Am J Stem Cells 2024;13(5):233-242
www.AJSC.us /ISSN:2160-4150/AJSC0158712

Review Article

The treatment and outcome prediction analysis of pediatric acquired severe aplastic anemia

Yi-Xian Li[1], Yun Chen[2], Jun-Bin Huang[1], Xiao-Yun Chen[3], Hong-Man Xue[1], Yu-Cai Cheng[1], Chun Chen[1]

[1]Division of Hematology/Oncology, Department of Pediatrics, The Seventh Affiliated Hospital of Sun Yat-Sen University, Shenzhen, Guangdong, PR China; [2]Research Center, The Seventh Affiliated Hospital of Sun Yat-Sen University, Shenzhen, Guangdong, PR China; [3]Outpatient Clinic of Pediatrics, The Seventh Affiliated Hospital of Sun Yat-Sen University, Shenzhen, Guangdong, PR China

Received June 24, 2024; Accepted September 16, 2024; Epub October 15, 2024; Published October 30, 2024

Abstract: Pediatric acquired severe aplastic anemia (SAA), a prevalent non-malignant hematological disorder, presents significant therapeutic challenges and carries considerable risks. Despite substantial progress in immunosuppressive therapy (IST) and allogeneic hematopoietic stem cell transplantation (allo-HSCT) in recent years, the protracted treatment duration, substantial costs, and significant disparities in long-term survival outcomes among patients remain problematic. Identifying predictors of treatment response before therapy initiation is crucial for optimal clinical decision-making and complication prevention. Recent studies has pinpointed predictive factors for IST and haploidentical hematopoietic stem cell transplantation (haplo-HSCT) efficacy in SAA, fostering the development and utilization of transplantation-based scoring systems for prognosis evaluation. This review summarizes advancements in treating pediatric SAA and discusses key elements that influence the outcomes of IST and haplo-HSCT, aiming to support clinical decision-making in diverse clinical scenarios.

Keywords: Severe aplastic anemia, pediatric, immunosuppressive therapy, allogeneic hematopoietic stem cell transplantation

Introduction

Aplastic anemia (AA), a bone marrow failure (BMF) syndrome, is characterized by pancytopenia and bone marrow hypoplasia. It is classified into congenital and acquired forms, with idiopathic acquired AA comprising 70% to 80% of cases [1]. Immune-mediated BMF, driven by hyperactive T-cell function, underlies the disease. AA's incidence displays a biphasic distribution, peaking in individuals aged 10-25 and over 60 years, without significant gender disparity. In Europe, the incidence is 2-3 per million, contrasting with East Asia's higher rates, such as China's 0.74 per 100,000 [2]. Such as in China, where the annual incidence is 0.74 per 100,000 [3]. SAA constitutes approximately 56% of pediatric AA cases [4]. Although SAA is non-malignant, its significant reduction in blood cell production can lead to frequent infections, bleeding, and anemia, necessitating long-term blood transfusions for survival. Severe, uncontrolled infections or hemorrhage can lead to multi-organ failure, impacting vital organs such as the heart, lungs, and kidneys, and pose significant health risks. Additionally, there is a progression risk in some patients toward malignant conditions, including myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML), which can complicate the therapeutic approach and elevate mortality rates.

For treating SAA, allo-HSCT stands as one of the most efficacious therapeutic approaches. The cornerstone of allo-HSCT lies in the successful engraftment of healthy hematopoietic stem cells and the establishment of immune tolerance, thereby averting rejection and immune attacks. Precise medication protocols are imperative both pre- and post-transplantation. This includes the administration of chemotherapy and immunosuppressants during the preconditioning phase to facilitate engraftment and prevent rejection. Furthermore, continuous

https://doi.org/10.62347/LACV8636

immunosuppression post-transplantation is crucial to prevent graft-versus-host disease (GVHD). Identical sibling donors, with perfect Human Leukocyte Antigen (HLA) matching, significantly diminish the risks of graft rejection and GVHD, allowing for reduced use of toxic preconditioning drugs and optimizing treatment outcomes. Matched sibling donor hematopoietic stem cell transplantation (MSD-HSCT) is widely considered the first-line treatment option for pediatric SAA due to its profound efficacy and low risks of long-term relapse and clonal disease transformation. However, less than 30% of patients can find an HLA-matched sibling donor, and due to the limited availability of fully matched donors, many patients are unable to receive this treatment promptly.

In their absence, the 2018 UK AA guidelines recommend immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and cyclosporine A (CsA) as the initial preferred therapy [1, 3]. IST aids bone marrow recovery by inhibiting the hyperactive T-cell function, alleviating symptoms. However, approximately one-third of SAA patients fail to achieve complete remission through IST, experiencing recurrent infections and iron overload from long-term blood transfusion support.

Recently, the rapid advancement of haplo-HSCT for treating SAA in China has led to new therapeutic recommendations by the expert panel of the Chinese Medical Doctor Association. For newly diagnosed pediatric SAA, a family haploidentical donor is now considered a viable first-line treatment [5, 6]. Haplo-HSCT extends treatment possibilities to a broader patient base by overcoming donor availability limitations. However, it remains challenged by high transplant-related mortality rates due to GVHD and infections associated with delayed immune reconstitution.

In summary, the primary therapeutic options for SAA include MSD-HSCT, IST, and haplo-HSCT, each presenting unique benefits and challenges, along with common issues like extended treatment durations, high costs, and substantial inter-individual efficacy variations. A critical focus of clinical research is the accurate assessment of treatment responses and risks in pediatric SAA patients prior to therapy, aiming to develop tailored treatment plans to minimize failures and complications.

This study reviews recent advancements in treating pediatric SAA, focusing on the efficacy and predictive factors of IST and haplo-HSCT. By analyzing research outcomes and identifying key factors affecting treatment strategies, we aim to equip clinicians with more scientific and rational decision-making tools for managing pediatric SAA under various clinical scenarios, ultimately aiming to optimize treatment strategies and improve patients' quality of life (QoL) and long-term prognosis.

Common therapeutic approaches for pediatric acquired SAA

MSD-HSCT

MSD-HSCT, an established therapeutic strategy, involves eradicating a patient's endogenous hematopoietic and immune systems and reconstituting them with hematopoietic stem cells from a fully HLA-matched sibling donor. This approach, characterized by easy engraftment into the patient's body, minimal rejection reactions, and a low incidence of complications, offers remarkable long-term survival rates above 90% for pediatric SAA patients [7]. However, with decreasing family sizes in China, over 70% of patients lack access to a matched sibling donor, highlighting the urgent need for alternative strategies and donor sources.

IST

The typical IST regimen for SAA includes the potent immunosuppressive agents ATG and CsA. ATG, a robust immunosuppressant, inhibits T lymphocyte function, while CsA prevents their proliferation and activation. Together, these agents promptly control abnormal immune responses while preserving residual hematopoietic function. The regimen involves administering rabbit ATG at 2.5-3.5 $mg \cdot kg^{-1} \cdot d^{-1}$ or pig ALG at 20-30 $mg \cdot kg^{-1} \cdot d^{-1}$ for five consecutive days, with cycles repeated every three months. CsA is administered concurrently, orally at 3-5 $mg \cdot kg^{-1} \cdot d^{-1}$, aiming for a target concentration of 100-150 μg/L, and continued for at least three months [3]. However, due to the scarcity and dysfunctional of residual hematopoietic stem cells, recovery is slow, with significant responses typically seen after six months and an efficacy rate of 55%-60% [4]. During this period, patients often require multiple hospital admissions for blood transfusions and anti-

infection treatments, which increases the complexity and burden of treatment. Additionally, the 10-year incidence of clonal evolution reaches 5%-6%, with a relapse rate of 15%, challenging long-term prognosis [8].

In recent years, the application of thrombopoietin (TPO) receptor agonists in AA treatment, particularly eltrombopag (EPAG), has attracted considerable attention. Multiple clinical trials have demonstrated its capacity to enhance platelet production and improve hematopoietic function. A phase II non-randomized trial by the National Institutes of Health (NIH) revealed that, after 12 weeks of EPAG treatment, 44% of patients with IST-resistant AA achieved a hematological response in at least one cell lineage, with some patients achieving transfusion independence and a gradual normalization of trilineage hematopoiesis [9]. This finding opens new possibilities for SAA treatment. Further research, including a multicenter, prospective, phase III randomized clinical trial organized by the European Society for Blood and Marrow Transplantation (EBMT), have confirmed the efficacy and safety of EPAG in combination with IST for treating SAA or very severe aplastic anemia (VSAA). Compared to standard IST, adding EPAG improved the complete remission rates, the response rates at 3 and 6 months, and the median time to first response, without significantly increasing the incidence of severe adverse effects [10]. Another prospective study indicated better outcomes with EPAG+IST compared to standard IST in pediatric SAA patients aged 2-18 years, excluding those with VSAA [11]. Collectively, these studies suggest that EPAG combined with IST can increase the response rate to 80% and complete remission rate to 50% in SAA, maintaining efficacy after discontinuing EPAG [8, 10, 12, 13]. Given these significant therapeutic benefits, in 2018, the FDA approved the combination of EPAG and IST as a first-line treatment for SAA patients, though vigilance for post-IST relapse and clonal evolution remains critical [12]. Future clinical practice must continue to explore and optimize treatment protocols to enhance therapeutic outcomes and the QoL for patients.

Alternative donor transplantation

For patients with SAA lacking a fully matched sibling donor and unresponsive to IST or experiencing relapse, current guidelines recommend alternative donor transplantation, including from HLA-matched unrelated donors, haploidentical donors, or umbilical cord blood stem cells [1, 3].

Mismatched unrelated donor hematopoietic stem cell transplantation (MUD-HSCT): MUD-HSCT involves selecting an unrelated donor who is a perfect HLA match from public bone marrow banks or stem cell donation centers, aiming to restore the recipient's hematopoietic and immune systems. Over the past two decades, MUD-HSCT has achieved significant progress in treating SAA, with studies demonstrating similar transplant outcomes between high-HLA-matched unrelated donors and matched sibling donors (MSD) [14]. For instance, a controlled study by Dufour et al. for pediatric SAA patients, divided into MUD-HSCT (n=29), MSD-HSCT (n=87), and IST groups (n=58), showed comparable 2-year overall survival (OS) and event-free survival (EFS) rates between MUD-HSCT and MSD-HSCT, both significantly superior to IST. Additionally, these rates were more favorable than those for salvage MUD-HSCT after IST failure [15]. Owing to its proven efficacy, advanced technology, and high safety profile, the 2015 British guidelines for AA now recommend MUD-HSCT as a first-line therapy for SAA [16].

Despite the advantages of MUD-HSCT, challenges include a low success rate of matching in bone marrow banks, long wait times, and risks of GVHD, graft rejection, treatment-related mortality, and infertility. Moreover, for those experiencing poor engraftment or transplant failure, securing donor cells for subsequent cellular therapies is often not feasible.

Haplo-HSCT: For patients without MSD or MUD, haplo-HSCT presents significant advantages in donor availability, ethical considerations, and high compliance, enabling flexible secondary collections and providing additional treatment options. However, due to the critical role of HLA in the human immune system, haploidentical transplants can induce severe rejection reactions, necessitating innovative preconditioning and immune modulation strategies beyond those used in fully matched transplants. Prominent among these are the "Beijing Protocol" and the "Baltimore Protocol".

The "Beijing Protocol", a preconditioning regimen incorporating granulocyte colony-stimulating factor (G-CSF), busulfan, and cyclophosphamide, was developed by Professor Huang Xiaojun's team at Peking University in 2001. The key to this protocol is the use of G-CSF, which reduces T-cell reactivity and fosters immune tolerance, proving crucial in conjunction with ATG and graft components (peripheral blood and bone marrow) for transplant success. After an Italian team validated it in 2011, the "Beijing Protocol" received widespread international recognition. Inspired by this, American researchers proposed the "Baltimore Protocol" in 2005, focusing on the use of post-transplant cyclophosphamide (PTCy) to manage T-cells cost-effectively, thereby achieving effective GVHD prevention and treatment in haploidentical transplantation for refractory AA. A meta-analysis by ElGohary et al. indicates that both protocols deliver comparable results in terms of OS and FFS, though they differ in primary graft failure (PGF) and GVHD rates [17], prompting ongoing efforts to integrate and optimize these methodologies across multiple centers.

In 2017, Peking University People's Hospital achieved encouraging outcomes by adopting the "Beijing Protocol" to perform haplo-HSCT on 52 pediatric patients diagnosed with SAA. The treatment resulted in a 3-year OS rate of (84.5±5.0)% and a 3-year FFS rate of (82.7±5.2)% [18]. Additionally, the incidences of grade II-IV and grade III-IV acute GVHD were recorded as (39.2±0.5)% and (13.7±0.2)%, respectively, with a chronic GVHD rate of (34.2±0.5)% [18]. In 2022, a multi-institutional retrospective study conducted in China explored the long-term outcomes of using haplo-HSCT and MSD-HSCT as primary therapies for SAA, revealing indistinguishable 9-year OS and FFS rates [19]. A 2023 meta-analysis examining the effectiveness and safety of allogeneic HSCT for SAA demonstrated similar 1-, 2-, and 3-year OS, FFS, and engraftment rates between Haplo-HSCT and MSD-HSCT. However, Haplo-HSCT was associated with a heightened occurrence of grade II-IV acute GVHD, chronic GVHD, and cytomegalovirus infections. Comparative assessments showed no discrepancies in 3- and 5-year OS, 3-year FFS, platelet engraftment, graft failure (GF), and acute GVHD rates between Haplo-HSCT and MUD-HSCT, yet Haplo-HSCT exhibited a reduced rate of chronic GVHD. Relative to IST, Haplo-HSCT offered better 3-year FFS and more rapid response rates [20, 21]. Additionally, a multicenter prospective study assessing the longitudinal QoL of SAA patients undergoing haplo-HSCT pre- and post-transplant confirmed satisfactory post-transplant QoL [22].

In summary, multiple clinical studies affirm haplo-HSCT as an effective and safe therapeutic option for SAA patients lacking fully matched donors, representing a viable alternative treatment [18, 19, 23, 24]. Future research should focus on optimizing preconditioning regimens, enhancing engraftment success, reducing GVHD incidence, and improving the rate of immune reconstitution, aiming for more precise and personalized treatment strategies.

Dual hematopoietic stem cell transplantation based on haplo-HSCT: Researchers have proposed a dual hematopoietic stem cell transplantation protocol, also known as third-party hematopoietic stem cell co-infusion, for the prevention of GVHD following haplo-HSCT. Umbilical cord blood (UCB) harbors a high concentration of hematopoietic stem/progenitor cells and various low-immunogenic immunomodulatory cells, which are tolerant to HLA mismatch and can decrease the incidence of GVHD [25]. Lei et al. reported on a multicenter retrospective study comparing SAA patients treated with haplo-HSCT combined with a single umbilical cord blood infusion to those receiving haplo-HSCT alone. The dual transplantation approach resulted in a higher five-year OS rate (84.0% vs 72.6%), higher GVHD-free and failure-free survival rate (72.4% vs 65.4%), and lower transplant-related mortality (16.4% vs 27.4%) [26]. Yao et al. found that children with SAA undergoing dual transplantation of haplo-HSCT and UCB experienced lower incidences of grade II-IV acute GVHD (16.1% vs 46.9%) and moderate to severe chronic GVHD (25.6% vs 51.3%) [27]. These findings suggest that UCB co-infusion may enhance survival rates in SAA patients undergoing haplo-HSCT, mitigate the incidence and severity of GVHD, and improve overall outcomes. The prospects for dual transplantation are promising, however, verification through large-scale randomized trials is needed, alongside further research into graft processing, stem cell engraftment, and interactions.

Autologous umbilical cord blood stem cell transplantation (ACBT): Since 2004, there have been reports of successful ACBT in treating childhood SAA, indicating that ACBT does not trigger rejection reactions. This method requires fewer cells compared to traditional HSCT, yet achieves stable hematopoietic reconstitution [28]. The therapy is safe, with no risk of GVHD associated with allo-HSCT, and patients typically experience a high QoL post-reconstruction. For children with AA who have stored their autologous cord blood, and where congenital BMF syndromes are ruled out, ACBT offers a safe and effective definitive treatment.

Choosing between IST and allo-HSCT

MSD-HSCT is considered the preferred treatment for SAA, known for its mature techniques, comprehensive efficacy, minimal complications, and high safety. However, the scarcity of donor sources is a challenge that also extends to MUD-HSCT, which additionally suffers from lengthy durations. For SAA patients lacking MSD and MUD, both IST and haplo-HSCT serve as conventional treatment options, though the choice between them requires consideration of multiple factors.

The British Committee for Standards in Haematology (BCSH) [16] and the Chinese Guidelines for Diagnosis and Treatment of AA (2022 Edition) [3] identify favorable prognostic factors for IST: (1) Including younger age with better hematopoietic reserve; (2) Less severe disease, non-very severe type; (3) Absolute reticulocyte count >25×10^9/L and an absolute lymphocyte count >1.0×10^9/L, indicating robust residual hematopoietic function and a low infection risk; (4) Chromosomal abnormalities such as +8 or del(13q); (5) Presence of PIGA gene mutations or small paroxysmal nocturnal hemoglobinuria (PNH) clones, suggesting immune abnormalities; (6) Long telomeres; (7) Mutations in BCOR and BCORL1 genes. Furthermore, the absence of class I HLA alleles has been identified as an independent predictor of clonal evolution from acquired AA to MDS/AML [29].

Clonal hematopoiesis is prevalent in AA, with approximately one-third of patients exhibiting somatic mutations in myeloid malignancy candidate genes. Specific mutations, such as in DNMT3A, ASXL1, TP53, RUNX1, and CSMD1, generally predict poor responses to IST, shorter survival, and higher risk of progression to MDS/AML [30]. SAA patients with large PNH clones (clone size >50%) are prone to hemolytic episodes, and IST is ineffective [16]. Patients with shortened telomeres experience high rates of recurrence, clonal evolution, and low overall survival post-IST [31]. Patients with abnormalities in chromosome 7 often face poor prognosis and an increased risk of progression to MDS/AML [32]. Li Y et al. found that VSAA patients with an ANC≤0.05×10^9/L had high early mortality rates, poor hematologic response, and low OS and EFS rates following first-line IST, likely due to extremely severe disease and uncontrollable infections [33]. Two multicenter studies evaluating the efficacy of allo-HSCT in patients with active infections and SAA patients without active infections demonstrated no statistical difference in OS and FFS between the groups. This finding supports the use of salvage allo-HSCT as a viable treatment option for refractory SAA patients with active infections who respond to existing antimicrobial therapy [34, 35]. In summary, poor prognostic factors for IST include adverse genetic mutations or chromosomal abnormalities, shortened telomeres, extremely severe disease, and concurrent uncontrollable active infections.

For achieving long-term disease-free survival and a high quality of life, SAA children with multiple favorable IST prognostic factors should primarily receive IST combined with EPAG, whereas those with multiple adverse factors should consider allo-HSCT when feasible [3].

Multiple factors impact the HSCT prognosis, such as the severity of the recipient's condition, the donor-recipient HLA match, the recipient's tolerance, and the source of the donor. SAA patients often have a history of multiple blood transfusions, leading to the production of donor-specific antibodies (DSA) and elevated serum ferritin (SF). A prospective study investigating the correlation between DSA and PGF revealed a significantly higher incidence of PGF in DSA-positive patients (MFI≥2000) compared to DSA-negative patients (MFI<2000) (27.3% vs 1.9%, P=0.003) [36]. Multivariate analysis indicates that an MFI>5000 in DSA is an independent risk factor for engraftment failure (P=0.006) [4]. Research has shown that iron overload (SF>2000 ng/mL) can lead to the

accumulation of reactive oxygen species (ROS) in cells, which inhibits the differentiation of the bone marrow microenvironment and human CD34+ cells, thereby suppressing hematopoiesis and serving as a risk factor for PGF [37]. Moreover, SAA patients with persistent, uncontrollable active infections or poor physical condition (ECOG≥3) prior to transplantation have lower success rates [34]. The intensity of preconditioning before transplantation for SAA patients is closely associated with the complications of Haplo-HSCT, such as GVHD and PGF.

In summary, the outcome of allo-HSCT is influenced by multiple variables, including the patient, donor, and the transplant center's experience level. Due to the high incidence of complications such as poor engraftment, GVHD, infection, and organ damage, the treatment-related mortality (TRM) of haplo-HSCT remains high, making pre-transplant risk assessment crucial.

Commonly used risk prediction models for HSCT patients prior to transplantation include the Hematopoietic Cell Transplantation-specific Comorbidity Index (HCT-CI), Karnofsky Performance Scale, EBMT Score, and Disease Risk Index-Comorbidity Index (DRCI). However, these models are used primarily for the assessment of hematologic malignancies and are unsuitable for SAA patients undergoing haplo-HSCT. In 2021, the Huang Xiaojun team developed a TRM prediction model for SAA patients receiving haplo-HSCT, which incorporates disease duration (representing disease-related factors), ECOG performance status score, and HCT-CI score to predict post-transplant TRM [38]. Time to transplantation after AA diagnosis, number of transfusions of red blood cells before transplant, ECOG performance status before HSCT, HCT-CI score before HSCT, and HLA difference are some of the potential predictors of three-year TRM that have been established using univariate analysis. Factors such as maternal grafts and ABO blood type incompatibility were found to be nonsignificant. Owing to incomplete data on pre-transplant red blood cell transfusion volume, this variable was excluded from the multivariate analysis. Ultimately, three independent predictors were found to be significantly associated with TRM, namely a prolonged interval from AA diagnosis to transplantation (≥12 months), reduced physical performance (ECOG score 2-3), and increased comorbidity burden (HCT-CI score ≥1). These factors contributed to the development of a TRM prediction model for haplo-HSCT, which could guide adjustments in preconditioning protocols for high-risk groups. However, due to the retrospective nature and single-center scope of this study, further validation and optimization with multi-center data are required.

Conclusion

Advancements in treatment technologies have diversified therapeutic options for pediatric acquired SAA, yielding significant improvements in outcomes. However, the main strategies, IST and haplo-HSCT, face limitations in efficacy. IST suffers from a relatively low overall survival rate, with substantial risks of relapse and clonal evolution. Meanwhile, haplo-HSCT, the predominant form of allo-HSCT in clinical practice, requires enhancements to boost engraftment success rates and reduce GVHD incidence [39]. The retrospective nature of most studies, combined with selection and recall biases, limited follow-up, and variable treatment standards across centers, complicates the determination of the more suitable first-line therapy between IST and allo-HSCT for pediatric SAA. This underscores the urgent need for more randomized and prospective studies to assess the efficacy and side effects of these modalities, and to better understand predictive factors for IST outcomes and HSCT models. A thorough evaluation of treatment options, accompanied by extended follow-up of treatment-related complications, is vital for improving success rates, especially in SAA patients without fully matched donors.

Furthermore, breakthroughs in basic research have opened new possibilities for SAA treatment. Wang H et al.'s research, which involved knocking out the Ythdf2 gene in mouse models or human umbilical cord blood (hUCB) cells, significantly enhanced the expression of transcription factors and increased the quantity of hematopoietic stem cells (HSCs) [40] without altering derived cell types or causing hematological malignancies. If successfully applied clinically, this gene knockout technique could markedly improve the limitations of current umbilical cord blood stem cell transplantation and potentially extend to other HSC-based ther-

apies. Envisioning the early-stage extraction and ex vivo expansion of autologous HSCs during the initial stages of AA, this strategy reserves valuable resources for salvage therapy following the failure of IST treatment, presenting immense clinical application prospects. We eagerly await the development of more innovative SAA treatment strategies and their prompt benefits for patients.

Acknowledgements

We thank Sanming Project of Medicine in Shenzhen (No. SZSM202011004) to support the design of the study, Shenzhen Healthcare Research Project (Grant No. SZLY2018001), and Shenzhen Science and Technology Innovation Commission (JCYJ20180307150419435 and JCYJ20210324123004011) to support the manuscript preparation and publication.

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Chun Chen, Yu-Cai Cheng and Hong-Man Xue, Department of Pediatrics, The Seventh Affiliated Hospital of Sun Yat-Sen University, No. 628 Zhenyuan Road, Guangming District, Shenzhen 518107, Guangdong, PR China. Tel: +86-0755-81206750; E-mail: chenchun@mail.sysu.edu.cn (CC); Tel: +86-13480201651; E-mail: chengyucai@sysush.com (YCC); Tel: +86-18938926545; E-mail: xuehongman@sysush.com (HMX)

References

[1] Kulasekararaj A, Cavenagh J, Dokal I, Foukaneli T, Gandhi S, Garg M, Griffin M, Hillmen P, Ireland R, Killick S, Mansour S, Mufti G, Potter V, Snowden J, Stanworth S, Zuha R and Marsh J; BSH Committee. Guidelines for the diagnosis and management of adult aplastic anaemia: a British Society for Haematology Guideline. Br J Haematol 2024; 204: 784-804.

[2] Shimano KA, Narla A, Rose MJ, Gloude NJ, Allen SW, Bergstrom K, Broglie L, Carella BA, Castillo P, de Jong JLO, Dror Y, Geddis AE, Huang JN, Lau BW, McGuinn C, Nakano TA, Overholt K, Rothman JA, Sharathkumar A, Shereck E, Vlachos A, Olson TS, Bertuch AA, Wlodarski MW, Shimamura A and Boklan J. Diagnostic work-up for severe aplastic anemia in children: consensus of the North American Pediatric Aplastic Anemia Consortium. Am J Hematol 2021; 96: 1491-1504.

[3] Red Blood Cell Disease (Anemia) Group, Chinese Society of Hematology, Chinese Medical Association. Guidelines for the diagnosis and management of aplastic anemia in China (2022). Zhonghua Xue Ye Xue Za Zhi 2022; 43: 881-888.

[4] Gao YY, Chen XJ and Luo RM. Research advances on haploidentical hematopoietic stem cell transplantation in the treatment of severe aplastic anemia in children. Zhongguo Dang Dai Er Ke Za Zhi 2021; 23: 854-859.

[5] Chang YJ, Pei XY and Huang XJ. Haematopoietic stem-cell transplantation in China in the era of targeted therapies: current advances, challenges, and future directions. Lancet Haematol 2022; 9: e919-e929.

[6] Zhang XH, Chen J, Han MZ, Huang H, Jiang EL, Jiang M, Lai YR, Liu DH, Liu QF, Liu T, Ren HY, Song YP, Sun ZM, Tang XW, Wang JM, Wu DP, Xu LP, Zhang X, Zhou DB and Huang XJ. The consensus from The Chinese Society of Hematology on indications, conditioning regimens and donor selection for allogeneic hematopoietic stem cell transplantation: 2021 update. J Hematol Oncol 2021; 14: 145.

[7] Pulsipher MA, Lehmann LE, Bertuch AA, Sasa G, Olson T, Nakano T, Gilio A, Burroughs LM, Lipton JM, Huang JN, Dickerson K, Bertaina A, Zhuang C, Malsch M, Fleming M, Weller E, Shimamura A and Williams DA. A study assessing the feasibility of randomization of pediatric and young adult patients between matched unrelated donor bone marrow transplantation and immune-suppressive therapy for newly diagnosed severe aplastic anemia: a joint pilot trial of the North American Pediatric Aplastic Anemia Consortium and the Pediatric Transplantation and Cellular Therapy Consortium. Pediatr Blood Cancer 2020; 67: e28444.

[8] Patel BA, Groarke EM, Lotter J, Shalhoub R, Gutierrez-Rodrigues F, Rios O, Quinones Raffo D, Wu CO and Young NS. Long-term outcomes in patients with severe aplastic anemia treated with immunosuppression and eltrombopag: a phase 2 study. Blood 2022; 139: 34-43.

[9] Olnes MJ, Scheinberg P, Calvo KR, Desmond R, Tang Y, Dumitriu B, Parikh AR, Soto S, Biancotto A, Feng X, Lozier J, Wu CO, Young NS and Dunbar CE. Eltrombopag and improved hematopoiesis in refractory aplastic anemia. N Engl J Med 2012; 367: 11-19.

[10] Peffault de Latour R, Kulasekararaj A, Iacobelli S, Terwel SR, Cook R, Griffin M, Halkes CJM, Recher C, Barraco F, Forcade E, Vallejo JC, Drexler B, Mear JB, Smith AE, Angelucci E, Raymakers RAP, de Groot MR, Daguindau E, Nur E, Barcellini W, Russell NH, Terriou L, Iori AP, La Rocca U, Sureda A, Sánchez-Ortega I, Xicoy B, Jarque I, Cavenagh J, Sicre de Fontbrune F,

Marotta S, Munir T, Tjon JML, Tavitian S, Praire A, Clement L, Rabian F, Marano L, Hill A, Palmisani E, Muus P, Cacace F, Frieri C, van Lint MT, Passweg JR, Marsh JCW, Socié G, Mufti GJ, Dufour C and Risitano AM; Severe Aplastic Anemia Working Party of the European Society for Blood and Marrow Transplantation. Eltrombopag added to immunosuppression in severe aplastic anemia. N Engl J Med 2022; 386: 11-23.

[11] Goronkova O, Novichkova G, Salimova T, Kalinina I, Baidildina D, Petrova U, Antonova K, Sadovskaya M, Suntsova E, Evseev D, Matveev V, Venyov D, Khachatryan L, Litvinov D, Pshonkin A, Ovsyannikova G, Kotskaya N, Gobadze D, Olshanskaya Y, Popov A, Raykina E, Mironenko O, Voronin K, Purbueva B, Boichenko E, Dinikina Y, Guseynova E, Sherstnev D, Kalinina E, Mezentsev S, Streneva O, Yudina N, Plaksina O, Erega E, Maschan M and Maschan A. Efficacy of combined immunosuppression with or without eltrombopag in children with newly diagnosed aplastic anemia. Blood Adv 2023; 7: 953-962.

[12] Jin Y, Li R, Lin S, Jia J, Yang Y, Zhang D, He G and Li J. A real-word experience of eltrombopag plus rabbit antithymocyte immunoglobulin-based IST in Chinese patients with severe aplastic anemia. Ann Hematol 2022; 101: 2413-2419.

[13] Ise M, Iizuka H, Kamoda Y, Hirao M, Kida M and Usuki K. Romiplostim is effective for eltrombopag-refractory aplastic anemia: results of a retrospective study. Int J Hematol 2020; 112: 787-794.

[14] Shimano KA, Rothman JA, Allen SW, Castillo P, de Jong JLO, Dror Y, Geddis AE, Lau BW, McGuinn C, Narla A, Overholt K, Pereda MA, Sharathkumar A, Sasa G, Nakano TA, Myers K, Gloude NJ, Broglie L and Boklan J. Treatment of newly diagnosed severe aplastic anemia in children: evidence-based recommendations. Pediatr Blood Cancer 2024; 71: e31070.

[15] Dufour C, Veys P, Carraro E, Bhatnagar N, Pillon M, Wynn R, Gibson B, Vora AJ, Steward CG, Ewins AM, Hough RE, de la Fuente J, Velangi M, Amrolia PJ, Skinner R, Bacigalupo A, Risitano AM, Socie G, Peffault de Latour R, Passweg J, Rovo A, Tichelli A, Schrezenmeier H, Hochsmann B, Bader P, van Biezen A, Aljurf MD, Kulasekararaj A, Marsh JC and Samarasinghe S. Similar outcome of upfront-unrelated and matched sibling stem cell transplantation in idiopathic paediatric aplastic anaemia. A study on behalf of the UK Paediatric BMT Working Party, Paediatric Diseases Working Party and Severe Aplastic Anaemia Working Party of EBMT. Br J Haematol 2015; 171: 585-594.

[16] Killick SB, Bown N, Cavenagh J, Dokal I, Foukaneli T, Hill A, Hillmen P, Ireland R, Kulasekararaj A, Mufti G, Snowden JA, Samarasinghe S, Wood A and Marsh JC; British Society for Standards in Haematology. Guidelines for the diagnosis and management of adult aplastic anaemia. Br J Haematol 2016; 172: 187-207.

[17] ElGohary G, El Fakih R, de Latour R, Risitano A, Marsh J, Schrezenmeier H, Gluckman E, Höchsmann B, Pierri F, Halkes C, Alzahrani H, De la Fuente J, Cesaro S, Alahmari A, Ahmed SO, Passweg J, Dufour C, Bacigalupo A and Aljurf M. Haploidentical hematopoietic stem cell transplantation in aplastic anemia: a systematic review and meta-analysis of clinical outcome on behalf of the severe aplastic anemia working party of the European group for blood and marrow transplantation (SAAWP of EBMT). Bone Marrow Transplant 2020; 55: 1906-1917.

[18] Xu LP, Zhang XH, Wang FR, Mo XD, Han TT, Han W, Chen YH, Zhang YY, Wang JZ, Yan CH, Sun YQ, Zuo SN and Huang XJ. Haploidentical transplantation for pediatric patients with acquired severe aplastic anemia. Bone Marrow Transplant 2017; 52: 381-387.

[19] Xu ZL, Xu LP, Wu DP, Wang SQ, Zhang X, Xi R, Gao SJ, Xia LH, Yang JM, Jiang M, Wang X, Liu QF, Chen J, Zhou M and Huang XJ. Comparable long-term outcomes between upfront haploidentical and identical sibling donor transplant in aplastic anemia: a national registry-based study. Haematologica 2022; 107: 2918-2927.

[20] Zhao J, Ma L, Zheng M, Su L and Guo X. Meta-analysis of the results of haploidentical transplantation in the treatment of aplastic anemia. Ann Hematol 2023; 102: 2565-2587.

[21] Liu L, Han B, Zhang Y, Lei M, Liu R, Lin Z, Jiao W, Zhang F, Fu R, Zhao X, Miao M, Zhang L and Wu D. First-line treatment of severe aplastic anemia: immunosuppressive therapy plus eltrombopag versus haploidentical hematopoietic stem cell transplantation, a multicenter prospective study. Bone Marrow Transplant 2024; [Epub ahead of print].

[22] Xu LP, Xu ZL, Wang SQ, Wu DP, Gao SJ, Yang JM, Xia LH, Liu QF, Jiang M, Bai H, Zhang X, Wang X and Huang XJ. Long-term follow-up of haploidentical transplantation in relapsed/refractory severe aplastic anemia: a multicenter prospective study. Sci Bull (Beijing) 2022; 67: 963-970.

[23] DeZern AE, Eapen M, Wu J, Talano JA, Solh M, Dávila Saldaña BJ, Karanes C, Horwitz ME, Mallhi K, Arai S, Farhadfar N, Hexner E, Westervelt P, Antin JH, Deeg HJ, Leifer E, Brodsky RA, Logan BR, Horowitz MM, Jones RJ and Pulsipher MA. Haploidentical bone marrow transplantation in patients with relapsed or re-

fractory severe aplastic anaemia in the USA (BMT CTN 1502): a multicentre, single-arm, phase 2 trial. Lancet Haematol 2022; 9: e660-e669.

[24] Passweg JR, Baldomero H, Chabannon C, Basak GW, de la Cámara R, Corbacioglu S, Dolstra H, Duarte R, Glass B, Greco R, Lankester AC, Mohty M, Peffault de Latour R, Snowden JA, Yakoub-Agha I and Kröger N; European Society for Blood and Marrow Transplantation (EBMT). Hematopoietic cell transplantation and cellular therapy survey of the EBMT: monitoring of activities and trends over 30 years. Bone Marrow Transplant 2021; 56: 1651-1664.

[25] Liu L, Zhang Y, Jiao W, Zhou H, Wang Q, Qiu H, Tang X, Han Y, Fu C, Jin Z, Chen S, Sun A, Miao M and Wu D. Combination of haploidentical haematopoietic stem cell transplantation with an unrelated cord-blood unit in patients with severe aplastic anemia: a report of 146 cases. Bone Marrow Transplant 2020; 55: 2017-2025.

[26] Lei M, Zhang Y, Jiao W, Li X, Zhou H, Wang Q, Qiu H, Tang X, Han Y, Fu C, Jin Z, Chen S, Sun A, Miao M, Liu L and Wu D. Comparison of haploidentical hematopoietic stem cell transplant with or without unrelated cord blood infusion in severe aplastic anemia: outcomes of a multicenter study. Front Immunol 2022; 13: 912917.

[27] Yao D, Tian Y, Li J, Li B, Lu J, Ling J, Zheng D, Yao Y, Xiao P, Meng L and Hu S. Association between haploidentical hematopoietic stem cell transplantation combined with an umbilical cord blood unit and graft-versus-host disease in pediatric patients with acquired severe aplastic anemia. Ther Adv Hematol 2022; 13: 20406207221134409.

[28] Fruchtman SM, Hurlet A, Dracker R, Isola L, Goldman B, Schneider BL and Emre S. The successful treatment of severe aplastic anemia with autologous cord blood transplantation. Biol Blood Marrow Transplant 2004; 10: 741-742.

[29] Hosokawa K, Mizumaki H, Yoroidaka T, Maruyama H, Imi T, Tsuji N, Urushihara R, Tanabe M, Zaimoku Y, Nguyen MAT, Tran DC, Ishiyama K, Yamazaki H, Katagiri T, Takamatsu H, Hosomichi K, Tajima A, Azuma F, Ogawa S and Nakao S. HLA class I allele-lacking leukocytes predict rare clonal evolution to MDS/AML in patients with acquired aplastic anemia. Blood 2021; 137: 3576-3580.

[30] Yoshizato T, Dumitriu B, Hosokawa K, Makishima H, Yoshida K, Townsley D, Sato-Otsubo A, Sato Y, Liu D, Suzuki H, Wu CO, Shiraishi Y, Clemente MJ, Kataoka K, Shiozawa Y, Okuno Y, Chiba K, Tanaka H, Nagata Y, Katagiri T, Kon A, Sanada M, Scheinberg P, Miyano S, Maciejewski JP, Nakao S, Young NS and Ogawa S. Somatic mutations and clonal hematopoiesis in aplastic anemia. N Engl J Med 2015; 373: 35-47.

[31] Scheinberg P, Cooper JN, Sloand EM, Wu CO, Calado RT and Young NS. Association of telomere length of peripheral blood leukocytes with hematopoietic relapse, malignant transformation, and survival in severe aplastic anemia. JAMA 2010; 304: 1358-64.

[32] Groarke EM, Patel BA, Shalhoub R, Gutierrez-Rodrigues F, Desai P, Leuva H, Zaimoku Y, Paton C, Spitofsky N, Lotter J, Rios O, Childs RW, Young DJ, Dulau-Florea A, Dunbar CE, Calvo KR, Wu CO and Young NS. Predictors of clonal evolution and myeloid neoplasia following immunosuppressive therapy in severe aplastic anemia. Leukemia 2022; 36: 2328-2337.

[33] Li Y, Wu Z, Zhao X, Zhang L, Jing L, Zhou K, Peng G, Ye L, Li Y, Li J, Fan H, Song L, Yang W, Jiang B and Zhang F. Outcomes of very severe aplastic anemia patients with different absolute neutrophil counts after frontline immnunosuppressive therapy. Zhonghua Xue Ye Xue Za Zhi 2016; 37: 329-333.

[34] Xu S, Wu L, Zhang Y, Mo W, Zhou M, Li Y, Pan S and Wang S. Allogeneic hematopoietic stem cell transplantation for the treatment of severe aplastic anemia patients with infection: a single-center retrospective study. Biol Blood Marrow Transplant 2018; 24: 2532-2539.

[35] Liu L, Miao M, Chen X, Zhang Y, Lei M, Li B, Zhou H, Wang Q, Qiu H, Tang X, Han Y, Fu C, Jin Z, Chen S, Sun A, Wang S and Wu D. Outcomes of severe aplastic anemia patients with infection proceeding with allogeneic hematopoietic stem cell transplantation, versus patients without infection. Bone Marrow Transplant 2021; 56: 2591-2594.

[36] Chang YJ, Zhao XY, Xu LP, Zhang XH, Wang Y, Han W, Chen H, Wang FR, Mo XD, Zhang YY, Huo MR, Zhao XS, Y K, Liu KY and Huang XJ. Donor-specific anti-human leukocyte antigen antibodies were associated with primary graft failure after unmanipulated haploidentical blood and marrow transplantation: a prospective study with randomly assigned training and validation sets. J Hematol Oncol 2015; 8: 84.

[37] Isidori A, Borin L, Elli E, Latagliata R, Martino B, Palumbo G, Pilo F, Loscocco F, Visani G and Cianciulli P. Iron toxicity - Its effect on the bone marrow. Blood Rev 2018; 32: 473-479.

[38] Xu LP, Yu Y, Cheng YF, Zhang YY, Mo XD, Han TT, Wang FR, Yan CH, Sun YQ, Chen YH, Wang JZ, Xu ZL, Tang FF, Han W, Wang Y, Zhang XH and Huang XJ. Development and validation of a mortality predicting scoring system for severe aplastic anaemia patients receiving hap-

loidentical allogeneic transplantation. Br J Haematol 2022; 196: 735-742.
[39] Scheinberg P. Acquired severe aplastic anaemia: how medical therapy evolved in the 20th and 21st centuries. Br J Haematol 2021; 194: 954-969.
[40] Wang H, Zuo H, Liu J, Wen F, Gao Y, Zhu X, Liu B, Xiao F, Wang W, Huang G, Shen B and Ju Z. Loss of YTHDF2-mediated m(6)A-dependent mRNA clearance facilitates hematopoietic stem cell regeneration. Cell Res 2018; 28: 1035-1038.