Arch. Immunol. Ther. Exp. (2012) 60:225–233
DOI 10.1007/s00005-012-0174-1

ORIGINAL ARTICLE

Matched Sibling Versus Matched Unrelated Allogeneic Hematopoietic Stem Cell Transplantation in Children with Severe Acquired Aplastic Anemia: Experience of the Polish Pediatric Group for Hematopoietic Stem Cell Transplantation

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Received: 1 April 2011 / Accepted: 10 February 2012 / Published online: 17 April 2012

Abstract In the study, 48 children with severe acquired aplastic anemia (SAA) transplanted from matched sibling donor (MSD) between 1991 and 2009, and 38 children with SAA transplanted from matched unrelated donor (MUD) between 2000 and 2009 were evaluated. Engraftment was achieved in 45 (93.75 %) patients after MSD-hematopoietic stem cell transplantation (HSCT) and in 33 (86.8 %) after MUD-HSCT. Transplant-related mortality rate after MSD-HSCT was 8 %, while 37 % after MUD-HSCT. After MSD-HSCT 44 (91.7 %) patients are alive for 1–216 months (median: 85 months), while after MUD-HSCT 24 (63.2 %) patients for 1–84 months (median: 16 months). The 5-year probability of event-free survival after MSD-HSCT and MUD-HSCT was 87 and 53 %, respectively, while 5 years of overall survival was 91 and 64 %, respectively. It was concluded that MSD-HSCT as the first line treatment for children with SAA is a safe therapeutic approach with a low rate of treatment failures and excellent outcome. Results of MUD-HSCT in pediatric patients with SAA who failed to respond to immunosuppressive therapy are still inferior than those of MSD-HSCT. Treatment failures of MUD-HSCT are mainly related to infectious complications and graft failure. It seems, however, that HLA-matching of unrelated donors at allelic level along with early MUD-HSCT after FCA (FLUDA, low-dose cyclophosphamide, and anti-thymocyte globulin) conditioning, perhaps using lower Thymoglobulin dose could enable further improvement of long-term results in children with SAA who lack MSD.

Keywords Severe acquired aplastic anemia · Children · Allogeneic hematopoietic stem cell transplantation · Matched sibling donor · Matched unrelated donor

Abbreviations

ALG	Anti-lymphocyte globulin
ANC	Absolute neutrophil counts
ATG	Anti-thymocyte globulin
BM	Bone marrow
CI	Cumulative incidence
CsA	Cyclosporine A
Cy	Cyclophosphamide
EBMT	European Group for Blood and Marrow Transplantation
EFS	Event-free survival
pEFS	Probability of event-free survival
FLUDA	Fludarabine

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FCA	Conditioning regimen consisting of FLUDA, low-dose cyclophosphamide, and anti-thymocyte globulin
GvHD	Graft versus host disease
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplantation
HLA	Human leukocyte antigen
IST	Immunosuppressive therapy
MSD	Matched sibling donor
MUD	Matched unrelated donor
MTX	Methotrexate
OS	Overall survival
pOS	Probability of overall survival
PB	Peripherial blood
PBSC	Peripherial blood stem cell
SAA	Severe acquired aplastic anemia
TBI	Total body irradiation
TREO	Treosulfan
TRM	Transplant-related mortality

Introduction

Severe acquired aplastic anemia (SAA) is a life-threatening disorder defined as marked (<25 %) hypocellularity, as estimated from bone marrow (BM) biopsy, and at least two of the following peripherial blood findings: neutrophil count $<0.5 \times 10^9$/l, platelet count $<20 \times 10^9$/l, or reticulocytes <1 % (International Agranulocytosis and Aplastic Anemia Study 1987).

Hematopoietic stem cell transplantation (HSCT) from a matched sibling donor (MSD) is the treatment of choice for children and young adults with SAA. The results of the Severe Aplastic Anemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT) indicate that 10-year survival in MSD transplants is 91 % for children (Locasciulli et al. 2007). Data from the International Bone Marrow Transplant Registry (http://www.cibmtr.org) showed a 3-year survival of 86 ± 2 % for 1,388 pediatric patients who underwent transplantation with MSD grafts between 1998 and 2008. However, the choice of this approach is limited by the availability of a MSD (only about 15–20 % of patients have a MSD; http://www.cibmtr.org).

The alternative treatment for patients without a MSD is immunosuppressive therapy (IST) with a combination of anti-thymocyte globulin and cyclosporine A (CsA) (Locasciulli et al. 2007). Survival rates after IST in children with SAA have been reported to be as high as 80 % (Locasciulli et al. 2007; http://www.cibmtr.org).

HSCT from matched unrelated donor (MUD) is indicated as salvage therapy for patients who fail to respond to one or more courses of IST and for patients who experienced recurrence of disease after IST (Locasciulli et al. 2007). Recent data from the International Bone Marrow Transplant Registry (http://www.cibmtr.org) for pediatric patients who received transplants from MUD showed a 3-year survival of 65 ± 2 %, thus significantly worse than after MSD-HSCT. Major causes of treatment failures after MUD-HSCT were: graft failure, graft versus host disease (GvHD), and infections. However, according to the Severe Aplastic Anemia Working Party of the EBMT, results of MUD-HSCT in children have recently significantly improved, i.e., 10-year overall survival (OS) was 75 % (Locasciulli et al. 2007).

This paper reports on the allogeneic HSCT procedure in 86 children with SAA, treated in Polish pediatric HSCT centers between 1991 and 2009, and compares treatment results and treatment failures in the MSD versus MUD group.

Materials and Methods

Patients

The results of allogeneic HSCT performed in Polish pediatric HSCT centers between January 1991 and December 2009 in 86 children with SAA (46 boys, 40 girls; median age: 11 years; range 2–23 years) were analyzed retrospectively. Patients' characteristics are listed in Table 1.

Donors' Characteristics, Stem Cell Source and Dose

In 48 (55.8 %) patients, HSCT was performed from MSD and in 38 (44.2 %) from MUD. Out of 48 patients transplanted from MSD, 46 (95.8 %) received BM and only 2 (4.2 %) hematopoietic stem cells (HSC) from peripherial blood (PBSC: peripherial blood stem cell). Among 38 children transplanted from MUD, 15 (39.5 %) received BM and 23 (60.5 %) PBSC. Recipients of HSC from MSD received 2.26×10^6 $CD34^+$ cells/kg (range 0.58–7.96) from BM, while those transplanted from MUD obtained 9.26×10^6 $CD34^+$ cells/kg (range 1.22–24.7) (including 8.1 (1.9–14.7) $\times 10^6$ $CD34^+$ cells/kg from BM and 10.7 (1.22–24.7) $\times 10^6$ $CD34^+$ cells/kg from peripherial blood). Data concerning the number of $CD34^+$ cells transplanted are available since 2001. Since 2001, 30 (34.9 %) MSD-HSCT and 37 (43 %) MUD-HSCT have been performed.

Preparative Regimens

For MSD-HSCT, 43 (89.6 %) recipients were conditioned with a preparative regimen consisting of cyclophosphamide (Cy) at a daily dose of 50 mg/kg for 4 days and anti-

Table 1 Patients characteristics

	MSD-HSCT *n* (%)	MUD-HSCT *n* (%)
Number	48 (55.8)	38 (44.2)
Age (years), range (median)	2–18 (12.1)	3–23 (10.5)
Recipient gender		
Female	21 (43.8)	19 (50)
Male	27 (56.2)	19 (50)
Stem cell source		
Bone marrow	46 (95.8)	15 (39.5)
Peripherial blood	2 (4.2)	23 (60.5)
Conditioning regimen		
CY + ATG	33 (68.8)	2 (5.2)
CY + ALG	10 (20.8)	0
FLUDA + ALG	2 (4.2)	0
TREO + CY + ATG	3 (6.3)	12 (31.6)
BU + FLUDA + ATG	1 (2.1)	0
FLUDA + CY + ATG	0	19 (50)
TBI + CY + ATG or Campath	0	2 (5.2)
TREO + FLUDA + ATG	0	1 (2.6)
FLUDA + CY + Campath	0	2 (5.2)
GvHD prophylaxis		
CsA + MTX	45 (93.7)	34 (89.5)
CsA + MTX + steroids	3 (6.3)	0
FK506 + steroids	0	2 (5.2)
MMF	0	1 (2.6)
MMF + steroids	0	1 (2.6)
$CD34^+$ cell dose ($\times 10^6$)/kg, median (range)	2.26 (0.58–7.96)	9.26 (1.22–24.7)

ALG anti-lymphocyte globulin, *ATG* anti-thymocyte globulin, *BU* busulphan, *CsA* cyclosporine A, *CY* cyclophosphamide, *FLUDA* fludarabine, *MMF* mycophenolan mofetil, *MSD-HSCT* matched sibling donor-hematopoietic stem cell transplantation, *MUD* matched unrelated donor hematopoietic stem cell transplantation, *MTX* methotrexate, *TBI* total body irradiation, *TREO* treosulfan

lymphocyte globulin (ALG; $n = 10$; before 1999) at a daily dose of 5 mg/kg intravenous (i.v.) for 4 days or thymoglobulin ($n = 33$; since 2000) at a daily dose of 2.5 mg/kg i.v. for 4 days (Table 1).

The majority of MUD recipients ($n = 31$; 81.6 %) were conditioned with thymoglobulin with Cy at a daily dose of 25 mg/kg i.v. for 4 days and fludarabine (FLUDA) at a daily dose of 30 mg/m^2 i.v. for 5 days or treosulfan (TREO) at a daily dose of 10 g/m^2 i.v. for 3 days (Table 1).

GvHD Prevention, Diagnosis and Grading

As GvHD prophylaxis, CsA plus "short" methotrexate (MTX) were given in about 90 % of recipients undergoing MSD-HSCT or MUD-HSCT. CsA was administered starting on day −1 of the transplant. Daily doses of CsA were adjusted to provide prophylactic blood levels (150–200 ng/ml) and therapeutic blood levels (200–250 ng/ml). MTX was given on days +1, 3, 6 and 11. Acute GvHD was diagnosed and graded according to clinical criteria established by Glucksberg et al. (1974) and modified by Deeg (1994). Chronic GvHD was diagnosed according to Schulman's grading (Shulman et al. 1980).

Minimal Requirements for Supportive Care

Central venous access was inserted in all patients. Patients were cared in a reverse isolation unit equipped with HEPA filters or laminar air-flow units. In addition, a low microbial diet, trimethoprim-sulfomethoxazol, and acyclovir were given. Children received i.v. substitution of 7-S immunoglobulins, and irradiated leukocyte-depleted blood products. Antiemetic prophylaxis, oral decontamination and mouth care, and monitoring for bacterial, fungal and viral infections were carried out according to local standards.

Outcome Measures

Outcome was measured as follows: (1) engraftment (absolute neutrophil counts (ANC) $\geq 0.5 \times 10^9$/l on 3 consecutive days and/or platelet count $\geq 20 \times 10^9$/l without transfusion on 7 consecutive days); (2) graft failure

Table 2 Occurrence of primary and secondary graft failure and characteristic of patients with graft failure

	MSD-HSCT ($n = 48$)	MUD-HSCT ($n = 38$)
Primary graft failure	$n = 1$ (2.1 %)	$n = 1$ (2.6 %)
Female/male	1/0	0/1
BM/PBSC	1/0	0/1
Cy + ATG	1	0
FLUDA + Cy + ATG	0	1
FLUDA + Cy + Campath	0	0
Secondary graft failure	$n = 2$ (4.2 %)	$n = 4$ (10.5 %)
Female/male	1/1	2/2
BM/PBSC	2/0	2/2
Cy + ATG	2	0
FLUDA + Cy + ATG	0	1
TREO + Cy + ATG	0	1
Other	0	2
Time after HSCT	30 and 145 months	2–4 months (median: 3)

ATG anti-thymocyte globulin, *BM* bone marrow, *CY* cyclophosphamide, *FLUDA* fludarabine, *MSD-HSCT* matched sibling donor hematopoietic stem cell transplantation, *MUD* matched unrelated donor hematopoietic stem cell transplantation, *PBSC* peripherial blood stem cell, *TREO* treosulfan

(primary failure: failure to reach ANC $\geq 0.5 \times 10^9$/l for at least 28 days after transplantation; secondary failure: decrease of ANC to below 0.2×10^9/l for at least 3 consecutive days after initial engraftment); (3) transplant-related mortality (TRM; the probability of dying without recurrence of SAA); (4) cumulative incidence (CI) of graft failure; (5) event-free survival (EFS; the time from HSCT to the event, i.e., death due to transplant-related complications, graft rejection, or graft failure); (6) OS (the time from HSCT to the last follow-up of surviving patients or death from any cause).

Statistical Analysis

Results of patients' demographic data and characteristics of the graft were given in the form of standard summary statistics. The probability of EFS and OS was estimated according to the Kaplan–Meier method. Univariate comparisons among various groups were made using the log-rank test. The following variables were evaluated: age of the patient, gender of patients, stem cell source, donor type, conditioning regimens and $CD34^+$ cell dose. Assessment of potential risk factors for outcomes of interest was performed in multivariate analyses using proportional hazard regression. Value of $p < 0.05$ indicates statistical significance. All data analyses were carried out using the Statistica 8.0 (StatSoft Inc.) and NCSS 2007 computer software packages.

Results

Engraftment and Graft Failure Incidence

Engraftment was achieved in 45 (93.8 %) recipients of MSD-HSCT and in 33 (86.8 %) of those after MUD-HSCT. The median duration of time to neutrophil ($>0.5 \times 10^9$/l) and platelet ($>20 \times 10^9$/l) engraftment after MSD-HSCT was 16 (range 11–27) and 34 (range 14–342) days, while for MUD-HSCT it was 16 (range 11–21) and 20 (range 12–216) days, respectively.

Primary graft failure occurred in one (2.1 %) patient after MSD-HSCT, and in one (2.6 %) after MUD-HSCT, while secondary graft failure in two (4.2 %) recipients of MSD-HSCT, and in four (10.5 %) after MUD-HSCT ($p = 0.6429$). Secondary graft failure was observed at 30 and 145 months after MSD-HSCT, and between 2 and 4 months (median: 3 months) after MUD-HSCT. Detailed characteristics of patients demonstrating graft failure are shown in Table 2. Patients with graft failure received 2×10^6 $CD34^+$ cells/kg (range 1.2–8.6; in one patient no data available).

The 2-, 5- and 7-year CI of graft failure in children after MSD-HSCT was 2.1 % (0.3–14.5 %), 4.9 % (1.2–19.2 %), and 4.9 % (1.2–19.2 %); after MUD-HSCT, the corresponding figures were: 19.5 % (9.5–40.2 %), 19.5 % (9.5–40.2 %) and 19.5 % (9.5–40.2 %).

Out of eight patients with graft failure, seven patients underwent a second HSCT, including three from MSD and four from MUD. Engraftment was achieved in four out of seven (57.1 %) second-HSCT patients, including two after MSD-HSCT, and two after MUD-HSCT. The remaining three patients died 18 days, 3 months and 3.5 months after second HSCT. Patient deaths after the second HSCT were all due to infectious complications (central nervous system (CNS) infection, $n = 2$; lymphoproliferative disease, $n = 1$).

Transplant-related Mortality

There were 4 (8 %) deaths after MSD-HSCT conditioned for transplantation with Cy + thymoglobulin (4/33, 12 %).

All four patients died due to infection (sepsis: $n = 3$, 75 % and pneumonia: $n = 1$, 25 %). The median duration of time to death was 69 days after HSCT (range 18–106 days).

After MUD-HSCT 14 (37 %) children died, including 6/19 (31.5 %) patients conditioned with FLUDA + Cy + thymoglobulin, 4/12 (33.3 %) with TREO + Cy + thymoglobulin, and 4/7 (57.1 %) who received other preparative regimens. Infections were the cause of death in 10 (71.4 %) patients (sepsis: $n = 6$, CNS infection: $n = 3$, pneumonia: $n = 1$), GvHD in 2 (14.2 %) (acute GvHD: $n = 1$, 7.1 %; chronic GvHD: $n = 1$, 7.1 %), and endothelial complications in 2 (14.2 %) (thrombotic microangiopathy: $n = 1$, 7.1 %; brain oedema in the course of a capillary leak syndrome: $n = 1$, 7.1 %). The median duration of time to death was 73 days after HSCT (range 9–198 days).

Event-Free Survival

The 5-year probability of EFS (pEFS) in children transplanted from MSD was 87 %, compared to 53 % in those after MUD-HSCT ($p = 0.001$; Fig. 1).

OS and Factors Influencing Survival

After MSD-HSCT 44 (91.7 %) patients are alive for 1–216 months (median: 85 months), while the corresponding data after MUD-HSCT are 24 (63.2 %) patients for 1–84 months (median: 16 months). The 5-year OS in children transplanted from MSD was 92 versus 65 % for children after MUD-HSCT ($p = 0.002$; Fig. 2).

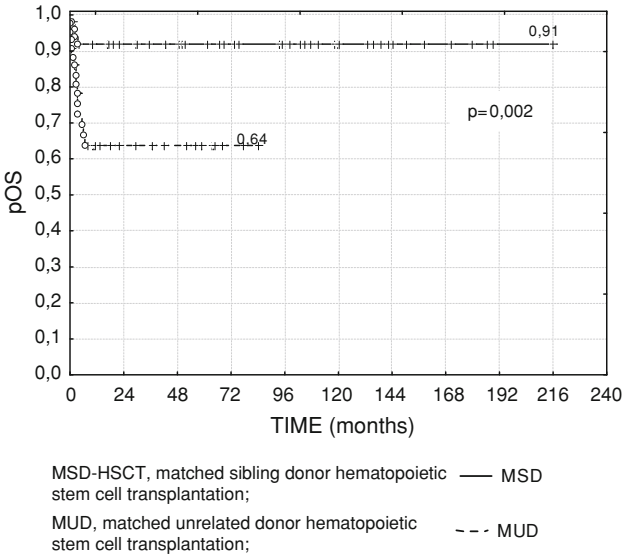

Fig. 2 pOS in children with SAA after MSD-HSCT and MUD-HSCT

There were no statistically significant differences between the probability of 5-year event-free and OS in patients transplanted from MSD and conditioned with Cy/ALG (pEFS 100 %, and probability of OS (pOS) 100 %) or Cy/ATG (pEFS 81 % and pOS 89 %), and similarly between children transplanted from MUD and conditioned with FLUDA + Cy + thymoglobulin (pEFS 58 % and pOS 68 %) or TREO + Cy + thymoglobulin (pEFS 56 % and pOS 65 %; Figs. 3, 4).

After second HSCT, four patients are alive for 22.3–102.7 months (median: 70.7 months).

The results of univariate analysis of the factors influencing survival are shown in Table 3. The donor type

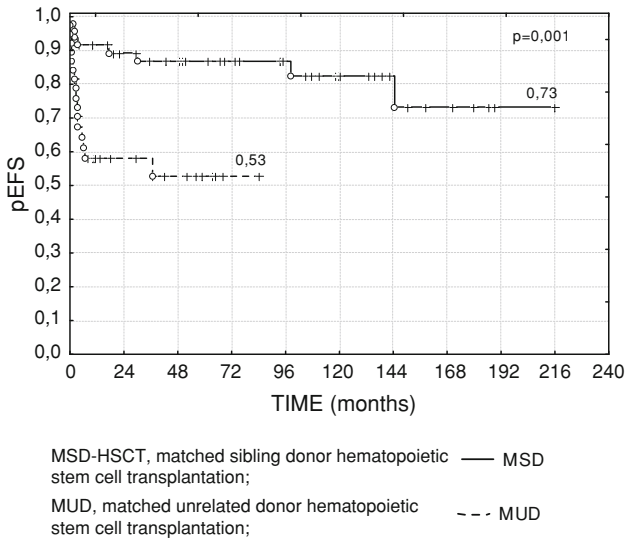

Fig. 1 pEFS in children with SAA after MSD-HSCT and MUD-HSCT

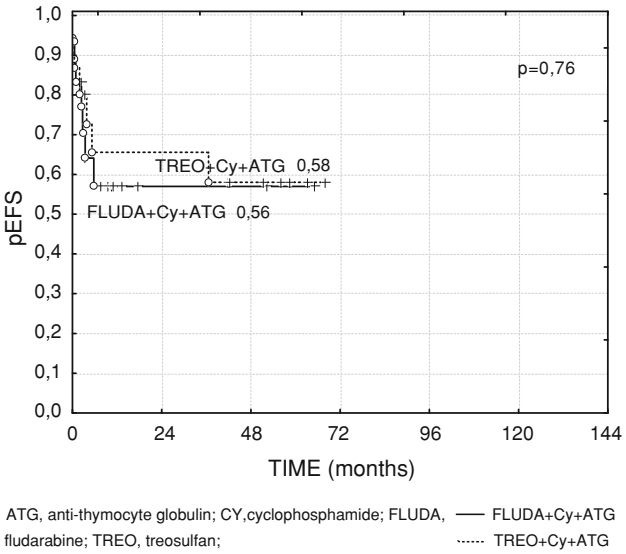

Fig. 3 pEFS according to preparative conditioning regimen in children with SAA after MUD-HSCT

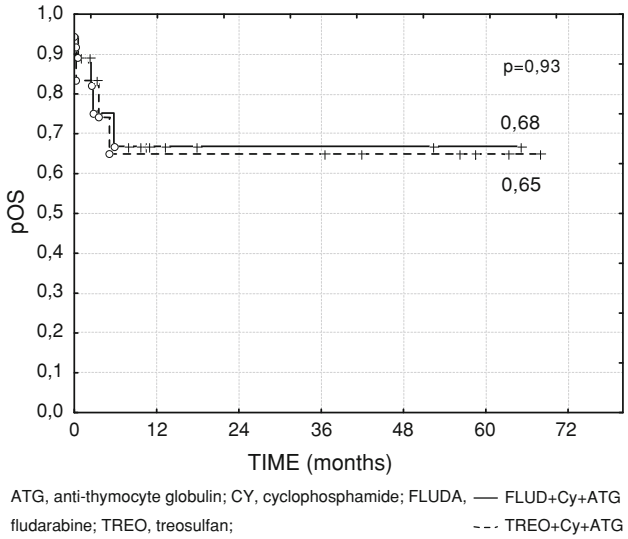

Fig. 4 pOS according to preparative conditioning regimen in children with SAA after MUD-HSCT

Table 3 Outcome following HSCT for severe aplastic anemia: univariate analysis

Covariates	5 years pOS (%)	*p* value
Recipient age		
<11 years $n = 34$ (39.5 %)	82	ns
≥11 years $n = 52$ (60.5 %)	74	
Recipient gender		
Female $n = 40$ (46.5 %)	81	ns
Male $n = 46$ (53.5 %)	74	
Stem cell source		
Bone marrow $n = 61$ (70.9 %)	77	ns
Peripherial blood $n = 25$ (29.1 %)	64	
CD 34^+ cell dose ($\times 10^6$)/kg		
<3 $n = 24$ (33.3 %)	61	ns
3–5 $n = 19$ (26.4 %)	75	
≥6 $n = 29$ (40.3 %)	85	
Donor type		
Matched sibling $n = 48$ (55.8 %)	92	0.003
Matched unrelated $n = 38$ (44.2 %)	65	
Preparative regimen for MUD-HSCT		
FLUDA + Cy + ATG $n = 19$ (50 %)	68	ns
TREO + Cy + ATG $n = 12$ (31.6 %)	65	
Time of MSD-HSCT		
1991–1999 $n = 14$ (29.2 %)	100	ns
2000–2009 $n = 34$ (70.8 %)	88	

ALG anti-lymphocyte globulin, *ATG* anti-thymocyte globulin, *CY* cyclophosphamide, *FLUDA* fludarabine, *MSD-HSCT* matched sibling donor hematopoietic stem cell transplantation, *TREO* treosulfan, *ns* not significant

($p = 0.003$) was the only one factor that was significantly related to overall survival, i.e., patients transplanted from MSD had higher pOS than children transplanted from MUD. Univariate analysis revealed no statistical differences in survival in relation to age, year of MSD-HSCT (≤1999 and ≥2000), gender of recipient, stem cell source, and $CD34^+$ cell dose within range used.

In multivariate analysis, donor type was an independent predictor of survival. The favorable donor type was MSD ($p = 0.021$).

Discussion

In children and young adults with SAA, MSD-HSCT is the first-line treatment (Locasciulli et al. 2007), while MUD-HSCT as well as MMD-HSCT are still considered as investigational treatment modalities, which are recommended only in those patients who demonstrate no response to IST (Führer 2008). Indeed, it was demonstrated that in children with SAA non-responding to the first course of IST, the MUD-HSCT demonstrates significantly better results than second course of IST (Kosaka et al. 2008), but there are still only several reports on MUD-HSCT results in children with SAA (Bunin et al. 2005; Kosaka et al. 2008; Perez-Albuerne et al. 2008). In addition, the reported groups of children were usually small with unrelated donors typed at the various resolution levels and conditioned with different preparative regimens, mostly containing low-dose total body irradiation (LD-TBI), which is not advocated in children (Führer 2008). Only two reports compared results of MSD-HSCT and MUD-HSCT in children with SAA, and in both studies children transplanted from MUD received TBI (Hsieh et al. 2010; Yagasaki et al. 2010). There is another comparative report (Kennedy-Nasser et al. 2006), but the cohort of 23 alternative donors was very heterogenous, and again LD-TBI was given within preparative regimen. In addition, only 15 children after MSD-HSCT were analyzed.

In our study, 48 children with SAA transplanted from MSD and 38 children with SAA transplanted from MUD were evaluated. The compared groups of children were not only larger than described in cited studies but also more homogenous in the context of many important aspects of HSCT for SAA. Namely, MSD-HSCT was exclusively used as the first-line treatment, while MUD-HSCT was used solely in children with SAA refractory to IST. All children in our study were conditioned for HSCT without LD-TBI. In addition, all patients were transplanted from fully matched sibling or fully MUDs, and the typing of all unrelated donors was performed at the allelic level. The same GvHD prophylaxis was given to the majority of children.

In contrast to the other reports (Hsieh et al. 2010; Yagasaki et al. 2010), in our study children transplanted from MUD demonstrated, when compared to those transplanted from MSD, the same time of neutrophil engraftment and faster platelet engraftment. This may be related to CD34 cells source, i.e., two-thirds of children undergoing MUD-HSCT received stem cells from peripheral blood, while those transplanted from MSD almost exclusively from BM and to a higher CD34 cells dose used in the case of MUD-HSCT.

Graft failure represents one of the major complications of allogeneic HSCT in patients with SAA. In the cohorts studied, the risk of graft failure after MUD-HSCT was higher than after MSD-HSCT, but it was comparable to the results reported by the majority of other authors (Bacigalupo et al. 2000, 2005, 2010; Kosaka et al. 2008; Passweg et al. 2006; Storb et al. 2001; Viollier et al. 2008) despite giving lower total dose of thymoglobulin (10 vs. 15 mg/kg). The higher risk of non-engraftment in children transplanted from MUD could be related to a higher number of unavoidable pre-transplant transfusions in children who had been first treated ineffectively with IST and then, after 6–12 months, qualified for MUD-HSCT (Kumar et al. 2006; Srinivasan et al. 2006; Young et al. 2006). In the context of graft failure, it is worth mentioning that the second transplantation is feasible and may offer rescue to these patients. In this study, four of seven patients with graft failure subjected to second HSCT achieved stable engraftment and are alive. Similar results of re-transplantation from the same unrelated donor have been reported in three adolescents by Führer et al. (2009).

Infectious complications were the sole cause of TRM in children transplanted from MSD and the major one in those who received MUD-HSCT. However, the TRM rate after MSD-HSCT was low (8 %), while it was significantly higher (37 %) after MUD-HSCT. Only a few children transplanted from MUD died due to acute or chronic GvHD, probably thanks to high-resolution human leukocyte antigen (HLA)-matching. It seems that high rate of lethal infections after MUD-HSCT could be related to extreme and long-lasting immunosuppression resulting from intensive IST given as first-line treatment, which is often complicated by severe infections, and then followed by an intense immunosuppressive conditioning regimen for transplantation. Thus, as postulated by Führer (2008) and Bacigalupo et al. (2010), to reduce the risk of life-threatening infectious complications and to improve results of MUD-HSCT as second-line treatment, the decision concerning the transplantation should be made earlier than now. In addition, earlier MUD-HSCT could also reduce the risk of non-engraftment related to alloimmunization resulting from pre-transplant transfusions. In order to decrease the risk of infectious complications, the use of thymoglobulin at total dose lower than 15 mg/kg could be also considered to facilitate post-transplant immunoreconstitution.

The probability of 5-year OS in children transplanted from MSD and from MUD was 91.7 and 63.2 %, respectively. Thus, it is comparable to OS rates in children transplanted during the last decade, as reported by the majority of authors (Locasciulli et al. 2007; http://www.cibmtr.org), and is still significantly less favorable in children who underwent MUD-HSCT (Kojima et al. 2002; Young et al. 2006). Nowadays, however, the probability of OS in the patients undergoing MUD-HSCT is significantly better than it was in the 1990s, i.e., before the implementation of high-resolution HLA typing (Deeg et al. 1999; Maury et al. 2009; Young et al. 2006). Recently, Bacigalupo et al. (2005, 2010) demonstrated that in SAA patients undergoing HSCT from alternative donors further improvement of 2-year OS is possible up to 73 %, with a trend for even better outcome in patients aged $\leq$14 years (84 %), when using conditioning regimen consisting of FLUDA, low-dose cyclophosphamide, and anti-thymocyte globulin (the FCA regimen). In our study, half of patients transplanted from MUD was conditioned according to this regimen, while about one-third received a regimen reported as safe and effective in adults with SAA (Giebel et al. 2006), consisting of TREO, cyclophosphamide and anti-thymocyte globulin, with comparable OS of 68 and 65 %, respectively.

So far the following factors have been recognized as predictors of better survival rates after allogeneic HSCT in patients with SAA: younger age (<16 years) (Locasciulli et al. 2007), short interval between diagnosis and HSCT (Locasciulli et al. 2007), minimum of blood products transfusion (Srinivasan et al. 2006; Young et al. 2006), no radiation in the conditioning regimen (Locasciulli et al. 2007), HLA-matching at the allelic level (Deeg et al. 2006; Maury et al. 2007), transplantation after 1996 (Locasciulli et al. 2007), MSD-HSCT (Locasciulli et al. 2007), and BM from MSD as stem cells source (Schrezenmeier et al. 2007). In our study, both in univariate and in multivariate analysis, the type of matched donor was identified as the only one independent factor significantly related to the probability of survival, with better prognosis in patients transplanted from MSD, but we had no appropriate data to evaluate the impact of time from diagnosis to transplantation.

Despite long-term observation, none of the children in the studied cohort developed so far a secondary malignancy, which may testify in favor of preparative regimens without TBI in patients with SAA undergoing allogeneic HSCT.

In conclusion, MSD-HSCT as the first-line treatment for children and adolescents with SAA is a safe therapeutic

approach with a low rate of treatment failures and excellent long-term outcome. Results of MUD-HSCT in pediatric patients with SAA who failed to respond to IST are still inferior than those of MSD-HSCT. Treatment failures of MUD-HSCT are mainly related to infectious complications and graft failure. It seems, however, that HLA matching of unrelated donors at the allelic level along with early MUD-HSCT after the FCA conditioning, perhaps using lower thymoglobulin dose could enable further improvement of long-term results in children with SAA who lack MSD.

Acknowledgments This work was supported by the University of Medical Sciences in Poznan (Poland) project no. 502-01-01104119-04448.

Conflict of interest The authors declare no potential conflict of interest.

References

Bacigalupo A, Oneto R, Bruno B et al (2000) Current results of bone marrow transplantation in patients with acquired severe aplastic anemia. Report of the European Group for Blood and Marrow transplantation. On behalf of the Working Party on Severe Aplastic Anemia of the European Group for Blood and Marrow Transplantation. Acta Haematol 103:19–25

Bacigalupo A, Locatelli F, Lanino E et al (2005) Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party. Bone Marrow Transplant 36:947–950

Bacigalupo A, Socie' G, Lanino E et al (2010) Fludarabine, cyclophosphamide, antithymocyte globulin, with or without low dose total body irradiation, for alternative donor transplants, in acquired severe aplastic anemia: a retrospective study from the EBMT-SAA working party. Haematologica 95:976–982

Bunin N, Aplenc R, Iannone R et al (2005) Unrelated donor bone marrow transplantation for children with severe aplastic anemia: minimal GVHD and durable engraftment with partial T cell depletion. Bone Marrow Transplant 35:369–373

Deeg HJ (1994) Prophylaxis and treatment of acute graft-versus-host disease: current state, implications of new immunopharmacologic compounds, and future strategies to prevent and treat acute GvHD in hight-risk patients. Bone Marrow Transplant 14(suppl 4):S56–S60

Deeg HJ, Seidel K, Casper J et al (1999) Marrow transplantation from unrelated donors for patients with severe aplastic anemia who have failed immunosuppressive therapy. Biol Blood Marrow Transplant 5:243–252

Deeg HJ, O'Donnell M, Tolar J et al (2006) Optimization of conditioning for marrow transplantation from unrelated donors for patients with aplastic anemia after failure of immunosuppressive therapy. Blood 108:1485–1491

Führer M (2008) Risk-adapted procedures for HSCT from alternative donor in children with severe aplastic anaemia. Bone Marrow Transplant 42(Suppl 2):S97–S100

Führer M, Claviez A, Klein B et al (2009) Re-transplantation from the same unrelated donor in three adolescents with severe aplastic anemia after graft rejection. Klin Padiatr 221:358–361

Giebel S, Wojnar J, Krawczyk-Kulis M et al (2006) Treosulfan, cyclophosphamide and antithymocyte globulin for allogeneic hematopoietic cell transplantation in acquired severe plastic anemia. Ann Tranplant 11:23–27

Glucksberg H, Storb R, Fefer A et al (1974) Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. Transplantation 18:295–303

Hsieh MY, Chiou TJ, Hung GY et al (2010) Outcomes of matched sibling and alternative donor stem cell transplantation for 26 children with severe aplastic anemia. Int J Hematol 91:54–60

International Agranulocytosis and Aplastic Anemia Study (1987) Incidence of aplastic anemia: the relevance of diagnostic criteria. Blood 70:1718–1721

Kennedy-Nasser AA, Leung KS, Mahajan A et al (2006) Comparable outcomes of matched-related and alternative donor stem cell transplantation for pediatric severe aplastic anemia. Biol Blood Marrow Transplant 12:1277–1284

Kojima S, Matsuyama T, Kato S et al (2002) Outcome of 154 patients with severe aplastic anemia who received transplants from unrelated donors: the Japan Marrow Donor Program. Blood 100:799–803

Kosaka Y, Yagasaki H, Sano K et al (2008) Prospective multicenter trial comparing repeated immunosuppressive therapy with stem-cell transplantation from an alternative donor as second-line treatment for children with severe and very severe aplastic anemia. Blood 111:1054–1059

Kumar R, Prem S, Mahapatra M et al (2006) Fludarabine, cyclophosphamide and horse antithymocyte globulin conditioning regimen for allogeneic peripherial blood stem cell transplantation performed in non-HEPA filter rooms for multiply transfused patients with severe aplastic anemia. Bone Marrow Transplant 37:745–749

Locasciulli A, Oneto R, Bacigalupo A et al (2007) Outcome of patients with acquired aplastic anemia given first line bone marrow transplantation or immunosuppressive treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation. Hematologica 92:e122

Maury S, Balère-Appert ML, Chir Z et al (2007) Unrelated stem cell transplantation for severe acquired aplastic anemia: improved outcome in the era of high-resolution HLA matching between donor and recipient. Haematologica 92:589–596

Maury S, Bacigalupo A, Anderlini P et al (2009) Improved outcome of patients older than 30 years receiving HLA-identical sibling hematopoietic stem cell transplantation for severe acquired aplastic anemia using fludarabine-based conditioning: a comparison with conventional conditioning regimen. Haematologica 94:1312–1315

Passweg JR, Pérez WS, Eapen M et al (2006) Bone marrow transplants from mismatched related and unrelated donors for severe aplastic anemia. Bone Marrow Transplant 37:641–649

Perez-Albuerne ED, Eapen M, Klein J et al (2008) Outcome of unrelated donor stem cell transplantation for children with severe aplastic anemia. Br J Haematol 141:216–223

Schrezenmeier H, Passweg JR, Marsh JC et al (2007) Worse outcome and more chronic GVHD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplants for young patients with severe acquired aplastic anemia. Blood 110:1397–1400

Shulman HM, Sullivan KM, Weiden PL et al (1980) Chronic graft-versus-host syndrome in man. A long term clinicopathologic study of 20 Seatle patients. Am J Med 69:204–217

Srinivasan R, Takahashi Y, McCoy JP et al (2006) Overcoming graft rejection in heavily transfused and allo-immunised patients with bone marrow failure syndromes using fludarabine-based haematopoietic cell transplantation. Br J Haematol 133:305–314

Storb R, Blume KG, O'Donnell MR et al (2001) Cyclophosphamide and antithymocyte globulin to condition patients with aplastic

anemia for allogeneic marrow transplantations: the experience in four centers. Biol Blood Marrow Transplant 7:39–44

Viollier R, Socié G, Tichelli A et al (2008) Recent improvement in outcome of unrelated donor transplantation for aplastic anemia. Bone Marrow Transplant 41:45–50

Yagasaki H, Takahashi Y, Hama A et al (2010) Comparison of matched-sibling donor BMT and unrelated donor BMT in children and adolescent with acquired severe aplastic anemia. Bone Marrow Transplant 45:1508–1513

Young NS, Calado RT, Scheinberg P (2006) Current concepts in the pathophysiology and treatment of aplastic anemia. Blood 108:2509–2519