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PROGRESS IN HEMATOLOGY

Aplastic anemia

Recent advances in the diagnosis and treatment of pediatric acquired aplastic anemia

Nao Yoshida[1]

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Abstract

Acquired aplastic anemia (AA) in children is a rare bone marrow failure that requires several special considerations for its diagnosis and treatment compared with that in adults. The most common issue is the differential diagnosis with refractory cytopenia of childhood and inherited bone marrow failure syndromes, which is crucial for making decisions on the appropriate treatment for pediatric AA. In addition to detailed morphological evaluation, a comprehensive diagnostic work-up that includes genetic analysis using next-generation sequencing will play an increasingly important role in identifying the underlying etiology of pediatric AA. When discussing treatment strategies for children with acquired AA, the long-term sequelae and level of hematopoietic recovery that affect daily or school life should also be considered, although the overall survival rate has reached 90% after immunosuppressive therapy or hematopoietic cell transplantation (HCT). Recent advances in HCT for pediatric patients with acquired AA have been remarkable, with the successful use of upfront bone marrow transplantation from a matched unrelated donor, unrelated cord blood transplantation or haploidentical HCT as salvage treatment, and fludarabine/melphalan-based conditioning regimens. This review discusses current clinical practices in the diagnosis and treatment of acquired AA in children based on the latest data.

Keywords Aplastic anemia · Inherited bone marrow failure syndrome · Refractory cytopenia of childhood · Hematopoietic cell transplantation · Pediatric

Introduction

Acquired aplastic anemia (AA) in children is a life-threatening disorder characterized by pancytopenia and hypocellular bone marrow (BM) [1]. Although patients are at risk for mortality because of bleeding or infection, the survival rate has reached 90% after immunosuppressive therapy (IST) or hematopoietic cell transplantation (HCT), which have been accepted as standard treatments in the past 3 decades [2, 3]. For optimal care and management, the underlying etiology of AA must be identified whenever possible, and the main differential diagnosis for children who meet the criteria of AA includes acquired AA, refractory cytopenia of childhood (RCC), inherited bone marrow failure syndromes (IBMFS), and other disorders. Thus, the usefulness of a central review system that includes detailed morphological evaluation and a comprehensive diagnostic work-up that includes genetic analysis have been highlighted [4–7]. In children, the choice of an appropriate treatment is influenced by the long-term sequelae of the disease and its therapy. The main challenges with IST are the lack of response, relapse, and clonal evolution, leading to a sustained remission rate of 40–60% [8–11]. Graft failure, graft versus host disease (GVHD), and secondary malignancies limit the success of HCT; however, recent advances in HCT have been remarkable, creating a paradigm shift in the treatment of pediatric acquired AA [12–14]. The latest topics include improved conditioning regimens aimed at overcoming late graft failure (LGF) such as donor-type aplasia or poor graft function, upfront usage of bone marrow transplantation (BMT) from a matched unrelated donor (MUD), which has been reserved for non-responders to IST, and the successful development of unrelated cord blood transplantation (UCBT) and haploidentical HCT.

✉ Nao Yoshida
nao-y@med.nagoya-u.ac.jp

[1] Department of Hematology and Oncology, Children's Medical Center, Japanese Red Cross Aichi Medical Center Nagoya First Hospital, 3-35 Michishita-cho, Nakamura-ku, Nagoya, Aichi 453-8511, Japan

This review aimed to describe recent advances in the diagnosis and therapeutic approaches and summarize current algorithm for the treatment of acquired AA in children based on latest data from recent studies.

Differential diagnosis of pediatric acquired AA

RCC

A definitive diagnosis of bone marrow failures (BMFs) in children is clinically challenging, with the most common issue being the differential diagnosis between AA and hypoplastic myelodysplastic syndromes (MDS), especially RCC, which is a provisional entity in the World Health Organization (WHO) classification of childhood MDS [15]. For the morphological diagnosis and evaluation of cellularity, BM biopsy, in addition to BM aspiration, is crucial, because BM cellularity can be patchy (Fig. 1). The new classification of RCC as a type of MDS raised some controversies in clinical practice because the BM of RCC is mostly hypoplastic and many of them have been diagnosed and successfully treated as AA [16–18]. Conversely, according to the WHO classification, the spectrum of RCC is broadly defined and supposed to include up to normo- or hypercellular BM that meets the criteria for MDS with multilineage dysplasia (MLD) as defined for adult MDS [15]. To determine the clinical significance of RCC, a recent prospective study conducted by the Japanese Society of Pediatric Hematology/Oncology evaluated BM morphology and its correlation with treatment outcomes in 252 children with acquired BMF [19]. The morphological distribution was AA in 25%, RCC without MLD in 52%, and RCC with MLD in 23%, and consistent with previous reports, no differences were found in their response rate to IST, incidence of clonal evolution, or overall survival (OS) among the three groups, although RCC with MLD was more likely to have chromosomal abnormalities at diagnosis. Thus, in clinical practice, the optimal care and treatment appear to be largely the same for AA and hypoplastic RCC [20].

Diagnostic approach incorporating comprehensive genetic testing

The differential diagnosis with IBMFS is also critical for the optimal treatment of acquired AA; if patients with IBMFS are treated as having acquired AA, they may not respond to the IST and can experience increased toxicity after a conditioning regimen used for HCT in acquired AA. IBMFS is more common in children, comprising approximately 10–20% of all pediatric BMF, but can also present in adolescent and young adults [21]. Possible IBMFS is supported by the presence of a family history and characteristic physical abnormalities; however, cryptic or atypical presentations may obscure the correct diagnosis. In other words, IBMFS

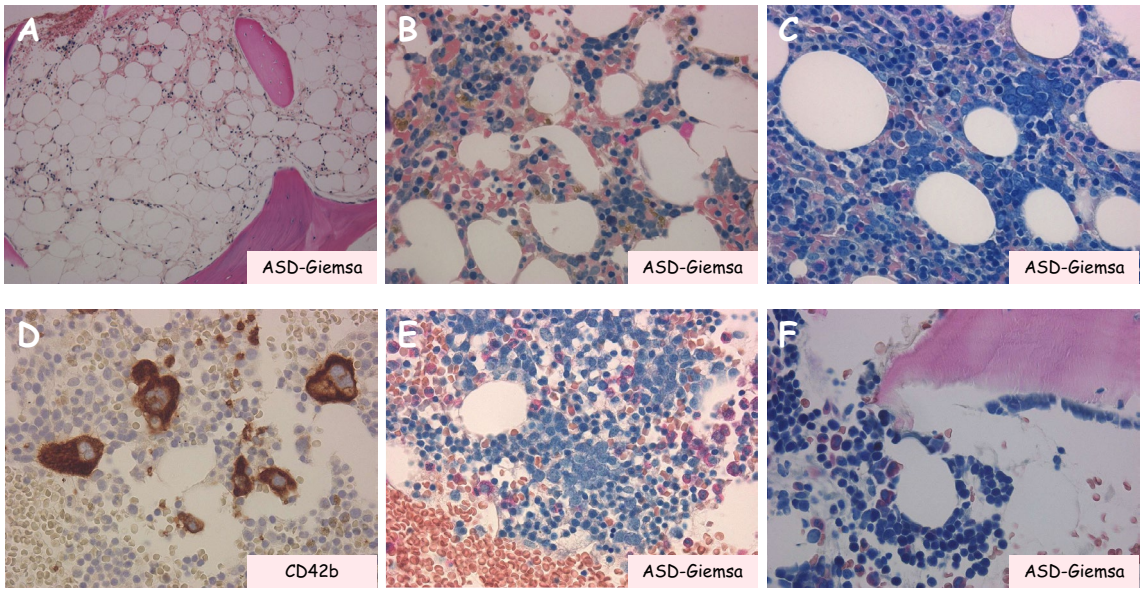

Fig. 1 Histopathological findings in pediatric bone marrow failures. **A** Severe hypocellular bone marrow in aplastic anemia (AA). **B** Mild hypocellular and patchy pattern bone marrow in refractory cytopenia of childhood (RCC). **C** Hypercellular bone marrow in RCC with multilineage dysplasia (MLD). **D** Decreased number of mature megakaryocytes and the presence of mononuclear small-sized megakaryocytes and micromegakaryocytes in RCC. **E** Megaloblastic changes of erythroblasts in RCC. **F** Abnormal localization of erythroblasts under the cortical bone in RCC

may be hidden even among patients diagnosed with acquired AA. Several consensus guidelines for the diagnosis of BMF in children describe the usefulness of a comprehensive diagnostic work-up, combining a detailed interview including family and present history, physical examination, blood and BM evaluations, screening tests to exclude IBMFS such as chromosome fragility test for Fanconi anemia and telomere length measurement by flow fluorescence in situ hybridization for dyskeratosis congenita, and genetic analyses [4–6, 22]. Especially, next-generation sequencing (NGS) is increasingly used for the definitive diagnosis of IBMFS and has been confirmed as an effective tool for differential diagnosis with acquired AA [7, 23]. A recent study in the US, including 732 children and adult HCT recipients clinically diagnosed with acquired AA, revealed that a comprehensive genetic analysis using NGS identified 48 (6.6%) patients with unrecognized IBMFS having 62 germline pathogenic variants in 22 IBMFS-associated genes [24]. Furthermore, patients with unrecognized IBMFS had inferior survival because of treatment-related mortality from organ failure. Thus, to tailor optimal intensity of conditioning regimens, genetic testing should be part of the diagnostic evaluation before HCT for all pediatric, adolescent, and may adult patients with AA. In addition, the diagnosis of IBMFS without overlooking it allows for appropriate long-term follow-up, with attention to the development of organ dysfunction and secondary malignancies that are specific and frequent in each IBMFS [25].

Conditioning regimens for acquired BMF in children

BMT has been an established treatment for acquired BMF, represented by AA, and the survival rate has reached 90% in pediatric patients [1–3, 26]. The standard conditioning regimen for BMT, as originally described by the Seattle group, is cyclophosphamide (CY) 200 mg/kg and antithymocyte globulin (ATG) [27]; however, the high-dose CY correlated with excess toxicities, such as cardiac dysfunction. Since the 2000s, as a strategy to reduce the dose of CY, fludarabine (FLU) has been incorporated into conditioning regimens for acquired AA [28, 29]. The European Group for Blood and Marrow Transplantation (EBMT) has recommended a regimen comprising FLU, lower dose CY (1200 mg/m^2, equivalent to approximately 40 mg/kg in children), and ATG for pediatric patients, and the same regimen combined with 2 Gy of total body irradiation (TBI) for adult patients [30]. However, the occurrence of graft failure including LGF with full donor chimerism, which is referred to as donor-type aplasia or poor graft function, remains a great concern, and the incidence has ranged from 12 to 17% [29–31]. To investigate the optimal dose of CY combined with FLU, a prospective study from the US compared the four doses of CY (0–150 mg/kg) [32, 33]. The arms receiving 0 and 150 mg/kg were quickly closed due to the high rates of primary graft failure and excess toxicity, respectively; thus, CY at a dose of 50–100 mg/kg was recommended. However, the incidence of graft failure, mainly LGF, was still high with 12–15%. Thus, another way to overcome graft failure other than adjusting the CY dose is required, and one possible way is to incorporate other alkylating agents to FLU.

A recent Japanese study of 367 children with acquired BMF used national registry data and showed that melphalan (MEL) has been successfully adopted for both patients with acquired AA and RCC receiving BMT, instead of CY [14]. In that study, the FLU/MEL-based regimen provided excellent outcomes; the OS and failure-free survival (FFS) reached 98% and 97%, respectively, the incidence of LGF decreased to 3%, and donor-type aplasia was not observed. In contrast, in patients receiving FLU/CY-based regimen, LGF was the most common cause of treatment failure at 11%, of which > 60% were donor-type aplasia. Donor-type aplasia is a severe complication that may require stem cell boost and/or re-transplantation in a significant proportion of patients [34, 35]. Considering the risk of re-transplantation, strategies to prevent this complication should be implemented at the time of initial transplantation. On the basis of the results, currently, a regimen comprising FLU, MEL (140 mg/m^2), and ATG with/without low-dose TBI as promising option for children with acquired BMF receiving BMT has been included in the treatment guidelines in Japan [20].

Upfront unrelated donor BMT

In children with acquired AA, BMT from a matched related donor (MRD) has been the treatment of choice [1, 2, 36], and recently, donors reported expanding to include one-locus mismatched related donors (1MMRD) [37]. IST using ATG and cyclosporine (CyA) has been considered the first-line therapy for patients who lack a MRD, with BMT from a MUD reserved as a salvage therapy for non-responders to IST. Based on the recent dramatic improvement in outcomes of BMT from a MUD, comparable to BMT from a MRD [38, 39], upfront BMT from a MUD has been considered an attractive option for children with acquired AA [40, 41].

In a UK study of HCT from MUD as first-line therapy in 29 patients aged < 20 years, excellent outcomes were reported with 2-year OS and FFS of 96% and 92%, respectively [40]. The most notable result was that outcomes of upfront HCT from a MUD were significantly better than those after HCT from a MUD performed as rescue after a failed response to IST. More recently, the EBMT analyzed the outcomes of 74 patients aged 1–76 years who received upfront unrelated HCT for acquired AA between 2010 and

2018 [41]. Considering that the population included 23% of patients who were > 40 years, the outcomes appeared promising. The 2-year OS and GVHD-free/relapse-free survival (GRFS) rates, defined as survival having engrafted without grade III–IV acute GVHD and extensive chronic GVHD, were 89% and 86%, respectively. When discussing the treatment strategies for children with AA, the long-term sequelae and level of hematopoietic recovery that affect daily or school life should be also considered. Importantly, when the analysis was limited to patients aged < 15 years, the 2-year GRFS exceeded 90%, indicating that BMT from a MUD is a promising first-line treatment for children with AA in terms of maintaining quality of life (QOL) after transplantation. These results led several guidelines to recommend that upfront MUD BMT can be a potential option in children with acquired AA who lack a MRD if a MUD is available within 2–3 months of diagnosis [30, 42, 43]. The upfront MUD BMT approach is highly dependent on the promptness of donor coordination and availability, with the risk of infectious and bleeding complications caused by unexpected donor delays. Previous retrospective studies have missed patients who were scheduled for upfront MUD BMT but were not performed because of unexpected complications or donor cancellation. Ongoing prospective intention-to-treat trials can address this limitation and may confirm this strategy as the standard of care for children with acquired AA.

Options for second-line treatment: UCBT and haploidentical HCT

Although treatment options for patients with acquired AA without a matched BM donor have been limited, advances in supportive care, improved conditioning regimens, and emergence of novel GVHD prophylaxis strategies over the past decade have led to vigorous development of UCBT and haploidentical HCT as feasible treatment options.

Early studies on UCBT for patients with acquired AA showed limited success, with approximately 50% of graft failure, which resulted in the OS rate of approximately 40% [44, 45]. A recent prospective French study (APCORD) that included 26 pediatric and young adult patients who underwent UCBT between 2011 and 2015 with a regimen comprising FLU, CY, ATG, and 2 Gy of TBI reported improved results, with the 2-year OS rate of 81% and engraftment of 88%, respectively [46]. A nationwide survey in Japan also reported remarkably improved UCBT outcomes in pediatric patients aged < 16 years, with a 5-year OS rate of 100% among 21 patients transplanted between 2011 and 2020 [26]. This was comparable to 5-year OS rates of 96% and 93% after related ($n = 136$) and unrelated BMT ($n = 137$), respectively. Another Japanese retrospective study revealed that ATG was significantly associated with poor survival when used with UCBT, and better outcomes were observed with a conditioning regimen comprising FLU, CY or MEL, and low-dose TBI without ATG ($n = 11$); the 2-year OS and FFS rates were both 100% [47]. These advances have the potential to change the treatment algorithm for pediatric AA, suggesting that UCBT is a useful treatment option for children who lack a MRD or MUD or for emergency cases.

Haploidentical HCT has emerged as another second-line treatment option for patients with AA, following its rapid spread use for hematological malignancies. In the setting of haploidentical HCT, bidirectional alloreactivity can lead to both GVHD and graft failure, which represents the main concerns with this strategy. However, significant progress to overcome the HLA barrier has been achieved in recent years, including ex vivo TCR-α/β-T cell depletion [48], in vivo T-cell depletion with ATG or alemtuzumab [49], and post-transplant CY (PTCY) approach [50–52]. Of these, the current mainstream of haploidentical HCT consists of two platforms for the prophylaxis of GVHD and graft failure, busulfan, CY, and high-dose ATG conditioning with a combination of BM and peripheral blood as stem cell source developed at Peking University [49], and PTCY approach originally proposed by Johns Hopkins University [52]. A multicenter phase II trial (BMT CTN 1502) on the PTCY approach that included 31 pediatric and adult patients who underwent haploidentical BMT as salvage therapy to IST between 2017 and 2020, with a regimen comprising FLU, reduced dose of CY, ATG, and 2 Gy of TBI was conducted in the US [50]. The 1-year OS rate was 81%, with no patients developing grade 3–4 acute GVHD or severe chronic GVHD, a promising outcome. However, 5 (16%) patients developed graft failure (4 primary and 1 secondary); all underwent salvage HCT, of which four resulted in transplantation-related mortality. Another retrospective analysis of haploidentical HCT using the Johns Hopkins University platform for 16 children with AA reported by the EBMT showed 2-year OS and 28-day neutrophil engraftment rates of 93% and 69%, respectively, suggesting that graft failure remains as the most concern with this approach [51]. Regarding the Peking University platform, a national registry-based study in China reported comparable long-term outcomes between haploidentical HCT and matched sibling donor (MSD) HCT as first-line therapy in 342 children and adult patients with AA. Neutrophil engraftment was observed in 97% of both groups, with 9-year FFS rates of 87% and 88% without significant difference, respectively, although higher incidence of GVHD was observed in the haploidentical setting [49]. Interestingly, the study also evaluated physical, mental, and social QOL before and after HCT, a measure of long-term outcomes not reflected in FFS, and found great improvements in the QOL after HCT, to the same extent between haploidentical and MSD HCT in both children and adults. These results will

encourage further widespread use of haploidentical transplantation for acquired AA.

Eltrombopag (EPAG) added to IST

Despite little progress in IST for AA for a long time, the thrombopoietin receptor agonists, EPAG, have recently been shown to have efficacy not only for refractory patients but also for untreated patients with AA. In a multicenter phase III trial (RACE) conducted by the EBMT, 197 patients aged ≥ 15 years with untreated severe AA were randomized to receive horse ATG plus CyA or with EPAG, and the overall response rates (ORR) at 6 months were 41% and 68%, respectively, indicating that the addition of EPAG to standard IST can improve short-term outcomes [53]. Regarding the long-term outcomes of IST with EPAG, the National Institutes of Health (NIH) reported high relapse rate in a prospective study with a median 4-year follow-up that included 178 pediatric and adult patients with AA: the 6-momth ORR to IST with EPAG was 81%; however, the relapse rate at 4 years among responders was as high as 43%, suggesting that its long-term therapeutic efficacy has not been satisfactory [54]. The incidence of clonal evolution, which is of greatest concern in the addition of EPAG, is reported to be approximately 15%, which was comparable to that of conventional IST. Even when limited to high-risk chromosomal abnormalities such as monosomy 7 and complex karyotypes, the incidence is not small, ranging from 5 to 10% [54–56]. Patients with high-risk chromosomal abnormalities have a poor prognosis, and careful observation with periodic BM evaluation remains essential, with or without the addition of EPAG.

Experience with the use of EPAG in children with newly diagnosed acquired AA is limited, with results from two prospective studies reported [56, 57]. A subgroup analysis of the NIH study described above, focusing on patients aged < 18 years, showed that the addition of EPAG to IST did not improve outcomes unlike in adult patients; no difference in the 6-month ORR was found between 87 control patients given conventional IST and 40 who received additional EPAG [56]. Notably, the relapse rate for responders was significantly higher in the EPAG group (43%) than in the control group (27%); consequently, the 4-year FFS was inferior in the EPAG group (44%) than in the control group (55%). Another concern is that during the 4-year follow-up, 5 (13%) patients in the EPAG group developed clonal evolution including three of monosomy 7, and one of them progressed to acute myeloid leukemia. Another prospective study of 98 children with AA in Russia also found no improvement in short- or long-term outcomes with the use of EPAG, that is, no difference was fond in the ORR, relapse rate, or FFS between IST with or without EPAG [57]. Currently, no evidence available recommends the addition of EPAG to IST with ATG and CyA for untreated pediatric AA. Although it is unclear whether EPAG use contributes to clonal evolution and further studies are warranted, EPAG should be used cautiously in children.

Conclusion

As AA is a rare disease in children, many physicians, including pediatric hematologists, have limited experience in its diagnosis and treatment. Thus, all children presenting with BMF should be referred to an institute or hospital with expertise in AA and other BMFs and offered an appropriate diagnostic work-up and follow-up. With the recognition of the possible underlying causes of IBMFS in the diagnosis of pediatric AA, several studies have demonstrated the importance of comprehensive genetic analysis using NGS. Currently, guidelines from various countries recommend comprehensive diagnostic work-up incorporating these genetic testing and will play an increasingly important role in making decisions for diagnosis and treatment of pediatric AA in the future [7, 25, 42]. The treatment algorithm currently used for the optimal therapeutic management of acquired AA in children, based on our recent experiences and those of others, is illustrated schematically in Fig. 2. BMT from a MRD/1MMRD is the treatment of choice. Given the recent advances in unrelated donor HCT, MUD, if readily available, could also be considered first-line therapy in the absence of a MRD/1MMRD. UCBT and haploidentical HCT are promising options for emergency cases and for patients without available matched donors, although these should be performed in experienced centers. The FLU/MEL-based regimen may be a standard conditioning for children with acquired AA that could overcome LGF, the most concerning complication of HCT [14]. In addition, long-term follow-up is essential to detect IST- or HCT-related complications and to evaluate QOL after treatment. Therefore, in the field of acquired AA in children, not only further development of diagnosis and treatment but also age-appropriate patient education and transition from pediatrics to adult medicine are important issues that should be addressed in future clinical and studies.

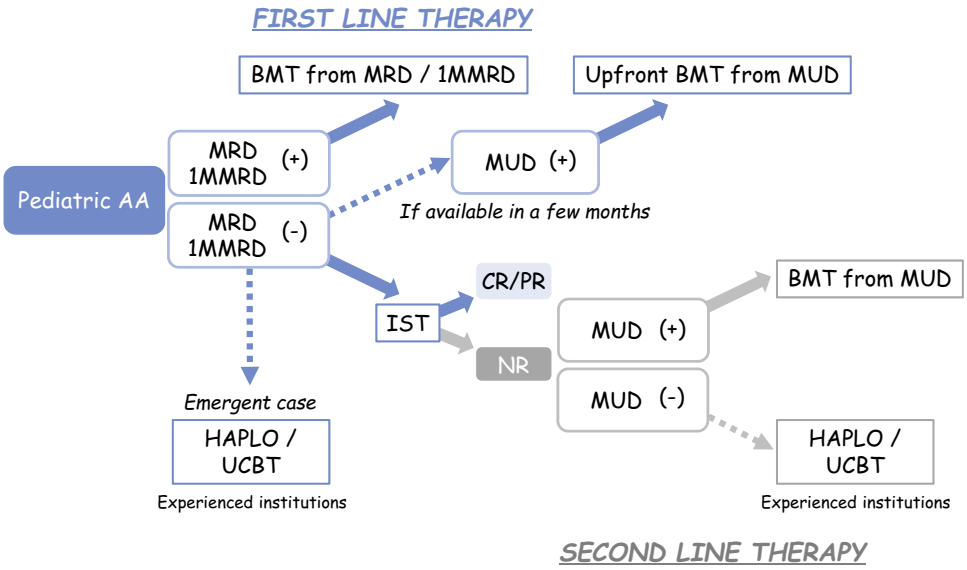

Fig. 2 Current treatment algorithm for children with acquired aplastic anemia. *AA*, aplastic anemia; *BMT*, bone marrow transplantation; *MRD*, matched related donor; *1MMRD*, one locus mismatched related donor; *MUD*, matched unrelated donor; *IST*, immunosuppressive therapy; *CR*, complete response; *PR*, partial response; *NR*, no response; *HAPLO*, haploidentical hematopoietic cell transplantation; *UCBT*, unrelated cord blood transplantation

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Data availability Data sharing is not applicable as no datasets were generated or analysed.

Declarations

Conflict of interest The author has no conflict of interest to declare.

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