Unrelated donor stem cell transplantation in acquired severe aplastic anemia: a systematic review

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ABSTRACT

Acquired severe aplastic anemia is a rare disease characterized by an immune-mediated functional impairment of hematopoietic stem cells. Transplantation of these cells from unrelated donors is a treatment option frequently offered to patients after failed immunosuppressive therapy. The aim was to investigate the outcome of these patients treated with unrelated donor transplants. Systematic literature searches were performed in *MEDLINE*, *EMBASE*, and *The Cochrane Library*. All databases were searched from inception to June 2009. Only full-text publications and studies including at least 10 patients were considered. The primary outcome was 5-year overall survival from the day of transplantation and the secondary outcomes were graft failure and graft-versus-host disease. A meta-analysis of survival estimates was conducted and heterogeneity was investigated. A total of 18 studies, one controlled trial and 17 case series were identified. The overall survival at five years and the corresponding confidence interval was stated in 8 studies and ranged from 28% to 94%. A meta-analysis revealed considerable heterogeneity between the studies that could not be explained and was also present in subgroups of the studies. The proportion of acute graft failure was 45% in one study using only umbilical cord blood, and it was reported to be 0-26% in 15 studies using mainly bone marrow as stem cell source after different follow-up periods. Acute GVHD grade II-IV was reported for 8-86% and extensive chronic GVHD for 0-38% of the evaluated patients in 16 studies. Recipient age, human leukocyte antigen match, performance status, year of transplantation, and conditioning with serotherapy were identified as significant factors for improved survival. Unrelated donor hematopoietic stem cell transplantation in patients with acquired severe aplastic anemia after failure to immunosuppressive therapy is a treatment option. A stable physical condition of the patients before receiving the transplant (for example, performance and age) may be associated with a better survival. Detailed HLA-matching facilitated by DNA-based typing, among other factors, may have contributed to recent improvements on survival after unrelated donor HSCT as a second-line treatment.

Key words: donor stem cell transplantation, aplastic anemia, systematic review.

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Introduction

Acquired severe aplastic anemia (SAA) is a rare[1] and potentially fatal disease which is characterized by hypocellular bone marrow and pancytopenia, and mainly affects young adults. The incidence rate was estimated at less than 4 per million per year in the general population.[2] The major signs and symptoms are severe infections, bleeding and exhaustion. The underlying pathophysiology is thought to be an aberrant immune response involving the T-cell mediated destruction of hematopoietic stem cells. In most cases, the cause is unknown, although various triggers such as drugs, toxins, and viruses have been reported.[3,4]

The treatment of SAA mainly includes immunosuppressive therapy (IST) with antithymocyte globulin and cyclosporine A, and allogeneic hematopoietic stem cell transplantation (HSCT).[3-5] Allogeneic HSCT is seen as the treatment of choice for selected patients with an HLA-matched related donor.[6,7] This donor type was documented for 66% (247 of 373) of the patients with an allogeneic HSCT who were registered in 2005 by the European Group for Blood and Marrow Transplantation (EBMT).[8] More than 70% of patients with SAA are not expected to have a matched related donor[3,9] and the question is whether or when to recommend allogeneic HSCT from an unrelated donor.

Clinical treatment algorithms have been suggested to find a

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decision that meets individual conditions, personal preferences and prognostic risk factors.[7] Allogeneic HSCT is associated with a high treatment-related morbidity and mortality. Potentially life-threatening adverse events are sepsis, acute and chronic graft-versus-host disease, bleeding and organ toxicities. During the 1980s and 1990s, survival rates of patients with unrelated donor HSCT were about half of those seen in related donor HSCT, with age, time from diagnosis and HLA-matching as strong predictors of survival.[3,4,10] Present data show that high-resolution molecular genetic HLA-matching may be an important factor for better survival in these patients.[11] Unrelated donor HSCT has been applied conservatively and usually offered as a second-line treatment after one or more failed IST. The search for an unrelated donor seems advisable if patients with SAA have failed one course of IST.[12,13]

This review summarizes the evidence available on unrelated donor HSCT after failed IST in SAA patients to answer the clinical question of whether the outcome has improved sufficiently for it to be acknowledged as an alternative for IST and used in future randomized trials.

The primary objective was to investigate the overall survival of patients treated with unrelated donor HSCT in SAA patients. The secondary objective was to consider adverse events.

Design and Methods

Study inclusion criteria

The study inclusion criteria are listed in Table 1. We included clinical controlled trials and case series that investigated patients with acquired severe aplastic anemia, including very severe forms, who received allogeneic HSCT from unrelated donors after failed IST. We arbitrarily set a minimum number of 10 transplanted patients per study to be considered, and required the proportion of relevant patients to be at least 80% per study if no stratification had been performed. Studies which did not present results separately from probands with other diseases and interventions were excluded from the studies. Only full-text publications were considered. All languages were included, as long as a title was available in English.

Search strategy

The sources used to search the studies are listed in *Online Supplementary Table S1*. MEDLINE (1950-2009), EMBASE (1980-2009) and The Cochrane Library (up to 2009) were searched without restrictions on study design, publication year, and language (final search 2nd June 2009). The terms and the syntax used for the search in MEDLINE/Ovid as shown in *Online Supplementary Table S2* were tailored to the requirements of the other 2 databases. Additional steps were taken to complement electronic database searches: online trial registers and citations from experts submitted to the German Federal Joint Committee and to the IQWiG were screened. Reference lists of retrieved original articles and systematic reviews, as well as conference proceedings were handsearched. Moreover, in cases of assumed unpublished trials, we contacted the authors by e-mail and by post using the published contact details of the relevant institutions and asked for further information.

Table 1. Inclusion criteria.

Inclusion criteria
• Participants: patients with acquired severe aplastic anemia including very severe forms
• Intervention: allogeneic hematopoietic stem cell transplantation with unrelated donors after failed IST
• Study types: clinical controlled trials and case series
• Other conditions: at least 10 transplanted patients; proportion of at least 80% relevant patients per study if no stratification had been performed; results had to be reported separately from other probands and other interventions; full-text publication available

Study quality

Study quality was evaluated by description of the study characteristics (design, inclusion criteria, location, observation period), the patients' characteristics (age, gender, number of failed IST, number of pre-transplantation IST courses, interval from diagnosis to transplant, cytomegalovirus and Epstein-Barr virus disease reactivation), the intervention (stem cell source, total body irradiation), the donor-recipient interaction (donor type, HLA-matching, HLA-typing, donor gender), and the outcome (method of survival analysis, follow-up, subgroup analysis, graft failure, acute and chronic GVHD, methods to investigate factors for improved survival).

Data extraction

Potentially relevant publications not in English were translated by medically trained native speakers. All steps of the literature screening and data extraction process were performed by two independent reviewers (*FP, SL*). Any disagreements were resolved by discussion.

Failed IST is a term used in this paper to describe patients who had failed pre-transplantation IST either as no response to IST (refractory patients), or as relapse after initial response

Data analysis

The primary outcome was survival from the day of transplantation based on Kaplan-Meier estimates as extracted directly from the text or deduced from the survival curve of the publication. The proportion of the number of survived patients at the end of observation was used to describe the outcome if Kaplan-Meier analyses were not reported.

The secondary outcomes were graft failure and graft-versus-host disease (GVHD), and they were documented according to the definition used in the individual publications. Graft failure included both primary and secondary types. Acute GVHD was considered if grade II-IV was stated and chronic GVHD was described in the present review if an extensive course was stated. Significant as well as non-significant factors for improved survival were searched in the identified studies provided that the statistical analysis method and the results of significance testing were reported.

Meta analysis

Survival estimates at five years and the corresponding 95% confidence intervals were extracted from the papers if present. If not explicitly stated, the survival estimates at five years were deduced from the survival curves if possible and the corresponding standard errors were estimated by using an approximate formula based on the survival probability and the number of patients at risk.[14] In case of insufficient information about the number of patients at risk, this was estimated assuming uniformly distributed censoring times in the time period from beginning to the end of follow-up. Meta analyses based on the 5-year survival estimates and the corresponding standard errors were conducted using the generic variance approach[15,16] and the random effects model.[17] Calculations were conducted using SAS version 9.1.3 (SAS Institute Inc., Cary, North Carolina, United States).

The results of the meta-analyses were graphically displayed by means of a forest plot. Heterogeneity of the results was visually assessed and quantified using the I^2 value.[18] In case of very large heterogeneity a pooled estimate is not sensible and was not calculated.[19] Heterogeneity was further explored according to the Cochrane Handbook guidelines by conducting several subgroup analyses.[18]

Results

Search results

Of 2,173 retrieved publications, 396 full-text papers were obtained for further assessment (Figure 1). Eighteen studies were identified that met the inclusion criteria.[10,11,15-17,20-32] Seventeen case series which investigated at least 10 patients treated with unrelated donor HSCT after failed IS could be included.[10,11,15-17,20-25,27-32] The transplantation arm of one controlled trial of unrelated donor HSCT versus IST in patients who had failed IST could also be included.[26]

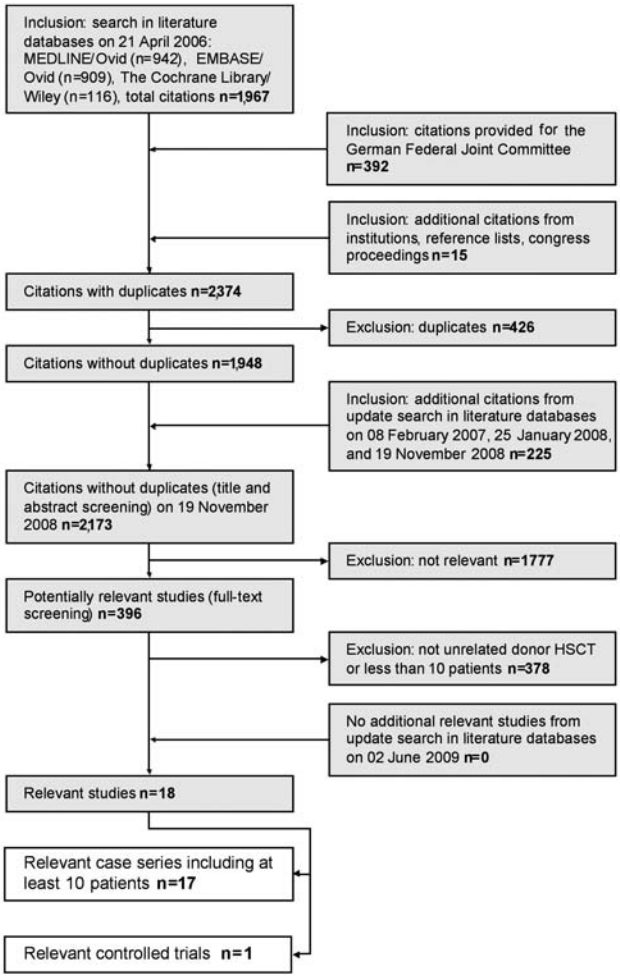


Figure 1. Results of the literature search.

Patients' and design characteristics

An overview of the study characteristics is presented in *Online Supplementary Table S3*. The participating centers are located in the United States, Western Europe, Japan, and Korea. Five to 142 centers collaborated in 9 multicenter studies, the number of centers was not stated in 2 other multicenter studies, and 7 studies were conducted in single centers. The study design was reported as prospective in one controlled trial[26] and in 4 case series.[15,16,20,24] A consecutive enrolment was stated in 3[23,25,27] of 13 retrospective case series.[10,11,17,21-23,25,27-32] Transplantations were performed between 1981 and 2006.

In 4 studies[10,20,24,30] survival estimates were stated for subgroups only. In one study,[20] patients younger than *versus* older than or equal to 20 years of age were investigated separately. Kim 2007[24] analyzed data in 2 groups with conditioning of 800 cGy *versus* 1000-1200 cGy total body irradiation. In one study,[10] 4 different donor types were investigated in subgroups according to the HLA status, although, only the 2 subgroups with HLA-matched unrelated donors *versus* HLA-mismatched unrelated donors were included in the present report. The year of transplant was investigated in subgroups in another study:[30] before *versus* after and in 1998.

An overview of the patients' characteristics is presented in *Online Supplementary Table S4*. A median of 32 patients (range 11-349, total 1,645) were investigated in the total study population of 14 studies[11,15-17,21-23,25-29,31,32] and in 8 relevant subgroups of 4 other studies.[10,20,24,30] The patients' median age in the individual studies was between eight and 27 years and the ratio of males : females ranged from a male (30 : 10) to a female preponderance (11 : 20). Most of the 1,645 patients whose data were used for survival estimates had received a pre-transplantation IST to which they had not responded or after which they had relapsed. In 7 studies, a failed IST was not clearly stated.[10,21,22,25,28,30,32] A few cases without SAA[11,16,27,29] or without previous IST were included in some studies. The number of IST courses before HSCT was specified in 3 studies.[20,24,26] The median interval from diagnosis to transplant was stated in 16 subgroups or studies with values per study spanning from six to 168 months.

An overview of the treatment characteristics is presented in Table 2. In 12 studies,[10,16,17,20-25,27,28,31] bone marrow was the exclusive stem cell source. In 2 studies,[11,15] 2 patients received peripheral blood stem cells, in one study,[26] 2 patients received umbilical cord blood stem cells, and the

Table 2. Treatment characteristics.

Study	Subgroup	TBI Treated/total (N. patients)	Stem cell source BM : PB : CB (N. patients)	Donor types MUD : MMUD : MMRD (N. patients)	Complete HLA genotyping Yes : no (N. patients)	Donor gender Male : female : unavailable (N. patients)	CMV disease reactivation Affected/total (N. patients)	EBV disease reactivation Affected/total (N. patients)
Bacigalupo 2005[15]	–	–	36 : 2 : 0	28 : 5 : 5	33 : 5	–	–	–
Bunin 2005[16]	–	12/12	12 : 0 : 0	4 : 8 : 0	12 : 0	–	–	–
Deeg 1999[17]	–	120/141	141 : 0 : 0	105 : 36 : 0	108 : 33	81 : 60 : 0	–	–
Deeg 2006[20]	< 20 years of age (Deeg 2006a)	47/47	47 : 0 : 0	–*	47 : 0	–*	–	–
	≥ 20 years of age (Deeg 2006b)	40/40	40 : 0 : 0	–*	40 : 0	–*	–	–
Hows 1992[21]	–	40/40	40 : 0 : 0	27 : 13 : 0	0 : 40	12 : 28 : 0	–	–
Inamoto 2008[22]	–	14/16	16 : 0 : 0	10 : 6 : 0	unclear	–	–	–
Kennedy-Nasser 2006[23]	–	22/22	22 : 0 : 0	12 : 7 : 3	0 : 22	12 : 10 : 0	6 / 22	1 / 22
Kim 2007[24]	800 cGy TBI (Kim 2007a)	26/26	26 : 0 : 0	–‡	–‡	–	–	–
	1000-1200 cGy TBI (Kim 2007b)	14/14	14 : 0 : 0	–‡	–‡	–	–	–
Kojima 2002[25]	–	107/154	154 : 0 : 0	79 : 75 : 0	142: 12	93 : 61 : 0	–	–
Kosaka 2008[26]	–	unclear	29 : 0 : 2	25 : 0 : 4	0 : 31	–	–	–
Margolis 1996[27]	–	28/28	28 : 0 : 0	8 : 20 : 0	0 : 28	17 : 11 : 0	–	–
Maury 2007[11]	–	36/89	87 : 2 : 0	31 : 58 : 0	44 : 45	–	–	–
Passweg 2006[10]	Matched unrelated donor (Passweg 2006a)	–	181 : 0 : 0	181 : 0 : 0	0 : 181	89 : 84 : 8	–	–
	Mismatched unrelated donor (Passweg 2006b)	–	51 : 0 : 0	0 : 51 : 0	0 : 51	30 : 20 : 1	–	–
Perez-Albuerne 2008[28]	–	148/195	195 : 0 : 0	129 : 66 : 0	195 : 0	109 : 86 : 0	–	–
Svenberg 2004[29]	–	9/12	–	12 : 0 : 0	12 : 0	6 : 6 : 0	–	–
Viollier 2008[30]	before 1998 (Viollier 2008a)	–	145 : 4 : 0	114 : 35 : 0	0 : 149	67 : 70 : 0	–	–
	after and in 1998 (Viollier 2008b)	–	250 : 99 : 0	287 : 62 : 0	0 : 349	214 : 110 : 0	–	–
Yagasaki 2007[31]	–	11 / 11	11 : 0 : 0	0 : 11 : 0	11 : 0	5 : 6 : 0	8/11	5/11
Yoshimi 2008[32]	–	24 / 31	0 : 0 : 31	6 : 25 : 0	22 : 9	14 : 17 : 0	9/31	1/31

*–: information not stated in the publication; BM: bone marrow; CB: cord blood; cGy: centi Gray; GVHD: graft-versus-host disease; HLA: human leukocyte angiten; IST: immunosuppressive therapy; MMRD: mismatched related donor; MMUD: mismatched unrelated donor; MUD: matched unrelated donor; PB: peripheral blood; TBI: total body irradiation. *Deeg 2006 stated the number of 62 : 25 : 0 patients who received the transplant from the donor types MUD : MMUD : MMRD, and the number of 51 : 34: 2 patients who received the transplants from donors with the gender male : female : unavailable from the whole study population of 87 patients. †Kennedy-Nasser 2006: Reduced conditioning in 19 of 23 transplanted patients. ‡Kim 2007 stated the number of 36 : 4 : 0 patients who received the transplant from the donor types MUD : MMUD : MMRD, the number of 24 : 16 patients who received a complete : not complete HLA genotyping from the whole study population of 40 patients.*

stem cell source was not stated in another study.[29] Viollier *et al*. reported that, for the subgroup of patients treated after and in 1998, 99 of 349 patients received peripheral blood stem cells and 250 of 349 patients received bone marrow stem cells.[30] Umbilical cord blood stem cells were the only stem cell source for all 31 patients in one study.[32] In 14 studies,[11,16,17,20-22,24,25,27-32] all patients received stem cell transplants from either HLA-matched or mismatched unrelated donors. Stem cell transplants from an HLA-mismatched related donor were included in 4 studies.[10,15,23,26] DNA-based HLA-typing was performed for all participants in 5 published studies,[16,20,28,29,31] and for the majority of participants in 5 other studies.[15,17,24,25,32] In 8 studies,[10,11,21-23,26,27,30] the HLA-typing was serology-based or unclear. Donor gender was reported in 12 studies.[10,17,20,21,23,25,27-32] Reactivation of disease caused by cytomegalovirus and Epstein-Barr virus was observed in 3 studies.[23,31,32]

In the controlled trial, 205 children and young people under 18 years of age with SAA were enrolled,[26] of whom 198 were treated with IST. Response rate was checked at least six months after the treatments were initiated. The 52 children who failed the first-line IST were assigned to 2 treatment groups: 31 were transplanted from alternative donors and 21 received a second-line IST. Of the 31 transplanted patients, 25 (81%) received a transplant from an HLA-matched unrelated donor, 2 (6%) received cord

Table 3. Survival.

Study	Subgroup	Overall survival from date of transplantation Kaplan-Meier estimate (%) [95% confidence inverval] 2 years	5 years	Proportion of patients; N. survived/ N. evaluated (%)	Follow-up of survived patients; median; range (months)
Bacigalupo 2005[15]	–	73 [–]	73* [–]	29/38 (76)	21 (6-74); deceased 4 (0-25)
Bunin 2005[16]	–	73* [–]	73* [–]	9/12 (75)	48 (13-153)
Deeg 1999[17]	–	37 [32-48]	34 [26-43]	51/141 (36)	36 (11-94)
Deeg 2006[20]	<20 years of age (Deeg 2006a)	–	73 [–]	–/ 33 (–)	–
	≥20 years of age (Deeg 2006b)	–	46 [–]	–/22 (–)	–
Hows 1992[21]	–	33* [–]	28 [13-43]	–/40 (–)	50 (9-130)
Inamoto 2008[22]	–	81* [–]	81* [–]	–/16 (–)	–
Kennedy-Nasser 2006[23]	–	89* [–]	89 [–] at 4-years	18 / 22 (81)	52 (6-99)
Kim 2007[24]	800 cGy TBI (Kim 2007a)	–	92* [–]	24 / 26 (92)	–†
	1000-1200 cGy TBI (Kim 2007b)	–	42* [–]	6 / 14 (43)	–†
Kojima 2002[25]	–	65* [–]	56 [34-78]	99/154 (64)	29 (3-82)
Kosaka 2008[26]	–	94* [–]	94 [90-98]	28/31 (90)	35 (4-83)
Margolis 1996[27]	–	54* [–]	54* [–]	15/28 (54)	33 (13-96)
Maury 2007[11]	Matched unrelated donor	–	42 [37-47]	38/89 (43)	37 (4-191)
	(Passweg 2006a)	–	39 [31-46]	72/181 (40)	62 (3-139)
Passweg 2006[10]	Mismatched unrelated donor (Passweg 2006b)	–	36 [23-50]	18/51 (35)	61 (8-136)
Perez-Albuerne 2008[28]	–	–	51 [44-58]	95/195 (49)	59 (6-162)
Svenberg 2004[29]	–	83* [–]	83 [–]	10/12 (83)	43 (10-174)§
Viollier 2008[30]	Before 1998 (Viollier 2008a)	37* [–]	32 [24-40]	49/149 (33)	86 (15-152)
	after and in 1998 (Viollier 2008b)	63* [–]	57 [49-65]	235/349 (67)	13 (3-83)
Yagasaki 2007[31]	–	–	–	11 / 11 (100)	33 (9-56)
Yoshimi 2008[32]	–	41 [24-58]	–	13 / 31 (42)	34 (6-77)

*–: information not stated in the publication; cGy: centi gray; IST immunosuppressive therapy; MMUD: mismatched unrelated donor; MUD: matched unrelated donor; TBI: total body irradiation. *Transferred from the Kaplan-Meier survival curve. †Kim 2007: the median (range) follow-up was 37 (7-100) months for the total number of 40 patients. ‡Kosaka 2008: second-line transplantation group: 84% failure-free survival; second-line IST group: 10% failure-free survival; p=0.001. §Svenberg 2004: including 12 patients of the SAA group and 13 patients of the inborn error of metabolism group.*

blood transplants from an unrelated donor, and 4 (13%) received a transplant from an HLA-mismatched related donor. This means a small fraction of the investigated transplanted patients did not have an unrelated transplantation.

Outcome

Primary outcome

An overview of the survival is presented in Table 3. The median follow-up of surviving patients in 15 studies [10,11,15-17,21,23,25-32] ranged from 13 to 86 months. The 5-year overall survival and the 95% corresponding confidence intervals for 8 studies[10,11,17,21,25,26,28,30] have been reported to be from 28% to 94% and could be estimated from the survival curve and follow-up data in 8 other studies[15,16,20,22-24,27,29] from 42% to 92% (Table 3).

Secondary outcomes

An overview of the adverse events is presented in Table 4. The proportion of graft failure was 45% in one study with all participants receiving stem cell transplants solely from umbilical cord blood[32] (Table 4). This proportion was reported to be 0-26% in 15 of the other studies.[10,11,15-17,21-23,25-31] The incidence of acute GVHD grade II-IV per study was 8-86% and the incidence of extensive chronic GVHD was 0-38% of the evaluated patients in 16 studies[10,11,15-17,21-23,25-32] (Table 4).

Factors for improved survival

One study reported the outcomes separately according to the different HLA donor status and the overall survival did not differ significantly.[10] In another study, the data were presented separately for two different time periods and the overall survival was statistically significantly higher in the late cohort (from 1998) compared to the early cohort (before 1998).[30]

In 11 studies,[10,11,15,17,20,21,24,25,28,30,32] factors for improved survival were stated (Table 5). Some factors, like irradiation dose, were reported in one study[24] and other factors, like recipient age, were reported in up to 8 studies (Table 6). The following 5 factors were reported frequently (at least 2 times) and had more significant than non-significant results: recipient age, HLA match, performance status, year of transplant, and conditioning with serotherapy (Table 6). The factor irradiation was analyzed in 4 studies.[11,17,24,32] Non-significant results were reported in 3 studies[11,17,32] which analyzed the inclusion *versus* non-inclusion of irradiation in the conditioning regimen. Another study[24]

Table 4. Adverse events.

Study	Subgroup	Patients with any graft failure; N. affected/ N. evaluable (%)	Patients with acute GVHD grade II-IV; N. affected/ N. evaluable (%)	Patients with extensive chronic GVHD; N. affected/ N. evaluable (%)
Bacigalupo 2005[15]	–	7/38 (18)	4/35 (11)	2/33 (6)
Bunin 2005[16]	–	0/12 (0)	4/12 (33) grade I-III	1/12 (8)
Deeg 1999[17]	–	15/131 (11)	60 / 116 (52)	24/77 (31)
Deeg 2006[20]	< 20 years of age (Deeg 2006a) ≥ 20 years of age (Deeg 2006b)	–* –*	–* –*	–* –*
Hows 1992[21]	–	(18)	(86)	–
Inamoto 2008[22]	–	1 / 16 (6)	(31)	(31)
Kennedy-Nasser 2006[23]	–	1 / 23 (4)	4 / 23 (17) grade III-IV	3 / 23 (13)
Kim 2007[24]	800 cGy TBI (Kim 2007a) 1000-1200 cGy TBI (Kim 2007b)	–† –†	–‡ –‡	–‡ –‡
Kojima 2002[25]	–	17 / 144 (11)	(29) at 100 days	(15) at 2 years
Kosaka 2008[26]	–	5 / 31 (16)	4 / 31 (13)	4/31 (13)‡
Margolis 1996[27]	–	3 / 28 (11)	7 / 25 (28)	2/19 (11)
Maury 2007[11]	–	(14)‖	(50)¶	(11)‖‡
Passweg 2006[10]	Matched unrelated donor (Passweg 2006a) Mismatched un-related donor (Passweg 2006b)	(15)¶ (18)¶	(48)¶ (37)¶	(26)**‡ (22)**‡
Perez-Albuerne 2008[38]	–	(15)**	84 / 195 (43)¶	63/195 (32)
Svenberg 2004[29]	–	0/12 (0)	1/12 (8)	0/12 (0)
Viollier 2008[30]	Before 1998 (Viollier 2008a) After and in 1998 (Viollier 2008b)	37/142 (26) 38/338 (11)	(37) (28)	32/85 at risk (38)‡‡ 53/245 at risk (22)‡‡
Yagasaki 2007[31]	–	1/11 (9)	2/10 (20)	1/10 (10)
Yoshimi 2008[32]	–	14/31 (45)	5/31 (16)	1/31 (3)

*–: information not stated in the publication; GVHD: graft-versus-host disease; IST immunosuppressive therapy; MMUD: mismatched unrelated donor; MUD: matched unrelated donor; MMRD: mismatched related donor (1MM/>1MM: 1-/>1-antigen mismatch). *Deeg 2006 stated the number of 4/82 (5) affected/evaluable (%) patients with any graft failure, the number of 58/81 (72) affected / evaluable (%) patients with acute GVHD grade II-IV, and the number of 37/69 (54) affected/evaluable (%) patients with extensive chronic GVHD for the whole study population of 87 patients. †Kim 2007 stated the number of 2/40 (5) affected/evaluable (%) patients with any graft failure, the number of 12/40 (30) affected/evaluable (%) patients with acute GVHD grade II, and the number of 8/40 (20) affected/evaluable (%) patients with extensive chronic GVHD for the whole study population of 40 patients. ‡Kosaka 2008; Maury 2007: extensive vs. limited chronic GVHD not differentiated. ‖Maury 2007: at two years. ¶Maury 2007; Passweg 2006: at 100 days. **Passweg 2006; Perez-Albuerne 2008: at one year. ††. ‡‡With any grade.*

analyzed different doses and found that a lower dose (800 cGy) is a significant factor for improved survival when compared to a higher dose (1000–1200 cGy). DNA-based HLA-typing and chronic GVHD category (limited *versus* extensive) were also significant factors for improved survival; both factors were reported once.

Comparative study

The estimated failure-free survival in the controlled trial[26] at five years from the beginning of second-line therapy was about 85% in the allogeneic HSCT group compared to about 10% in the IST group (*data not shown*). There was no difference in the overall survival rate between the allogeneic HSCT and the IST group and was about 95% (*data not shown*).

Meta analysis

The 5-year survival probabilities and 95% confidence intervals have been stated in 8 studies.[10,11,17,21,25,26,28,30] In 2 of these studies, estimates were reported only for the subgroups HLA matched (*Passweg 2006a*) *versus* mismatched (*Passweg 2006b*) unrelated donors in one study[10] and for the subgroups date of transplantation before (*Viollier 2008a*) *versus* after or in (*Viollier 2008b*) the year 1998 in another study.[30] Five-year survival probabilities and the corresponding standard errors were deduced for another 9 studies.[15,16,20,22-24,27,29,32] In 2 of these studies, again estimates were reported only for the subgroups younger (*Deeg 2006a*) *versus* older or equal to (*Deeg 2006b*) 20 years of age in one study[20] and for the subgroups total body irradiation dose 800 cGy (*Kim 2007a*) *versus* 1000-1200 cGy (*Kim 2007b*) in another study.[24] Survival probabilities were not stated in one study.[31]

The meta analysis of the 5-year survival probabilities in 17 studies revealed a very high heterogeneity of I^2=96% (Figure 2) and calculation of a pooled estimate was not justified. Weights were based on the random effects model. This level of heterogeneity did not change after removal of the 4 studies from the analysis which showed only subgroup results (13 studies analyzed; I^2=96%; *data not shown*). Further, heterogeneity did not change after additional removal of the deduced estimates from 7 studies (6 studies analyzed; I^2=98%; *data not shown*). The results of the following subgroup analyses did not explain the heterogeneity either (*data not shown*):

- Median age younger *vs.* older than or equal to 16;
- Unicenter *vs.* multicenter;
- Prospective *vs.* retrospective design;

Table 5. Factors for improved survival.

Study	Factors for statistically significant better survival	Factors for statistically NOT significant better survival
Bacigalupo 2005[15]	–	Recipient age ≤ 14 years *vs.* > 14 years*
Bunin 2005[16]	–	–
Deeg 1999[17]	Interval time from diagnosis to transplant ≤3 years (*vs.* >3 years, p=0.02†; p=0.01‡), HLA-matched *vs.* mismatched, p=0.03)†	Recipient age <20 years (*vs.* ≥20 years)† Donor age <35 years (*vs.* ≥35 years)† Donor *vs.* recipient gender†, CMV status negative (*vs.* positive)†, Conditioning irradiation (no *vs.* yes)† GVHD prophylaxis cyclosporine A and methotrexate (*vs.* other)†, T-cell depletion yes (*vs* no)†
Deeg 2006[20]	Recipient age ≤20 years (*vs.* >20 years, p=0.05)*	*Interval time from diagnosis to transplant ≤1 year (*vs.* >1 year, p=0.67)*
Hows 1992[21]	Recipient age <15 years (*vs.* ≥15 years, p=0.03)†	HLA-matched (*vs.*mismatched)†
Inamoto 2008[22]	–	–
Kennedy-Nasser 2006[23]	–	–
Kim 2007[24]	Total body irradiation dose 800 cGy (*vs.* 1000–1200 cGy, p=0.001)*, Chronic GVHD limited (*vs.* extensive, p=0.013)*, DNA-based HLA-typing (*vs.* serologic, p=0.006)* Transfusion amount ≤90 units (*vs.* >90 units, p=0.020)*	Recipient age*, HLA match* Interval time from diagnosis to transplant*
Kojima 2002[25]	Recipient age <20 years (*vs.* >=20 years, p=0.005†; p=0.03‡) Donor age <35 years (*vs.* >=35 years, p=0.02)† Donor gender female (*vs*. male, p=0.02)† Donor recipient gender interaction (p=0.02)† Interval time from diagnosis to transplant <3 (*vs.* ≥3, p=0.04†; 0.02‡), HLA-matching by DNA typing (*vs.* mismatch, p=0.009)† HLA-A or -B match (*vs.* mismatch, p=0.04)‡	Recipient gender† Red blood cell transfusions† GVHD prophylaxis† Marrow cell dose† Conditioning regimen†, Donor gender‡
Kosaka 2008[26]	–	–
Margolis 1996[27]	–	–
Maury 2007[11]	Year of transplant 1999-2004 (*vs.* 1989-1998, p=0.008*; p<0.01†) Recipient age ≤17 years (*vs.* >17 years, p<0.01)‡ Serotherapy in conditioning: yes (*vs.* no, p<0.05)‡	Donor recipient gender mismatch‡ Cell dose ≤2.6×10^8 nucleated cells/kg‡ Irradiation-based conditioning‡ Interval time from diagnosis to transplant <1 year‡ Fludarabine in conditioning‡
Passweg 2006[10]	Recipient age <21 years (*vs.* ≥21 years, p=0.05)‡ Karnofsky performance status ≥90% (*vs.* <90%, p<0.001)‡	Type of donor MUD *vs.* MMUD *vs.* MMRD 1MM *vs.* MMRD >1MM, p (overall)=0.52
Perez-Albuerne 2008[28]	Interval time from diagnosis to transplant ≤4 years (*vs.* >4 years, p<0.001)‡ Performance score 90-100 (*vs.* <90, p=0.001)‡ HLA match (*vs.* mismatch, p=0.006)‡	–
Svenberg 2004[29]	–	–
Viollier 2008[30]	Year of transplant ≥1998 (*vs.* <1998, p=0.001†; p=0.001‡) Recipient age in years 0-10 (*vs.* 10-20 *vs.* 20-30 *vs.* 30-40 *vs.* >40, p=0.009)‡ Karnofsky performance status good (*vs.* poor *vs.* missing, p=0.0001)‡	Interval time from diagnosis to transplant‡
Yagasaki 2007[31]	–	–
Yoshimi 2008[32]	Conditioning regimen: total body irradiation + cyclophosphamide + fludarabine (*vs.* total body irradiation + melphalan + fludarabine *vs.* others, p=0.02)† Conditioning regimen: Serotherapy no (*vs.* yes, p=0.007)†	Recipient age† Interval time from diagnosis to transplant† Red blood cell transfusions before transplant† Platelet transfusions before transplant† Serologic typing: HLA match† GVHD prophylaxis (methotrexate)† Mononuclear cell count† CD34 count†

*–: information not stated in the publication. CD34: cluster of differentiation leukocyte cell surface molecule n. 34; CMV: cytomegalovirus; GVHD: graft-versus-host disease; HLA: human leukocyte angiten; IST: immunosuppressive therapy; MMUD: mismatched unrelated donor; MUD: matched unrelated donor; MMRD: mismatched related donor (1 MM/>1MM: 1- />1-antigen mismatch). *Kaplan-Meier method and log-rank test. †Univariate analysis. ‡Multivariate analysis.*

• HLA genotyping complete *vs.* partial or none;
• Median interval from diagnosis to transplant shorter *vs.* longer than or equal to 14 months;
• Study population less than *vs.* greater than or equal to 50 participants;
• Transplantation was performed before *vs.* after or in 1998.

Data quality

Characteristics

The patients' characteristics were described with varying details in the available publications. A clear and consistent definition of response criteria was lacking. In many studies, mixed populations of patients with a lack of response to IST (refractory SAA) and those with a recurrence of disease after initial treatment success (relapsed SAA) were investigated. Response was determined at various time points from three to six months after the beginning of the treatment where stated. The number of repeats of pre-transplantation IST varied considerably in one study and 87 patients received 1-11 IST repeats (median 3).[20] This detailed information was not available for the other 17 studies, although it might be a considerable risk factor for long-term morbidity and mortality.

Comparative study

In the study by Kosaka *et al.*,[26] the allocation of the patients to the transplantation group and the IST group was probably dependent on donor availability. This might be accepted as a qualified allocation, so called *genetic randomization*. However, the allocation was not described adequately in the paper. The patients' characteristics of the transplantation group *versus* the IST group were different regarding the proportion of very severe aplastic anemia (32% *versus* 67%, p=0.03, statistically significant according to our calculation), and male gender (45% *versus* 67%, p=0.21, not statistically significant according to our calculation). Furthermore, the median follow-up was 35 (range 4-83) months *versus* 66 (9-80) months. These differences between the groups were not considered in the analysis and not discussed in the publication. In addition, the source of anti-thymocyte globulin was rabbit in the transplantation group and horse in the IST group. The median age (eight *versus* nine years) and the time from diagnosis to second-line therapy (eight *versus* seven months) were comparable. The response was evaluated at three and six months after the treatment. In the IST group, 6 patients had a response within 6-12 months and were not considered in the analysis.

Discussion

Outcomes

The outcome data varied considerably between the studies and the 5-year overall survival estimates ranged from 28% to 94%. This range is similar to data published in recent reviews, such as Young *et al.*[4] (4-year or 5-year overall survival of 58-100% for alternative donor HSCT) and Marsh *et al.*[7] (5-year overall survival of 36-73% for unrelated donor HSCT). The values in the present analysis of the cumulative incidence for treatment-associated complications, such as graft failure, acute and chronic GVHD, are also similar to the ranges stated in these articles provided that bone marrow was used as a stem cell source. Only one study relied solely on umbilical cord blood as the stem cell source.[32] Merely 55% of 31 recipients achieved a sustained engraftment and 23% died because of graft failure. Chan *et al.*[33] reported unrelated cord blood transplantation in 9 children of whom 89% (8 of 9 patients) engrafted, 2 after the second transplantation, and 78% were alive at a median follow-up of 34 months.

Factors for improved survival

Factors for improved survival were addressed in 11 studies and the results were inconsistent. Lower irradiation dose, DNA-based HLA-typing, and limited chronic GVHD were significant factors, but each factor was analyzed in merely one study. The following factors were reported to be significant in at least 2 studies and were significant in the majority of the studies reporting the

Table 6. Comparison of potential prognostic factors for improved survival.

Potential prognostic factors*	N. studies reporting the factor	N. studies with significant results
Recipient age	8	5
Interval time from diagnosis to transplant	8	3
HLA match	4	3
Performance status	3	3
GVHD prophylaxis	3	–
Conditioning: irradiation	3	–
Year of transplant	2	2
Conditioning: serotherapy	2	2
Donor age	2	1
Donor gender	2	1
Donor recipient gender interaction	2	1
Transfusion amount	2	1
Red blood cell transfusions before transplant	2	–
DNA-based HLA-typing	1	1
Conditioning: irradiation dose	1	1
Chronic GVHD category	1	1
Recipient gender	1	–
Donor type (MUD, MMUD, MMRD)	1	–
CMV status	1	–
Mononuclear cell count	1	–
CD34 count	1	–
Platelet transfusions before transplant	1	–
Conditioning: fludarabine	1	–
T-cell depletion	1	–
Marrow cell dose	1	–

*–: information not stated in the publication. CD34: cluster of differentiation leukocyte cell surface molecule n. 34; CMV: cytomegalovirus; GVHD: graft-versus-host disease; HLA: human leukocyte antigen; MMUD: mismatched unrelated donor; MUD: matched unrelated donor; MMRD: mismatched related donor. *Ordered according to the number of studies reporting the factor (column 2).*

respective factor: recipient age, HLA match, performance status, year of transplant, and conditioning with serotherapy. We gave the priority to the latter factors because of frequent reporting. However, this was not intended to detract from the importance of the previous factors reported.

Passweg *et al.*[10] studied patients transplanted up to 1998 and did not find a significant effect of year of transplantation. Viollier *et al.*[30] studied patients transplanted from 1990 to 1998 and from 1998 to 2005 and found significantly improved survival for patients transplanted after 1998, speculating that this result may be due to better donor matching.

Outcome assessment criteria

The considerable shortcomings of the available data mean that interpretation is limited. Although criteria for the quality of response after immunosuppressive treatment have been defined by the *European Group for Blood and Marrow Transplantation (EBMT)*,[34] it should be emphasized that authors use different definitions, not only in terms of response but also in terms of relapsed patients, refractory patients, and of the time interval from the beginning of treatment to ascertaining the response.[35-37] For example, the more the IST is repeated in relapsed patients, the more the patient will be exposed to blood products. An association of the number of applied blood products with reduced survival estimates has been shown, and it was concluded that the number of IST repeats in turn can be a prognostic factor for survival.[38] In addition, it was concluded that patients who fail 2 courses of treatment have almost always been heavily transfused and frequently have significant infections that make transplantation less likely to be successful.[9] The number of IST courses was stated in merely 3 of 18 studies and the possible impact of this factor was not investigated.

Lack of controlled trials

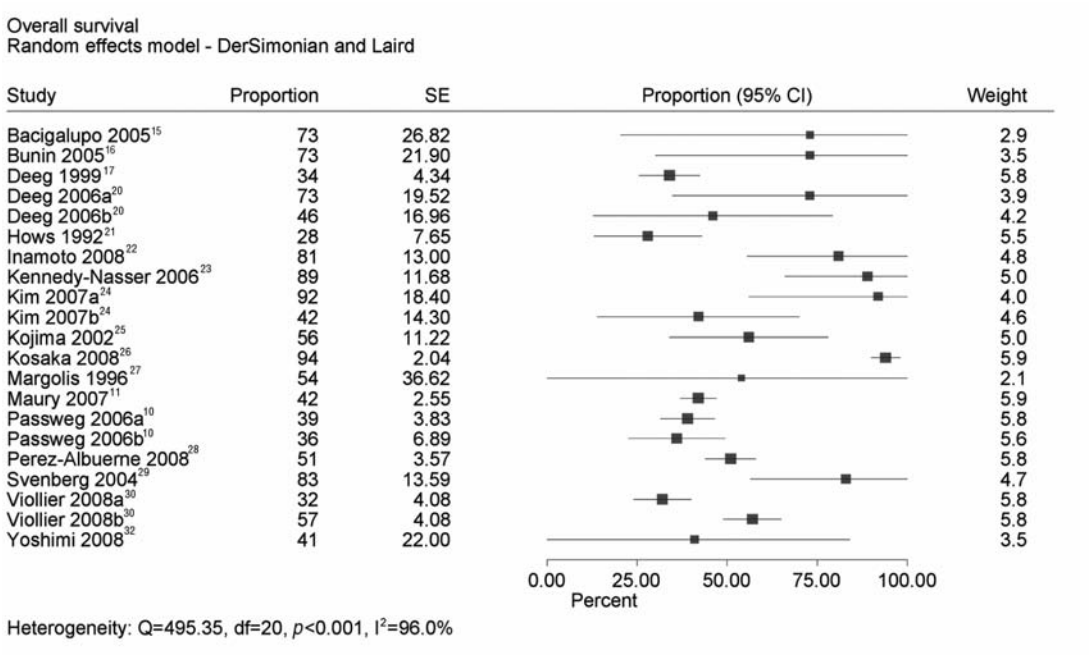
According to recent recommendations,[6] HLA-matched and HLA-mismatched unrelated donor HSCT, as well as HLA-mismatched related donor HSCT, are associated with significant morbidity but can be a clinical option when other therapies have failed. Unrelated donor HSCT is regarded by most clinicians as a sequential option which, by definition, could not be compared with a preceding treatment. On the other hand, it was stated that "*a prospective comparison of unrelated donor transplantation versus a second trial of immunosuppression is needed to address this issue*",[9] and it was also stated that "*an ongoing IBMTR/EBMT study will compare the outcome after MUD BMT versus second course of IST in patients failing the first course of IST*".[34]

Evidence base for second-line unrelated donor HSCT after failed IST

In 2008, the results were published[26] of the first prospective multicenter study to compare the efficacy of repeated IST with HSCT from an alternative donor in children with acquired SAA who failed to respond to an initial course of IST. There was no difference in the estimated overall survival after five years between the two treatment groups. The estimated failure-free survival after five years was significantly increased in the allogeneic HSCT group and may indicate that the transplanted patients may have an additional benefit.

The evidence for second-line unrelated donor HSCT after failed IST remains unclear. Well-performed controlled trials with stringent eligibility criteria need to be conducted to evaluate the true benefit. The documentation of data from all patients regardless of the type of intervention is feasible due to the very low prevalence of a disease like SAA. We believe that a disease-related rather than a procedure-related documentation in transplanta-

Figure 2. Meta analysis of 5-year survival estimates.



tion registers would provide data for additional comparative studies eligible for inclusion in future systematic reviews.

Second-line IST after failed first-line IST

Second-line IST after failed first-line IST may remain a treatment option. There are some uncontrolled studies providing results after second-line IST. The overall survival after a median follow-up of 30 months in 30 patients not responding to first IST was reported to be 93% by Di Bona *et al*.[39] Scheinberg *et al*. reported an overall survival after 2.7 years of 70% for 22 refractory patients and of 83% for 21 relapsed patients.[40] Tichelli *et al*. reported a long-term follow-up:[41] The overall survival after ten years was 55% for 25 refractory patients and was 51% for 18 relapsed patients. However, the proportion of late clonal complications at 20 years was 53%.

Strengths and limitations of the present review

The strengths of this review are the broadness of the search strategy and the comprehensiveness of the published data included. Significant as well as non-significant factors that may influence the survival of the patients were considered in the present report. While the results of the present descriptive review may not be conclusive, they can provide useful summaries of the state of knowledge and be used for future design and data collections to obtain reliable comparative results.

This review has limitations. We described case series with heterogeneous clinical characteristics, apart from one controlled trial with methodological flaws, and we did not consider a systematic evaluation of case series on IST. While investigating factors that may influence survival, we considered results that were included in subgroup analyses, which increase the likelihood of false-positive results. Patients with disease other than SAA and patients not treated with pre-transplant IST were included. The time interval from diagnosis to transplant varied between one month and 28 years across the studies, and the number of IST courses were not stated clearly for any individual patient. We arbitrarily did not include studies with less than 10 participants and we did not consider asking the authors for individual patient data.

Heterogeneity was explored by conducting a meta analysis of the survival estimates at five years. The forest plot demonstrated a considerable difference between the studies and this heterogeneity was quantified by a very high I^2 value. Therefore, a pooled estimate was not justified.

Conclusions

Unrelated donor hematopoietic stem cell transplantation in patients with acquired severe aplastic anemia after failure to immunosuppressive therapy is a treatment option. A stable physical condition of the patients before receiving the transplant (for example, performance and age) may be associated with a better survival. Detailed HLA-matching facilitated by DNA-based typing, among other factors, may have contributed to recent improvements in survival after unrelated donor HSCT as a second-line treatment. The results are based mainly on bone marrow as a stem cell source. Transplants from peripheral blood were used infrequently and transplants from umbilical cord blood were observed with an unusually large proportion of graft failure. The results of additional controlled trials comparing unrelated stem cell transplantation with further immunsuppressive therapy are needed for a meaningful analysis.

Authorship and Disclosures

FP: principal investigator and takes primary responsibility for the paper; FP: coordinated the study, conducted the literature search, and also screened and analyzed the retrievals; SL: initiated the study; FP and SL: extracted the data; FP: drafted the manuscript; UG and FP: conducted the meta analyses; NK: provided a clinical perspective; MP: provided general advice; BZ: provided a patient-oriented perspective. All authors interpreted the data and made an intellectual contribution to the manuscript. All authors reviewed and approved the final version. The authors reported no potential conflicts of interest.

Five authors (FP, UG, MP, BZ, SL) are IQWiG full-time employees, SL is deputy director of IQWiG; one author (NK) is deputy director of the Interdisciplinary Clinic for Stem Cell Transplantation, University Hospital Hamburg-Eppendorf.

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