was 24.500 months, 2.133 months in the BMF-1 group, and 1.367 months in the BMF-2/3 group. The 3-year OS rate of AML patients without BMF was 39.2% and that of AML patients with BMF-1 was 16.7%, with BMF-2/3 being 0 (Figure 4B).

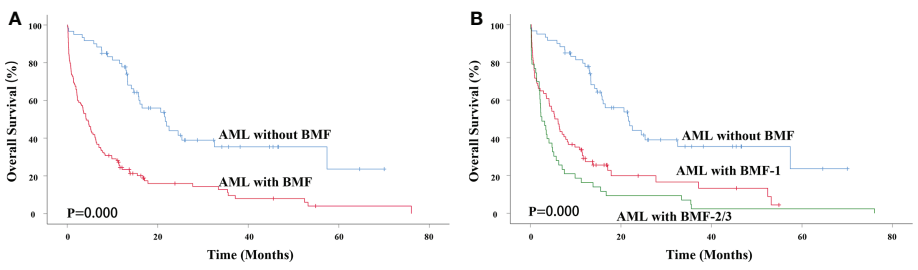

FIGURE 2
Impact of BMF on OS in primary AML patients. **(A)** Kaplan-Meier curves comparing the OS of patients without (blue) or with BMF (red) (21.667 months vs. 4.200 months, P = 0.000). **(B)** Kaplan-Meier curves comparing the OS of patients without (blue), with BMF-1 (red) or BMF-2/3 (green) (21.667 months vs. 5.400 months vs. 2.533 months, P = 0.000). AML, acute myeloid leukemia; BMF, bone marrow fibrosis; OS, overall survival.

TABLE 6 Cox regression analysis for overall survival in newly diagnosed primary AML.

Covariates	Univariate		Multivariate	
	HR (95% CI)	P	HR (95% CI)	P
Age[a]	2.628 (1.824-3.786)	0.000	2.495 (1.708-3.644)	0.000
Gender (male vs. female)	1.050 (0.756-1.460)	0.770		
WBC[b]	0.980 (0.704-1.365)	0.906		
Blasts in PB[c]	1.291 (0.655-2.542)	0.461		
Blasts in BM[d]	0.812 (0.330-2.001)	0.651		
Cytogenetics[e]	1.508 (0.827-2.753)	0.180		
Prognostic level[f]	1.761 (1.261-2.458)	0.001	1.004 (0.694-1.451)	0.984
Bone marrow fibrosis		0.000		0.000
BMF-0 VS BMF-1	2.853 (1.850-4.400)	0.000	2.556 (1.602-4.078)	0.000
BMF-0 VS BMF-2/3	2.053 (1.626-2.591)	0.000	2.203 (1.661-2.924)	0.000
BMF-1 VS BMF-2/3	1.531 (1.029-2.278)	0.036	1.577 (1.051-2.366)	0.028

HR, hazard ratio; CI, confidence interval; [a]Age < 60 years old versus ≥ 60 years old; [b]WBC ≤10×10^9/L versus WBC >10×10^9/L; [c]Blasts in peripheral blood versus without blasts; [d]Blasts in bone marrow >20% versus = 20%; [e]Low risk versus intermediate and high risk; [f]Low and intermediate risk versus high risk; AML, acute myeloid leukemia.

For AML patients with an intermediate risk, the AML with BMF group also had a lower OS rate (3-year OS rate: 33.5% vs. 15.7%, χ^2 = 7.571, P = 0.006). The median survival time of AML without BMF was 21.933 months and that of AML with BMF was 4.100 months (Figure 4C). The OS of the BMF-1 group and the BMF-2/3 group was compared with that of the AML without BMF group, and the results showed statistically significant differences among the three groups (χ^2 = 10.452, P = 0.005). The median survival of the AML without BMF group was 21.933 months, 5.400 months in the BMF-1 group, and 1.933 months in the BMF-2/3 group. The 3-year OS rate of AML patients without BMF was 33.5% while that of AML patients with BMF-1 was 26.3%, with BMF-2/3 being 0 (Figure 4D).

For AML patients with high risk, the AML with MF group also had a lower OS rate (3-year OS rate: 36.4% vs. 7.2%, χ^2 = 5.161, P = 0.023). The median survival time of AML without

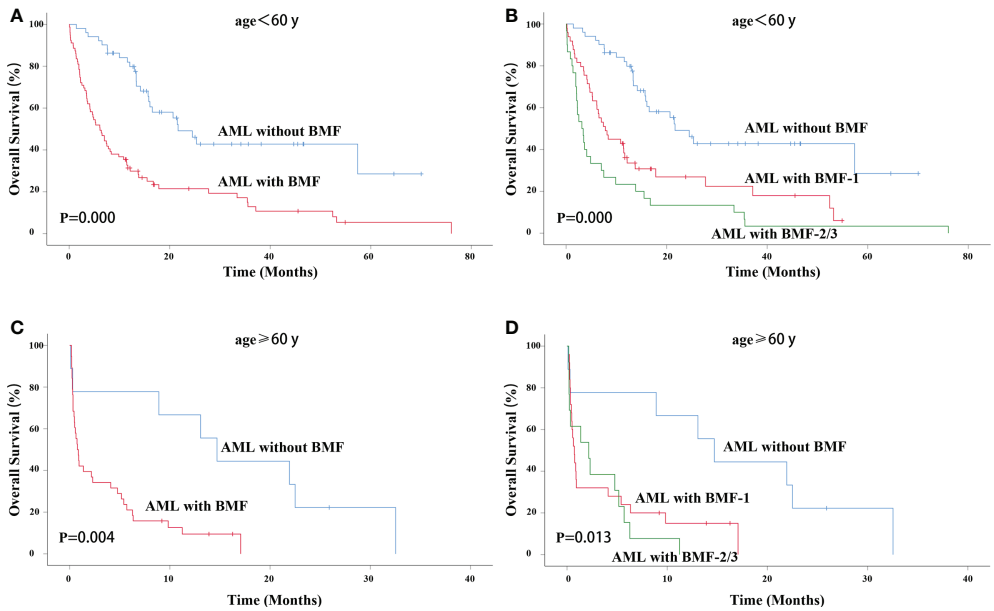

FIGURE 3
OS in different age groups. OS of primary AML patients (age < 60 years old) with or without BMF **(A)** or BMF subgroups **(B)**. OS of primary AML patients (age ≥ 60 years old) with or without BMF **(C)** or BMF subgroups **(D)**. AML, acute myeloid leukemia; BMF, bone marrow fibrosis; OS, overall survival.

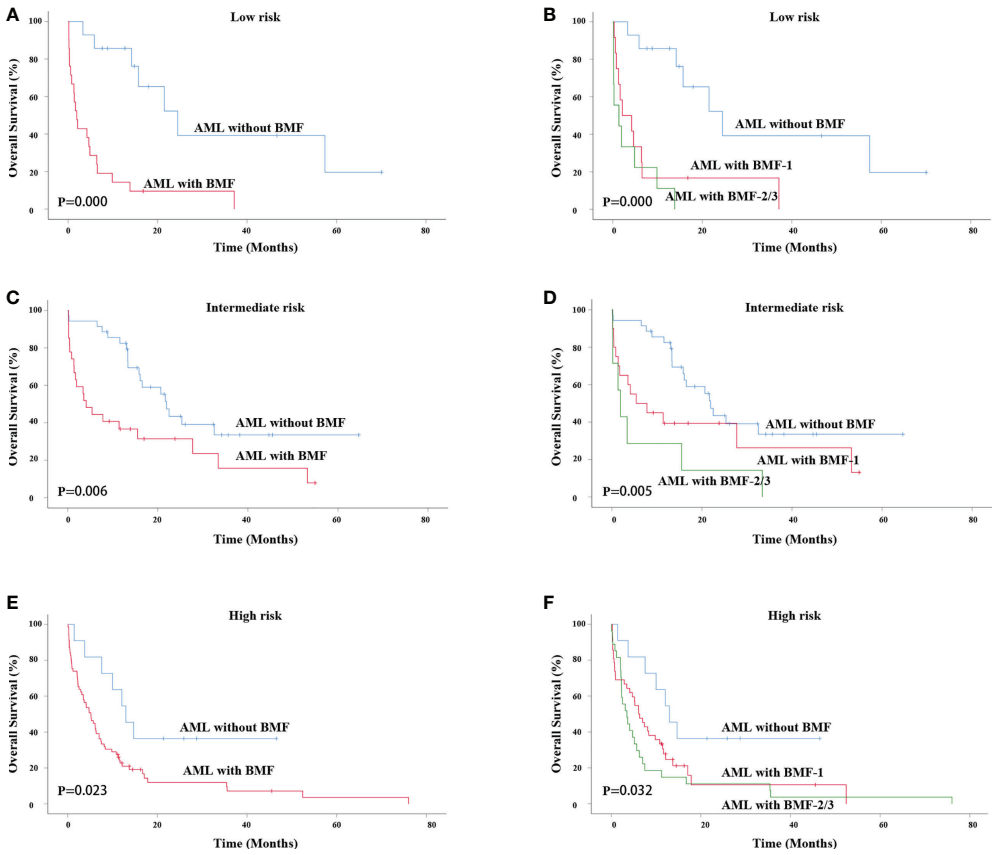

FIGURE 4
Survival analysis of patients with different risk stratifications. OS of primary AML patients (low risk) with or without BMF **(A)** or BMF subgroups **(B)**. OS of primary AML patients (intermediate risk) with or without BMF **(C)** or BMF subgroups **(D)**. OS of primary AML patients (intermediate risk) with or without BMF **(E)** or BMF subgroups **(F)**. AML, acute myeloid leukemia; BMF, bone marrow fibrosis; OS, overall survival.

BMF was 12.967 months while that of AML with BMF was 5.167 months (Figure 4E). The OS of the BMF-1 group and the BMF-2/3 group was compared with that of the AML without BMF group, there were statistically significant differences among the three groups (χ^2 = 6.910, P = 0.032). The median survival of the AML without BMF group was 12.967 months, 6.133 months in the BMF-1 group, and 3.500 months in the BMF-2/3 group. The 3-year OS rate of AML patients without BMF was 36.4%, while that of the AML patients with BMF-1 was 10.6%, with BMF-2/3 being 3.7% (Figure 4F).

Survival by HSCT

However, due to the influence of patient status, family economic status, donor availability and other factors, not all of the AML patients with intermediate and high risk received allogeneic transplant. A total of 8 AML patients with BMF and 17 without BMF underwent transplantation. BMF had no effect on OS in AML patients undergoing HSCT (P = 0.256). The median survival time of AML without BMF was 23.100 months while that of AML with BMF was15.433 months. The 3-year OS rate of AML patients without BMF was 36.4%, while that of AML patients with BMF being 0 (Figure 5).

Discussion

The most frequent type of leukemia associated with the syndrome of bone marrow fibrosis is acute megakaryoblastic leukemia (AMKL) (8, 24, 25), but as has been shown in this study, it is also present in other types of AML. Islam et al. reviewed the clinical features of 34 patients with AML, approximately one-third (12/34) of whom had various degrees of BMF at the time of their diagnosis with AML. In addition, a previous study showed that fibrosis did not affect the regeneration of the hematopoietic system (26). However, another study showed that engraftment was significantly delayed in MDS patients with fibrosis. Overall, bone marrow fibrosis had no significant effect on the OS of MDS patients with

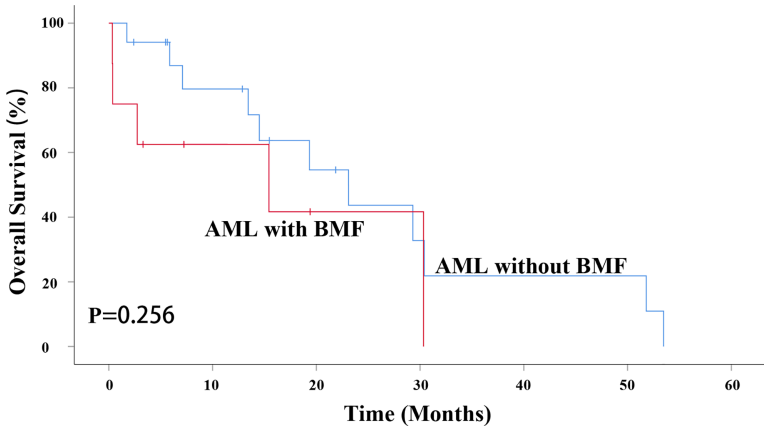

FIGURE 5
BMF had no effect on OS in AML patients undergoing HSCT (P = 0.256). AML, acute myeloid leukemia; BMF, bone marrow fibrosis; OS, overall survival; HSCT, hematopoietic stem cell transplantation.

HSCT with a low International Prognostic Scoring System (IPSS) score, but the OS, relapse-free survival (RFS), and non-relapse mortality (NRM) between MDS patients (int-2 or high-risk disease) with and without fibrosis were inferior (12).

The pathogenesis of AML with BMF remains unclear. It has been suggested that the abnormal proliferation of BMF is a secondary reaction to the clonal proliferation of hematopoietic cells (27). Bone marrow stromal cells consist of endothelial cells, adipocytes, macrophages and reticular cells. The deposition of reticulin and collagen fibrosis in the bone marrow of patients with BMF is mediated by bone marrow fibrosis hematopoietic stem/progenitor cells, resulting in an impaired hematopoietic microenvironment that is conducive to malignant and abnormal hematopoiesis (6). Dilly et al. found that stromal cells such as reticular cells and vascular endothelial cells were increased in both acute and chronic granulocyte tumors, and most granulocyte tumors increased the synergistic stimulation of stromal cells and tumor cells (28). Although the exact mechanism of myelofibrotic progression in AML is unclear, one study suggested that certain factors are released by proliferating megakaryocytes because they are unable to store these factors (platelet-derived growth factor, fibroblast growth factor, platelet factor-4, transforming growth factor-β and beta-thromboglobulin) in defective α particles, which promote the growth of bone marrow fibroblasts (29). Other studies have also confirmed platelet-derived growth factor modified by malignant megakaryocytes and its leakage into the BMM promotes fibroblast activity (30, 31). Collagenase inhibitor, platelet factor 4 (32) and transforming growth factor, which promote collagen synthesis (33), play an important role in the progression of BMF (34). Leukemia cells express specific growth factor proteins, platelet-derived growth factor, transforming growth factor and fibronectin in extramedullary tumors and may selectively regulate tumor formation (34).

There are many studies on AMKL (34–36) but few studies on AML patients with or without BMF. In terms of clinical characteristics, AML patients with BMF tended to be older. AML patients with BMF were more prone to hepatosplenomegaly, which is consistent with previous reports (34, 36). β2-MG was higher in AML patients with BMF than in those without BMF. Newly diagnosed AML patients with BMF often have poor prognosis karyotypes. We compared the two groups of patients according to their different prognostic subgroups. The proportion of AML patients without BMF was high in the intermediate-risk group and low in the high-risk group. There was no significant difference in white blood cell count, hemoglobin and platelet count, LDH, AST, ALT, α1-MG, D dimer, ferritin, blood type and AML diagnosis.

AML patients with epigenetic modification gene ASXL1 mutations, considered an independent predictor of a poor outcome, affect 5-11% of AML patients and are especially common in older, male and secondary AML patients (37–39). A TET2 mutation, an unfavorable prognostic factor in AML patients with intermediate-risk cytogenetics, especially when it is combined with other adverse molecular markers [other than CEBPA (+)], occurred in 13.2% of primary AML patients and was closely associated with older age, intermediate-risk cytogenetics, NPM1 mutation and ASXL1 mutation (40). In our study, ASXL1 and TET2 were present at higher levels in the AML with BMF group, which predicted poor prognosis. Mutations of the FMS-like tyrosine kinase 3 (FLT3) gene appear in approximately 30% of AML cases, which is the only druggable molecular abnormality today that help patients achieve longer and more durable remissions. FLT3 with

internal tandem duplication (FLT3-ITD) is the most common type of FLT3 mutation in AML, which presents with a high leukemic burden and a poor prognosis. While FLT3 mutation in the tyrosine kinase domain (FLT3-TKD) has a lower incidence, and the prognostic value of FLT3-TKD is uncertain (41). Our study showed the mutation frequencies of FLT3-ITD and FLT3-TKD had no statistically significant differences between the two groups. CCAAT/enhancer-binding protein-alpha (CEBPA), a transcription factor, regulates the proliferation and differentiation of myeloid progenitors. One study found that patients with the CEBPA mutation had favorable outcomes in the absence of any other prognostic factors indicating a poor outcome. Systematic analysis of CEBPA mutations, as well as changes in hematopoietic master genes, may be helpful in assessing the prognosis of AML, especially for patients in the "intermediate" prognosis subgroup (42). The mutation frequency of CEBPA in our study was significantly lower in the AML with BMF group. Therefore, we hypothesized that AML patients with BMF had a poor prognosis. However, accurate assessment of prognosis and risk stratification of AML patients requires the consideration of coexisting mutations. A study performed by Papaemmanuil et al. showed that there were interactions among genes, so the commutation-identified groups determined a favorable or adverse prognosis (43). Therefore, the effect of the coexistence of multiple gene mutations should be fully considered when assessing the risk stratification of AML, and the impact of complex genomic changes on clinical prognosis should not be ignored.

The CR/CRi rate and OS of AML patients with different induction therapies were statistically analyzed. Our study showed that there was no significant difference in CR/CRi rate and OS among the IA, DA and CAG groups, which excludes their influence on our study.

Multivariate analysis showed that BMF had independent prognostic significance. AML patients without BMF had a higher CR/CRi rate, and the time of hematopoietic recovery in patients achieving CR/CRi was longer in BMF group. The degree of BMF, prognostic level and blasts in peripheral blood were independent risk factors for CR/CRi in newly diagnosed AML patients. Therefore, early screening of AML patients with BMF, genetic and chromosomal examinations are of great significance for the development of individualized treatment regimens, improvement of clinical efficacy and outcome.

The correlation between BMF and the prognosis in newly diagnosed AML patients is controversial. Manoharan et al. thought that increased marrow reticulin did not change the overall prognosis of acute leukemia and that effective antileukemia therapy could reduce bone marrow reticulin (17). However, another study reported that moderate to marked marrow reticulin in patients with acute leukemia predicted a poor outcome, which was attributed to the persistence of marrow reticulin and possible interference with the normal hematopoietic reconstruction of the bone marrow after chemotherapy (44). Wu et al. confirmed that BMF was an independent risk factor for the survival of AML patients (18). Our research showed that AML patients with BMF had a lower OS rate, especially AML patients with BMF ≥ 2, indicating that BMF was an independent prognostic factor affecting the OS of AML patients. These results suggest that AML and BMF jointly affect the prognosis of AML patients with BMF.

In our study, Cox multivariate analysis showed that age had independent prognostic significance for the OS of primary AML patients. Therefore, AML with or without BMF was divided into two groups aged less than 60 years and greater than or equal to 60 years. AML with the BMF group had a lower OS rate, regardless of age < 60 or ≥ 60 years old. However, in AML patients younger than 60 years old, the higher the degree of BMF was, the shorter the median survival time and the lower the OS rate. In the age ≥ 60 group, the median survival time in the BMF-1 and the BMF-2/3 groups was shorter.

In addition, survival analysis by different risk stratifications was performed. For primary AML patients with low, intermediate and high risk, there was always a lower OS rate in patients with BMF. The median survival of AML patients decreased with an increasing degree of fibrosis in different risk stratifications, which suggested that the conventional chemotherapy regimen could not effectively improve the OS of AML patients with BMF. Therefore, we need to optimize the chemotherapy regimen to improve the survival time of AML patients with BMF.

However, bone marrow biopsy was not a routine examination for newly diagnosed AML. In addition, bone marrow biopsy was performed only when the presence of BMF was considered at the initial diagnosis of AML in the past. Therefore, the data collected included more patients with BMF than those without BMF. In addition, because our study was a retrospective and monocentric study, the conclusions may be biased. In the future, we will conduct bone marrow biopsy for each newly diagnosed patient to further expand the sample size and further verify our conclusions.

Conclusion

In conclusion, our study showed that AML patients with BMF have a poor prognosis. We found that BMF and age were independent prognostic factors affecting the OS of AML patients. Hence, bone marrow biopsy should be a routine examination during the diagnosis of AML. More studies are needed to confirm that BMF could be used as an important predictor of risk stratification in AML patients. Further research on the pathophysiological mechanism of bone marrow is of great significance for determining the prognostic risk stratification of AML patients with BMF, developing appropriate chemotherapy regimens and improving the clinical efficacy of 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.

Ethics statement

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

XZ and ZJ conceived and designed the experiments. JY, XZ, and FW collected clinical samples and data. XZ wrote the manuscript. All authors contributed to the article and approved the submitted version.

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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

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Comparison of characteristics and outcomes on ETP-ALL/LBL and non-ETP ALL patients receiving allogeneic hematopoietic stem cell transplantation

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Objective: This study aims to compare the characteristics of early T-cell precursor acute lymphoblastic leukemia/lymphoma (ETP-ALL/LBL) and non-ETP ALL patients and the outcomes of these patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT).

Method: A total of 57 patients with T-cell acute lymphoblastic leukemia/lymphoma receiving allo-HSCT at our center between January 2016 and March 2022 were enrolled in the study. Twenty-eight patients were diagnosed as ETP-ALL/LBL (28/57, 49.12%) in the cohort.

Results: The baseline characteristic was not significantly different between the two groups. The median time for myeloid engraftment was 14 days (ranged from 11 to 21) *versus* 14 days (ranged from 10 to 20) (P = 0.067) and 18 days (ranged from 12 to 27) *versus* 15.5 days (ranged from 12 to 72) (P = 0.183) for platelet engraftment in the ETP-ALL/LBL and non-ETP ALL groups, respectively. There was no significant difference in 5-year overall survival (54.74% ± 10.33% *vs*. 64.20% ± 10.30%, P = 0.786), relapse-free survival (56.22% ± 10.11% *vs*. 57.17% ± 12.71%, P = 0.841), cumulative incidence of relapse (30.14% ± 9.85% *vs*. 22.79% ± 8.24%, P = 0.774), and non-relapse mortality (19.52% ± 8.99% *vs*. 25.95% ± 14.44%, P = 0.967) between the two groups. The incidence of acute graft *versus* host disease (aGVHD) (P = 0.922),

II–IV aGVHD (P = 0.940), III–IV aGVHD (P = 0.664), cytomegalovirus infection (P = 0.862), Epstein–Barr virus infection (P = 0.610), and severe bacterial infection (P = 0.145) was also similar.

Conclusion: The prognosis of patients with ETP-ALL/LBL was similar to non-ETP ALL patients when they received allo-HSCT.

KEYWORDS

early T-cell precursors, acute lymphoblastic leukemia, allogeneic hematopoietic stem cell transplantation, characteristics, prognosis

Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy which accounts for 15% and 25% of childhood and adult ALL cases, respectively (1). Early T-cell precursor lymphoblastic leukemia/lymphoma (ETP-ALL/LBL) is a special subtype of T-ALL first recognized in 2009 (2), which is characterized by arrested early T-cell differentiation, with some myeloid and stem cell characteristics remaining at the immunophenotypic and also genetic levels (3, 4). The incidence of ETP-ALL reported in previous studies is 11%–16% of T-ALL cases in children and 7.4%–32% in adults (5–7), respectively. In a large cohort study in Chinese adult T-ALL (n = 112), ETP-ALL accounts for 47.3% of all patients (8). Some studies suggested that the prognosis of ETP-ALL/LBL was worse than that of typical T-ALL (2, 5, 9–11). However, other studies have found that the prognosis of ETP-ALL and non-ETP was not significantly different (8, 12–14).

Although many efforts have been made to uncover the genetic aberrations and molecular pathogenesis of ETP-ALL (15–18), the management of ETP-ALL is still challenging. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an important potentially curative treatment for ETP-ALL/LBL. In this study, we aim to assess the efficacy of allo-HSCT on ETP-ALL/LBL patients and compare the outcomes of ETP-ALL/LBL and non-ETP patients.

Methods

Patients and definitions

We retrospectively analyzed the data of 57 patients who received allo-HSCT in our center from January 2016 to March 2022. The final date of follow-up was June 30, 2022 for patients without events. Of the 57 patients, 28 were diagnosed as ETP-ALL/LBL (one patient was diagnosed as ETP-LBL) according to the diagnosis criteria. ETP was diagnosed by the immunophenotype of the positive expression of CD7 but lack of CD1a and CD8, weak expression of CD5 (with <75% positive blasts), and positive expression of one or more stem cell or myeloid markers including CD117, HLA-DR, CD13, CD33, CD11b, or CD65 (3). The initial induction chemotherapy was VDCLP or Hyper-CVAD. After complete remission, we conducted three to six courses of consolidation chemotherapy before allo-HSCT. Minimal residual disease (MRD) analysis was detected by flow cytometry, and MRD <0.01% ($1 * 10^{-4}$) of nucleated cells was defined as negative. All patients and donors provided written informed consent for this protocol. For patients younger than 18 years old in the cohort, the consent was carried out by their parents. This study was approved by the Ethics Review Committee of the Institute of Hematology, Chinese Academy of Medical Science and Peking Union Medical College, and was in compliance with the Declaration of Helsinki.

Treatment

All the patients received a myeloablative conditioning regimen before allo-HSCT, including total body irradiation/Cy-based regime [(3.33 Gy, -9 to -7 days) + Cy (cyclophosphamide) (40 mg/kg/day, -6 to -5 days) + Ara-c (cytarabine) (2 g/m^2/day, -4 to -2 days) + Flu (fludarabine) (30 mg/m^2/day, -4 to -2 days)] and Bu/Cy-based regime [Bu (busulfan) (3.2 mg/kg/day, -6 to -4 days) + CTX (cyclophosphamide) (40 mg/kg/day, -6 to -5 days) + VP-16 (etoposide) (20 mg/kg/day, -9 to -7 days). For patients who received grafts from HLA-haploidentical related donor and unrelated donor, additional anti-thymocyte globulin/anti-lymphocyte globulin (anti-thymocyte globulin 2.5 mg/kg/day, -5 to -2 days/anti-lymphocyte 20 mg/kg/day, -4 to -2 days) was added in the conditioning regimen.

Graft *versus* host disease (GVHD) prophylaxis and supportive care were as described previously (19).

Criteria of outcomes

Engraftment was defined as absolute neutrophil counts (ANC) ≥0.5 × 10^9/L for 3 consecutive days and platelet count ≥20 × 10^9/L without transfusion for seven consecutive days. The Mount Sinai Acute GVHD International Consortium criteria were used to diagnose and grade acute GVHD (aGVHD) (20). Cytomegalovirus (CMV) and Epstein–Barr virus (EBV) viremia was defined as before (19). Severe bacterial infection referred to bacteremia or severe tissue infections. Complete remission (CR) referred to no blasts in blood, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, <5% bone marrow blasts, and no extramedullary leukemia. Overall survival (OS) was calculated from HSCT to death of any cause or last follow-up. Relapse-free survival (RFS) was defined as the time from HSCT to relapse, censoring at death or last follow-up. Cumulative incidence of relapse (CIR) was defined as relapse after HSCT.

Statistical analysis

The data were analyzed by the software GraphPad Prism 8 (version 8, supplied by GraphPad Software, Inc.) and IBM SPSS statistics 25 (version 25, supplied by IBM). The descriptive statistics for continuous variables and chi-square test and Fisher's exact test for categorical variables were used to compare incidence in univariate analysis. The Kaplan–Meier method was used to estimate the cumulative survival/incidence, and differences were compared by the log-rank/Wilcoxon test. A two-sided *P*-value <0.05 was considered as statistically significant.

Results

Characteristics of patients

There are 28 and 29 patients in the ETP group and non-ETP group, respectively. The baseline characteristics of patients in the two groups are listed in Table 1. Gender, age, WBC/HB/PLT at diagnosis, BM blast, chromosome karyotype, and interval from diagnosis to HSCT did not differ among the two groups. There are 26 and 27 patients in the ETP group and non-ETP group who underwent next-generation sequencing. The top mutated gene in both groups was NOTCH1 (12/26, 46.2% in the ETP group and 15/27, 55.6% in the non-ETP group), followed by NRAS, JAK3, WT1, EZH2 in the ETP group and FBXW7, NRAS, DNMT3A, and PHF6 in the non-ETP group (Figure 1).

Transplantation details

The transplantation-associated details including donor type, chemotherapy before CR, MRD status before transplantation, GVHD prophylaxis, and dose of MNC and $CD34^+$ cells between

TABLE 1 Characteristics of patients.

Characteristics	ETP (*N* = 28)	Non-ETP (*N* = 29)	*P*-value
Gender			
Male	24 (85.7%)	19 (65.5%)	0.077
Female	4 (14.3%)	10 (34.5%)	
Age (years)			
Median (range)	26 (16–48)	22 (11–56)	0.570
WBC (×10^9/L) at diagnosis			
Median (range)	26.00 (1.48–305.64)	51.93 (2.14–461.63)	0.158
HB (g/L) at diagnosis			
Median (range)	105.5 (53.0–165.0)	114.5 (64.2–161.0)	0.157
PLT (×10^9/L) at diagnosis			
Median (range)	80.0 (25.0–327.0)	53.5 (10.0–270.0)	0.173
BM blast (%)			
Median (range)	87.00 (6.02–96.31)	78.20 (40.00–93.61)	0.866
Chromosome karyotype			
Normal	14 (50.0%)	19 (65.5%)	0.308
Abnormal	11 (39. 3%)	6 (20.7%)	
Unknown	3 (10.7%)	4 (13.8%)	
Interval from diagnosis to hematopoietic stem cell transplantation (days)			
Median (range)	218 (116–380)	209 (48–352)	0.297

ETP, early T-cell precursor; WBC, white blood cell; HB, hemoglobin; PLT, platelet; BM, bone marrow.

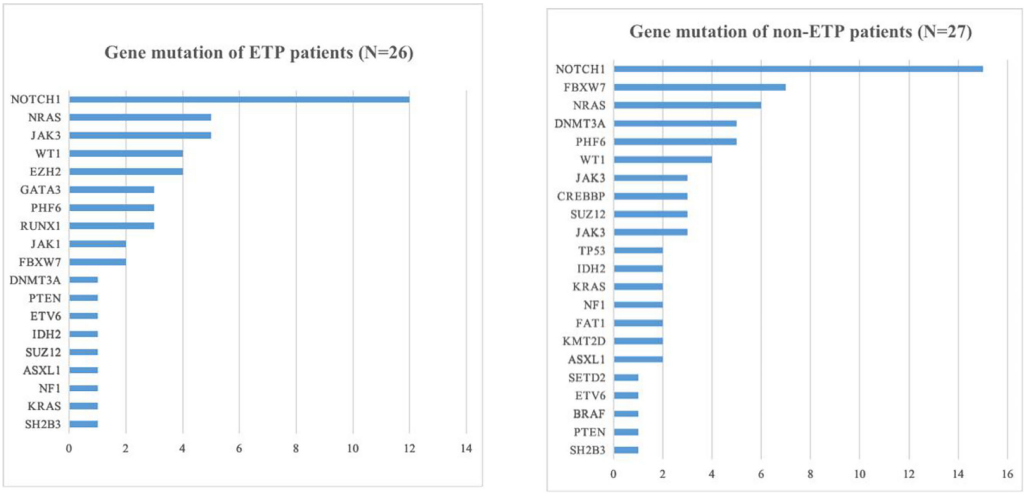

FIGURE 1
Gene mutation of early T-cell precursor (ETP) and non-ETP patients.

the two groups were similar (Table 2). The median dose of infused MNC and $CD34^+$ cells in the ETP group was 10.83 × 10^8/kg (range, 6.76–21.10) and 3.05 × 10^6/kg (range, 1.56–5.90), which was not significantly different from the non-ETP group [MNC: 11.79 × 10^8/kg (range, 7.00–23.84) and $CD34^+$ cells: 3.00 × 10^6/kg (range, 2.00–9.48)]. Moreover, there was one patient in each group who received additional cord blood infusion due to insufficient infused cell dose.

Engraftment

All patients had ANC engraftment, whereas 25 patients (89.3%) in the ETP group and 28 patients (96.6%) in the non-ETP group had platelet engraftment in 100 days post-transplantation. The median time of ANC recovery in the ETP group and non-ETP group was 14 days (ranged from 11 to 21) and 14 days (ranged from 10 to 20), respectively. For platelet

TABLE 2 Transplantation details.

	ETP (*N* = 28)	Non-ETP (*N* = 29)	*P*-value
Donor type			
MSD	10 (35.7%)	11 (37.9%)	0.104
HRD	14 (50.0%)	18 (62.1%)	
MUD	4 (14.3%)	0 (0.0%)	
Cycles of chemotherapy before CR			
1	18 (64.3%)	23 (79.3%)	0.207
≥2	10 (35.7%)	6 (20.7%)	
MRD status before transplantation			
Positive	7 (25.0%)	6 (20.7%)	0.698
Negative	21 (75.0%)	23 (79.3%)	
GVHD prophylaxis			
CSA	9 (32.1%)	13 (44.8%)	0.325
FK506	19 (67.9%)	16 (55.2%)	
Dose of MNC (*10^8/kg)			
Median (range)	10.83 (6.76–21.10)	11.79 (7.00–23.84)	0.260
Dose of CD34+ cells (*10^6/kg)			
Median (range)	3.05 (1.56–5.90)	3.00 (2.00–9.48)	0.503

ETP, early T-cell precursor; MSD, matched sibling donor; HRD, HLA-haploidentical related donor; MUD, matched unrelated donor; CR complete remission; MRD, minimal residual disease; GVHD, graft versus host disease; MNC, mononuclear cell.

recovery, the median time was 18 days (ranged from 12 to 27) and 15.5 days (ranged from 12 to 72), respectively.

Infection and GVHD

The incidence of CMV viremia and EBV viremia was not significantly different in the ETP group and the non-ETP group (64.3% *vs*. 62.1%, P = 0.862; 10.7% *vs*. 6.9%, P = 0.610, respectively). In total, 14 patients in the ETP group and nine patients in the non-ETP group developed severe infection (50.0% *vs*. 31.0%, P = 0.431). The incidence of I–IV, II–IV, and III–IV aGVHD was similar in the two groups (P = 0.922; P = 0.940; P = 0.664).

Deaths and survival

The median time from HSCT to death or last follow-up was 424 days (ranged from 46 to 1841). The estimated 5-year OS of the total cohort was 55.40% ± 7.90% (Figure 2A). Until the last follow-up, there were 11 patients who died in the ETP group, seven had a relapse, two had infection or aGVHD, and one had graft failure. A total of 10 patients died in the non-ETP group, six had a relapse and four had infection or aGVHD. In the ETP group, at a median follow-up of 435 days (ranges from 93 to 1,841), 17 patients survived, and the 5-year OS was 54.74% ± 10.33%. In the non-ETP group, at a median follow-up of 419 days (ranged from 46 to 1,434), 19 patients survived, and the 5-year OS was 64.20% ± 10.30%. There was no significant difference in terms of the 5-year OS between the two groups (P = 0.786), and so were the 5-year RFS, CIR, and non-relapse mortality (NRM) (P = 0.841; P = 0.774; P = 0.697) (Table 3 and Figures 2B–E). Moreover, we compared the survival of MRD-positive and MRD-negative patients. Patients who were MRD-negative before transplantation had a higher 5-year OS than the MRD-positive patients (59.79% ± 9.04% *vs*. 43.08% ± 14.67%, P = 0.048) (Figure 3).

Discussion

ETP-ALL/LBL was first recognized in 2009 and defined by World Health Organization classification 2016 version as a distinct subtype of ALL due to its unique immunophenotypic and genomic profile (2, 3). Since then, many clinicians and researchers started to pay attention to the subtype. ETP-ALL is characterized by early differentiation arrest and distinct genetic and transcriptional features and thought to be a high-risk subgroup of ALL. ETP-ALL tends to be resistant to chemotherapy, and novel agents such as BCL-2 inhibitors have shown a good response for this disease. Another important and potentially curative treatment is allo-HSCT (21, 22). In this study, we summarized the characteristics of 57 T-ALL patients who received allo-HSCT at our center and compared the heterogeneity between ETP and non-ETP.

The percentage of ETP-ALL/LBL was 49.12% (28/57) in the cohort, which was higher than those in some international studies (16%–32%) (5, 12, 14, 23) but comparable with Chinese data (8, 24) (47.3%–47.6%). This may be caused by ethnic differences and may be partially due to ETP-ALL/LBL patients being more inclined to undergo allo-HSCT as a high-risk subtype.

The majority of ETP-ALL/LBL patients was male, which was consistent with previous studies (7, 8, 17, 24, 25). WBC at diagnosis was reported to be lower in ETP patients than in non-ETP patients, while the platelet count was higher (8, 14, 26). In our study, there was a trend of lower WBC and higher platelet count in ETP patients compared with non-ETP patients, but it was not significantly different [26.00 × 10^9/L (1.48–305.64) *vs*. 51.93 × 10^9/L (2.14–461.63), P = 0.158; 80.0 × 10^9/L (25.0–327.0) *vs*. 53.5 × 10^9/L (10.0–270.0), P = 0.173; respectively]. The top mutated gene in the ETP group was NOTCH1 (12/26, 46.2%), followed by NRAS, JAK3, WT1, and EZH2, while in the non-ETP group, the top five mutated genes were NOTCH1, FBXW7, NRAS, DNMT3A, and PHF6. The top mutated genes were mainly related to cytokine and RAS signaling.

A study conducted by St. Jude Children's Research Hospital demonstrated that, with standard intensive chemotherapy, the 10-year overall survival for patients with ETP-ALL was significantly lower than for the non-ETP patients (19% *vs*. 84%, P < 0.0001) (2). Other two studies by MD Anderson Cancer Center and Pediatric Blood Diseases Center in our hospital also indicated inferior prognosis of ETP-ALL (5, 26). However, the Group for Research on Adult Acute Lymphoblastic Leukemia—2003 and —2005 studies showed that the 5-year overall survival for patients with ETP-ALL was not inferior to that of the non-ETP-ALL group (59.6%, 95% CI: 44.2% to 72.0% *vs*. 66.5%, 95% CI: 58.7% to 73.2%; P = 0.33) (14). A recent study in Chinese ALL patients also suggested a similar 2-year overall survival between the ETP and non-ETP patients (40.7% ± 8.2% *vs*. 37.9% ± 7.0%, P > 0.05) (8). A proportion of patients in the latter two studies received allo-HSCT other than chemotherapy alone, indicating that allo-HSCT could overcome the poor prognosis of ETP patients. In this study, we focused on ALL patients undergoing allo-HSCT and found that the 5-year OS, RFS, CIR, and NRM were not significantly different between the ETP and non-ETP patients (54.74% ± 10.33% *vs*. 64.20% ± 10.30%, hazard ratio (HR): 1.125, P = 0.786; 56.22% ± 10.11% *vs*. 57.17% ± 12.71%, HR: 1.091, P = 0.841; 30.14% ± 9.85% *vs*. 22.79% ± 8.24%, HR: 1.173, P =

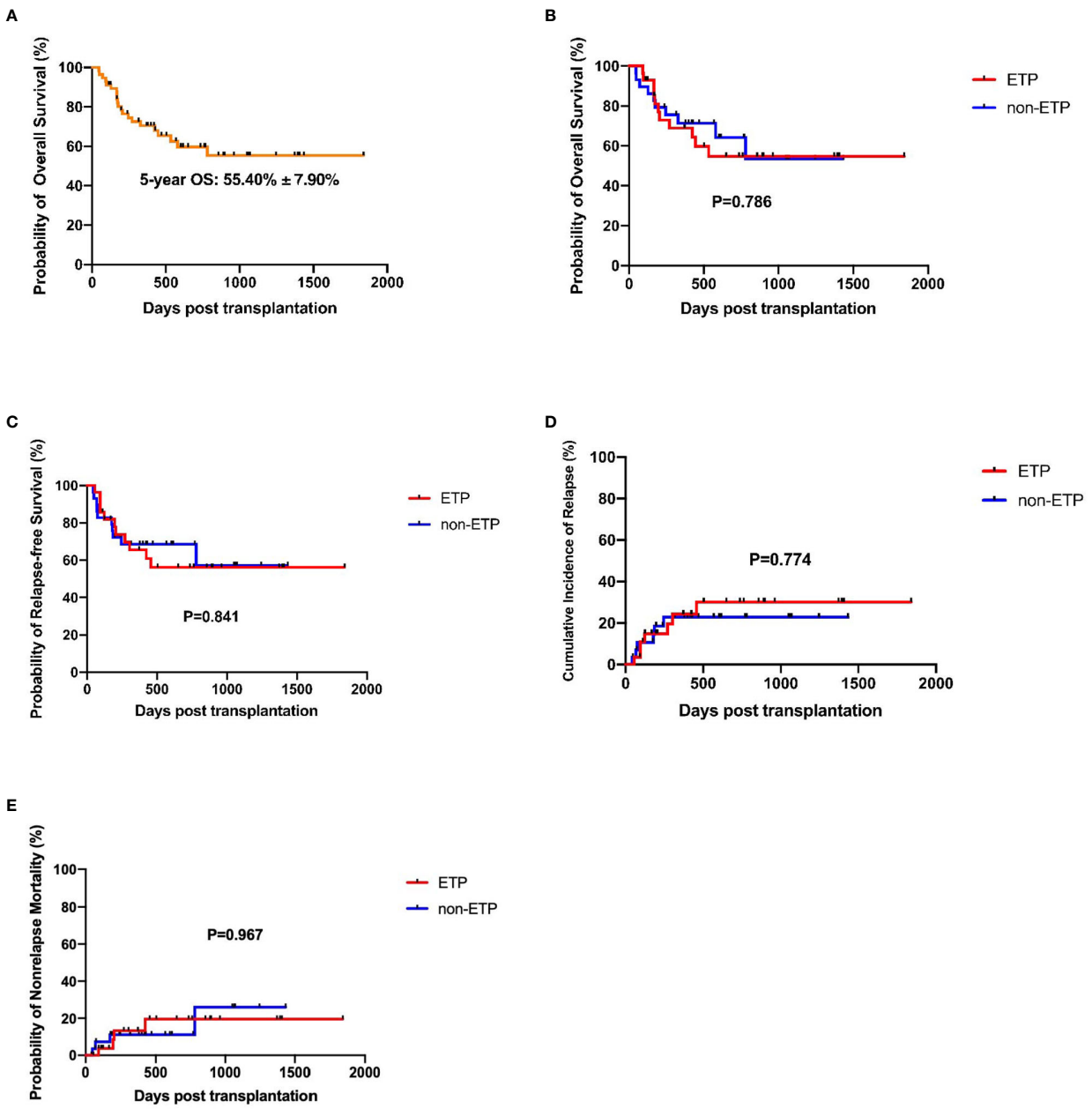

FIGURE 2
Survival analysis of early T-cell precursor (ETP) and non-ETP patients. **(A)** Overall survival of the total cohort; **(B)** Overall survival of ETP and non-ETP patients; **(C)** Relapse-free survival of ETP and non-ETP patients; **(D)** Cumulative incidence of relapse of ETP and non-ETP patients; **(E)** Non-relapse mortality of ETP and non-ETP patients.

0.774; 19.52% ± 8.99% *vs.* 25.95% ± 14.44%, HR: 0.971, P = 0.967). The survival of ETP-ALL/LBL patients was similar with or superior to other studies. Moreover, the OS in our study was calculated from HSCT, while in most of the previous studies it was calculated from diagnosis. Thus, our survival data was better than that of the previous studies as the median interval from diagnosis to HSCT was approximately 200 days, suggesting that allo-HSCT was an effective treatment for these patients and should be considered. However, due to the retrospective origin and small sample size, future prospective, large-scaled clinical trials are needed to investigate and confirm the results.

TABLE 3 Outcomes of patients.

	ETP (N = 28)	Non-ETP (N = 29)	P-value
Time of engraftment			
Absolute neutrophil count, days (range)	14 (11–21)	14 (10–20)	0.067
Platelet, days (range)	18 (12–27)	15.5 (12–72)	0.183
Infection			
	CMV		
Yes	18 (64.3%)	18 (62.1%)	0.862
No	10 (35.7%)	11 (37.9%)	
	EBV		
Yes	3 (10.7%)	2 (6.9%)	0.610
No	25 (89.3%)	27 (93.1%)	
	Severe bacterial infection		
Yes	14 (50.0%)	9 (31.0%)	0.145
No	14 (50.0%)	20 (69.0%)	
aGVHD			
I–IV	10 (35.7%)	10 (34.5%)	0.922
II–IV	7 (25.0%)	7 (24.1%)	0.940
III–IV	5 (17.9%)	3 (10.3%)	0.664
5-year OS, %	54.74 ± 10.33	64.20 ± 10.30	0.786
5-year RFS, %	56.22 ± 10.11	57.17 ± 12.71	0.841
5-year CIR, %	30.14 ± 9.85	22.79 ± 8.24	0.774

ETP, early T-cell precursor; CMV, cytomegalovirus; EBV, Epstein–Barr virus; aGVHD, acute graft versus host disease; OS, overall survival; RFS, relapse-free survival; CIR, cumulative incidence of relapse.

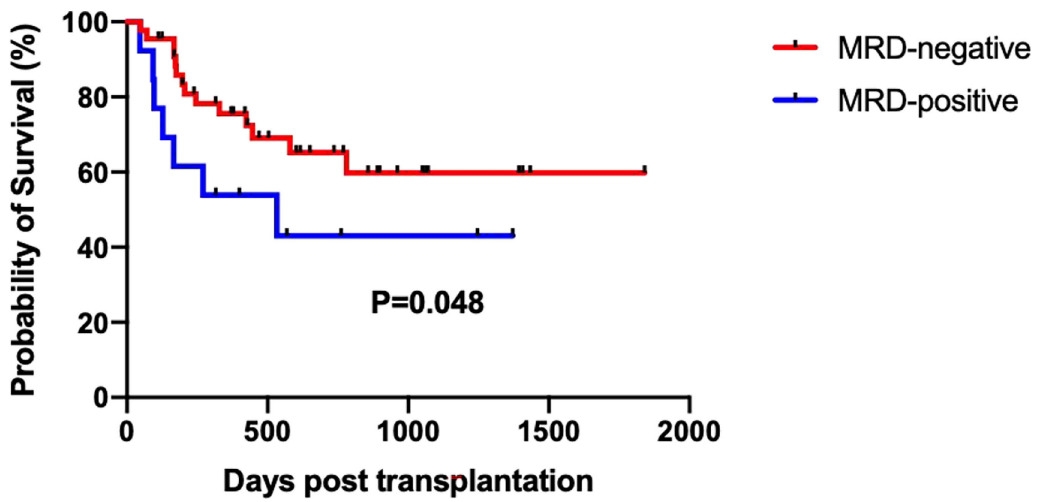

FIGURE 3
Survival of minimal residual disease (MRD)-positive and MRD-negative patients.

Furthermore, MRD status was associated with the prognosis reported by many studies (7, 12, 27). In the study, we also compared the survival of MRD-positive and MRD-negative patients and found that the 5-year OS was significantly lower in the MRD-positive patients (43.08% ± 14.67% *vs.* 59.79% ± 9.04%, P = 0.048).

In conclusion, in the setting of allo-HSCT, ETP-ALL/LBL and non-ETP patients could achieve similar survival. Moreover, MRD-negativity before transplantation was associated with better prognosis. Allo-HSCT should be considered for ETP patients and novel treatment strategies (such BCL-2 inhibitors, *etc.*) to eliminate MRD before transplantation could further improve the efficacy.

Data availability statement

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

Ethics statement

This study was approved by the Ethics Review Committee of the Institute of Hematology, Chinese Academy of Medical Science and Peking Union Medical College and was in compliance with the Declaration of Helsinki. All patients and donors provided written informed consent for this protocol. For patients younger than 18 years old in the cohort, the consent was carried out by their parents.

Author contributions

SF conceived and designed the study. JC analyzed the data and drafted the manuscript. SF secured financing of the study. LL, RM, AP, DY, XC, JW, YH, RZ, WZ, QM, EJ, and MH contributed to the review and editing. All authors contributed to the article and approved the submitted version.

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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

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Case report: Ponatinib as a bridge to CAR-T cells and subsequent maintenance in a patient with relapsed/refractory Philadelphia-like acute lymphoblastic leukemia

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Philadelphia (Ph)-like acute lymphoblastic leukemia (ALL) constitutes a heterogeneous subset of ALL with a uniformly unfavorable prognosis. The identification of mutations amenable to treatment with tyrosine kinase-inhibitors (TKIs) represents a promising field of investigation. We report the case of a young patient affected by relapsed/refractory Ph-like ALL treated with chimeric antigen receptor T (CAR-T) cells after successful bridging with compassionate-use ponatinib and low-dose prednisone. We restarted low-dose ponatinib maintenance three months later. Twenty months later, measurable residual disease negativity and B-cell aplasia persist. To the best of our knowledge, this is the first case reporting the use of ponatinib in Ph-like ALL as a bridge to and maintenance after CAR-T cell therapy.

KEYWORDS

ponatinib, CAR-T, Philadelphia-like ALL, bridge therapy, maintenance therapy

Introduction

Ph-like ALL, a subtype of B-cell ALL with adverse clinical features and unfavorable prognosis (1, 2), represents up to 15% of childhood ALL and 15-25% of adolescent and young adult ALL (3). They exhibit higher rates of measurable residual disease (MRD) persistence, higher rates of relapse, even in case of a MRD clearance, and a shorter survival compared to patients with non-Ph-like ALL (4). Patients with Ph-like ALL are often young males with hyperleukocytosis and a normal karyotype (5).

The pathophysiology of Ph-like ALL accounts for a plethora of kinase-activating mutations, affecting in up to 90% of cases either the tyrosine kinase super-family (*ABL1, ABL2, CSF1R* and *PDGFRB*) or the cytokine receptor pathway (*JAK1, JAK2, IL7-R, CRLF2, EPOR*), but lacking a classical *BCR::ABL1* rearrangement. Ph-like and Ph-positive ALL have a partly overlapping gene expression profile and are both often associated with deletions or mutations of the transcription factor *IKZF1* (6, 7).

The identification of actionable lesions in Ph-like ALL has paved the way towards targeted therapies (8). The efficacy of TKIs in Ph-like ALL has already been established (9, 10). In addition, other small molecule inhibitors, such as ruxolitinib, sirolimus and gedatolisib, have shown promising results in pre-clinical models of *JAK2*-mutated subtypes and are under evaluation (11). Moreover, the introduction of immunotherapy and CAR-T cells in the clinical practice may represent a valuable option to impact on the negative prognosis harboured by the Ph-like signature. As the treatment paradigm in ALL is undergoing a major shift, new efforts are warranted to define the proper place for each drug within the therapeutic algorithm for different subgroups of patients.

Case presentation

We report the case of a 19-year-old female diagnosed with Ph-negative B-cell ALL in February 2019, who presented with hyperleukocytosis (WBC count 317 x 10^9/L). Flow cytometry on a bone marrow (BM) aspirate showed that 93% of cells were positive for CD45, CD10, CD19 and CD22, and had an aberrant expression of CD33. FISH performed using the Cytocel probe detected a deletion at 6q21/SEC63 in 43.5% of the analysed nuclei. The *BCR::ABL1*-like predictor was positive and showed a *CRLF2* upregulation and an *IKZF1* deletion (7). Molecular-cytogenetic analysis, performed using the ZytoLight® SPEC *CRLF2* Dual Color Break Apart probe, and the LSI *IGH* Dual Color, Break Apart Rearrangement Probe (Vysis-Abbott) showed hybridization patterns consistent with the presence of an *IGH : CRLF2* rearrangement. Targeted RNA sequencing detected no mutations or rearrangements.

The patient was enrolled in the chemo-immunotherapy GIMEMA LAL2317 protocol, which exploits a risk-oriented strategy based on disease characteristics and MRD evaluation at fixed time-points, intercalating a maximum of two cycles of blinatumomab into a pediatric-like chemotherapy backbone (clinicaltrial.gov NCT03367299).

Our patient, classified as very high risk, underwent three cycles of chemotherapy, obtaining a complete morphologic remission (CR) after induction. Central nervous system (CNS) prophylaxis was carried out as per protocol. MRD assessment by RQ-PCR Ig gene rearrangement remained strongly positive (>10^{-2}) after all three chemotherapy cycles. After a single cycle of blinatumomab which induced the molecular remission (<10^{-5}), the patient underwent an allogeneic hematopoietic stem cell transplant (HSCT) from an HLA-identical sister. Conditioning consisted of treosulfan, fludarabine and TBI 4 Gy, and the graft versus host disease (GvHD) prophylaxis included post-transplantation cyclophosphamide and sirolimus (12). A post-transplant aspirate documented a CR with full donor chimerism, FISH and a molecular MRD negativity. Sirolimus was discontinued six months later. The patient never developed GvHD.

In January 2021, 18 months after the HSCT, a BM evaluation detected a relapse (5% blasts). The patient had also a palpable mass in her right breast, whose histology was compatible with an ALL localization. No CNS disease was detected.

The patient was deemed fit for anti-CD19 CAR-T cell therapy with tisagenlecleucel. After lymphapheresis in early February, we started a bridging treatment with ponatinib 45 mg daily for 30 days on compassionate use and 1 mg/kg prednisone for 14 days. No cardiac, hepatic or hematologic toxicity was reported. In mid-February, we repeated a BM aspirate that confirmed a morphologic relapse (12% blasts). A third aspirate after a month of ponatinib showed a stable disease (8% blasts) and on physical examination a reduction of the palpable breast nodule was documented. We withdrew bridging drugs and started lymphodepletion with fludarabine-cyclophosphamide, followed by a CAR-T cell infusion on March 2021. The patient developed a grade 4 neutropenia and received three doses of tocilizumab for grade 1 cytokine release syndrome (CRS). No neurotoxicity occurred. Three months later the patient was in CR with a full donor chimerism and a MRD negativity. A breast ultrasound revealed a regression of her nodule. In June 2021, maintenance with ponatinib at a lower dose (15 mg/day) was initiated. In November 2022, 20 months after the CAR-T cell infusion the patient is in good clinical conditions and in persistent molecular CR. She still receives ponatinib maintenance with excellent tolerance, except for a 10-day discontinuation due to a transient G4 neutropenia. Figure 1 summarizes the case timeline. We longitudinally monitored the patient's CAR-T cell expansion and their subsequent persistence by flow cytometry and plotted the data over time in Figure 2A. After a marked expansion peak at day 7 after infusion (1819.6/mcl), CAR-T cell counts decreased, though persisting over time. At late time-points (day 180 and 270), the patient still had circulating anti-CD19 CAR-T cells (4.0 and 4.3/mcl, respectively). At the last available follow-up (365 days, April 2022),

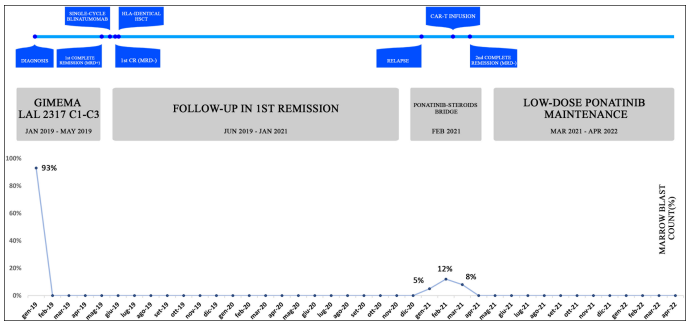

FIGURE 1
Case report timeline. The table offers an overview of the patient's history, treatment lines **(A)** and corresponding marrow blast count **(B)**. The percentage of bone marrow blasts was evaluated at significant time-points, namely at diagnosis, post-induction, post-transplant, at disease relapse, during bridging and after CAR-T infusion. At the last follow-up, the patient is still in molecular remission and in persistent B-cell aplasia.

circulating CAR-T cells were no longer detectable. However, a concomitant B-cell aplasia is ongoing and we are monitoring it as a decisional tool for future treatment. Peak levels of inflammatory cytokines and chemokines occurred within the first week after the CAR T-cell infusion, with particularly high levels of monocyte chemoattractant protein-1 (MCP-1), interferon γ-induced protein 10 kDa (IP-10), interleukin-6 (IL-6) and interferon-γ (IFN-γ, Figure 2B).

Discussion

The present case illustrates how ponatinib might represent a valid therapeutic option to be explored in Ph-like ALL. Even though TKIs have to date no standardized place, it seems reasonable to incorporate them in treatment schemes given their safety and potential effectiveness. The patient was initially enrolled into a sequential chemo-immunotherapy protocol (clinicaltrial.gov NCT03367299) and obtained a MRD negativity only after a cycle of blinatumomab, suggesting a possible role of this drug in Ph-like patients, whose long-term efficacy is still debatable.

Upon relapse, anti-CD19 CAR-T cells were considered the best salvage option. Considering the fast disease kinetics, the risk of major complications while waiting for the CAR-T cells and the widely accepted notion that a lower disease burden upon lymphodepletion correlates with an improved outcome, it seemed imperative to choose a safe and effective bridging option. Based on the assumption that *CRLF2* hyperexpression might be amenable to treatment with ponatinib, our patient received 45 mg ponatinib daily for a month and steroids for two weeks: follow-up BM aspirate before lymphodepletion demonstrated a persistent disease stability in spite of the rapidly progressive nature of Ph-like ALL. Even though it is difficult to discriminate between the role of ponatinib and the role of steroids due to their synergistic effect, the combination proved effective.

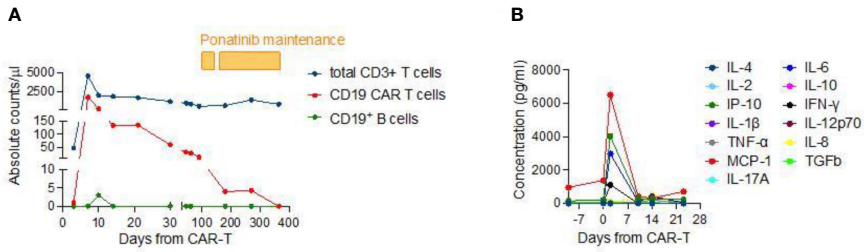

FIGURE 2
(A) Longitudinal evaluation of total CD3$^+$ T cells, anti-CD19 CAR-T cells, CD19$^+$ B cells and released cytokines in the patient's peripheral blood. Absolute counts were evaluated by flow cytometry at several time-points for up to 1 year after CAR-T cell infusion. The pharmacokinetics shows a remarkable CAR-T cell expansion in the first week following infusion (coinciding with CRS onset and resolution), with engineered cells representing nearly 40% of overall T cells, and a subsequent drop in CAR-T cells over time. At day 270, the patient still has a subset of CAR-T cells accounting for around 1% of the total T-cell count. At the time of last follow-up, circulating CAR-T cells had decreased below the detection limit of the assay but B-cell aplasia persists. **(B)** Evaluation of serum cytokines/chemokines concentrations in the first three weeks after CAR-T cell infusion. The analysis shows a significant peak occurring within the first week after treatment.

Whereas the efficacy of TKIs in *ABL1*-mutated ALL is demonstrated by a growing number of studies (13), there is still uncertainty on to their role in cases lacking such mutations, the rationale of its efficacy lying in the broad-spectrum of its kinase-inhibiting activity. Interestingly, ponatinib might represent the most promising of all TKIs based on studies highlighting its efficacy regardless of the patient's mutational status, both *in vitro* and *in vivo* (7, 14). Recently, *Lunghi et al.* (15) reported a patient with relapsed/refractory Ph-like ALL with *BCR::JAK2* rearrangement who achieved a CR2 and a first MRD clearance with ponatinib.

Another open issue is how to consolidate and maintain the results obtained with CAR-T cells. Even though HSCT consolidation seems beneficial in specific cases, clear indications are missing. Due to the major toxicities and the poor outcome associated with a second HSCT, we decided to strictly monitor the MRD status and ongoing B-cell aplasia, while pursuing a maintenance therapy with lower-dose ponatinib. Pre-clinical data show that TKIs might affect T-cell receptor signaling. It is already established that dasatinib inhibits Src family kinase activity, potentially affecting the effectiveness of immunotherapies. More recently, it has been reported that dasatinib may also ablate CAR-mediated signaling, by interfering with LCK and inhibiting the phosphorylation of CD3z and ZAP70 (16). This activity can induce a reversible function-off state in CAR-T cells that can be exploited to mitigate CRS (16) and to improve CAR-T cell fitness by preventing exhaustion and promoting the acquisition of a memory-like phenotype (17). However, little is known about ponatinib immunomodulating properties. Small clinical series suggest that coadministration of ponatinib or dasatinib with immunotherapies do not affect their effectiveness and might be beneficial in disease control (18).

To the best of our knowledge, this is one of the first reports of successful treatment of non *ABL*-mutated Ph-like ALL with ponatinib and the very first report of ponatinib being used as a bridge to and maintenance after CAR-T cell therapy.

The current treatment landscape in ALL is rapidly evolving. Unfortunately, some subsets of ALL are lagging behind and still retain a poor prognosis. Several open issues require settling in Ph-like ALL, such as the role of TKIs, which inhibitor to prefer, the appropriate timing of its introduction, and the outcomes of combination therapies. There is also an urgent need to define a standardized bridging strategy to CAR-T cells and post-CAR-T cell management in ALL. Future studies, preferably prospective and randomized, are warranted in order to re-define the appropriate therapeutic algorithm for patients with Ph-like ALL.

Data availability statement

The datasets presented in this article are not readily available because of ethical/privacy restrictions. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the patient has been treated according to current institutional guidelines, upon written informed consent for all the treatment procedures, the review of medical records and the use of immunological monitoring for patients undergoing allogeneic HSCT within the non-interventional "ALMON study", approved by San Raffaele Institutional Ethical Committee on 19/10/2007. Bone marrow and peripheral blood samples were collected and stocked at San Raffaele Hospital upon written informed consent for future potential studies within our Institutional observational "biobanking protocol", approved by San Raffaele Institutional Ethical Committee on 04/05/2006.The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

FG conceived the study and wrote the paper; EC wrote the paper and provided all clinical and hematological data; FL, EX, RG, LL, AB, ML-S, MGC, JP, FC provided all clinical and hematological data; MN and CB performed and evaluated CAR-T cell monitoring; RS performed and evaluated cytogenetic analysis; MC performed and evaluated serum cytokines/chemokines concentrations; SC evaluated and performed the BCR/ABL1-like predictor. RLS, SC, RF, FC supervised and wrote the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

CB is inventor on different patents on cancer immunotherapy and genome editing Use of common g-chain cytokines for the visualization, isolation and genetic modification of memory T lymphocytes, Patent family PCT/IT2006/000600; Targeted disruption of T cell receptor genes using engineered zinc finger protein nucleases, Patent family US N. 12/927,292 and PCT/US2014/031360; WT1-TCRs, Patent family N. PCT/EP2018/060477 and N. PCT/EP2019/079916; Compositions and methods for immunotherapy, PCT/US2019/056399. CB has been a member of Advisory Boards and a Consultant for Intellia Therapeutics, TxCell, Novartis, GSK, Allogene, Kite/Gilead, Miltenyi, Kiadis, Janssen and received research support from Intellia Therapeutics.

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.

Publisher's note

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High-dose cytarabine monotherapy is superior to standard-dose cytarabine-based multiagent sequential treatment cycle for consolidation treatment in adult (14-59 years) AML patients according to European Leukemia Net 2022 risk stratification

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Introduction: We firstly investigate based on 2022 European Leukemia Net (ELN) risk stratification, whether standard-dose cytarabine based multiagent sequential chemotherapy (SDMSC) is more beneficial than high-dose cytarabine (HDAC) monotherapy in consolidation for the survival of adult acute myeloid leukemia (AML) patients.

Methods: One hundred and eighty-three AML patients with complete remission (CR) were evaluated.

Results and discussion: The 3-year relapse rate was 33.4% in the HDAC group and 50.5% in the SDMSC group (p=0.066). The 3-year overall survival (OS) and event-free survival (EFS) rates in the HDAC group (69.2%, 60.7%) were significantly higher than that in the SDMSC group (50.8%, 42.1%) (p=0.025, 0.019). For patients in the intermediate risk group, the 3-year OS and EFS rates in the HDAC group (72.5%, 56.7%) were higher than that in the SDMSC group

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(49.1%, 38.0%) (p=0.028, 0.093). This study indicates that for young adult AML patients, HDAC consolidation achieves a higher long-term survival than SDMSC, especially for patients in the intermediate-risk group according to the 2022 ELN risk stratification.

KEYWORDS

acute myeloid leukemia, 2022 European Leukemia Net, high-dose cytarabine, multiagent sequential chemotherapy, consolidation

Introduction

The standard treatment of acute myeloid leukemia (AML) consists of one or two cycles of chemotherapy to induce complete remission (CR) followed by post-remission treatment to improve the duration of long-term remission. Only about 35 to 40% of CR patients can achieve long-term survival without disease recurrence (1), and post-remission therapy is mandatory to prevent relapse. Multiple cycles of high-dose cytarabine (HDAC) have been commonly used as standard consolidation treatment for AML patients who achieved CR in Europe and the United States (2). However, sequential multiagent chemotherapy using non–cross-resistant agents were also commonly used in Asian countries such as Japan; the JALSG AML 201 study (3) demonstrated that the multiagent chemotherapy regimen is as effective as HDAC regimen for consolidation, however, the HDAC regimen was accompanied with more severe and longerlasting neutropenia leading to more frequent infectious events.

To further investigate based on 2022 ELN risk stratification (4), whether standard-dose cytarabine based multiagent sequential chemotherapy (SDMSC) is more beneficial than HDAC monotherapy in consolidation for the survival of adult AML patients. We retrospectively analyzed the clinical features of newly diagnosed AML patients under 60 years from June 2015 to December 2020 in our center who achieved CR after the first induction therapy with IA (3 + 7) regimen, and compared the efficacy of SDMSC with HDAC regimens for consolidation therapy, focusing on the disease relapse and long-term survival (follow-up to June 2022).

Patients and methods

Patients

This study retrospectively analyzed 213 patients with newly diagnosed AML who achieved CR after first induction therapy with the IA (3 + 7) regimen at the First Affiliated Hospital of the University of Science and Technology of China (Anhui Provincial Hospital) from June 2015 to December 2020. The screening criteria were 14 years or older and 59 years or younger, complete remission after the first induction with the IA (3 + 7) regimen; and patients with acute promyelocytic leukemia were excluded. Among the patients studied, 30 patients were excluded due to these patients received other consolidation therapy. The remaining 183 patients were divided into two groups, including 127 patients received SDMSC and the other 56 patients received HDAC (Figure 1). This study protocol was approved by the ethics

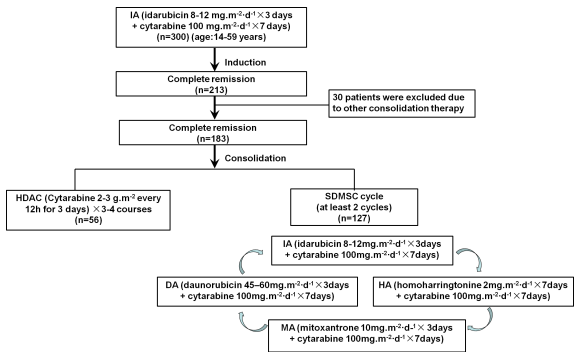

FIGURE 1
Scheme of the study protocol.

committee of Anhui Provincial Hospital and was conducted in accordance with the Declaration of Helsinki (2022-RE-329).

Treatment

All patients in CR after induction therapy with IA (3 + 7) (idarubicin 8-12mg/m^2/day for 3 days + cytarabine 100mg/m^2/day for 7 days) followed by consolidation therapy, either 3-4 courses of HDAC (2-3g/m^2 every 12h for 3 days) or SDMSC. SDMSC consisted of at least 2 cycles of multiagent sequential chemotherapy, and each cycle were conducted in the following order: IA (idarubicin 8-12 mg/m2/day for 3 days + cytarabine 100 mg/m2/day for 7 days), followed by HA (homoharringtonine 2 mg/m2/day for 7 days + cytarabine 100 mg/m2/day for 7 days), MA(mitoxantrone 10mg/m2/day for 3 days + cytarabine 100 mg/m2/day for 7 days), DA (daunorubicin 45-60mg/m2/day for 3 days + cytarabine 100 mg/m2/day for 7 days). Patients at intermediate or adverse risk are eligible for further evaluation of hematopoietic stem cell transplantation (HSCT).

Definitions and statistical analysis

Risk- stratification was derived from the 2022 European Leukemia Net (ELN) recommendations on diagnosis and management of AML in adults (4). The definition of CR, relapse, overall survival (OS) and event-free survival (EFS) were defined as reported elsewhere (5). Cumulative incidence of relapse (CIR) was defined as time from remission to relapse for all patients who achieved CR, and patients who died without relapse were considered competing causes of failure. Patient-, disease-, and transplant-related variables were measured using chi-square test (categorical variables), Mann-Whitney U-test (continuous variables). Relapse was generated by the cumulative-incidence function method, taking competing risks into account. The probabilities of OS and EFS were generated by the Kaplan-Meier method. R statistical software was used for statistical analysis (R Foundation for Statistical Computing). Differences were considered statistically significant at $p< 0.05$.

Results

Clinical characteristics

One hundred and eighty-three CR patients were evaluated. Among them, 56 patients received treatment of HDAC (HDAC group), and 127 patients received treatment of SDMSC (SDMSC group). There were no significant differences in age, sex, 2022 ELN risk stratification, underlying disease, white blood cell count at first diagnosis, minimal residual disease (MRD) after induction, MRD prior HSCT, and molecular abnormalities between two consolidation groups (Table 1).

Overall results

The 3-year CIR was 33.4% (95% CI:22.5%-47.7%) in the HDAC group and 50.5% (95% CI:41.6%-60.2%) in the SDMSC group (p=0.066) (Figure 2A). The 3-year OS rate in the HDAC group was significantly higher than that in the SDMSC group, 69.2% (95% CI:55.1%-79.6%) vs 50.8% (95% CI:41.4%-59.4%), respectively(p=0.025) (Figure 2B). The 3-year-EFS in the HDAC group was significantly higher than that in the SDMSC group, 60.7% (95% CI:46.7%-72.1%) vs 42.1% (95% CI:33.3%-50.7%) (p=0.019) (Figure 2C).

Results according to 2022 ELN risk stratification

For patients in the favorable risk group, the 3-year CIR in the HDAC group was 22.6% (95%CI: 9.1%-49.7%), which was similar to that in the SDMSC group, 38.5% (95%CI:24.8%-56.3%) (p=0.265) (Figure 3A). The 3-year OS rate of patients in the HDAC group was 66.7% (95%CI:40.4%-83.4%) and in the SDMSC group was 58.8% (95%CI:41.7%-72.5%) (p=0.618) (Figure 3B). The 3-year EFS rate in the HDAC group was 66.7%(95%CI:40.4%-83.4%), which was slightly higher than that in the SDMSC group 46.8%(95%CI:30.8%-61.4%) (p=0.148) (Figure 3C).

For patients in the intermediate risk group, the 3-year CIR was 41.2% (95%CI:25.9%-61.1%) in the HDAC group and 57.3% (95%CI:44.4%-70.9%) in the SDMSC group (p=0.278) (Figure 3D). The 3-year OS rate of patients in the HDAC group was 72.5%(95%CI:52.3%-85.3%), which was significantly higher than that in the SDMSC group 49.1%(95%CI:35.5%-61.3%) (p=0.028) (Figure 3E). The 3-year EFS rate was 56.7% (95%CI:37.3%-72.1%) in the HDAC group, which was slightly higher than that in the SDMSC group 38.0%(95%CI:25.7%-50.3%) (p=0.093) (Figure 3F).

For patients in the adverse-risk group, there were no significant differences between patients in the HDAC group and in the SDMSC group, in terms of 3-year CIR [27.1%(95% CI:7.5%-72.4%) vs 53.1%(95%CI:34.7%-74.0%), p=0.301] (Figure 3G), 3-year OS rate[62.5%(95%CI:22.9%-86.1%) vs 42.1%(95%CI:22.4%-61.2%), p=0.468] (Figure 3H), and 3-year EFS rate[62.5%(95%CI:22.9%-86.1%) vs 45%(95%CI:25.2%-63.0%), p=0.434] (Figure 3I).

TABLE 1 Baseline clinical characteristics of patients.

Characteristics	SDMSC (n=127)	HDAC (n=56)	*p* -value
Age (years), Median (range)	44 (16-58)	43 (14-58)	*p*=0.426
Sex ratio, M/F, no. (%)	58/69 (45.7/54.3)	28/28 (50.0/50.0)	*p*=0.632
ELN risk assessment, no. (%)			*p*=0.610
Favorable	40 (31.5)	18 (32.1)	
Intermediate	61 (48.0)	30 (53.6)	
Adverse	26 (20.5)	8 (14.3)	
Underlying disease, no. (%)			*p*=0.701
Hypertension	11 (8.7)	4 (7.1)	
Diabetes	8 (6.3)	2 (3.6)	
Rheumatism	4 (3.1)	1 (1.8)	
Virus hepatitis	5 (3.9)	1 (1.8)	
Malignant tumor	1 (0.8)	1 (1.8)	
Pregancy	4 (3.1)	2 (3.6)	
Myeloid sarcoma	0	1 (1.8)	
Stoke	1 (0.8)	1 (1.8)	
Arhythmia	1 (0.8)	1 (1.8)	
Parkinson's disease	1 (0.8)	0	
Hyperthyroidism	2 (1.6)	0	
Hypothyroidism	1 (0.8)	1 (1.8)	
Pulmonary embolism	0	1 (1.8)	
WBC at first diagnosis ($\times10^9$/L), Median (range)	12.76 (0.96-477.26)	10.11 (1.12-383.22)	*p*=0.790
Molecular biology			*p*=0.073
CEBPA mutation, no. (%)	24 (18.9)	9 (16.1)	
FLT3-ITD mutation, no. (%)	8 (6.3)	1 (1.8)	
NPM1 and FLT3-ITD mutation, no. (%)	2 (1.6)	5 (8.9)	
NPM1 mutation, no. (%)	11 (8.7)	8 (14.3)	
Negative detection, no. (%)	44 (34.6)	13 (23.2)	
Others	13 (10.2)	10 (17.9)	
Not available, no. (%)	25 (19.7)	10 (17.9)	
MRD after induction			*p*=0.704
Negative, no. (%)	80 (63.0)	29 (51.8)	
Positive, no. (%)	35 (27.6)	15 (26.8)	
Not available, no. (%)	12 (9.4)	12 (21.4)	
Transplantation, no. (%)	29 (22.8)	20 (35.7)	*p*=0.102
MRD before transplantation			*p=0.552*
Negative, no. (%)	17 (58.6)	8 (40.0)	

(Continued)

TABLE 1 Continued

Characteristics	SDMSC (n=127)	HDAC (n=56)	*p* -value
Positive, no. (%)	4 (13.8)	0	
Not available, no. (%)	8 (27.6)	12 (60.0)	
Relapse, no. (%)	59 (46.5)	18 (32.1)	*p*=0.076
Follow-up time (months), median (range)	26 (2-84)	30 (5-74)	*p*=0.241

Impact of transplantation

Allogenetic hematopoietic stem cell transplantation (HSCT) was performed in 20(35.7%) and 29(22.8%) patients in the HDAC and SDMSC groups, respectively. Among them, 15 and 19 patients in the HDAC and SDMSC groups, respectively, underwent cord blood transplantation, 5 and 10 patients underwent haploidentical HSCT(*p*=0.542). The 3-year OS rate of the transplantation patients reached 76.2%(95%CI:59.7%-86.6%), while the 3-year OS rate of patients without transplantation was only 49.0% (95% CI:40.1%– 57.3%) (*p*<0.001) (Figure 4A). Similarly, the 3-year EFS rate of the transplantation patients was 58.6%(95%CI:43.4%-71.1%), which was higher than that patients without transplantation 43.8% (95%CI:35.1%-52.2%) (*p*=0.039) (Figure 4B).

For the transplantation patients, the 3-year OS rate in the HDAC group and in the SDMSC group were 77.3%(95% CI:49.0%-91.1%) and 69.8%(95%CI:46.0%-84.6%) (*p*=0.742), respectively (Figure 4C). And the 3-year EFS rate in the HDAC group and in the SDMSC group were 65.0%(95% CI:40.3%-81.5%) and 54.0%(95%CI:34.1%-70.3%) (*p*=0.563), respectively (Figure 4D).

For patients without transplantation, the 3-year OS rate in the HDAC group was 60.1%(95%CI:41.9%-74.2%), which was slightly higher than that in the SDMSC group 45.0%(95% CI:34.8%-54.7%) (*p*=0.051) (Figure 4E). The 3-year EFS rate was 58.3% (95% CI:40.7%-72.4%) in the HDAC group, which was significantly higher than that of 38.7% (95% CI:28.9%-48.4%) in the SDMSC group (*p*=0.034) (Figure 4F).

Discussion

This retrospective study demonstrated that for young adult AML patients, HDAC (2-3 g/m2 every 12 hours on d1-3) achieves a higher long-term survival than SDMSC regimens based on standard-dose cytarabine (cytarabine 100 mg.m-2·d-1×7 days), especially for patients in the intermediate-risk group according to the 2022 ELN risk stratification.

Regarding the consolidation strategies, HDAC was commonly used in countries such as the United States since the landmark of Cancer and Leukemia Group B-8525 trial (CALGB-8525) was reported (6). This study indicated a significant dose-dependent effect of cytarabine in the postremission treatment for AML. Patients 60 years of age or younger who received HDAC (3 g/m2 every 12 hours on D1, D3, and D5) had lower relapse and higher long-term survival rates than patients who received lower doses of cytarabine (100 mg/m2 on D1-5 or 400 mg/m2 on D1-5). However, the CALGB-8525 trial only demonstrated that HDAC alone was superior to lower-dose cytarabine alone and did not assess the effects of combination regimens in consolidation. The Acute Leukemia

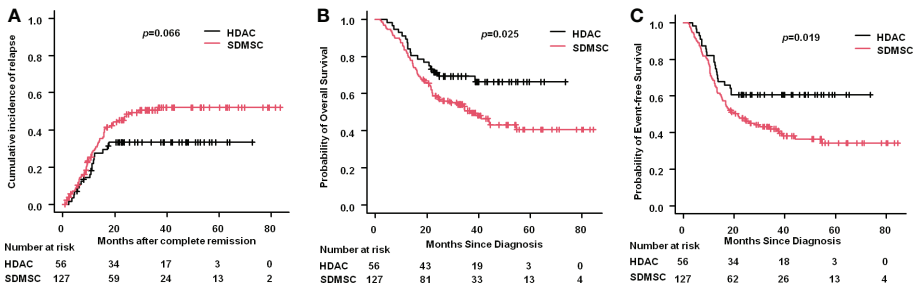

FIGURE 2
Relapse and survival in patients of aml. Cumulative incidence of relapse for the whole cohort **(A)**. Probability of overall survival for the whole cohort **(B)**. Probability of event-free survival for the whole cohort **(C)**.

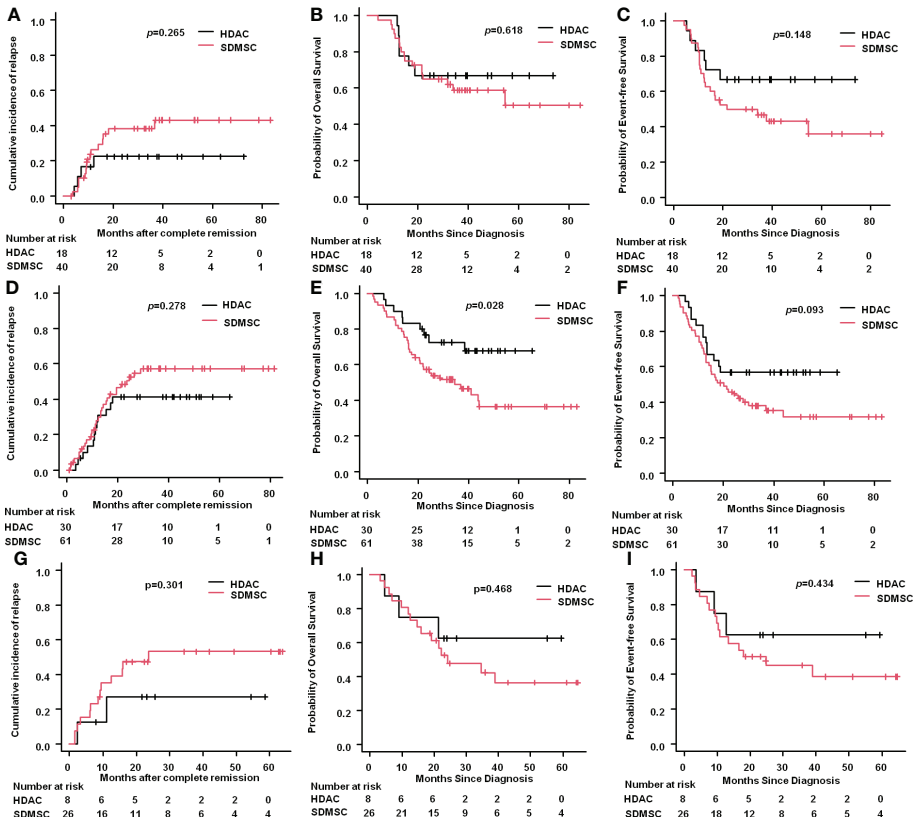

FIGURE 3
Results according to 2022 ELN risk stratification. Cumulative incidence of relapse for favourable-risk patients **(A)**. Probability of overall survival for favourable-risk patients **(B)**. Probability of event-free survival for favourable-risk patients **(C)**. Cumulative incidence of relapse for intermediate-risk patients **(D)**. Probability of overall survival for intermediate-risk patients **(E)**. Probability of event-free survival for intermediate-risk patients **(F)**. Cumulative incidence of relapse for adverse-risk patients **(G)**. Probability of overall survival for adverse-risk patients **(H)**. Probability of event-free survival for adverse-risk patients **(I)**.

French Association Group trial (ALFA-9802) (7) compared HDAC (3 g/m^2) consolidation regimen with a timed-sequential consolidation regimen consisting of etoposide, mitoxantrone, and cytarabine (500 mg/m^2, d1-d5) and showed the HDAC regimen was more beneficial for patient survival.

However, not all studies support that HDAC consolidation is superior to multi-agent combination regimens. The JALSG AML201 trial (3) demonstrated that the lower-dose cytarabine (200 mg/m2 d1-d5) regimen combined with mitoxantrone, daunorubicin, aclarubicin, or etoposide was as effective as HDAC (2 g/m2 twice daily for 5 days) in postremission consolidation, and recommended that the conventional multiagent chemotherapy may be suitable for the AML patients in intermediate or adverse cytogenetic risk groups. The Cancer and Leukemia Group B 9222 trial (CALGB-9222) (8) showed that sequential multiagent chemotherapy had similar disease-free survival to HDAC for post-remission treatment of adults under 60 years of age. The Medical Research Council AML15 Trial (9) indicated that multiagent consolidiation regimens [amsacrine, cytarabine, and etoposide (MACE) followed by mitoxantrone and cytarabine (MidAC)] achieved similar results to HDAC for patients with favorable and intermediate risk but superior in patients with high-risk disease, although it was associated with more hematologic toxicity.

This study aimed to explore whether the SDMSC using non–cross-resistant agents might improve long-term survival. First, in this study, the 3-year OS rate and EFS rate of patients in the HDAC group (69.2% and 60.7%) were significantly higher than that of SDMSC group (50.8% and 42.1%), especially for patients in the intermediate risk group (72.5% and 56.7% in the HDAC group vs 49.1% and 38.0% in the SDMSC group). These results indicated that HDAC (2-3 g/m2 every 12 hours on d1-3) with 3~4 courses is the preferred consolidation regimen for young adult AML patients. In addition, we investigated whether patients with allo-HSCT had an OS or EFS benefit in comparison to those without allo-HSCT. As previously reported (10, 11), among patients at intermediate or adverse risk, an allogeneic transplant had an improved long-term

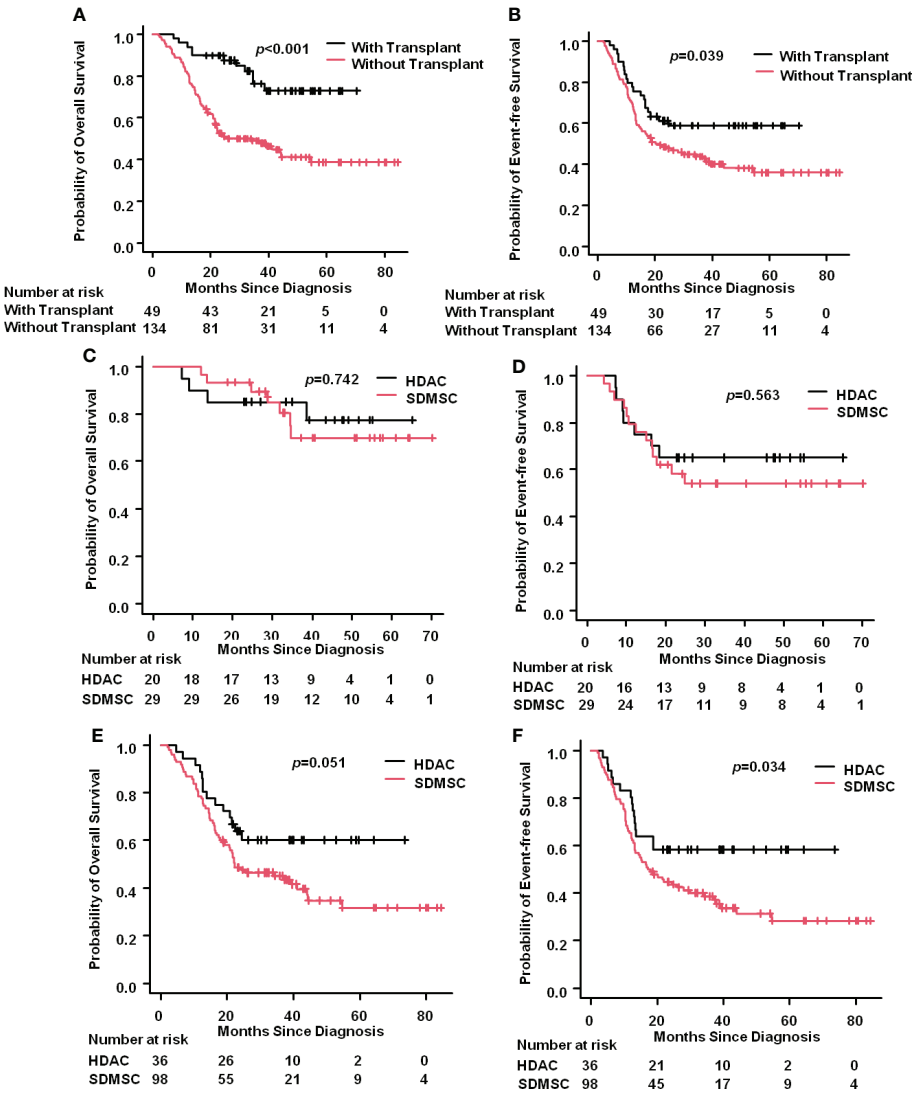

FIGURE 4
Impact of transplantation. The 3-year OS rate of AML patients with or without transplantation **(A)**. The 3-year EFS rate of AML patients with or without transplantation **(B)**. For the transplantation patients, the 3-year OS rate in the HDAC group and in the SDMSC group **(C)**, and the 3-year EFS rate in the HDAC group and in the SDMSC group **(D)**. For patients without transplantation, the 3-year OS rate in the HDAC group and in the SDMSC group **(E)**, and the 3-year EFS rate in the HDAC group and in the SDMSC group **(F)**.

survival, suggesting a positive impact of allo-HSCT in these patients. Although the most effective post-remission treatment is allo-HSCT, it is not available to all patients with intermediate or high-risk disease because of high rates of treatment-related complications and lacking suitable donors. Interestingly, for patients with transplantation, 3-year OS or EFS in the HDAC group was similar with that in the SDMSC group, suggesting that for patients undergoing transplantation, there was no survival differences for either receiving HDAC or SDMSC regimens prior to transplantation. However, for patients who do not receive transplantation, 3-year OS and EFS in the HDAC group were significantly higher than that in the SDMSC group, suggesting that HDAC consolidation is the preferred regimen for AML patients who have no opportunity to receive allo-HSCT.

Finally, to our knowledge, our study is the first to investigate the impact of consolidation treatment options for AML according to the ELN-2022 risk stratification system. Recently, the ELN published the revised risk stratification system for AML (ELN-2022) and several modifications have been made (4); AML with *FLT3*-ITD are now categorized in the intermediate-risk group, AML with myelodysplasia-related gene mutations is now categorized in the adverse-risk group, the presence of adverse-risk cytogenetic abnormalities in *NPM1*-mutated AML now defines adverse risk, etc. In this study, we demonstrated that

for patients in the intermediate risk group, the 3-year OS rate of patients in the HDAC group was significantly higher than that in the SDMSC group (72.5% vs 49.1%, p=0.028); however, for patients in the favorable risk and adverse-risk groups, there were no significant differences between patients in the HDAC group and in the SDMSC group, in terms of 3-year CIR, OS and EFS.

In summary, this study indicates that for young adult AML patients, HDAC consolidation achieves a higher long-term survival than SDMSC, especially for patients in the intermediate-risk group according to the 2022 ELN risk stratification. Allo-HSCT is preferred for selected patients with intermediate and adverse prognosis, while both HiDAC regimen and SDMSC can be used prior to transplantation. However, this study has inherited limitations, such as single center, retrospective study, small sample size (especially in the adverse risk group), lack of detailed data on the genetic characteristics of patients at diagnosis and on MRD after consolidation, and failure to compare treatment-related toxicities between regimens. Therefore, prospective randomized multicenter clinical trials are needed to confirm the results of this study.

Data availability statement

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

Author contributions

XW and DL collected data, XW wrote the original paper, analyzed data and completed the tables and figures. CZ designed research, performed research, analyzed and interpreted data, critically reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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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

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Real-world experiences of CNS-directed chemotherapy followed by autologous stem cell transplantation for secondary CNS involvement in relapsed or refractory diffuse large B-cell lymphoma

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Introduction: Secondary central nervous system (CNS) involvement is a rare but fatal event in patients with diffuse large B cell lymphoma (DLBCL). Some studies have suggested autologous stem cell transplantation (ASCT) for patients responding to salvage therapies, although its role is not clear.

Methods: We analyzed DLBCL patients with secondary CNS involvement who received salvage therapies with curative intent and who underwent high-dose chemotherapy followed by ASCT. We analyzed the post-ASCT outcome in terms of CNS and/or systemic relapse and overall survival (OS) according to type of secondary CNS involvement and salvage treatment.

Results: A total of 43 patients who achieved complete or partial response after salvage treatments, mainly high-dose methotrexate (MTX)-containing chemotherapy, was treated with busulphan-thiotepa followed by ASCT between 2009 to 2019. Fifteen patients experienced grade III/IV febrile neutropenia, but all adverse events were manageable. At the median follow-up of 14.7 months after ASCT, 17 patients did not relapse, however, 26 patients had relapsed, comprising isolated CNS relapse (n = 12), systemic relapse (n = 12), and both (n = 2). Patients with systemic relapse had significantly shorter OS than those with isolated CNS relapse (42.7 vs, 11.1 months, p = 0.002). Of the 26 patients who relapsed after ASCT, six patients were rescued by subsequent salvage treatments. Finally, 21 patients were alive at the time of analysis.

Discussion: In conclusion, consolidative ASCT might be beneficial for secondary CNS involvement in relapsed or refractory DLBCL patients if they responded to CNS-directed salvage chemotherapy and were eligible for transplantation.

KEYWORDS

secondary CNS involvement, autologous stem cell transplantation, diffuse large B-cell lymphoma, salvage treatment, high-dose methotrexate (HD-MTX)

Introduction

Secondary central nervous system (CNS) involvement is an uncommon complication of diffuse large B cell lymphoma (DLBCL) with around 5% incidence, but it carries a poor prognosis (1, 2). Secondary CNS involvement can be observed as a sign of disease progression during treatment for systemic DLBCL or as a relapse disease with or without systemic relapse of DLBCL. The clinical outcome of DLBCL patients with secondary CNS involvement is dismal because the CNS is a chemotherapy sanctuary site and secondary CNS involvement demonstrates the aggressiveness of systemic DLBCL (3–5). Although there is no consensus on optimal treatment strategies for secondary CNS involvement, treatments targeting CNS lesions have been widely used, such as treatment for primary CNS lymphoma. Thus, high-dose methotrexate (MTX)-containing chemotherapies might be effective for patients with secondary CNS involvement. However, the majority of patients fail to be cured by these therapeutic approaches because the response duration is relatively short and systemic disease progression can occur outside the CNS (6, 7).

Nevertheless, CNS-directed high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) has been conducted for transplant-eligible patients because previous phase II studies demonstrated ASCT as a feasible consolidative treatment to improve the survival outcome of secondary CNS involvement (8–12). However, due to the rarity of the disease, those studies have small and heterogeneous populations with different histologies, first-line therapies, salvage therapies, and conditioning regimens. Furthermore, a substantial number of patients with secondary CNS involvement might not be enrolled in prospective studies because they are usually in poor health condition. Therefore, real-world data representing the efficacy and feasibility of ASCT in clinical practice are needed. Previous retrospective studies have shown a prolonged duration of response in a small number of patients who received high-dose busulfan/thiotepa-based conditioning and ASCT (13, 14). In addition, an international multicenter retrospective study analyzing 291 DLBCL patients with secondary CNS involvement also reported favorable outcome of patients who underwent ASCT after intensive salvage chemotherapy with curative intent (15). However, only 25 of those patients received ASCT after heterogeneous conditioning regimens. Thus, there are limited data regarding the efficacy of ASCT in DLBCL patients with secondary CNS involvement. This single-center real-world data study analyzed the duration of response and survival outcome of DLBCL patients with secondary CNS involvement who underwent ASCT as a consolidation treatment and compared the outcome after ASCT based on the pattern of secondary CNS involvement.

Methods

Patients

We retrospectively reviewed the medical records of patients who were diagnosed with DLBCL from 2009 to 2019, and selected patients according to the following criteria: 1) CNS relapse or progression during or after R-CHOP chemotherapy; 2) underwent ASCT after salvage treatments for secondary CNS involvement; and 3) data available for analysis of disease relapse and survival status. We excluded patients who had CNS involvement at diagnosis. As this study aimed to evaluate the role of ASCT in DLBCL patients with secondary CNS involvement, patients who did not undergo ASCT were also excluded. Patient demographics and clinical characteristics at initial presentation and at the time of secondary CNS involvement were collected, and information about the type of salvage therapy after secondary CNS involvement and ASCT was gathered. For patients who relapsed after ASCT, the patterns of relapse and post-relapse outcomes were analyzed, and survival status was last updated in April 2022. The Institutional Review Board of Samsung Medical Center approved this study (IRB No: 2022-05-035), and informed consent was waived due to the retrospective nature.

Diagnosis of secondary CNS involvement

The evaluation of CNS was conducted for patients having signs and/or symptoms of suspicious CNS involvement during or after R-CHOP chemotherapy. The diagnosis of CNS involvement was based on the combination of neurologic manifestations, magnetic resonance imaging (MRI), and/or histological findings of the brain parenchyma or cerebrospinal fluid (CSF). The patterns of CNS involvement were parenchymal, leptomeningeal, and combined involvement. Parenchymal involvement was diagnosed on brain MRI or brain biopsy. If the cytologic examination of CSF showed the presence of lymphoma cells or suspicious lymphoma cells with increased protein levels, leptomeningeal involvement was diagnosed. In addition, when leptomeningeal enhancement compatible with leptomeningeal involvement was observed in brain or spine MRI of patients with

neurologic symptoms or signs, leptomeningeal involvement was diagnosed.

ASCT and response evaluation

ASCT was carried out for patients who responded to salvage chemotherapy after CNS involvement. Peripheral blood stem cells were collected during salvage chemotherapy or mobilization chemotherapy with etoposide as we previously reported for patients with primary CNS lymphoma (16). The target number of CD34+ cells was more than 3.0×10^6 per kilogram of the recipient's body weight. The conditioning regimen performed prior to ASCT was the same as that for primary CNS lymphoma, consisting of busulfan and thiotepa (busulfan 3.2 mg/kg days –9 to –5, and thiotepa 5 mg/kg days –4 to –3) as we previously reported (16, 17). Treatment response was assessed according to the involved sites. CNS response evaluation was assessed by contrast-enhanced MRI with neuroradiologic review according to the international guidelines for primary CNS lymphoma (18). If a patient had simultaneous systemic disease involvement, systemic response evaluation was also performed according to the Lugano response criteria (19).

Statistical analysis

The clinical features and treatment outcomes were analyzed, and categorical variables were evaluated using Fisher's exact test. The time to secondary CNS involvement was defined as the time between the initial diagnosis of DLBCL to the date of secondary CNS involvement. The post-ASCT overall survival (OS) was defined as the time from the date of stem cell infusion to death from any cause, and living patients were censored at the time of analysis. The post-ASCT progression-free survival (PFS) was defined as the time from the date of stem cell infusion to the date of systemic or CNS progression or relapse or death. Survival was estimated based on Kaplan–Meier curves and compared using a log-rank test. Two-sided statistical tests yielding a P value < 0.05 were considered significant. Survival analyses were performed using IBM PASW version 24.0 software (SPSS Inc., Chicago, IL, USA).

Results

Patients with secondary CNS involvement

A total of 92 patients had secondary CNS involvement at our institute between 2009 and 2019 according to the review of medical records. As the ASCT was only reimbursed for patients who were younger than 65 years old during that period, 70 patients were determined to be eligible for ASCT based on the reimbursement criteria. However, out of 70 patients who were potentially eligible for ASCT, 43 patients (43/70, 61.4%) underwent ASCT after they achieved complete or partial response to CNS-directed salvage chemotherapy and the remaining patients (n = 27) could not receive ASCT because they failed to respond to salvage therapy. Thus, we analyzed 43 patients who underwent ASCT after secondary CNS involvement. Their median age at initial diagnosis of DLBCL was 53 years (range: 23 – 66 years), and 23 patients were male (54%). The majority of patients had ECOG 0/1 (n = 38, 88%) at diagnosis, and initial stages were as follows: stage II (n = 13, 30%), III (n = 6, 14%), and IV (n = 24, 56%). However, extranodal involvement was found in most patients, and bone marrow was involved in 15 patients (35%) at diagnosis. The presence of at least one extranodal involvement was found in 43 patients (95%), and 19 patients (44%) had two or more extranodal involvements (Figure 1). The involvement of a head area adjacent to the brain such as nasal cavity and paranasal sinuses was most common (n = 16) compared to other organs including liver (n = 7), gastrointestinal tract (n = 5), thorax (n = 5), soft tissue/muscle (n = 5), breast (n = 4), and kidney (n = 2). However, out of 43 patients who underwent ASCT, there was no case of DLBCL that initially had testicular involvement at diagnosis. All patients received R-CHOP as the primary treatment, and the response was as follows: CR (n = 36, 84%), PR (n = 5, 12%), and PD (n = 2, 5%). During R-CHOP chemotherapy, only eight patients received CNS prophylaxis according to physicians' discretion in case of breast involvement or high tumor burden. Thus, six patients received IT MTX (intrathecal administration of 12 mg of MTX every three weeks) whereas two patients received high-dose MTX prophylaxis (MTX $3.5 g/m^2$ intravenous infusion every three weeks for two cycles after the completion of R-CHOP chemotherapy). However, CNS involvement occurred even in patients with stage II completely responding to R-CHOP (Patient No. 8, 25, 35, Figure 1). Secondary CNS involvement occurred as an isolated CNS relapse or progression in 27 patients (63%), whereas 16 patients (37%) showed concomitant systemic disease progression (Figure 1). The diagnosis of secondary CNS involvement was based on various manifestations suspicious of CNS involvement, and most patients presented multiple neurologic symptoms (Figure 2A). Secondary CNS involvement occurred most commonly within 12 months after initial diagnosis of DLBCL (Figure 2B); thus, the median age at the time of secondary CNS involvement was 55.7 years (range: 23.4 – 66.8 years) as the median time to secondary CNS involvement was 10.2 months (95% CI: 5.8 – 14.7 months, Figure 2C). At diagnosis of CNS involvement, 40% of patients had poor performance status (≥ ECOG 2), and the primarily involved sites of CNS were brain parenchyma (n = 20), leptomeninges (n = 11), both parenchyma and leptomeninges (n = 10), and spinal cord only (n =2). Patients received CNS-directed chemotherapy, mainly high-dose MTX-containing regimens as a salvage regimen. The salvage treatments that patients received after secondary CNS involvement were R-MVP (rituximab, methotrexate, vincristine, and procarbazine, n = 16), MVP (methotrexate, vincristine, and procarbazine, n = 15), ICED (ifosfamide, carboplatin, etoposide, and dexamethasone, n = 8), and other MTX-containing regimens (Figure 2D). The R-MPV treatment protocol consisted of rituximab ($375 mg/m^2$ intravenous on day 1), MTX ($3.5 g/m^2$ intravenous over 6 hours on day 2), leucovorin ($15 mg/m^2$ intravenous four times per day, total 12 times during three days), procarbazine ($100 mg/m^2$ orally administered for seven days at odd-numbered chemotherapy sessions), and vincristine ($1.4 mg/m^2$ intravenous on day 2) for a total of five cycles at two-week intervals. Among patients who received high-dose MTX, 28 received the planned dosage ($3.5 g/m^2$), while three received less than $3.5 g/m^2$. The ICED treatment protocol consisted of intravenous ifosfamide $1670 mg/m^2$ on days 1-3, carboplatin 5 x AUC on day 1, intravenous etoposide $100 mg/m^2$ on days 1-3, and oral or intravenous dexamethasone 40mg on days 1–4 every three weeks, for four cycles.

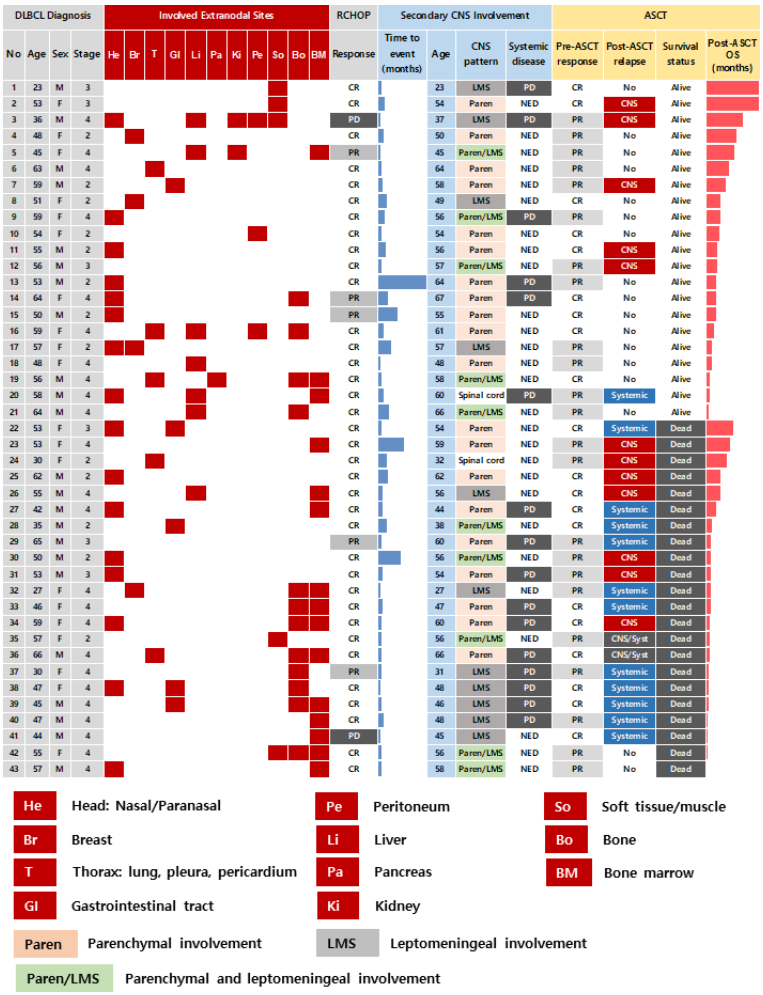

FIGURE 1
Summary of the 43 patients in the study.

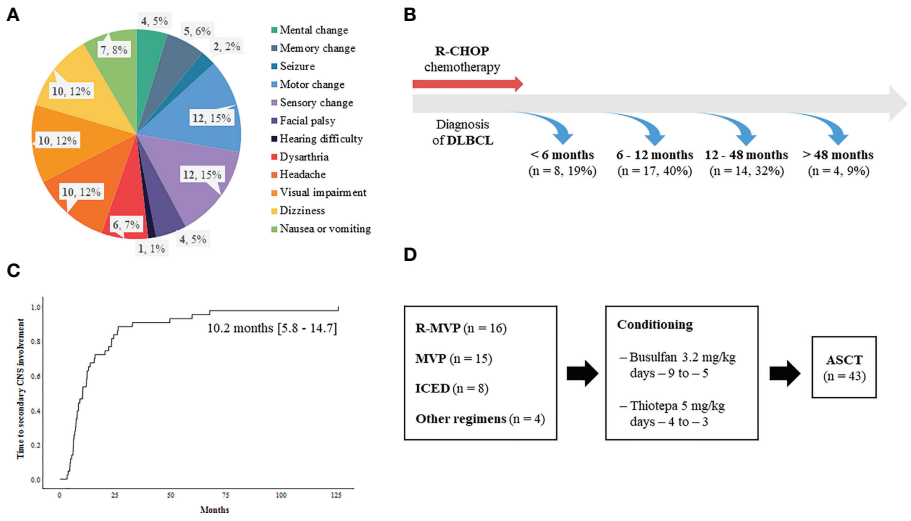

FIGURE 2
Characteristics of secondary CNS involvement in relapsed or refractory DLBCL patients **(A)** Signs and symptoms suspicious of secondary CNS involvement leading to CNS evaluation **(B)** Onset of secondary CNS involvement **(C)** Time to secondary CNS involvement **(D)** Treatment summary of 43 patients.

Outcome of ASCT

The median number of infused CD34+ cells per body weight was 4.1 × 10^6 cells per kg (range: 2.0 – 34.9 × 10^6). Fifteen patients experienced grade III/IV febrile neutropenia including one patient requiring treatment in the intensive care unit. Sinus obstruction syndrome occurred in one patient, however, all those adverse events were manageable. Neutrophil engraftment (the first of three days on which the neutrophil count was maintained at 500 × 10^6/L) and platelet engraftment (the first day free from platelet transfusion following at least seven days with a platelet count >20 × 10^9/L) occurred around nine days after ASCT. At the median follow-up of 14.7 months (95% CI: 3.7 – 25.7) after ASCT, 17 patients did not relapse. However, the remaining 26 patients (60.5%) relapsed, and more than 60% of relapses were found (n = 16, 61.5%) within six months after ASCT. Thus, the median PFS after ASCT was 12.0 months (95% CI: 2.7 – 21.3 months, Figure 3A). The patterns of relapse were isolated CNS, systemic, and combined relapses, and 20 patients died after relapse. Out of 26 relapsed patients, six patients were alive after subsequent salvage treatments (Figure 3B). Thus, the median OS after ASCT was 32.3 months (95% CI: 4.4 – 60.2, Figure 3C). The number of survivors was significantly higher in patients receiving R-MVP after secondary CNS involvement (Figure 3D). Thus, the OS of patients receiving R-MVP or MVP was better than that of those receiving ICED after secondary CNS involvement, although the difference was not statistically significant (Figure 3E). The IT MTX was administered simultaneously to 15 patients including 11 patients with leptomeningeal involvement, however, the use of IT MTX for patients with leptomeningeal involvement did not have any impact on their survival outcome. Thus, the OS of patients who received IT MTX plus R-MVP or MVP was not significantly different from that of patients who received R-MVP or MVP alone (p = 0.255, data not shown). In case of isolated CNS relapse, R-MVP or MVP were mainly used as salvage treatments without whole-brain radiotherapy. Radiotherapy including whole-brain radiotherapy or gamma-knife was done in 13 patients, especially for concomitant systemic and CNS progression and patients who were symptomatic for their brain lesion.

Post-ASCT relapse

The post-ASCT OS was compared by the time of onset of secondary CNS involvement after initial diagnosis. Patients with early secondary CNS involvement within 12 months showed worse OS than patients with secondary CNS involvement 12 months later, although the difference was not significant (Figure 4A). The comparison of survival outcomes according to the type of CNS involvement showed that the presence of leptomeningeal involvement had a negative effect on survival, although it was not statistically significant (Figure 4B). In addition, patients who experienced an isolated CNS relapse after ASCT showed significantly better OS than patients with systemic or combined relapse (Figure 4C). The onset of relapse after ASCT was also related to prognosis after ASCT, thus, post-ASCT relapse within six months after ASCT showed poor OS, and the majority of those patients with early relapse experienced systemic relapse (Figures 1, 4D).

Discussion

Our study evaluated the outcomes of 43 DLBCL patients with secondary CNS involvement who underwent successful ASCT after salvage chemotherapy with curative intent. As secondary CNS involvement is not a common event in DLBCL, and only patients responding to salvage chemotherapy could undergo ASCT, the proportion of patients receiving ASCT is small even among patients with secondary CNS involvement (13–15). Indeed, the feasibility of ASCT is limited due to the failure of salvage treatment, toxicity, and unsuccessful stem cell harvest. Thus, ASCT has been reported as being applicable only to selected patients with secondary CNS involvement (20). Therefore, this study analyzed a relatively large

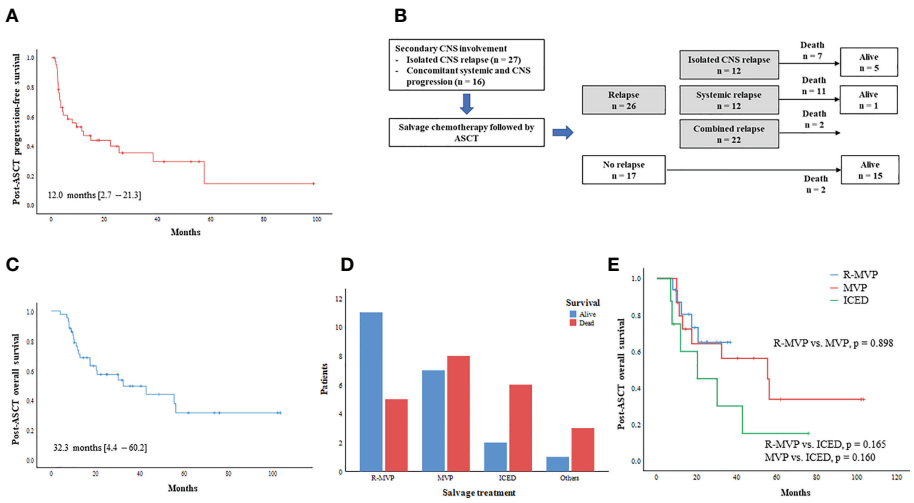

FIGURE 3
Outcome of ASCT for secondary CNS involvement **(A)** Progression-free survival after ASCT **(B)** Outcome after ASCT and type of post-ASCT relapse **(C)** Overall survival after ASCT **(D)** Comparison of survivors according to type of salvage CNS-directed treatment **(E)** Post-ASCT overall survival based on type of CNS-directed chemotherapy.

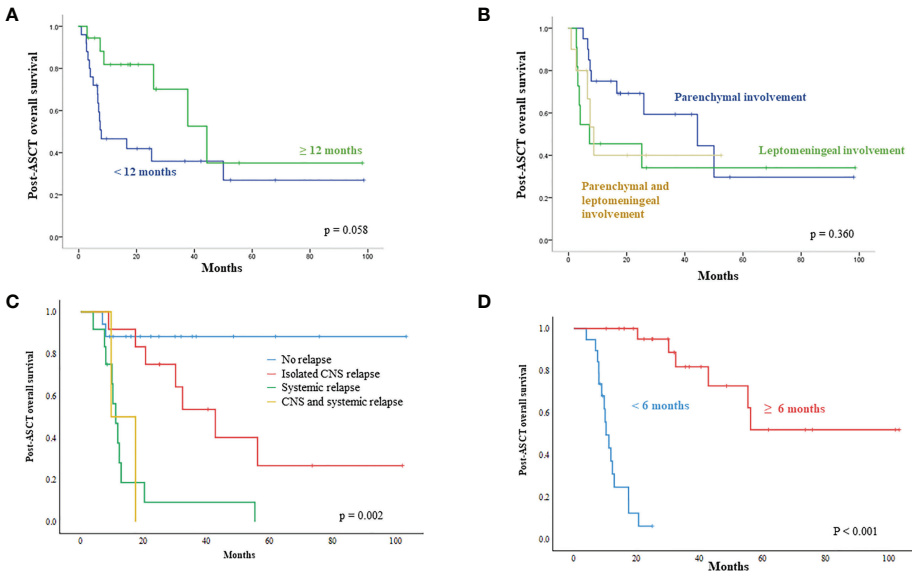

FIGURE 4
Unfavorable parameters influencing survival outcome after ASCT **(A)** Patients demonstrating secondary CNS involvement within 12 months after initial diagnosis showed worse survival after ASCT **(B)** Patients having leptomeningeal involvement show a tendency for poor survival after ASCT **(C)** Systemic relapse after ASCT has a negative impact on post-ASCT survival **(D)** Early relapse within six months after ASCT showed worse overall survival than relapse occurring after six months.

number of patient outcomes according to onset and type of secondary CNS involvement.

Secondary CNS involvement could occur as isolated CNS relapse or progression or concomitant with systemic progression. The previous study that gathered the largest number of DLBCL patients with secondary CNS involvement showed a median time from initial diagnosis of DLBCL to secondary CNS involvement of nine months (range: 1 – 132 months) (15). A previous multicenter prospective study on secondary CNS involvement reported a median time of 10.4 months (range: 3.4 – 29.2 months) from diagnosis to secondary CNS involvement (21). Our study likewise showed 10.2 months of median time to CNS involvement, and around 60% of cases occurred within 12 months after the initial diagnosis of DLBCL. Although it was not statistically significant, OS after ASCT was inferior when secondary CNS involvement occurred within 12 months of initial diagnosis compared to when CNS relapse occurred after 12 months. The pattern of CNS involvement was also associated with the prognosis of secondary CNS involvement. Brain parenchyma is the most commonly reported site with a better outcome than leptomeninges (15). Our study also showed the negative impact of leptomeningeal involvement on the outcome of patients after ASCT, although it was not significant.

As high-dose MTX is the mainstay of CNS-directed treatment, the majority of patients (83.7%) in our study received high-dose MTX-containing chemotherapy with curative intent. Especially, 16 patients received treatment in combination with rituximab, such as R-MVP, because R-MVP followed by ASCT has been widely used for the treatment of primary CNS DLBCL (16, 22). The outcome after ASCT was better in patients receiving R-MVP or MVP than in patients receiving ICED. Likewise, a retrospective study of 113 patients with isolated CNS relapse of systemic NHL reported better OS in the MTX

TABLE 1 Summary of previous studies.

	Ferreri et al. (12)	Doorduijn et al. (11)	Ferreri et al. (10)	Korfel et al (8)	Our study
No. of patients	75	36	38	30	92
Median age (range)	58 (23-70)	57 (23-65)	59 (36-70)	58 (29-65)	51 (23-66)
Median time to CNS involvement (month, range)	Not reported	12 (2-186)	1 (0-69)	9 (3-80)	10 (3-126)
CNS involvement at relapse (%)	57	100	58	100	100
Transplant patients (%)	49	42	53	80	46
PFS after ASCT	1y 58%	1y 19%	2y 50%	2y 49%	2y 58.6%
Median OS after CNS relapse (month)	29	7	Not reported	Not reported	32
OS of transplant patients	2y 83%	1y 32%	2y 68%	2y 68%	2y 57%
Conditioning regimen	Carmustine Thiotepa	Busulfan Cyclophosphamide	BCNU Thiotepa	Carmustin Thiotepa Etoposide	Busulfan Thiotepa

group (p=0.007) (23). Our study showed that 15 patients maintained their response without evidence of relapse, demonstrating a plateau in the survival curve. Nevertheless, 26 patients experienced relapse after ASCT, and relapses occurred in the CNS, systemically, or both. In particular, patients who had systemic relapse showed significantly shorter OS than those who had isolated CNS relapse. The median time to relapse after ASCT was 12.0 months (range; 2.1-21.3 months). The patients that relapsed within six months after ASCT tended to have a systemic relapse; only four of them had isolated CNS relapse (Figure 1). This might be why early relapsed patients had shorter OS. Our survival outcome after ASCT was comparable to that of previous prospective studies analyzing patients with secondary CNS involvement (Table 1). Among previous studies, one study reported 32% OS at one year, after the use of busulfan/cyclophosphamide as a conditioning regimen (11). Considering that the thiotepa-based conditioning regimen was associated with longer OS in primary CNS lymphoma (24), the type of conditioning could influence the post-ASCT outcome.

Although our results showed that high-dose MTX-based salvage therapy and ASCT might be beneficial for patients with secondary CNS involvement if they qualify for transplantation, our findings could be influenced by selection bias because we only analyzed patients receiving ASCT who might have a better prognosis than those who did not receive ASCT. Thus, the role of ASCT in DLBCL patients with secondary CNS involvement in this study should be cautiously interpreted given the limitation of our study. However, active application of intensified treatment followed by ASCT should be considered for patients who are eligible for transplantation, especially for cases with isolated CNS relapse 12 months after initial DLBCL diagnosis. However, considering that the prognosis of patients with relapse within six months after ASCT and those with concomitant systemic relapse was extremely poor, careful selection of those who may benefit from more intensive therapy is important, and additional therapeutic approaches should be explored in the future.

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 study was approved by the Institutional Review Board of Samsung Medical Center (IRB number 2022-05-035).

Author contributions

SJ and SK wrote the manuscript. DC and EK performed targeted sequencing and analysis. SJ and SK analyzed the data. SY, WK, and SK reviewed the clinical data. JC reviewed the pathology, and SK designed the study. All authors contributed to the article and approved the submitted version.

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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

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Lipid profile as a novel prognostic predictor for patients with acute myeloid leukemia

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Purpose: This study investigated the relationship between serum lipid levels and clinical outcomes in acute myeloid leukemia (AML) by establishing a predictive risk classification model.

Method: A total of 214 AML patients who were pathologically diagnosed and treated with standard induction chemotherapy at Sun Yat-Sen University Cancer Center were included. The patients were randomly divided into the training (n = 107) and validation (n=107) cohorts. Univariate and multivariate Cox analyses were used to assess the value of triglyceride (TG), Apolipoprotein B (Apo B), Apo Apolipoprotein A-I (Apo A-I), cholesterol (CHO), and high-density lipoprotein (HDL) as prognostic factors for AML.

Results: After a series of data analyses, a five-factor model was established to divide the patients into high- and low-risk groups. Kaplan-Meier survival analysis showed that the high-risk group had a poor prognosis (P<0.05). The area under the curve of the novel model for five-year OS was 0.737. A nomogram was constructed to integrate the model with age and the 2017 ELN cytogenetic classification, with the merged model showing improved accuracy with an area under the curve of 0.987 for five-year OS.

Conclusion: A novel model was constructed using a combination of the serum lipid profile and clinical characteristics of AML patients to enhance the predictive accuracy of clinical outcomes. The nomogram used the lipid profile which is routinely tested in clinical blood biochemistry and showed both specific prognostic and therapeutic potential.

KEYWORDS

acute myeloid leukemia, lipid profile, blood chemical analysis, prognostic factors, predictive modeling, dyslipdemia

Introduction

Acute myeloid leukemia (AML) is known for its complicated cytogenetics and pathological heterogeneity, its strikingly high relapse tendency, and is lethal in ~50% of young adults and ~80% of older adults (1). Owing to its poor prognosis and easy recurrence, efforts have been made to improve the standardization of chemotherapy and risk classification, such as the risk classification of the 2017 European LeukemiaNet (ELN) (2). However, the currently used prognostic risk stratification criteria for AML focus mainly on mutations seen in cytogenetic analysis and are, therefore limited in terms of accurate prognostic prediction. Improvement in accurate and individualized risk assessment is thus required.

Lipids are important components of cell membranes and have been associated with the underlying mechanisms of cancer progression, including excess proliferation and aberrant signaling. In addition, lipid metabolism plays a key role in cellular energy supply and signaling, as well as other essential aspects of tumor cell proliferation. Due to the increased metabolism and proliferation of tumor cells, dyslipidemia is typically observed in various tumor patients with a variety of tumor types (3). A series of recent studies have illustrated that the serum lipid profile (including apolipoprotein [Apo] A-1, Apo B, cholesterol [CHO], triglycerides [TG], high-density lipoprotein [HDL], and low-density lipoprotein (LDL]) is valuable in tumor prognosis prediction (4–6) and, as a result, the lipid profile has been considered as a promising therapeutic target (7–9). These studies include a report by our colleagues that demonstrated an association between lipid metabolism and the prognosis of patients with multiple myeloma (10), which made us wonder about the role of lipid metabolism in other hematological tumors. As far as we know, few studies have focused on the relationship between serum lipid levels and survival outcomes in patients with AML.

In the present study, we investigated the role of lipid and apolipoprotein profiles as prognostic indicators in AML. Not only did we retrospectively analyze the lipid characteristics of AML patients and explore their value in predicting disease prognosis when combined with current clinical indicators, but we also developed a lipid profile-based model which was found to have improved prognostic precision.

Materials and methods

We performed a retrospective analysis of the clinical data of 273 AML patients. The inclusion criteria were patients who had been pathologically diagnosed with AML and were first treated in the Sun Yat-sen University Cancer Center (SYSUCC) between Dec 2000 and May 2021. The exclusion criteria were: a) patients with acute promyelocytic leukemia; b) patients who had previously taken or were regularly taking lipid-lowering medication, or who had a combination of chronic metabolic diseases such as diabetes mellitus, chronic renal failure, or abnormal liver function at the time of initial diagnosis; c) patients with missing lipid profile data; d) patients with incomplete follow-up data or whose survival periods were too short to analyze; e) patients with other malignant diseases. After the collection of information on treatment, a further 17 cases were excluded as they had not received the standard induction remission chemotherapy with cytarabine and DNR (Daunorubicin)/IDR(Idarubicin) (DNR 50 mg/m2, d1–3 + Ara-C 100 mg/m2, d1–7; or IDA 12 mg/m2, d1–3 + Ara-C 100 mg/m2, d1–7). Finally, 214 patients were selected for analyses (10) and were arbitrarily separated into the training (TC, n = 107) and validation cohorts (VC, n = 107).

Clinical information and serum lipid characterization

Patient data were collected after diagnosis and before treatment. The following baseline demographics were obtained and analyzed: age, sex, white blood cell count (WBC), apolipoprotein AI (Apo A-1), apolipoprotein B (Apo B), cholesterol (CHO), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), lactate dehydrogenase (LDH), body mass index (BMI), dates of diagnosis, death, or last follow-up, and cytogenetic risk classification based on the 2017 ELN criteria along with information on initial therapy. Laboratory examinations were performed on fresh blood samples obtained from patients after overnight (ON) fasting. The median duration between blood collection and treatment initiation was 14 days (3-25 days). This duration was not based on the patients' OS.

Follow-up and study endpoints

Following treatment, patients were monitored *via* hospital outpatient appointments or telephone conversations. Interviews were conducted once in six months for the first three years, and then annually to assess relapse or death. The last follow-up visit was dated July 31, 2021, to verify the final status of the study participants and to exclude those who could not be contacted. The primary endpoint was overall survival (OS), described as the duration of time between diagnosis and death for patients who had died or between diagnosis and the last follow-up for those that had survived.

Threshold identification for prognostic indicators

To assess possible prognostic indicators among lipid and apolipoprotein profiles, X-tile software (3.6.1)16 was used to identify the optimal OS-based threshold. The patients were then stratified into low- (LR) and high-risk (HR) sub-cohorts. Univariate and multivariate Cox regression analyses were then used to identify potential independent prognostic values associated with the lipid profile. Optimum cutoff values were determined by X-tile as follows: Age (60 years), WBC (76×10^9/L), Apo-A1 (0.7 g/L), Apo B (0.65 g/L), CHO (2.67 mmol/L), TG (2.55 mmol/L), HDL (0.45 mmol/L), LDL (1.43 mmol/L), LDH (187.9 U/L), and BMI (19.3).

Statistical analysis

SPSS version 26 (IBM Corporation, Armonk, NY, USA) was used for all data analyses. Continuous variables were analyzed using one-way ANOVA and categorical variables by χ^2 or Fisher exact tests. Continuous data are presented as the mean ± standard deviation. After determining the optimal cutoff value for classifying continuous variables as categorical variables by X-tile software, univariate and

multivariate Cox regression hazard models were employed to identify the independent prognostic indicators for AML patients.

Subsequently, optimal weighting coefficients for the stand-alone prognostic indicators were identified using least absolute shrinkage and selection operator (LASSO) regression analysis. OS analyses were conducted using Kaplan-Meier (KM) curves. The prognostic power of the model was determined using time-dependent receiver operating characteristic (ROC) curves and areas under the curve (AUCs). These analyses were initially conducted in the TC followed by verification in the VC. A two-tailed P-value <0.05 was set as the significance threshold. R software (version 3.6.3 for Windows, http://www.R-project.org) was used for significance estimation.

Results

Subject recruitment and demographics

A total of 214 AML patients who fulfilled the criteria for lipid and survival information were enrolled and separated into the TC (n=107) and VC (n=107) cohorts as described in the flow chart (Figure 1); the baseline clinical data of the patients are provided in Supplemental Table 1. The median age was 45 years. Ninety-one patients (42.5%) were male and 123 (57.5%) were female. According to the 2017 ELN cytogenetic risk classification, 48 patients (22.4%) were categorized as favorable, 49 patients (22.9%) as intermediate, and 63 patients (29.4%) as adverse, while the cytogenetic information on 54 patients was not available. Patients with positive mutations in the TP53 or RUNX1 genes were classified as high-risk, patients with mutations in NPM1 or CEPBA were classified as low-risk, and the remaining patients were classified as intermediate risk. The distribution of the patients' lipid and other clinical characteristics are shown in Table 1. Approximately 51% of cases died before the date of the last follow-up.

Identification of independent prognostic features

The clinical characteristics were divided into categorical variables based on the optimum cut-off values, and univariate and multivariate analyses were performed to identify indices of prognostic value. Univariate analysis showed that the indices of the lipid profile, namely, Apo A-I, Apo B, CHO, TG, LDH, HDL, and LDL were

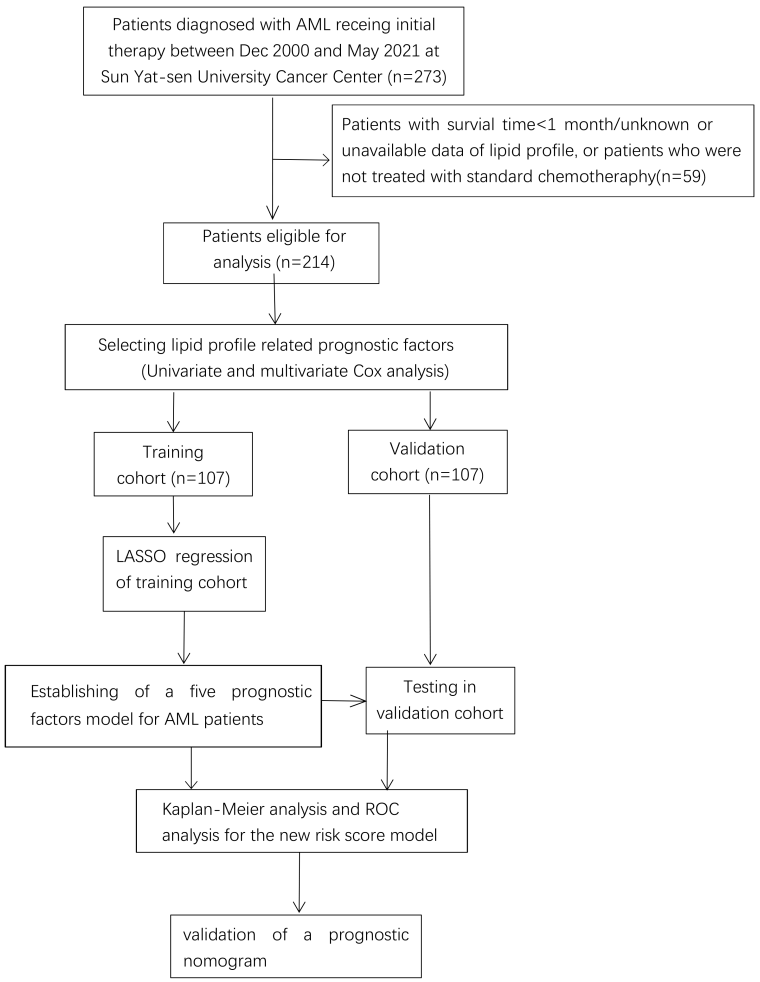

FIGURE 1
Flow chart of data collection and analysis.

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TABLE 1 Univariate and multivariate COX analysis of OS in AML patients.

Variables	Hazard Ratio	Std. Err.	p	[95% Conf. Interval]	
Univariate analysis					
Gender (male vs. female)	1.188	0.187	0.356	0.824	1.712
Age (≥60 vs. <60)	3.637	0.207	**0.000**	2.426	5.452
Apo A-I (>0.7 vs. ≤0.7g/L)	0.310	0.188	**0.000**	0.214	0.448
Apo B (>0.65 vs. ≤0.65 vs.)	0.373	0.185	**0.000**	0.373	0.537
CHO (>2.67 vs.≤2.67mmol/L)	0.275	0.198	**0.000**	0.183	0.405
TG (>2.55mmol/L vs.≤2.55)	3.052	0.212	**0.000**	2.013	4.628
HDL (>0.45 vs. ≤0.45 mmol/L)	0.191	0.215	**0.000**	0.126	0.292
LDL (>1.43 vs. ≤1.43mmol/L)	0.373	0.212	**0.000**	0.246	0.565
LDH(≥250 vs. <250 U/L)	1.081	0.184	0.673	0.753	1.552
WBC (>76 vs. ≤76*10^9/L)	1.487	0.205	0.053	0.995	2.222
Multivariate analysis					
Age(≥60 vs. <60)	2.545	0.239	**0.000**	1.592	4.068
Apo A-I (>0.7 vs.≤0.7 g/L)	0.471	0.229	**0.001**	0.301	0.739
Apo B (>0.65 vs.≤0.65 g/L)	0.556	0.261	**0.024**	0.317	0.825
CHO (>2.67 vs.≤2.67 mmol/L)	0.525	0.371	**0.042**	0.272	0.867
TG (>2.55 vs.≤2.55 mmol/L)	2.170	0.271	**0.004**	1.275	3.694
HDL (>0.45 vs.≤0.45 mmol/L)	0.359	0.290	**0.000**	0.203	0.634
LDL (>1.43 vs.≤1.43 mmol/L)	1.605	0.330	0.152	0.840	3.065

OS, overall survival; LDH, lactate dehydrogenase; TC, total cholesterol; TG, tri-glyceride; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; Apo B, Apolipoprotein B; Apo A-I, Apo Apolipoprotein A-I.
Bold fonts indicate that the p-value is statistically significant.

significant prognostic factors (p<0.05) while multivariate analysis showed that Apo A-I, Apo B, TG, CHO, and HDL were independent prognostic factors (Table 1, p<0.05).

To verify the definitions of the optimal cutoff values, KM OS analysis was used to compare the differences in OS associated with reduced and elevated lipid and apolipoprotein levels, respectively (Figures 2A–E). This showed a significant reduction in OS in patients with low Apo A-I, Apo B, HDL, and CHO, compared with patients with elevated levels (P<0.05) (Figures 2A–D), while significantly longer OS was observed in patients with lower TG levels compared with patients with high TG (p<0.05) (Figure 2E).

Generation and verification of lipid profile risk scores (RS)

Cox analyses were first used to identify factors that were significantly associated with the prediction of prognosis. Next, LASSO regression analysis was used to generate a prognostic model using the five identified prognostic factors (Apo A-I, Apo B, TG, HDL, and CHO) in the TC. After calculation of the best weighting coefficients by the regularization parameter lambda and the 1-SE criteria (Supplement Figure 1), a five-factor prognostic model was selected to be included using the equation: RS= - 0.23× serum Apo A-I levels - 0.84×serum Apo B levels - 0.93×serum HDL levels -0.63× serum CHO levels + 0.93 × serum TG levels, and the RS for each AML patient was calculated as described above. The patients were then assigned to low- (LR) and high-risk (HR) groups according to the median threshold of the lipid-profile RS calculated using the TC data. After grouping, the differences between the HR and LR cohorts were analyzed by one-way ANOVA and χ^2 tests (Table 2). K-M curves showed that patients with low RS values had significantly longer OS (p<0.01) in the entire group (Figure 2F), the TC (Figure 3A), and the VC (Figure 3B).

In terms of the evaluation of the prognostic efficiency, in the TC, the AUCs for the one-, three-, and five-year survival were 0.811, 0.774, and 0.783, respectively (Figure 3C), and were 0.860, 0.838, and 0.857, respectively, in the VC (Figure 3D), indicative of predictive significance. We also used dot plots to compare the distribution of the lipid profile RSs in patients with different OS times. The findings revealed that OS was lengthened in the low-risk group but reduced in the high-risk group (Figures 3E, F) in both cohorts.

Univariate and multivariate analyses

After the measurement of the RS, we transformed the clinical features into categorical variables and repeated the univariate and

multivariate analyses by incorporating the RS and other identified prognostic factors. The univariate analysis indicated that the RS independently predicted OS length and, after the removal of confounders in the multivariate analysis, the RS remained an independent predictor of OS in both cohorts (Figure 4).

Comparison of the prognostic factors

The AUC of the RS model for the whole group for five-year OS was 0.737 and 0.723 for 10-year OS (Figure 5A). The consistency indices (C indices) were assessed for the prognostic co-variates and RSs alone and in combination. A higher C-index represents more accurate assessment results. It was found that the nomogram performed the best (Figure 5B). When compared with other clinical indicators, the RS had the best predictive accuracy: the AUCs of the RS for five-year OS were 0.767 and 0.744 in the TC and VC, respectively, while those for age were 0.620 and 0.641, and for WBC were 0.544 and 0.578. (Figures 5C, D). Overall presentation of the distribution the five-lipid prognostic factor and other clinicopathological characteristics in different risk scores for the TC and the VC were displayed by the heatmap (Figures 5E, F).

To create a better means of evaluation, the cytogenetic results, age, and the metabolic model were integrated into a nomogram (Figure 6A). The calibration plots exhibited satisfactory nomogram performance in estimating the one-, three-, five-, and seven-year OS (Figure 6B). The AUCs of the overall scores for the one-, three-, five-, and seven-year OS were 0.881, 0.908, 0.987, and 0.988, respectively, which were significantly higher than the 2017 ELN cytogenetic classification or the age alone, indicating that the nomogram was more effective in predicting OS than traditional prognostic markers (Figures 6C–F).

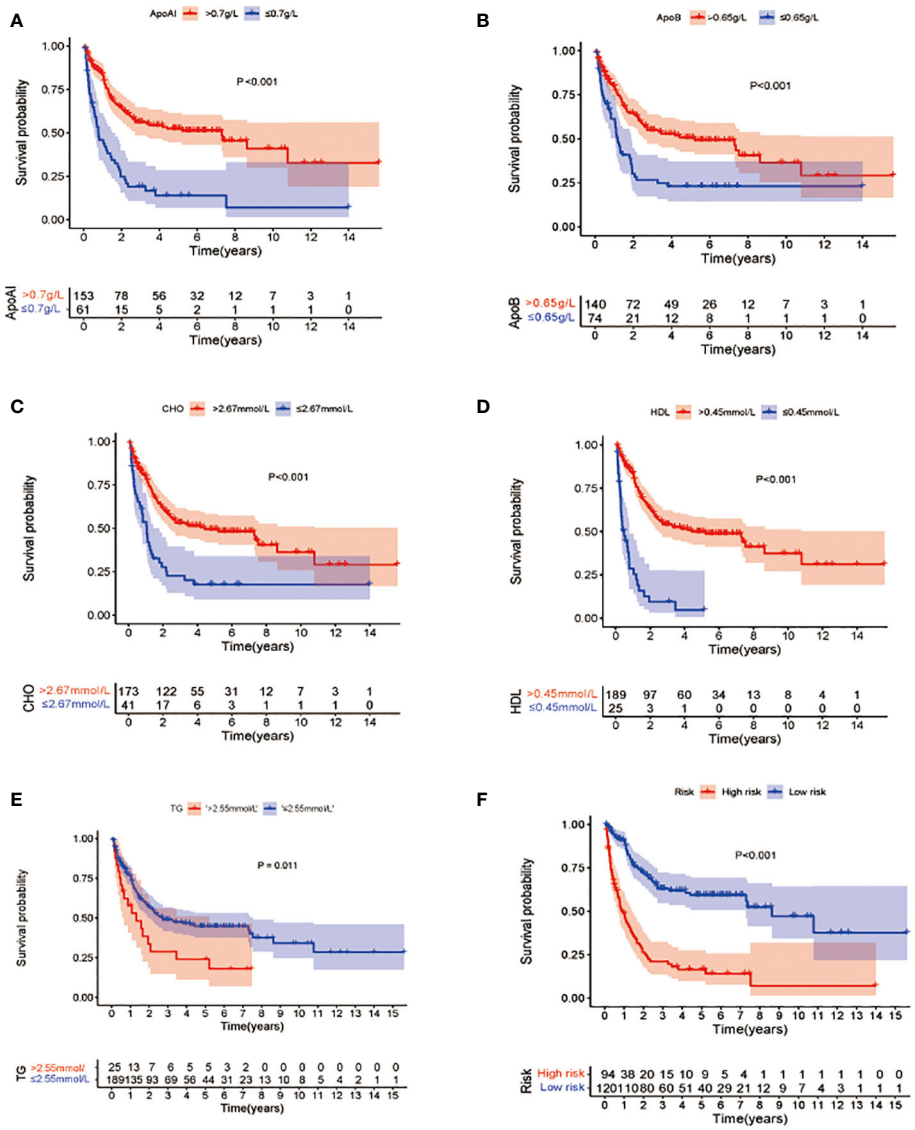

FIGURE 2
Kaplan-Meier survival analysis of overall survival between patients groups with low and high levels of lipid profile biomarkers **(A–E)**. **(A)** OS stratified by the level of Apo A-I ≤0.7 vs >0.7 g/L (p<0.001). **(B)**: OS stratified by the level of Apo B ≤0.65 vs >0.65 g/L (p<0.001). **(C)** OS stratified by the level of CHO ≤2.67 vs >2.67 mmol/L(p<0.001). **(D)** OS stratified by the level of HDL ≤0.45 vs >0.45 mmol/L (p <0.001). **(E)** OS stratified by the level of TG ≤2.55 vs >2.55 mmol/L (p<0.001). **(F)** Survival differences between high- and low-risk groups in the whole cohort.

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TABLE 2 The detailed characteristics of patients and correlation between clinicopathological features and risk score level in the training and validation cohorts.

Characteristics	training cohort(n=107)		P-value	Validating cohort(n=107)		P-value
	High risk, n(%)	Low risk, n(%)		High risk, n(%)	Low risk, n(%)	
Patient	48	59		53	54	
age			0.077			**0.042**
<60	36 (75.0)	52 (88.1)		39 (73.6)	48 (88.9)	
≥60	12 (25.0)	7 (11.9)		14 (26.4)	6 (11.1)	
gender			0.175			0.365
female	25 (52.1)	23 (39.0)		19 (35.8)	24 (44.4)	
male	23 (47.9)	36 (61.0)		34 (64.2)	30 (55.6)	
BMI			0.384			0.711
<19.3	15 (31.3)	14 (23.7)		14 (26.4)	16 (29.6)	
≥19.3	33 (68.8)	45 (76.3)		39 (73.6)	38 (70.4)	
WBC			0.408			0.314
<76	33 (68.8)	46 (78.0)		40 (75.5)	45 (83.3)	
≥76	15 (31.3)	13 (22.0)		13 (24.5)	9 (16.7)	
Apo B			**0.000**			**0.000**
≤0.65	32 (66.7)	11 (18.6)		37 (69.8)	10 (18.5)	
>0.65	16 (33.3)	48 (81.4)		16 (30.2)	44 (81.5)	
Apo A1			**0.000**			**0.001**
≤0.7	25 (52.1)	6 (10.2)		34 (64.2)	7 (13.0)	
>0.7	23 (47.9)	53 (89.8)		19 (35.8)	47 (87.0)	
CHO			**0.000**			**0.000**
≤2.67	15 (31.2)	2 (3.4)		27 (24.5)	8(14.8)	
>2.67	33 (68.8)	57 (96.6)		26 (75.5)	46(85.1)	
TG			**0.035**			**0.000**
≤2.55	31 (64.6)	59 (100.0)		34 (64.2)	54 (100.0)	
>2.55	17 (35.4)	0 (0.0)		19 (35.8)	0 (0)	
HDL			0.063			**0.000**
≤0.45	17 (35.4)	0 (0.0)		23 (43.4)	0(0.0)	
>0.45	31 (64.6)	59 (59.0)		30 (56.6)	54 (100.0)	
LDH			0.065			0.933
<187.9	25 (52.1)	41 (69.5)		30 (56.6)	31 (57.4)	56
≥187.9	23 (47.9)	18 (30.5)		23 (43.4)	23 (42.6)	
PLR			0.131			**0.015**
<60	29 (60.4)	27 (45.8)		35 (66.0)	23 (42.6)	
≥60	19 (39.6)	32 (54.2)		18 (34.0)	31 (57.4)	
NLR			0.621			0.775
<3.3	5 (10.4)	8 (13.6)		5 (84.4)	6 (11.1)	
≥3.3	43 (89.6)	51 (86.4)		48 (15.6)	48 (88.9)	
Cytogenetic risk classification			0.591			0.116
Favorable	8 (26.7)	16 (37.2)		6 (15.4)	18 (33.3)	

(Continued)

TABLE 2 Continued

Characteristics	training cohort(n=107)		P-value	Validating cohort(n=107)		P-value
	High risk, n(%)	Low risk, n(%)		High risk, n(%)	Low risk, n(%)	
Intermediate	14 (46.7)	11 (25.6)		8 (20.5)	16 (29.6)	
Adverse	8 (26.7)	16 (37.2)		25 (64.1)	10 (18.5)	

Apo B, apolipoprotein BI; Apo A-I, apolipoprotein A-I; CHO, cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; LDH, lactate dehydrogenase; Cytogenetic risk classification refers to European LeukemiNet (ELN) 2017 risk classification; NLR, Neutrophil-to-Lymphocyte ratio; PLR, Platelet-to-Lymphocyte ratio.
Bold fonts indicate that the p-value is statistically significant.

Discussion

In this study, we focused on the relationship between cellular dyslipidemia and clinical outcome in AML patients. A model for predicting survival based on retrospective biochemical and clinical data was constructed and confirmed. The model provided an RS calculated from four lipid indices (Apo A-I, Apo B, CHO, TG, and HDL) which are routinely measured in blood biochemical tests. After patients were stratified into HR and LR groups according to the lipid results, it was found that the HR group had poor prognoses. Due to

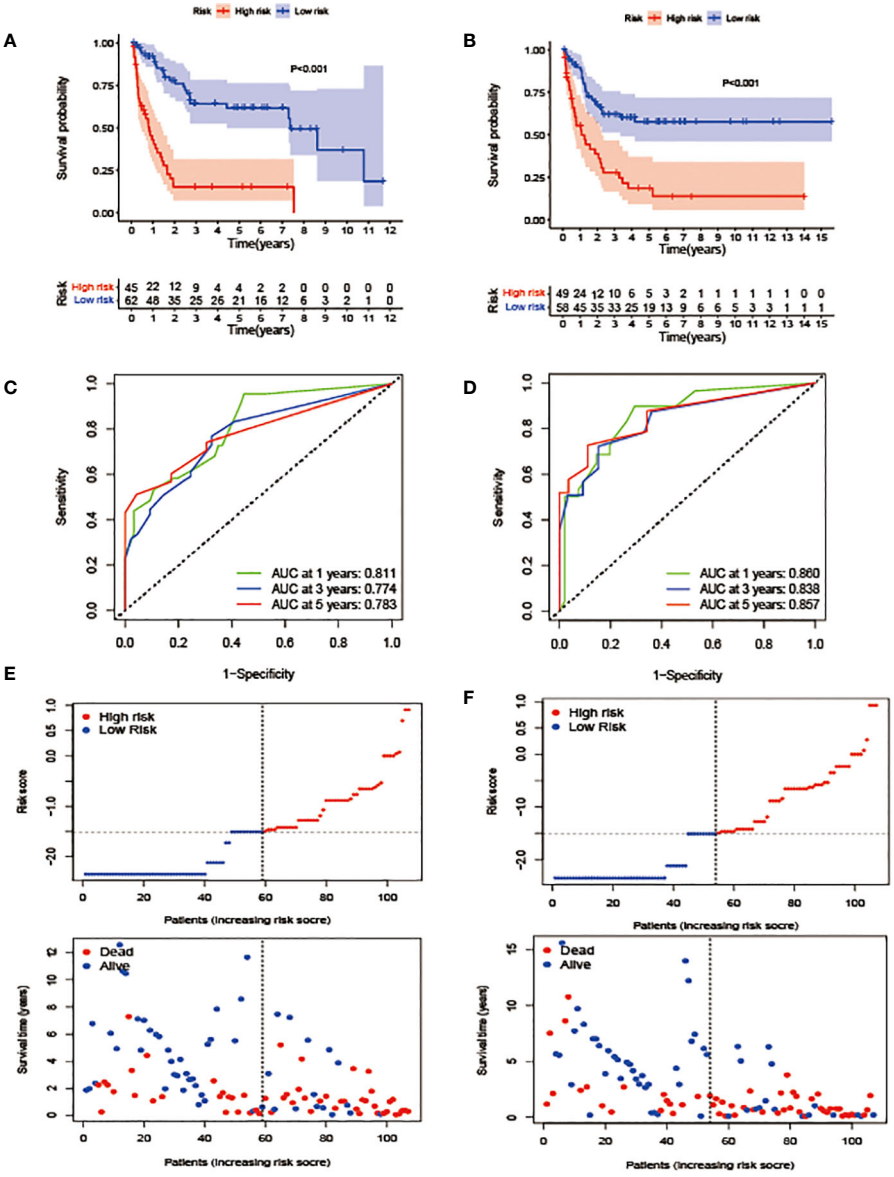

FIGURE 3
Construction and validation of the prognostic model. **(A, B)**. OS stratified by the new lipid profile risk score in the training **(A)** and validation **(B)** cohorts. **(C, D)** Areas under the curve (AUCs) of a receiver-operator characteristic (ROC) curve were compared among the one-, three-, and five-year OS of the prognostic model in the training **(C)** and validation **(D)** cohorts. Higher AUC values indicate greater prediction accuracy. **(E, F)** Risk score analysis of the signature in the high- and low-risk groups in the training and validation cohorts.

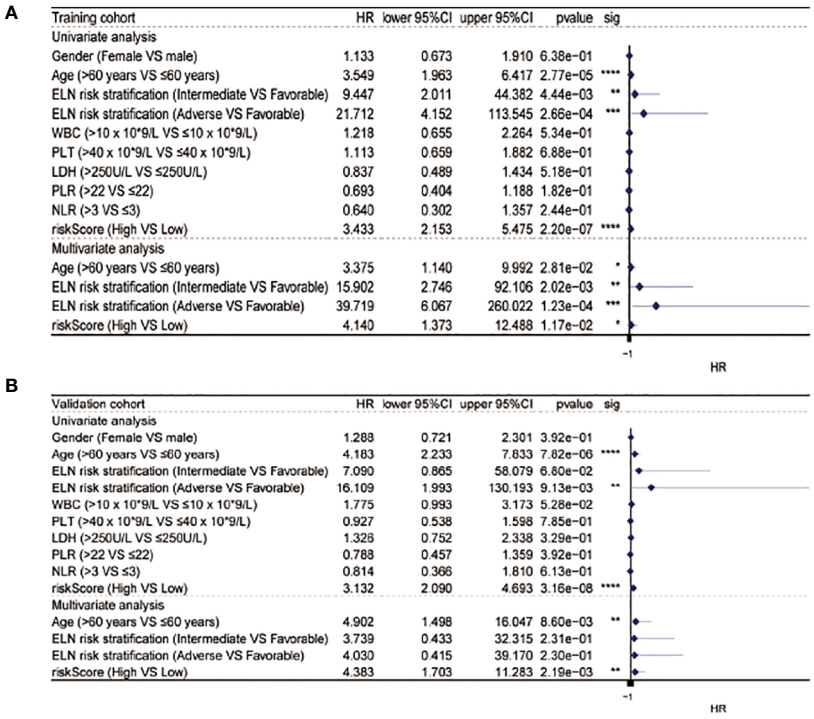

FIGURE 4
Univariate and multivariate Cox analysis in the training **(A)** and validation **(B)** cohorts. * equals p<0.05; ** equals p<0.01; *** equals p<0.001; **** equals p<0.0001.

the physiological characteristics of disease-sustaining leukemic stem cells, we further combined the RS with other factors including the broadly applied 2017 ELN cytogenetic classification to generate a nomogram. The time-dependent ROC curve illustrated that the merged model was more effective and that the merge significantly enhanced the predictive accuracy.

Several studies have reported abnormalities in the plasma lipid profiles of leukemia patients (8, 9); however, none of these has systematically demonstrated a link between the lipid profile and prognosis. In this study, we sought to use a layer-by-layer analysis to elucidate the association between lipid profiles and OS of leukemia patients, finding a specific connection. To reduce the influence of variations in treatment, we included only patients who had been treated with the standard induction regimen for AML. We created a new risk classification model based on clinical metabolic data and combined it with other prognostic factors for clinical application. Further validation demonstrated that the RS showed significant prognostic differentiation.

After many years of investigation, the diagnosis and treatment of leukemia have matured and been systematized, with hematopoietic stem cell transplantation significantly extending the survival of patients. However, leukemia still has an extremely high mortality rate (1). More comprehensive and precise risk stratification and treatment strategies are urgently needed. The unchecked proliferation of malignant tumor cells creates disordered metabolism in the cells, and recent *in vitro* studies have suggested that certain genetic changes in leukemia cells are associated with enhanced dynamics and metabolism of lipid species in AML (11). The pathogenesis and chemoresistance of leukemia are closely related to abnormal tumor cell metabolic microenvironments including disorders in the lipid profile reflected by serum lipid levels, which has both prognostic and therapeutic target value.

Our study analyzed the relationship between cholesterol, triglycerides, apolipoproteins, and other indicators of body lipid metabolism, and clinical survival outcomes in acute myeloid leukemia from the perspective of macroscopic laboratory indicators. The formula for calculating the risk score derived from the LASSO analysis showed that the triglyceride (TG) level had a greater impact on the assigned score. Triglycerides play a pivotal role in the synthesis and utilization of fatty acids, which play important roles in the use of cellular energy, and an intermediate product of TG synthesis and utilization, diacylglycerol, acts as a second messenger in cellular signaling (12). It has been verified that AML cancer cells are dependent on very long-chain fatty acids for their energy supply (13), which may be related to the abnormal micro-metabolic environment of tumor cells. Increased serum TG levels are frequently observed in leukemia patients, including pediatric patients (14). ApoA-1 is the main component of HDL, which is critical for lipid metabolism and inflammation. Apo B is also synthesized by the liver and is the main structural protein of LDL-CHOL, accounting for about 97% of the total protein content of LDL-CHOL (15). It has been proposed that the products derived from lipoprotein-peroxide interactions could contribute to mutagenicity and carcinogenicity in cells (16). In the present study, we found that the serum Apo A-1 and Apo B levels were positively associated with OS in AML patients. Significant reductions in serum LDL levels have also been reported in AML patients (17). The results of these studies are generally in good agreement with the findings of our analysis.

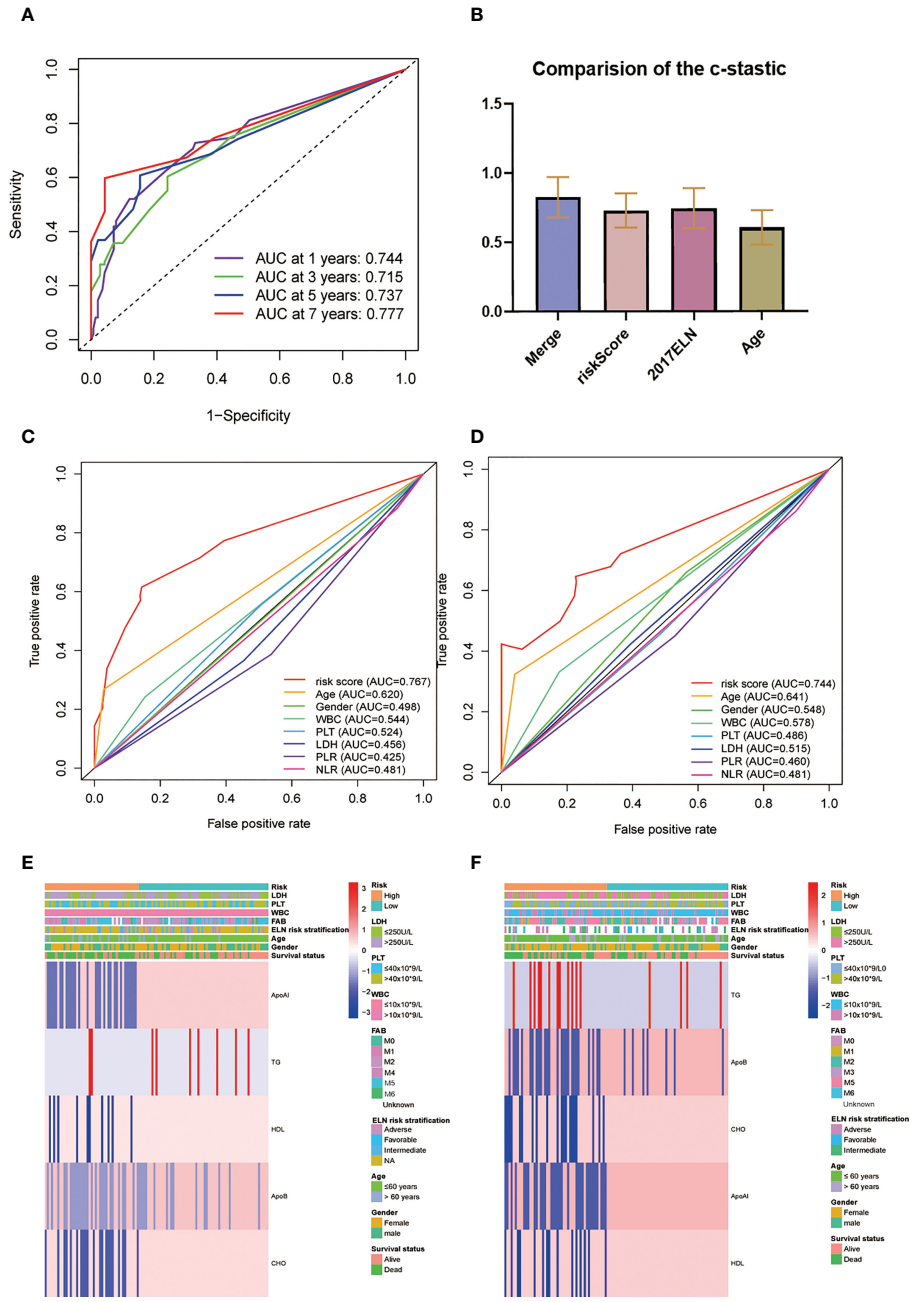

FIGURE 5
(A) Time-dependent ROC analysis for one-, three-, five-, and seven-year OS for the lipid prognostic model. **(B)** C-indices were compared by combining the lipid prognostic model with the 2017 ELN cytogenetic risk classification and others. A higher C-index indicates greater precision in prediction. **(C, D)** AUCs of the five-year risk score model in the training **(C)** and validation **(D)** cohorts were significantly different from other clinical indicators. **(E, F)** Heatmap of the five-lipid prognostic factor and other clinicopathological characteristics in different risk scores for the training **(E)** and the validation **(F)** cohorts.

Several studies have suggested that leukemia stem cells (LSCs) with the potential for self-renewal are responsible for disease sustainability, and traditional cyto-cycling or cytotoxic drugs are unable to target these relapse-related stem cells (18). It has been shown that after administration of drugs that disrupt cellular lipid homeostasis, it is possible to specifically kill LSCs without affecting normal hematopoietic stem cells (19). This may be related to the observation that LCSs from patients with relapsed AML are able to undergo oxidative phosphorylation for energy supply through fatty acid metabolism, whereas this process only occurs through amino acid metabolism in LSCs from novo AML patients (20). These discoveries suggest new directions for precision-targeting of the leukemic metabolic microenvironment, while several studies have reported re-normalization of serum lipid level after standard chemotherapy (21). At the same time, several investigations into the targeting of lipid metabolism at the molecular level and the identification of novel therapeutic targets in AML cells have confirmed a specific association between abnormal metabolism, such as lipid peroxidation, and the biological behavior of tumor cells. The form of cell death associated with lipid peroxidation has also been linked to ferroptosis in tumor cells (22). These results have led us to speculate about the role that targets related to metabolic

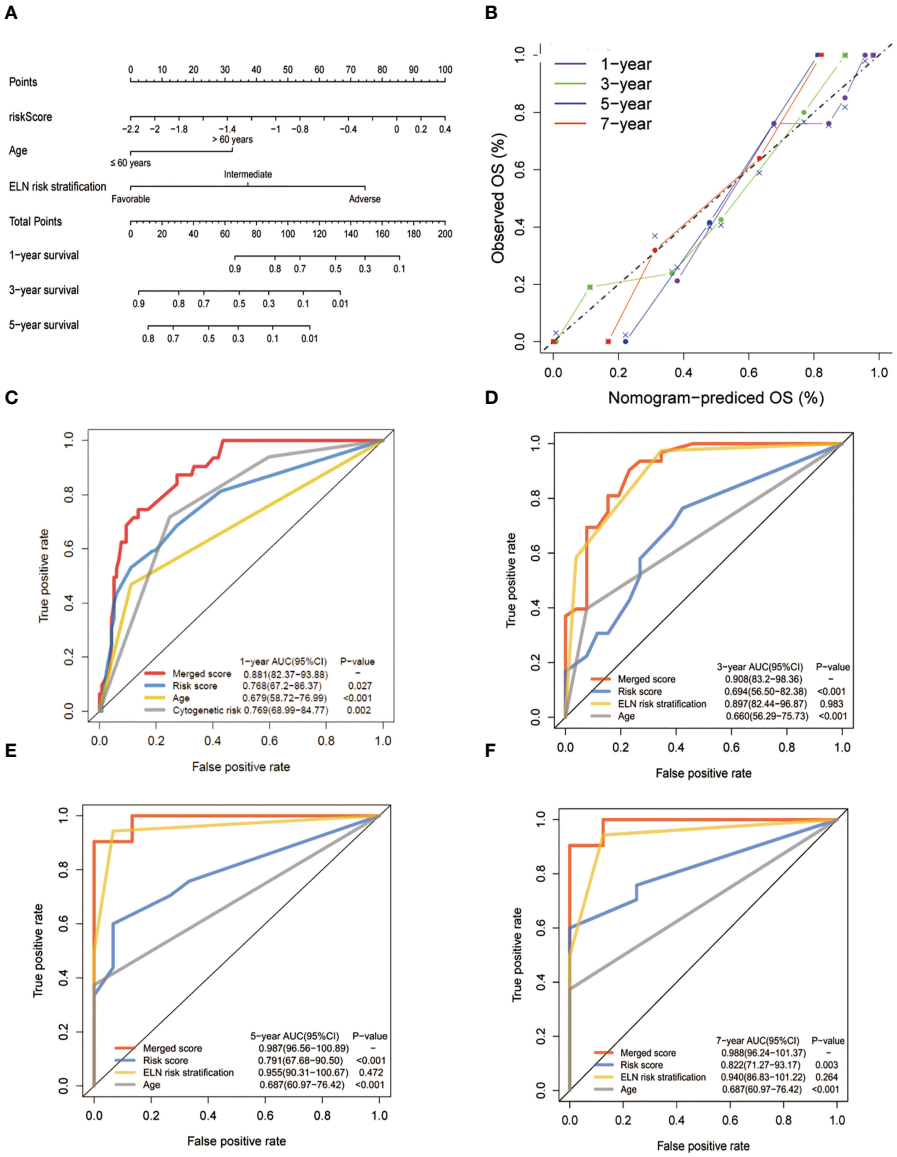

FIGURE 6
Construction and validation of the nomogram for prediction of OS in patients with AML. **(A)** The nomogram plot was constructed based on the lipid risk score and the 2017 ELN cytogenetic risk classification. **(B)** Calibration plot of the nomogram. **(C-F)** AUCs of one-,three-, five-, and seven-year OS. The lipid profile risk score showed greater predictive accuracy than the 2017 ELN classification for one-year OS while the merged model showed greater predictive accuracy for all observed years.

processes such as fatty acid energy supply and lipid peroxidation could play in the future treatment of AML.

On the other hand, since most of the chemotherapy drugs used for treating leukemia are highly toxic, their effects on liver and kidney functions cannot be ignored (23). The standard induction chemotherapy regimen for AML patients is based on high doses of anthracyclines and cytarabine. Whether the effects of abnormal lipid levels before treatment combined with the application of cytotoxic chemotherapeutic agents on the metabolic function of the body influences patient survival outcomes require further investigation. Our analysis of the data failed to elucidate clear differences in serum lipid markers before and after treatment due to some missing data. Research to further clarify the relationships and mechanisms between them is required.

As described above, although the significance of lipid parameters in predicting survival has been confirmed, there were, nevertheless, some limitations to this process. When time-dependent ROC curves were applied in the entire patient cohort, we noticed that the AUCs of the three- and five-year RS values were below those of the 2017 ELN risk classification (0.761 vs. 0.903 and 0.763 vs. 0.909, respectively). ELN staging incorporates a variety of AML prognosis-related genes, including gene mutations related to lipid metabolism, and has been demonstrated to be a complete acute myeloid prognosis-stratified management system (24, 25). while the intention of our RS is to illustrate the relationship between serum lipid levels and prognosis in AML patients from a macroscopic blood biochemistry perspective. In general, this novel RS model supports the 2017 ELN cytogenetic risk classification through integration into a nomogram. Research

conducted in Japan has reported that statins used for the control of blood lipid levels reduced the transcription of AML-1A, a MIP-1α transcription factor (26), suggesting there are many associations between serum lipid levels and AML prognosis that are worth exploring. Recent advances in high-throughput sequencing (next-generation sequencing) technology have made it possible to detect precise mutations associated with AML, not only for the highly sensitive detection of molecular measurable residual disease (MRD) after chemotherapy but also for the detection of mutations at loci that can determine the prognosis of the disease, such as FLT3 and EVI1 (27). After further research to elucidate the mechanisms linking cellular oxidative energy supply processes such as lipid metabolism to the biological behaviors of AML tumor cells, perhaps next-generation sequencing could also be applied to the detection of metabolism-related gene mutations, including lipids, to guide precision therapy and early clinical intervention in AML patients.

Despite the interesting data, this research has certain limitations. First, the data were collected over an extended period and it was not possible to evaluate them systematically using a unified assessment program. Second, all patient data were from a single institution, which may introduce potential bias. The sample population was relatively small and additional studies are warranted to assess whether the optimal threshold is applicable on a wider scale. Additional large, prospective, multicenter investigations would be needed to confirm our conclusions, and a quantifiable, clinically guided, and simply operationalized risk scoring system is currently lacking. Lastly, the possible molecular biological mechanisms involving lipid metabolism in AML development and progression and its therapeutic value remain to be systematically explored in a more specific manner.

Conclusion

Using real-world clinical data as a foundation, a model was constructed using data on serum lipid profiles to estimate OS in AML patients. Lipid profiles can thus be used as new prognostic indicators to enhance the predictive precision of traditional factors including the revised 2017 ELN genetic risk stratification of AML and may promote the future study of incorporating lipid metabolism in the precision regulation of treatment regimens for patients with AML.

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 authors.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committees of Sun Yat-sen University Cancer Center. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

Study concept and design: HZW, SB, and RS; data collecting: JW and SL; statistical analysis: HZW, RS, and SB; figure and tables preparation: SB, HZW, and RS; writing-original draft: SB and HZW; data and tables inspection and validation: SB and BF; project administration: YL and HW; work supervision: YL and HW; Writing-review and editing: YL and HW; Funding acquisition; YL. Critical revision of the manuscript for important intellectual content: all authors. All authors contributed to the article and approved the submitted version.

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.950732/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Construction of the lipid profile-related model. A. 1000 bootstrap replicates by LASSO regression analysis for variable selection. B. LASSO coefficients of lipids and apolipoproteins. Each curve represents a factor.

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Agents contributing to secondary immunodeficiency development in patients with multiple myeloma, chronic lymphocytic leukemia and non-Hodgkin lymphoma: A systematic literature review

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Introduction: Patients with hematological malignancies (HMs), like chronic lymphocytic leukemia (CLL), multiple myeloma (MM), and non-Hodgkin lymphoma (NHL), have a high risk of secondary immunodeficiency (SID), SID-related infections, and mortality. Here, we report the results of a systematic literature review on the potential association of various cancer regimens with infection rates, neutropenia, lymphocytopenia, or hypogammaglobulinemia, indicative of SID.

Methods: A systematic literature search was performed in 03/2022 using PubMed to search for clinical trials that mentioned in the title and/or abstract selected cancer (CLL, MM, or NHL) treatments covering 12 classes of drugs, including B-lineage monoclonal antibodies, CAR T therapies, proteasome inhibitors, kinase inhibitors, immunomodulators, antimetabolites, anti-tumor antibiotics, alkylating agents, Bcl-2 antagonists, histone deacetylase inhibitors, vinca alkaloids, and selective inhibitors of nuclear export. To be included, a publication had to report at least one of the following: percentages of patients with any grade and/or grade ≥3 infections, any grade and/or grade ≥3 neutropenia, or hypogammaglobulinemia. From the relevant publications, the percentages of patients with lymphocytopenia and specific types of infection (fungal, viral, bacterial, respiratory [upper or lower respiratory tract], bronchitis, pneumonia, urinary tract infection, skin, gastrointestinal, and sepsis) were collected.

Results: Of 89 relevant studies, 17, 38, and 34 included patients with CLL, MM, and NHL, respectively. In CLL, MM, and NHL, any grade infections were seen in 51.3%, 35.9% and 31.1% of patients, and any grade neutropenia in 36.3%, 36.4%, and 35.4% of patients, respectively. The highest proportion of patients with grade ≥3 infections across classes of drugs were: 41.0% in patients with MM treated with a B-lineage monoclonal antibody combination; and 29.9% and 38.0% of patients with CLL and NHL treated with a kinase inhibitor combination, respectively. In the limited studies, the mean percentage of patients with lymphocytopenia was 1.9%, 11.9%, and 38.6% in CLL, MM, and NHL, respectively. Two studies reported the proportion of patients with hypogammaglobulinemia: 0–15.3% in CLL and 5.9% in NHL (no studies reported hypogammaglobulinemia in MM).

Conclusion: This review highlights cancer treatments contributing to infections and neutropenia, potentially related to SID, and shows underreporting of hypogammaglobulinemia and lymphocytopenia before and during HM therapies.

KEYWORDS

secondary immunodeficiency, hematological malignances, neutropenia, hypogammaglobulinemia, secondary antibody deficiency, B-lineage monoclonal antibodies, Bruton kinase inhibitors

1 Introduction

1.1 Secondary immunodeficiency (SID) in patients with hematological malignancies (HMs)

SID is a group of disorders in which cell-mediated immunity and/or humoral immune responses are compromised by non-inherited factors, increasing the risk of infections (1, 2). SID can be caused by several factors, including non-genetic metabolic diseases (e.g., protein-losing enteropathy, diabetes mellitus, chronic kidney disease, etc.), malnutrition, medications and malignancies, among others (2–4). Patients with HMs, including chronic lymphocytic leukemia (CLL), multiple myeloma (MM), and non-Hodgkin lymphoma (NHL), have a higher risk of SID, SID-related infections, and mortality compared with immunocompetent individuals (3, 5). Their risk of developing SID and SID-related infections is influenced by the distinct intrinsic pathophysiology of the disease, the use of and exposure time to different cancer treatments, and the presence of certain comorbidities, such as chronic lung or heart disease, kidney failure, diabetes, chronic obstructive pulmonary disease, and hypertension, of which some are caused or aggravated by cancer treatments (1, 3, 5–8). Interestingly, differences exist in both the sites and pathogen spectrum associated with certain HMs and their treatments (9–13), which might also be different from those observed in primary immunodeficiency (PID) (14). Additionally, there is a growing body of evidence that suggests that PID-related genes might influence the development of certain HMs and the likelihood of SID development in cohorts of patients with HMs (15–19).

1.2 Agents contributing to SID development in patients with HMs

Various agents used to treat HMs have been reported to increase the risk of infection due to their mode of action or as associated adverse effects on the immune system that are not clearly related to the pharmacologic activity of the molecule (2, 5, 8). These agents can affect the innate and/or adaptive immune systems in different ways, depending on which component they target (e.g., neutrophils, dendritic cells [DC], granulocytes, monocytes, and macrophages, which regulate the innate immune response; antibodies, B and T cells, which regulate the adaptive immune response; natural killer cells [NK], which are involved in both the innate and adaptive immune responses) (20, 21).

Anti-cancer monoclonal antibodies can be detrimental to both the innate and the adaptive immune systems based on the antigens they target. For instance, anti-CD20 antibodies primarily induce B-cell depletion, since CD20 is expressed by B cells only. However, since CD52 is expressed by T cells, B cells, granulocytes, monocytes, macrophages, NK cells, and DC, monoclonal antibodies directed against CD52 will impact both the innate and adaptive immune systems (22, 23). In addition, monoclonal antibodies can lead to infections, neutropenia, and sometimes cause a prolonged delay of functional recovery of the targeted cell population (24–28). In a similar way, the effects of chimeric antigen receptor T-cell (CAR T) therapies on the immune systems are influenced by which antigens the T cells are engineered to target; but can also lead to other adverse events related directly to its mode of action (e.g., cytokine release syndrome and hypogammaglobulinemia) and other adverse effects considered 'on-target off-tumor', like infections, neutropenia, and fatigue (22, 24, 29).

Proteasome inhibitors can induce neutropenia, reduce the number of T cells, NK cells and DC, alter NK-cell and CD8+ T-cell function, and cytokine production, therefore affecting both innate and adaptive processes (1, 30). Several kinases are involved in both the proliferation, activation, and survival of malignant cells, as well as the regulation of signaling pathways of immune cells (e.g., granulocytes, monocytes, DC, and NK cells for the regulation of the innate immune response; antibody production, T and B cells for the regulation of the adaptive immune response) (31).

Kinase inhibitors have drastically helped manage HMs; however, they can compromise the correct functioning of different immune cells, leading to infections and neutropenia (24, 31). For instance, ibrutinib inhibits the Bruton's tyrosine kinase (BTK), which regulates granulocyte and monocyte function, DC maturation and activation, and B-cell development (31–33).

The precise mode of action of immunomodulatory imide drugs (IMiDs) remains unclear and current hypotheses are mainly based on *in vitro* studies. The immune modulation of IMiDs has been linked to both the innate and adaptive immune responses, including CD4+ and CD8+ T-cell co-stimulation, NK-cell activation, regulatory T-cell (T_{reg}) suppression, cytokine production, neutropenia, and increased antibody-dependent cellular cytotoxicity (1, 34). Finally, several drugs with a systemic mode of action not directly linked with the immune system have been used to treat patients with HMs and have adverse events associated with immune system dysfunction. For instance, cytotoxic conventional chemotherapeutics have been shown to affect both the innate and adaptive immune systems by targeting DC, T_{reg}, NK cells, cytokine production, and neutrophil and macrophage activity (35). Therefore, physicians need to be aware of the likely immunodeficiency resulting from the combined use of these agents, which affect the correct functioning of multiple immune cell types.

1.3 Secondary antibody deficiency (SAD), neutropenia, lymphocytopenia, and hypogammaglobulinemia

Various sub-types of SID have been described based on the components of both the innate and adaptive immune systems that are missing and/or are impaired/malfunctioning (4). For instance, neutropenia, loss of skin and mucosal barrier function, as well as reduced phagocytosis and cytotoxicity are examples of SID related to the innate immune response (4). On the other hand, compromised antibody function and production, and impaired T cells are examples of SID related to the adaptive immune response (4). In an increasing number of cases, defects in T, B, and NK cells may be present at the same time resulting in a combined immunodeficiency (CID) (15).

In this systematic literature review, we will focus on SAD, neutropenia, and lymphocytopenia or diminished lymphocyte function. SAD is defined as a reduction in serum immunoglobulin (Ig) concentration and/or diminished Ig function/quality (3), with hypogammaglobulinemia specifically referring to the aspect of reduction in serum Ig concentration rather than loss of functionality (36). Several cut-offs for hypogammaglobulinemia are used in the literature, suggesting a potential lack of consistency across studies (37–40). The authors agree with the recent expert consensus review published in *Blood Reviews*, where mild (4–6 g/L) and severe (<4 g/L) definitions of hypogammaglobulinaemia are suggested (41).

Neutropenia is a reduction in the absolute number of neutrophils circulating in the blood, graded per the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 5.0 as grade 1, less than the lower limit of normal–1,500 per mm^3; grade 2, 1,499–1,000 per mm^3; grade 3, 999–500 per mm^3; grade 4, <500 per mm^3 (42, 43). Lymphocytopenia is a reduction in the total lymphocyte count (i.e., T cells, B cells, and NK cells) graded per NCI-CTAE, version 5.0 as grade 1, less than the lower limit of normal–800 per mm^3; grade 2, 799–500 per mm^3; grade 3, 499–200 per mm^3; grade 4, <200 per mm^3 or a decreased function of these cells (42–44). However, different institutions may use slightly different reference ranges to determine grading. Lymphocytopenia might not be an ideal marker of a dysfunctional immune system in patients with CLL due to lymphocytosis; however, it could be useful in patients with NHL and MM to define the risk of infections (44–46).

1.4 Unmet needs

Epidemiological data on the prevalence of infections and infection-related mortality in patients with HMs suggest infections may account for up to 50% of deaths in CLL, and up to 22% and 33% of deaths in MM and NHL, respectively (7, 47–49). However, it is difficult to confirm whether these infections are linked to hypogammaglobulinemia, impacts on other immune components, comorbidities, or a combination thereof. Furthermore, data are lacking regarding differences in rates of hypogammaglobulinemia and hypogammaglobulinemia-related infections across HMs and across classes of drugs, rates of lymphocytopenia and related infections, and types of infections across HMs. The lack of data may result in a lesser awareness of the issue of hypogammaglobulinemia and infections within this population and therefore a lower uptake in assessment and management strategies for SID in HMs (Table 1).

1.5 Scope of the systematic literature review

In this systematic literature review, we aim to provide insights into the cancer agents used to treat HMs that are associated with SID, including differences in incidence of SID and infections among patients undergoing systemic treatment for CLL, MM, and NHL.

2 Methods

This review is reported in accordance with PRISMA guidelines on reporting reviews of the literature. On March 16th, 2022, a systematic literature search was performed from the PubMed database, searching for studies that mentioned in the title and/or abstract the following categories of drugs (licensed to treat CLL, MM, or NHL in the EU and US) divided per class of drug (Table 2): monoclonal antibodies, CAR T therapies, proteasome inhibitors, kinase inhibitors, IMiDs, corticosteroids, antimetabolites, anti-tumor antibiotics, alkylating agents, Bcl-2 antagonism through Bcl-2 homology 3 (BH3) mimetic, histone deacetylase (HDAC) inhibitors, vinca alkaloids, or selective inhibitors of nuclear export (SINE). In addition, the search strings included the MeSH terms for three types of HMs that are more indolent

TABLE 1 Current knowledge gaps in the management of SID.

Lack of data
•Differences in rates of hypogammaglobulinemia-related infections •Rates of hypogammaglobulinemia and infections across classes of drugs (e.g., B-lineage monoclonal antibody, CAR T therapies, etc.) •Types of infections across HMs •Development of improved markers of cellular immunodeficiency
Lack of protocol-based approaches
•Testing and monitoring Ig levels in patients with HMs (e.g., when; how often) •Testing lymphocyte count before and during therapy as first step in identifying CID •Determining the functional status of the immune system (e.g., test immunization to assess the response to polysaccharide and polypeptide vaccine challenge)
Lack of awareness
•The impact of cancer agents in developing SID •The risk of death due to SID-related infections

CAR T, chimeric antigen receptor T-cell; CID, combined immunodeficiency; HM, hematological malignancy; Ig, immunoglobulin; SID, secondary immunodeficiency.

than others, in which SID is known to be a current unresolved challenge, and for which sufficient studies were expected to be found in order to carry out the analysis: CLL, or MM, or NHL. Finally, the following studies were included: interventional, or observational, or retrospective, or cohort, or meta-analysis, or prospective, or database, or multicenter, or case-control. Further inclusion criteria were applied to identify articles written in English, including humans, labeled as clinical trials in PubMed, and published between 2011 and 2022. Based on agreement among the authors, this period reflects the rapid evolution of the treatment landscape over the last decade.

This initial search resulted in 738 publications, which were then further refined to include phase III, phase IV and observational studies only, excluding phase I and phase II studies to avoid considering doses or settings that might not reflect the approved labels and are more likely to have fewer patients enrolled compared with phase III and phase IV studies. We obtained 243 publications in total (Supplementary Figure 1) that were then screened for relevance by type of HM, drug regimen, number of patients, year of publication, and class of drug. The screening was performed in parallel to minimize the risk of bias. Double counting was avoided by using the numerical identifier unique to each article and the Excel functionality called 'distinct count'. In order to be included in this systematic literature review, a publication had to report at least one of the following details related to adverse events (defined per the CTCAE): percentages of patients with any grade or grade ≥3 infections; percentages of patients with any grade or grade ≥3 neutropenia; and percentages of patients with hypogammaglobulinemia. These types of infections were selected as they were the most frequently reported and comparable across all studies. In addition, studies that reported grade 1 and/or grade 2 adverse events only were excluded because of incompatibility with the any grade or grade ≥3 events criteria used in our paper.

Of the 243 studies evaluated, 89 were considered relevant. From the relevant publications, the percentages of patients with lymphocytopenia (composition of lymphocytopenia was not specified) and specific types of infection (fungal, viral, bacterial,

TABLE 2 Selected drugs used for the search criteria in PubMed.

Classes of drugs	Agents
Alkylating agents	Bendamustine, chlorambucil, cisplatin, cyclophosphamide, ifosfamide, and melphalan
Antimetabolites	Cladribine, cytarabine, fludarabine, methotrexate, nelarabine, pentostatin, and pralatrexate
Anti-tumor antibiotics	Doxorubicin and pixantrone
BH3 mimetic	Venetoclax
CAR T therapies	Axicabtagene ciloleucel, brexucabtagene autoleucel, idecabtagene vicleucel, and tisagenlecleucel
Corticosteroids	Prednisone and dexamethasone
HDAC inhibitors	Panobinostat and vorinostat
IMiDs	Lenalidomide, pomalidomide, and thalidomide
Kinase inhibitors	Acalabrutinib, duvelisib, ibrutinib, and idelalisib
Monoclonal antibodies	Alemtuzumab, belantamab mafodotin, brentuximab vedotin, daratumumab, elotuzumab, isatuximab, obinutuzumab, ofatumumab, and rituximab
Proteasome inhibitors	Bortezomib, carfilzomib, and ixazomib
SINE	Selinexor
Vinca alkaloids	Vincristine

BH3, Bcl-2 antagonism through Bcl-2 homology 3; CAR T, chimeric antigen receptor T-cell; HDAC, histone deacetylase; IMiDs, immunomodulatory imide drugs; SINE, selective inhibitors of nuclear export.

lower respiratory tract infection [LRTI], upper respiratory tract infection [URTI], sinusitis, nasopharyngitis, respiratory, bronchitis, pneumonia, urinary tract infection [UTI], skin, gastrointestinal [GI], *Candida*, and sepsis) were collected if available. Of note, not all studies reported values for both any grade and grade ≥3 events; this has led to the situation where in some categories, individual studies only reporting grade ≥3 results reported higher levels of grade ≥3 events than other studies did for any grade events, leading to the average of grade ≥3 events being higher than the average for any grade events. These instances are highlighted in the analysis for clarity.

2.1 Types of infection analyses

Further analyses were performed on sinopulmonary bacterial infections and the types of infections that were most reported in the studies evaluated as part of the systematic literature review. The mean percentage of patients with the following types of infections were collected for these analyses: fungal, viral, bacterial, bacteremia, staphylococcal bacteremia, varicella-zoster virus (VZV) reactivation, LRTI, URTI, sinusitis, nasopharyngitis, respiratory, bronchitis, pneumonia, lung, UTI, skin, GI, herpes simplex virus, *Candida* only, and sepsis. Due to some of these descriptors overlapping (e.g., respiratory, lung, LRTI, and URTI), we categorized herpes simplex virus and VZV reactivation within the herpes group viral subtype; sinusitis and nasopharyngitis within the URTI subtype; bacteremia and staphylococcal bacteremia within the bacterial subtype; and lung with the respiratory subtype. Sinopulmonary bacterial infections were calculated by including LRTI, URTI, sinusitis, nasopharyngitis, bronchitis, and/or pneumonia.

3 Results

3.1 Infection and neutropenia rates in patients with CLL, MM, and NHL

Of the 89 relevant publications, 17 included patients with CLL (50–66), 38 with MM (67–99), and 34 with NHL (100–134) (Table 3). The mean proportion of patients who had any grade or grade ≥3 infections was 51.3% and 19.8% in CLL, 35.9% and 16.3% in MM, and 31.1% and 11.3% in NHL, respectively (Table 3). The mean percentage of patients who had any grade neutropenia was 36.3% in CLL, 36.4% in MM, and 35.4% in NHL. The mean percentage of patients with grade ≥3 neutropenia was 29.8% in patients with CLL, 23.2% in patients with MM, and 38.7% in patients with NHL.

In addition, rates of any grade and grade ≥3 infections, neutropenia, and hypogammaglobulinemia were divided into two timeframe groups to reflect changes in the treatment landscape, 2011–2016 and 2017–2022 (Supplementary Table 1). The rates of grade ≥3 infections were higher in the 2017–2022 group versus the 2011–2016 group across CLL, MM, and NHL.

The high variability across studies resulted in extremely wide ranges of neutropenia and infection rates. For this reason, box and whisker plots (Figure 1) were created to locate each percentage of patients within the ranges. As shown in the box and whisker plots, patients with CLL seem to be more susceptible to any grade and grade ≥3 infections than patients with MM and NHL.

3.2 Drug class-related analyses

Drug class-related analyses were performed and included all studies where a B-lineage monoclonal antibody, a proteasome inhibitor, a kinase inhibitor, or immunomodulatory drugs were used either as monotherapy or in combination with different classes of drugs as a doublet or triplet regimen (Table 4). The sum of the number of studies for monotherapy and doublet/triplet regimens in Table 4 may be higher than the total number of studies reported in Table 3 as some studies may have both monotherapy and doublet/triplet arms, therefore may have been counted twice. Only one study on the use of CAR T therapies in patients with NHL resulted from the systematic literature review; the proportion of patients with any grade infection was 29.5% (104).

3.2.1 B-lineage monoclonal antibodies

B-lineage monoclonal antibodies (anti-CD20: rituximab, ofatumumab, and obinutuzumab; anti-CD38: isatuximab and daratumumab; anti-CD30: brentuximab vedotin; anti-CD52: alemtuzumab; anti-CD269: belantamab mafodotin; or anti-CD319:

TABLE 3 Percentages of patients with CLL, MM, and NHL who had infections (any grade and grade ≥3), neutropenia (any grade or grade ≥3), or hypogammaglobulinemia.

Malignancies		Any grade neutropenia*		Grade ≥3 neutropenia*		Any grade infections*		Grade ≥3 infections*		Hypogamma*
Studies (n)		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Range
CLL	17	36.3	9.4–64.0	29.8	3.0–60.0	51.3	14.4–69.1	19.8	6.4–39.0	0.0–15.3
MM	38	36.4	9.8–85.5	23.2	2.0–80.0	35.9	0.0–68.0	16.3	0.0–50.2	–
NHL	34	35.4	3.2–87.5	38.7	0.0–100.0	31.1	4.0–81.0	11.3	0.9–38.0	5.9
Total	89	36.0	3.2–87.5	29.6	0.0–100.0	36.7	0.0–81.0	15.9	0.0–50.2	0.0–15.3

*The reporting criteria for time to adverse events differed across studies.
Neutropenia grades: grade 1, less than the lower limit of normal–1,500 per mm3; grade 2, 1,499–1,000 per mm^3; grade 3, 999–500 per mm^3; grade 4, <500 per mm^3; grade 5, death.
Infection grades: grade 1, –; grade 2, localized, local intervention indicated; grade 3, IV antibiotic, antifungal, or antiviral intervention indicated, interventional radiology or operative intervention indicated; grade 4, life-threatening consequences e.g., septic shock, hypotension, acidosis, or necrosis; grade 5, death.
CLL, chronic lymphocytic leukemia; hypogamma, hypogammaglobulinemia; IV, intravenous; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; –, not reported.

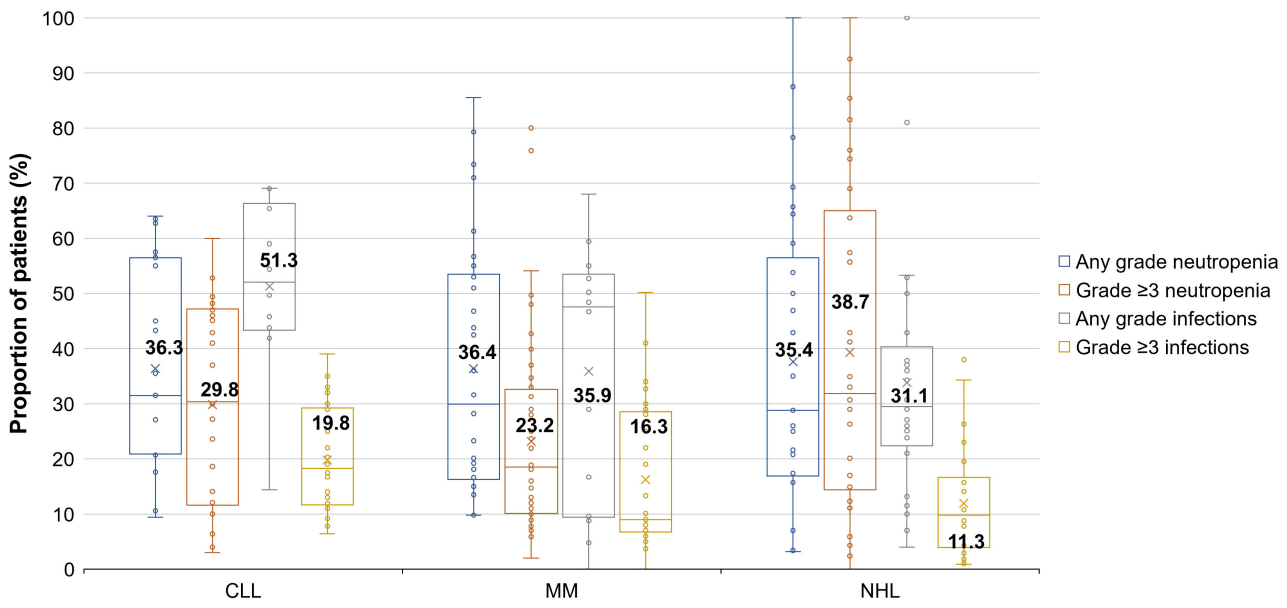

FIGURE 1
Proportions of patients with CLL, MM, and NHL who had infections (any grade and grade ≥3) or neutropenia (any grade or grade ≥3). Each box displays data distribution through their quartile (i.e., upper quartile, median, and lower quartile), with the bars representing the variability outside the upper and lower quartile (i.e., upper extreme and lower extreme). A dot outside the bars represents an outlier. The x symbols and corresponding data label represent the mean values for each data set. Not all studies reported values for both any grade and grade ≥3 events; this has led to the situation where in some categories, individual studies reported higher levels of grade ≥3 events than other studies did for any grade events, leading to the average of grade ≥3 events being higher than the average for any grade events Neutropenia grades: grade 1, less than the lower limit of normal–1,500 per mm^3; grade 2, 1,499–1,000 per mm^3; grade 3, 999–500 per mm^3; grade 4, <500 per mm^3; grade 5, death. Infection grades: grade 1, –; grade 2, localized, local intervention indicated; grade 3, IV antibiotic, antifungal, or antiviral intervention indicated, interventional radiology or operative intervention indicated; grade 4, life-threatening consequences e.g., septic shock, hypotension, acidosis, or necrosis; grade 5, death. CLL, chronic lymphocytic leukemia; IV, intravenous; MM, multiple myeloma; NHL, non-Hodgkin lymphoma.

elotuzumab) were used in three studies in CLL (54, 59, 66), two in MM (68, 91), and seven in NHL (110, 113, 122, 124, 127, 128, 134) as monotherapy. In addition, 14 studies in CLL (50–58, 61–65), five studies in MM (87, 90, 92, 97, 99), and 18 studies in NHL (105–107, 111, 112, 114, 115, 117–121, 124, 127, 130–133) reported use of B-lineage monoclonal antibodies in combination with other agents (Table 4).

The mean proportion of patients treated with a B-lineage monoclonal antibody as monotherapy who had any grade infections was 50.2% in MM and 23.0% in NHL. In patients with CLL, no publications reported the rate of any grade infections when a B-lineage monoclonal antibody was used as monotherapy. Grade ≥3 infections were similar in patients with CLL and NHL regardless of using a B-lineage monoclonal antibody as monotherapy (19.7% in CLL and 3.6% in NHL) or in combination with other agents (20.6% in CLL and 8.5% in NHL); however, grade ≥3 infections were numerically lower in patients with MM treated with a B-lineage monoclonal antibody as monotherapy (13.3%) compared with patients treated with doublet/triplet regimen that included a B-lineage monoclonal antibody (41.0%; Table 4). The mean proportion of patients who reported any grade and grade ≥3 neutropenia was often numerically lower in patients treated with a B-lineage monoclonal antibody as monotherapy compared with patients treated with doublet/triplet regimen in patients with CLL, MM, and NHL (Table 4).

3.2.2 Proteasome inhibitors

Proteasome inhibitors used as monotherapy were reported in nine studies in MM (69, 70, 80, 81, 83, 94, 95, 98, 135) and three in NHL (109, 123, 126). In addition, 18 studies in MM (71, 76–78, 82, 84–87, 89, 90, 93, 96–99, 136) and two studies in NHL (107, 124) reported the use in combination with other agents (Table 4). No data were reported on the use of proteasome in patients with CLL. Data on infections were not reported in patients treated with proteasome inhibitor monotherapy in patients with NHL. In patients with MM, any grade infections were numerically lower in patients treated with a proteasome inhibitor as monotherapy compared with doublet/triplet regimen; however, grade ≥3 infections were numerically higher in patients who received mono versus combination therapy (Table 4). The mean proportion of patients with grade ≥3 neutropenia was lower in patients treated with proteasome inhibitor monotherapy compared with patients treated with doublet/triplet regimen (Table 4). However, fewer studies reported the use of a proteasome inhibitor as monotherapy compared with combination therapy, which might have skewed the results.

3.2.3 Kinase inhibitors

Kinase inhibitors were reported in three studies in CLL (51, 60, 62) and one in NHL (123) when used as monotherapy, and in five studies in CLL (50, 51, 56, 61, 62) and two studies in NHL (100, 127) in combination with other therapies. The mean proportion of patients treated with a kinase inhibitor as monotherapy who had any grade and grade ≥3 infections was 57.6% and 14.0% in CLL, respectively. Data on any grade and grade ≥3 infections were not reported in patients with MM or NHL treated with a kinase inhibitor. The mean proportion of patients treated with a kinase inhibitor in combination

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TABLE 4 Percentages of patients with CLL, MM, and NHL treated with different classes of drugs as monotherapy or in combination with other therapies who had infections (any grade and grade ≥3), neutropenia (any grade or grade ≥3), or hypogammaglobulinemia.

Malignancies		Any grade neutropenia*		Grade ≥3 neutropenia*		Any grade infections*		Grade ≥3 infections*		Hypogamma*
Studies (n)		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Range
B-lineage monoclonal antibodies (as doublet, triplet etc.)										
CLL	14	44.9	20.9–64.0	35.2	3.0–60.0	50.2	14.4–69.1	20.6	6.4–39.0	15.3
MM	5	27.5	9.8–61.3	20.5	5.9–54.1	–	–	41.0	41.0	–
NHL	18	51.5	17.4–87.5	50.8	11.1–92.5†	33.0	10.0–53.3	8.5	0.9–23.0	–
Total	37	44.1	9.8–87.5	38.9	3.0–92.5	40.6	10.0–69.1	16.7	0.9–41.0	15.3
B-lineage monoclonal antibodies (as monotherapy)										
CLL	3	24.4	20.9–27.8	15.3	7.0–23.6	–	–	19.7	11.0–35	–
MM	2	16.4	13.5–19.2	10.4	7.8–13.1	50.2	50.2	13.3	13.3	–
NHL	7	14.1	3.4–22.0	8.5	2.4–14.9	23.0	7.0–36.1	3.6	2.0–4.4	5.9
Total	12	16.9	3.4–27.8	10.7	2.4–23.6	27.5	7.0–50.2	11.9	2.0–35	5.9
Proteasome inhibitors (as doublets, triplets etc.)										
MM	18	34.3	14.0–73.4	21.1	7.0–42.7	25.9	9.6–48.4	8.5	3.8–13.5	–
NHL	2	32.2	17.4–46.9	23.0	11.1–34.9	53.3	53.3	6.9	2.9–10.8	–
Total	20	33.9	14.0–73.4	21.4	7.0–42.7	31.4	9.6–53.3	8.1	2.9–13.5	–
Proteasome inhibitors (as monotherapy)										
MM	9	50.0	28.2–79.3	13.2	2.0–25.0	8.8	8.8	16.9	3.7–30.0†	–
NHL	3	25.5	25.0–26.0	11.5	5.9–17.0	–	–	–	–	–
Total	12	40.2	25.0–79.3	12.7	2.0–25.0	8.8	8.8	16.9	3.7–30.0	–
Kinase inhibitors (as doublets, triplets etc.)										
CLL	5	39.0	20.9–64.0	30.4	6.4–60.0	69.1	69.0–69.1	29.9	20.8–39.0	15.3
NHL	2	31.9	20.8–42.9	24.3	15.6–33	42.9	42.9	38.0	38.0	–
Total	7	37.0	20.8–64.0	29.4	6.4–60.0	60.3	42.9–69.1	32.6	20.8–39.0	15.3
Kinase inhibitors (as monotherapy)										
CLL	3	13.6	9.4–20.7	8.7	4.1–12.1	57.6	49.7–65.4	14.0	14.0	0.0
NHL	1	16.0	16.0	13.0	13.0	–	–	–	–	–
Total	4	14.2	9.4–20.7	9.8	4.1–13.0	57.6	49.7–65.4	14.0	14.0	0.0
IMiDs (as doublets, triplets etc.)										
MM	12	25.9	15.0–43.8	19.2	8.0–37.0	44.8	16.7–59.4	12.0	6.0–29.0	–
NHL	1	36.1	22.1–50.0	–	–	–	–	–	–	–
Total	13	28.8	15.0–50.0	19.2	8.0–37.0	44.8	16.7–59.4	12.0	6.0–29.0	–
IMiDs (lenalidomide and thalidomide as monotherapy)										
MM	5	51.3	31.6–71.0	29.7	13.0–43.0	–	–	21.1	5.0–50.2	–
NHL	2	15.7	15.7	20.1	20.1	29.0	29.0	10.8	10.8	–
Total	7	39.4	15.7–71.0	27.3	13.0–43.0	29.0	29.0	19.0	5.0–50.2	–
Non-specific agents (immunomodulators, antimetabolites, anti-tumor antibiotics, alkylating agents, mitotic inhibitors)										
CLL	1	17.6	17.6	14.1	14.1	45.8	45.8	11.9	11.9	–
MM	2	85.5	85.5	78.0	75.9–80.0	4.8	4.8	11.5	4.0–19.0	–

(Continued)

TABLE 4 Continued

Malignancies		Any grade neutropenia*		Grade ≥3 neutropenia*		Any grade infections*		Grade ≥3 infections*		Hypogamma*
Studies (n)		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Range
NHL	5	48.2	28.8–65.7	46.6	26.3–76.0†	19.4	4.0–34.3	19.6	8.8–34.3	–
Total	8	49.5	17.6–85.5	50.9	14.1–80.0	21.4	4.0–45.8	15.6	4.0–34.3	–

*The reporting criteria for time to adverse events differed across studies.
†Not all studies reported values for both any grade and grade ≥3 events; this has led to the situation where in some categories, individual studies reported higher levels of grade ≥3 events than other studies did for any grade events, leading to the average of grade ≥3 events being higher than the average for any grade events.
Neutropenia grades: grade 1, less than the lower limit of normal–1,500 per mm^3; grade 2, 1,499–1,000 per mm^3; grade 3, 999–500 per mm^3; grade 4, <500 per mm^3; grade 5, death.
Infection grades: grade 1, –; grade 2, localized, local intervention indicated; grade 3, IV antibiotic, antifungal, or antiviral intervention indicated, interventional radiology or operative intervention indicated; grade 4, life-threatening consequences e.g., septic shock, hypotension, acidosis, or necrosis; grade 5, death.

with other therapies who had any grade and grade ≥3 infections was 69.1% and 29.9% in CLL, and 42.9% and 38.0% in NHL, respectively. The mean proportion of patients with any grade/grade ≥3 infections and neutropenia was numerically lower in patients with CLL treated with a kinase inhibitor as monotherapy versus combination therapy (any grade and grade ≥3 infections: 57.6% and 14.0% versus 69.1% and 29.9%, respectively; any grade and grade ≥3 neutropenia: 13.6% and 8.7% versus 39.0% and 30.4%, respectively, Table 4).

3.2.4 IMiDs

IMiDs were used as monotherapy in five studies in MM (69, 73, 75, 79, 135) and two in NHL (108, 125). In addition, IMiDs were used in combination with other therapies in 12 studies in MM (67, 71–74, 77, 79, 84, 89, 92, 136, 137) and one study in NHL (111). No studies reported data on IMiDs in CLL. In patients with MM treated with monotherapy, only grade ≥3 infections were reported, and the mean rate was 21.1%. The mean proportion of patients treated with monotherapy who had any grade and grade ≥3 neutropenia was 51.3% and 29.7% in MM, respectively. In patients with NHL, the mean proportion of patients with any grade and grade ≥3 infections was 29.0% and 10.8%, respectively, and the mean proportion of patients who had any grade and grade ≥3 neutropenia was 15.7% and 20.1%, respectively. When used as monotherapy in patients with MM, IMiDs led to a numerically higher rate of grade ≥3 infections compared with IMiDs used in combination with other therapies (Table 4).

3.2.5 Non-specific agents

Additional analyses for non-specific agents were performed and included only corticosteroids, antimetabolites, anti-tumor antibiotics, alkylating agents, and mitotic inhibitors regardless of whether these were monotherapy or combination regimen (Table 4). These analyses did not include B-lineage monoclonal antibodies, tyrosine kinase inhibitors, proteasome inhibitors, or IMiDs. Non-specific agents were used in eight studies, one in CLL (65), two in MM (79, 82), and five in NHL (102, 115, 116, 119, 129). In CLL, the proportion of patients with any grade and grade ≥3 infections was 45.8% and 11.9%, respectively. The mean percentage for any grade and grade ≥3 infections was 4.8% and 11.5% in patients with MM, respectively (this is due to individual studies reporting only grade ≥3 results that were higher than other studies reported for any grade events), and 19.4% and 8.8% in patients with NHL, respectively. The mean proportion of patients who had any grade and grade ≥3 neutropenia are shown in Table 4.

3.3 Specific drug analyses

Analyses were performed to estimate the ranges of patients with infections, neutropenia or hypogammaglobulinemia associated with specific drug use (Table 5). These analyses included the use of drugs as monotherapy or in combination with other agents. Only the drugs with the highest number of studies in each class of drug were selected for these analyses.

3.3.1 Rituximab

In the nine studies that evaluated the anti-CD20 agent rituximab as monotherapy [two in CLL (54, 66) and seven in NHL (110, 113, 115, 122, 124, 127, 128)], the mean percentage for any grade and grade ≥3 infections was 23% and 3.6% in patients with NHL, respectively (Table 5). Only grade ≥3 infections and neutropenia were reported in patients with CLL, and the rates were 15.0% and 7.0%, respectively. The mean percentage for any grade and grade ≥3 neutropenia was 14.1% and 8.5% in patients with NHL, respectively.

Twenty-four studies evaluated rituximab [seven in CLL (50, 53, 54, 56–58, 63) and 17 in NHL (105–107, 111, 112, 114, 115, 117, 118, 120, 121, 124, 127, 130–133)] in combination with other therapies (Table 5).When rituximab was used as monotherapy, the rates of any grade and ≥3 infections and neutropenia were numerically lower across CLL, MM, and NHL compared with rituximab used in combination with other therapies (Table 5).

3.3.2 Bortezomib

Bortezomib was evaluated in nine studies as monotherapy [seven in MM (69, 81, 83, 94, 95, 98, 135) and two in NHL (109, 126)] and in 15 studies (14 in MM (71, 74, 76–78, 82, 84–86, 89, 90, 96, 98, 136) and one in NHL) in combination with other therapies. No studies reported the use of bortezomib in patients with CLL. When used as monotherapy in patients with MM, bortezomib led to a higher rate of grade ≥3 infections compared with its use in combination with other therapies (Table 5).

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TABLE 5 Percentages of patients with CLL, MM, and NHL treated with rituximab, bortezomib, ibrutinib, lenalidomide, or dexamethasone as monotherapy or in combination with other therapies who had infections (any grade and grade ≥3), neutropenia (any grade or grade ≥3), or hypogammaglobulinemia.

Malignancies	Studies (n)	Any grade neutropenia*		Grade ≥3 neutropenia*		Any grade infections*		Grade ≥3 infections*		Hypogamma*
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Range
Rituximab (as doublets, triplets etc.)										
CLL	7	46.6	20.9–64.0	36.2	3.0–60.0	64.0	59.0–69.0	23.8	6.4–39.0	–
NHL	17	53.0	17.4–87.5	51.9	11.1–92.5	33.0	10.0–53.3	8.5	0.9–23.0	–
Total	24	51.6	17.4–87.5	46.7	3.0–92.5	38.7	10.0–69.0	15.8	0.9–39.0	–
Rituximab (as monotherapy)										
CLL	2	20.9	20.9	7.0	7.0	–	–	15.0	11.0–19.0	–
NHL	7	14.1	3.4–22.0	8.5	2.4–14.9	23.0	7.0–36.1	3.6	2.0–4.4	5.9
Total	9	15.2	3.4–22.0	8.2	2.4–14.9	23.0	7.0–36.1	8.1	2.0–19	5.9
Bortezomib (as doublets, triplets etc.)										
MM	14	36.2	18.1–73.4	24.8	9.2–42.7	25.9	9.6–48.4	8.5	3.8–13.5	–
NHL	1	17.4	17.4	11.1	11.1	53.3	53.3	10.8	10.8	–
Total	15	33.1	17.4–73.4	23.7	9.2–42.7	31.4	9.6–53.3	8.8	3.8–13.5	–
Bortezomib (as monotherapy)										
MM	7	42.5	42.5	10.6	2.0–25.0	8.8	8.8	16.9	3.7–30.0†	–
NHL	2	25.0	25.0	5.9	5.9	–	–	–	–	–
Total	9	33.8	25.0–42.5	9.6	2.0–25.0	8.8	8.8	16.9	3.7–30.0	–
Ibrutinib (as doublets, triplets etc.)										
CLL	2	39.4	35.5–43.3	27.8	18.6–37.0	–	–	–	–	15.3
NHL	1	42.9	42.9	33.0	33.0	42.9	42.9	38.0	38.0	–
Total	3	40.6	35.5–43.3	29.5	18.6–37.0	42.9	42.9	38.0	38.0	15.3
Ibrutinib (as monotherapy)										
CLL	2	15.1	9.4–20.7	8.1	4.1–12.1	49.7	49.7	–	–	0.0
NHL	1	16.0	16.0	13.0	13.0	–	–	–	–	–
Total	3	15.4	9.4–20.7	9.7	4.1–13.0	49.7	49.7	–	–	0.0
Lenalidomide (as doublets, triplets etc.)										
MM	10	32.5	15.0–61.3	23.0	8.0–54.1	52.5	46.7–59.4	19.5	6.0–41.0	–
NHL	1	36.1	22.1–50.0	–	–	–	–	–	–	–
Total	11	33.4	15.0–61.3	23.0	8.0–54.1	52.5	29.0–59.4	19.5	6.0–41.0	–
Lenalidomide (as monotherapy)										
MM	3	71.0	71.0	29.7	13.0–43.0	–	–	25.7	5.0–50.2	–
NHL	2	15.7	15.7	20.1	20.1†	29.0	29.0	10.8	10.8	–
Total	5	43.4	15.7–71.0	27.3	13.0–43.0	29.0	29.0	22.0	5.0–50.2	–
Dexamethasone (as doublets, triplets etc.)										
MM	21	34.2	9.8–73.4	22.5	5.9–54.1	46.8	9.6–68.0	18.4	6.0–41.0	–
NHL	3	10.8	3.2–22.1	34.8	0.0–100.0†	51.6	36.0–81.0	12.7	11.3–14.1	–
Total	24	30.5	3.2–73.4	23.3	0.0–100.0	48.6	9.6–81.0	17.7	6.0–41.0	–

(Continued)

TABLE 5 Continued

Malignancies	Studies (n)	Any grade neutropenia*		Grade ≥3 neutropenia*		Any grade infections*		Grade ≥3 infections*		Hypogamma*
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Range
Dexamethasone (as monotherapy)										
MM	1	20.1	20.1	16	16	52.7	52.7	32.7	32.7	–

*The reporting criteria for time to adverse events differed across studies.
†Not all studies reported values for both any grade and grade ≥3 events; this has led to the situation where in some categories, individual studies reported higher levels of grade ≥3 events than other studies did for any grade events, leading to the average of grade ≥3 events being higher than the average for any grade events.
Neutropenia grades: grade 1, less than the lower limit of normal–1,500 per mm^3; grade 2, 1,499–1,000 per mm^3; grade 3, 999–500 per mm^3; grade 4, <500 per mm^3; grade 5, death.
Infection grades: grade 1, –; grade 2, localized, local intervention indicated; grade 3, IV antibiotic, antifungal, or antiviral intervention indicated, interventional radiology or operative intervention indicated; grade 4, life-threatening consequences e.g., septic shock, hypotension, acidosis, or necrosis; grade 5, death.
CLL, chronic lymphocytic leukemia; hypogamma, hypogammaglobulinemia; IV, intravenous; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; –, not reported.

3.3.3 Ibrutinib

Three studies evaluated the use of ibrutinib as monotherapy, two in CLL (60, 62) and one in NHL (123), and three studies in combination with other therapies [two in CLL (61, 62) and one in NHL (100)]. In patients treated with ibrutinib monotherapy, only the mean percentage for any grade infections was reported and only in patients with CLL (49.7%). When used in combination with other therapies, only the mean percentage for any grade and grade ≥3 infections was reported in patients with NHL, and the rates were 42.9% and 38.0%, respectively (Table 5).

Both any grade and grade ≥3 neutropenia were numerically lower in both patients with CLL and NHL when treated with ibrutinib monotherapy compared with ibrutinib included in doublet/triplet regimen (Table 5).

3.3.4 Lenalidomide

Lenalidomide was used as monotherapy in five studies [three in MM (69, 75, 79) and two in NHL (108, 125)], and in combination with other therapies in 11 studies [10 in MM (67, 71–74, 77, 79, 92, 93, 137) and one in NHL (111)]. When used as monotherapy, the mean percentage for grade ≥3 infections was 25.7% in patients with MM, and the mean percentage for any grade and grade ≥3 infections was 29% and 10.8% in patients with NHL, respectively. Data on any grade infections were not reported in patients with MM treated with lenalidomide monotherapy (Table 5). The mean percentage for any grade and grade ≥3 neutropenia was 71% and 29.7% in patients with MM, respectively, and 15.7% and 20.1% in patients with NHL, respectively. Data for combination with other therapies, are shown in Table 5.

3.3.5 Dexamethasone

The use of dexamethasone as monotherapy was reported in only one MM study (88). Combination with other therapies was reported in 24 studies, 21 in MM (67, 71–74, 77–79, 82, 84, 86–88, 92, 93, 96, 97, 99, 136–138) and three in NHL (101, 103, 111).

For combination regimens, in which dexamethasone was used with a diverse range of agents, the mean percentage for any grade and grade ≥3 infections was 46.8% and 18.4% in patients with MM, and 51.6% and 12.7% in patients with NHL, respectively. The mean percentage for any grade and grade ≥3 neutropenia was 34.2% and 22.5% in patients with MM, and 10.8% and 34.8% in patients with NHL, respectively.

3.4 Infection and neutropenia rates in patients receiving regimen combinations commonly used in clinical practice

The drugs with the highest number of studies in each class of drug were selected for the drug specific analyses. However, in clinical practice, certain specific drug combinations are more commonly used than others, such as those recommended by the European Society for Medical Oncology (ESMO) (139–141).

When assessing these more commonly used combinations, seven studies reported the use of rituximab in combination with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) in patients with NHL (106, 107, 114, 120, 130, 131, 133). In this population, the mean percentage for any grade and grade ≥3 infections was 34.9% and 11.3%, respectively; and the mean percentage for any grade and grade ≥3 neutropenia was 67.1% and 64.5%, respectively.

In patients with CLL, the use of chlorambucil in combination with obinutuzumab was reported in five studies (G-Clb); fludarabine, cyclophosphamide, and rituximab (FCR) in three studies; and bendamustine plus rituximab in four studies. None of the selected studies reported data on the use of venetoclax in combination with obinutuzumab. In the G-Clb group, the mean percentage for any grade and grade ≥3 infections was 29.1% and 11.1%, respectively; and the mean percentage for any grade and grade ≥3 neutropenia was 46.5% and 38.4%, respectively (51, 52, 61, 63, 64). In the FCR group, only the mean percentage for grade ≥3 infections and neutropenia was reported: 24.1% and 26.0%, respectively (53, 54, 57). In patients who received bendamustine plus rituximab, the mean percentage for any grade and grade ≥3 infections was 42.2% and 18.0%, respectively; and the mean percentage for any grade and grade ≥3 neutropenia was 59.7% and 43.4%, respectively (54, 56, 58, 63).

In one study that investigated the use of daratumumab in combination with lenalidomide and dexamethasone in patients with MM, the rate for grade ≥3 infections was 41.0% and the rates for any grade and grade ≥3 neutropenia were 61.3% and 54.1%, respectively (92). Only the rate for grade ≥3 neutropenia (39.9%) was reported in patients with MM who received daratumumab in combination with bortezomib, melphalan, and prednisone (90). None of the selected studies reported data on both the use of bortezomib in combination with lenalidomide and dexamethasone (VRd) and the use of daratumumab in combination with bortezomib, thalidomide, and dexamethasone (daraVTD) in patients with MM.

None of these studies reported data on the rates of hypogammaglobulinemia, highlighting the need for further reporting on immunoglobulin G (IgG) levels, especially in regimen combinations including drugs known to have a mode of action likely to impact IgG levels directly, such as B-lineage monoclonal antibodies like daratumumab.

3.5 Lymphocytopenia in patients with CLL, MM, and NHL

The rates of lymphocytopenia were reported in a limited number of studies only: one in patients with CLL (64), seven in MM (68, 70, 78, 91, 92, 97, 138), and three in NHL (116, 120, 127) (data not shown). The mean percentage of patients with lymphocytopenia was 1.9% in patients with CLL, 11.9% in MM, and 38.6% in NHL.

3.6 Hypogammaglobulinemia and sinopulmonary bacterial infection analyses

Only two of the evaluated studies reported data on hypogammaglobulinemia (Table 3). In patients with CLL, one study reported hypogammaglobulinemia in 15.3% of patients who received combination therapy with ublituximab (anti-CD20) and ibrutinib, and in 0.0% of patients who received ibrutinib monotherapy (62). In patients with NHL, one study reported hypogammaglobulinemia in 5.9% of patients who received rituximab maintenance therapy for up to 2 years (122). Neither study had a confirmed definition of what was classed as hypogammaglobulinemia nor was testing reported prior to the initiation of treatment.

Patients with hypogammaglobulinemia commonly present with recurrent bacterial sinopulmonary infections (e.g., otitis, sinusitis, pneumonia, nasopharyngitis), which often are due to encapsulated bacteria such as *S. pneumoniae* (3–5, 26, 142). In this systematic literature review, we classed sinopulmonary bacterial infections to include LRTI, URTI, sinusitis, nasopharyngitis, bronchitis, and/or pneumonia, which were collected from 17 studies in CLL, 38 in MM, and 34 in NHL. However, not all relevant studies reported all types of infections used to calculate the rate of sinopulmonary bacterial infections (e.g., no relevant studies reported data on the percentages of patients with LRTI and sinusitis in MM; as a result, the data for sinopulmonary bacterial infections in patients with MM did not include LRTI and sinusitis values). The mean proportion of patients with sinopulmonary bacterial infections was 7.6%, 14.4%, and 6.3% in patients with CLL, MM, and NHL, respectively. Sinopulmonary bacterial infections were reported in 15.7% (54, 59, 66), 8.5% (68, 91), and 7.8% (110, 113, 115, 122, 124, 127, 128, 134) of patients with CLL, MM, and NHL, respectively, when treated with B-lineage monoclonal antibodies as monotherapy (Table 6). In patients who received proteasome inhibitor as monotherapy, sinopulmonary bacterial infections were reported in 7.7% (69, 70, 80, 81, 83, 94, 95, 98, 135) of patients with MM. In patients treated with kinase monotherapy, sinopulmonary bacterial infections were reported in 8.0% of patients with CLL (51, 60, 62). In those patients who received non-specific agents as monotherapy and/or double/triplet regimen, sinopulmonary bacterial infections were reported in 9.9% (102, 115, 116, 119, 129) of patients with NHL.

In this systematic literature review, the most common types of infections reported in patients with CLL and MM were related to the respiratory system, whereas in patients with NHL they were bacterial infections, pneumonia, and viral infections (data not shown). Other less common types of infections included viral and UTI infections in CLL, viral and skin infections in MM, and UTI and *Candida* infections in NHL (data not shown).

4 Discussion

Despite infections related to SID accounting for 22–50% of deaths in patients with HMs (7, 47–49), we still observed a lack of data

TABLE 6 Proportions of patients with CLL, MM, and NHL treated with different classes of drugs as monotherapy who had sinopulmonary bacterial infections.

Malignancies	Studies (n)	Mean (%)*
B-lineage monoclonal antibodies		
CLL	3	15.7
MM	2	8.5
NHL	7	7.8
Proteasome inhibitors		
MM	9	8.8
Kinase inhibitors		
CLL	3	7.7
Non-specific agents		
NHL	5	9.9

*The reporting criteria for time to adverse events differed across studies.
Neutropenia grades: grade 1, less than the lower limit of normal–1,500 per mm^3; grade 2, 1,499–1,000 per mm^3; grade 3, 999–500 per mm^3; grade 4, <500 per mm^3; grade 5, death.
Infection grades: grade 1, –; grade 2, localized, local intervention indicated; grade 3, IV antibiotic, antifungal, or antiviral intervention indicated, interventional radiology or operative intervention indicated; grade 4, life-threatening consequences e.g., septic shock, hypotension, acidosis, or necrosis; grade 5, death.
CLL, chronic lymphocytic leukemia; IV, intravenous; MM, multiple myeloma; NHL, non-Hodgkin lymphoma.

reported on hypogammaglobulinemia, lymphocytopenia, and consistent infection reporting in phase III, phase IV, and observational studies, suggesting a likely underestimate of hypogammaglobulinemia and cellular immunodeficiency in the development of recurrent and fatal infections in patients with HMs. In addition, there is still a lack of data in the literature regarding differences in rates of hypogammaglobulinemia-related infections, rates of hypogammaglobulinemia and infections across classes of drugs, and types of infections across HMs (Table 1).

This level of granularity in reporting rates of specific subtypes of SID and related infections might not be a priority for hematologists and hemato-oncologists with the main focus on treating the malignancy. However, we believe that collecting these data might help highlight trends and possible correlations that could inform changes in the management of HMs and related infections in everyday clinical practice, such as improving supportive care and serving as a stimulus for development of approaches that include the early testing and detection of immunodeficiency alongside prevention and treatment of infection as part of the routine management of these HMs (41). Therefore, we undertook this systematic literature review to provide insight into the cancer treatments associated with SID, including the incidence of infections, neutropenia, and hypogammaglobulinemia among patients undergoing systemic treatment for CLL, MM, and NHL.

In this systematic literature review, the highest proportion of patients with grade ≥3 infections across classes of drugs was 41.0% in patients with MM treated with a B-lineage monoclonal antibody combination; and 29.9% and 38.0% of patients with CLL and NHL treated with a kinase inhibitor combination, respectively. As expected, the incidence of neutropenia did not always correlate with the incidence of infections. Interestingly, the higher rates of grade ≥3 infections in the 2017–2022 group versus the 2011–2016 group across all the selected HMs might be due to numerous factors such as the concomitant use of old and novel therapeutic agents (e.g., B-lineage monoclonal antibodies, tyrosine kinase inhibitors, and proteasome inhibitors) and HM therapies becoming increasingly more potent and correspondingly more immunosuppressive, as well as longer survival and more comorbidities.

As many CAR T therapies are still in phase I or II clinical development (143–145) (and were therefore excluded from this systematic literature review) SID data associated with CAR T therapies is still emerging and not fully represented in this systematic literature review. For example, lisocabtagene maraleucel and ciltacabtagene autoleucel were not included in our analyses because these drugs were not approved in both the US and EU markets by March 16, 2022, and therefore there is a relatively small number of patients treated with these agents. Further work is needed in the rapidly evolving field of CAR T to report data on SID-related and hypogammaglobulinemia-related infections (144, 146).

Notably, the use of monotherapy was mostly associated with a numerically lower risk of infection or neutropenia. For instance, the mean proportion of patients with any grade infections was numerically lower when rituximab was used as monotherapy across patients with CLL, MM, and NHL compared with its use in combination with other agents. The use of ibrutinib as monotherapy led to a numerically lower mean percentage of patients with any grade and grade ≥3 neutropenia versus combination therapies. On the contrary, bortezomib used as monotherapy was associated with a numerically higher mean percentage of patients with grade ≥3 infections and a numerically lower mean percentage of patients with any grade infections; as already mentioned, this is due to individual studies reporting only grade ≥3 results that were higher than other studies did for any grade events. Unfortunately, further analyses to compare anti-CD20 versus anti-CD38 agents could not be undertaken due to sample sizes and mismatched disease cohorts.

The infection spectrum observed in this patient population has some similarities with those observed in primary antibody deficiency (PAD) but also some differences. While sinopulmonary infections are common in HMs and PAD, infection sites that are less common in PAD were also observed in this systematic literature review, such as the urinary tract and skin (with herpes group viral reactivation/infection in particular). The occurrence of viral and fungal, as well as bacterial pathogen groups, speaks to a potential CID phenotype in many patients with HM. The variability in the types of infection across patients with CLL, MM, and NHL might be due to both the disease and different related treatments that influence the infection profile of patients with HMs. Future data highlighting the differences between bacterial, fungal, and viral infection distribution with higher statistical power might be useful to predict patients' infection risk and inform clinical decision making. While coronavirus disease 2019 (COVID-19) infection data were not collected in the studies analyzed, it is recognized that patients with HMs are at risk for severe COVID-19. In addition, the information gained from the use of vaccines against COVID-19 in these patients has been extremely informative in terms of providing functional vaccine response data to refine risk stratification.

Interestingly, despite both B-lineage monoclonal antibodies against CD20 and tyrosine kinase inhibitors being detrimental to B-cell development, hypogammaglobulinemia was detected only in patients with CLL who received ublituximab and ibrutinib (BTK) combination therapy compared with patients treated with ibrutinib monotherapy who did not show a decrease in their IgG serum concentration. Notably, neutropenia, pneumonia, bronchitis, and *Herpes zoster* infections were also higher in patients treated with ublituximab and ibrutinib combination therapy compared with ibrutinib monotherapy (62). It is possible that monotherapy has been used in less severe disease settings and that a balance exists between immunosuppression from the therapy on normal immune cells and reduction in tumor-related immunosuppression due to the therapy.

This systematic literature review has several limitations: i) as not all the studies analyzed specified precise definitions for hypogammaglobulinemia, infections and SAD, this might have influenced the data as slightly different outcomes may have been captured; ii) systematic literature reviews are not powered to have statistical significance; therefore, data should be considered as

exploratory. However, they can help highlight trends and possible correlations that lay the foundation for further studies; iii) most of the data came from phase III clinical trials, which do not necessarily reflect real-life clinical practice (147). Some investigators recognize the pivotal role of real-world data and evidence that can be optimized (148, 149). Meta-analysis of data from hematological databases is one avenue that could provide insightful follow-up to extrapolate information on the rate of patients with HMs and hypogammaglobulinemia due to various cancer treatments in real-life settings; iv) finally, these drugs may be used at various times throughout a disease course and as induction or maintenance therapy. Therefore, as the risk of infection can vary depending on both the timing from diagnosis and severity of disease, direct comparison of infections rates between drugs must be undertaken with caution since data were not normalized for time exposure to agents and infection reporting. Future analyses will be crucial in evaluating the rates of hypogammaglobulinemia and infections in early versus late disease course. Moreover, distinction between BTK and phosphoinositide 3-kinase (PI3K) inhibitors were not performed due to the low number of studies that tested PI3K inhibitors, therefore an overall kinase inhibitor category was used, which may limit practical application of data from this category.

With this systematic literature review, the authors wish to shed light on which treatments might contribute to the development of SID in this rapidly evolving therapeutic area and to highlight the importance of reporting data on hypogammaglobulinemia, both before and during therapy in patients with HMs. The authors believe that, while treatment of the malignancy is clearly of primary importance, there are still several knowledge gaps on the management of SID (Table 1); therefore, efforts need to be undertaken to improve awareness of how to diagnose and treat patients with hypogammaglobulinemia, CID, and infections in HMs, as well as optimize treatments to prevent recurrent and severe infections. Without increased recording and reporting of Ig levels in this patient population, the benefits of a range management strategies such as infection exposure mitigation strategies, vaccination, antibiotics, antiviral drugs, and immunoglobulin replacement therapy (IgRT) cannot be fully evaluated (41).

Data availability statement

The datasets presented in this study are the results of analyses conducted with data taken from online publications. The name of the publications can be found in the reference list.

Author contributions

All authors contributed equally to the review approach/design, consensus meetings, recommendations, interpretation of literature, writing, and critical review of this article. All authors reviewed the final manuscript and agreed on the decision to submit it for publication.

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Conflict of interest

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Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1098326/full#supplementary-material

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Real-world experience of sorafenib maintenance after allogeneic hematopoietic stem cell transplantation for FLT3-ITD AML reveals high rates of toxicity-related treatment interruption

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Sorafenib significantly improves survival of FLT3-ITD mutated AML patients when used as a post-allogeneic HSCT maintenance. Importantly, clinical trials reported a low rate of toxicities requiring sorafenib discontinuation. The aim of our analysis was to evaluate the real-world experience in patients treated with post-allogeneic HSCT sorafenib maintenance therapy for FLT3-ITD AML with a particular focus on tolerability and toxicity-related treatment interruption. We conducted a single-center retrospective study on 30 FLT3-ITD AML patients undergoing allogeneic HSCT in complete remission between 2017 and 2020 and who received sorafenib maintenance. 26 patients (87%) experienced toxicities leading to dose reduction (n=9) or direct interruption (n=17). Average time on sorafenib was 125 days (range 1-765). Most common toxicities were skin, gastrointestinal, and hematologic. Among patients who had a dose reduction, 4 eventually interrupted the drug and 5 were able to continue. Among patients who interrupted sorafenib because of toxicities, 7 were re-challenged with good tolerance in 3 cases. Overall, 18 patients (60% of the entire cohort) definitively discontinued sorafenib because of toxicities. 14 patients were thereafter switched to midostaurin. Importantly, with a median follow-up of 12 months, the median overall survival was not reached suggesting a positive impact of sorafenib maintenance despite the high rates of treatment interruption. In conclusion, our real-world analysis reveals high rates of toxicity-related interruption of sorafenib maintenance after allogeneic HSCT. Interestingly, our results suggest the feasibility of re-challenging with sorafenib and/or of switching to other maintenance approaches in case of intolerance.

KEYWORDS

sorafenib, maintenance, HSCT = hematopoietic stem cell transplant, acute myeloid leukemia, drug toxicity and adverse effect

Introduction

FLT3-Internal Tandem Duplication (FLT3-ITD) mutations of the gene encoding the FLT3 tyrosine kinase receptor are found in 25-30% of AML patients. It is associated with a high risk of relapse and therefore with poor prognosis despite intensive chemotherapy and allogeneic HSCT (1). FLT3 tyrosine kinase inhibitors (TKI) recently emerged as an efficient boost to conventional AML induction chemotherapy, significantly improving survival of FLT3 mutated AML patients in large prospective trials (2). In patients who relapsed after allogeneic HSCT, several studies showed that sorafenib, a broad-spectrum TKI with strong activity against FLT3, induced durable remissions (3). Post-HSCT maintenance with sorafenib emerged in recent years as a way to improve prognosis by diminishing relapse risk of FLT3-ITD AML, as reported in early studies (4–6) as well as phase II (7) and III (8) clinical trials. Overall, retrospective studies and clinical trials reported relatively low rates of drug interruption or reduction suggesting this treatment is well tolerated in the post-transplant setting. Based on these promising outcomes, maintenance with sorafenib is routinely used in many centers for patients with FLT3-ITD AML after allogeneic HSCT, starting as early as hematological reconstitution. The aim of our single-center retrospective analysis was to evaluate the real-world experience in patients treated with post-allogeneic HSCT sorafenib maintenance therapy for FLT3-ITD AML with a particular focus on tolerability and toxicity-related treatment interruptions.

Methods

Study design

Our study included 30 patients who received transplantation at our center between 2017 and 2021 for AML with FLT3-ITD in complete hematological remission. Clinical data were retrospectively extracted from the medical records. Written informed consent was obtained from all patients included in the study. All patients included received Sorafenib maintenance therapy starting at time of hematological reconstitution after transplantation. Sorafenib was started at 200 mg BID and increased at 400 mg BID after a week in case of good tolerance. Treatment was planned for two years after transplant, if well tolerated. In case of toxicity, the drug was either reduced to 200 mg BID, or stopped depending on the severity of the toxicity.

Statistical analysis and data visualization

Baseline characteristics were descriptively reported. Categorical variables were expressed as proportions. Continuous variables were expressed as median with range. Overall survival (OS) was calculated from the date of transplant to death or last follow-up. Progression-free survival (PFS) was calculated from the date of HSCT until disease relapse/progression, death or last follow-up. Probability of OS and PFS were calculated using the Kaplan-Meier estimator. Statistical analyses were performed using R version 3.5.1 with R studio version 1.1.453.

Results

High rates of toxicity leading to Sorafenib dose reduction or drug interruption

Patient characteristics are reported in Table 1. Median age at transplant in our cohort was 55 years (29–68). All patients had a FLT3-ITD mutation and 23 (77%) had a NPM1 mutation. Twenty-seven (90%) were transplanted in first complete remission (CR) and 3 (10%) in second CR. At the time of transplant molecular Measurable Residual Disease (MRD) was positive in 13 (43%) and negative in 17 (57% patients. Twenty-one (70%) patients had a comorbidity index of 0 to 2 points and 9 (30%) of 3 points or more. 9 (30%) patients received a graft from an HLA identical donor, 17 (57%) patients received a graft from a matched unrelated donor (MUD), and 4 (13%) from a haplo-identical donor. Sixteen (53%) patients received myeloablative conditioning (MAC) mostly fludarabine (150 mg/m^2) and treosulfan (42 mg/m^2). Fourteen (47%) patients received reduced intensity conditioning (RIC) mostly fludarabine (150 mg/m2) and treosulfan (10 g/m^2). GVHD prophylaxis consisted of Calcineurin Inhibitors (CNI) and Mycophenolate Mofetil (MMF) in 3 (10% patients), CNI and Methotrexate in 19 (64%) patients, CNI, MMF and post-transplant Cyclophosphamide in 6 (20%) patients, CNI, sirolimus and MMF in 2 (6%) patients. Twenty (68%) patients received *in vivo* T-cell depletion before transplantation, with antithymocyte globuline. Eight (27%) patients received ex-vivo T-cell depletion with anti-CD52 antibody in the bag. Median follow-up was 324 (62–1099) days.Median time from transplant to sorafenib initiation was 63 (41–213) days. At sorafenib start, median hemoglobin was 112 g/dl (77–152), median platelet count was 170 G/l (49–278), median leucocyte count was 4.8 G/l (1.55-14.3), median renal clearance measured with GFR was 80 ml/min/m^2 (49–117). Twenty-six (87%) patients experienced toxicities leading to drug interruption in 17 patients and dose reduction in 9 patients (Figure 1).

TABLE 1 Patient characteristics.

Median age (range), years	55 (29–68)
Sex, *n* (%)	
Male	15 (50)
Female	15 (50)
Mutational status, *n* (%)	
FLT3-ITD mutation	30 (100)
NPM1	23 (77)

(Continued)

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TABLE 1 Continued

Median age (range), years	55 (29–68)
Status at transplant	
1st CR, *n* (%)	27 (90)
2nd CR, *n* (%)	3 (10)
Molecular MRD status at transplant	
MRD positive	13 (43)
MRD negative	17 (57)
HCT-CI, *n* (%)	
0-2pts	21 (70)
≥ 3pts	9 (30)
Conditioning Type, *n* (%)	
MAC	16 (53)
RIC	14 (47)
GVHD prophylaxis regimen	
CNI, NMF	3 (10)
CNI, MTX	19 (64)
CNI, MMF, PTCy	6 (20)
CNI, sirolimus, MMF	2 (6)
T-cell depletion	
ATG	20 (68)
Ex-vivo T-cell depletion	8 (27)
No T-cell depletion	7 (24)
Donor type, *n* (%)	
Sibling donor	9 (13)
Matched-unrelated	17 (57)
Haplo-identical	4 (13)
Stem cell source	
Peripheral blood	29 (97)
Bone marrow	1 (3)
Median time to sorafenib initiation(range), days	63 (41-213)
Laboratory values at sorafenib start	
Median WBC (range), G/I	4.8 (1.5-14.3)
Median Platelets (range), G/I	170 (49-278)
Median hemoglobin (range), g/dl	112 (77-152)
Median renal eGFR (range), ml/min/m^{2}73	80 (49-117)

FLT3, fms-like tyrosine kinase 3; NPM1, nucleophosmin 1; CR, complete remission; MRD, Measurable Residual Disease; HCT-CI, hematopoietic cell transplant comorbidity index; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; CNI, calcineurin inhibitors; MMF, mycophenolate mofetil; MTX, methotrexate, ATG, Antithymocyte globulin; HLA, human leucocyte antigen; WBC, white blood cell; eGFR, estimated glomerular filtation rate; PTCy, post-transplant cyclophosphamide.

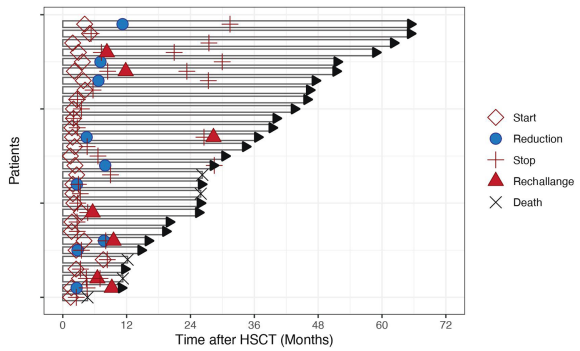

FIGURE 1
Length of Sorafenib administration in 30 patients over time. Each bar represents the time from transplant date to last follow-up. Symbols on each line indicate sorafenib start, reduction, stop, rechallenge and death dates, if applicable.

Heterogeneous profile of toxicities requiring sorafenib dose reduction or interruption

In the 26 patients with reported toxicities, most common toxicities were skin (n=5, grade II), gastrointestinal (n=7, 27%, grade II and III), and hematological (n=7, 27%, grade III). One patient experienced concomitant uveitis (grade III) and pneumonia (grade IV), both resolved after sorafenib interruption. 3 (11%) patients experienced hypertension (grade II in 2 patients and III in 1 patient), 2 (7%) had hepatitis (1 grade II and 1 grade III) and one (4%) patient had a PRESS (posterior reversible encephalopathy syndrome) grade IV possibly related to sorafenib (Figure 2). Skin biopsies were obtained in three patients who presented with an erythematous and papular rash with follicular hyperkeratosis. In the three of them, a lymphocytic infiltrate was present surrounding the

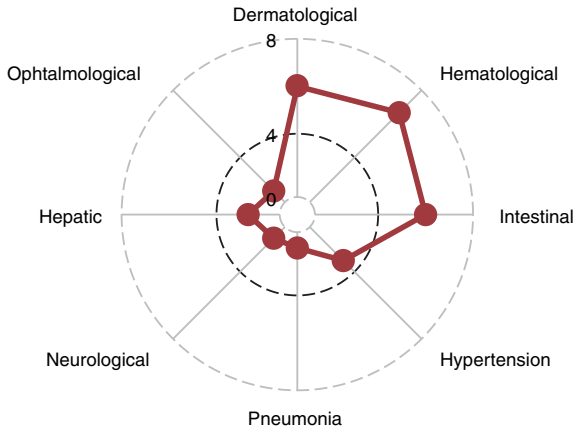

FIGURE 2
Spider Plot of sorafenib toxicities. Each point on the disc indicates the number of patients in the cohort who experienced each type of toxicity.

hair follicules, with presence of polynuclear granular leucocytes. In one patient eosinophils infiltration and keratinocyte necrosis were present. For this reason, acute skin GvHD could not be excluded in this patient but the condition rapidly improved after sorafenib interruption. Of note, we observed no hand-foot syndrome nor stomatitis, which are the most common cutaneous side-effects reported with sorafenib (9). Hematological adverse events were grade II and III thrombocytopenia in 6 patients and grade III neutropenia in 1 patient. Gastro-intestinal symptoms included diarrhea in 2 patients, abdominal discomfort in 1, dysgeusia in 1 patients, nausea in the other patients. No digestive biopsies were performed in any of the patients because symptoms were rapidly resolved after sorafenib interruption or dose adjustment.

Patient care and outcome after sorafenib interruption or reduction

Of the 9 patients (30% of entire cohort) who had a dose reduction, 4 eventually stopped because of toxicity and 5 continued the drug. Median time on sorafenib before interruption in the whole cohort was 41 days (range 1-765).

Among 21 patients (70% of entire cohort) who interrupted (either directly for 17 patients or after reduction attempt for 4 patients) sorafenib because of toxicities, 7 were re-challenged with good tolerance in 3 cases and 4 eventually stopped because of toxicity recurrence.

In the end, definitive discontinuation because of toxicities happened in 18 patients (60% of entire cohort). Non-toxicity-related causes of sorafenib discontinuation were relapse in 3 patients, including FLT3-ITD negative relapse in 1 patient, and end of scheduled maintenance in 5 patients. Among patients who discontinued the drug because of toxicities, 14 patients were switched to midostaurin. Among them, 5 are still taking the drug, 3 completed the 2-year maintenance and 6 interrupted midostaurin because of toxicities.

Importantly, the analysis of patients' outcome showed a favorable progression-free survival (24-month PFS 73% (58%-91%); Figure 3A) and overall survival (24-month OS was 90% (79%-100%); Figure 3B) despite the high rate of treatment interruption.

Discussion

Relapse remains the first cause of death after allogeneic HSCT in AML patients. The rationale of maintenance therapy is to reduce the risk of relapse without impeding the graft-versus-leukemia (GvL) effect. Based on impressive survival benefits demonstrated in phase II and III studies, the use of sorafenib maintenance has rapidly expanded in recent years. Sorafenib is a first-generation multi-target FLT3 tyrosine kinase inhibitor that has been used since 2007 in oncology, for treatment of advanced hepatocellular carcinoma and renal carcinoma. Besides FLT3, it mainly targets vascular endothelial growth factors (VEGFR1-3) and Raf kinases, but additional reported targets include KRAS or BRAF (10–12). Most common treatment related toxicities include hypertension, diarrhea, fatigue, hand-food skin reaction (13). In trials for advanced solid cancers, these side-effects were mostly mild, with less than 10% of high-grade side effects. In one phase III trials that included 903 patients with renal carcinoma (14) and two phase III trials that included 602 and 226 hepatocarcinoma patients treatment discontinuation rates ranged from 10 to 38% (15, 16).

Early retrospective studies on sorafenib maintenance as well as the phase I clinical trial reported a fairly good tolerability, with dose reduction rates ranging from 15 to 27% and interruption rates ranging from 18-31% (4–6). In a retrospective series, Chappell et al.

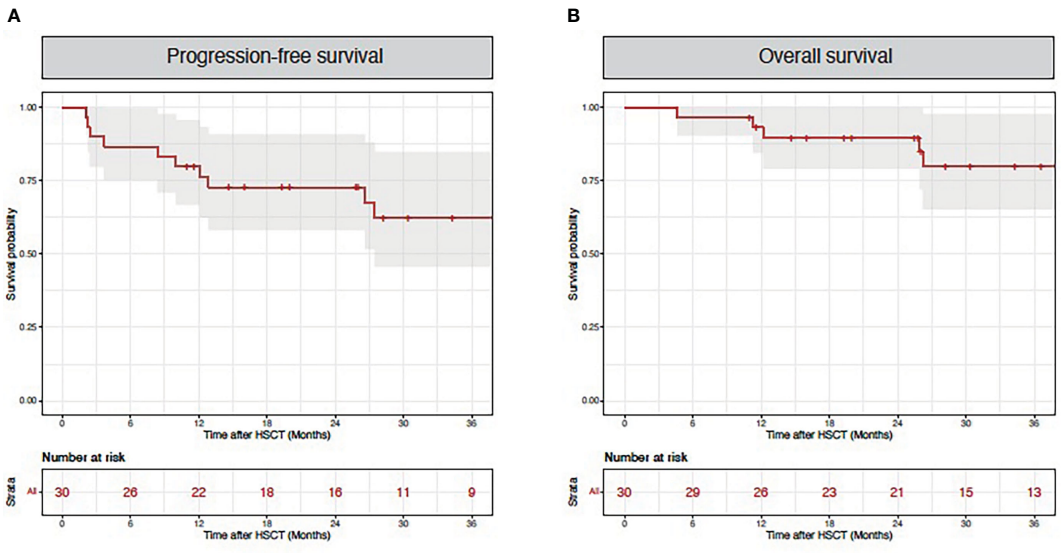

FIGURE 3
Patient outcomes. Red lines indicate progression-free survival **(A)** and overall survival **(B)** for the entire cohort.

reported a higher rate of dose reduction (66%) with a low (14%) rate of drug interruption (17).

In the two randomized trials, dose reduction rates happened in nearly half of patients but drug interruption and discontinuation rates were much less frequent than in our experience. In the phase II randomized, placebo-controlled phase II SORMAIN trial dose reduction was performed in 48.8% of patients and discontinuation in 22% of patients in sorafenib group (7). In the phase III trial conducted by Xuan et al, dose reduction rate was 42% in sorafenib group while dose interruption rate was 12% and definitive discontinuation rate was only 5% (8).

In our real-world analysis of sorafenib maintenance after allogeneic HSCT, dose modifications, especially interruption, because of toxicities, were particularly high: reduction rate was 30% of entire cohort, interruption rate (direct or after reduction attempt) was 70% of entire cohort and definitive discontinuation rate was 60% of entire cohort. Such high rates of toxicity-related dose adjustments or interruptions are closer to the ones reported by Pratz et al. in their single-arm pilot study where sorafenib dosing was individualized, starting at a dose of 200 mg/day and titrated based on tolerability and toxicities (18). In this study, which included 44 patients (median age 52 years, very close to our own cohort) treated with sorafenib post-transplant, most patients (40/44, 90%) were unable to escalate the dose to reach 400 mg BID, with only 4 patients able to tolerate 400 mg BID. The authors also performed elegant pharmacokinetics and pharmacodynamics studies where they measured sorafenib concentrations at different timepoints (accounting for sorafenib active metabolite) and assessed FLT-ITD inhibition with a plasma inhibitory activity (PIA) assay. Interestingly, these correlative studies found consistent inhibition of FLT3 at all tolerability-determined dosing levels. Based on these results, the authors recommend an individualized dosing for patients after transplantation, according to tolerability. In our cohort, sorafenib was started at 200 mg bid and, after a week, increased at 400 mg. After this rather quick dose increase, in case of suspected toxicities, the drug was more frequently interrupted than decremented first. A first explanation for this is a low tolerance to side-effects in this heavily pre-treated population of transplanted patients. In addition, the high rate of drug interruption we found in the real-world setting may be due to the fact that two of the most frequent side effects of sorafenib we observed were gastro-intestinal and cutaneous, both of which are very frequent sites of acute GVHD. One common strategy when suspecting this complication is to stop any medications that could be causing the symptom and, if persistent, proceed to biopsies to document GVHD.

Only 7/21 patients interrupting sorafenib in our series were rechallenged thereafter, while the 14 remaining patients were not rechallenged because of fear of recurrence of side-effects and relatively easy access to midostaurin as an alternative FLT3 inhibitor. Although among the 14 patients who switched to midostaurin the majority of them experienced adverse events when exposed to this alternative multitargeted TKI, nearly half were able to continue the drug. Although this drug has been proven to be effective in first-line therapy, data supporting its use as a post-allogeneic HSCT maintenance are still limited. In the RADIUS trial, a phase II randomized study to investigate the role of midostaurin maintenance, an early report found a benefit to adding midostaurin to SOC as a maintenance treatment in patients with FLT3-ITD AML after allogenic HSCT (19). The estimated 24-month RFS was 85% (64-94%) and the estimated 24-month OS was 85% (65-94%) in the midostaurin arm. Dose adjustments occurred in 63% of patients (related to adverse events in 84% cases) and treatment discontinuation occurred in 27% of patients, mostly due to gastro-intestinal adverse events (nausea and vomiting) or liver enzyme elevation. Gilteritinib is a newer generation TKI with specific and potent activity against FLT3-ITD and AXL1-kinases (20), currently under investigation as a maintenance treatment in the BMT CTN Protocol 1506, and results are awaited regarding efficacy and tolerance in the post-transplant maintenance setting to see if it can replace sorafenib in this indication. Importantly, the analysis of patients' outcome in our cohort confirmed the previously reported positive impact of sorafenib maintenance on overall survival despite high rates of treatment interruption: 24-month PFS was 73% (58%-91%) and 24 months OS was 90% (79%-100%). These outcomes are comparable to what was found in the randomized trials: in the SORMAIN trial 24-months RFS was 85% and 24-month OS was 90.5% in the sorafenib group (7). In phase III trial by Xuan et al, the authors found comparable outcomes 24-months RFS of 78.9% and 24-months OS of 82.% (8). We can hypothesize that sorafenib impact, despite the relatively short treatment course in most patients, could be due to a long-lasting immune-mediated effect. In a preclinical study, sorafenib was shown to promote a graft-versus-leukemia effect by inducing the secretion of T and NK cells growth factors, namely IL-15 by AML cells (21). Subgroup analysis done in the phase III trial revealed that patients who received allo-HCT from matched sibling donor and in patients without GVHD, retained the strongest benefit from sorafenib, also suggesting an immunomodulatory role (8). A non-mutually exclusive hypothesis is that the positive outcome observed can be due to alternative maintenance therapies in patients who were unable to tolerate sorafenib.

In conclusion, our real-world experience with sorafenib maintenance therapy after allogeneic HSCT reveals higher rates of toxicity-related dose reduction and drug interruption than previously reported in clinical trials. Importantly, we confirmed the benefit of the drug, despite high-interruption rates potentially as a consequence of the immunomodulatory role of sorafenib and/or of the feasibility of switching to midostaurin in case of intolerance.

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 Commission Cantonale d'Ethique de la Recherche sur

l'être humain de Genève. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

SM, FS, and YC designed the study. SM, FG, A-CM, AP, and SM-L collected the clinical data. SM and FS analyzed the data, performed statistical analysis, and prepared figures. SM wrote the manuscript. FS and YC provided overall guidance and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

FS: consulting fees from BMS/Celgene, Incyte, Kite/Gilead; speaker fees from Kite/Gilead, Incyte; travel support from Kite/Gilead, Novartis, AstraZeneca, Neovii, Janssen; research funding from Kite/Gilead, Novartis, BMS/Celgene. YC: consulting fees from MSD, Novartis, Incyte, BMS, Pfizer, Abbvie, Roche, Jazz, Gilead, Amgen, Astra-Zeneca, Servier; Travel support from MSD, Roche, Gilead, Amgen, Incyte, Abbvie, Janssen, Astra-Zeneca, Jazz.

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A novel inflammation-related prognostic model for predicting the overall survival of primary central nervous system lymphoma: A real-world data analysis

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Background: Primary central nervous system lymphoma (PCNSL) is a type of extranodal non-Hodgkin lymphoma. Although there are widely used prognostic scores, their accuracy and practicality are insufficient. Thus, a novel prognostic prediction model was developed for risk stratification of PCNSL patients in our research.

Methods: We retrospectively collected 122 patients with PCNSL from two medical centers in China from January 2010 to June 2022. Among them, 72 patients were used as the development cohort to construct a new model, and 50 patients were used for the validation. Then, by using univariate and multivariate Cox regression analsis and Lasso analysis, the Xijing model was developed and composed of four variables, including lesion number, β2-microglobulin (β2-MG), systemic inflammation response index (SIRI) and Karnofsky performance status (KPS). Finally, we evaluated the Xijing model through internal and external validation.

Results: Compared with the original prognostic scores, the Xijing model has an overall improvement in predicting the prognosis of PCNSL according to the time-dependent area under the curve (AUC), Harrell's concordance index (C-index), decision curve analysis (DCA), integrated discrimination improvement (IDI) and continuous net reclassification index (NRI). For overall survival (OS) and progression-free survival (PFS), the Xijing model can divide PCNSL patients into three groups, and shows more accurate stratification ability. In addition, the Xijing model can still stratify and predict prognosis similarly better in the elderly with PCNSL

and subgroups received high-dose methotrexate (HD-MTX) or Bruton's tyrosine kinase inhibitors (BTKi). Finally, external validation confirmed the above results.

Conclusions: Integrating four prognostic factors, including imaging findings, tumor burden, systemic inflammation response index, and comprehensive physical condition, we provided a novel prognostic model for PCNSL based on real-world data and evaluated its predictive capacity.

KEYWORDS

PCNSL, prognostic model, nomogram, risk stratification, SIRI

1 Introduction

Primary central nervous system lymphoma (PCNSL) is a rare extranodal non-Hodgkin lymphoma, which accounts for approximately 3-4% of intracranial malignancies and is localized to the cerebral parenchyma, leptomeninges, spinal cord and eyes, without peripheral involvement (1, 2). About 95% of PCNSL pathological types are diffuse large B-cell lymphoma (DLBCL), and the remaining rare pathological types include T-cell, Burkitt, lymphoblastic, and marginal zone lymphomas (3–6). PCNSL is characterized by strong aggressiveness, rapid disease progression, and poor prognosis, and the overall survival time of untreated PCNSL patients is only 1.5 months (7). Therefore, risk stratification and prognostic prediction of PCNSL patients are particularly important.

At present, the most widely used prediction models are the IELSG prognostic score and the MSKCC prognostic score. The former model, developed by the International Extranodal Lymphoma Study Group, contains five variables, age, deep brain lesions, Eastern Cooperative Oncology Group (ECOG) performance status, lactate dehydrogenase (LDH), and cerebrospinal fluid (CSF) protein level, each assigned 1 score, thus classifying PCNSL patients into low-risk (0-1 score), medium-risk (2-3 score), and high-risk (4-5 score) groups (8).. The latter was proposed by Memorial Sloan-Kettering Cancer Center and consists of only two variables: age and Karnofsky performance status (KPS). Patients can be divided into three groups according to age ≤ 50 years old, age>50 years old and KPS≥70, and age>50 years old and KPS<70 (9).

In recent years, the treatment of PCNSL is mainly based on high-dose methotrexate (HD-MTX). With the development of new therapeutic strategies such as immunotherapy for novel molecular targets, autologous hematopoietic stem cell transplantation, and CART therapy, the PFS and OS of patients with PCNSL have been improved (10–14), which may lead to changes in the predictive efficacy of previously developed IELSG and MSKCC scores. Therefore, there is an urgent need for a reliable predictive model suitable for the current stage to predict the survival outcome of PCNSL patients, carry out fine risk stratification, and provide a basis for clinical decision-making.

In this retrospective study, we collected data from 122 patients with PCNSL from two medical centers in Northwest China to construct a new predictive model, which was externally validated. The newly constructed model can more accurately predict the prognosis of PCNSL patients, stratify the risk of patients and provide clinical decision-making guidance.

2 Materials and methods

2.1 Cohort selection

A total of 122 newly diagnosed PCNSL patients were retrospectively collected from Xijing Hospital and Tangdu Hospital from January 2010 to June 2022. The diagnostic criteria were according to the 2003 Classification of Tumors of the Central Nervous System 2021, and no peripheral involvement was found by PET-CT, bone marrow cell morphology and puncture biopsy (15). The follow-up time was up to June 2022. All patients were at least 18 years old, and treatment and survival data were available. We selected PCNSL patients in Xijing Hospital as the development cohort (N=72), and PCNSL patients in Tangdu Hospital as the validation cohort (N=50). The flow chart of patient inclusion is shown in Figure 1.

2.2 Data collection

Basic and specific clinical information of patients was collected when PCNSL was first diagnosed. Patients' basic characteristics include age, sex, and history of underlying diseases, while specific clinical characteristics contain peripheral blood neutrophil count (NEU, $\times10^9$/L), lymphocyte count (LYM, $\times10^9$/L), mononuclear cell count, (MONO, $\times10^9$/L), platelet count (PLT, $\times10^9$/L), β_2-microglobulin (β_2-MG, mg/L), albumin (ALB, g/L), LDH (IU/L), CSF protein (g/L), KPS, ECOG performance status (ECOG-PS), number and location of lesions, immunohistochemical (IHC) results of pathologic tissue, IELSG and MSKCC score. In addition, immune inflammation index and prognostic nutritional index were calculated respectively based on β2-MG, LDH, ALP,

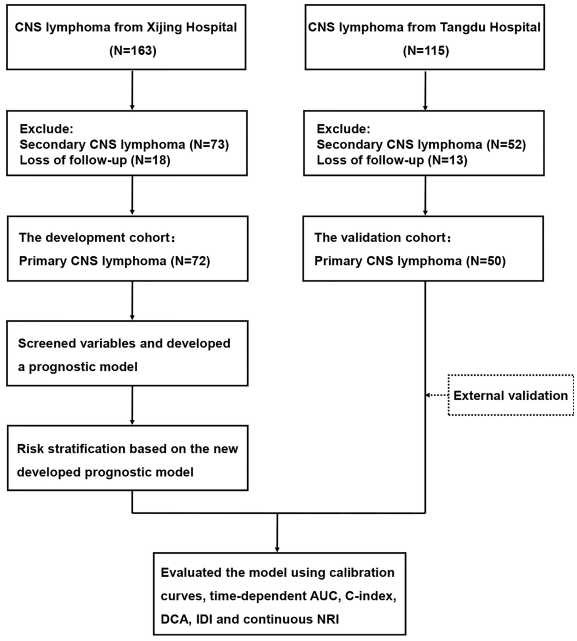

FIGURE 1
Flow chart of patient enrollment.

ALB, and complete blood cell count for new prognostic model development, including systemic inflammation response index (SIRI), systemic immune inflammation index (SII), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), β_2-microglobulin-to-lymphocyte ratio (βLR), lactate dehydrogenase-to-lymphocyte ratio (LLR), lymphocyte-to-monocyte ratio (LMR), serum albumin-to-alkaline phosphatase ratio (AAPR) and prognostic nutritional index (PNI). Their calculation formula is as follows (7): SIRI= NEU count ×MONO count/LYM count, SII= PLT count ×NEU count/LYM count, NLR= NEU count/LYM count, PLR= PLT count/LYM count, βLR= β2-MG/LYM count, LLR= LDH/LYM count, LMR= LYM count/MONO count, AAPR= ALB/ALP, PNI= ALB+5×LYM count.

2.3 Ending event definitions

The last follow-up was up to June 2022. The primary end point event was overall survival (OS), defined as the time from the diagnosis of PCNSL to all-cause death or the last follow-up. The secondary end point was progression-free survival (PFS), defined as the time from the diagnosis of PCNSL to disease progression or all-cause death or until the last follow-up.

2.4 Variables selection

Before screening the variables used to construct the new model, continuous variables with reference ranges are transformed into categorical variables according to normal values, while other continuous variables without normal reference ranges are transformed to categorical variables based on calculated cut-off values. As for the cut-off values of pathological indicators, we refer to previous published papers (16, 17). Besides, we perform multivariate imputation on missing data.

Univariate Cox regression was utilized to analyze and evaluate the variables in the development cohort, and P<0.1 was used as the criterion for screening candidate variables. To prevent overfitting, we performed Lasso regression on the selected candidate variables (18). Considering the clinical practicality, we finally selected four variables for the construction of a new predictive model for PCNSL, and wholly evaluated the model by multivariate Cox regression analysis.

2.5 Validation of the new developed model

We conducted internal and external validation of the developed model respectively, and adopted the following indicators to evaluate and verify the predictive ability of the model in the development and validation cohort. (1) Time-dependent area under the curve (AUC) and Harrell's concordance index (C-index): The discrimination of the new model was tested by time-dependent AUC of the receiver operator characteristic (ROC) and C-index (19). (2) Calibration curve: Bootstrap was used to conduct 1000 times resamples to draw the calibration curve. The coincidence degree between the curve and the 45° diagonal reflects the degree of agreement between the predicted probability and the actual result (20). (3) Decision curve analysis (DCA): It reflects the clinical usefulness of the new model as well as the range of risk thresholds

and net benefits, which shows if the model was the best choice for patients with PCNSL (21). (4) Integrated discrimination improvement (IDI) and continuous net reclassification index (NRI): These two indicators reflect whether the predictive capacity of the new model is improved compared with the original IELSG and MSKCC scores (22, 23).

2.6 Statistical methods

R version 4.1.0 and SPSS version 26.0 were used for statistical analysis, and a two-sided P<0.05 is statistically significant. Qualitative variables were analyzed by chi-square test or Fisher exact test, and quantitative variables were analyzed by Mann-Whitney U test. Kaplan-Meier method was used to draw survival curves, and Log-rank was used to test the differences between groups. Besides, univariate and multivariable Cox proportional hazard models were used to assess the prognostic variables and calculate hazard ratios (HR) with 95% confidence intervals (CI). The R packages used in the above statistical analysis are detailed in the Supplementary Materials.

3 Results

3.1 Baseline characteristics

The median ages of patients in the development cohort (N=72) and validation cohort (N=50) are 57.5(48.25-63) years and 61(51-67) years, respectively, and the male-to-female ratios are 1.32:1 and 1.08:1, respectively. In all collected patients, most of patients received chemotherapy, and other patients received treatments including surgery, whole brain radiation therapy (WBRT), surgery combined with WBRT, and palliative care. Among them, 46 patients (63.9%) in the development cohort received HD-MTX-based chemotherapy regimen (MTX or R ± MA) and 23 patients (31.9%) received immuno-targeted therapy containing Bruton's tyrosine kinase inhibitors (BTKi). Similarly, the number and proportion of patients in the validation cohort are 28(56%) and 10(20%), respectively. The remaining baseline characteristics are shown in Table 1. The duration of follow-up is 138 months as of June 1, 2022, with a median follow-up of 40 months (range from 1 to 126 months) and 48 months (range from 3 to 90 months) for the development and validation cohort. The median OS is 21 months

TABLE 1 Baseline characteristics of development and validation cohorts.

Characteristics	Development (n=72) n (%)	Validation (n=50) n (%)	P
Patient specific			
Age>60	46 (63.9)	30 (60)	0.663
Median age (IQR)	57.5 (48.25-63)	61 (51-67)	0.067
Male	41 (56.9)	26 (52)	0.589
Underlying disease			
Hypertension	22 (30.6)	13 (26)	0.584
Diabetes	13 (18.1)	8 (16)	0.767
CHD	5 (6.9)	2 (4)	0.492
Disease specific			
Deep brain lesions	43 (59.7)	37 (26)	0.103
Multiple lesions	43 (59.7)	28 (56)	0.682
ECOG-PS≥2	62 (86.1)	39 (78)	0.243
Median KPS (IQR)	60 (50-70)	60 (50-70)	0.685
CSF protein			0.740
>0.45 g/L	37 (51.4)	30 (60)	
Missing	7 (9.7)	—	
LDH>250 IU/L	19 (26.4)	22 (44)	0.043
β_2-MG>2.5 mg/L	42 (58.3)	32 (64)	0.529
D-Dimer>0.6 mg/L	43 (59.7)	32 (64)	0.633
RDW>0.15	16 (22.2)	19 (38)	0.058
ALB≥40 g/L	56 (77.8)	25 (50)	0.001
Bcl-2≥60%	37 (51.4)	24 (48)	0.713

(Continued)

TABLE 1 Continued

Characteristics	Development (n=72) n (%)	Validation (n=50) n (%)	P
Bcl-6≥40%	32 (44.4)	26 (52)	0.411
C-myc≥40%	54 (75)	35 (70)	0.541
MUM1≥40%	22 (30.6)	20 (40)	0.280
Ki67≥80%	50 (69.4)	37 (74)	0.584
SIRI≥3.3	19 (26.4)	14 (28)	0.844
SII≥97.4	17 (23.6)	33 (66)	<0.001
NLR≥5.1	29 (40.3)	18 (36)	0.633
PLR≥78.9	62 (86.1)	46 (92)	0.316
LLR≥220.3	24 (33.3)	17 (34)	0.939
βLR≥4.1	10 (13.9)	8 (16)	0.746
LMR≥2.7	48 (66.7)	29 (58)	0.329
PNI≥40	45 (62.5)	33 (66)	0.393
AAPR≥0.6	31 (43.1)	13 (26)	0.054
IELSG stratification			0.015
Low-risk	13 (18.1)	12 (24)	
Median-risk	35 (48.6)	14 (28)	
High-risk	17 (23.6)	24 (48)	
Missing	7 (9.7)	—	
MSKCC stratification			0.507
Low-risk	21 (29.2)	10 (20)	
Median-risk	19 (26.4)	14 (28)	
High-risk	32 (44.4)	26 (52)	
Chemotherapy regimen			
HD-MTX-based	46 (63.9)	28 (56)	0.380
Treatment containing BTKi	23 (31.9)	10 (20)	0.144
AHSCT	5 (6.9)	—	—

IQR, interquartile range; CHD, coronary heart disease; ECOG-PS, Eastern Cooperative Oncology Group performance status; KPS, Karnofsky performance status; CSF, cerebrospinal fluid; LDH, lactate dehydrogenase; β2-MG, β2-microglobulin; RDW, red blood cell volume distribution width; ALB, albumin; SIRI, systemic inflammation response index; SII, systemic immune inflammation index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LLR, lactate dehydrogenase-to-lymphocyte ratio; βLR, β2-microglobulin-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PNI, prognostic nutritional index; AAPR, serum albumin-to-alkaline phosphatase ratio; IELSG, International Extranodal Lymphoma Study Group; MSKCC, Memorial Sloan Kettering Cancer Center; HD-MTX, high-dose methotrexate; BTKi, Bruton's tyrosine kinase inhibitors; AHSCT, Autologous haematopoietic stem cell transplantation; -, none.

and 17 months, and the median PFS is 6 months and 7 months, respectively.

3.2 Development and evaluation of the Xijing model

We first performed univariate Cox regression analysis on all the variables in the development cohort and filtered out 16 variables with P<0.1, including age, number of lesions, ECOG-PS≥2, KPS, LDH, β2-MG, ALB, RDW, D-Dimer, Bcl-2, C-myc, SIRI, PLR, LLR, βLR, and PNI (Table 2). Lasso analysis was then performed on above variables to identify six candidate variables: age, Bcl-2, number of lesions, β2-MG, KPS, and SIRI (Supplemental Figure S1). However, based on clinical experience, two candidate variables, age and Bcl-2, were excluded, and we then determined four variables, number of lesions, β2-MG, KPS and SIRI, to be included in the multivariate Cox regression analysis. Finally, a new prediction model was constructed by multivariate Cox proportional hazard model (Table 3), in which the P values of the three tests (Likelihood ratio, Wald, and Score) of the model were all less than 0.001, indicating a good fit of the Xijing model. The nomogram of the Xijing model is shown in Figure 2A, of which the points of each variable and the 1-year, 2-year and 5-year survival probability corresponding to the total points in the nomogram are displayed in Supplemental Tables S1; S2.

In addition, we compared the Xijing model with the widely used IELSG and MSKCC scores to assess the predictability of the Xijing

TABLE 2 Univariate Cox regression analysis in the development cohort.

Characteristics	HR	95% CI	P
Age>60	1.254	0.661-2.379	0.489
Female	1.253	0.677-2.322	0.473
Deep brain lesions	1.633	0.862-3.094	0.133
Multiple lesions	2.694	1.336-5.431	0.006*
ECOG-PS≥2	2.830	1.506-5.314	0.001*
KPS	0.966	0.951-0.981	<0.001*
LDH>250 IU/L	2.252	1.174-4.320	0.015*
CSF protein>0.45 g/L	1.459	0.781-2.727	0.237
β_2-MG>2.5 mg/L	3.054	1.463-6.415	0.003*
ALB≥40 g/L	2.793	0.990-7.876	0.052
RDW>0.15	1.963	0.946-4.075	0.070
D-Dimer>0.6 mg/L	1.736	0.904-3.332	0.097
Bcl-2≥60%	2.158	1.159-4.017	0.015*
Bcl-6≥40%	0.819	0.442-1.518	0.527
MUM1≥40%	0.733	0.390-1.377	0.334
C-myc≥40%	2.057	1.041-4.065	0.038*
Ki-67≥80%	1.362	0.665-2.793	0.399
SIRI≥3.3	2.898	1.551-5.415	<0.001*
SII≥97.4	1.579	0.787-3.170	0.199
NLR≥5.1	1.405	0.759-2.601	0.279
PLR≥78.9	0.471	0.216-1.025	0.058
LLR≥220.3	1.924	1.029-3.598	0.041*
βLR≥4.1	3.038	1.402-6.582	0.005*
LMR≥2.7	0.637	0.339-1.197	0.192
PNI≥40	0.394	0.210-0.741	0.004*
AAPR≥0.6	0.653	0.341-1.249	0.198

ECOG-PS, Eastern Cooperative Oncology Group performance status; KPS, Karnofsky performance status; LDH, lactate dehydrogenase; CSF, cerebrospinal fluid; β2-MG, β2-microglobulin; ALB, albumin; RDW, red blood cell volume distribution width; SIRI, systemic inflammation response index; SII, systemic immune inflammation index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LLR, lactate dehydrogenase-to-lymphocyte ratio; βLR, β2-microglobulin-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PNI, prognostic nutritional index; AAPR, serum albumin-to-alkaline phosphatase ratio; CI, confidence interval; HR, hazard ratio; *Statistically significant (P<0.05).

model. In the development cohort, we took 24 samples as a group, conducted bootstrap with 1000 times resample to draw calibration curves, and evaluated the calibration degree of the Xijing model. The calibration curve of 1-year OS indicated that predictive probability was basically consistent with the actual observed probability (Figure 2B). In terms of discrimination, the 1, 2 and 5-year ROC curves of Xijing model are shown in the Figure 2C. Both time-dependent AUC and C-index of the Xijing model were overall higher than those of the existing IELSG and MSKCC scores (Table 4; Figures 3A, B). We also assessed the clinical net benefit of the Xijing model by decision curve analysis (DCA) (Figure 3C), which demonstrated that the Xijing model could achieve more positive net benefit and larger area under the decision curve (AUDC) over a wider range of risk threshold than that of IELSG and MSKCC scores (Table 4). Moreover, the 2-year and 5-year OS calibration curves and DCA of the Xijing model are presented in the Supplemental Figure S2.

We also calculated the IDI and continuous NRI (Table 4) of the Xijing model to evaluate whether there was an improvement in the prediction efficiency between the Xijing model and the two existing prognostic scores. Compared with IELSG score, the Xijing model improved the predictive efficiency of 1-year and 2-years OS in PCNSL patients. The model indicated that the IDI of 1-year OS was 16.5% (P=0.03, Figure 3D), and the IDI of 2-year OS was 14.4% (P=0.02, Supplemental Figure S3A). The difference was significant. The IDI of 5-year OS was -3.9%, no statistical difference (P=0.905, Supplemental Figure S3C). However, the 1-year, 2-year, and 5-year continuous NRI of the Xijing model were 33.7%, 23.1%, and -8.6%,

TABLE 3 Multivariate Cox regression analysis in the development cohort.

Characteristics	Coefficient	HR	95% CI	P
Lesion number				
Single vs. Multiple	0.937	2.553	1.213-5.377	0.014*
β_2-MG (mg/L)				
<2.5vs.≥2.5	0.903	2.468	1.158-5.258	0.019*
SIRI				
<3.3vs.≥3.3	0.602	1.826	0.940-3.550	0.076
KPS	-0.028	0.972	0.956-0.988	<0.001*
Statistical analysis of the prognostic model				
Likelihood ratio test				<0.001*
Wald test				<0.001*
Score (log-rank) test				<0.001*

β2-MG, β2-microglobulin; SIRI, systemic inflammation response index; KPS, Karnofsky performance status; CI, confidence interval; HR, hazard ratio; *Statistically significant (P<0.05).

respectively, with no statistical difference (P=0.06, 0.119, and 0.965, respectively).

Additionally, compared with MSKCC score, the 1-year and 2-year IDI of the Xijing model was 24.9% (P<0.001, Figure 3E) and 19.3% (P=0.03, Supplemental Figure S3B), respectively, which indicated the improvement of predictive efficiency for 1-year OS and 2-year OS. However, 5-year IDI was 1%, showing no statistical difference (P=0.826, Supplemental Figure S3D). The continuous

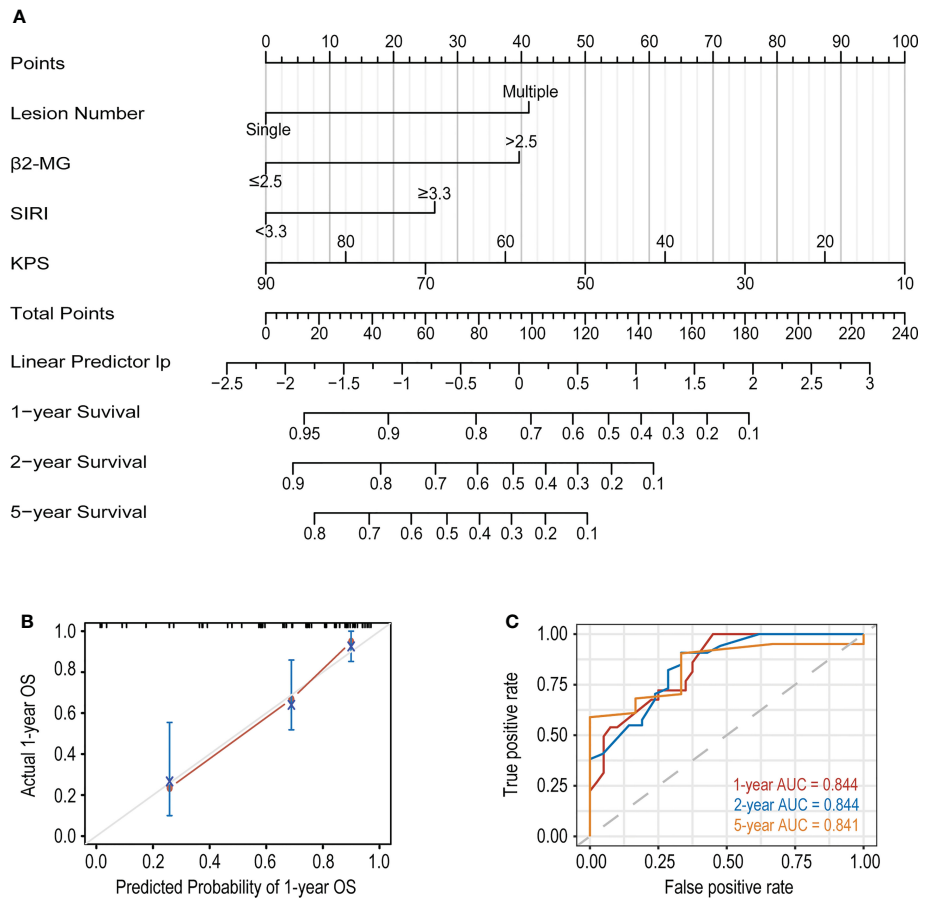

FIGURE 2
(A) The nomogram based on the development cohort to predict OS of PCNSL patients. **(B)** The calibration curve of the Xijing model for predicting 1-year OS. **(C)** The 1, 2 and 5-year ROC curves of the Xijing model.

TABLE 4 Comprehensive evaluations of different models in the development cohort.

OS	12 months	24 months	60 months
AUC, n (95% CI)			
Xijing Model	0.844 (0.750-0.938)	0.844 (0.740-0.948)	0.841 (0.694-0.989)
IELSG	0.776 (0.676-0.876)	0.747 (0.626-0.867)	0.806 (0.624-0.989)
MSKCC	0.672 (0.539-0.804)	0.720 (0.590-0.850)	0.762 (0.551-0.973)
C-index, n			
Xijing Model	0.814	0.794	0.769
IELSG	0.735	0.696	0.683
MSKCC	0.648	0.636	0.640
Range, n(%)			
Xijing Model	3.63%-98.42%	8.01%-99.99%	13.69%-80.58%
IELSG	15.40%-60.98%	28.32%-54.60%	40.71%-71.07%
MSKCC	19.23%-48.95%	34.25%-73.29%	48.96%-69.68%
AUDC, n			
Xijing Model	0.1288	0.2649	0.3098
IELSG	0.0564	0.1060	0.1423
MSKCC	0.0319	0.0730	0.1248
IDI, n (95% CI), P value			
vs. IELSG	16.5% (1.5%-31.9%) P=0.030*	14.4% (1.4%-33.8%) P=0.020*	-3.9% (-28.6%-26.2%) P=0.905
vs. MSKCC	24.9% (12.0%-40.9%) P<0.001*	19.3% (1.8%-39.7%) P=0.030*	1.0% (-24.9%-32%) P=0.826
Continuous NRI, n (95% CI), P value			
vs. IELSG	33.7% (-0.3%-57.5%) P=0.06	23.1% (-5.0%-61.7%) P=0.119	-8.6% (-55.4%-61.2%) P=0.965
vs. MSKCC	40.6% (11.1%-69.0%) P<0.001*	38.2% (-5.1%-67.4%) P=0.090	48.8% (-43.2%-76.3%) P=0.577

OS, overall survival; AUC, area under the curve; C-index, Harrell's concordance index; Range, range of risk threshold to get a positive net benefit in the decision curve analysis; AUDC, area under the decision curve analysis; IDI, integrated discrimination improvement; NRI, net reclassification index; IELSG, International Extranodal Lymphoma Study Group; MSKCC, Memorial Sloan Kettering Cancer Center; *Statistically significant (P<0.05).

NRI of 1-year, 2-year and 5-year OS was 40.6%, 38.2% and 48.8%, respectively, and only the continuous NRI of 1-year OS was statistically significant (P<0.001).

3.3 Risk stratification of the Xijing model

First of all, we calculated the points of each patient based on nomogram of the Xijing model (Table S1), and then used X-tile software to calculate the optimal cut-off value (24) based on all patients' points. According to the cut-off value, patients were divided into three groups: low-risk group (≤93), medium-risk group (>93 and <141), and high-risk group (≥141).

In the development cohort (N=72), there were 37(51.4%) patients at low-risk group, 21(29.2%) patients at medium-risk group, and 14(19.4%) patients at high-risk group, with the medium OS of 48, 19 and 5 months and the median PFS of 10, 5.5 and 3 months, respectively (Table 5). Both the medium OS and PFS of each group were shorter than that of the corresponding stratification in the IELSG and MSKCC scores, suggesting that the Xijing model may have better performance in finer prognostic stratification. Subsequently, we used the Xijing model, IELSG and MSKCC scores to stratify the patients in the development cohort

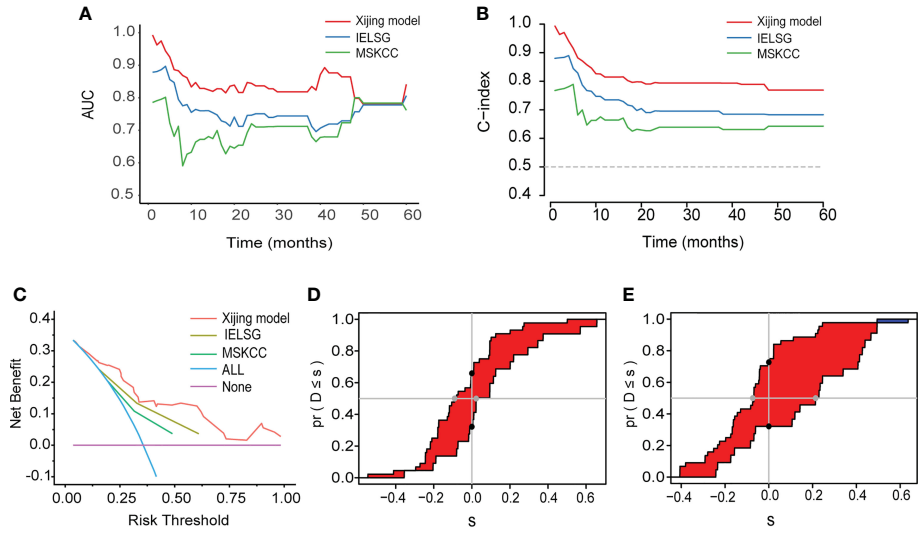

FIGURE 3
(A) The time-dependent AUC of the three models. **(B)** The time-dependent Harrell's C-index of the three models. **(C)** The DCA was used to estimate clinical usefulness of the three models for predicting 1-year OS. **(D, E)** Compared with the IELSG and MSKCC scores, the IDI and continuous NRI indicated the improvement of prediction ability of the Xijing model. The IDI is the value of the difference in area between red and blue zones. The continuous NRI is the value of the distance between two black dots.

respectively, and plotted survival curves (Figures 4A-F) as well as the distribution and co-occurrence graph of the three stratifications (Figure 5A). We found that there existed overlapping survival curves between low and medium-risk groups of the MSKCC score, suggesting poor differentiation of patients in the low and medium-risk groups. Furthermore, the distribution and co-occurrence graph of the patients displayed that there were 54 patients in the medium and high-risk groups of the IELSG score, of which 34 patients were stratified inconsistently with the Xijing model, accounting for 62.7%. While, the stratification of the MSKCC score was inconsistent with that of Xijing model in 19 (47.5%) of the 40 patients in the low and medium-risk groups. These above suggest that the Xijing model can further identify specific groups of another two prognostic scores precisely, including the medium and high-risk groups of the IELSG score, as well as the low and medium-risk groups of the MSKCC score. The survival curves shown in Figures 5B, C suggest that the specific groups of the IELSG and MSKCC mentioned above can be reclassified into three groups more precisely, and the Log-rank test showed statistical significance between groups (P<0.0001 and P=0.0004).

TABLE 5 The OS and PFS of different stratifications in the development cohort.

Stratification	Median OS (months)	Median PFS (months)
Xijing Model		
Low-risk	48	10
Median-risk	19	5.5
High-risk	5	3
IELSG		
Low-risk	89	18
Median-risk	23	7
High-risk	6	4
MSKCC		
Low-risk	NR	8.5
Median-risk	23	8.5
High-risk	12	4.5

OS, overall survival; PFS, progression-free survival; IELSG, International Extranodal Lymphoma Study Group; MSKCC, Memorial Sloan Kettering Cancer Center; NR, not reached.

3.4 Subgroup analysis

Researches have shown that age is one of the important prognostic factors for patients with PCNSL (8–10). In the newly developed Xijing model, we also explored its applicability for specific populations with age>60 years old. The survival curves (Figures 6A, B) showed that the Xijing model also had the ability of prognostic prediction in elderly patients with PCNSL.

The treatment for PCNSL is mainly based on standard HD-MTX chemotherapy nowadays. Many patients have already accepted HD-MTX-based induction chemotherapy regimens at the time of admission. Therefore, as a prognostic model, the Xijing model need to be confirmed in the modern combination chemotherapy regimens based on HD-MTX. We collected data from 46 patients with PCNSL after the first course of HD-MTX treatment (Table S3), and further explored the stratifying capacity of the Xijing model for these patients by plotting survival curves (Figure 6C). We found the model is able to perform risk stratification in the specific subgroup treated with HD-MTX similarly better.

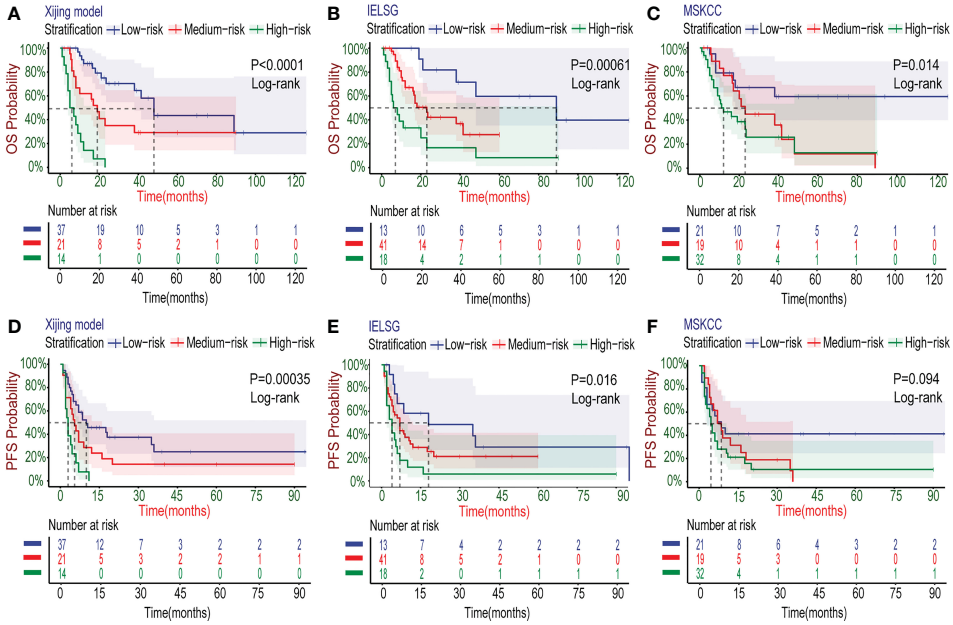

FIGURE 4
Kaplan–Meier survival curves in the development cohort. **(A-C)** For OS, the PCNSL patients were stratified into three groups by the Xijing Model, IELSG and MSKCC prognostic scores. **(D-F)** The PFS was also classified by above three risk stratifications.

It is worth noting that the Xijing model is equally applicable for PCNSL patients who have been treated with BTKi (detailed treatment regimens including BTKi are shown in Table S4) in the development cohort (Figure 6D), which suggests that the Xijing model may still perform better in the era of immune targeted therapy for PCNSL, compared to the original scores.

3.5 External validation of the Xijing model

In the validation cohort, we verified the predictive performance of the Xijing model comprehensively. Specifically, the calibration curves of the Xijing model for 1-year, 2-year and 5-year OS are all close to 45° diagonal line, indicating that the predictive probability of Xijing model is roughly consistent with actual observation results (Figure 7A; Supplemental Figures S4A, B).

Table 6 displays the results of other comprehensive evaluation of the Xijing model in the validation cohort, in which both the time dependence AUC and C-index of Xijing model are overall higher than those of the IELSG and MSKCC scores (Figures 7B, C). Besides, the DCA of the Xijing model has a larger net benefit among a wider range of risk thresholds than the other two existing prognostic scores (Figure 7D; Supplemental Figures S4C, D). According to IDI, the ability of the Xijing model to predict 1-year OS in PCNSL patients was improved, compared to the IELSG and MSKCC prognostic scores (29.6%, P=0.01 and 26.1%, P=0.01, Figures 7E, F). Particularly, the Xijing model still has a tendency to perform better than the MSKCC score in predicting 2-year OS (18%, P=0.06, Supplemental Figure S5B). But compared with the IELSG score, there was no statistical difference about the predictive improvement shown by 2-year IDI of the Xijing model (10%, P=0.398, Supplemental Figure S5A). As for the continuous NRI, the Xijing model showed an improvement in 1-year and 2-year OS prediction compared with MSKCC score (44.1%, P=0.04 and 48.3%, P=0.05), and also showed a better trend in 1-year OS prediction compared with IELSG score (53.3%, P=0.07). However, there is no statistical difference in the improvement of continuous NRI in 2-year OS prediction (31.4%, P=0.199). For the 5-year OS prediction of PCNSL patients, there was no statistical difference in the improvement of prediction efficiency of IDI and continuous NRI in the Xijing model (Supplemental Figures S5C, D). Finally, we used the nomogram mentioned above to calculate the total points of each patient in the validation cohort and divided those patients into three groups according to the risk-stratification criteria of the Xijing model. The groups were as follows: 19(38%) patients in the low-risk group, 14(28%) patients in the medium-risk group, and 17(34%) patients in the high-risk group, with median OS of 30 months, 17 months, and 7 months and median PFS of 14 months, 10 months and 3 months, respectively. Subsequently, the OS and PFS survival curves of the validation cohort were plotted (Figures 8A-F) with statistical difference existing in both the Xijing model and the above mentioned two prognostic scores by the Log-rank test.

4 Discussion

PCNSL refers to a class of rare malignancies originating in the CNS without peripheral involvement. The annual incidence of PCNSL has increased in recent decades, which is about 0.48/100,000 (25). More than 80% of patients with PCNSL have intracranial lesions, and only a few have leptomeninges and eyes involvement (26). The clinical symptoms of PCNSL mostly include

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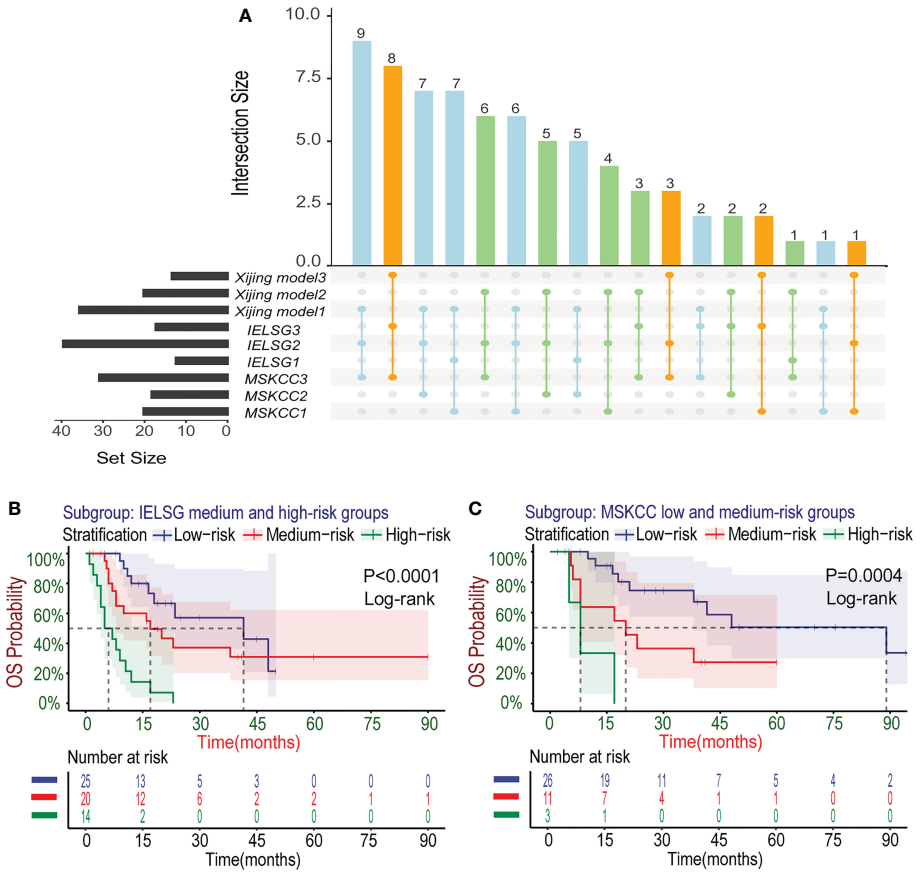

FIGURE 5
Subgroup analysis about the three risk stratifications in the development cohort. **(A)** The distribution and co-occurrence of the patients respectively classified by the Xijing Model, IELSG and MSKCC scores in the development cohort were shown. Dots and their connected lines indicate that the patients coexisted in different stratifications and the vertical bar graphs reflect the number of these patients. Also, the blue, orange and green dots respectively represent the co-occurrence of the patients who were classified into low, median and high-risk groups by the Xijing Model and other two scores. **(B)** Subgroup analysis with the survival curves for OS in the medium and high-risk groups of IELSG score stratified by the Xiijing model. **(C)** Subgroup analysis with the survival curves for OS in the low and medium-risk groups of MSKCC score stratified by the Xijing model.

consciousness disturbance, headache, hemiplegia, epilepsy, aphasia, and visual abnormalities, which are easy to be misdiagnosed because of specificity lacking (2). At present, there is no clear and unified standard for the treatment of PCNSL, and the major treatments include MTX-based combined chemotherapy, surgical resection, whole brain radiation therapy, etc. (27). In recent years, with the introduction of new strategies, such as molecular-targeted drugs, autologous hematopoietic stem cell transplantation (AHSCT) and chimeric antigen receptor T-cell immunotherapy (CAR-T), though the survival rate of PCNSL patients has improved, the overall prognosis of PCNSL is still unsatisfactory. It has been reported that the 5-year OS rate of PCNSL patients is only 15-30%, which undoubtedly increases the economic burden on both patients and society (10, 14, 28).Therefore, a reliable prognostic prediction model which can stratify accurately and guide clinical decisions is of particular importance for patients with PCNSL.

The existing IELSG and MSKCC prognostic scores are widely used in clinical practice, but they both have their own limitations. Although the research that developed the IELSG score was based on multi-center and had a large sample size (N=378), data on LDH and CSF proteins were missing in 2/3 of the samples (8). Besides, we found that CSF protein is usually difficult to obtain in clinical practice due to the contraindications of lumbar puncture, the non-cooperation of patients and unnecessary of examination. Therefore, it is difficult for some patients to effectively predict the prognosis by IELSG score, which also limits the clinical application of IELSG score (29–31). As for the MSKCC score, though external validation of its predictive validity has been confirmed in the original study, there still be bias in the risk stratification based on the two variables of age and KPS only. Some relevant studies have not found a strong association between the MSKCC score and the OS of patients with PCNSL (32, 33), which caused controversy over the reliability of the MSKCC score. Thus, some researches attempted to improve the predictive capacity of the MSKCC score by adding some prognosis-related factors, such as SII and TBIL (34, 35). Unfortunately, all the subjects of the improved MSKCC score were from single-center and received high-dose MTX chemotherapy, and some of them received glucocorticoids before diagnosis, which may affect prognostic prediction and lead to bias. In addition, the lack of external validation also limits the universality of the improved MSKCC score.

Based on 122 patients with PCNSL from two medical centers in China, our research has developed a novel and simple prognostic

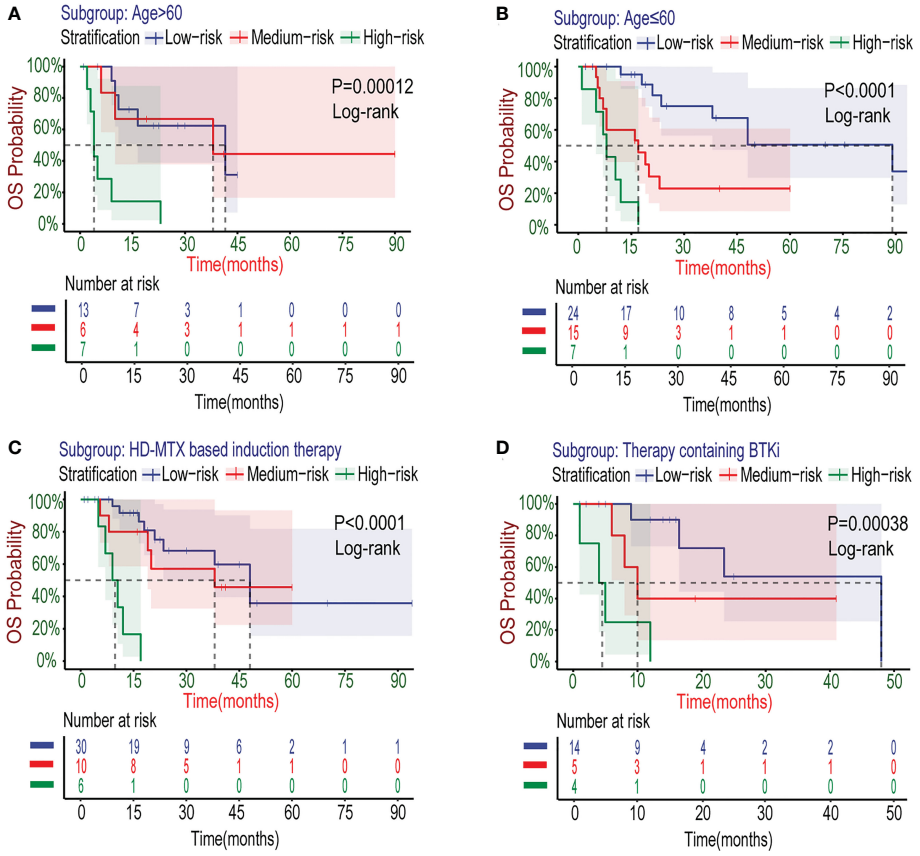

FIGURE 6
Kaplan–Meier survival curves of specific subgroups stratified by the Xijing Model in the development cohort. The OS curves for subgroups with different characteristics of age **(A, B)** and therapy **(C, D)** were displayed.

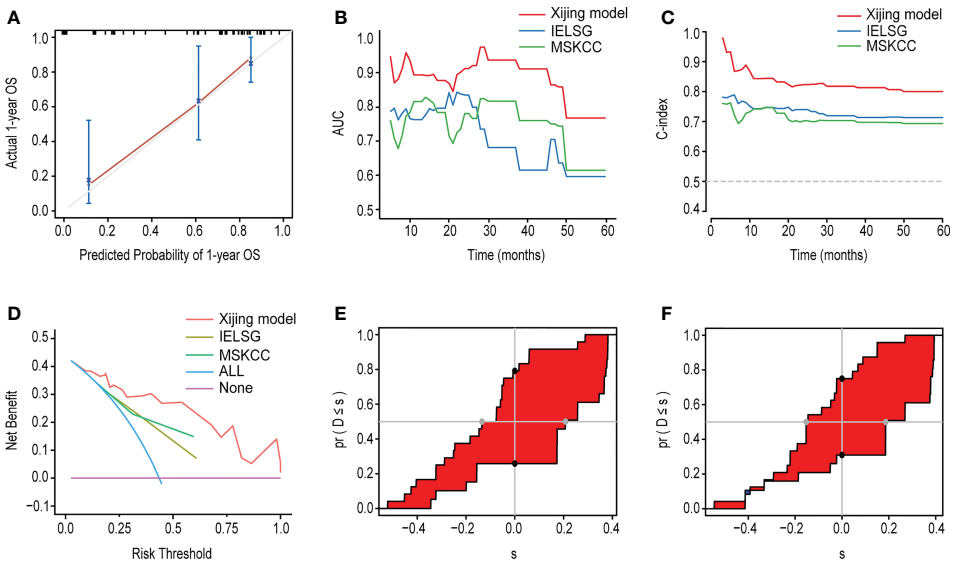

FIGURE 7
The performance of the Xijing Model, IELSG and MSKCC scores for predicting OS in the validation cohort. **(A)** The time-dependent AUC of the three models. **(B)** The time-dependent Harrell's C-index of the three models. **(C)** The DCA was used to estimate clinical usefulness of the three models for predicting 1-year OS. **(D)** The improvement in prediction of the Xijing model compared to the IELSG **(E)** and MSKCC **(F)** scores. The IDI is the value of the difference in area between red and blue zones. The continuous NRI is the value of the distance between two black dots.

TABLE 6 Comprehensive evaluations of different models in the validation cohort.

OS	12 months	24 months	60 months
AUC, n (95% CI)			
Xijing Model	0.895 (0.801-0.989)	0.913 (0.829-0.997)	0.769 (0.623-0.914)
IELSG	0.764 (0.641-0.887)	0.836 (0.675-0.997)	0.598 (0.198-0.998)
MSKCC	0.817 (0.709-0.924)	0.782 (0.624-0.940)	0.616 (0.236-0.996)
C-index, n			
Xijing Model	0.843	0.823	0.80
IELSG	0.743	0.740	0.713
MSKCC	0.742	0.702	0.693
Range, n(%)			
Xijing Model	2.54%-100%	8.21%-100%	22.82%-100%
IELSG	34.17%-64.90%	37.36%-90.56%	58.97%-86.48%
MSKCC	14.25%-59.60%	29.89%-87.68%	53.82%-84.68%
AUDC, n			
Xijing Model	0.2367	0.4710	0.5486
IELSG	0.0865	0.1988	0.1864
MSKCC	0.1022	0.1974	0.1871
IDI, n (95% CI), P value			
vs. IELSG	29.6% (5.20%-49.8%) P=0.01*	10.0% (-12.9%-32.6%) P=0.398	-5.5% (-34.0%-40.1%) P=0.915
vs. MSKCC	26.1% (3.7%-44.9%) P=0.01*	18.0% (-1.1%-38.9%) p=0.06	-8.8% (-44.3%-39.8%) P=0.796
Continuous NRI, n (95% CI), P value			
vs. IELSG	53.3% (-1.8%-79.0%) P=0.07	31.4% (-1.0%-57.2%) P=0.199	25.4% (-42.0%-78.9%) P=0.557
vs. MSKCC	44.1% (1.0%-79.4%) P=0.04*	48.3% (0%-71.9%) P=0.05	20.5% (-46.3%-82.4%) P=0.627

OS, overall survival; AUC, area under the curve; C-index, Harrell's concordance index; Range, range of risk threshold to get a positive net benefit in the decision curve analysis; AUDC, area under the decision curve analysis; IDI, integrated discrimination improvement; NRI, net reclassification index; IELSG, International Extranodal Lymphoma Study Group; MSKCC, Memorial Sloan Kettering Cancer Center; *Statistically significant (P<0.05).

model and plot the nomogram. Considering that the median OS of the development cohort was 21 months (roughly 2 years) and most patients achieved primary end event within 2 years (2-year survival probability 45.6%), we paid more attention to 2-year OS rather than the usual 3-year OS, which was also similar to a Singaporean retrospective study by Lo YT et al. (36). Thus, the Xijing model is used to predict the 1,2 and 5-year OS for PCNSL. First of all, we screened out six prognosis-related candidate variables by univariate Cox regression analysis and Lasso analysis, including age, Bcl-2, the number of lesions, KPS, β2-MG and SIRI. However, Bcl-2 is only expressed on the cell surface of B-cell lymphomas, and the pathological types of PCNSL include peripheral T-cell lymphomas in addition to B-cell lymphomas. Besides, the evaluation of Bcl-2 IHC positive results depends on the judgment of professional pathologists, which is greatly affected by clinical experience and other personal factors and is prone to bias. Therefore, Bcl-2 is not a suitable candidate variable for all patients with PCNSL. As for age, with the development of treatment, the OS of PCNSL patients has been improved, and the influence of age on the prognosis of PCNSL has a downward trend. To sum up, given the clinical relevance and statistical significance, the remaining four variables, the number of lesions, β2-MG, KPS and SIRI, were incorporated eventually to

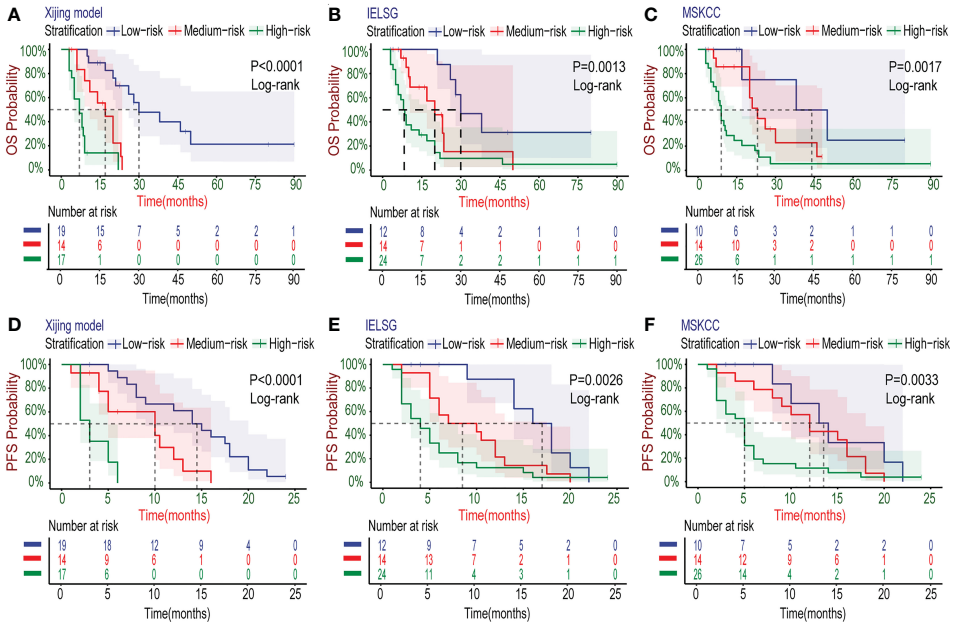

FIGURE 8
Kaplan–Meier survival curves in the validation cohort. **(A-C)** The OS curves of PCNSL patients stratified by the Xijing Model, IELSG and MSKCC scores. **(D-F)** The PFS curves of PCNSL patients classified by the three risk stratifications.

develop the prognostic model and ensure parsimony of the final model. Thus, the Xijing model predicts 1,2 and 5-year survival probability for PCNSL.

Then, we drew calibration curves, DCA and calculated time-dependent AUC, time-dependent C-index, IDI and continuous NRI, and compared the Xijing model with two widely used existing scores to evaluate the prediction capability of the Xijing model. The results of our research show that the above evaluation indicators of the Xijing model have an overall improvement, compared with the IELSG and MSKCC scores. The Xijing model performs better in terms of discrimination, calibration, clinical net benefit and predictive efficiency, and has greater prognostic prediction value for PCNSL patients. We also used the nomogram of Xijing model to score the patients in the development cohort, and divided them into low-risk (≤93), medium-risk (>93 and <141) and high-risk group (≥141) by the cut-off value. The survival curves were drawn and statistically tested, and there were statistical differences among the groups. Finally, we completed the external validation of the Xijing model in the validation cohort.

In addition to the improvement of predictive capacity, compared with the two existing prognostic scores, the advantages of the Xijing model are that the four variables it contains are more easily to be obtained in clinical practice and the process of prognostic assessment is visual. Among them, the number of lesions can be achieved by imaging examination and reflects the extent of tumor involvement. KPS is generally completed at the time of admission assessment, which can easily reflect the patients' physical condition. β2-MG, which reflects the patient's tumor burden, can be obtained by peripheral blood tests (37–39), while SIRI can be calculated by complete blood count. Our study has shown that the four variables of Xijing model are all related to the prognosis of PCNSL patients. Compared with the only two variables of MSKCC score, a more comprehensive judgment can be made from the four aspects of the number of lesions, tumor burden, systemic inflammatory response and physical condition. It is worth mentioning that SIRI is an emerging indicator of systemic inflammation in recent years. More and more evidence suggesting that tumor-related inflammatory response promotes the proliferation, invasion and metastasis of tumor cells (40–42), and more studies have confirmed that SIRI is an independent prognostic risk factor for kinds of malignant tumors, including breast cancer, hepatocellular carcinoma, glioblastoma, pancreatic cancer as well as PCNSL (7, 43–47).

Xijing model can more comprehensively predict the 1-year, 2-year and 5-year OS probability of PCNSL patients from four aspects: imaging findings, tumor burden, systemic inflammatory response index and comprehensive physical condition. In addition, the Xijing model can further stratify the medium and high-risk groups of IELSG as well as the low and medium-risk groups of MSKCC, indicating that the Xijing model performs better on detailed stratification and accurate prediction for patients with PCNSL. Similarly, the elderly with PCNSL and specific populations who have accepted HD-MTX or BTKi treatment can also be stratified by the Xijing model, which validates the utility of the Xijing model in specific subgroups. Finally, due to the fact that the source of samples for the development and validation cohort were inevitably different, the Xijing model showed good predictive ability in both cohorts, suggesting the universality of the Xijing model.

However, our model still exists some limitations. First, PCNSL is rare, and it is difficult to recruit a large number of patients in

clinical practice. Therefore, the sample size for the new model is small, and a larger sample size from multiple medical centers is needed for validation in the future. Second, the study was based on a Chinese population, which may affect how the Xijing model performs in other populations.

5 Conclusion

In summary, we developed a new PCNSL prognostic model based on real-world data and visualized it by nomogram. The variables in the model are easy to obtain and strongly practical. The validation results demonstrate that the Xijing model has better prediction ability, universality, and higher clinical application value.

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 Xijing and Tangdu hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZWu and CW conceived the present study, participated in its methodology design, drafted the manuscript and interpreted the data. RL, GG, MH and ZWa conceptualized the study and participated in methodology design. JW, XD, NZ, JG and YZ enrolled the patients. YaL, ZL, ML, SW, BW, NY and YeL acquired the data. Data analysis was performed by ZWu and CW. All authors contributed to the article and approved the submitted version.

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1104425/full#supplementary-material

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Radiotherapy or chemotherapy: a real-world study of the first-time relapsed and refractory primary central nervous system lymphoma

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Background: Primary central nervous system lymphoma (PCNSL) is an uncommon variant of non-Hodgkin lymphoma (NHL) with high aggressiveness and poor prognosis. Although complete remission (CR) could be achieved with therapy, some patients remain refractory or recurrently with a worse response to salvage treatment and poor prognosis. No consensus on rescue therapy has been established currently. This study is aimed to evaluate the efficacy of radiotherapy or chemotherapy in first-time relapsed or refractory progressed PCNSL (R/R PCNSL) and analysis the prognostic factors, to explore differences between relapsed and refractory PCNSL.

Methods: Totally 105 R/R PCNSL patients from Huashan Hospital between 1 January 2016 and 31 December 2020 were enrolled, underwent salvage radiotherapy or chemotherapy and received response assessments after each course. PFS1 was defined as the time from diagnosis to the first time of recurrence or refractory progression. Statistical analysis was performed with SPSS version 26.0.

Results: Response and survival were analyzed over a 17.5months (median) follow-up. Compared to relapsed PCNSL (n = 42), refractory PCNSL (n = 63) had a shorter median PFS1 related to deep lesions. 82.4% of cases were discovered as the second relapse or progression. ORR and PFS were both higher in relapsed PCNSL than those in refractory PCNSL. ORR of radiotherapy in both relapsed and refractory PCNSL was higher than that of chemotherapy. Elevated CSF protein and ocular involvement were related to PFS and OS after recurrence respectively in relapsed PCNSL. Age ≥ 60y was unfavorable to OS-R (OS after recurrence or progression) in refractory PCNSL.

Conclusions: Our results indicate that relapsed PCNSL responds well to inducing and salvage therapy and has a better prognosis compared to refractory PCNSL. Radiotherapy is effective for PCNSL after the first relapse or progression. Age, CSF protein level, and ocular involvement could be potential factors to predict prognosis.

KEYWORDS

relapsed or refractory primary central nervous system lymphoma, radiotherapy, chemotherapy, prognosis, salvage therapy

Introduction

Primary central nervous system lymphoma (PCNSL) is a rare type of extranodal non-Hodgkin lymphoma (NHL), accounting for less than 3% of NHL and about 2%-4% of primary intracranial tumors (1, 2). PCNSL originates in the central nervous system, with lesions confined to the brain parenchyma, soft meninges, cerebrospinal fluid (CSF), spine, and eyes, and rarely involved other systems.

PCNSL is prevalent in the elderly population over 60 years of age, and a rising incidence has been recognized over the past two decades, reaching 0.5 per 100,000 person-years (2–4). Compared with system lymphomas outside the CNS, the prognosis for PCNSL is usually poor, with a 5-year survival rate of only 30-40%, a median progression-free survival (PFS) of 24 months, and a median overall survival (OS) of 36.9-46 months (5–7). Nearly a century of clinical experience and research have proven that the first-line treatment for newly-diagnosed PCNSL patients is chemotherapy based on high-dose methotrexate (HD-MTX) (>3g/m^2). However, there are still 10%-35% of refractory PCNSL remain insensitive to HD-MTX, and even among patients who achieve remission with first-line therapy, 35%-60% eventually experience relapse (8). Moreover, the prognosis for PCNSL that has failed first-line therapy remains even worse, although new therapeutic approaches have improved survival (9, 10).

Many studies have been conducted nationally and internationally on salvage therapy for recurrent or refractory PCNSL (R/R PCNSL), but most of them have focused only on either recurrent or refractory PCNSL, or have discussed both groups simultaneously. However, there are significant differences in the overall outcomes of the two groups, and the choice of salvage treatment options is also focused on differently. Therefore, there is still some heterogeneity between the two groups of patients, and the salvage treatment options and the evaluation of their efficacy cannot be generalized.

Thus, the purpose of this study was to compare the efficacy of chemotherapy and radiotherapy for patients with recurrent or refractory PCNSL after the first time of relapse/progression and explore the prognostic factors of R/R PCNSL.

Methods

Study design

This retrospective study involved 105 patients with relapsed or refractory PCNSL admitted to Huashan Hospital, Fudan University between 1 January 2016 and 31 December 2020, and was approved by the ethical review boards of Huashan Hospital (KY2017-014). All participants provided informed consent before enrollment.

Relapsed PCNSL was defined as the re-emergence of a new lesion in a patient with PCNSL after achieving CR. Since there was no uniform definition currently, in this study refractory PCNSL was defined as failing to achieve PR after 3 courses or developing PD in 2 courses, referring to clinical experience and diagnostic criteria of other hematologic malignancies (11, 12). The specific evaluation criteria were based on the Lugano criteria for malignant lymphoma (13).

After being evaluated as the first-time relapse or refractory PCNSL, the patient accepted comprehensive evaluation, including but not limited to whole-body positron emission tomography/computed tomography (PET-CT), cranial enhanced magnetic resonance imaging (MRI) with gadolinium enhancer, chest and abdominal CT, ultrasound of superficial lymph nodes, bone marrow examination (smear and biopsy), blood tests, as well as cerebrospinal fluid examination and ophthalmologic examination. Patients with other malignancies, contraindications to radiotherapy or chemotherapy, or active Hepatitis B or C had been excluded.

After being recruited, patients continued to be divided into radiotherapy with or without adjuvant chemotherapy group (hereinafter referred to as radiotherapy group, RT group) and chemotherapy alone group (hereinafter referred to as chemotherapy group, CT group) according to the salvage treatment option chosen after the first relapse/progression. Patients had response assessments after each course. The study process is shown in Figure 1. Near-term response to salvage therapy was measured at 1 month after radiotherapy or in 3 courses of chemotherapy. The primary endpoints were overall response rate (ORR) and progression-free survival (PFS). The secondary endpoints were overall survival (OS) and OS after recurrence or progression (OS-R). PFS1 was defined as the time from diagnosis to the first time of recurrence or refractory progression.

Statistic methods

SPSS 26.0 was used for statistical analysis. The t-test of independent samples and the chi-square test were used to compare differences in measurement data or categorical data between groups, respectively. The Mann-Whitney U test was used to compare nonparametric variables between the 2 groups. Log-rank test and Cox regression model were used to analyze survival data. Variables with p-value < 0.2 were included in multivariate analysis as potential prognostic factors. Data with a p-value < 0.05 are considered statistically significant.

Results

Patient characteristics

A total of 105 patients with R/R PCNSL were enrolled and evaluated, with 42 in the relapse group (RL) and 63 in the refractory group (RF). The age at diagnosis was 56 years, 53 years for RL, and 58 years for RF. The study groups were well-balanced in patients and tumor characteristics (Table 1). Different induction treatments were performed before this study. The distribution of induction treatment is summarized in Supplemental Table 1.

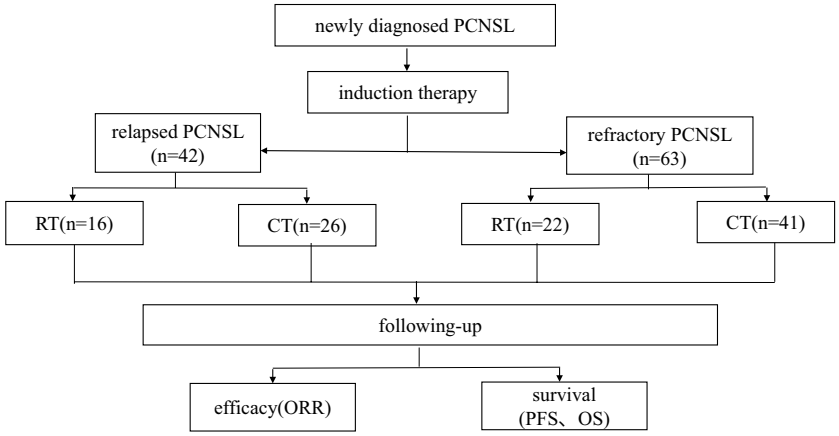

FIGURE 1
The flowchart of this study.

TABLE 1 Clinical and tumor characteristics.

Characteristics	All (*n*)	RL (*n*)	RF (*n*)	χ^2	*p*
Gender					
Male	62	27	35	0.794	0.373
Female	43	15	28		
Median age					
≥60 y	44	14	30	2.113	0.146
<60 y	61	28	33		
Pathology					
DLBCL	93	36	57	3.065	0.216
B-cell lymphoma	10	4	6		
NHL	2	2	0		
KPS					
≥70	58	23	35	0.006	1.000
<70	47	19	28		
Deep lesions					
Present	70	24	46	2.857	0.097
Absent	35	18	17		
Ocular lymphoma*					
Present	14	8	6	1.754	0.244
Absent	86	33	53		
Biopsy					
Resection	36	16	20	1.669	0.434
Puncture	67	26	41		
CSF	2	0	2		

*Five patients failed to receive ocular examinations because of poor condition or consciousness disorders.

Differences of PFS1 in R/R PCNSL

Since all R/R PCNSL patients had at least one recurrence/progression event, we defined the time from diagnosis to the first time of recurrence or refractory progression as PFS1. The median PFS1 of all recruited patients was 6.2 months (*95% CI*, 4.1 to 8.3), 14.9 months in the RL group, and 3.4 months in the RF group (*95% CI*, 9.0 to 20.8; *95% CI*, 2.8 to 4.0, respectively, $p < 0.01$) (Figure 2A). In the RL group, there were 13 patients with PFS1 ≤ 12 months and 29 patients with PFS1 > 12 months. No patients relapsed in 6 months. In the RF group, there were 4 patients with PFS1 > 12 months and 59 patients with PFS1 ≤ 12 months, including 52 patients who had PFS1 ≤ 6 months. The difference proportion of PFS1 ≤ 12 months in the two groups differed significantly ($\chi^2 = 45.967$, $p < 0.01$).

Prognostic factors of PFS1

Table 2 and Figures 2B–F show multivariate analysis and survival curves of prognostic factors for initial recurrence or progression. Univariate analysis suggested patients with deep lesions are more likely to acquire shorter PFS1 (5.4m vs 7.6m, $p = 0.032$). The HR for PFS1 in all recruited patients with R/R PCNSL was 1.62 for deep lesions compared to non-deep lesions in cox regression analysis (*95%CI*, 1.050 to 2.499, $p = 0.029$). However, no significant independent factors were discovered in subgroups RL and RF. Detailed univariate analysis is summarized in Supplemental Table 2.

Response of salvage therapy

The distribution of salvage therapy received was shown in Supplemental Table 3. In the RT group, one relapsed patient received stereotactic radiosurgery (SRS) while the other patients received whole brain radiotherapy (WBRT). Among patients received WBRT with detailed record, 12 received a total dose of 20-30 Gy (5 in RL group and 7 in RF group), 4 received a total dose of 36-48 Gy (2 in RL group and 2 in RF group), in fractionation of 1.8 to 2 Gy. In the CT group, treatment programs included HD-MTX reuse, rituximab, idarubicin, cytarabine, Bruton's tyrosine kinase inhibitors and et al. (Supplemental Figure 2). No significant difference was observed in therapy strategy choice ($\chi^2 = 3.184$, $p = 0.203$).

Table 3 shows the clinical efficacy in enrolled subjects with R/R PCNSL. Five losing patients were excluded in this section but were included in the analysis at the last follow-up. The rate of CR was 35% (14 of 40) in the RL group, with 18.3% (11 of 60) in the RF group. The objective response rates (ORR) were 52.5% and 36.7% for RL and RF groups, respectively ($p = 0.043$).

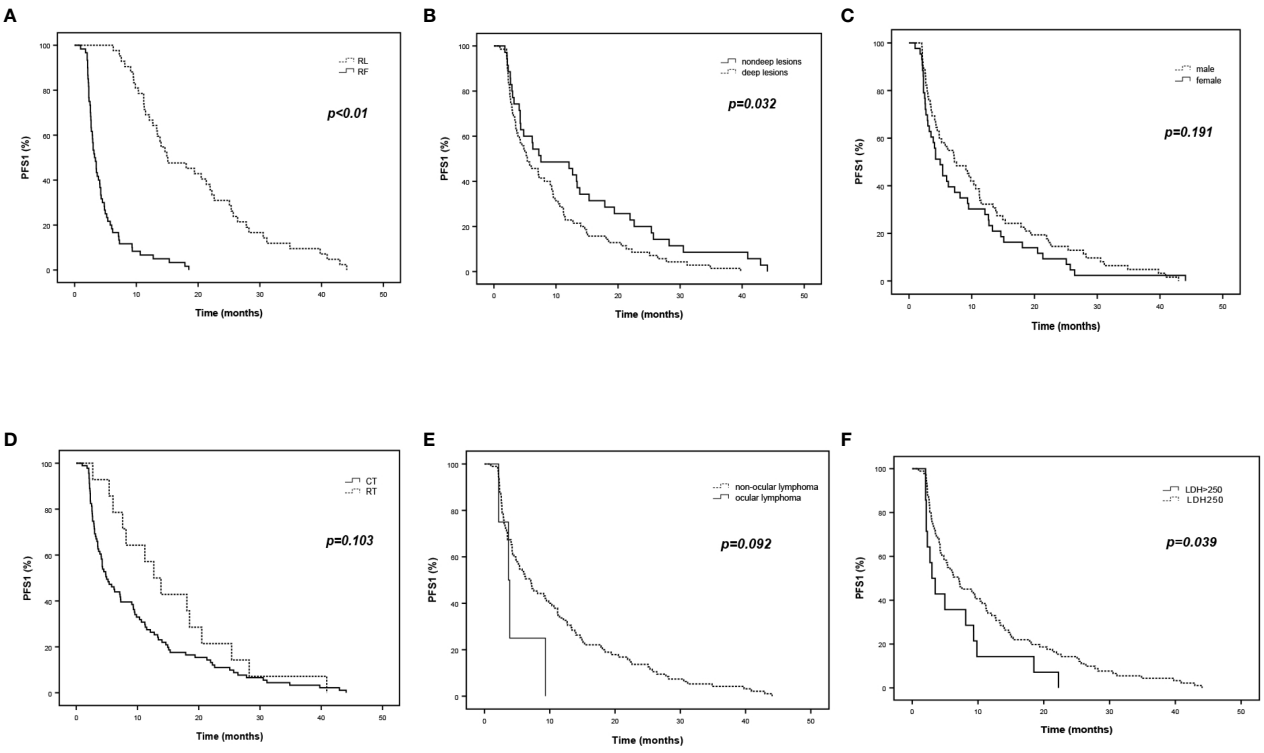

FIGURE 2
Survival curves of PFS1 in R/R PCNSL. **(A)**. PFS1 (the time from diagnosis to the first time of recurrence or refractory progression) of the RL and RF group. B-F. Prognostic factors for initial recurrence or progression according to PFS1. **(B)** Position of lesions. **(C)** Gender. **(D)** Induction therapy. **(E)** Ocular lymphoma. **(F)** Level of serum LDH.

TABLE 2 Multivariate analysis of prognostic factors for initial recurrence or progression.

R/R PCNSL			
Factors	HR	95% CI	p
Deep lesions	1.429	0.913-2.237	0.118
Male	0.790	0.519-1.202	0.270
Induction-RT	1.558	0.869-2.793	0.137
Ocular lymphoma	0.708	0.235-2.136	0.540
LDH>250U/L	1.559	0.796-3.053	0.195
RL group			
Factors	HR	95% CI	p
≥60y	0.516	0.195-1.366	0.183
Deep lesions	1.387	0.624-3.081	0.422
CSF protein>0.45g/L	0.438	0.177-1.083	0.074
LDH>250U/L	0.270	0.051-1.425	0.123
RF group			
Factors	HR	95% CI	p
Male	0.654	0.393-1.091	0.104
Induction-RT	3.019	0.929-9.807	0.066

*All factors were measured at diagnosis.

Furthermore, subgroup analysis of the effect of treatment strategy on the near-term outcomes (Supplemental Table 4) showed increased ORR in radiotherapy with or without adjuvant chemotherapy as compared with chemotherapy only, either in RL or RF group ($p < 0.05$).

Outcome and survival after recurrence and progression

The present analysis was done on data frozen on 31 May 2021. Three patients in the RF group were not followed up to a second progressive or relapsed outcome but only to a survival outcome. Totally 10 patients were lost to follow-up about the end-point, including 3 from the RL group and 7 from the RF group. The median follow-up for 95 patients was 17.5 months (range, 2.5m to 65.7m). Table 4 shows 15 (14.7%) patients with re-recurrence after obtaining CR with salvage therapy, 69 (67.6%) patients with progression, and 18 (17.6%) patients without events. A total of 41 patients died as a result of advanced tumors or severe complications. The event rate and death rate were similar in RL and RF groups: 81.0% vs. 83.3%, p=0.796; 38.1% vs. 41.7%, p =0.747, respectively.

TABLE 3 Response of salvage therapy in R/R PCNSL.

	RL (%)	RF (%)	All (%)	z	p
CR	35.0	18.3	25.0		
PR	17.5	18.3	18.0		
SD	10.0	6.3	8.0	-2.021	0.043
PD	35.7	56.7	49.0		
ORR	52.5	36.7	43.0		

Among R/R PCNSL patients, median progression-free survival (PFS) was 3.1 months (*95%CI*, 1.3 to 4.8), which was 5.3 months (*95%CI*, 2.2 to 8.5) in the RL group and 2.2 months (*95%CI*, 1.6 to 2.8) in RF group (p=0.034) (Figure 3A), respectively.

The median overall survival (OS) was 46 months (*95%CI*, 35.1 to 57.0) among all enrolled patients, 53.6 months (*95%CI*, 39.3 to 67.8) in the RL group and 30.8 months (*95%CI*, 15.8 to 45.8) in RF group (p = 0.009) (Figure 3B), respectively. However, after removing the effect of PFS1 on OS, no significant difference was observed in the median OS after salvage therapy between the RL group and the RF group (38.7 months vs. 21.3 months, p=0.291) (Figure 3C).

Besides, relapse patients in the RT group showed inferior PFS compared to those in the CT group. The PFS rates were 40.0% vs. 13,5% at 6 months (p = 0.005) (Figure 3D) (Supplemental Figure 1).

Prognostic factors of R/R PCNSL

Table 5 shows the multivariate analysis of prognostic factors for relapsed and refractory PCNSL related to the progression of disease and death. The HR for re-progression of relapsed PCNSL, adjusted for major prognostic factors, was 3.531 for CSF protein > 0.45g/L compared to ≤0.45g/L (*95%CI*, 1.141 to 10.922, p = 0.029). The HR for death of relapsed PCNSL was 4.415 for ocular involvement at recurrence compared to non-ocular involvement(*95%CI*, 1.221 to 15.957, p =0.024), which of refractory PCNSL was 2.535 for age ≥60years compared to <60 years (*95%CI*, 1.060 to 6.066, p =0.037) (Figure 4).

Discussion

PCNSL, as a highly heterogeneous hematologic malignancy with high aggressiveness, easy recurrence, and poor prognosis, has always been a hot spot of concern in the field of hematology. Newly diagnosed PCNSL with solid pathology diagnosis at our institution accepted high-dose MTX with or without rituximab as initial therapy, or whole brain radiotherapy while accompanied with contraindication of encephalic biopsy or chemotherapy. However, somatic disorders caused by the nervous system and ocular involvement greatly affect the patient's normal social role and reduce the quality of life, especially in the population of R/R PCNSL patients. Therefore, the exploration of effective salvage treatment for R/R PCNSL and the delay of disease progression are major challenges in clinical work.

Most of the previously conducted clinical studies related to R/R PCNSL have studied either recurrent PCNSL or refractory PCNSL (14), or have discussed both patients as a whole (15, 16). However, our results revealed the heterogeneity between the two groups of patients. According to the definition in this paper, recurrent PCNSL

TABLE 4 End-point of R/R PCNSL after salvage therapy.

	RL (n)	RF (n)	n/N (%)	χ^2	p
Primary end-point					
recurrence/progression	9/25	6/44	15/69(82.4)	0.096	0.796
Non-event	8	10	18(17.6)		
Secondary end-point					
Alive	23	31	54(56.8)	0.584	0.747
Death	16	25	41(43.2)		

could achieve CR after induction therapy, whereas refractory PCNSL fails to achieve PR or even develops PD early. Obviously, the differences in PFS1 indicate that the response to induction therapy differed significantly between relapsed and refractory patients. As the choice of salvage treatment options was not different between RL and RF groups, the difference in response to salvage treatment instead emerged with a significantly higher ORR in the relapsed PCNSL. It can be hypothesized that after the initial relapse/progression, relapsed PCNSL remains higher sensitive to salvage therapy than refractory PCNSL. However, specific mechanisms remain to be explored.

According to the results of previous studies, the median PFS of PCNSL after relapse/progression is only 2-5 months (17–19), which is consistent with our results (3.1 m of all patients, 5.3m of RL, 2.2m

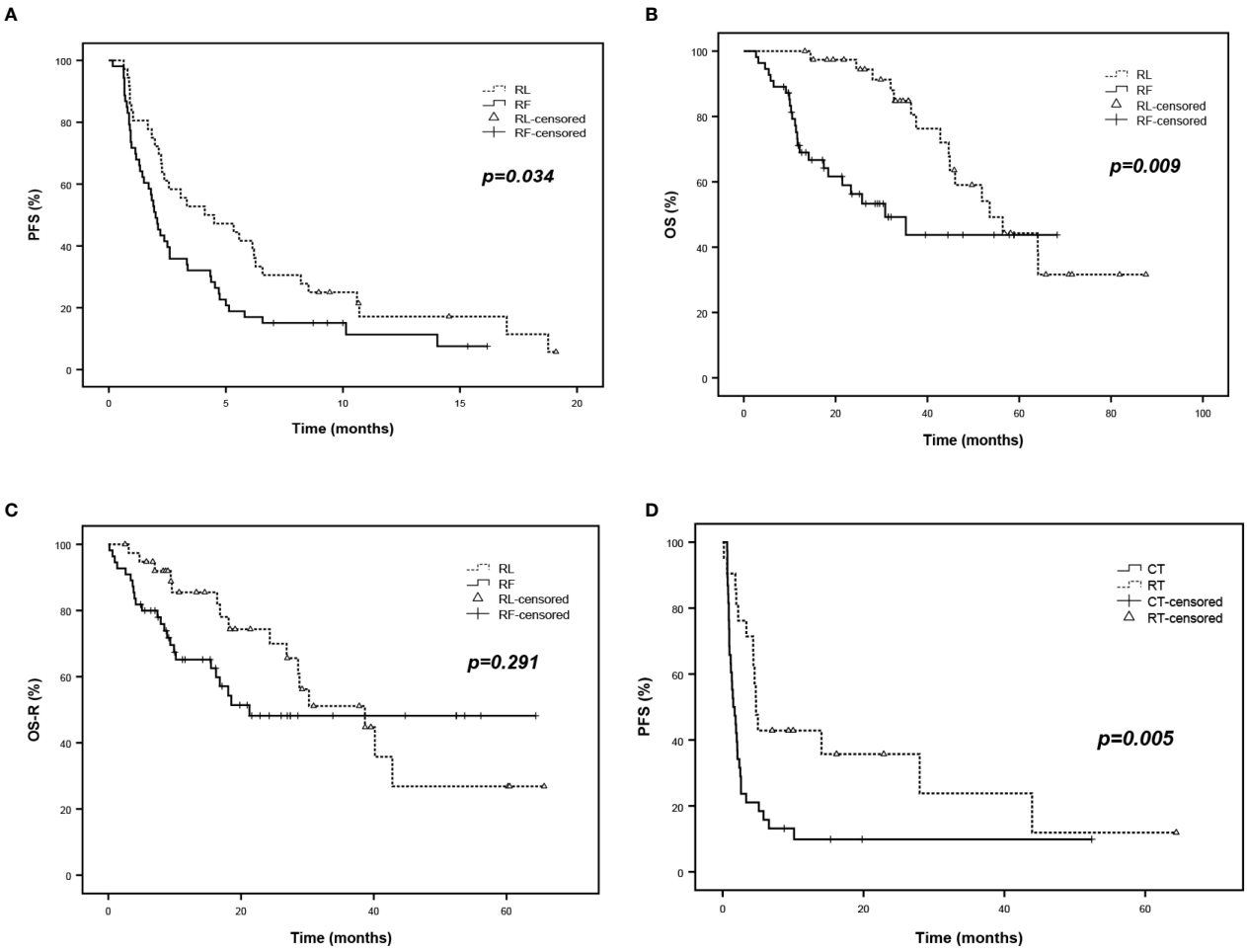

FIGURE 3
Survival analysis of R/R PCNSL. **(A)** PFS of R/R PCNSL patients after salvage treatment according to RL and RF group. **(B)** OS of R/R PCNSL patients according to RL and RF group. **(C)** OS-R (OS after salvage treatment) of R/R PCNSL patients according to RL and RF group. **(D)** PFS of refractory PCNSL patients after salvage treatment according to RT and CT subgroup.

TABLE 5 Multivariate analysis of prognostic factors for relapsed and refractory PCNSL.

RL group							
	PFS				OS-R		
Factors	HR	95% CI	p	Factors	HR	95% CI	p
≥60y	0.702	0.269-1.834	0.471	Male	4.312	0.876-21.219	0.072
KPS<70	0.742	0.293-1.880	0.529	≥60y	1.076	0.324-3.582	0.904
CSF protein>0.45g/L	3.531	1.141-10.922	0.029	KPS<70	0.629	0.166-2.383	0.495
CSF cells>8×10^6/L	0.825	0.205-3.316	0.787	Ocular lymphoma	4.415	1.221-15.957	0.024
				PFS1<12m	2.342	0.603-9.089	0.219
RF group							
	PFS				OS-R		
Factors	HR	95% CI	p	Factors	HR	95% CI	p
≥60y	2.302	0.151-1.246	0.121	Male	1.942	0.797-4.732	0.144
KPS<70	1.058	0.342-3.273	0.921	≥60y	2.535	1.060-6.066	0.037
Deep lesions	1.089	0.364-3.255	0.879	KPS<70	1.696	0.713-4.035	0.232
LDH >250U/L	1.117	0.361-3.454	0.848	PFS1<6m	1.743	0.620-4.900	0.292
CSF protein>0.45g/L	1.352	0.392-4.659	0.633	LDH >250U/L	1.178	0.422-3.291	0.755
CSF cells>8×10^6/L	2.263	0.689-7.438	0.179				

*KPS, CSF protein, CSF cells, and ocular lymphoma were measured at recurrence.
*KPS, LDH, CSF protein, CSF cells, and ocular lymphoma were measured at progression.

of RF). With the limitations of retrospective analysis, the treatment regimen showed high heterogeneity. We briefly divided patients who received salvage chemotherapy into MTX-based group and non-MTX group, no significance was observed. Furthermore, Ferreri and colleagues reported a new chemoimmunotherapy, MATRix, with higher complete remission rate (49%) compared with methotrexate-cytarabine alone (23%) or plus rituximab (30%), which encourage newly combination to apply in newly diagnosed PCNSL and relapsed/refractory PCNSL (20).

Radiotherapy is often considered as consolidation therapy or deferred until relapse. Further analysis of the efficacy of the salvage regimen in R/R PCNSL in this article revealed that the response rate for radiotherapy was significantly higher than that for chemotherapy in both the relapse and refractory groups. In refractory PCNSL, radiotherapy also resulted in a longer duration of disease remission. Another retrospective cohort study showed that salvage WBRT results in longer PFS and higher CR rates compared with high-dose cytarabine, with 10 months of median PFS and 54% for 1-year OS rate (21). The safety and efficacy of salvage WBRT had been evaluated by Hottinger, showing 79% for response rate (22). Furthermore, the occurrence of delayed neurotoxic events may also be greatly reduced with unimpaired disease control (5-year failure-free survival, 51% vs. 50%) when the total WBRT dose is controlled to less than 36Gy according to Ferreri and the International Extranodal Lymphoma Study Group (23, 24). A case series from National Cancer Institute of Colombia also supports the benefit of radiotherapy with effective local control and long term survival up to 10 years (25). However, radiotherapy did not show a significant advantage in OS in this paper, although numerically the median post-relapse/progression OS was longer in the CT group than in the RT group. These results suggest that we can use radiotherapy in the early stages of relapse/progression as an access to delay progression in the short term, improve patients' quality of life to some extent, and gain the opportunity for patients to try more treatments. However, further studies are needed to design better treatment strategies to give patients the benefit of long-term survival.

To predict prognosis as accurately as possible and select more appropriate treatment options, new prognostic factors need to be explored. In this study, we verified that age ≥60 years at diagnosis was an independent adverse prognostic factor for OS after recurrence/progression, which is consistent with other studies and grading criteria (26–28). Besides, cox regression analysis shows patients with abnormally elevated CSF protein are more likely to undergo progression. It has been suggested that cerebrospinal fluid cells and protein levels are important prognostic assessment factors (29, 30) because they both reflect the extent of meningeal involvement and intracranial tumor load to some extent. But in this paper results were not matched in univariate and multivariate analyses, caused by patients admitted to other hospitals for treatment without administering CSF examination in our institution.

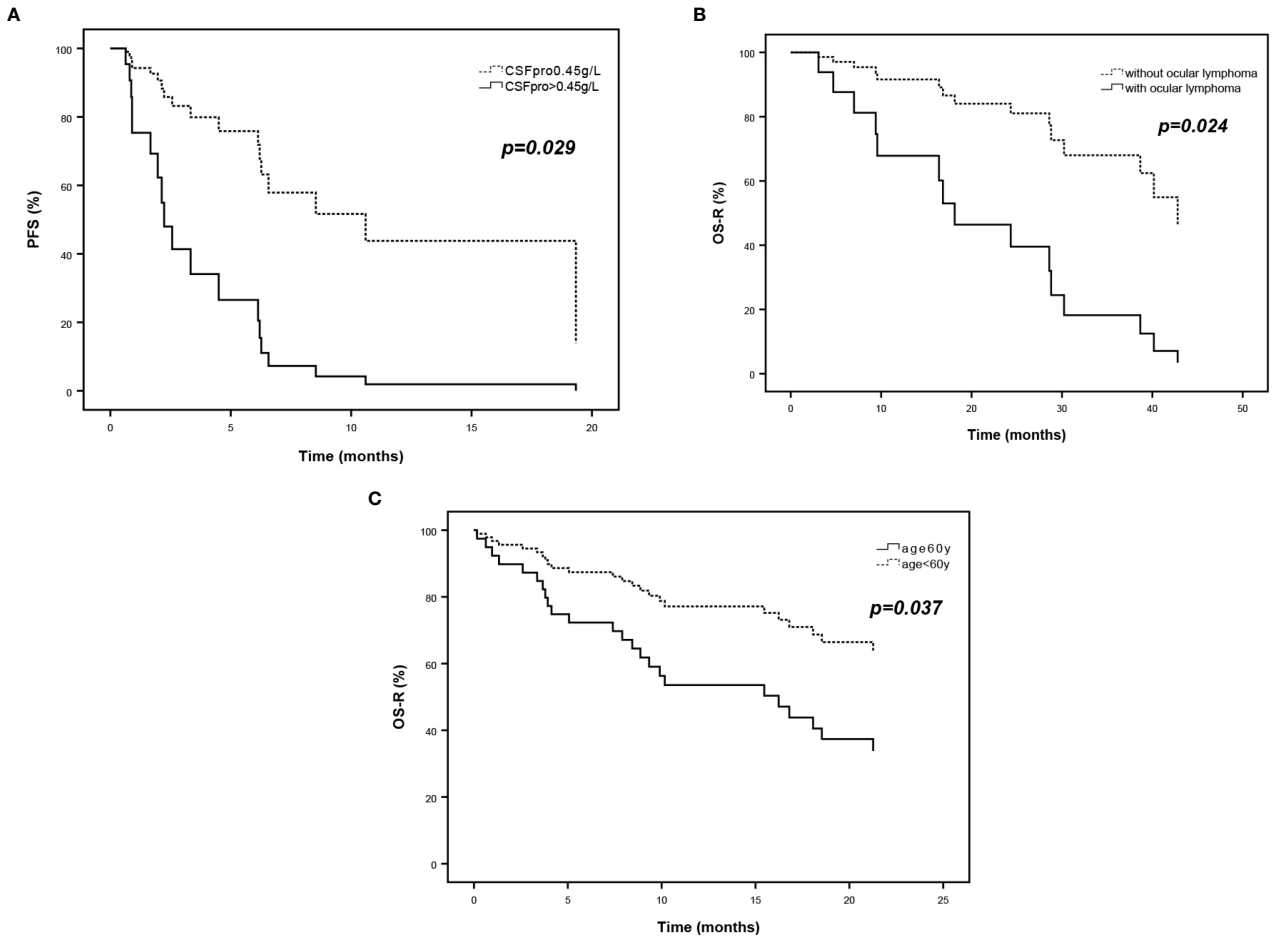

FIGURE 4
Survival curves of multivariate analysis of prognostic factors for relapsed and refractory PCNSL. **(A)** PFS curves according to CSF protein and **(B)** OS-R curves according to ocular lymphoma of relapsed PCNSL. **(C)** OS-R curves according to age at diagnosis of refractory PCNSL.

The results of our previous study (31) showed that patients with concomitant intraocular lymphoma were more likely to relapse compared to patients without intraocular lymphoma (relapse rates, 71.4% vs. 46.3%), whereas in this study we found that concomitant intraocular involvement at the time of relapse was associated with shorter post-recurrence OS. Survival could be affected when patients accepted intraocular MTX injection, which is also a treatment regimen adjustment. Intraocular lymphoma is an important branch of PCNSL, and clinicians can continue to explore the relationship between intraocular involvement and survival in multidisciplinary collaboration with the ophthalmology department.

In conclusion, radiotherapy could be a viable salvage treatment option for R/R PCNSL patients with initial recurrence or progression, demonstrating better antitumor effects and allowing for longer disease remission, at least in the early stages. Age, ocular involvement, and level of CSF protein may serve as potential prognostic predictors. However, multicenter, large-sample, and prospective studies are still needed to explore who benefits more in overall survival with radiotherapy versus chemotherapy after relapse/progression.

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 authors.

Ethics statement

The studies involving human participants were reviewed and approved by the ethical review boards of Huashan Hospital, Fudan University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YY, YM, and BC designed the research; YY acquired patients, analyzed data, and wrote the manuscript; YM and BC reviewed and revised the manuscript. QL, JM, HK, and ZL participated in the implementation of chemotherapy treatment. YW participated in

the implementation of radiotherapy. All authors contributed to the article and approved the submitted version.

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1098785/full#supplementary-material

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