Outcome of aplastic anemia in adolescence: a survey of the Severe Aplastic Anemia Working Party of the European Group for Blood and Marrow Transplantation

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ABSTRACT

We analyzed the outcome of 537 adolescents (age 12-18 years) with idiopathic aplastic anemia included in the database of the Severe Aplastic Anemia Working Party of the European Group for Blood and Marrow Transplantation comparing: i) matched family donor hematopoietic stem cell transplantation performed as first-line treatment with ii) front-line immunosuppressive therapy not followed by subsequent transplant given for failure and with iii) hematopoietic stem cell transplantation performed after failed front-line immunosuppressive therapy. Overall survival was 86% in the matched family donor hematopoietic stem cell transplantation group, 90% in patients given front-line immunosuppressive alone (those who did not fail this treatment and who did not receive subsequent rescue with hematopoietic stem cell transplantation) and 78% in subjects who underwent hematopoietic stem cell transplantation post failed front-line immunosuppressive therapy (P=0.14). Event-free survival in the same groups was respectively 83%, 64% and 71% (P=0.04). Cumulative incidence of rejection was 8% in matched family donor hematopoietic stem cell transplantation and 9% in transplants post failed front-line immunosuppression (P=0.62). Cumulative incidence of acute graft-*versus*-host disease was 12% in matched family donor transplants and 18% in transplants post failed immunosuppression (P=0.18). Chronic graft-*versus*-host disease was higher in matched family donor hematopoietic stem cell transplantation (8%) than in transplants post failed immunosuppressive therapy (20%) (P=0.0009). Cumulative incidence of post-therapy malignancies was 0.7% in matched family donor transplantations, 7% in transplantations post failed immunosuppression and 21% after front-line immunosuppression (P=0.0017). In the whole cohort, under multivariate analysis, the diagnosis to treatment interval of two months or under positively affected overall survival whereas up-front immunosuppression alone (with no subsequent rescue transplants) negatively affected event-free survival. In transplanted patients an interval from diagnosis to treatment of 2 months or under, bone marrow as source of cells and first-line matched family donor transplants provided a significant advantage in overall and event-free survival. Aplastic anemia in adolescents has a very good outcome. If a matched family donor is available, hematopoietic stem cell transplantation using bone marrow cells is the first choice treatment. If such a donor is not available, immunosuppressive treatment may still be an acceptable second choice, also because, in case of failure, hematopoietic stem cell transplantation is a very good rescue option.

Introduction

Idiopathic aplastic anemia (AA) is a rare disease characterized by peripheral blood cytopenias due to failure of the bone marrow (BM) to produce blood cells. In a significant proportion of cases, marrow function is damaged by an autoimmune attack to the progenitor cells and this explains the success of immunosuppressive therapy.[1] However, immunosuppressive therapy, although successful, does not completely restore hematopoiesis[2] and is complicated by a relapse rate of approximately 30%.[3,4] Thus, if a matched family donor (MFD) is available, hematopoietic stem cell trans-

 doi:10.3324/haematol.2014.106096
The online version of this article has a Supplementary Appendix.
Manuscript received on February 27, 2014. Manuscript accepted on July 21, 2014.
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plantation (HSCT) is the preferred first-line treatment.[3,5]

Although many studies have investigated the outcome of AA in different age groups,[6-8] none have focused on adolescents. Interestingly, adolescence is a peak age of incidence of AA[9] and other autoimmune diseases such as insulin-dependent diabetes mellitus, polyarticular juvenile rheumatoid arthritis and thyroiditis[10-15] that frequently occur at this time of life. In addition, protocols and registration studies often have a cut off at the age of 18 years without further subdivisions into lower age groups.[16] Based on these reasons, and on the increasing interest shown towards adolescent medicine in general, and in hematology in particular,[17] we focused our attention on the outcome of AA in adolescence.

To this end, we analyzed the records of 537 adolescents diagnosed with AA held in the database of the Severe Aplastic Anemia Working Party (SAAWP) of the European Group for Blood and Bone Marrow Transplantation (EBMT), to evaluate the outcome of the disease according to different treatments, the rate of post-therapy late tumors, and to identify prognostic factors that could affect disease outcome.

Methods

Patients

Records of 537 consecutive adolescents (310 males and 227 females) included in the database of the SAAWP of the EBMT with a diagnosis of AA in the period from 1st January 2000 to 31st December 2009, were analyzed. Constitutional marrow failure diseases were excluded based on the currently available laboratory and clinical diagnostic tools. The age limits that the World Health Organization (WHO)[18] provides for adolescence (10-19 years) includes the period of life stretching from puberty to adulthood. We arbitrarily set the lower limit at 12 years of age given that at this age most individuals have already started puberty and the upper limit at 18 years of age, this being the universally recognized start of adulthood. Thus, in this study, adolescents are all the individuals aged 12 years or over and under 18 years of age. Median age at diagnosis was 15 years (range 12-17.5). Median follow up of survivors was 2.3 years (range 0.02-11.3). Eighteen of 537 patients (3.3%), all before HSCT, showed a paroxysmal nocturnal hemoglobinuria (PNH) clone.

Patients were first analyzed according to the first-line treatment received (either MFD HSCT or immunosuppressive therapy). In this cohort, patients who underwent transplant for failure of this treatment were also included. In order to evaluate the outcome of this latter group (defined as HSCT post IST), we extracted these subjects from the original population of front-line IST (defined as IST) and compared their outcome with that of MFD HSCT and of IST with no subsequent transplant (defined as IST alone). Thus, results data will refer to the following populations: MFD HSCT (patients transplanted front line from MFD), IST (patients receiving IST front line and including subjects who were transplanted after IST failure), IST alone (patients receiving IST upfront but excluding those subsequently transplanted for treatment failure), and patients transplanted after having failed IST (HSCT post IST).

Definition of adult, mixed and pediatric centers

In order to evaluate the effect on the outcome, centers in which patients were transplanted were arbitrarily divided into 3 categories: 'Adult', 'Mixed' and 'Pediatric'. This definition was based on JACIE criteria[19] that consider as 'Mixed' those centers who perform at least 5 HSCTs per year in patients aged under 18 years of age over the time-span of the accreditation.

Statistical analysis

In order to evaluate the effect of treatments on patient outcome, the probability of overall survival (OS) and event-free survival (EFS) was estimated. OS was defined as the time from diagnosis to death from any cause. EFS was calculated from the date of diagnosis to the first event or to the last follow up. Events were death, lack of response, relapse, occurrence of clinical PNH, malignancies occurring over follow up and transplant for patients receiving IST front line (IST). The same events, with the exclusion of transplant, were considered in the group receiving IST upfront with no subsequent transplant (IST alone). Patients lost to follow up were censored at the time of their withdrawal. The log rank test[20] was used for univariate analysis. Multivariate Cox model analysis was planned for variables with log rank $P<0.1$.[21] Differences in the distribution of various parameters were compared using χ^2 or Fisher's exact tests as appropriate. Statistical analysis was carried out using the SAS statistical program (SAS-PC, v.9.23; SAS Institute Inc., Cary, NC, USA).

Cumulative incidences of malignancies, chronic and acute graft-*versus*-host disease (GvHD) and rejection were estimated in the competing risk model, with relapse or death (not related to late malignancies and GvHD, respectively) as the competing event. Cumulative incidence curves were compared using the Gray test.[22,23]

Results

Treatments

Front-line IST was received by 143 patients. Treatment consisted of cyclosporine A (CsA) plus either horse or rabbit ATG according to the period. Since after 2007 horse ATG (Lymphoglobulin) was withdrawn from the European market and only rabbit ATG was available, after this date patients were treated with rabbit ATG. One hundred and eighteen patients were treated before and 25 after 2007. Sixty-four of 143 patients treated with up-front IST did not receive any additional treatment (IST alone) (Table 1A). Seventy-nine patients who failed IST underwent subsequent HSCT (HSCT post IST), the majority from a matched unrelated donor (MUD), followed by MFD and from mismatched family (MMFD) or unrelated donor (MMUD). The most widely used conditioning regimen was cyclophosphamide plus fludarabine followed by cyclophosphamide alone and by cyclophosphamide plus fludarabine plus a third agent (busulphan, melphalan or campath). ATG was present in 51% of conditioning regimens. GVHD prophylaxis was mostly methotrexate (MTX) and CsA followed by CsA plus micophenolate mofetil (MMF) (Table 1B).

Front-line MFD HSCT was given to 394 adolescents. Unfortunately, the data set was incomplete for conditioning regimen and GvHD prophylaxis. Based on available data, cyclophosphamide alone was the most frequent conditioning regimen followed by cyclophosphamide plus fludarabine (Table 1B). GvHD prophylaxis was with MTX and CsA in the majority of available cases and CsA alone was the second most used scheme. ATG was present in 58% of conditioning regimens.

Overall survival and event-free survival according to different treatments

The 3-year probability of OS and EFS of the whole population was 85% (SE 2%) and 78% (SE 2%), respectively.

The 3-year OS was 86% (SE 2%) after MFD HSCT *versus* 82% (SE 4%) after IST (*P*=0.53) (Figure 1A). The 3-year EFS was significantly better in the group of patients who underwent front-line MFD HSCT than in that treated with IST upfront (IST) (83% -SE 2%- *vs.* 37% -SE 4%-; *P*<0.0001) (Figure 1A).

Events occurring in the latter group are reported in *Online Supplementary Table S1A*. Interestingly, of the 143 patients initially treated with IST, 79 who failed this treatment underwent HSCT and most were still alive at follow up (OS of 78%; *see below*) (Figure 2A).

Since we aimed to collect information about the outcome of subjects transplanted after having failed IST as a front-line treatment, we extracted this group from that of subjects initially treated with IST and compared it with the group of IST alone and with that of HSCT from MFD front-line (see Methods for definitions). The 3-year probability of OS after IST alone was 90% (SE 4%), after MFD HSCT 86% (SE 2%) and after HSCT post failed IST 78% (SE 5%) (*P*=0.14) (Figure 2A).

Causes of death are reported in *Online Supplementary Table S1B*. The 3-year probability of EFS was 83% (SE 2%) after MFD HSCT, 71% (SE 5%) after HSCT post failed IST and 64% (SE 7%) after IST alone (*P*=0.04) that includes patients surviving with response, without MDS/AML/PNH, transplant and secondary malignancies after front-line IST (Figure 2B). In subgroup analysis, MFD HSCT did significantly better than IST alone (*P*=0.003) and than HSCT post failed IST (*P*=0.039), whereas there was no significant difference between IST alone and HSCT post failed IST (*P*=0.17) (Figure 2B). In the group of subjects transplanted upfront from MFD, bone marrow (BM) as cell source provided a 3-year OS of 93% (SE 2%) *versus* 70% (SE 5%) of peripheral blood (PB) (*P*<0.0001). Also EFS was significantly better with BM over PB (90% -SE 2%- *vs.* 67% -SE 5%-; *P*=<0.0001). Interestingly, in patients transplanted with PB cells, acute GvHD was significantly higher than in those transplanted with BM cells (16.5% *vs.* 9.5%; *P*=0.047) and this was also observed for chronic GvHD (11.6% in PB *vs.* 5% in BM; *P*=0.018). In patients treated with IST alone, the severity of the disease (non-severe AA *vs.* severe AA *vs.* very severe AA) defined according to international criteria[24] was not associated with differences in either OS (*P*=0.08) or EFS (*P*=0.89).

Table 1. Characteristics of 537 adolescents diagnosed with AA included in the study.

A

Front-line IST (defined as IST)	143 pts
Pre 2007	118 pts (82%)
Post 2007	25 pts (18%)
No further treatment after IST (defined as IST alone in Methods)	64 pts (45%)

B

Transplanted population	HSCT after failed IST	Front-line MFD HSCT
473 pts	79 pts	394 pts
Donor		
MUD	64%	
MFD	23%	100%
MMF or MMUD	13%	
Conditioning		
Cy +Flu	41%	17%*
Cy alone	20%	72%*
Cy +Flu+others	18%	2.5%*
Cy+others	14%	6%*
Flu ± others	7%	2.5%*
ATG no/yes	49%/51%	42%/58%
GVHD PROPH		
CsA+Mtx	56%≠	73% ≠
CsA	16%≠	18%≠
CsA +MMF	21%≠	5%
Others	15%≠	4%≠

*MUD: matched unrelated donor; MFD: matched family donor; MMFD: mismatched family donor; MMUD: mismatched unrelated donor. *percentages are calculated from 321 of 394 patients of whom data were available.Cy: cyclophosphamide; Flu: fludarabine; ATG: globulin; CsA: cyclosporine A; MTX: methotrexate; MMF: mycophenolatemofetil. ≠percentages are calculated from 318 of 394 patients for whom data were available for MFD HSCT group and from 66 of 79 patients for whom data were available for HSCT post failed IST group.*

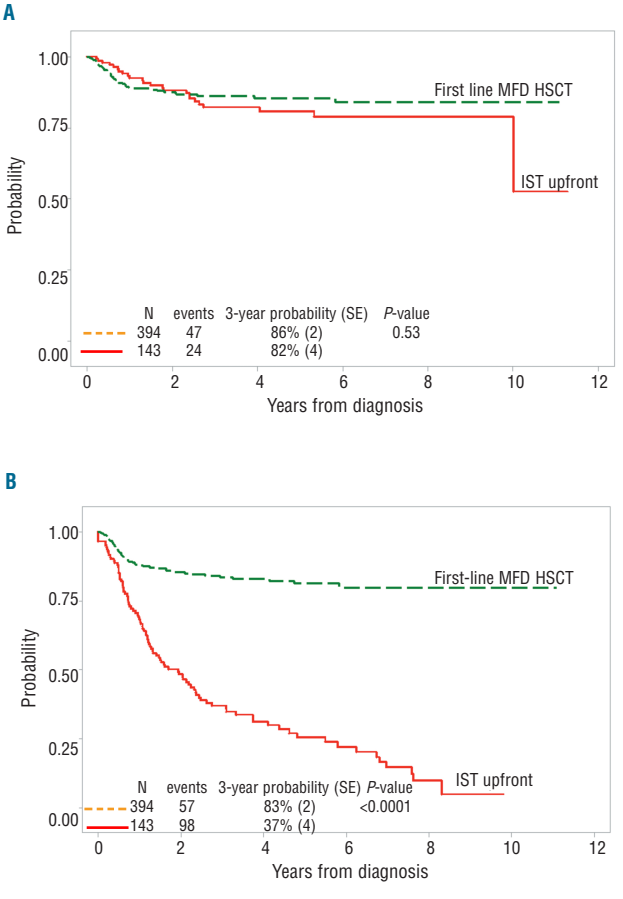


Figure 1. (A) Probability of 3-years OS (Kaplan-Meier method) for the whole population of 537 patients stratified according to treatment. IST means patients receiving IST front line including subjects who were transplanted after IST failure. (B) Probability of 3-years EFS (Kaplan-Meier method) for the whole population of 537 patients stratified by treatment. IST means patients receiving IST front line including subjects who were transplanted after IST failure.

Rejection and graft-versus-host disease

There was no significant difference in 5-year cumulative incidence (CI) of rejection in up-front MFD HSCT (8%, SE 2%) *versus* HSCT post failed IST group (9%, SE 4%) (*P*=0.62) (Figure 3A). Secondary rejection was not reported. Since acute and chronic GvHD affect the quality of life of survivors after transplant, we also looked at this parameter in patients receiving first-line HSCT from MFD and HSCT post failed IST. There was no significant difference in 100-day CI of acute GvHD grade II-IV between MFD HSCT (12%, SE 2%) and HSCT post failed IST (18%, SE 4%) (*P*=0.18) (Figure 3B). The 2-year CI of chronic GvHD was significantly higher in HSCT after failed IST patients (20%, SE 5%) than in MFD HSCT subjects (8%, SE 2%) (*P*=0.0009) (Figure 3C). This might also reflect the high proportion of MM and MUD over MFD in the post IST transplant group.

Malignancies occurring during follow up

Out of 537 patients, a total of 10 late malignancies (7 acute leukemias, 2 advanced myelodyspasias and one lymphoma for which no information on the EBV status is available) occurred giving an overall frequency of 2%, were distributed as follows: 4 of 64 (6%) in the group of

Table 2. Results of univariate and multivariate analyses considering the whole population of 537 patients.

Characteristics	Pts (n)	Events (n)	3-year OS (%) SE (%)	Univariate P	Multivariate P	HR (95% CI)	Events (n)	3-year EFS (%) SE (%)	Univariate P	Multivariate P	HR (95% CI)
Sex											
F	227	27	87 (3)				39	81 (3)	0.45		
M	310	44	83 (2)	0.47			62	77 (3)			
Median age (years)											
≤15	267	35	85 (2)	0.86			43	81 (3)	0.1		
> 15	270	36	84 (3)				58	75 (3)			
Interval (months)[c]											
≤2	313	27	90 (2)	0.0005	0.0007	0.4 (0.3-.7)	51	81 (3)	0.09		
>2	224	44	78 (3)				50	75 (3)			
IST without HSCT											
Yes	64	5	90 (4)	0.22			19	64 (7)	0.0064	0.007	2.0 (1.2-3.3)
No	473	66	84 (2)				82	80 (2)			

N: number; Pts: patients; yrs: years; F: female; M: male; IST: immunosuppressive therapy; HSCT: hematopoietic stem cell transplantation; [c]Interval between diagnosis and treatment.

Table 3. Results of univariate and multivariate analyses considering the 473 patients who underwent HSCT.

Characteristics	Pts (n)	Events (n)	3-year OS (%) SE (%)	Univariate P	Multivariate P	HR (95% CI)	Events (n)	3-year EFS (%) SE (%)	Univariate P	Multivariate P	HR (95% CI)
Sex											
F	204	27	85 (3)	0.71			34	82 (3)	0.74		
M	269	39	83 (3)				48	79 (3)			
Median age (years)											
≤15	235	31	85 (3)	0.52			35	84 (3)	0.12		
> 15	238	35	82 (3)				47	77 (3)			
Center Jacie*°											
P or A	243	27	87 (2)	0.10			35	84 (3)	0.13		
M	229	39	80 (3)				47	76 (3)			
Center 20%^°											
P or A	338	38	87 (2)	0.02			51	82 (3)	0.1		
M	134	28	78 (4)				31	77 (4)			
Interval (months)[c]											
≤2	268	24	89 (2)	0.0006	0.0016	0.4(0.3-0.7)	37	84 (3)	0.03	0.028	0.6 (0.4-0.9)
>2	205	42	77 (3)				45	75 (4)			
1st line MFD HSCT											
Yes	394	47	86 (2)	0.13	0.032	0.5(0.3-0.9)	57	83 (2)	0.04	0.031	0.6 (0.31-0.19)
No	79	19	78 (5)				25	71 (5)			
Source of cells											
BM	308	27	90 (2)	<0.0001	<0.0001	0.4(0.2-0.6)	37	86 (2)	<0.0001	0.0002	0.4 (0.3-0.7)
Others	155	39	71 (4)				45	67 (4)			
Family donor											
Yes	403	51	85 (2)	0.14			62	82 (2)	0.06		
No	58	15	77 (6)				19	70 (6)			

*N: number; Pts: patients; F: female; M: male; P: pediatric center; A: adult center; M: mixed center; HSCT: hematopoietic stem cell transplantation; MFD: matched family donor; BM: bone marrow. *Centers defined based on Jacie criteria. ^Centers defined by 20% arbitrary criteria. °1 missing value. [c]Interval between diagnosis and treatment.*

IST alone, 4 of 79 (5%) in the group of transplants after failed IST, and 2 of 394 (0.5%) in the MFD HSCT group. In all patients who received a transplant, tumors occurred after rejection thus implying that malignancy did not originate from donor cells. The 7-year CI of malignancies in the whole cohort of patients was 4% (SE 1%) while it was 21% (SE 13%) in the IST alone group, 7% (SE 4%) in HSCT after failed IST, and 0.7% (SE 0.5%) in MDF HSCT group (P=0.0017) (Figure 3D).

Effect of center on outcome of HSCT

Since adolescence is a transitional age between childhood and adulthood, and since policies on age limits beyond which centers are entitled to transplant adolescent patients are not homogeneous across Europe, we assessed the effect of the center on transplant outcome. There was no significant difference in OS and EFS between 'Adult', 'Mixed' or 'Pediatric' centers defined according to JACIE-based criteria (*Online Supplementary Figure S1A and B*). It is noteworthy that when we applied an arbitrary criterion identifying as 'Adult' centers those performing less than 20% of transplants per year in patients under 18 years of age, as 'Pediatric' those performing more than 80% of grafts in patients under 18 years or age and 'Mixed' all the others, there was no still significant difference in OS and EFS between different centers (*data not shown*).

Prognostic factors

In the whole population of 537 adolescents, amongst the assessed variables, diagnosis to treatment interval of two months or under was associated to a better OS both in univariate and multivariate analysis. IST without subsequent HSCT was associated to worse EFS in both univariate and multivariate analysis (Table 2).

When considering the population of 473 patients who underwent a transplant (MFD HSCT plus HSCT post IST), diagnosis to treatment interval of two months or under and BM as source of cells were favorably associated to OS in univariate analysis. In multivariate analysis, an interval from diagnosis to treatment of two months or under, BM as source of cells and MFD HSCT were all associated to a better OS. The same variables were associated to better EFS in both univariate and multivariate analysis. Interestingly, family donor was not associated to better OS or to EFS under univariate analysis (Table 3).

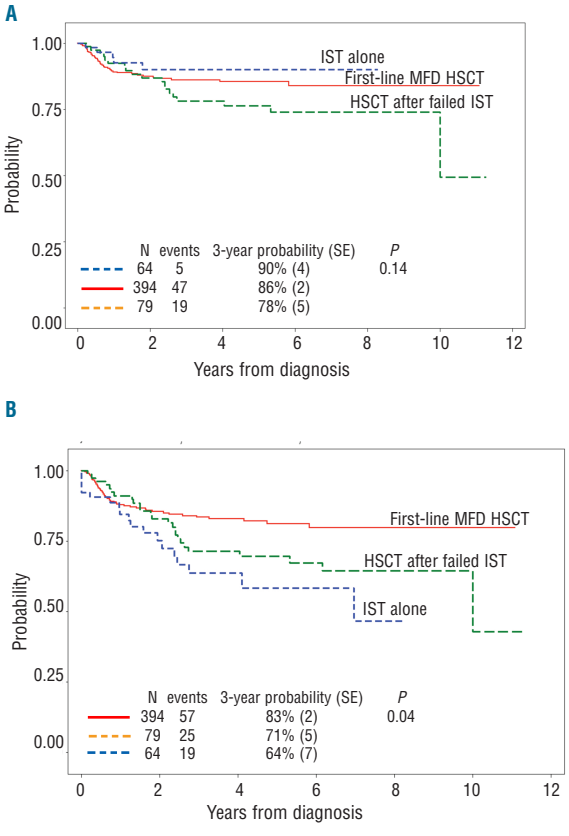


Figure 2. (A) **Probability of 3-years OS (Kaplan-Meier method) for the whole population of 537 patients stratified by treatment. IST alone means patients receiving IST upfront with the exclusion of those subsequently transplanted. (B) Probability of 3-years EFS (Kaplan-Meier method) for the whole population of 537 patients stratified according to treatment. IST alone means patients receiving IST up front with the exclusion of those subsequently transplanted. Subgroup analysis: First-line MFD HSCT *vs.* IST alone: P=0.003. First-line MFD HSCT *vs.* HSCT post-failed IST: P=0.039. IST alone *vs.* HSCT post-failed IST: P=0.17.**

Discussion

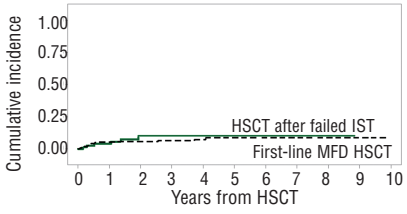
To the best of our knowledge, this is the first report investigating the outcome of AA in the specific age group of adolescence. Our survey indicates very satisfactory OS and EFS rates of 85% and 78%, respectively. Similar to other published data[4,7,24] in different age groups, a shorter time from diagnosis to treatment was significantly associated with improved OS and EFS in both univariate and multivariate analysis. This observation points to the need for an early referral of AA patients, irrespective of their age, to specialist centers for appropriate diagnosis and correct therapeutic decision-making.

There was no significant difference in OS between HSCT from MFD and IST, but EFS was far inferior in the latter group (Figure 1B) where more than half of patients (79 of 143) failed IST offered as front-line option and required a transplant. Of note, however, these patients could be effectively rescued by subsequent transplant as shown by their OS that was comparable to that of MFD HSCT and IST alone groups (Figure 2A). On the contrary, EFS, a qualitative indicator of survival, was significantly better with MFD HSCT *versus* HSCT post IST and *versus* IST alone (Figure 2B). In keeping with this, chronic GvHD, an event also affecting the quality of life of transplant survivors, was significantly lower in the MFD HSCT with respect to HSCT post IST.

Overall, based on the far superior EFS and the rather low chronic GvHD rate, the choice of MFD HSCT as a first choice option seems justified. In addition, MFD HSCT has two other advantages. The first is the protection against post-therapy cancers and the second is that it provides a more complete long-term reconstitution of hematopoiesis[2] which is very important in adolescents who may suffer from restrictions to their sporting and other activities due to subnormal platelet and/or Hb values or from higher risks for infection due to suboptimal neutrophil count and prolonged CsA treatment.

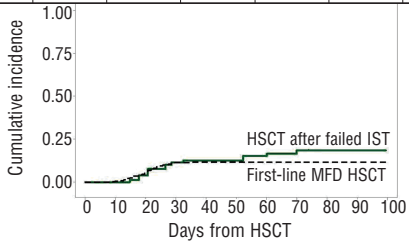
In cases in which an MFD is not available, our findings show that IST as front-line therapy provides an excellent

A



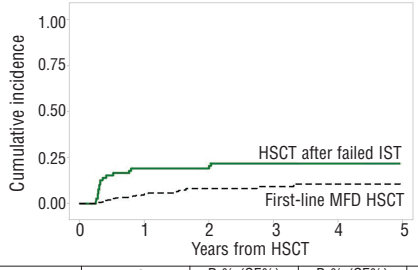
Rejection	Pr% (SE%) 3-mos	Pr% (SE%) 6-mos	Pr% (SE%) 1-yr	Pr% (SE%) 2-yr	Pr% (SE%) 3-yr	Pr% (SE%) 5-yr	Pr% (SE%) 10-yr
MFD HSCT	2 (0.7)	4 (1)	5 (1)	6 (1)	6 (1)	8 (2)	8 (2)
HSCT post failed IST	1 (1)	3 (2)	4 (2)	9 (4)	9 (4)	9 (4)	–
P 0.62							

B



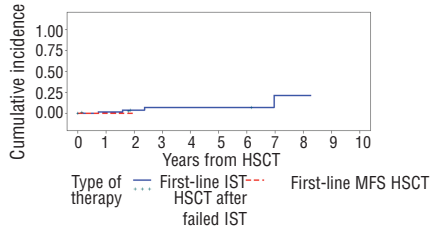
Acute GvHD	Pr% (SE%) 1 month	Pr% (SE%)	Pr% (SE%)	*P*-value (Gray's test)
MFD HSCT	11 (2)	11 (2)	12 (2)	0.18
HSCT after failed IST	11 (4)	15 (4)	18 (4)	

C



Chronic GvHD	Pr% (SE%) 6 months	Pr% (SE%) 1-year	Pr% (SE%) 2-year	*P* (Gray's test)
MFD HSCT	2 (1)	5 (1)	8 (2)	0.0009
HSCT post failed IST	15 (4)	19 (4)	20 (5)	

D



Second malignancy	Pr% (SE%) 1-year	Pr% (SE%) 2-year	Pr% (SE%) 5-year	Pr% (SE%) 7-year	*P*
IST alone	2 (2)	4 (3)	7 (4)	21 (3)	0.0017
HSCT post failed IST	1 (1)	3 (2)	4 (2)	7 (4)	
First line MFD HSCT	0.3 (0.3)	0.7 (0.5)	0.7 (0.5)	0.7 (0.5)	

Figure 3. (A) Cumulative Incidence of rejection in patients receiving MFD HSCT *versus* those receiving HSCT post failed IST. (B) Cumulative Incidence of acute GvHD grade II-III-IV in patients transplanted front line from MSD and after failed IST. Most of HSCT post failed IST were from MM (13%) and MUD (64%) donors. (C) Cumulative Incidence of chronic GvHD in patients transplanted front line from MSD and after failed IST. Most of HSCT post failed IST were from MM (13%) and MUD (64%) donors. (D) Cumulative Incidence of secondary malignancies in patients receiving IST alone front line, MDF HSCT front line and HSCT post failed IST. It is of note that secondary tumors only occurred in patients who had transplant rejection.

OS, comparable to that obtained with MFD HSCT, but a high rate of failure and a rather poor quality of survival, as expressed by the far lower EFS, that includes the risk of relapse and a weaker reconstitution of hematopoiesis (often CsA-dependent). However, in case of failure of IST upfront, HSCT offers a very good rescue option as shown by OS and EFS rates comparable to IST alone.

Overall, given the excellent OS and the chance of salvage by HSCT, for the moment, IST, although suboptimal, can be still considered an acceptable front-line option if no MSD donor is available.[25,26] In the group of transplant post failed IST, most transplants (64%) were from MUD. In keeping with this, in a recent study, MUD HSCT in children after failed IST showed a failure-free survival rate of up to 95%.[27] Our HSCT post failed IST group included a mixture of donors and so we can not draw definite occlusions on the position of MUD HSCT in the algorithm of treatment of AA. However, our finding supports the increasing consideration of the use of front-line MUD HSCT in children and young adults in cases in which an MFD is not available. This issue is under investigation in an analysis being carried out by the SAAWP of the EBMT. When compared with other ages, adolescents fared better than adults (>20 years) for MFD (OS 86% *vs.* 70%, respectively[8]) and had similar OS rates to younger patients for IST (82% *vs.* 87-92% of the Asian study of Jeong *et al.*[28]). Similar to other studies[8,9,29] also in adolescents, the use of BM as stem cell source resulted in significantly better OS and EFS compared to PB. Interestingly, both acute and chronic GvHD were more frequent in the PB group, thus reinforcing the need to use BM as the preferred stem cell source. Adolescence is by definition a transitional age between childhood and adulthood and there are no data on which centers are more suitable for transplanting adolescent patients in the literature. Our analysis indicates that, regardless of which criteria was adopted for identifying the most suitable centers, there are no substantial differences in outcome if adolescent patients are transplanted in 'Adult', 'Mixed' or 'Pediatric' centers.

The short follow up and group imbalance make data on post-therapy tumors difficult to interpret and preclude definitive conclusion. The significantly increased risk in IST-treated patients is expected on review of previous literature that showed a higher cumulative incidence of cancers after IST as compared to other therapies.[30] Moreover, the low occurrence of tumors after MFD transplant is in keeping with that seen in recent studies with a longer follow up.[31] However, given the young age of our patients, tumor occurrence needs to be carefully monitored during long-term follow up. Reports in literature indicate that a

second course of IST after previous failure provides an overall success rate of 30-70%[3,32-34] but a far weaker hematopoietic reconstitution than after HSCT.[2] A prospective Japanese study showed a far better outcome with MUD HSCT over second IST in children relapsing/non-responders after an initial course of IST.[35] Given the higher risk of secondary malignancies we found after IST, if the first course of IST fails, the option of MUD HSCT rather than a second immunosuppressive cycle seems justified, and so does the recommendation to start the donor search at diagnosis for those patients who may not benefit from an MFD transplant.

Our study has both limitations and strengths. The limitations are represented by its retrospective nature, by the incomplete data set regarding certain fields and by the numerical imbalance of different treatment subgroups. These limitations are to a certain extent inherent to large registry studies and reflect the bias of a transplant group registry. However, they do not affect the main end point: the outcome of AA in adolescence according to different types of treatments and not to the type of conditioning and GvHD prophylaxis. Study strengths are its design and the large number of patients, accounting for the largest adolescent cohort with AA ever studied. It should be noted that AA is a rare disease with an estimated incidence in Western countries of around 2 per million of the general population per year[3] and that its occurrence in different age subgroups is obviously far lower. Therefore, our study offers a unique source of information on a rare disease in a specific age group and provides robust evidence for treatment recommendations considering as end points not only survival in itself but also its qualitative aspects.

In summary, this study demonstrates that AA in adolescents has a very good outcome. If an MFD is available, HSCT performed either in an adult or in a pediatric center using BM cells within two months of diagnosis is the first treatment choice. If an MFD is not available, for the moment, IST using the combination of ATG and CSA is still an acceptable second therapeutic choice. This is largely because if IST fails, HSCT represents a very good rescue alternative both in terms of OS and EFS. Previous IST increases the risk of post-therapy tumors that must be monitored during long-term follow up.

Acknowledgments

ERG spa., Rimorchiatori Riuniti, Cambiaso & Risso, SAAR Depositi Oleari Portuali and UC Sampdoria are acknowledged for supporting the activity of the Clinical and Experimental Haematology Unit of G.Gaslini Institute. No specific funding was received for this study.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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