Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review

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Subject: Transplantation for SAA

Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review*

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Keywords

severe aplastic anemia, stem cell transplantation, bone marrow transplantation, immunosuppressive therapy, systematic review

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Abstract

Objectives: Acquired severe aplastic anemia is a rare and potentially fatal disease. The aim of this Cochrane review was to evaluate the effectiveness and adverse events of first-line allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors compared to first-line immunosuppressive therapy.

Setting: Specialised stem cell transplantations units in primary care hospitals

Participants: We included 302 participants with newly diagnosed acquired severe aplastic anemia. The age ranged from early childhood to young adulthood. We excluded studies on participants with secondary aplastic anemia.

Interventions: We included allogeneic haematopoietic stem cell transplantation as the test intervention harvested from any source of matched sibling donor and serving as a first-line therapy. We included immunosuppressive therapy as comparator with either antithymocyte/-antilymphocyte globulin or ciclosporin or a combination of the two.

Primary and secondary outcome measures planned and finally measured: The primary outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-host disease, no response to immunosuppressive therapy, relapse after initial successful treatment, secondary clonal disease or malignancies, health-related quality of life, and performance scores.

Results: We identified three prospective non-randomized controlled trials with a study design that was consistent with the principle of 'Mendelian randomization' in allocating patients to treatment groups. All studies had a high risk of bias due to the study design and were conducted more than 15 years. The pooled hazard ratio for overall mortality for the

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transplanted group versus the not transplanted group was 0.95 (95% confidence interval 0.43 to 2.12, P = 0.90).

Conclusions: There are insufficient and biased data that do not allow any firm conclusions to be made about the comparative effectiveness of first-line allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors and first-line immunosuppressive therapy of patients with acquired severe aplastic anemia.

Strengths and limitations of this study

- We conducted a comprehensive literature search and strictly adhered to the projected methodology.
- We restricted the study design to randomized controlled trials and prospective non-randomized controlled trials and the studies had to be compatible with 'Mendelian Randomization' to avoid excess risk of bias.
- The included data are too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST.
- The included data were collected 15 to more than 30 years ago. Thus, the results may not be applicable to current modern standard care.

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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; it mainly affects young adults. The estimated incidence rate of SAA ranges from 0.7 to 4.1 per million people per year [2]. The underlying pathophysiology is thought to be an aberrant immune response involving the T-cell mediated destruction of hematopoietic stem cells [3]. Major signs and symptoms are severe infections, bleeding, and exhaustion and patients may experience paleness, weakness, fatigue, and shortness of breath.

According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of the British Committee for Standards in Haematology [4], first-line allogeneic hematopoietic stem cell transplantation (HSCT) from the bone marrow of an human leukocyte antigen (HLA)-matched sibling donor (MSD) is regarded as the initial treatment of choice for newly diagnosed patients with severe aplastic anemia. Graft failure may lead to early death and the conditioning regimen may lead to severe non-hematological organ toxicities. First-line ciclosporin and/or antithymocyte or antilymphocyte globulin denoted as first-line immunosuppressive therapy (IST) is indicated for patients where no MSD is available, which can be expected for 70% of patients with SAA [3]. Some patients do not respond well or show no response at all. Frequent transfusions increase the risk of adverse events such as iron overload and early death. If a diagnosis of SAA is established at an early patient age, then it is crucial to know which treatment promises more benefit and less harm in the long run. We aimed to evaluate the effectiveness and severe adverse events of MSD-HSCT compared to IST in patients with SAA.

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Methods

This article is based on a Cochrane Systematic Review published in The Cochrane Library [5]. Publication of this work is in agreement with the policy of The Cochrane Collaboration [6]. While preparing this systematic review and meta-analysis, we endorsed the PRISMA statement, adhered to its principles and conformed to its checklist [7].

Study inclusion criteria

We included randomized controlled trials (RCTs) and prospective non-randomized controlled trials as long as the study design was consistent with the principle of 'Mendelian randomization' in allocating patients to treatment groups. We required a minimum of 80% of relevant patients per group and we set a minimum sample size of five participants per group. We set no limits on language, year of publication, or year of treatment. We included participants with newly diagnosed acquired severe aplastic anemia [4]. We did not set any age limits for participants. We excluded studies on participants with secondary aplastic anemia. We included HSCT as the test intervention harvested from any source of MSD and serving as a first-line therapy [8]. That means, no other HSCT or IST has been offered to the patients before. We included IST as comparator with either antithymocyte/antilymphocyte globulin or ciclosporin or a combination of the two [8]. The primary outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-host disease, no response to IST, relapse after initial successful treatment, secondary clonal disease or malignancies, health-related quality of life, and performance scores.

Search strategy and selection of studies

We conducted an electronic literature database search in MEDLINE (Ovid), EMBASE (Ovid), and Cochrane Library CENTRAL (Wiley) including articles published from inception to

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22 April 2013. The corresponding search strategies are depicted in the original Cochrane Review [5]. We retrieved all titles and abstracts by electronic searching and downloaded them to the reference management database EndNote Version X3 [9]. Two authors assessed the eligibility of retrieved papers independently. We considered studies written in languages other than English. We judged studies to be prospective if an explicit statement was reported or there were clues suggesting a prospective design (e.g. prior approval of treatment, informed consent). We judged studies to be retrospective if an explicit statement was reported or it was implied by description that data were reviewed from an existing source. We regarded each of the following items as an indication of a retrospective design: registry reports and reviewing of medical records. Gray 1991 and Wheatley 2004 described the potential of 'Mendelian randomization' to minimize bias when comparing MSD-HSCT with an alternative therapy [10 11]. We judged studies as consistent with the principle of 'Mendelian randomization' if all transplant donors were clearly siblings and if the allocation of patients to treatment groups was not based on age. We regarded studies as not consistent with the principle of 'Mendelian randomization' if age was not balanced between groups, indicating that age played a role in group assignment. Example for imbalance: distribution of age categories was statistically not comparable (P value less than 0.05).

Assessment of risk of bias in included studies

Two review authors independently assessed the risk of bias in the included studies using six criteria. We have used four criteria from The Cochrane Collaboration's tool for assessing risk of bias [12]: blinding of outcome assessment, complete outcome data such as missing data, selective reporting such as not reporting pre-specified outcomes, and other sources of bias such as bias related to the specific study design and competing interest. We extended the Cochrane tool for assessing risk of bias with two additional criteria that are specific to the

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inclusion criteria for the present review and critical for confidence in results: comparable baseline characteristics and concurrent control. We applied The Cochrane Collaboration's criteria for judging risk of bias [13].

Data synthesis

One review author entered the data into Review Manager [14]. Another review author checked the entered data. We synthesized data on mortality (MSD-HSCT versus IST) by using the hazard ratio (HR) for time-to-event data as the primary effect measure with a random-effects model. If the hazard ratio was not directly given in the publication, we estimated hazard ratios according to methods proposed by [15] and [16].

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Results

Search results

We identified three non-randomized, prospective, parallel, controlled clinical trials (**Figure 1**). Bayever 1984 [17] and Gratwohl 1981 [18] reported their results in a single original article, respectively. Führer 1998 reported five publications including one original article [19], a follow up article [20], one protocol [21], and two abstracts [22 23]. We did not identify any RCTs.

Characteristics of included articles

The main study, patients and interventions characteristics are shown in **Table 1**. The patients were treated and observed between 1976 and 1997. Thus, the reported data were collected more than 15 years ago. Median follow up was not reported. Median age, fraction of males, and median days of time interval between diagnosis and begin of treatment were roughly comparable between the treatment groups within each study. The age ranged from early childhood to young adulthood. In the study by Führer 1998, all patients were less than 17 years old by definition of the inclusion criteria [19]. Bone marrow was used as source for all transplants. All three included studies had a high risk of bias due to the study design (**Table 2**).

Effects of intervention

The pooled hazard ratio estimate for overall mortality was 0.95 with a 95% confidence interval of 0.43 to 2.12 (P value = 0.90) (**Figure 2**). According to the meta-analysis based on data from all three included studies, overall mortality was not statistically significantly different between MSD-HSCT and IST. Overall survival ranged from 47% to 84% in the MSD-HSCT

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group and from 45% to 87% in the IST group (**Table 3**). The results for the secondary outcomes including treatment-related mortality after MSD-HSCT, graft failure after MSD-HSCT, graft-versus-host-disease (GVHD) after MSD-HSCT, no response to IST, and relapse after IST are shown in **Table 4**. With respect to secondary clonal disease or malignancies, Bayever 1984 reported one patient who developed T-cell lymphoma after MSD-HSCT and Führer 1998 reported 4 patients who developed acute myelogenous leukemia after IST. Health-related quality of life questionnaires were not used in any of the included studies. Bayever 1984 reported that almost all evaluable patients in the MSD-HSCT group (92%) and less than half of the patients in the IST group had a Karnofsky Performance Status of higher than 70%.

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Discussion

Interpretation of main results

We identified three prospective, non-randomized controlled trials [17-19] including 302 participants; 121 received MSD-HSCT and 181 received IST. Based on these trials we found insufficient evidence to clarify whether MSD-HSCT leads to better overall survival than IST. Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in the IST group and in a meta-analysis, overall mortality was not statistically significantly different between the treatment groups. Treatment-related mortality in the MSD-HSCT group was considerable ranging from 20% to 42%. The graft failure rate was variable and caused the death of 3% to 16% of transplanted patients. GVHD affected a quarter to a half of transplanted patients. More than half of patients in one study did not respond to IST. Relapse affected up to one in eight patients after IST in one study. Secondary clonal disease or malignancies were detected rarely in both treatment groups. Marsh 2009 estimated that allogeneic bone marrow transplantation from an HLA-identical sibling donor provides a 75% to 90% chance of long-term cure in patients younger than 40 years of age [4]. Similarly, in a review of studies with younger patients, Guinan 2009 reported an overall survival between 75% and 95% at three to five years [24]. Sangiolo 2010 concluded that their data favors extending MSD-HSCT to patients older than 40 years of age who are without significant co-morbidities [25]. The results of the studies included in the present systematic review appear to roughly match the recent estimates reported by others.

Recent therapeutic improvement

Bacigalupo 2008 reported that the outcome has improved since 1996 for HSCT but not for IST. Peinemann 2011 identified three studies that reported a statistically significant improve-

ment of overall survival in the group of matched related donor transplants but not in the IST group [26]. Several factors may have contributed to recent improvements in HSCT, such as detailed HLA-matching and less irradiation-based conditioning. The Third Consensus Conference on the treatment of aplastic anemia agreed in 2010 that bone marrow should be used as the source of stem cells and that the upper age limit should be 50 years and that the combination of antithymocyte globulin and ciclosporin remains the gold standard for immunosuppressive therapy [27]. Scheinberg 2012 provided an overview and update of various treatment options for severe aplastic anemia including immunosuppressive therapy and transplantation [28].

Strengths and limitations

One of the strengths of this review is the broadness of the search strategy such that study retrieval bias is very unlikely. We restricted the inclusion to studies to randomized controlled trials and prospective non-randomized controlled trials that were compatible with 'Mendelian Randomization' to avoid excess risk of bias. Nevertheless, the included data are too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST. The rates of adverse events, such as treatment-related mortality, graft failure, no response to IST, and GVHD, are unusually high, which may be explained by the age of the studies (starting in 1976). All data were collected about 15 up to more than 30 years ago. Thus, the results may not be applicable to current modern standard care. Use of 'Mendelian randomization' is no guarantee that bias is minimized and Nitsch 2006 described the limits to causal inference based on 'Mendelian randomization' [29].

Conclusions

There are insufficient and biased data that do not allow any firm conclusions to be made about the comparative effectiveness of MSD-HSCT and IST. Patients should be made aware of the

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early treatment-related mortality and the burden of GVHD after HSCT. Patients treated with IST should also be made aware that the disease may recur after initial successful treatment, and that life-threatening late clonal and malignant disease after IST may occur in a higher percentage compared to HSCT.

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Acknowledgments

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Ethics statement

An ethics statement was not required for this work.

Financial Disclosure

Provision of fulltexts by the University of Cologne, Germany. No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest Statement

No authors have any competing interests.

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Figure legends

Figure 1. Study flow

Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia

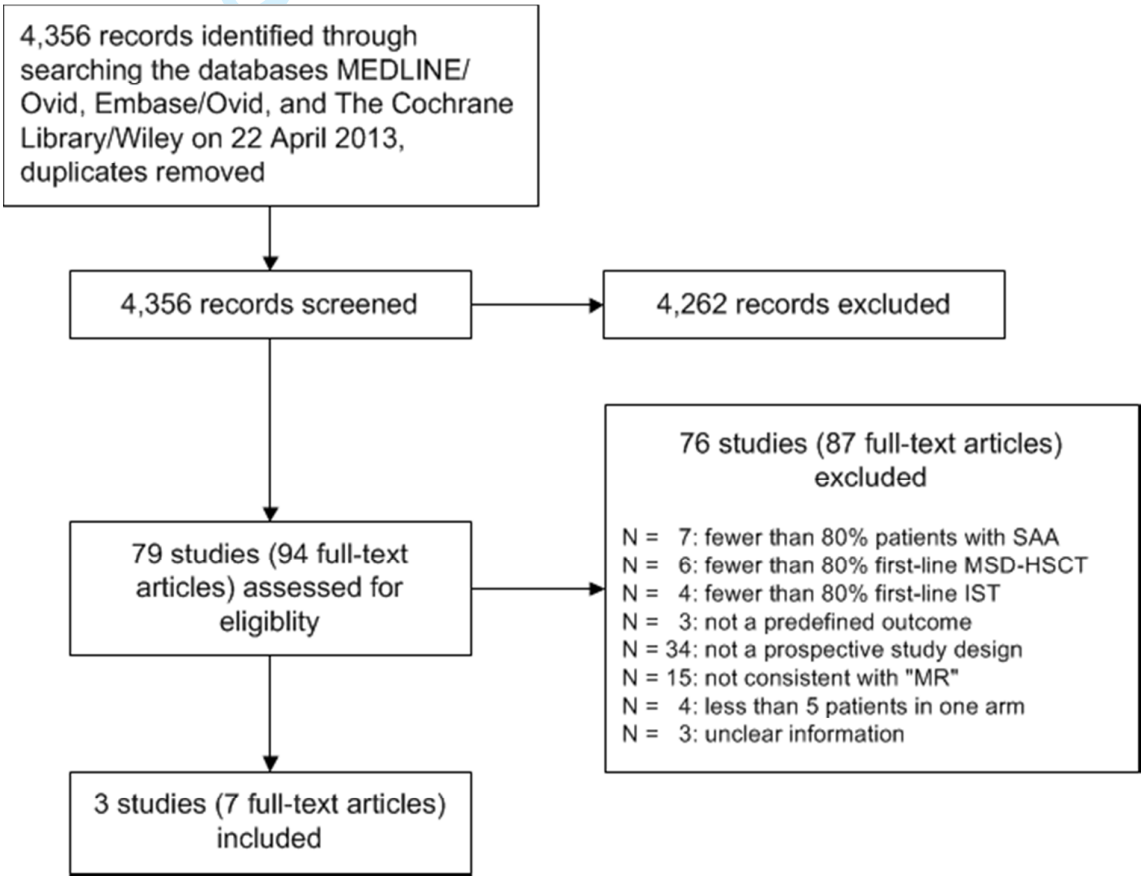

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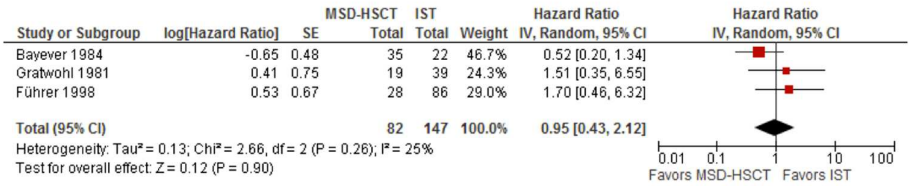
Figure 2. Mortality (MSD-HSCT vs. IST); effect: hazard ratio; random-effects model.

Standard error calculated from data presented in the Kaplan-Meier graph of the article.

Abbreviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error



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Tables

Table 1. Characteristics of included studies

Study ID	Duration of study	Median follow up	Setting, center, country	Patients, no.[1]	Median age, years (range)[1]	Fraction of males, %[1]	Median interval, days[1,2]	Stem cell source	IST compo-nents	ATG source
Bayever 1984	1977 to 1982	N.R.	Single, United States	35 vs. 22	17 (2 to 24) vs. 15 (1 to 23)	67 vs. 68	60 vs. 58	bone marrow	ATG	horse
Führer 1998	1993 to 1997	N.R.	Multi, Germa-ny, Austria	28 vs. 86	10.1 (2.3 to 15.8) vs. 9.1 (0.9 to 15.2)	43 vs. 62	49 vs. 23	bone marrow	ATG Ciclosporin	horse
Gratwohl 1981	1976 to 1980	N.R.	Single, Swit-zerland	19 vs. 13	18 (4 to 29) vs. 23 (7 to 37)	53 vs. 54	105 vs. 180	bone marrow	ATG Ciclosporin	N.R.

[1]MSD-HSCT vs. IST
[2]Median time interval between diagnosis and begin of treatment
Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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Table 2. Risk of bias of included studies

Study ID	Blinding of out-come assessment	Incomplete outcome data	Selective reporting	Other bias	Comparable base-line characteristics	Concurrent control	Overall judgement of bias
Bayever 1984	High	Low	Unclear	High[1]	Low	Low	High
Führer 1998	High	High	High[2]	High[3]	Low	Low	High
Gratwohl 1981	High	High	Unclear	Unclear	Low	Low	High

[1]Bayever 1984: The authors reported the study results at an early time point before all planned data had been gathered: "We present this interim report(...)".
[2]Führer 1998: In the 2005 update, overall survival, secondary clonal disease or malignancies, and also relapse were not reported separately for the 2 distinct treatment groups. Rather, the results were presented for 2 subgroups according to disease severity. This was different from the earlier report of the same study published in 1998 covering the study period from 1993 to 1997. See Fig. 1 and Tab. 1 of the article.
[3]Führer 1998: Financial support was provided by 2 pharmaceutical companies.
Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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Table 3. Overall survival

Study ID	MSD-HSCT		IST		FU[1]	P value
	N	OS (95% CI)	N	OS (95% CI)	Year	
Bayever 1984	35	72% (64 to 80)	22	45% (29 to 61)	2	0.18
Führer 1998	28	84% (N.R.)	86	87% (N.R.)	4	0.43
Gratwohl 1981	19	47% (N.R.)	13	69%[2] (N.R.)	5	0.56[3]

[1]Time point of Kaplan-Meier estimate.
[2]Gratwohl 1981: Two of 13 patients were eligible for MSD-HSCT but donors were not available in the first place; the two patients died after they received a second-line HSCT from the then again available MSD that was offered after the patients showed no response to IST.
[3]The P value was not reported and we calculated the P value using Fisher's exact test.
Abbreviations: CI: confidence interval; FU: follow up; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N: number of analyzed patients; N.R.: not reported; OS: overall survival

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Table 4. Secondary outcomes

Study ID	TRM after MSD-HSCT[1,2]	Graft failure after MSD-HSCT[1]	GVHD after MSD-HSCT[1]	No response to IST[1]	Relapse at 5 years after IST[1]
Bayever 1984	20% (7 of 35)	3% (1 of 35)	51% (17 of 33)	64% (14 of 22)	12.5% (1 of 8)
Führer 1998	N.R.	N.R.	N.R.	N.R.	N.R.
Gratwohl 1981	42% (8 of 19)	16% (3 of 19)	26% (5 of 19)	15% (2 of 13)	N.R.

[1]In parenthesis: number of affected of number of evaluable patients

[2]Treatment-related mortality was not reported for IST.

Abbreviations. GVHD: graft-versus-host disease; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N.R.: not reported; TRM: treatment-related mortality

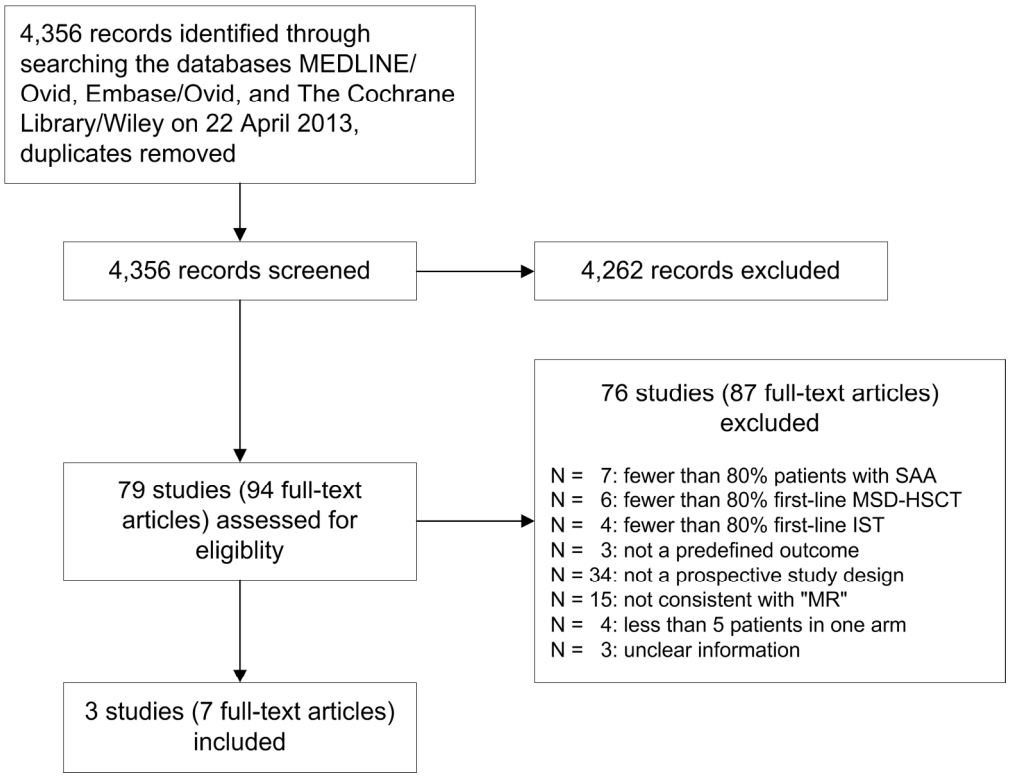

Figure 1. Study flow
Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia
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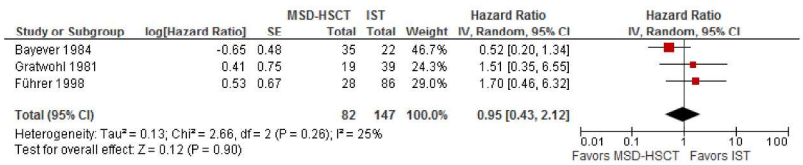

Figure 2. Mortality (MSD-HSCT vs. IST); effect: hazard ratio; random-effects model.
Standard error calculated from data presented in the Kaplan-Meier graph of the article. Ab-breviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error
254x190mm (300 x 300 DPI)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	in the Cochrane review
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6 to 7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7

PRISMA 2009 Checklist

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	in the Cochrane review
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8, table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8 to 9, table 3 to 4, figure 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	figure 1
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	table 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	none
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12
FUNDING			

PRISMA 2009 Checklist

Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	12

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review*

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Keywords

severe aplastic anemia, stem cell transplantation, bone marrow transplantation, immunosuppressive therapy, systematic review

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Abstract

Objectives: Acquired severe aplastic anemia is a rare and potentially fatal disease. The aim of this Cochrane review was to evaluate the effectiveness and adverse events of first-line allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors compared to first-line immunosuppressive therapy.

Setting: Specialised stem cell transplantations units in primary care hospitals

Participants: We included 302 participants with newly diagnosed acquired severe aplastic anemia. The age ranged from early childhood to young adulthood. We excluded studies on participants with secondary aplastic anemia.

Interventions: We included allogeneic haematopoietic stem cell transplantation as the test intervention harvested from any source of matched sibling donor and serving as a first-line therapy. We included immunosuppressive therapy as comparator with either antithymocyte/-antilymphocyte globulin or ciclosporin or a combination of the two.

Primary and secondary outcome measures planned and finally measured: The primary outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-host disease, no response to immunosuppressive therapy, relapse after initial successful treatment, secondary clonal disease or malignancies, health-related quality of life, and performance scores.

Results: We identified three prospective non-randomized controlled trials with a study design that was consistent with the principle of 'Mendelian randomization' in allocating patients to treatment groups. All studies had a high risk of bias due to the study design and were conducted more than 15 years. The pooled hazard ratio for overall mortality for the

transplanted group versus the not transplanted group was 0.95 (95% confidence interval 0.43 to 2.12, P = 0.90).

Conclusions: There are insufficient and biased data that do not allow any firm conclusions to be made about the comparative effectiveness of first-line allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors and first-line immunosuppressive therapy of patients with acquired severe aplastic anemia.

Strengths and limitations of this study

- We conducted a comprehensive literature search and strictly adhered to the projected methodology.
- We restricted the study design to randomized controlled trials and prospective non-randomized controlled trials and the studies had to be compatible with 'Mendelian Randomization' to avoid excess risk of bias.
- The included data are too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST.
- The included data were collected 15 to more than 30 years ago. Thus, the results may not be applicable to current modern standard care.

Introduction

Acquired severe aplastic anemia (SAA) is a rare [1] and potentially fatal disease which is characterized by hypocellular bone marrow and pancytopenia; it mainly affects young adults. The estimated incidence rate of SAA ranges from 0.7 to 4.1 per million people per year [2]. The underlying pathophysiology is thought to be an aberrant immune response involving the T-cell mediated destruction of hematopoietic stem cells [3]. Major signs and symptoms are severe infections, bleeding, and exhaustion and patients may experience paleness, weakness, fatigue, and shortness of breath.

According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of the British Committee for Standards in Haematology [4], first-line allogeneic hematopoietic stem cell transplantation (HSCT) from the bone marrow of an human leukocyte antigen (HLA)-matched sibling donor (MSD) is regarded as the initial treatment of choice for newly diagnosed patients with severe aplastic anemia. Graft failure may lead to early death and the conditioning regimen may lead to severe non-hematological organ toxicities.

According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of the British Committee for Standards in Haematology [4], first-line immunosuppressive therapy (IST) is a combination of antithymocyte globulin (ATG) and ciclosporin. First-line IST is indicated for patients where no MSD is available, which can be expected for 70% of patients with SAA [3]. Ciclosporin is an immunosuppressant drug that is not lymphocytotoxic but has specific inhibitory effects on T-lymphocyte function [5]. In the past, antilymphocyte globulin (ALG) was reported interchangeably in the literature alongside ATG, therefore, ALG is reported in the present study on equal terms. ATG as well as ALG are polyclonal antibodies that recognize a variety of human lymphocyte cell surface antigens, reduce the number of lymphocytes and induce an immunosuppressive effect. They originate in animals immunized

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with either normal human thymocytes, collected at pediatric cardiac surgery or thoracic duct lymphocytes, collected during therapeutic cannulation [5]. Concerning the hematologic response and the survival of patients after a first treatment for severe aplastic anemia, it may be crucial in what type of animal ATG originates, as a randomized study showed that rabbit ATG was inferior in this respect to horse ATG [6]. The currently recommended combination of ciclosporin with ATG in the treatment of severe aplastic anemia is based on their separate and potentially complementary modes of action[5]. Some patients do not respond well to IST or show no response at all. Frequent transfusions increase the risk of adverse events such as iron overload and early death. If a diagnosis of SAA is established at an early patient age, then it is crucial to know which treatment promises more benefit and less harm in the long run. We aimed to evaluate the effectiveness and severe adverse events of MSD-HSCT compared to IST in patients with SAA.

Methods

This article is based on a Cochrane Systematic Review published in The Cochrane Library [7]. Publication of this work is in agreement with the policy of The Cochrane Collaboration [8]. While preparing this systematic review and meta-analysis, we endorsed the PRISMA statement, adhered to its principles and conformed to its checklist [9].

Study inclusion criteria

We included randomized controlled trials (RCTs) and prospective non-randomized controlled trials as long as the study design was consistent with the principle of 'Mendelian randomization' in allocating patients to treatment groups. We required a minimum of 80% of relevant patients per group and we set a minimum sample size of five participants per group. We set no limits on language, year of publication, or year of treatment. We included participants with newly diagnosed acquired severe aplastic anemia [4]. We did not set any age limits for participants. We excluded studies on participants with secondary aplastic anemia. We included HSCT as the test intervention harvested from any source of MSD and serving as a first-line therapy [10]. That means, no other HSCT or IST has been offered to the patients before. We included IST as comparator with ciclosporin combined with ATG as the current mode of IST [10]. To accommodate also former modes of IST, we also included ciclosporin combined with ALG, cyclosporine alone, ATG alone, and ALG. Other agents such as corticosteroids and androgens were not considered. The primary outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-host disease, no response to IST, relapse after initial successful treatment, secondary clonal disease or malignancies, health-related quality of life, and performance scores.

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Principle of 'Mendelian randomization'

There are ethical concerns around randomization of patients with severe aplastic anemia to transplantation versus non-transplantation. In general, MSD-HSCT is a life-threatening treatment that can lead to early severe adverse events including death. Gray 1991 [11] and Wheatley 2004 [12] described the potential of 'Mendelian randomization' to minimize bias when comparing MSD-HSCT with an alternative therapy. The base concept has been ascribed to Katan 1986 [13]. 'Mendelian randomization' means the view that nature itself has already 'randomized' the paternal and maternal part of a gene given that donor and recipient are siblings. Thus, 'Mendelian randomization' by definition accepts only siblings as transplant donors and these sibling donors are required to have 'identical' or matched features of specific transplant-relevant HLA sites when compared with the transplant recipient. Therefore, patients with an HLA-matched sibling will be allocated to the MSD-HSCT group. On the other hand, patients with siblings that are not HLA compatible will be allocated to the immunosuppressive therapy group. The term 'Mendelian randomization' refers to the fact that the genetic distribution of paternal and maternal alleles follows a random process and is determined before birth. This concept takes advantage of an instrumental variable for allocating the patients to treatment groups and, at the same time, this variable is neither associated with the treatment nor associated with the outcome.

Search strategy and selection of studies

We conducted an electronic literature database search in MEDLINE (Ovid), EMBASE (Ovid), and Cochrane Library CENTRAL (Wiley) including articles published from inception to 22 April 2013. The corresponding search strategies are depicted in the original Cochrane Review [7]. We retrieved all titles and abstracts by electronic searching and downloaded them to the reference management database EndNote Version X3 [14]. Two authors assessed the eli-

gibility of retrieved papers independently. We considered studies written in languages other than English. We judged studies to be prospective if an explicit statement was reported or there were clues suggesting a prospective design (e.g. prior approval of treatment, informed consent). We judged studies to be retrospective if an explicit statement was reported or it was implied by description that data were reviewed from an existing source. We regarded each of the following items as an indication of a retrospective design: registry reports and reviewing of medical records. We judged studies as consistent with the principle of 'Mendelian randomization' if all transplant donors were clearly siblings and if the allocation of patients to treatment groups was not based on age. We regarded studies as not consistent with the principle of 'Mendelian randomization' if age was not balanced between groups, indicating that age played a role in the group assignment. Example for imbalance: distribution of age categories was statistically not comparable (P value less than 0.05).

Assessment of risk of bias in included studies

Two review authors independently assessed the risk of bias in the included studies using six criteria. We have used four criteria from The Cochrane Collaboration's tool for assessing risk of bias [15]: blinding of outcome assessment, complete outcome data such as missing data, selective reporting such as not reporting pre-specified outcomes, and other sources of bias such as bias related to the specific study design and competing interest. We extended the Cochrane tool for assessing risk of bias with two additional criteria that are specific to the inclusion criteria for the present review and critical for confidence in results: comparable baseline characteristics and concurrent control. We applied The Cochrane Collaboration's criteria for judging risk of bias [16].

Data synthesis

One review author entered the data into Review Manager [17]. Another review author

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checked the entered data. We synthesized data on mortality (MSD-HSCT versus IST) by using the hazard ratio (HR) for time-to-event data as the primary effect measure with a random-effects model. If the hazard ratio was not directly given in the publication, we estimated hazard ratios according to methods proposed by [18] and [19].

Results

Search results

We identified three non-randomized, prospective, parallel, controlled clinical trials (**Figure 1**). Bayever 1984 [20] and Gratwohl 1981 [21] reported their results in a single original article, respectively. Führer 1998 reported five publications including one original article [22], a follow up article [23], one protocol [24], and two abstracts [25 26]. We did not identify any RCTs.

Characteristics of included articles

The main study, patients and interventions characteristics are shown in **Table 1**. The patients were treated and observed between 1976 and 1997. Thus, the reported data were collected more than 15 years ago. Median follow up was not reported. Median age, fraction of males, and median days of time interval between diagnosis and begin of treatment were roughly comparable between the treatment groups within each study. The age ranged from early childhood to young adulthood. In the study by Führer 1998, all patients were less than 17 years old by definition of the inclusion criteria [22]. Bone marrow was used as source for all transplants. All three included studies had a high risk of bias due to the study design (**Table 2**). We judged a low risk of bias for blinding the assessment of overall mortality. Blinding or lack of blinding is not expected to make a difference concerning overall mortality. The authors of all included studies did not report that 'Mendelian randomization' was planned and the authors did not report the size of the involved families. The authors did not report the numbers of siblings and the results of the individual genetic analyses.

Effects of intervention

The pooled hazard ratio estimate for overall mortality was 0.95 with a 95% confidence interval of 0.43 to 2.12 (P value = 0.90) (**Figure 2**). According to the meta-analysis based on data from all three included studies, overall mortality was not statistically significantly different between MSD-HSCT and IST. Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in the IST group (**Table 3**). The results for the secondary outcomes including treatment-related mortality after MSD-HSCT, graft failure after MSD-HSCT, graft-versus-host-disease (GVHD) after MSD-HSCT, no response to IST, and relapse after IST are shown in **Table 4**. With respect to secondary clonal disease or malignancies, Bayever 1984 reported one patient who developed T-cell lymphoma after MSD-HSCT and Führer 1998 reported 4 patients who developed acute myelogenous leukemia after IST. Health-related quality of life questionnaires were not used in any of the included studies. Bayever 1984 reported that almost all evaluable patients in the MSD-HSCT group (92%) and less than half of the patients in the IST group had a Karnofsky Performance Status of higher than 70%.

Discussion

Interpretation of main results

We identified three prospective, non-randomized controlled trials [20-22] including 302 participants; 121 received MSD-HSCT and 181 received IST. Based on these trials we found insufficient evidence to clarify whether MSD-HSCT leads to better overall survival than IST. Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in the IST group and in a meta-analysis, overall mortality was not statistically significantly different between the treatment groups. Treatment-related mortality in the MSD-HSCT group was considerable ranging from 20% to 42%. The graft failure rate was variable and caused the death of 3% to 16% of transplanted patients. GVHD affected a quarter to a half of transplanted patients. More than half of patients in one study did not respond to IST. Relapse affected up to one in eight patients after IST in one study. Secondary clonal disease or malignancies were detected rarely in both treatment groups. Marsh 2009 estimated that allogeneic bone marrow transplantation from an HLA-identical sibling donor provides a 75% to 90% chance of long-term cure in patients younger than 40 years of age [4]. Similarly, in a review of studies with younger patients, Guinan 2009 reported an overall survival between 75% and 95% at three to five years [27]. Sangiolo 2010 concluded that their data favors extending MSD-HSCT to patients older than 40 years of age who are without significant co-morbidities [28]. The results of the studies included in the present systematic review appear to roughly match the recent estimates reported by others.

Recent therapeutic improvement

Bacigalupo 2008 reported that the outcome has improved since 1996 for HSCT but not for IST. Peinemann 2011 identified three studies that reported a statistically significant improve-

ment of overall survival in the group of matched related donor transplants but not in the IST group [29]. Several factors may have contributed to recent improvements in HSCT, such as detailed HLA-matching and less irradiation-based conditioning. The Third Consensus Conference on the treatment of aplastic anemia agreed in 2010 that bone marrow should be used as the source of stem cells and that the upper age limit should be 50 years and that the combination of antithymocyte globulin and ciclosporin remains the gold standard for immunosuppressive therapy [30]. Scheinberg 2012 provided an overview and update of various treatment options for severe aplastic anemia including immunosuppressive therapy and transplantation [31].

Strengths and limitations

One of the strengths of this review is the broadness of the search strategy such that study retrieval bias is very unlikely. We restricted the inclusion to studies to randomized controlled trials and prospective non-randomized controlled trials that were compatible with 'Mendelian Randomization' to avoid excess risk of bias. Nevertheless, the included data are too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST. The rates of adverse events, such as treatment-related mortality, graft failure, no response to IST, and GVHD, are unusually high, which may be explained by the age of the studies (starting in 1976). We did not separate horse ATG from rabbit ATG, although the type of animal as the origin of ATG was reported as a serious effect modifier [6]. All data were collected about 15 up to more than 30 years ago. Thus, the results may not be applicable to current modern standard care. Use of 'Mendelian randomization' is no guarantee that bias is minimized. This may be because tissue typing data may not be accurate. Patients may have only one sibling either in the donor or in the no donor group. Large families have a greater chance of finding a donor. Therefore, designing a non-randomized controlled trial by applying 'Mendelian ran-

domization' requires careful thought to effectively reduce bias and control for potential confounders. There is a time lag in patients with siblings because tissue typing and readiness for assignment to treatment group may possibly take several months [12]. On the other hand, patients with no siblings can be assigned immediately and are at earlier risk for adverse events. Nitsch 2006 described the limits to causal inference based on 'Mendelian randomization' [32].

Conclusions

There are insufficient and biased data that do not allow any firm conclusions to be made about the comparative effectiveness of MSD-HSCT and IST. Patients should be made aware of the early treatment-related mortality and the burden of GVHD after HSCT. Patients treated with IST should also be made aware that the disease may recur after initial successful treatment, and that life-threatening late clonal and malignant disease after IST may occur in a higher percentage compared to HSCT.

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Ethics statement

An ethics statement was not required for this work.

Financial Disclosure

Provision of fulltexts by the University of Cologne, Germany. No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest Statement

No authors have any competing interests.

Data Sharing Statement

No additional data available.

Contributorship Statement

FP: design, search strategy, study selection, data extraction, data analysis, writing the manuscript

AL: methodological perspective, reviewing the manuscript

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Figure legends

Figure 1. Study flow

Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia

Figure 2. Mortality (MSD-HSCT vs. IST); effect: hazard ratio; random-effects model. Standard error calculated from data presented in the Kaplan-Meier graph of the article. Abbreviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error

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Tables

Table 1. Characteristics of included studies

Study ID	Duration of study	Median follow up	Setting, center, country	Patients, no.[1]	Median age, years (range)[1]	Fraction of males, %[1]	Median interval, days[1,2]	Stem cell source	IST components	ATG source
Bayever 1984	1977 to 1982	N.R.	Single, United States	35 vs. 22	17 (2 to 24) vs. 15 (1 to 23)	67 vs. 68	60 vs. 58	bone marrow	ATG	horse
Führer 1998	1993 to 1997	N.R.	Multi, Germany, Austria	28 vs. 86	10.1 (2.3 to 15.8) vs. 9.1 (0.9 to 15.2)	43 vs. 62	49 vs. 23	bone marrow	ATG Ciclosporin	horse
Gratwohl 1981	1976 to 1980	N.R.	Single, Switzerland	19 vs. 13	18 (4 to 29) vs. 23 (7 to 37)	53 vs. 54	105 vs. 180	bone marrow	ATG Ciclosporin	N.R.

[1]MSD-HSCT vs. IST
[2]Median time interval between diagnosis and begin of treatment
Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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Table 2. Risk of bias of included studies

Study ID	Blinding of assessment of overall mortality	Incomplete outcome data	Selective reporting	Other bias	Comparable baseline characteristics	Concurrent control	Overall judgement of bias
Bayever 1984	Low	Low	Unclear	High[1]	Low	Low	High
Führer 1998	Low	High	High[2]	High[3]	Low	Low	High
Gratwohl 1981	Low	High	Unclear	Unclear	Low	Low	High

[1]Bayever 1984: The authors reported the study results at an early time point before all planned data had been gathered: "We present this interim report(...)".
[2]Führer 1998: In the 2005 update, overall survival, secondary clonal disease or malignancies, and also relapse were not reported separately for the 2 distinct treatment groups. Rather, the results were presented for 2 subgroups according to disease severity. This was different from the earlier report of the same study published in 1998 covering the study period from 1993 to 1997. See Fig. 1 and Tab. 1 of the article.
[3]Führer 1998: Financial support was provided by 2 pharmaceutical companies.
Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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Table 3. Overall survival

Study ID	MSD-HSCT		IST		FU[1]	P value
	N	OS (95% CI)	N	OS (95% CI)	Year	
Bayever 1984	35	72% (64 to 80)	22	45% (29 to 61)	2	0.18
Führer 1998	28	84% (N.R.)	86	87% (N.R.)	4	0.43
Gratwohl 1981	19	47% (N.R.)	13	69%[2] (N.R.)	5	0.56[3]

[1]Time point of Kaplan-Meier estimate.
[2]Gratwohl 1981: Two of 13 patients were eligible for MSD-HSCT but donors were not available in the first place; the two patients died after they received a second-line HSCT from the then again available MSD that was offered after the patients showed no response to IST.
[3]The P value was not reported and we calculated the P value using Fisher's exact test.
Abbreviations: CI: confidence interval; FU: follow up; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N: number of analyzed patients; N.R.: not reported; OS: overall survival

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Table 4. Secondary outcomes

Study ID	TRM after MSD-HSCT[1,2]	Graft failure after MSD-HSCT[1]	GVHD after MSD-HSCT[1]	No response to IST[1]	Relapse at 5 years after IST[1]
Bayever 1984	20% (7 of 35)	3% (1 of 35)	51% (17 of 33)	64% (14 of 22)	12.5% (1 of 8)
Führer 1998	N.R.	N.R.	N.R.	N.R.	N.R.
Gratwohl 1981	42% (8 of 19)	16% (3 of 19)	26% (5 of 19)	15% (2 of 13)	N.R.

[1]In parenthesis: number of affected of number of evaluable patients
[2]Treatment-related mortality was not reported for IST.
Abbreviations. GVHD: graft-versus-host disease; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N.R.: not reported; TRM: treatment-related mortality

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Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review*

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Subject: bmjopen-2014-005039-R1: SAA-MSD

Keywords

severe aplastic anemia, stem cell transplantation, bone marrow transplantation, immunosuppressive therapy, systematic review

Strengths and limitations of this study

- We conducted a comprehensive literature search and strictly adhered to the projected methodology.
- We restricted the study design to randomized controlled trials and prospective non-randomized controlled trials and the studies had to be compatible with 'Mendelian Randomization' to avoid excess risk of bias.
- The included data are too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST.
- The included data were collected 15 to more than 30 years ago. Thus, the results may not be applicable to current modern standard care.

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Abstract

Background

Acquired severe aplastic anemia is a rare and potentially fatal disease. The aim of this Cochrane review was to evaluate the effectiveness and adverse events of first-line allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors compared to first-line immunosuppressive therapy.

Procedure

We searched the electronic databases MEDLINE (Ovid), EMBASE (Ovid), and The Cochrane Library CENTRAL (Wiley) for published articles from 1946 to 22 April 2013. We included randomized controlled trials and prospective non-randomized controlled trials as long as the study design was consistent with the principle of 'Mendelian randomization' in allocating patients to treatment groups.

Results

We identified three prospective non-randomized controlled trials with 302 participants. We did not identify a randomized controlled trial. All studies had a high risk of bias due to the study design and were conducted more than 15 years ago and may not be applicable to the standard of care of today. The pooled hazard ratio for overall mortality for the transplanted group versus the not transplanted group was 0.95 (95% confidence interval 0.43 to 2.12, P = 0.90).

Conclusions

There are insufficient and biased data that do not allow any firm conclusions to be made about the comparative effectiveness of first-line allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors and first-line immunosuppressive therapy of patients with acquired severe aplastic anemia.

Introduction

Acquired severe aplastic anemia (SAA) is a rare [1] and potentially fatal disease which is characterized by hypocellular bone marrow and pancytopenia; it mainly affects young adults. The estimated incidence rate of SAA ranges from 0.7 to 4.1 per million people per year [2]. The underlying pathophysiology is thought to be an aberrant immune response involving the T-cell mediated destruction of hematopoietic stem cells [3]. Major signs and symptoms are severe infections, bleeding, and exhaustion and patients may experience paleness, weakness, fatigue, and shortness of breath.

According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of the British Committee for Standards in Haematology [4], first-line allogeneic hematopoietic stem cell transplantation (HSCT) from the bone marrow of an human leukocyte antigen (HLA)-matched sibling donor (MSD) is regarded as the initial treatment of choice for newly diagnosed patients with severe aplastic anemia. Graft failure may lead to early death and the conditioning regimen may lead to severe non-hematological organ toxicities.

According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of the British Committee for Standards in Haematology [4], first-line immunosuppressive therapy (IST) is a combination of antithymocyte globulin (ATG) and ciclosporin. First-line IST ~~ciclosporin and/or antithymocyte or antilymphocyte globulin denoted as first-line immunosuppressive therapy (IST)~~ is indicated for patients where no MSD is available, which can be expected for 70% of patients with SAA [3].

Ciclosporin is an immunosuppressant drug that is not lymphocytotoxic but has specific inhibitory effects on T-lymphocyte function [5]. In the past, antilymphocyte globulin (ALG) was reported interchangeably in the literature alongside ATG, therefore, ALG is reported in

the present study on equal terms. ATG as well as ALG are polyclonal antibodies that recognize a variety of human lymphocyte cell surface antigens, reduce the number of lymphocytes and induce an immunosuppressive effect. They originate in animals immunized with either normal human thymocytes, collected at pediatric cardiac surgery or thoracic duct lymphocytes, collected during therapeutic cannulation [5]. Concerning the hematologic response and the survival of patients after a first treatment for severe aplastic anemia, it may be crucial in what type of animal ATG originates, as a randomized study showed that rabbit ATG was inferior in this respect to horse ATG [6]. The currently recommended combination of ciclosporin with ATG in the treatment of severe aplastic anemia is based on their separate and potentially complementary modes of action[5]. Some patients do not respond well to IST or show no response at all. Frequent transfusions increase the risk of adverse events such as iron overload and early death. If a diagnosis of SAA is established at an early patient age, then it is crucial to know which treatment promises more benefit and less harm in the long run. We aimed to evaluate the effectiveness and severe adverse events of MSD-HSCT compared to IST in patients with SAA.

Methods

This article is based on a Cochrane Systematic Review published in The Cochrane Library [7]. Publication of this work is in agreement with the policy of The Cochrane Collaboration [8]. While preparing this systematic review and meta-analysis, we endorsed the PRISMA statement, adhered to its principles and conformed to its checklist [9].

Study inclusion criteria

We included randomized controlled trials (RCTs) and prospective non-randomized controlled trials as long as the study design was consistent with the principle of 'Mendelian randomization' in allocating patients to treatment groups. We required a minimum of 80% of relevant patients per group and we set a minimum sample size of five participants per group. We set no limits on language, year of publication, or year of treatment. We included participants with newly diagnosed acquired severe aplastic anemia [4]. We did not set any age limits for participants. We excluded studies on participants with secondary aplastic anemia. We included HSCT as the test intervention harvested from any source of MSD and serving as a first-line therapy [10]. That means, no other HSCT or IST has been offered to the patients before. We included IST as comparator with ~~either~~ ciclosporin combined with ATG as the current mode of IST [10]. To accommodate also former modes of IST, we also included ciclosporin combined with ALG, cyclosporine alone, ATG alone, and ALG. Other agents such as corticosteroids and androgens were not considered. ~~antithymocyte/antilymphocyte globulin or ciclosporin or a combination of the two~~ The primary outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-host disease, no response to IST, relapse after initial successful treatment, secondary clonal disease or malignancies, health-related quality of life, and performance scores.

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Principle of 'Mendelian randomization'

There are ethical concerns around randomization of patients with severe aplastic anemia to transplantation versus non-transplantation. In general, MSD-HSCT is a life-threatening treatment that can lead to early severe adverse events including death. Gray 1991 [11] and Wheatley 2004 [12] described the potential of 'Mendelian randomization' to minimize bias when comparing MSD-HSCT with an alternative therapy. The base concept has been ascribed to Katan 1986 [13]. 'Mendelian randomization' means the view that nature itself has already 'randomized' the paternal and maternal part of a gene given that donor and recipient are siblings. Thus, 'Mendelian randomization' by definition accepts only siblings as transplant donors and these sibling donors are required to have 'identical' or matched features of specific transplant-relevant HLA sites when compared with the transplant recipient. Therefore, patients with an HLA-matched sibling will be allocated to the MSD-HSCT group. On the other hand, patients with siblings that are not HLA compatible will be allocated to the immunosuppressive therapy group. The term 'Mendelian randomization' refers to the fact that the genetic distribution of paternal and maternal alleles follows a random process and is determined before birth. This concept takes advantage of an instrumental variable for allocating the patients to treatment groups and, at the same time, this variable is neither associated with the treatment nor associated with the outcome.

Search strategy and selection of studies

We conducted an electronic literature database search in MEDLINE (Ovid), EMBASE (Ovid), and Cochrane Library CENTRAL (Wiley) including articles published from inception to 22 April 2013. The corresponding search strategies are depicted in the original Cochrane Review [7]. We retrieved all titles and abstracts by electronic searching and downloaded them to

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the reference management database EndNote Version X3 [14]. Two authors assessed the eligibility of retrieved papers independently. We considered studies written in languages other than English. We judged studies to be prospective if an explicit statement was reported or there were clues suggesting a prospective design (e.g. prior approval of treatment, informed consent). We judged studies to be retrospective if an explicit statement was reported or it was implied by description that data were reviewed from an existing source. We regarded each of the following items as an indication of a retrospective design: registry reports and reviewing of medical records. ~~Gray 1991 and Wheatley 2004 described the potential of 'Mendelian randomization' to minimize bias when comparing MSD HSCT with an alternative therapy~~ [11 12]~~.~~ We judged studies as consistent with the principle of 'Mendelian randomization' if all transplant donors were clearly siblings and if the allocation of patients to treatment groups was not based on age. We regarded studies as not consistent with the principle of 'Mendelian randomization' if age was not balanced between groups, indicating that age played a role in the group assignment. Example for imbalance: distribution of age categories was statistically not comparable (P value less than 0.05).

Assessment of risk of bias in included studies

Two review authors independently assessed the risk of bias in the included studies using six criteria. We have used four criteria from The Cochrane Collaboration's tool for assessing risk of bias [15]: blinding of outcome assessment, complete outcome data such as missing data, selective reporting such as not reporting pre-specified outcomes, and other sources of bias such as bias related to the specific study design and competing interest. We extended the Cochrane tool for assessing risk of bias with two additional criteria that are specific to the inclusion criteria for the present review and critical for confidence in results: comparable

baseline characteristics and concurrent control. We applied The Cochrane Collaboration's criteria for judging risk of bias [16].

Data synthesis

One review author entered the data into Review Manager [17]. Another review author checked the entered data. We synthesized data on mortality (MSD-HSCT versus IST) by using the hazard ratio (HR) for time-to-event data as the primary effect measure with a random-effects model. If the hazard ratio was not directly given in the publication, we estimated hazard ratios according to methods proposed by [18] and [19].

Results

Search results

We identified three non-randomized, prospective, parallel, controlled clinical trials (**Figure 1**). Bayever 1984 [20] and Gratwohl 1981 [21] reported their results in a single original article, respectively. Führer 1998 reported five publications including one original article [22], a follow up article [23], one protocol [24], and two abstracts [25 26]. We did not identify any RCTs.

Characteristics of included articles

The main study, patients and interventions characteristics are shown in **Table 1**. The patients were treated and observed between 1976 and 1997. Thus, the reported data were collected more than 15 years ago. Median follow up was not reported. Median age, fraction of males, and median days of time interval between diagnosis and begin of treatment were roughly comparable between the treatment groups within each study. The age ranged from early childhood to young adulthood. In the study by Führer 1998, all patients were less than 17 years old by definition of the inclusion criteria [22]. Bone marrow was used as source for all transplants. All three included studies had a high risk of bias due to the study design (**Table 2**). We judged a low risk of bias for blinding the assessment of overall mortality. Blinding or lack of blinding is not expected to make a difference concerning overall mortality. The authors of all included studies did not report that 'Mendelian randomization' was planned and the authors did not report the size of the involved families. The authors did not report the numbers of siblings and the results of the individual genetic analyses.

Effects of intervention

The pooled hazard ratio estimate for overall mortality was 0.95 with a 95% confidence interval of 0.43 to 2.12 (P value = 0.90) (**Figure 2**). According to the meta-analysis based on data from all three included studies, overall mortality was not statistically significantly different between MSD-HSCT and IST. Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in the IST group (**Table 3**). The results for the secondary outcomes including treatment-related mortality after MSD-HSCT, graft failure after MSD-HSCT, graft-versus-host-disease (GVHD) after MSD-HSCT, no response to IST, and relapse after IST are shown in **Table 4**. With respect to secondary clonal disease or malignancies, Bayever 1984 reported one patient who developed T-cell lymphoma after MSD-HSCT and Führer 1998 reported 4 patients who developed acute myelogenous leukemia after IST. Health-related quality of life questionnaires were not used in any of the included studies. Bayever 1984 reported that almost all evaluable patients in the MSD-HSCT group (92%) and less than half of the patients in the IST group had a Karnofsky Performance Status of higher than 70%.

Discussion

Interpretation of main results

We identified three prospective, non-randomized controlled trials [20-22] including 302 participants; 121 received MSD-HSCT and 181 received IST. Based on these trials we found insufficient evidence to clarify whether MSD-HSCT leads to better overall survival than IST. Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in the IST group and in a meta-analysis, overall mortality was not statistically significantly different between the treatment groups. Treatment-related mortality in the MSD-HSCT group was considerable ranging from 20% to 42%. The graft failure rate was variable and caused the death of 3% to 16% of transplanted patients. GVHD affected a quarter to a half of transplanted patients. More than half of patients in one study did not respond to IST. Relapse affected up to one in eight patients after IST in one study. Secondary clonal disease or malignancies were detected rarely in both treatment groups. Marsh 2009 estimated that allogeneic bone marrow transplantation from an HLA-identical sibling donor provides a 75% to 90% chance of long-term cure in patients younger than 40 years of age [4]. Similarly, in a review of studies with younger patients, Guinan 2009 reported an overall survival between 75% and 95% at three to five years [27]. Sangiolo 2010 concluded that their data favors extending MSD-HSCT to patients older than 40 years of age who are without significant co-morbidities [28]. The results of the studies included in the present systematic review appear to roughly match the recent estimates reported by others.

Recent therapeutic improvement

Bacigalupo 2008 reported that the outcome has improved since 1996 for HSCT but not for IST. Peinemann 2011 identified three studies that reported a statistically significant improve-

ment of overall survival in the group of matched related donor transplants but not in the IST group [29]. Several factors may have contributed to recent improvements in HSCT, such as detailed HLA-matching and less irradiation-based conditioning. The Third Consensus Conference on the treatment of aplastic anemia agreed in 2010 that bone marrow should be used as the source of stem cells and that the upper age limit should be 50 years and that the combination of antithymocyte globulin and ciclosporin remains the gold standard for immunosuppressive therapy [30]. Scheinberg 2012 provided an overview and update of various treatment options for severe aplastic anemia including immunosuppressive therapy and transplantation [31].

Strengths and limitations

One of the strengths of this review is the broadness of the search strategy such that study retrieval bias is very unlikely. We restricted the inclusion to studies to randomized controlled trials and prospective non-randomized controlled trials that were compatible with 'Mendelian Randomization' to avoid excess risk of bias. Nevertheless, the included data are too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST. The rates of adverse events, such as treatment-related mortality, graft failure, no response to IST, and GVHD, are unusually high, which may be explained by the age of the studies (starting in 1976). We did not separate horse ATG from rabbit ATG, although the type of animal as the origin of ATG was reported as a serious effect modifier [6]. All data were collected about 15 up to more than 30 years ago. Thus, the results may not be applicable to current modern standard care. Use of 'Mendelian randomization' is no guarantee that bias is minimized. This may be because tissue typing data may not be accurate. Patients may have only one sibling either in the donor or in the no donor group. Large families have a greater chance of finding a donor. Therefore, designing a non-randomized controlled trial by applying 'Mendelian ran-

domization' requires careful thought to effectively reduce bias and control for potential confounders. There is a time lag in patients with siblings because tissue typing and readiness for assignment to treatment group may possibly take several months [12]. On the other hand, patients with no siblings can be assigned immediately and are at earlier risk for adverse events. ~~and~~ Nitsch 2006 described the limits to causal inference based on 'Mendelian randomization' [32].

Conclusions

There are insufficient and biased data that do not allow any firm conclusions to be made about the comparative effectiveness of MSD-HSCT and IST. Patients should be made aware of the early treatment-related mortality and the burden of GVHD after HSCT. Patients treated with IST should also be made aware that the disease may recur after initial successful treatment, and that life-threatening late clonal and malignant disease after IST may occur in a higher percentage compared to HSCT.

Acknowledgments

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Ethics statement

An ethics statement was not required for this work.

Financial Disclosure

Provision of fulltexts by the University of Cologne, Germany. No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest Statement

No authors have any competing interests.

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Figure legends

Figure 1. Study flow

Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia

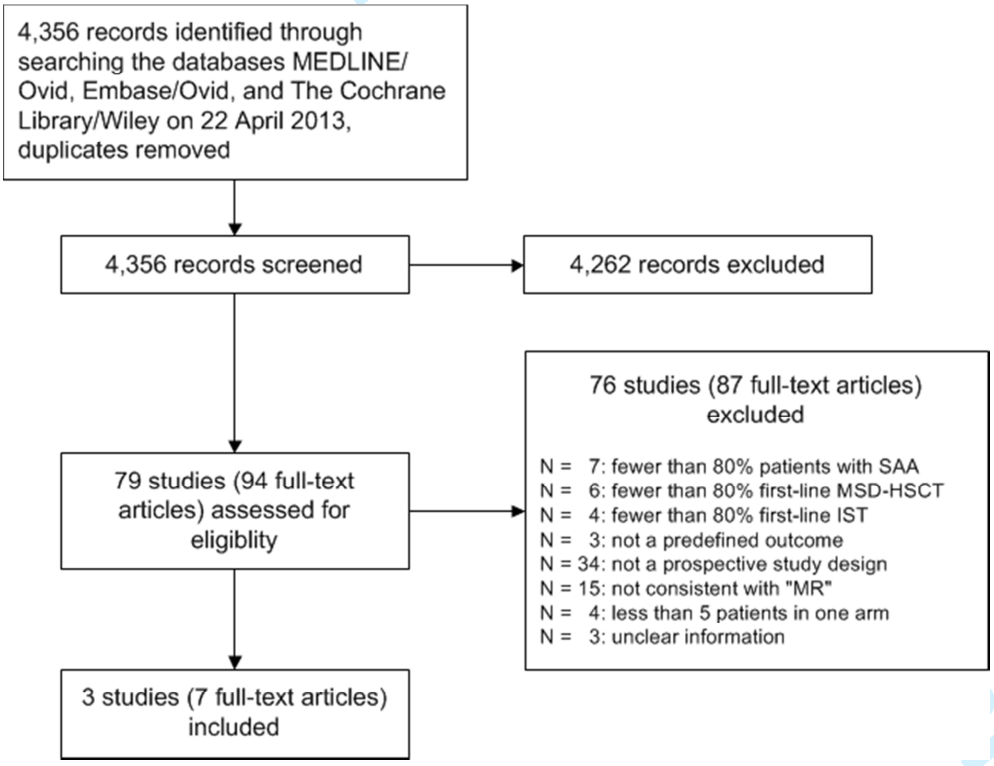

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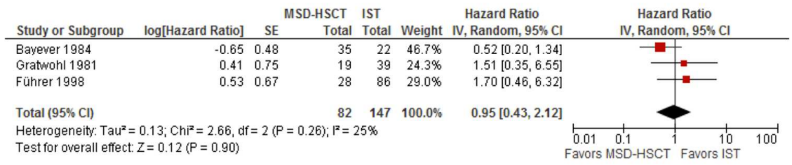
Figure 2. Mortality (MSD-HSCT vs. IST); effect: hazard ratio; random-effects model.

Standard error calculated from data presented in the Kaplan-Meier graph of the article.

Abbreviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error



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Tables

Table 1. Characteristics of included studies

Study ID	Duration of study	Median follow up	Setting, center, country	Patients, no.[1]	Median age, years (range)[1]	Fraction of males, %[1]	Median interval, days[1,2]	Stem cell source	IST components	ATG source
Bayever 1984	1977 to 1982	N.R.	Single, United States	35 vs. 22	17 (2 to 24) vs. ~~vs.~~ 15 (1 to 23)	67 vs. 68	60 vs. 58	bone marrow	ATG	horse
Führer 1998	1993 to 1997	N.R.	Multi, Germany, Austria	28 vs. 86	10.1 (2.3 to 15.8) vs. ~~vs.~~ 9.1 (0.9 to 15.2)	43 vs. 62	49 vs. 23	bone marrow	ATG Ciclosporin	horse
Gratwohl 1981	1976 to 1980	N.R.	Single, Switzerland	19 vs. 13	18 (4 to 29) vs. ~~vs.~~ 23 (7 to 37)	53 vs. 54	105 vs. 180	bone marrow	ATG Ciclosporin	N.R.

[1]MSD-HSCT vs. IST
[2]Median time interval between diagnosis and begin of treatment
Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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Table 2. Risk of bias of included studies

Study ID	Blinding of assessment of overall mortality~~out-come assessment~~	Incomplete outcome data	Selective reporting	Other bias	Comparable baseline characteristics	Concurrent control	Overall judgement of bias
Bayever 1984	~~High~~Low	Low	Unclear	High[1]	Low	Low	High
Führer 1998	~~High~~Low	High	High[2]	High[3]	Low	Low	High
Gratwohl 1981	~~High~~Low	High	Unclear	Unclear	Low	Low	High

[1]Bayever 1984: The authors reported the study results at an early time point before all planned data had been gathered: "We present this interim report(...)".
[2]Führer 1998: In the 2005 update, overall survival, secondary clonal disease or malignancies, and also relapse were not reported separately for the 2 distinct treatment groups. Rather, the results were presented for 2 subgroups according to disease severity. This was different from the earlier report of the same study published in 1998 covering the study period from 1993 to 1997. See Fig. 1 and Tab. 1 of the article.
[3]Führer 1998: Financial support was provided by 2 pharmaceutical companies.
Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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Table 3. Overall survival

Study ID	MSD-HSCT		IST		FU[1]	P value
	N	OS (95% CI)	N	OS (95% CI)	Year	
Bayever 1984	35	72% (64 to 80)	22	45% (29 to 61)	2	0.18
Führer 1998	28	84% (N.R.)	86	87% (N.R.)	4	0.43
Gratwohl 1981	19	47% (N.R.)	13	69%[2] (N.R.)	5	0.56[3]

[1]Time point of Kaplan-Meier estimate.
[2]Gratwohl 1981: Two of 13 patients were eligible for MSD-HSCT but donors were not available in the first place; the two patients died after they received a second-line HSCT from the then again available MSD that was offered after the patients showed no response to IST.
[3]The P value was not reported and we calculated the P value using Fisher's exact test.
Abbreviations: CI: confidence interval; FU: follow up; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N: number of analyzed patients; N.R.: not reported; OS: overall survival

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Table 4. Secondary outcomes

Study ID	TRM after MSD-HSCT[1,2]	Graft failure after MSD-HSCT[1]	GVHD after MSD-HSCT[1]	No response to IST[1]	Relapse at 5 years after IST[1]
Bayever 1984	20% (7 of 35)	3% (1 of 35)	51% (17 of 33)	64% (14 of 22)	12.5% (1 of 8)
Führer 1998	N.R.	N.R.	N.R.	N.R.	N.R.
Gratwohl 1981	42% (8 of 19)	16% (3 of 19)	26% (5 of 19)	15% (2 of 13)	N.R.

[1]In parenthesis: number of affected of number of evaluable patients
[2]Treatment-related mortality was not reported for IST.
Abbreviations. GVHD: graft-versus-host disease; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N.R.: not reported; TRM: treatment-related mortality

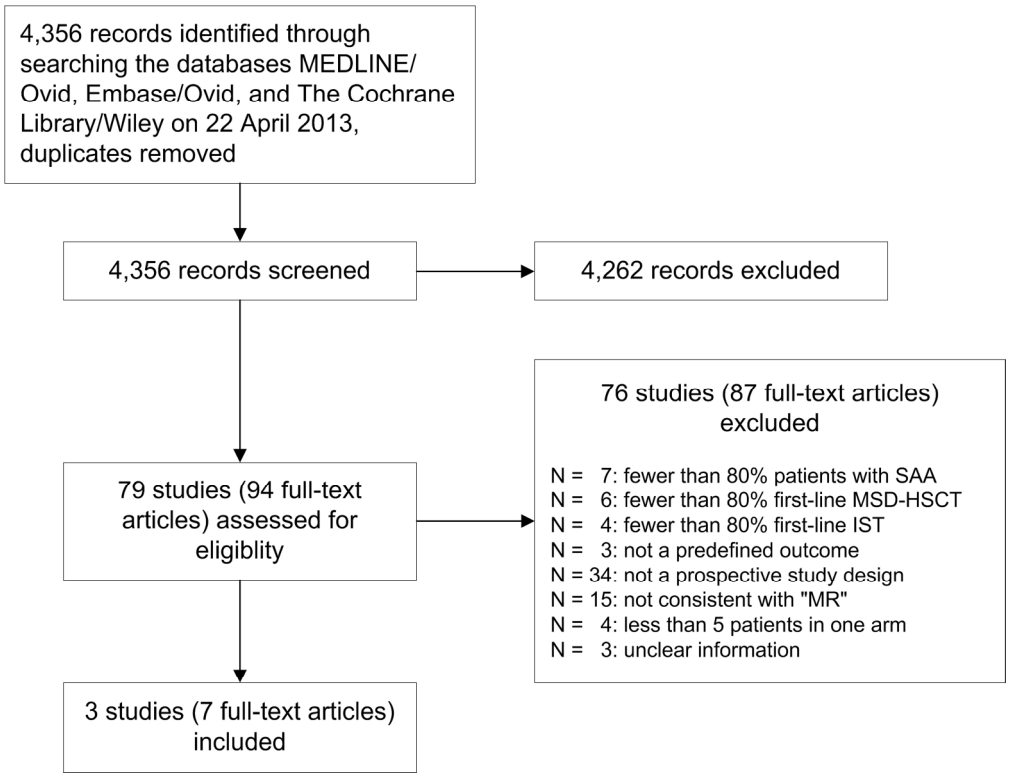

Figure 1. Study flow
Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia
170x130mm (300 x 300 DPI)

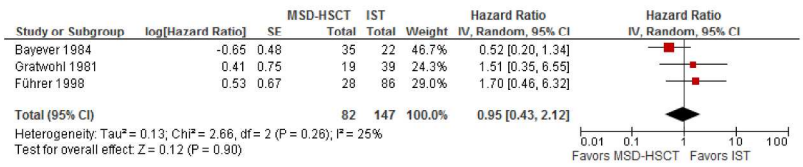

Figure 2. Mortality (MSD-HSCT vs. IST); effect: hazard ratio; random-effects model.
Standard error calculated from data presented in the Kaplan-Meier graph of the article. Ab-breviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error
254x190mm (300 x 300 DPI)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	in the Cochrane review
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6 to 7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7

PRISMA 2009 Checklist

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	in the Cochrane review
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8, table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8 to 9, table 3 to 4, figure 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	figure 1
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	table 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	none
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12
FUNDING			

PRISMA 2009 Checklist

Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	12

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

BMJ Open

Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review

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Subject: bmjopen-2014-005039-R2: SAA-MSD

Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review*

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Keywords

severe aplastic anemia, stem cell transplantation, bone marrow transplantation, immunosuppressive therapy, systematic review

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Abstract

Objectives: Acquired severe aplastic anemia is a rare and potentially fatal disease. The aim of this Cochrane review was to evaluate the effectiveness and adverse events of first-line allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors compared to first-line immunosuppressive therapy.

Setting: Specialised stem cell transplantations units in primary care hospitals

Participants: We included 302 participants with newly diagnosed acquired severe aplastic anemia. The age ranged from early childhood to young adulthood. We excluded studies on participants with secondary aplastic anemia.

Interventions: We included allogeneic haematopoietic stem cell transplantation as the test intervention harvested from any source of matched sibling donor and serving as a first-line therapy. We included immunosuppressive therapy as comparator with either antithymocyte/-antilymphocyte globulin or ciclosporin or a combination of the two.

Primary and secondary outcome measures planned and finally measured: The primary outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-host disease, no response to immunosuppressive therapy, relapse after initial successful treatment, secondary clonal disease or malignancies, health-related quality of life, and performance scores.

Results: We identified three prospective non-randomized controlled trials with a study design that was consistent with the principle of 'Mendelian randomization' in allocating patients to treatment groups. All studies had a high risk of bias due to the study design and were conducted more than 15 years. The pooled hazard ratio for overall mortality for the donor group versus the no donor group was 0.95 (95% confidence interval 0.43 to 2.12, P = 0.90).

Conclusions: There are insufficient and biased data that do not allow any firm conclusions to be made about the comparative effectiveness of first-line allogeneic hematopoietic stem cell

transplantation of HLA-matched sibling donors and first-line immunosuppressive therapy of patients with acquired severe aplastic anemia.

Strengths and limitations of this study

- We conducted a comprehensive literature search and strictly adhered to the projected methodology.
- We restricted the study design to randomized controlled trials and prospective non-randomized controlled trials and the studies had to be compatible with 'Mendelian Randomization' to avoid excess risk of bias.
- The included data are too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST.
- The included data were collected 15 to more than 30 years ago. Thus, the results may not be applicable to current modern standard care.

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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; it mainly affects young adults. The estimated incidence rate of SAA ranges from 0.7 to 4.1 per million people per year [2]. The underlying pathophysiology is thought to be an aberrant immune response involving the T-cell mediated destruction of hematopoietic stem cells [3]. Major signs and symptoms are severe infections, bleeding, and exhaustion and patients may experience paleness, weakness, fatigue, and shortness of breath.

According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of the British Committee for Standards in Haematology [4], first-line allogeneic hematopoietic stem cell transplantation (HSCT) from the bone marrow of an human leukocyte antigen (HLA)-matched sibling donor (MSD) is regarded as the initial treatment of choice for newly diagnosed patients with severe aplastic anemia. Graft failure may lead to early death and the conditioning regimen may lead to severe non-hematological organ toxicities.

According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of the British Committee for Standards in Haematology [4], first-line immunosuppressive therapy (IST) is a combination of antithymocyte globulin (ATG) and ciclosporin. First-line IST is indicated for patients where no MSD is available, which can be expected for 70% of patients with SAA [3]. Ciclosporin is an immunosuppressant drug that is not lymphocytotoxic but has specific inhibitory effects on T-lymphocyte function [5]. In the past, antilymphocyte globulin (ALG) was reported interchangeably in the literature alongside ATG, therefore, ALG is reported in the present study on equal terms. ATG as well as ALG are polyclonal antibodies that recognize a variety of human lymphocyte cell surface antigens, reduce the number of

lymphocytes and induce an immunosuppressive effect. They originate in animals immunized with either normal human thymocytes, collected at pediatric cardiac surgery or thoracic duct lymphocytes, collected during therapeutic cannulation [5]. Concerning the hematologic response and the survival of patients after a first treatment for severe aplastic anemia, it may be crucial in what type of animal ATG originates, as a randomized study showed that rabbit ATG was inferior in this respect to horse ATG [6]. The currently recommended combination of ciclosporin with ATG in the treatment of severe aplastic anemia is based on their separate and potentially complementary modes of action[5]. Some patients do not respond well to IST or show no response at all. Frequent transfusions increase the risk of adverse events such as iron overload and early death. If a diagnosis of SAA is established at an early patient age, then it is crucial to know which treatment promises more benefit and less harm in the long run. We aimed to evaluate the effectiveness and severe adverse events of MSD-HSCT compared to IST in patients with SAA.

Methods

This article is based on a Cochrane Systematic Review published in The Cochrane Library [7]. Publication of this work is in agreement with the policy of The Cochrane Collaboration [8]. While preparing this systematic review and meta-analysis, we endorsed the PRISMA statement, adhered to its principles and conformed to its checklist [9].

Study inclusion criteria

We included randomized controlled trials (RCTs) and prospective non-randomized controlled trials as long as the study design was consistent with the principle of 'Mendelian randomization' in allocating patients to treatment groups. We required a minimum of 80% of relevant patients per group and we set a minimum sample size of five participants per group. We set no limits on language, year of publication, or year of treatment. We included participants with newly diagnosed acquired severe aplastic anemia [4]. We did not set any age limits for participants. We excluded studies on participants with secondary aplastic anemia. We included HSCT as the test intervention harvested from any source of MSD and serving as a first-line therapy [10]. That means, no other HSCT or IST has been offered to the patients before. We included IST as comparator with ciclosporin combined with ATG as the current mode of IST [10]. To accommodate also former modes of IST, we also included ciclosporin combined with ALG, cyclosporine alone, ATG alone, and ALG. Other agents such as corticosteroids and androgens were not considered. The primary outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-host disease, no response to IST, relapse after initial successful treatment, secondary clonal disease or malignancies, health-related quality of life, and performance scores.

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Principle of 'Mendelian randomization'

There are ethical concerns around randomization of patients with severe aplastic anemia to transplantation versus non-transplantation. In general, MSD-HSCT is a life-threatening treatment that can lead to early severe adverse events including death. Gray 1991 [11] and Wheatley 2004 [12] described the potential of 'Mendelian randomization' to minimize bias when comparing MSD-HSCT with an alternative therapy. The base concept has been ascribed to Katan 1986 [13]. 'Mendelian randomization' means the view that nature itself has already 'randomized' the paternal and maternal part of a gene given that donor and recipient are siblings. Thus, 'Mendelian randomization' by definition accepts only siblings as transplant donors and these sibling donors are required to have 'identical' or matched features of specific transplant-relevant HLA sites when compared with the transplant recipient. Therefore, patients with an HLA-matched sibling will be allocated to the MSD-HSCT group. On the other hand, patients with siblings that are not HLA compatible will be allocated to the immunosuppressive therapy group. The term 'Mendelian randomization' refers to the fact that the genetic distribution of paternal and maternal alleles follows a random process and is determined before birth. This concept takes advantage of an instrumental variable for allocating the patients to treatment groups and, at the same time, this variable is neither associated with the treatment nor associated with the outcome.

Search strategy and selection of studies

We conducted an electronic literature database search in MEDLINE (Ovid), EMBASE (Ovid), and Cochrane Library CENTRAL (Wiley) including articles published from inception to 22 April 2013. The corresponding search strategies are depicted in the original Cochrane Review [7]. We retrieved all titles and abstracts by electronic searching and downloaded them to the reference management database EndNote Version X3 [14]. Two authors assessed the eli-

gibility of retrieved papers independently. We considered studies written in languages other than English. We judged studies to be prospective if an explicit statement was reported or there were clues suggesting a prospective design (e.g. prior approval of treatment, informed consent). We judged studies to be retrospective if an explicit statement was reported or it was implied by description that data were reviewed from an existing source. We regarded each of the following items as an indication of a retrospective design: registry reports and reviewing of medical records. We judged studies as consistent with the principle of 'Mendelian randomization' if all transplant donors were clearly siblings and if the allocation of patients to treatment groups was not based on age. We regarded studies as not consistent with the principle of 'Mendelian randomization' if age was not balanced between groups, indicating that age played a role in the group assignment. Example for imbalance: distribution of age categories was statistically not comparable (P value less than 0.05).

Assessment of risk of bias in included studies

Two review authors independently assessed the risk of bias in the included studies using six criteria. We have used four criteria from The Cochrane Collaboration's tool for assessing risk of bias [15]: blinding of outcome assessment, complete outcome data such as missing data, selective reporting such as not reporting pre-specified outcomes, and other sources of bias such as bias related to the specific study design and competing interest. We extended the Cochrane tool for assessing risk of bias with two additional criteria that are specific to the inclusion criteria for the present review and critical for confidence in results: comparable baseline characteristics and concurrent control. We applied The Cochrane Collaboration's criteria for judging risk of bias [16].

Data synthesis

One review author entered the data into Review Manager [17]. Another review author

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checked the entered data. We synthesized data on mortality for the donor group (MSD-HSCT) versus the no donor group (IST) by using the hazard ratio (HR) for time-to-event data as the primary effect measure with a random-effects model. If the hazard ratio was not directly given in the publication, we estimated hazard ratios according to methods proposed by [18] and [19].

Results

Search results

We identified three non-randomized, prospective, parallel, controlled clinical trials (**Figure 1**). Bayever 1984 [20] and Gratwohl 1981 [21] reported their results in a single original article, respectively. Führer 1998 reported five publications including one original article [22], a follow up article [23], one protocol [24], and two abstracts [25 26]. We did not identify any RCTs.

Characteristics of included articles

The main study, patients and interventions characteristics are shown in **Table 1**. The patients were treated and observed between 1976 and 1997. Thus, the reported data were collected more than 15 years ago. Median follow up was not reported. Median age, fraction of males, and median days of time interval between diagnosis and begin of treatment were roughly comparable between the treatment groups within each study. The age ranged from early childhood to young adulthood. In the study by Führer 1998, all patients were less than 17 years old by definition of the inclusion criteria [22]. Bone marrow was used as source for all transplants. All three included studies had a high risk of bias due to the study design (**Table 2**). We judged a low risk of bias for blinding the assessment of overall mortality. Blinding or lack of blinding is not expected to make a difference concerning overall mortality. The authors of all included studies did not report that 'Mendelian randomization' was planned and the authors did not report the size of the involved families. The authors did not report the numbers of siblings and the results of the individual genetic analyses.

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Effects of intervention

The pooled hazard ratio estimate for overall mortality was 0.95 with a 95% confidence interval of 0.43 to 2.12 (P value = 0.90) (**Figure 2**). According to the meta-analysis based on data from all three included studies, overall mortality was not statistically significantly different between MSD-HSCT and IST. Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in the IST group (**Table 3**). The results for the secondary outcomes including treatment-related mortality after MSD-HSCT, graft failure after MSD-HSCT, graft-versus-host-disease (GVHD) after MSD-HSCT, no response to IST, and relapse after IST are shown in **Table 4**. With respect to secondary clonal disease or malignancies, Bayever 1984 reported one patient who developed T-cell lymphoma after MSD-HSCT and Führer 1998 reported 4 patients who developed acute myelogenous leukemia after IST. Health-related quality of life questionnaires were not used in any of the included studies. Bayever 1984 reported that almost all evaluable patients in the MSD-HSCT group (92%) and less than half of the patients in the IST group had a Karnofsky Performance Status of higher than 70%.

Discussion

Interpretation of main results

We identified three prospective, non-randomized controlled trials [20-22] including 302 participants; 121 received MSD-HSCT and 181 received IST. Based on these trials we found insufficient evidence to clarify whether MSD-HSCT leads to better overall survival than IST. Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in the IST group and in a meta-analysis, overall mortality was not statistically significantly different between the treatment groups. Treatment-related mortality in the MSD-HSCT group was considerable ranging from 20% to 42%. The graft failure rate was variable and caused the death of 3% to 16% of transplanted patients. GVHD affected a quarter to a half of transplanted patients. More than half of patients in one study did not respond to IST. Relapse affected up to one in eight patients after IST in one study. Secondary clonal disease or malignancies were detected rarely in both treatment groups. Marsh 2009 estimated that allogeneic bone marrow transplantation from an HLA-identical sibling donor provides a 75% to 90% chance of long-term cure in patients younger than 40 years of age [4]. Similarly, in a review of studies with younger patients, Guinan 2009 reported an overall survival between 75% and 95% at three to five years [27]. Sangiolo 2010 concluded that their data favors extending MSD-HSCT to patients older than 40 years of age who are without significant co-morbidities [28]. The results of the studies included in the present systematic review appear to roughly match the recent estimates reported by others.

Recent therapeutic improvement

Bacigalupo 2008 reported that the outcome has improved since 1996 for HSCT but not for IST. Peinemann 2011 identified three studies that reported a statistically significant improve-

ment of overall survival in the group of matched related donor transplants but not in the IST group [29]. Several factors may have contributed to recent improvements in HSCT, such as detailed HLA-matching and less irradiation-based conditioning. The Third Consensus Conference on the treatment of aplastic anemia agreed in 2010 that bone marrow should be used as the source of stem cells and that the upper age limit should be 50 years and that the combination of antithymocyte globulin and ciclosporin remains the gold standard for immunosuppressive therapy [30]. Scheinberg 2012 provided an overview and update of various treatment options for severe aplastic anemia including immunosuppressive therapy and transplantation [31].

Strengths and limitations

One of the strengths of this review is the broadness of the search strategy such that study retrieval bias is very unlikely. We restricted the inclusion to studies to randomized controlled trials and prospective non-randomized controlled trials that were compatible with 'Mendelian Randomization' to avoid excess risk of bias. We assumed a possible 'Mendelian Randomization' in the three included studies, though, the authors did not mention this approach and did not report what proportion of patients with a matched sibling donor actually received the transplant. Thus, crucial information is lacking to judge the compliance of patients and the significance of the assumed concept of natural allocation. Nevertheless, the included data are too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST. The rates of adverse events, such as treatment-related mortality, graft failure, no response to IST, and GVHD, are unusually high, which may be explained by the age of the studies (starting in 1976). We did not separate horse ATG from rabbit ATG, although the type of animal as the origin of ATG was reported as a serious effect modifier [6]. All data were collected about 15 up to more than 30 years ago. Thus, the results may not be applicable to

current modern standard care. Use of 'Mendelian randomization' is no guarantee that bias is minimized. This may be because tissue typing data may not be accurate. Patients may have only one sibling either in the donor or in the no donor group. Large families have a greater chance of finding a donor. Therefore, designing a non-randomized controlled trial by applying 'Mendelian randomization' requires careful thought to effectively reduce bias and control for potential confounders. There is a time lag in patients with siblings because tissue typing and readiness for assignment to treatment group may possibly take several months [12]. On the other hand, patients with no siblings can be assigned immediately and are at earlier risk for adverse events. Nitsch 2006 described the limits to causal inference based on 'Mendelian randomization' [32].

Conclusions

There are insufficient and biased data that do not allow any firm conclusions to be made about the comparative effectiveness of MSD-HSCT and IST. Patients should be made aware of the early treatment-related mortality and the burden of GVHD after HSCT. Patients treated with IST should also be made aware that the disease may recur after initial successful treatment, and that life-threatening late clonal and malignant disease after IST may occur in a higher percentage compared to HSCT.

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Contributorship Statement

FP: design, search strategy, study selection, data extraction, data analysis, writing the manuscript

AL: methodological perspective, reviewing the manuscript

Conflict of Interest Statement

No authors have any competing interests.

Data Sharing Statement

No additional data available

Ethics statement

An ethics statement was not required for this work.

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Figure legends

Figure 1. Study flow

Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia

Figure 2. Mortality in the donor group (MSD-HSCT) vs. the no donor group (IST); effect: hazard ratio; random-effects model.

Standard error calculated from data presented in the Kaplan-Meier graph of the article.

Abbreviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error

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Tables

Table 1. Characteristics of included studies

Study ID	Duration of study	Median FU	Setting, center, country	Patients, no.[1]	Median age, years (range)[1]	Fraction of males, %[1]	Median interval, days[1,2]	Stem cell source	IST components	ATG source
Bayever 1984	1977 to 1982	NR	Single, United States	35 vs. 22	17 (2 to 24) vs. 15 (1 to 23)	67 vs. 68	60 vs. 58	Bone marrow	ATG	horse
Führer 1998	1993 to 1997	NR	Multi, Germany, Austria	28 vs. 86	10.1 (2.3 to 15.8) vs. 9.1 (0.9 to 15.2)	43 vs. 62	49 vs. 23	Bone marrow	ATG Ciclosporin	horse
Gratwohl 1981	1976 to 1980	NR	Single, Switzerland	19 vs. 13	18 (4 to 29) vs. 23 (7 to 37)	53 vs. 54	105 vs. 180	Bone marrow	ATG Ciclosporin	N.R.

[1]Donor group (MSD-HSCT) versus No donor group (IST)
[2]Median time interval between diagnosis and begin of treatment
Abbreviations. ATG: anti-thymocyte globulin; FU: follow-up; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; NR: not reported; no.: number

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Table 2. Risk of bias of included studies

Study ID	Blinding of assessment of overall mortality	Incomplete outcome data	Selective reporting	Other bias	Comparable baseline characteristics	Concurrent control	Overall judgement of bias
Bayever 1984	Low	Low	Unclear	High[1]	Low	Low	High
Führer 1998	Low	High	High[2]	High[3]	Low	Low	High
Gratwohl 1981	Low	High	Unclear	Unclear	Low	Low	High

[1]Bayever 1984: The authors reported the study results at an early time point before all planned data had been gathered: "We present this interim report(...)".
[2]Führer 1998: In the 2005 update, overall survival, secondary clonal disease or malignancies, and also relapse were not reported separately for the 2 distinct treatment groups. Rather, the results were presented for 2 subgroups according to disease severity. This was different from the earlier report of the same study published in 1998 covering the study period from 1993 to 1997. See Fig. 1 and Tab. 1 of the article.
[3]Führer 1998: Financial support was provided by 2 pharmaceutical companies.
Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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Table 3. Overall survival

Study ID	Donor group (MSD-HSCT)		No donor group (IST)		FU[1]	P value
	N	OS (95% CI)	N	OS (95% CI)	Year	
Bayever 1984	35	72% (64 to 80)	22	45% (29 to 61)	2	0.18
Führer 1998	28	84% (N.R.)	86	87% (N.R.)	4	0.43
Gratwohl 1981	19	47% (N.R.)	13	69%[2] (N.R.)	5	0.56[3]

[1]Time point of Kaplan-Meier estimate.
[2]Gratwohl 1981: Two of 13 patients were eligible for MSD-HSCT but donors were not available in the first place; the two patients died after they received a second-line HSCT from the then again available MSD that was offered after the patients showed no response to IST.
[3]The P value was not reported and we calculated the P value using Fisher's exact test.
Abbreviations: CI: confidence interval; FU: follow up; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N: number of analyzed patients; N.R.: not reported; OS: overall survival

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Table 4. Secondary outcomes

Study ID	TRM after MSD-HSCT[1,2]	Graft failure after MSD-HSCT[1]	GVHD after MSD-HSCT[1]	No response to IST[1]	Relapse at 5 years after IST[1]
Bayever 1984	20% (7 of 35)	3% (1 of 35)	51% (17 of 33)	64% (14 of 22)	12.5% (1 of 8)
Führer 1998	N.R.	N.R.	N.R.	N.R.	N.R.
Gratwohl 1981	42% (8 of 19)	16% (3 of 19)	26% (5 of 19)	15% (2 of 13)	N.R.

[1]In parenthesis: number of affected of number of evaluable patients
[2]Treatment-related mortality was not reported for IST.
Abbreviations. GVHD: graft-versus-host disease; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N.R.: not reported; TRM: treatment-related mortality

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Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review*

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Keywords

severe aplastic anemia, stem cell transplantation, bone marrow transplantation, immunosuppressive therapy, systematic review

Strengths and limitations of this study

- We conducted a comprehensive literature search and strictly adhered to the projected methodology.
- We restricted the study design to randomized controlled trials and prospective non-randomized controlled trials and the studies had to be compatible with 'Mendelian Randomization' to avoid excess risk of bias.
- The included data are too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST.
- The included data were collected 15 to more than 30 years ago. Thus, the results may not be applicable to current modern standard care.

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Abstract

Background

Acquired severe aplastic anemia is a rare and potentially fatal disease. The aim of this Cochrane review was to evaluate the effectiveness and adverse events of first-line allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors compared to first-line immunosuppressive therapy.

Procedure

We searched the electronic databases MEDLINE (Ovid), EMBASE (Ovid), and The Cochrane Library CENTRAL (Wiley) for published articles from 1946 to 22 April 2013. We included randomized controlled trials and prospective non-randomized controlled trials as long as the study design was consistent with the principle of 'Mendelian randomization' in allocating patients to treatment groups.

Results

We identified three prospective non-randomized controlled trials with 302 participants. We did not identify a randomized controlled trial. All studies had a high risk of bias due to the study design and were conducted more than 15 years ago and may not be applicable to the standard of care of today. The pooled hazard ratio for overall mortality for the ~~transplanted~~ donor group versus the ~~not transplanted~~no donor group was 0.95 (95% confidence interval 0.43 to 2.12, P = 0.90).

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Conclusions

There are insufficient and biased data that do not allow any firm conclusions to be made about the comparative effectiveness of first-line allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors and first-line immunosuppressive therapy of patients with acquired severe aplastic anemia.

Introduction

Acquired severe aplastic anemia (SAA) is a rare [1] and potentially fatal disease which is characterized by hypocellular bone marrow and pancytopenia; it mainly affects young adults. The estimated incidence rate of SAA ranges from 0.7 to 4.1 per million people per year [2]. The underlying pathophysiology is thought to be an aberrant immune response involving the T-cell mediated destruction of hematopoietic stem cells [3]. Major signs and symptoms are severe infections, bleeding, and exhaustion and patients may experience paleness, weakness, fatigue, and shortness of breath.

According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of the British Committee for Standards in Haematology [4], first-line allogeneic hematopoietic stem cell transplantation (HSCT) from the bone marrow of an human leukocyte antigen (HLA)-matched sibling donor (MSD) is regarded as the initial treatment of choice for newly diagnosed patients with severe aplastic anemia. Graft failure may lead to early death and the conditioning regimen may lead to severe non-hematological organ toxicities.

According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of the British Committee for Standards in Haematology [4], first-line immunosuppressive therapy (IST) is a combination of antithymocyte globulin (ATG) and ciclosporin. First-line IST is indicated for patients where no MSD is available, which can be expected for 70% of patients with SAA [3]. Ciclosporin is an immunosuppressant drug that is not lymphocytotoxic but has specific inhibitory effects on T-lymphocyte function [5]. In the past, antilymphocyte globulin (ALG) was reported interchangeably in the literature alongside ATG, therefore, ALG is reported in the present study on equal terms. ATG as well as ALG are polyclonal antibodies that recognize a variety of human lymphocyte cell surface antigens, reduce the number of lymphocytes and induce an immunosuppressive effect. They originate in animals immunized

with either normal human thymocytes, collected at pediatric cardiac surgery or thoracic duct lymphocytes, collected during therapeutic cannulation [5]. Concerning the hematologic response and the survival of patients after a first treatment for severe aplastic anemia, it may be crucial in what type of animal ATG originates, as a randomized study showed that rabbit ATG was inferior in this respect to horse ATG [6]. The currently recommended combination of ciclosporin with ATG in the treatment of severe aplastic anemia is based on their separate and potentially complementary modes of action[5]. Some patients do not respond well to IST or show no response at all. Frequent transfusions increase the risk of adverse events such as iron overload and early death. If a diagnosis of SAA is established at an early patient age, then it is crucial to know which treatment promises more benefit and less harm in the long run. We aimed to evaluate the effectiveness and severe adverse events of MSD-HSCT compared to IST in patients with SAA.

Methods

This article is based on a Cochrane Systematic Review published in The Cochrane Library [7]. Publication of this work is in agreement with the policy of The Cochrane Collaboration [8]. While preparing this systematic review and meta-analysis, we endorsed the PRISMA statement, adhered to its principles and conformed to its checklist [9].

Study inclusion criteria

We included randomized controlled trials (RCTs) and prospective non-randomized controlled trials as long as the study design was consistent with the principle of 'Mendelian randomization' in allocating patients to treatment groups. We required a minimum of 80% of relevant patients per group and we set a minimum sample size of five participants per group. We set no limits on language, year of publication, or year of treatment. We included participants with newly diagnosed acquired severe aplastic anemia [4]. We did not set any age limits for participants. We excluded studies on participants with secondary aplastic anemia. We included HSCT as the test intervention harvested from any source of MSD and serving as a first-line therapy [10]. That means, no other HSCT or IST has been offered to the patients before. We included IST as comparator with ciclosporin combined with ATG as the current mode of IST [10]. To accommodate also former modes of IST, we also included ciclosporin combined with ALG, cyclosporine alone, ATG alone, and ALG. Other agents such as corticosteroids and androgens were not considered. The primary outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-host disease, no response to IST, relapse after initial successful treatment, secondary clonal disease or malignancies, health-related quality of life, and performance scores.

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Principle of 'Mendelian randomization'

There are ethical concerns around randomization of patients with severe aplastic anemia to transplantation versus non-transplantation. In general, MSD-HSCT is a life-threatening treatment that can lead to early severe adverse events including death. Gray 1991 [11] and Wheatley 2004 [12] described the potential of 'Mendelian randomization' to minimize bias when comparing MSD-HSCT with an alternative therapy. The base concept has been ascribed to Katan 1986 [13]. 'Mendelian randomization' means the view that nature itself has already 'randomized' the paternal and maternal part of a gene given that donor and recipient are siblings. Thus, 'Mendelian randomization' by definition accepts only siblings as transplant donors and these sibling donors are required to have 'identical' or matched features of specific transplant-relevant HLA sites when compared with the transplant recipient. Therefore, patients with an HLA-matched sibling will be allocated to the MSD-HSCT group. On the other hand, patients with siblings that are not HLA compatible will be allocated to the immunosuppressive therapy group. The term 'Mendelian randomization' refers to the fact that the genetic distribution of paternal and maternal alleles follows a random process and is determined before birth. This concept takes advantage of an instrumental variable for allocating the patients to treatment groups and, at the same time, this variable is neither associated with the treatment nor associated with the outcome.

Search strategy and selection of studies

We conducted an electronic literature database search in MEDLINE (Ovid), EMBASE (Ovid), and Cochrane Library CENTRAL (Wiley) including articles published from inception to 22 April 2013. The corresponding search strategies are depicted in the original Cochrane Review [7]. We retrieved all titles and abstracts by electronic searching and downloaded them to the reference management database EndNote Version X3 [14]. Two authors assessed the eli-

gibility of retrieved papers independently. We considered studies written in languages other than English. We judged studies to be prospective if an explicit statement was reported or there were clues suggesting a prospective design (e.g. prior approval of treatment, informed consent). We judged studies to be retrospective if an explicit statement was reported or it was implied by description that data were reviewed from an existing source. We regarded each of the following items as an indication of a retrospective design: registry reports and reviewing of medical records. We judged studies as consistent with the principle of 'Mendelian randomization' if all transplant donors were clearly siblings and if the allocation of patients to treatment groups was not based on age. We regarded studies as not consistent with the principle of 'Mendelian randomization' if age was not balanced between groups, indicating that age played a role in the group assignment. Example for imbalance: distribution of age categories was statistically not comparable (P value less than 0.05).

Assessment of risk of bias in included studies

Two review authors independently assessed the risk of bias in the included studies using six criteria. We have used four criteria from The Cochrane Collaboration's tool for assessing risk of bias [15]: blinding of outcome assessment, complete outcome data such as missing data, selective reporting such as not reporting pre-specified outcomes, and other sources of bias such as bias related to the specific study design and competing interest. We extended the Cochrane tool for assessing risk of bias with two additional criteria that are specific to the inclusion criteria for the present review and critical for confidence in results: comparable baseline characteristics and concurrent control. We applied The Cochrane Collaboration's criteria for judging risk of bias [16].

Data synthesis

One review author entered the data into Review Manager [17]. Another review author

checked the entered data. We synthesized data on mortality for the donor group (MSD-HSCT) versus the no donor group (IST) by using the hazard ratio (HR) for time-to-event data as the primary effect measure with a random-effects model. If the hazard ratio was not directly given in the publication, we estimated hazard ratios according to methods proposed by [18] and [19].

Results

Search results

We identified three non-randomized, prospective, parallel, controlled clinical trials (**Figure 1**). Bayever 1984 [20] and Gratwohl 1981 [21] reported their results in a single original article, respectively. Führer 1998 reported five publications including one original article [22], a follow up article [23], one protocol [24], and two abstracts [25 26]. We did not identify any RCTs.

Characteristics of included articles

The main study, patients and interventions characteristics are shown in **Table 1**. The patients were treated and observed between 1976 and 1997. Thus, the reported data were collected more than 15 years ago. Median follow up was not reported. Median age, fraction of males, and median days of time interval between diagnosis and begin of treatment were roughly comparable between the treatment groups within each study. The age ranged from early childhood to young adulthood. In the study by Führer 1998, all patients were less than 17 years old by definition of the inclusion criteria [22]. Bone marrow was used as source for all transplants. All three included studies had a high risk of bias due to the study design (**Table 2**). We judged a low risk of bias for blinding the assessment of overall mortality. Blinding or lack of blinding is not expected to make a difference concerning overall mortality. The authors of all included studies did not report that 'Mendelian randomization' was planned and the authors did not report the size of the involved families. The authors did not report the numbers of siblings and the results of the individual genetic analyses.

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Effects of intervention

The pooled hazard ratio estimate for overall mortality was 0.95 with a 95% confidence interval of 0.43 to 2.12 (P value = 0.90) (**Figure 2**). According to the meta-analysis based on data from all three included studies, overall mortality was not statistically significantly different between MSD-HSCT and IST. Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in the IST group (**Table 3**). The results for the secondary outcomes including treatment-related mortality after MSD-HSCT, graft failure after MSD-HSCT, graft-versus-host-disease (GVHD) after MSD-HSCT, no response to IST, and relapse after IST are shown in **Table 4**. With respect to secondary clonal disease or malignancies, Bayever 1984 reported one patient who developed T-cell lymphoma after MSD-HSCT and Führer 1998 reported 4 patients who developed acute myelogenous leukemia after IST. Health-related quality of life questionnaires were not used in any of the included studies. Bayever 1984 reported that almost all evaluable patients in the MSD-HSCT group (92%) and less than half of the patients in the IST group had a Karnofsky Performance Status of higher than 70%.

Discussion

Interpretation of main results

We identified three prospective, non-randomized controlled trials [20-22] including 302 participants; 121 received MSD-HSCT and 181 received IST. Based on these trials we found insufficient evidence to clarify whether MSD-HSCT leads to better overall survival than IST. Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in the IST group and in a meta-analysis, overall mortality was not statistically significantly different between the treatment groups. Treatment-related mortality in the MSD-HSCT group was considerable ranging from 20% to 42%. The graft failure rate was variable and caused the death of 3% to 16% of transplanted patients. GVHD affected a quarter to a half of transplanted patients. More than half of patients in one study did not respond to IST. Relapse affected up to one in eight patients after IST in one study. Secondary clonal disease or malignancies were detected rarely in both treatment groups. Marsh 2009 estimated that allogeneic bone marrow transplantation from an HLA-identical sibling donor provides a 75% to 90% chance of long-term cure in patients younger than 40 years of age [4]. Similarly, in a review of studies with younger patients, Guinan 2009 reported an overall survival between 75% and 95% at three to five years [27]. Sangiolo 2010 concluded that their data favors extending MSD-HSCT to patients older than 40 years of age who are without significant co-morbidities [28]. The results of the studies included in the present systematic review appear to roughly match the recent estimates reported by others.

Recent therapeutic improvement

Bacigalupo 2008 reported that the outcome has improved since 1996 for HSCT but not for IST. Peinemann 2011 identified three studies that reported a statistically significant improve-

ment of overall survival in the group of matched related donor transplants but not in the IST group [29]. Several factors may have contributed to recent improvements in HSCT, such as detailed HLA-matching and less irradiation-based conditioning. The Third Consensus Conference on the treatment of aplastic anemia agreed in 2010 that bone marrow should be used as the source of stem cells and that the upper age limit should be 50 years and that the combination of antithymocyte globulin and ciclosporin remains the gold standard for immunosuppressive therapy [30]. Scheinberg 2012 provided an overview and update of various treatment options for severe aplastic anemia including immunosuppressive therapy and transplantation [31].

Strengths and limitations

One of the strengths of this review is the broadness of the search strategy such that study retrieval bias is very unlikely. We restricted the inclusion to studies to randomized controlled trials and prospective non-randomized controlled trials that were compatible with 'Mendelian Randomization' to avoid excess risk of bias. We assumed a possible 'Mendelian Randomization' in the three included studies, though, the authors did not mention this approach and did not report what proportion of patients with a matched sibling donor actually received the transplant. Thus, crucial information is lacking to judge the compliance of patients and the significance of the assumed concept of natural allocation. Nevertheless, the included data are too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST. The rates of adverse events, such as treatment-related mortality, graft failure, no response to IST, and GVHD, are unusually high, which may be explained by the age of the studies (starting in 1976). We did not separate horse ATG from rabbit ATG, although the type of animal as the origin of ATG was reported as a serious effect modifier [6]. All data were collected about 15 up to more than 30 years ago. Thus, the results may not be applicable to

current modern standard care. Use of 'Mendelian randomization' is no guarantee that bias is minimized. This may be because tissue typing data may not be accurate. Patients may have only one sibling either in the donor or in the no donor group. Large families have a greater chance of finding a donor. Therefore, designing a non-randomized controlled trial by applying 'Mendelian randomization' requires careful thought to effectively reduce bias and control for potential confounders. There is a time lag in patients with siblings because tissue typing and readiness for assignment to treatment group may possibly take several months [12]. On the other hand, patients with no siblings can be assigned immediately and are at earlier risk for adverse events. Nitsch 2006 described the limits to causal inference based on 'Mendelian randomization' [32].

Conclusions

There are insufficient and biased data that do not allow any firm conclusions to be made about the comparative effectiveness of MSD-HSCT and IST. Patients should be made aware of the early treatment-related mortality and the burden of GVHD after HSCT. Patients treated with IST should also be made aware that the disease may recur after initial successful treatment, and that life-threatening late clonal and malignant disease after IST may occur in a higher percentage compared to HSCT.

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Ethics statement

An ethics statement was not required for this work.

Financial Disclosure

Provision of fulltexts by the University of Cologne, Germany. No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest Statement

No authors have any competing interests.

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Figure legends

Figure 1. Study flow

Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia

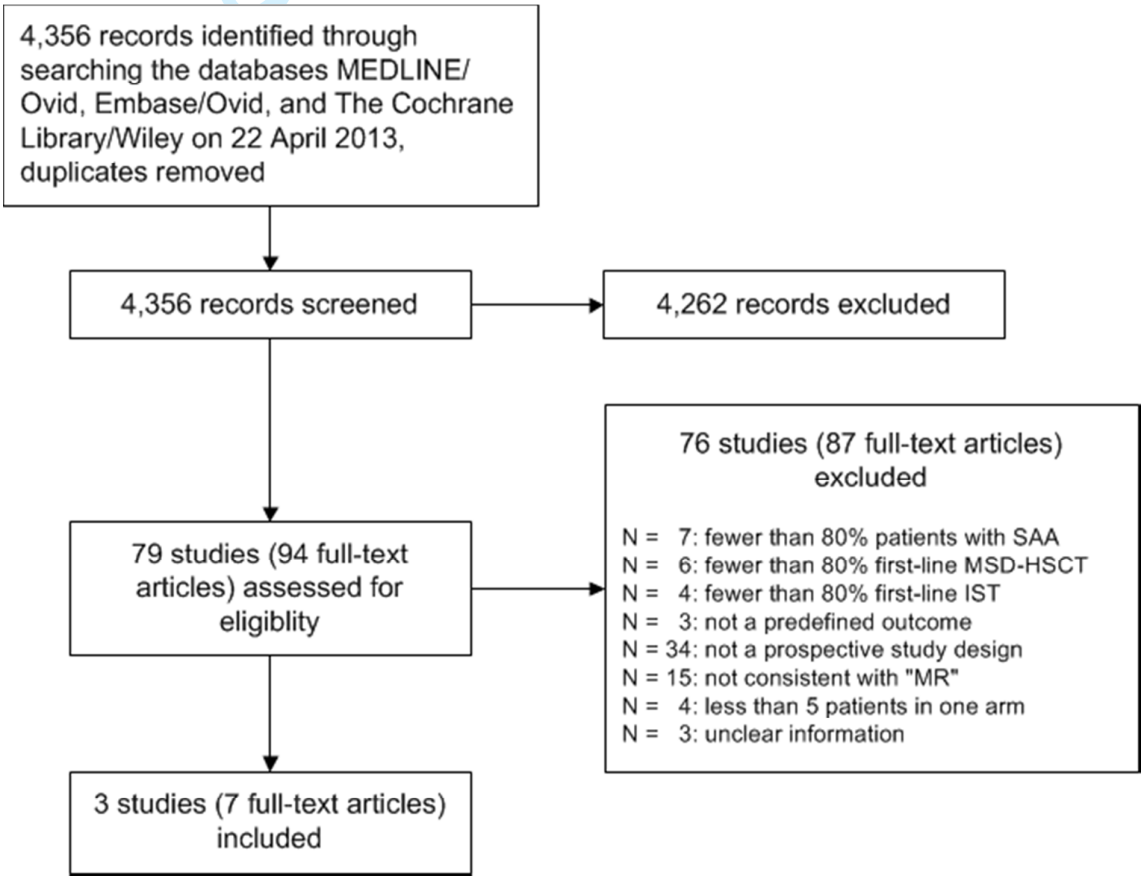

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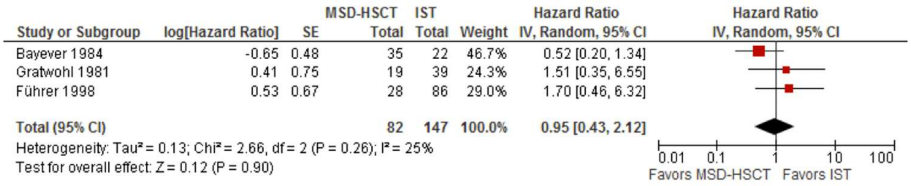
Figure 2. Mortality in the donor group (MSD-HSCT) vs. the no donor group (IST); effect: hazard ratio; random-effects model.

Standard error calculated from data presented in the Kaplan-Meier graph of the article.

Abbreviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error



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Tables

Table 1. Characteristics of included studies

Study ID	Duration of study	Median FU	Setting, center, country	Patients, no.[1]	Median age, years (range)[1]	Fraction of males, %[1]	Median interval, days[1,2]	Stem cell source	IST components	ATG source
Bayever 1984	1977 to 1982	NR	Single, United States	35 vs. 22	17 (2 to 24) vs. 15 (1 to 23)	67 vs. 68	60 vs. 58	Bone marrow	ATG	horse
Führer 1998	1993 to 1997	NR	Multi, Germany, Austria	28 vs. 86	10.1 (2.3 to 15.8) vs. 9.1 (0.9 to 15.2)	43 vs. 62	49 vs. 23	Bone marrow	ATG Ciclosporin	horse
Gratwohl 1981	1976 to 1980	NR	Single, Switzerland	19 vs. 13	18 (4 to 29) vs. 23 (7 to 37)	53 vs. 54	105 vs. 180	Bone marrow	ATG Ciclosporin	N.R.

[1]Donor group (MSD-HSCT) versus No donor group (IST)

[2]Median time interval between diagnosis and begin of treatment

Abbreviations. ATG: anti-thymocyte globulin; FU: follow-up; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; NR: not reported; no.: number

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Table 2. Risk of bias of included studies

Study ID	Blinding of assessment of overall mortality	Incomplete outcome data	Selective reporting	Other bias	Comparable baseline characteristics	Concurrent control	Overall judgement of bias
Bayever 1984	Low	Low	Unclear	High[1]	Low	Low	High
Führer 1998	Low	High	High[2]	High[3]	Low	Low	High
Gratwohl 1981	Low	High	Unclear	Unclear	Low	Low	High

[1]Bayever 1984: The authors reported the study results at an early time point before all planned data had been gathered: "We present this interim report(...)".
[2]Führer 1998: In the 2005 update, overall survival, secondary clonal disease or malignancies, and also relapse were not reported separately for the 2 distinct treatment groups. Rather, the results were presented for 2 subgroups according to disease severity. This was different from the earlier report of the same study published in 1998 covering the study period from 1993 to 1997. See Fig. 1 and Tab. 1 of the article.
[3]Führer 1998: Financial support was provided by 2 pharmaceutical companies.
Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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Table 3. Overall survival

Study ID	Donor group (MSD-HSCT)		No donor group (IST)		FU[1]	P value
	N	OS (95% CI)	N	OS (95% CI)	Year	
Bayever 1984	35	72% (64 to 80)	22	45% (29 to 61)	2	0.18
Führer 1998	28	84% (N.R.)	86	87% (N.R.)	4	0.43
Gratwohl 1981	19	47% (N.R.)	13	69%[2] (N.R.)	5	0.56[3]

[1]Time point of Kaplan-Meier estimate.
[2]Gratwohl 1981: Two of 13 patients were eligible for MSD-HSCT but donors were not available in the first place; the two patients died after they received a second-line HSCT from the then again available MSD that was offered after the patients showed no response to IST.
[3]The P value was not reported and we calculated the P value using Fisher's exact test.
Abbreviations: CI: confidence interval; FU: follow up; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N: number of analyzed patients; N.R.: not reported; OS: overall survival

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Table 4. Secondary outcomes

Study ID	TRM after MSD-HSCT[1,2]	Graft failure after MSD-HSCT[1]	GVHD after MSD-HSCT[1]	No response to IST[1]	Relapse at 5 years after IST[1]
Bayever 1984	20% (7 of 35)	3% (1 of 35)	51% (17 of 33)	64% (14 of 22)	12.5% (1 of 8)
Führer 1998	N.R.	N.R.	N.R.	N.R.	N.R.
Gratwohl 1981	42% (8 of 19)	16% (3 of 19)	26% (5 of 19)	15% (2 of 13)	N.R.

[1]In parenthesis: number of affected of number of evaluable patients

[2]Treatment-related mortality was not reported for IST.

Abbreviations. GVHD: graft-versus-host disease; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N.R.: not reported; TRM: treatment-related mortality

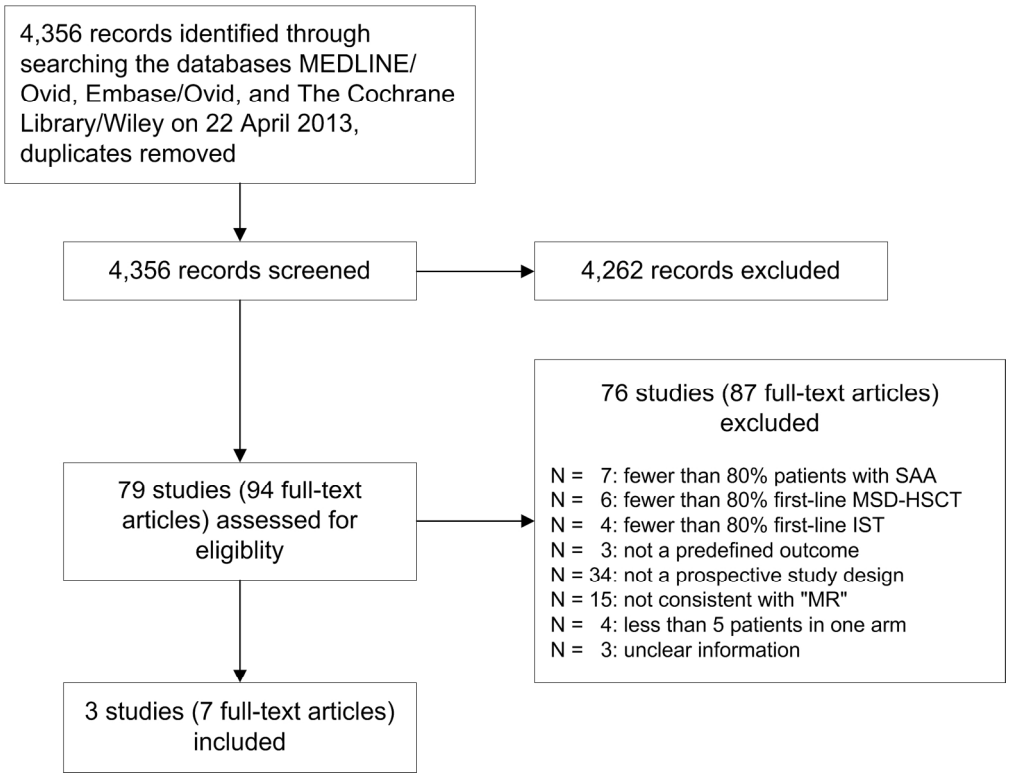

Figure 1. Study flow
Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia
170x130mm (300 x 300 DPI)

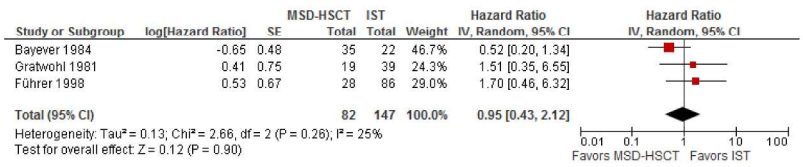

Figure 2. Mortality (MSD-HSCT vs. IST); effect: hazard ratio; random-effects model.
Standard error calculated from data presented in the Kaplan-Meier graph of the article. Ab-breviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error
254x190mm (300 x 300 DPI)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	in the Cochrane review
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6 to 7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7

PRISMA 2009 Checklist

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	in the Cochrane review
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8, table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8 to 9, table 3 to 4, figure 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	figure 1
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	table 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	none
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12
FUNDING			

PRISMA 2009 Checklist

Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	12

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

BMJ Open

Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review

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Secondary Subject Heading:	Paediatrics, Pharmacology and therapeutics
Keywords:	CHEMOTHERAPY, Anaemia < HAEMATOLOGY, Bone marrow transplantation < HAEMATOLOGY, Paediatric oncology < ONCOLOGY, STATISTICS & RESEARCH METHODS, TRANSPLANT MEDICINE

Subject: bmjopen-2014-005039-R3: SAA-MSD

Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review*

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Subject: bmjopen-2014-005039-R3: SAA-MSD

Keywords

severe aplastic anemia, stem cell transplantation, bone marrow transplantation, immunosuppressive therapy, systematic review

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Abstract

Objectives: Acquired severe aplastic anemia is a rare and potentially fatal disease. The aim of this Cochrane review was to evaluate the effectiveness and adverse events of first-line allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors compared to first-line immunosuppressive therapy.

Setting: Specialised stem cell transplantations units in primary care hospitals

Participants: We included 302 participants with newly diagnosed acquired severe aplastic anemia. The age ranged from early childhood to young adulthood. We excluded studies on participants with secondary aplastic anemia.

Interventions: We included allogeneic haematopoietic stem cell transplantation as the test intervention harvested from any source of matched sibling donor and serving as a first-line therapy. We included immunosuppressive therapy as comparator with either antithymocyte/-antilymphocyte globulin or ciclosporin or a combination of the two.

Primary and secondary outcome measures planned and finally measured: The primary outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-host disease, no response to immunosuppressive therapy, relapse after initial successful treatment, secondary clonal disease or malignancies, health-related quality of life, and performance scores.

Results: We identified three prospective non-randomized controlled trials with a study design that was consistent with the principle of 'Mendelian randomization' in allocating patients to treatment groups. All studies had a high risk of bias due to the study design and were conducted more than 15 years. The pooled hazard ratio for overall mortality for the donor

group versus the no donor group was 0.95 (95% confidence interval 0.43 to 2.12, P = 0.90).

Conclusions: There are insufficient and biased data that do not allow any firm conclusions to be made about the comparative effectiveness of first-line allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors and first-line immunosuppressive therapy of patients with acquired severe aplastic anemia.

Strengths and limitations of this study

- We conducted a comprehensive literature search and strictly adhered to the projected methodology.
- We restricted the study design to randomized controlled trials and prospective non-randomized controlled trials and the studies had to be compatible with 'Mendelian Randomization' to avoid excess risk of bias.
- The included data are too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST.
- The included data were collected 15 to more than 30 years ago. Thus, the results may not be applicable to current modern standard care.

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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; it mainly affects young adults. The estimated incidence rate of SAA ranges from 0.7 to 4.1 per million people per year [2]. The underlying pathophysiology is thought to be an aberrant immune response involving the T-cell mediated destruction of hematopoietic stem cells [3]. Major signs and symptoms are severe infections, bleeding, and exhaustion and patients may experience paleness, weakness, fatigue, and shortness of breath.

According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of the British Committee for Standards in Haematology [4], first-line allogeneic hematopoietic stem cell transplantation (HSCT) from the bone marrow of an human leukocyte antigen (HLA)-matched sibling donor (MSD) is regarded as the initial treatment of choice for newly diagnosed patients with severe aplastic anemia. Graft failure may lead to early death and the conditioning regimen may lead to severe non-hematological organ toxicities.

According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of the British Committee for Standards in Haematology [4], first-line immunosuppressive therapy (IST) is a combination of antithymocyte globulin (ATG) and ciclosporin. First-line IST is indicated for patients where no MSD is available, which can be expected for 70% of patients with SAA [3]. Ciclosporin is an immunosuppressant drug that is not lymphocytotoxic but has specific inhibitory effects on T-lymphocyte function [5]. In the past, antilymphocyte globulin (ALG) was reported interchangeably in the literature alongside ATG, therefore, ALG is reported in the present study on equal terms. ATG as well as ALG are polyclonal antibodies that recognize a variety of human lymphocyte cell surface antigens, reduce the number of lymphocytes and induce an immunosuppressive effect. They originate in animals immunized

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with either normal human thymocytes, collected at pediatric cardiac surgery or thoracic duct lymphocytes, collected during therapeutic cannulation [5]. Concerning the hematologic response and the survival of patients after a first treatment for severe aplastic anemia, it may be crucial in what type of animal ATG originates, as a randomized study showed that rabbit ATG was inferior in this respect to horse ATG [6]. The currently recommended combination of ciclosporin with ATG in the treatment of severe aplastic anemia is based on their separate and potentially complementary modes of action[5]. Some patients do not respond well to IST or show no response at all. Frequent transfusions increase the risk of adverse events such as iron overload and early death. If a diagnosis of SAA is established at an early patient age, then it is crucial to know which treatment promises more benefit and less harm in the long run. We aimed to evaluate the effectiveness and severe adverse events of MSD-HSCT compared to IST in patients with SAA.

Methods

This article is based on a Cochrane Systematic Review published in The Cochrane Library [7]. Publication of this work is in agreement with the policy of The Cochrane Collaboration [8]. While preparing this systematic review and meta-analysis, we endorsed the PRISMA statement, adhered to its principles and conformed to its checklist [9].

Study inclusion criteria

We included randomized controlled trials (RCTs) and prospective non-randomized controlled trials as long as the study design was consistent with the principle of 'Mendelian randomization' in allocating patients to treatment groups. We required a minimum of 80% of relevant patients per group and we set a minimum sample size of five participants per group. We set no limits on language, year of publication, or year of treatment. We included participants with newly diagnosed acquired severe aplastic anemia [4]. We did not set any age limits for participants. We excluded studies on participants with secondary aplastic anemia. We included HSCT as the test intervention harvested from any source of MSD and serving as a first-line therapy [10]. That means, no other HSCT or IST has been offered to the patients before. We included IST as comparator with ciclosporin combined with ATG as the current mode of IST [10]. To accommodate also former modes of IST, we also included ciclosporin combined with ALG, cyclosporine alone, ATG alone, and ALG. Other agents such as corticosteroids and androgens were not considered. The primary outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-host disease, no response to IST, relapse after initial successful treatment, secondary clonal disease or malignancies, health-related quality of life, and performance scores.

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Principle of 'Mendelian randomization'

There are ethical concerns around randomization of patients with severe aplastic anemia to transplantation versus non-transplantation because the risk of early death is expected to be higher in the transplantation group than in the non-transplantation group. The reason is the potentially life-threatening graft-versus-host disease occuring only in the transplanted patients. Gray 1991 [11] and Wheatley 2004 [12] described the potential of 'Mendelian randomization' to minimize bias when comparing MSD-HSCT with an alternative therapy. The base concept has been ascribed to Katan 1986 [13]. 'Mendelian randomization' means the view that nature itself has already 'randomized' the paternal and maternal part of a gene given that donor and recipient are siblings. Thus, 'Mendelian randomization' by definition accepts only siblings as transplant donors and these sibling donors are required to have 'identical' or matched features of specific transplant-relevant HLA sites when compared with the transplant recipient. Therefore, patients with an HLA-matched sibling will be allocated to the MSD-HSCT group. On the other hand, patients with siblings that are not HLA compatible will be allocated to the immunosuppressive therapy group. The term 'Mendelian randomization' refers to the fact that the genetic distribution of paternal and maternal alleles follows a random process and is determined before birth. This concept takes advantage of an instrumental variable for allocating the patients to treatment groups and, at the same time, this variable is neither associated with the treatment nor associated with the outcome.

Search strategy and selection of studies

We conducted an electronic literature database search in MEDLINE (Ovid), EMBASE (Ovid), and Cochrane Library CENTRAL (Wiley) including articles published from inception to 22 April 2013. The corresponding search strategies are depicted in the original Cochrane Review [7]. We retrieved all titles and abstracts by electronic searching and downloaded them to

the reference management database EndNote Version X3 [14]. Two authors assessed the eligibility of retrieved papers independently. We considered studies written in languages other than English. We judged studies to be prospective if an explicit statement was reported or there were clues suggesting a prospective design (e.g. prior approval of treatment, informed consent). We judged studies to be retrospective if an explicit statement was reported or it was implied by description that data were reviewed from an existing source. We regarded each of the following items as an indication of a retrospective design: registry reports and reviewing of medical records. We judged studies as consistent with the principle of 'Mendelian randomization' if all transplant donors were clearly siblings and if the allocation of patients to treatment groups was not based on age. We regarded studies as not consistent with the principle of 'Mendelian randomization' if age was not balanced between groups, indicating that age played a role in the group assignment. Example for imbalance: distribution of age categories was statistically not comparable (P value less than 0.05).

Assessment of risk of bias in included studies

Two review authors independently assessed the risk of bias in the included studies using six criteria. We have used four criteria from The Cochrane Collaboration's tool for assessing risk of bias [15]: blinding of outcome assessment, complete outcome data such as missing data, selective reporting such as not reporting pre-specified outcomes, and other sources of bias such as bias related to the specific study design and competing interest. We extended the Cochrane tool for assessing risk of bias with two additional criteria that are specific to the inclusion criteria for the present review and critical for confidence in results: comparable baseline characteristics and concurrent control. We applied The Cochrane Collaboration's criteria for judging risk of bias [16].

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Data synthesis

One review author entered the data into Review Manager [17]. Another review author checked the entered data. We synthesized data on mortality for the donor group (MSD-HSCT) versus the no donor group (IST) by using the hazard ratio (HR) for time-to-event data as the primary effect measure with a random-effects model. If the hazard ratio was not directly given in the publication, we estimated hazard ratios according to methods proposed by [18] and [19].

Results

Search results

We identified three non-randomized, prospective, parallel, controlled clinical trials (**Figure 1**). Bayever 1984 [20] and Gratwohl 1981 [21] reported their results in a single original article, respectively. Führer 1998 reported five publications including one original article [22], a follow up article [23], one protocol [24], and two abstracts [25 26]. We did not identify any RCTs.

Characteristics of included articles

The main study, patients and interventions characteristics are shown in **Table 1**. The patients were treated and observed between 1976 and 1997. Thus, the reported data were collected more than 15 years ago. Median follow up was not reported. Median age, fraction of males, and median days of time interval between diagnosis and begin of treatment were roughly comparable between the treatment groups within each study. The age ranged from early childhood to young adulthood. In the study by Führer 1998, all patients were less than 17 years old by definition of the inclusion criteria [22]. Bone marrow was used as source for all transplants. All three included studies had a high risk of bias due to the study design (**Table 2**). We judged a low risk of bias for blinding the assessment of overall mortality. Blinding or lack of blinding is not expected to make a difference concerning overall mortality. The authors of all included studies did not report that 'Mendelian randomization' was planned and the authors did not report the size of the involved families. The authors did not report the numbers of siblings and the results of the individual genetic analyses.

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Effects of intervention

The pooled hazard ratio estimate for overall mortality was 0.95 with a 95% confidence interval of 0.43 to 2.12 (P value = 0.90) (**Figure 2**). According to the meta-analysis based on data from all three included studies, overall mortality was not statistically significantly different between MSD-HSCT and IST. Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in the IST group (**Table 3**). The results for the secondary outcomes including treatment-related mortality after MSD-HSCT, graft failure after MSD-HSCT, graft-versus-host-disease (GVHD) after MSD-HSCT, no response to IST, and relapse after IST are shown in **Table 4**. With respect to secondary clonal disease or malignancies, Bayever 1984 reported one patient who developed T-cell lymphoma after MSD-HSCT and Führer 1998 reported 4 patients who developed acute myelogenous leukemia after IST. Health-related quality of life questionnaires were not used in any of the included studies. Bayever 1984 reported that almost all evaluable patients in the MSD-HSCT group (92%) and less than half of the patients in the IST group had a Karnofsky Performance Status of higher than 70%.

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Discussion

Interpretation of main results

We identified three prospective, non-randomized controlled trials [20-22] including 302 participants; 121 received MSD-HSCT and 181 received IST. Based on these trials we found insufficient evidence to clarify whether MSD-HSCT leads to better overall survival than IST. Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in the IST group and in a meta-analysis, overall mortality was not statistically significantly different between the treatment groups. Treatment-related mortality in the MSD-HSCT group was considerable ranging from 20% to 42%. The graft failure rate was variable and caused the death of 3% to 16% of transplanted patients. GVHD affected a quarter to a half of transplanted patients. More than half of patients in one study did not respond to IST. Relapse affected up to one in eight patients after IST in one study. Secondary clonal disease or malignancies were detected rarely in both treatment groups. Marsh 2009 estimated that allogeneic bone marrow transplantation from an HLA-identical sibling donor provides a 75% to 90% chance of long-term cure in patients younger than 40 years of age [4]. Similarly, in a review of studies with younger patients, Guinan 2009 reported an overall survival between 75% and 95% at three to five years [27]. Sangiolo 2010 concluded that their data favors extending MSD-HSCT to patients older than 40 years of age who are without significant co-morbidities [28]. The results of the studies included in the present systematic review appear to roughly match the recent estimates reported by others.

Recent therapeutic improvement

Bacigalupo 2008 reported that the outcome has improved since 1996 for HSCT but not for IST. Peinemann 2011 identified three studies that reported a statistically significant improve-

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ment of overall survival in the group of matched related donor transplants but not in the IST group [29]. Several factors may have contributed to recent improvements in HSCT, such as detailed HLA-matching and less irradiation-based conditioning. The Third Consensus Conference on the treatment of aplastic anemia agreed in 2010 that bone marrow should be used as the source of stem cells and that the upper age limit should be 50 years and that the combination of antithymocyte globulin and ciclosporin remains the gold standard for immunosuppressive therapy [30]. Scheinberg 2012 provided an overview and update of various treatment options for severe aplastic anemia including immunosuppressive therapy and transplantation [31].

Strengths and limitations

One of the strengths of this review is the broadness of the search strategy such that study retrieval bias is very unlikely. We restricted the inclusion to studies to randomized controlled trials and prospective non-randomized controlled trials that were compatible with 'Mendelian Randomization' to avoid excess risk of bias. We assumed a possible 'Mendelian Randomization' in the three included studies, though, the authors did not mention this approach and did not report what proportion of patients with a matched sibling donor actually received the transplant. Thus, crucial information is lacking to judge the compliance of patients and the significance of the assumed concept of natural allocation. Nevertheless, the included data are too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST. The rates of adverse events, such as treatment-related mortality, graft failure, no response to IST, and GVHD, are unusually high, which may be explained by the age of the studies (starting in 1976). We did not separate horse ATG from rabbit ATG, although the type of animal as the origin of ATG was reported as a serious effect modifier [6]. All data were collected about 15 up to more than 30 years ago. Thus, the results may not be applicable to

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current modern standard care. Use of 'Mendelian randomization' is no guarantee that bias is minimized. This may be because tissue typing data may not be accurate. Patients may have only one sibling either in the donor or in the no donor group. Large families have a greater chance of finding a donor. Therefore, designing a non-randomized controlled trial by applying 'Mendelian randomization' requires careful thought to effectively reduce bias and control for potential confounders. There is a time lag in patients with siblings because tissue typing and readiness for assignment to treatment group may possibly take several months [12]. On the other hand, patients with no siblings can be assigned immediately and are at earlier risk for adverse events. Nitsch 2006 described the limits to causal inference based on 'Mendelian randomization' [32].

Conclusions

There are insufficient and biased data that do not allow any firm conclusions to be made about the comparative effectiveness of MSD-HSCT and IST. Patients should be made aware of the early treatment-related mortality and the burden of GVHD after HSCT. Patients treated with IST should also be made aware that the disease may recur after initial successful treatment, and that life-threatening late clonal and malignant disease after IST may occur in a higher percentage compared to HSCT.

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Ethics statement

An ethics statement was not required for this work.

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Provision of fulltexts by the University of Cologne, Germany. No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest Statement

No authors have any competing interests.

Contributorship Statement

FP: design, search strategy, study selection, data extraction, data analysis, writing the manuscript
AL: methodological perspective, reviewing the manuscript

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No additional data available.

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Tables

Table 1. Characteristics of included studies

Study ID	Duration of study	Median FU	Setting, center, country	Patients, no.[1]	Median age, years (range)[1]	Fraction of males, %[1]	Median interval, days[1,2]	Stem cell source	IST components	ATG source
Bayever 1984	1977 to 1982	NR	Single, United States	35 vs. 22	17 (2 to 24) vs. 15 (1 to 23)	67 vs. 68	60 vs. 58	Bone marrow	ATG	horse
Führer 1998	1993 to 1997	NR	Multi, Germany, Austria	28 vs. 86	10.1 (2.3 to 15.8) vs. 9.1 (0.9 to 15.2)	43 vs. 62	49 vs. 23	Bone marrow	ATG Ciclosporin	horse
Gratwohl 1981	1976 to 1980	NR	Single, Switzerland	19 vs. 13	18 (4 to 29) vs. 23 (7 to 37)	53 vs. 54	105 vs. 180	Bone marrow	ATG Ciclosporin	N.R.

[1]Donor group (MSD-HSCT) versus No donor group (IST)
[2]Median time interval between diagnosis and begin of treatment
Abbreviations. ATG: anti-thymocyte globulin; FU: follow-up; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; NR: not reported; no.: number

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Table 2. Risk of bias of included studies

Study ID	Blinding of assessment of overall mortality	Incomplete outcome data	Selective reporting	Other bias	Comparable baseline characteristics	Concurrent control	Overall judgement of bias
Bayever 1984	Low	Low	Unclear	High[1]	Low	Low	High
Führer 1998	Low	High	High[2]	High[3]	Low	Low	High
Gratwohl 1981	Low	High	Unclear	Unclear	Low	Low	High

[1]Bayever 1984: The authors reported the study results at an early time point before all planned data had been gathered: "We present this interim report(...)".
[2]Führer 1998: In the 2005 update, overall survival, secondary clonal disease or malignancies, and also relapse were not reported separately for the 2 distinct treatment groups. Rather, the results were presented for 2 subgroups according to disease severity. This was different from the earlier report of the same study published in 1998 covering the study period from 1993 to 1997. See Fig. 1 and Tab. 1 of the article.
[3]Führer 1998: Financial support was provided by 2 pharmaceutical companies.
Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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Table 3. Overall survival

Study ID	Donor group (MSD-HSCT)		No donor group (IST)		FU[1]	P value
	N	OS (95% CI)	N	OS (95% CI)	Year	
Bayever 1984	35	72% (64 to 80)	22	45% (29 to 61)	2	0.18
Führer 1998	28	84% (N.R.)	86	87% (N.R.)	4	0.43
Gratwohl 1981	19	47% (N.R.)	13	69%[2] (N.R.)	5	0.56[3]

[1]Time point of Kaplan-Meier estimate.
[2]Gratwohl 1981: Two of 13 patients were eligible for MSD-HSCT but donors were not available in the first place; the two patients died after they received a second-line HSCT from the then again available MSD that was offered after the patients showed no response to IST.
[3]The P value was not reported and we calculated the P value using Fisher's exact test.
Abbreviations: CI: confidence interval; FU: follow up; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N: number of analyzed patients; N.R.: not reported; OS: overall survival

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Table 4. Secondary outcomes

Study ID	TRM after MSD-HSCT[1,2]	Graft failure after MSD-HSCT[1]	GVHD after MSD-HSCT[1]	No response to IST[1]	Relapse at 5 years after IST[1]
Bayever 1984	20% (7 of 35)	3% (1 of 35)	51% (17 of 33)	64% (14 of 22)	12.5% (1 of 8)
Führer 1998	N.R.	N.R.	N.R.	N.R.	N.R.
Gratwohl 1981	42% (8 of 19)	16% (3 of 19)	26% (5 of 19)	15% (2 of 13)	N.R.

[1]In parenthesis: number of affected of number of evaluable patients
[2]Treatment-related mortality was not reported for IST.
Abbreviations. GVHD: graft-versus-host disease; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N.R.: not reported; TRM: treatment-related mortality

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Figure legends

Figure 1. Study flow

Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia

Figure 2. Mortality in the donor group (MSD-HSCT) vs. the no donor group (IST); effect: hazard ratio; random-effects model.

Standard error calculated from data presented in the Kaplan-Meier graph of the article. Abbreviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error

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Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review*

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Keywords

severe aplastic anemia, stem cell transplantation, bone marrow transplantation, immunosuppressive therapy, systematic review

Strengths and limitations of this study

- We conducted a comprehensive literature search and strictly adhered to the projected methodology.
- We restricted the study design to randomized controlled trials and prospective non-randomized controlled trials and the studies had to be compatible with 'Mendelian Randomization' to avoid excess risk of bias.
- The included data are too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST.
- The included data were collected 15 to more than 30 years ago. Thus, the results may not be applicable to current modern standard care.

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Abstract

Background

Acquired severe aplastic anemia is a rare and potentially fatal disease. The aim of this Cochrane review was to evaluate the effectiveness and adverse events of first-line allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors compared to first-line immunosuppressive therapy.

Procedure

We searched the electronic databases MEDLINE (Ovid), EMBASE (Ovid), and The Cochrane Library CENTRAL (Wiley) for published articles from 1946 to 22 April 2013. We included randomized controlled trials and prospective non-randomized controlled trials as long as the study design was consistent with the principle of 'Mendelian randomization' in allocating patients to treatment groups.

Results

We identified three prospective non-randomized controlled trials with 302 participants. We did not identify a randomized controlled trial. All studies had a high risk of bias due to the study design and were conducted more than 15 years ago and may not be applicable to the standard of care of today. The pooled hazard ratio for overall mortality for the donor group versus the no donor group was 0.95 (95% confidence interval 0.43 to 2.12, P = 0.90).

Conclusions

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There are insufficient and biased data that do not allow any firm conclusions to be made about the comparative effectiveness of first-line allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors and first-line immunosuppressive therapy of patients with acquired severe aplastic anemia.

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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; it mainly affects young adults. The estimated incidence rate of SAA ranges from 0.7 to 4.1 per million people per year [2]. The underlying pathophysiology is thought to be an aberrant immune response involving the T-cell mediated destruction of hematopoietic stem cells [3]. Major signs and symptoms are severe infections, bleeding, and exhaustion and patients may experience paleness, weakness, fatigue, and shortness of breath.

According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of the British Committee for Standards in Haematology [4], first-line allogeneic hematopoietic stem cell transplantation (HSCT) from the bone marrow of an human leukocyte antigen (HLA)-matched sibling donor (MSD) is regarded as the initial treatment of choice for newly diagnosed patients with severe aplastic anemia. Graft failure may lead to early death and the conditioning regimen may lead to severe non-hematological organ toxicities.

According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of the British Committee for Standards in Haematology [4], first-line immunosuppressive therapy (IST) is a combination of antithymocyte globulin (ATG) and ciclosporin. First-line IST is indicated for patients where no MSD is available, which can be expected for 70% of patients with SAA [3]. Ciclosporin is an immunosuppressant drug that is not lymphocytotoxic but has specific inhibitory effects on T-lymphocyte function [5]. In the past, antilymphocyte globulin (ALG) was reported interchangeably in the literature alongside ATG, therefore, ALG is reported in the present study on equal terms. ATG as well as ALG are polyclonal antibodies that recognize a variety of human lymphocyte cell surface antigens, reduce the number of lymphocytes and induce an immunosuppressive effect. They originate in animals immunized

with either normal human thymocytes, collected at pediatric cardiac surgery or thoracic duct lymphocytes, collected during therapeutic cannulation [5]. Concerning the hematologic response and the survival of patients after a first treatment for severe aplastic anemia, it may be crucial in what type of animal ATG originates, as a randomized study showed that rabbit ATG was inferior in this respect to horse ATG [6]. The currently recommended combination of ciclosporin with ATG in the treatment of severe aplastic anemia is based on their separate and potentially complementary modes of action[5]. Some patients do not respond well to IST or show no response at all. Frequent transfusions increase the risk of adverse events such as iron overload and early death. If a diagnosis of SAA is established at an early patient age, then it is crucial to know which treatment promises more benefit and less harm in the long run. We aimed to evaluate the effectiveness and severe adverse events of MSD-HSCT compared to IST in patients with SAA.

Methods

This article is based on a Cochrane Systematic Review published in The Cochrane Library [7]. Publication of this work is in agreement with the policy of The Cochrane Collaboration [8]. While preparing this systematic review and meta-analysis, we endorsed the PRISMA statement, adhered to its principles and conformed to its checklist [9].

Study inclusion criteria

We included randomized controlled trials (RCTs) and prospective non-randomized controlled trials as long as the study design was consistent with the principle of 'Mendelian randomization' in allocating patients to treatment groups. We required a minimum of 80% of relevant patients per group and we set a minimum sample size of five participants per group. We set no limits on language, year of publication, or year of treatment. We included participants with newly diagnosed acquired severe aplastic anemia [4]. We did not set any age limits for participants. We excluded studies on participants with secondary aplastic anemia. We included HSCT as the test intervention harvested from any source of MSD and serving as a first-line therapy [10]. That means, no other HSCT or IST has been offered to the patients before. We included IST as comparator with ciclosporin combined with ATG as the current mode of IST [10]. To accommodate also former modes of IST, we also included ciclosporin combined with ALG, cyclosporine alone, ATG alone, and ALG. Other agents such as corticosteroids and androgens were not considered. The primary outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-host disease, no response to IST, relapse after initial successful treatment, secondary clonal disease or malignancies, health-related quality of life, and performance scores.

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Principle of 'Mendelian randomization'

There are ethical concerns around randomization of patients with severe aplastic anemia to transplantation versus non-transplantation because the risk of early death is expected to be higher in the transplantation group than in the non-transplantation group. The reason is the potentially life-threatening graft-versus-host disease occuring only in the transplanted patients. ~~In general, MSD HSCT is a life threatening treatment that can lead to early severe adverse events including death.~~ Gray 1991 [11] and Wheatley 2004 [12] described the potential of 'Mendelian randomization' to minimize bias when comparing MSD-HSCT with an alternative therapy. The base concept has been ascribed to Katan 1986 [13]. 'Mendelian randomization' means the view that nature itself has already 'randomized' the paternal and maternal part of a gene given that donor and recipient are siblings. Thus, 'Mendelian randomization' by definition accepts only siblings as transplant donors and these sibling donors are required to have 'identical' or matched features of specific transplant-relevant HLA sites when compared with the transplant recipient. Therefore, patients with an HLA-matched sibling will be allocated to the MSD-HSCT group. On the other hand, patients with siblings that are not HLA compatible will be allocated to the immunosuppressive therapy group. The term 'Mendelian randomization' refers to the fact that the genetic distribution of paternal and maternal alleles follows a random process and is determined before birth. This concept takes advantage of an instrumental variable for allocating the patients to treatment groups and, at the same time, this variable is neither associated with the treatment nor associated with the outcome.

Search strategy and selection of studies

We conducted an electronic literature database search in MEDLINE (Ovid), EMBASE (Ovid), and Cochrane Library CENTRAL (Wiley) including articles published from inception to

22 April 2013. The corresponding search strategies are depicted in the original Cochrane Review [7]. We retrieved all titles and abstracts by electronic searching and downloaded them to the reference management database EndNote Version X3 [14]. Two authors assessed the eligibility of retrieved papers independently. We considered studies written in languages other than English. We judged studies to be prospective if an explicit statement was reported or there were clues suggesting a prospective design (e.g. prior approval of treatment, informed consent). We judged studies to be retrospective if an explicit statement was reported or it was implied by description that data were reviewed from an existing source. We regarded each of the following items as an indication of a retrospective design: registry reports and reviewing of medical records. We judged studies as consistent with the principle of 'Mendelian randomization' if all transplant donors were clearly siblings and if the allocation of patients to treatment groups was not based on age. We regarded studies as not consistent with the principle of 'Mendelian randomization' if age was not balanced between groups, indicating that age played a role in the group assignment. Example for imbalance: distribution of age categories was statistically not comparable (P value less than 0.05).

Assessment of risk of bias in included studies

Two review authors independently assessed the risk of bias in the included studies using six criteria. We have used four criteria from The Cochrane Collaboration's tool for assessing risk of bias [15]: blinding of outcome assessment, complete outcome data such as missing data, selective reporting such as not reporting pre-specified outcomes, and other sources of bias such as bias related to the specific study design and competing interest. We extended the Cochrane tool for assessing risk of bias with two additional criteria that are specific to the inclusion criteria for the present review and critical for confidence in results: comparable

baseline characteristics and concurrent control. We applied The Cochrane Collaboration's criteria for judging risk of bias [16].

Data synthesis

One review author entered the data into Review Manager [17]. Another review author checked the entered data. We synthesized data on mortality for the donor group (MSD-HSCT) versus the no donor group (IST) by using the hazard ratio (HR) for time-to-event data as the primary effect measure with a random-effects model. If the hazard ratio was not directly given in the publication, we estimated hazard ratios according to methods proposed by [18] and [19].

Results

Search results

We identified three non-randomized, prospective, parallel, controlled clinical trials (**Figure 1**). Bayever 1984 [20] and Gratwohl 1981 [21] reported their results in a single original article, respectively. Führer 1998 reported five publications including one original article [22], a follow up article [23], one protocol [24], and two abstracts [25 26]. We did not identify any RCTs.

Characteristics of included articles

The main study, patients and interventions characteristics are shown in **Table 1**. The patients were treated and observed between 1976 and 1997. Thus, the reported data were collected more than 15 years ago. Median follow up was not reported. Median age, fraction of males, and median days of time interval between diagnosis and begin of treatment were roughly comparable between the treatment groups within each study. The age ranged from early childhood to young adulthood. In the study by Führer 1998, all patients were less than 17 years old by definition of the inclusion criteria [22]. Bone marrow was used as source for all transplants. All three included studies had a high risk of bias due to the study design (**Table 2**). We judged a low risk of bias for blinding the assessment of overall mortality. Blinding or lack of blinding is not expected to make a difference concerning overall mortality. The authors of all included studies did not report that 'Mendelian randomization' was planned and the authors did not report the size of the involved families. The authors did not report the numbers of siblings and the results of the individual genetic analyses.

Effects of intervention

The pooled hazard ratio estimate for overall mortality was 0.95 with a 95% confidence interval of 0.43 to 2.12 (P value = 0.90) (**Figure 2**). According to the meta-analysis based on data from all three included studies, overall mortality was not statistically significantly different between MSD-HSCT and IST. Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in the IST group (**Table 3**). The results for the secondary outcomes including treatment-related mortality after MSD-HSCT, graft failure after MSD-HSCT, graft-versus-host-disease (GVHD) after MSD-HSCT, no response to IST, and relapse after IST are shown in **Table 4**. With respect to secondary clonal disease or malignancies, Bayever 1984 reported one patient who developed T-cell lymphoma after MSD-HSCT and Führer 1998 reported 4 patients who developed acute myelogenous leukemia after IST. Health-related quality of life questionnaires were not used in any of the included studies. Bayever 1984 reported that almost all evaluable patients in the MSD-HSCT group (92%) and less than half of the patients in the IST group had a Karnofsky Performance Status of higher than 70%.

Discussion

Interpretation of main results

We identified three prospective, non-randomized controlled trials [20-22] including 302 participants; 121 received MSD-HSCT and 181 received IST. Based on these trials we found insufficient evidence to clarify whether MSD-HSCT leads to better overall survival than IST. Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in the IST group and in a meta-analysis, overall mortality was not statistically significantly different between the treatment groups. Treatment-related mortality in the MSD-HSCT group was considerable ranging from 20% to 42%. The graft failure rate was variable and caused the death of 3% to 16% of transplanted patients. GVHD affected a quarter to a half of transplanted patients. More than half of patients in one study did not respond to IST. Relapse affected up to one in eight patients after IST in one study. Secondary clonal disease or malignancies were detected rarely in both treatment groups. Marsh 2009 estimated that allogeneic bone marrow transplantation from an HLA-identical sibling donor provides a 75% to 90% chance of long-term cure in patients younger than 40 years of age [4]. Similarly, in a review of studies with younger patients, Guinan 2009 reported an overall survival between 75% and 95% at three to five years [27]. Sangiolo 2010 concluded that their data favors extending MSD-HSCT to patients older than 40 years of age who are without significant co-morbidities [28]. The results of the studies included in the present systematic review appear to roughly match the recent estimates reported by others.

Recent therapeutic improvement

Bacigalupo 2008 reported that the outcome has improved since 1996 for HSCT but not for IST. Peinemann 2011 identified three studies that reported a statistically significant improve-

ment of overall survival in the group of matched related donor transplants but not in the IST group [29]. Several factors may have contributed to recent improvements in HSCT, such as detailed HLA-matching and less irradiation-based conditioning. The Third Consensus Conference on the treatment of aplastic anemia agreed in 2010 that bone marrow should be used as the source of stem cells and that the upper age limit should be 50 years and that the combination of antithymocyte globulin and ciclosporin remains the gold standard for immunosuppressive therapy [30]. Scheinberg 2012 provided an overview and update of various treatment options for severe aplastic anemia including immunosuppressive therapy and transplantation [31].

Strengths and limitations

One of the strengths of this review is the broadness of the search strategy such that study retrieval bias is very unlikely. We restricted the inclusion to studies to randomized controlled trials and prospective non-randomized controlled trials that were compatible with 'Mendelian Randomization' to avoid excess risk of bias. We assumed a possible 'Mendelian Randomization' in the three included studies, though, the authors did not mention this approach and did not report what proportion of patients with a matched sibling donor actually received the transplant. Thus, crucial information is lacking to judge the compliance of patients and the significance of the assumed concept of natural allocation. Nevertheless, the included data are too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST. The rates of adverse events, such as treatment-related mortality, graft failure, no response to IST, and GVHD, are unusually high, which may be explained by the age of the studies (starting in 1976). We did not separate horse ATG from rabbit ATG, although the type of animal as the origin of ATG was reported as a serious effect modifier [6]. All data were collected about 15 up to more than 30 years ago. Thus, the results may not be applicable to

current modern standard care. Use of 'Mendelian randomization' is no guarantee that bias is minimized. This may be because tissue typing data may not be accurate. Patients may have only one sibling either in the donor or in the no donor group. Large families have a greater chance of finding a donor. Therefore, designing a non-randomized controlled trial by applying 'Mendelian randomization' requires careful thought to effectively reduce bias and control for potential confounders. There is a time lag in patients with siblings because tissue typing and readiness for assignment to treatment group may possibly take several months [12]. On the other hand, patients with no siblings can be assigned immediately and are at earlier risk for adverse events. Nitsch 2006 described the limits to causal inference based on 'Mendelian randomization' [32].

Conclusions

There are insufficient and biased data that do not allow any firm conclusions to be made about the comparative effectiveness of MSD-HSCT and IST. Patients should be made aware of the early treatment-related mortality and the burden of GVHD after HSCT. Patients treated with IST should also be made aware that the disease may recur after initial successful treatment, and that life-threatening late clonal and malignant disease after IST may occur in a higher percentage compared to HSCT.

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Acknowledgments

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Ethics statement

An ethics statement was not required for this work.

Financial Disclosure

Provision of fulltexts by the University of Cologne, Germany. No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest Statement

No authors have any competing interests.

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Figure legends

Figure 1. Study flow

Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia

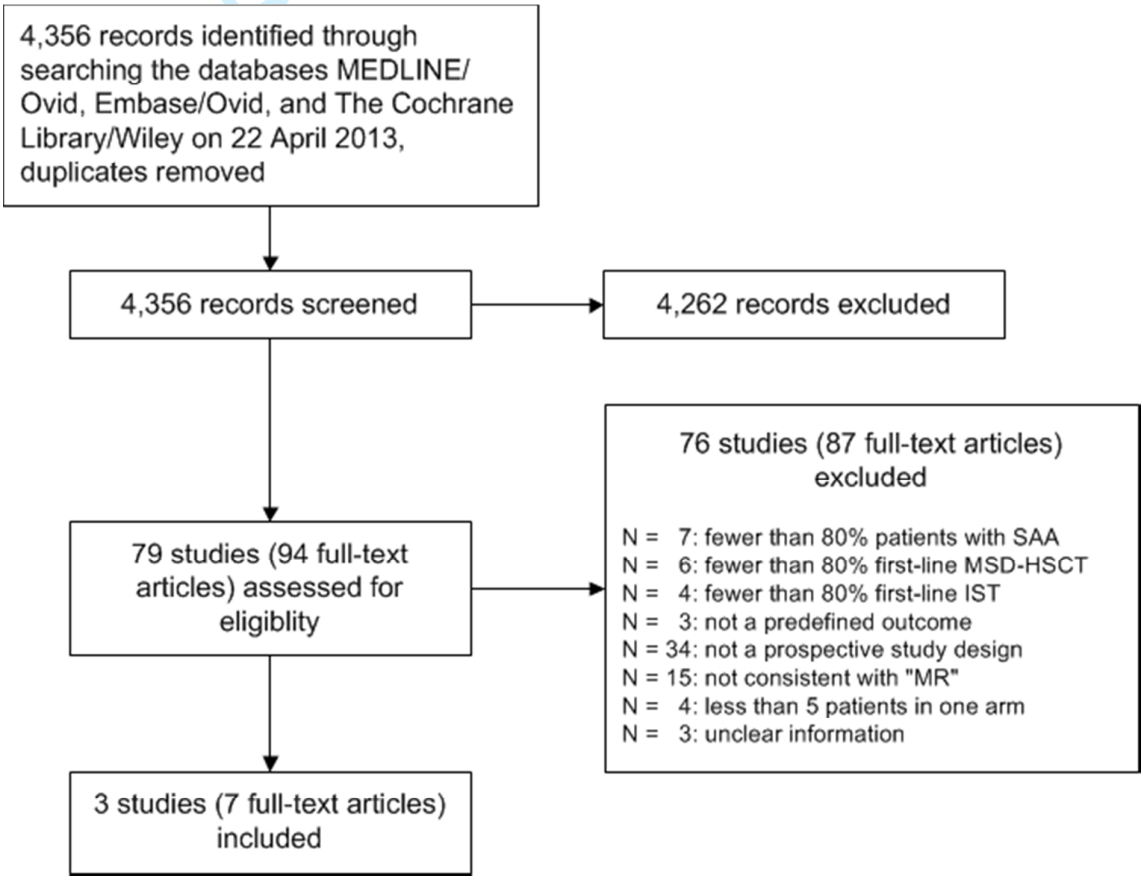

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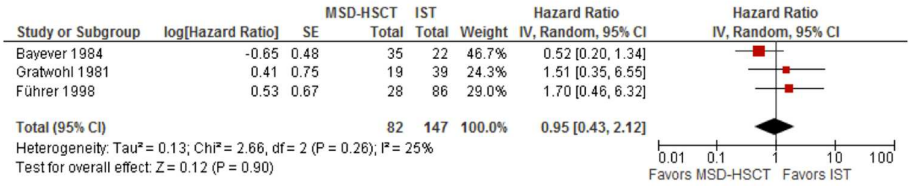
Figure 2. Mortality in the donor group (MSD-HSCT) vs. the no donor group (IST); effect: hazard ratio; random-effects model.

Standard error calculated from data presented in the Kaplan-Meier graph of the article.

Abbreviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error



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Tables

Table 1. Characteristics of included studies

Study ID	Duration of study	Median FU	Setting, center, country	Patients, no.[1]	Median age, years (range)[1]	Fraction of males, %[1]	Median interval, days[1,2]	Stem cell source	IST components	ATG source
Bayever 1984	1977 to 1982	NR	Single, United States	35 vs. 22	17 (2 to 24) vs. 15 (1 to 23)	67 vs. 68	60 vs. 58	Bone marrow	ATG	horse
Führer 1998	1993 to 1997	NR	Multi, Germany, Austria	28 vs. 86	10.1 (2.3 to 15.8) vs. 9.1 (0.9 to 15.2)	43 vs. 62	49 vs. 23	Bone marrow	ATG Ciclosporin	horse
Gratwohl 1981	1976 to 1980	NR	Single, Switzerland	19 vs. 13	18 (4 to 29) vs. 23 (7 to 37)	53 vs. 54	105 vs. 180	Bone marrow	ATG Ciclosporin	N.R.

[1]Donor group (MSD-HSCT) versus No donor group (IST)
[2]Median time interval between diagnosis and begin of treatment
Abbreviations. ATG: anti-thymocyte globulin; FU: follow-up; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; NR: not reported; no.: number

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Table 2. Risk of bias of included studies

Study ID	Blinding of assessment of overall mortality	Incomplete outcome data	Selective reporting	Other bias	Comparable baseline characteristics	Concurrent control	Overall judgement of bias
Bayever 1984	Low	Low	Unclear	High[1]	Low	Low	High
Führer 1998	Low	High	High[2]	High[3]	Low	Low	High
Gratwohl 1981	Low	High	Unclear	Unclear	Low	Low	High

[1]Bayever 1984: The authors reported the study results at an early time point before all planned data had been gathered: "We present this interim report(...)".
[2]Führer 1998: In the 2005 update, overall survival, secondary clonal disease or malignancies, and also relapse were not reported separately for the 2 distinct treatment groups. Rather, the results were presented for 2 subgroups according to disease severity. This was different from the earlier report of the same study published in 1998 covering the study period from 1993 to 1997. See Fig. 1 and Tab. 1 of the article.
[3]Führer 1998: Financial support was provided by 2 pharmaceutical companies.
Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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Table 3. Overall survival

Study ID	Donor group (MSD-HSCT)		No donor group (IST)		FU[1]	P value
	N	OS (95% CI)	N	OS (95% CI)	Year	
Bayever 1984	35	72% (64 to 80)	22	45% (29 to 61)	2	0.18
Führer 1998	28	84% (N.R.)	86	87% (N.R.)	4	0.43
Gratwohl 1981	19	47% (N.R.)	13	69%[2] (N.R.)	5	0.56[3]

[1]Time point of Kaplan-Meier estimate.
[2]Gratwohl 1981: Two of 13 patients were eligible for MSD-HSCT but donors were not available in the first place; the two patients died after they received a second-line HSCT from the then again available MSD that was offered after the patients showed no response to IST.
[3]The P value was not reported and we calculated the P value using Fisher's exact test.
Abbreviations: CI: confidence interval; FU: follow up; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N: number of analyzed patients; N.R.: not reported; OS: overall survival

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Table 4. Secondary outcomes

Study ID	TRM after MSD-HSCT[1,2]	Graft failure after MSD-HSCT[1]	GVHD after MSD-HSCT[1]	No response to IST[1]	Relapse at 5 years after IST[1]
Bayever 1984	20% (7 of 35)	3% (1 of 35)	51% (17 of 33)	64% (14 of 22)	12.5% (1 of 8)
Führer 1998	N.R.	N.R.	N.R.	N.R.	N.R.
Gratwohl 1981	42% (8 of 19)	16% (3 of 19)	26% (5 of 19)	15% (2 of 13)	N.R.

[1]In parenthesis: number of affected of number of evaluable patients
[2]Treatment-related mortality was not reported for IST.
Abbreviations. GVHD: graft-versus-host disease; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N.R.: not reported; TRM: treatment-related mortality

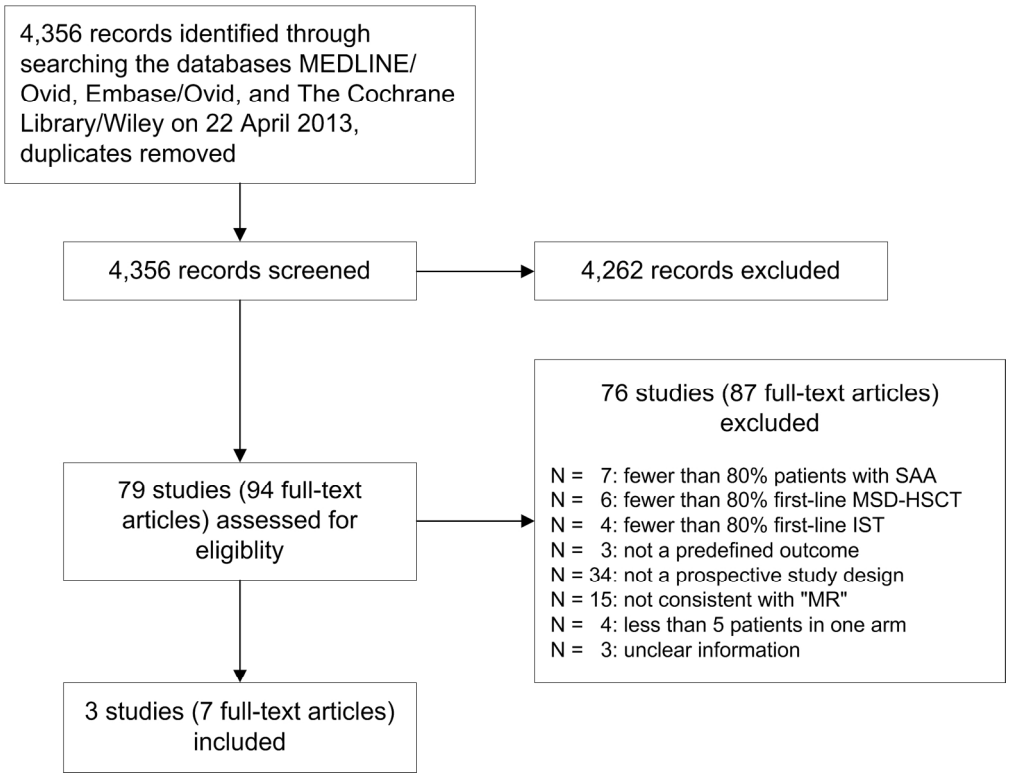

Figure 1. Study flow
Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia
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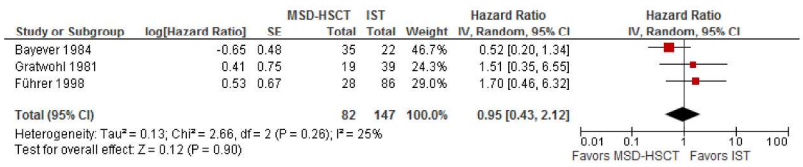

Figure 2. Mortality (MSD-HSCT vs. IST); effect: hazard ratio; random-effects model.
Standard error calculated from data presented in the Kaplan-Meier graph of the article. Ab-breviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error
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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	in the Cochrane review
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6 to 7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7

PRISMA 2009 Checklist

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	in the Cochrane review
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8, table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8 to 9, table 3 to 4, figure 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	figure 1
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	table 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	none
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12
FUNDING			

PRISMA 2009 Checklist

Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	12

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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