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REGULAR ARTICLE

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Severe aplastic anaemia in children: Impact of histopathology profile and treatment on very long-term outcomes

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Abstract

Aim: To assess very long-term outcomes of children with severe aplastic anaemia (SAA) and impact of histopathology and of different treatments over time.

Methods: We conducted a retrospective study of 57 consecutive patients with SAA during 1973-2019. According to period, treatment consisted of androgens, immunosuppressive treatment (IST) and haematopoietic cell transplantation (HCT) in 14, 31 and 13 patients, respectively. Histopathology immune profiles were studied on bone marrow (BM).

Results: Response rate (RR) to androgens was 35%, with long-term survivorship in 4 of 5 responders. RR and 10-year overall survival (OS) after IST was 65% and 80%, respectively. RR was higher in girls (92% vs 43% in boys, $P = .02$). Mean baseline BM values of CD34 + and of B-lymphocytes in responders vs non-responders were 1.3% vs 0 ($P = .08$) and 14.1% vs 9.7% ($P = .07$), respectively. After IST, BM cellularity gradually increased and cytotoxic T-lymphocytes decreased (time variation $P = .003$ and 0.07, respectively). Outcome did not differ between patients with IST or frontline HCT. Ten-year OS improved over time, increasing from 35.3% to 77.1% and 77% during 1973-1985, 1986-2003 and 2004-2019, respectively.

Conclusion: Histopathology may refine response prediction to IST. The course of SAA in children, a previously fatal disease, was altered in recent times.

KEYWORDS

androgens, aplastic anaemia, haematopoietic stem cell transplantation, histopathology, immunosuppressive treatment

Abbreviations: ANOVA, Analysis of variance; ATG, Antithymocyte globulin; BM, Bone marrow; CBC, Complete blood count; CR, Complete response; CSA, Cyclosporine A; EBMT, European Society for Blood and Marrow Transplantation; EFS, Event-free survival; EWOG, European Working Group; G-CSF, Granulocyte colony-stimulating factor; GPR, Good partial response; GVHD, Graft-versus-host disease; HCT, Haematopoietic cell transplantation; HLA, Human leukocyte antigen; IST, Immunosuppressive treatment; MMF, Mycophenolate mofetil; MSD, Matched sibling donor; MUD, Matched unrelated donor; OS, Overall survival; PNH, Paroxysmal nocturnal haemoglobinuria; PPR, Poor partial response; RR, Response rate; SAA, Severe aplastic anaemia; VSAA, Very severe aplastic anaemia.

1 | INTRODUCTION

Severe aplastic anaemia (SAA) is a rare, immune-mediated, acquired bone marrow (BM) failure syndrome characterised by peripheral blood cytopenias and hypocellular BM without signs of dysplasia.[1] The natural course of the disease leads to life-threatening cytopenia-related complications, in particular infection and haemorrhage. Historically, the treatment of SAA consisted of androgens, corticosteroids and supportive care, with mediocre short- and long-term results.

The modern therapeutic algorithm of severe aplastic anaemia in childhood comprises allogeneic haematopoietic cell transplantation (HCT) if a matched sibling donor (MSD) is available and, otherwise, immunosuppressive treatment (IST), which combines antithymocyte globulin (ATG) and cyclosporine A (CSA).[2,3] Salvage HCT from alternative donors is indicated upon IST failure.

Very long-term outcome results, combined with diagnostic and follow-up morphology and histopathology data in SAA in childhood, using evolving treatments in the course of time, are scarce. We retrospectively studied cases consecutively diagnosed and treated in a reference centre, and report BM data and very long-term outcomes over more than 4 decades of management and follow-up of children with SAA.

Key Notes

- Most paediatric and adult studies of aplastic anaemia have limited follow-up.
- We report on very long outcomes of children with severe aplastic anaemia over a period spanning the androgens era as well as that of modern treatment with immunosuppressive treatment and bone marrow transplantation.
- By serial measurements of immune cell populations, we found that bone marrow haematopoietic progenitor cells and B-lymphocytes may serve as predictive factors of response to immunosuppressive treatment

2 | PATIENTS AND METHODS

The Department of Pediatric Hematology-Oncology (TAO) of 'Aghia Sophia' Children's Hospital is a major referral centre for childhood haematological and oncological diseases in Greece, with the longest national experience in SAA treatment. However, the present study was not a population-based study, strictly speaking. Based on the assumption of annual incidence 1.2-2.2/10^6 children,[4-6] approximately 2-4 children are diagnosed with aplastic anaemia per year in Greece. Records of all children with acquired SAA who were referred, diagnosed and treated in the Department during a period of 46 years (1973-2019) were reviewed. Acquired aplastic anaemia was diagnosed in 60 children. Three patients had moderate aplastic anaemia and were excluded from analysis. Overall, 57 patients with SAA/VSAA were included. All patients who did no longer convened for regular consultation or their caregivers were reached by telephone to assess outcomes.

2.1 | Diagnosis and histopathology

Diagnosis and severity classification were made according to Camitta's criteria.[7] BM morphology of BM aspiration was assessed by the same person (KT), on 1000 cells or, in cases of very low cellularity, at least 500 cells. Minimal and maximal cellularity, myeloid, erythroid and megakaryocytic lineages, numbers of total T-lymphocytes (CD3), B-lymphocytes (CD79a), cytotoxic T-lymphocytes (CD8), haematopoietic progenitor cells (CD34), and macrophages/monocytes (CD68), and signs of dysplasia were evaluated on BM biopsy by the same pathologist (KS). BM biopsies and cytogenetics were performed at diagnosis, every 6 months for the first 2 years after IST, and then annually for various lengths of time. Patients with diagnosis of hypoplastic myelodysplastic syndrome defined by hypocellular BM and dysplasia in one or more lineages were excluded. Constitutional genetic defects were excluded by clinical examination, family history, absence of organ dysfunction, and, negative mitomycin C chromosome breakage test. In particular, Fanconi anaemia was excluded in all patients by at least one negative mitomycin C chromosome breakage test. Karyotype and fluorescent in situ hybridisation for chromosomes 5, 7, 8 and 20 abnormalities were performed in all patients after 2000. Paroxysmal nocturnal haemoglobinuria (PNH) clones were detected by flow cytometry on peripheral blood.

2.2 | Treatment

Immunosuppressive treatment and HCT were introduced in our Hospital in 1985 and 1993, respectively. Upfront HCT was determined by MSD availability but was offered only in Transplantation centres abroad before 1993. Before IST, treatment of SAA consisted of corticosteroids, prednisolone 2 mg/kg/d or analogue, and androgens, oxymetholone 2-5 mg/kg/d. IST consisted of horse ATG (Lymphoglobulin 0.75 mL/kg/d for 5 days or during the recent years, ATGAM 40 mg/kg/d for 4 days) or rabbit ATG (Thymoglobulin 3.75 mg/kg/d for 5 days), depending on availability of products in Europe. CSA was started on day 1 of ATG at the dose of 5 mg/kg/d divided to 2 doses, for at least 2 years aiming at trough levels of 100-200 μg/L. Corticosteroids were administered from day 1 of ATG and for 14 days, and tapered thereafter. Four patients received G-CSF in adjunction to IST as part of the protocol of a clinical trial.[8]

2.3 | Response assessment

Response to IST was defined according to the European Working Group for Severe Aplastic Anemia in childhood (EWOG-SAA) criteria. In detail, complete response (CR) was defined as transfusion

independence and normal complete blood count (CBC), good partial response (GPR) as transfusion independence and haemoglobin >6 g/dL and neutrophil count 1000-1500 × 10^6/L and platelet count 50 000-150 000 × 10^6/L, and poor partial response (PPR) as transfusion independence and haemoglobin >6 g/dL and neutrophil count 500-1000 × 10^6/L and platelet count 20 000-50 000 × 10^6/L. Patients with CR/GPR were defined as responders. Relapse was defined as loss of the above-defined response.

2.4 | Statistics

Predictive factors of response were tested by logistic regression. Cell percentages per high-power field were examined as continuous variables. Assumption of Normality was tested using the Shapiro-Wilk test. Parametric and non-parametric variables were compared using the t test and the Mann-Whitney *U* test. Cumulative incidence of response was estimated taking into account the competing risk of death and HCT and compared using the Gray test.[9] Death, HCT and second course of IST were registered as events for calculation of event-free survival (EFS) after IST. OS and EFS were calculated with the Kaplan-Meier method.[10] Survival times were compared using the Cox model.[11] Repeated-measures analysis of variance (ANOVA) was used to test differences of quantitative histological measures, with time as the within-subject factor and response to IST as the between-subject factor (responders vs non-responders). Type I error was fixed at 5%. All tests were two-tailed. All calculations were performed using R version 3.3.2.

3 | RESULTS

3.1 | Patients characteristics

Characteristics of 57 patients with SAA/VSAA are shown in Table 1. Newly diagnosed cases were evenly distributed through the study period (17%, 22%, 17%, 27%, 17% in the 1970s, 1980s, 1990s, 2000s and 2010s, respectively). Median follow-up was 13 years. Seven patients were lost to follow-up after a median time of 8.5 years (range 0.3-27 years). In addition, 4 patients died early during the early era and 1 patient was lost to follow-up before receiving any treatment. Upfront treatment included IST, HCT and androgens in 31 (60%), 7 (13%) and 14 (27%) patients, respectively. Among patients who received IST, 6 (19%) underwent HCT as salvage/second-line treatment.

3.2 | Androgens

A total of 19 patients received androgens, of whom 14 without IST, during 1973-1986, and 5 with IST. RR in patients treated solely by androgens was 35%, including GPR and CR in 1 and 4 patients,

TABLE 1 Characteristics of the patients and treatment

	Number (%), median [IQR]
Patients	57
Age (years)	8.5 [2.5-14.2]
Boys/girls	37/22
Leucocytes (×10^6/L)	2300 [1400-3800]
Neutrophils (×10^6/L)	390 [210-500]
Haemoglobin (g/dL)	7.5 [6-8.7]
Reticulocytes (×10^9/L)	12 [6-33]
Platelets (×10^6/L)	10 000 [4000-13 500]
Severity	
SAA	32 (56%)
VSAA	20 (35%)
SAA or VSAA[a]	5 (9%)
Aetiology	
Seronegative hepatitis	4 (7%)
Benzene exposure	1 (2%)
Idiopathic	52 (91%)
PNH clone[b]	1 (2%)
Treatment[c]	
Androgens only	14 (25%)
Age (years)	9 [5.8-11]
Boys/girls	11/3
SAA	10 (70%)
VSAA	2 (15%)
SAA or VSAA[a]	2 (15%)
IST	31 (52%)
Age (years)	8 [4.9-9.6]
Boys/girls	17/14
SAA	18 (58%)
VSAA	13 (42%)
Additional IST	9/31 (29%)
Second course ATG	7/9 (77%)
Mycophenolate mofetil	1/9 (6%)
Campath	1/9 (6%)
HCT	13 (23%)
Age (years)	8 [4.8-10]
Boys/girls	9/4
SAA	4 (31%)
VSAA	8 (61%)
SAA or VSAA[a]	1 (8%)

Abbreviations: ATG, Antithymocyte globulin; HCT, haematopoietic cell transplantation; IST, immunosuppressive treatment; PNH, Paroxysmal nocturnal haemoglobinuria; SAA, severe aplastic anaemia; VSAA, Very severe aplastic anaemia.

[a]Distinction between SAA and VSAA not possible due to insufficient data;

[b]A small PNH clone <5% was detected in 1 patient with no signs of haemolysis, treated with IST;

[c]Sum of treatments higher than 100% because of concomitant or consecutive treatments in some patients.

respectively, all with SAA. One patient had PPR. Median time to best response was 12 months. Among 5 responders, one relapsed at 6 months and of note, 4 were long-term survivors. None of the patients underwent HCT. None of non-responders survived. Two late deaths occurred 11 and 15 years after diagnosis, in non-responders, from post-transfusion iron overload and haemorrhage, respectively. Of note, patient-reported outcomes among 4 responders, who were contacted 30 years after diagnosis, showed excellent quality of life and personal achievements in domains like marriage, reproduction and profession.

3.3 | Immunosuppressive treatment

Between 1985 and 2019, 31 patients received upfront IST. Median follow-up was 9 years (range 0.5-29 years). Median time from diagnosis to treatment was 24 days (range 1-549 days). Horse and rabbit ATG were used in 1/3 and 2/3 of cases, respectively. RR to ATG was 65%. Cumulative incidence of response among evaluable cases was 29%, 37%, 42% and 53% at 3, 6, 12 and 24 months after IST, respectively. Type of response according to time of assessment is represented in Figure 1. Of note, among responders, the proportion of patients with CR rose from 10% to 50%, 70% and 80% at 3, 6, 12 and 24 months after IST, respectively (Figure 1). Among patients with missing assessments at the above timepoints, 3 patients had normal CBC at 5, 16 and 22 years after a single course of IST, and therefore they were considered as additional long-term responders.

An additional course of IST was administered in 8 patients (Table 1). Median time to second IST was 6 months. One patient treated with IST + eltrombopag, relapsed at 9 months with no response to eltrombopag. RR to additional IST was 66%, including in 5 of 7 patients with a second course of ATG and 1 patient treated with Campath (alemtuzumab, a humanised monoclonal antibody directed against the glycoprotein CD52). In total, only 6/31 patients treated with IST eventually underwent HCT after a median time of 6 months, including 2 after additional IST. Ten-year EFS after IST was 56.9%.

Among pre-treatment characteristics, only gender and, as a trend, severity, were predictive of response to upfront IST: RR to IST was 92% vs 43% in girls vs boys, respectively, ($P = .02$) and in 81% vs 46% of patients with SAA vs VSAA, respectively ($P = .11$) (Table S1).

Ten-year OS was 80%. Ten-year OS in SAA vs VSAA was 89% vs 67%, respectively ($P = .15$) (Figure 2). Six deaths occurred during follow-up, all in non-responders. Two early deaths, that is ≤3 months from IST, were due to bleeding and occurred during the earliest period of the study. One patient died from infection after a repeated ATG course. Three deaths occurred during the most recent period, that is after 2000, from transplant-related causes after matched unrelated donor (MUD) HCT 7, 7 and 9 months after a unique IST course. Median OS was 2 years in non-responders as compared to not reached in responders, who all survived long-term ($P = .0001$).

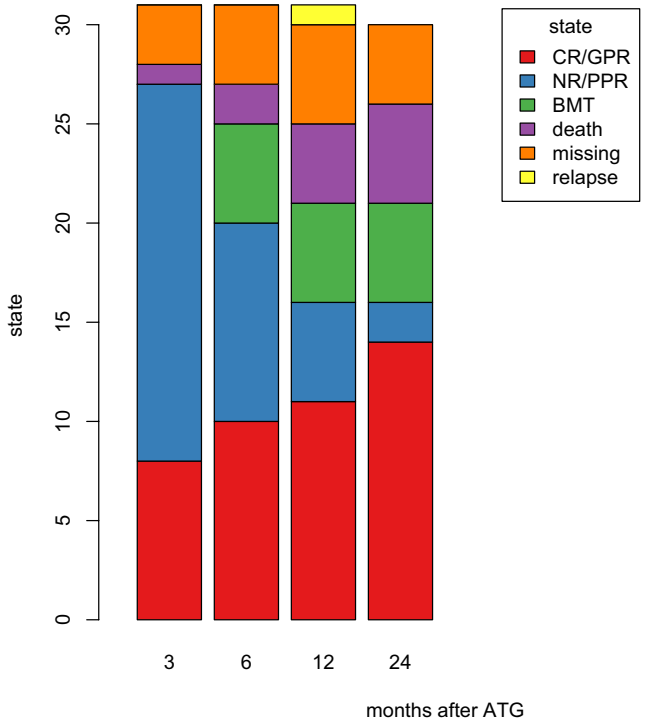

FIGURE 1 Response according to time of assessment after immunosuppressive treatment (IST)

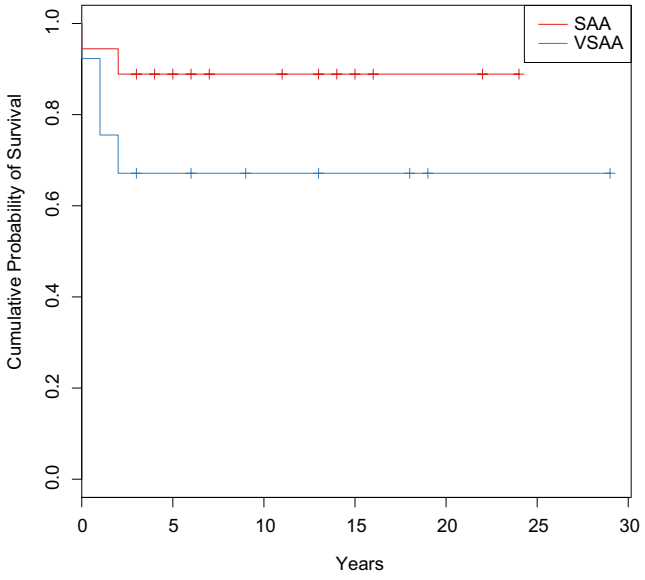

FIGURE 2 Overall survival according to severity of aplastic anaemia in patients treated with immunosuppressive treatment (IST)

Among non-responders who were long-term survivors, 3 patients had undergone HCT and one patient had no further treatment. Low-grade cervical intraepithelial neoplasia was recorded in one responder 10 years after IST. One patient had hypertension at an adult age.

Baseline karyotype was performed in 30 patients. Two patients had baseline pre-IST abnormalities, one patient each with del(5)(p13;p15), del(12)(p11;p13) and constitutional t(3;10)(p13-21;p15). In total, 3/31 patients developed transient cytogenetic abnormalities during follow-up. Those 3 patients were responders after 1 or 2 courses of IST. One patient each acquired del(6)(q21;q25), del(6)(p21) and t(7;14)(p22;q11), detected on at least two consecutive banding karyotypes at 1 month interval, 24, 20 and 15 months after IST, respectively, and no longer detected thereafter. Rabbit ATG was used in all patients with transient cytogenetic abnormalities. Baseline del(5)(p13;p15) and del(12)(p11;p13) disappeared after IST.

3.4 | Histopathology findings at diagnosis and after IST

At diagnosis, 28 BM biopsy samples from 25 patients were centrally reviewed. Of those, 21 belonged to 18 patients who subsequently received IST and 7 to patients who were treated upfront with HCT.

Median baseline BM minimal and maximal cellularity was 20% (range 3%-40%) and 30 (range 3%-50%), respectively. Megakaryocytes were absent or very rare. Median value of CD34 + haematopoietic progenitor cells was 0 (range 0%-5%). Median values of immune cell populations were 17.5% for CD3 + T-lymphocytes, 12.5% for CD8 + T-lymphocytes, 12.5% for CD79a + B-lymphocytes and 7.5% for CD68 + macrophages/monocytes.

We sought associations between response to IST and pre-treatment characteristics in BM biopsies of 18 patients, of whom 12 were responders and 6 non-responders, with quantitative measures of cell populations. In total, 20 samples were centrally reviewed. A borderline positive correlation between the number of CD34 + cells and CD79 + B-lymphocytes and RR was noted: Mean number of CD34 + cells per visual field was 1.3% in responders vs 0 in non-responders (Mann-Whitney *U* test, $P = .08$), and mean number of CD79 + B-lymphocytes was 14.1% in responders vs 9.7% in non-responders ($P = .07$). No other parameter among those tested was significantly associated with response rate (Table S1).

Then, we studied temporal associations between treatment impact and response in 18 patients, of whom 13 were responders and 5 were non-responders. In total, 20 diagnostic and 51 follow-up samples were centrally reviewed, with a median of 2 follow-up samples per patient (range 1-6). Median duration of BM follow-up was 7.5 months (range 1-90 months). Responders had serial examinations for longer periods than non-responders (mean duration of follow-up 36 months vs 4.8 months, respectively).

Minimal and maximal BM cellularity increased over time in the whole group (within-subject ANOVA $P = .04$ and .003, respectively). Increase of maximal BM cellularity was higher in responders as compared to non-responders (between-subject $P = .002$). Increase in minimal BM cellularity did not reach statistical significance (between-subject $P = .08$). BM CD8 + T-lymphocytes decreased with time as compared to baseline levels ($P = .07$). Responders tended to have a more profound decrease of BM CD8 + T-lymphocytes as compared to non-responders ($P = .13$). No other differences were found with reference to BM cellular parameters between responders and non-responders.

3.5 | Haematopoietic cell transplantation

A total of 13 patients underwent allogeneic HCT, upfront and in the salvage setting after IST failure, in 7 and 6 cases, respectively. All but one upfront HCT were performed from a MSD, whereas all transplants performed as salvage after IST failure, used a MUD with 9/10 or 10/10 HLA-compatibility. Median time from diagnosis to transplant was 60 days (range 23-297 days) and 278 days (range 142-751 days) for upfront and salvage HCT, respectively. Conditioning regimens generally included cyclophosphamide/ATG for MSD transplants and fludarabine/cyclophosphamide/ATG for MUD transplants. The source of stem cells was BM in all cases. Graft failure was documented in 2 cases. Ten-year OS after HCT was 47%. A total of 6 deaths were due to GVHD, graft failure, cyclophosphamide-induced acute cardiotoxicity, and infection in 2, 1, 1 and 2 patients, respectively. Three of the deaths occurred in the upfront and 3 in the salvage setting. No difference in survival was found between the upfront and salvage setting (10-year OS 45.7% vs 57.1%, $P = .66$). However, time period and preferential use of MSD or MUD in upfront or salvage HCT, respectively, according to HCT indication, were major confounding parameters when comparing those modalities.

3.6 | Immune-mediated events

Immune-mediated events included immune thrombocytopenia after IST, with normalisation of platelet count after splenectomy, Miller-Fisher syndrome after HCT, treated with rituximab and intravenous immunoglobulin, and autoimmune haemolytic anaemia after HCT, in 1 patient each.

3.7 | Outcomes according to time period and allocation of treatment

In order to detect differences in outcomes according to study period, we used 3 time-points: 1973, year of diagnosis of the first patient included, 1985, year of first use of ATG, and 2004, year of widespread use of HCT for SAA within our organisation. Periods 1, 2 and 3 were defined from 1973 to 1985, 1986 to 2004 and 2004 to present time, respectively. A total of 17, 22 and 18 patients were diagnosed during periods 1, 2 and 3, respectively. Overall survival at 10 years was 35.3%, 77.1% and 77% during periods 1, 2 and 3, respectively (Figure 3) ($P = .002$). Outcomes of patients of periods 2 and 3 treated with IST or upfront HCT were not different (P = not significant).

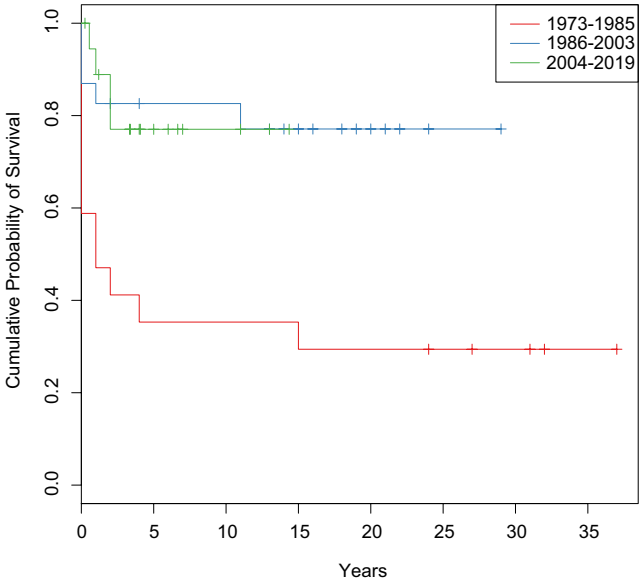

FIGURE 3 Overall survival according to period of the study

4 | DISCUSSION

We studied histopathology features and very long-term follow-up data of a single centre patient cohort, illustrating the evolution of therapeutic algorithms in SAA in children, and studying the impact of their implementation over time in consecutive or parallel ways, on the final outcome. OS approximated 80% and remained stable over the last 35 years by using IST unless a MSD was available. The European Society for Blood and Marrow Transplantation (EBMT) and the European Working Group (EWOG) for SAA in childhood endorse this algorithm, whereas the role of universal upfront MUD transplantation is debated.[2,3]

In use before the advent of IST and the wide availability of HCT, androgens generated good quality, long-term responses in 4 out of 14 patients. Those findings are in agreement with larger studies on androgens in acquired aplastic anaemia of children.[7]

Cytogenetic abnormalities with low risk of clonal evolution were found both at diagnosis and after IST, as previously described.[12] We documented the transient character of del(6q) and del(6p) and sustained response after IST in one patient each. The observation that rabbit ATG was used in patients who developed transient cytogenetic abnormalities could suggest that less robust responses are associated with clonal evolution.

Quantitation of histological parameters and of in situ immune cell populations on BM biopsies showed gradual restoration of cellularity to normal in responders to IST. Detectable BM CD34 + and higher BM B-lymphocytes at diagnosis were, as a trend, predictive of higher RR. Of note, none of the statistical tests was significant; however, the number of subjects was low, possibly due to the rarity of the disease and low number of patients. Moreover, we observed temporal associations with histological changes of disease activity. Decrease of cytotoxic T-lymphocytes after IST in responders was more pronounced than that of other immune cell populations. Those findings suggest that immunohistochemistry and in situ study of lymphocyte populations could be used as surrogate marker of response prediction and monitoring.

In this study, we found that only female gender and SAA vs VSAA were additional favourable predictive factors for response to IST, considering limited numbers of patients and the retrospective nature of the study. Other predictive factors of response in children include interval between diagnosis and treatment, telomere length, baseline lymphocyte counts and lymphocyte depletion early after IST.[1,13-15]

Overall survival in IST studies for SAA in children is usually reported after relatively limited follow-up, with the notable exception of National Institutes of Health and EBMT series.[13-20] Here, we report favourable very long outcomes with a median follow-up of more than 10 years. Late and very late responses were seen after IST. In the long term, health outcomes were not influenced by the choice of upfront treatment, that is IST vs HCT. In particular, improvement of response to IST with time in terms of RR and quality was continuing mostly up to one year after initiation of treatment, and even up to 24 months in 10% of the patients. We did not record any haematopoietic malignancy and only one case of in situ neoplasia after IST.

Direct comparison between IST and HCT was not possible because of different patterns of treatment allocation during this long retrospective study. Published paediatric studies show that the two modalities result in similar OS but lower EFS in patients treated with IST as compared to upfront HCT.[21-26] However, paediatric studies measuring GVHD-free/failure-free survival and quality of life are rare. In a recent study using modern conditioning regimens in children, 10-year GVHD-free/failure-free survival was 51.2%, which is similar to that after IST in most studies, including ours (10-year EFS 56.9%).[27] Judicious use of HCT as per the established algorithm, with use of IST when MSD is not available, results in excellent outcomes. Clearly, more studies are needed in order to explore the place of upfront HCT, from alternative, including haploidentical, donors.

Distinction between refractory cytopenia of childhood and SAA is essential in order to adapt therapeutic algorithms to the precise hypocellular BM failure syndrome. The use of EWOG's histological criteria[2] and molecular findings such as SAMD9/SAMD9L mutations[28] may be useful for precise diagnosis and accurate prognosis. Recognising inherited conditions predisposing to SAA, better response prediction to IST, and optimal use of upfront HCT, may render possible further outcome improvement.[29,30]

In conclusion, in this retrospective study, we present the progress of clinical practice in treating SAA and the evolution over time of histopathological findings, in response to treatment. Long-term outcomes with IST followed by HCT when needed were favourable. Rapid diagnosis and early initiation of either treatment are key issues to succeed in altering the disease course.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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