Unrelated alternative donor transplantation for severe acquired aplastic anemia: a study from the French Society of Bone Marrow Transplantation and Cell Therapies and the EBMT Severe Aplastic Anemia Working Party

Raynier Devillier,[1] Jean-Hugues Dalle,[2] Austin Kulasekararaj,[3] Maud D'aveni,[4] Laurence Clément,[4,5] Alicja Chybicka,[6] Stéphane Vigouroux,[5] Patrice Chevallier,[7] Mickey Koh,[8] Yves Bertrand,[9] Mauricette Michallet,[10] Marco Zecca,[11] Ibrahim Yakoub-Agha,[12] Jean-Yves Cahn,[13] Per Ljungman,[14] Marc Bernard,[15] Pascale Loiseau,[16,17] Valérie Dubois,[18] Sébastien Maury,[19] Gérard Socié,[20] Carlo Dufour,[21]* and Regis Peffault de Latour[20]*

[1]Hematology Department, Institut Paoli Calmettes, Marseille, France; [2]Pediatric Hematology Unit, Robert Debré University Hospital, Paris, France; [3]Hematology Department, King's College Hospital, London, UK; [4]Pediatric Hematology Unit, University Hospital, Nancy, France; [5]Hematology Department, Haut-Leveque Hospital and Bordeaux University Hospital, Pessac, France; [6]Hematology Department, Wroclaw Medical University, Poland; [7]Hematology Department, University Hospital, Nantes, France; [8]Hematology Department, St. George's Hospital, London, UK; [9]Pediatric Hematology Unit, University Hospital, Lyon, France; [10]Hematology Department, University Hospital, Lyon, France; [11]Hematology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [12]Hematology Department, University Hospital, Lille, France; [13]Hematology Department, University Hospital, Grenoble, France; [14]Hematology Department, Karolinska University Hospital, Stockholm, Sweden; [15]Hematology Department, University Hospital, Rennes, France; [16]HLA Laboratory, Saint-Louis University Hospital, Paris, France; [17]French Society of Histocompatibility and Immunogenetics (SFHI), France; [18]HLA Laboratory, Etablissement Français du Sang, Lyon, France; [19]Hematology Department, Henri Mondor Hospital, Créteil, France; [20]BMT Department, Saint-Louis Hospital, Diderot Paris 7 University, Paris, France; and [21]Clinical and Experimental Haematology Unit, Giannina Gaslini Children's Hospital, Genova, Italy

*CD and RPdL contributed equally to this work.

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Correspondence:
regis.peffaultdelatour@aphp.fr

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ABSTRACT

Unrelated allogeneic transplantation for severe aplastic anemia is a treatment option after immunosuppressive treatment failure in the absence of a matched sibling donor. Age, delay between disease diagnosis and transplantation, and HLA matching are the key factors in transplantation decisions, but their combined impact on patient outcomes remains unclear. Using the French Society of Bone Marrow Transplantation and Cell Therapies registry, we analyzed all consecutive patients (n=139) who underwent a first allogeneic transplantation for idiopathic severe aplastic anemia from an unrelated donor between 2000 and 2012. In an adjusted multivariate model, age over 30 years (Hazard Ratio=2.39; *P*=0.011), time from diagnosis to transplantation over 12 months (Hazard Ratio=2.18; *P*=0.027) and the use of a 9/10 mismatched unrelated donor (Hazard Ratio=2.14; *P*=0.036) were independent risk factors that significantly worsened overall survival. Accordingly, we built a predictive score using these three parameters, considering patients at low (zero or one risk factors, n=94) or high (two or three risk factors, n=45) risk. High-risk patients had significantly shorter survival (Hazard Ratio=3.04; *P*<0.001). The score was then confirmed on an independent cohort from the European Group for Blood and Marrow Transplantation database of 296 patients, with shorter survival in patients with at least 2 risk factors (Hazard Ratio=2.13; *P*=0.005) In conclusion, a simple score using age, transplantation timing and HLA matching would appear useful to help physicians in the daily care of patients with severe aplastic anemia.

Introduction

In the absence of a matched sibling donor for patients with severe aplastic anemia (SAA), allogeneic hematopoietic stem cell transplantation (allo-HSCT) from an unrelated donor (UD) is considered to be the standard treatment after immune suppressive therapy (IST) failure. The graft-*versus*-host disease (GvHD), morbidity, and mortality after UD allo-HSCT observed in early reports partly explain this strategy.[1,2] Although IST provides high long-term overall survival and reasonable response rates, patients are still exposed to clonal evolution toward paroxysmal nocturnal hemoglobinuria, myelodysplastic syndrome and acute leukemias, whereas the latter complications are rarely observed following allo-HSCT. In the last two decades, optimized HLA typing, along with better defined conditioning regimens and GvHD prophylaxis approaches have drastically improved outcomes after UD allo-HSCT.[3-6] Recently, the Severe Aplastic Anemia Working Party (SAAWP) of the European Group for Blood and Marrow Transplantation (EBMT) reported 1500 allo-HSCTs for SAA performed between 2005 and 2009, with no differences between matched sibling and UD allo-HSCT when stratified by independent risk factors such as time from diagnosis to allo-HSCT, age at the time of allo-HSCT, and the use of peripheral blood stem cells as graft source.[7] Moreover, excellent UD allo-HSCT results were reported in children receiving *in vivo* T-cell depletion as part of their conditioning regimen, in the setting of both IST failure[8] and up-front treatment.[9] Together, these recent results suggest considering early UD allo-HSCT as a preferred option for younger patients who lack HLA-identical siblings, provided the graft be performed early after diagnosis. However, although the age limit for this procedure, the timing of UD allo-HSCT and the impact of HLA mismatch have been tested as singular factors, the combined effect of these variables during the natural history of the disease remains unclear.[3,5,10] We, therefore, analyzed all patients who received an initial allo-HSCT for idiopathic SAA in France from a UD between 2000 and 2012, with the particular aim of evaluating the predictive value of the combination of age, timing of allo-HSCT and HLA matching on overall survival (OS) after UD allo-HSCT.

Methods

Design and selection criteria

The study population was made up of all patients who received a first allo-HSCT for idiopathic SAA from a UD in France between 2000 and 2012. Clinical data were prospectively collected using ProMISe (Project Manager Internet Server), an internet-based data registry system shared by all centers of the French Society of Bone Marrow Transplantation and Cell Therapies (SFGM-TC). Data concerning HLA typing were collected and cross-validated using the French Society of Histocompatibility and Immunogenetics (SFHI) and the French Biomedical Agency (ABM). Patients were separated into two groups (10/10 and 9/10) by HLA matching at 10 loci at high-level resolution (HLA-A, -B, -C, -DRB1 and -DQB1). Patients with more than one mismatch at these 10 loci, as well as those for whom allele-level HLA typing was unavailable, were excluded from the study (n=5). All conditioning regimens and GvHD prophylaxes were considered for analysis. All graft sources were accepted, with the exception of cord blood.

We analyzed a different cohort of patients used as a validation set. Clinical data of these patients were collected from the SAAWP of EBMT using the standard minimal required forms. One hundred and eleven EBMT centers participated in this study (a complete list of participating centers is available in the Online Supplementary Appendix). Inclusion criteria were: 1) first allo-HSCT for idiopathic SAA between 2000 and 2012; 2) patients transplanted in France were excluded; 3) HLA matched or mismatched unrelated donor (10 loci HLA-A, -B, -C, -DRB1 and -DQB1, high resolution). The study was approved by the scientific committees of both the SFGM-TC and the Severe Aplastic Anemia Working Party of EBMT, and was conducted in accordance with the Declaration of Helsinki for clinical research. All patients provided written signed informed consent for clinical data collection (entered in ProMISe database) and participation in retrospective database analysis.

Statistical analyses

Overall survival was the primary end point, estimated from the date of allo-HSCT using the Kaplan-Meier method, and univariate comparisons were made using the log rank test.[11] GvHD was assessed as previously described.[12,13] The cumulative incidence of chronic GvHD (cGvHD) was calculated considering death before GvHD as a competing event, and univariate comparisons were made using the Gray test.[14] In the study population, we analyzed the impact of our 3 main parameters: age at the time of allo-HSCT (≤ 30 *vs.* > 30 years), the time between diagnosis and allo-HSCT (≤ 12 *vs.* > 12 months) and allele-level HLA matching (9/10 *vs.* 10/10) on outcome. We tested the impact on outcome of following potential confounding factors: transplantation period (2000-05 *vs.* 2006-12), donor age at the time of allo-HSCT (≤ 35 *vs.* > 35 years), cytomegalovirus (CMV) serostatus (donor and recipient: negative *vs.* other combinations), graft source [bone marrow *vs.* peripheral blood stem cells, (PBSC)], conditioning regimen [without total body irradiation (TBI) *vs.* with TBI], GvHD prophylaxis [cyclosporine A (CSA) + methotrexate (MTX) *vs.* other] and the use of *in vivo* T-cell depletion as part of the conditioning regimen (no *vs.* yes). Among them, those with $P<0.150$ were selected to adjust the impact of the 3 main variables of interest (age, timing and HLA matching) in a multivariate Cox model.[15] Subsequently, a score was calculated based on the three variables of interest (age, timing of allo-HSCT and HLA matching) in which each of them carried a weight defined by Cox model hazard ratios. The impact of this score was then evaluated in univariate and multivariate analyses (the latter adjusted for the same co-variables). Finally, the score was tested on the validation independent set of patients in both univariate and multivariate analyses.

Results

Characteristics of patients of the training set (SFGM-TC database)

One hundred and thirty-nine consecutive patients (64 male; 46%) met the selection criteria. Median age was 23 years (range 1-66) (Table 1). Median follow up was 51 months (range 2-140), and 124 patients (89%) had a minimal follow up of 24 months. All patients were in failure of first-line of treatment by IST. Forty-six and 93 patients were transplanted in the periods 2000-05 and 2006-12, respectively. In the more recent period, patients were significantly older (median age 2000-05 *vs.* 2006 12: 18 years *vs.* 25 years, respectively; $P=0.030$), more frequently

received *in vivo* T-cell depletion (2000-05 *vs.* 2006-12: 57% *vs.* 91%; *P*<0.001, respectively) and were more prone to receive PBSC as graft source (2000-05 *vs.* 2006-12: 7% *vs.* 19%, respectively; *P*=0.074). There was no difference in interval between diagnosis and allo-HSCT (as continuous variable: Mann-Whitney test: *P*=0.312; as categorical variables ≤12 *vs.* >12 months: *P*=0.855), HLA matching and GvHD prophylaxis between the two transplantation periods.

Transplantation-related events

Ten patients (7%) experienced primary graft failure. Forty-eight patients developed grade II-IV acute GvHD, leading to a day-100 cumulative incidence of 35% (95CI: 26-42) (grade II 32 of 48, 66%; grade III 10 of 48, 21%; grade IV 6 of 48, 13%). The use of PBSC was associated with a higher incidence of grade II-IV acute GvHD (PBSC *vs.* bone marrow: 52% *vs.* 31%; *P*=0.044) (Table 2) but not with higher incidence of chronic GvHD. The cumulative incidence of chronic GvHD at four years was 24% (95CI: 17-31) (extensive GvHD 8%). Chronic GvHD occurred at a median time of six months after allo-HSCT (range 3-24). No specific risk factor was identified (Table 2).

Overall survival

With a median follow up of 51 months (range 2-140), 4-year OS was 66% (95CI: 58-75). Median time from allo-HSCT to death was four months (range 1-46). Forty-one of the 45 deaths (91%) occurred within the first two years after allo-HSCT. Causes of death were GvHD (n=17, 37%), severe infections without GvHD (n=14, 31%), graft failure (n=8, 18%), secondary malignancy (n=2, 4%), and other causes (n=4, 9%).

Univariate analyses of OS by age at the time of allo-HSCT, interval from diagnosis of SAA to allo-HSCT, and HLA matching (10 loci) are illustrated in Table 3. To select factors for adjustment in the multivariate model, we tested the impact of these confounding factors in univariate analyses and found that the transplantation period significantly influenced OS (2000-05 *vs.* 2006-12: 52% *vs.* 74%; *P*=0.018). Moreover, CMV sero status (D-/R- *vs.* other: 71% *vs.* 61%; *P*=0.125) and the use of *in vivo* T-cell depletion (yes *vs.* no: 67% *vs.* 55%; *P*=0.145) were selected for the adjusted model, having *P* values <0.150. The remaining factors (donor age, graft source, conditioning regimen and GvHD prophylaxis) did not significantly influence outcome and were not considered to adjust the multivariate model (*Online Supplementary Table S1*).

In multivariate analyses adjusted for the transplantation period, CMV sero status and the use of *in vivo* T-cell depletion, we found that age over 30 years [HR=2.39 (1.23-4.66); *P*=0.011], time from diagnosis to allo-HSCT over 12 months [HR=2.18 (1.09-4.37); *P*=0.027] and the use of a 9/10 mismatched UD [HR=2.14 (1.05-4.38) *P*=0.036] were independent risk factors that significantly influenced OS (Table 3).

Predictive score for overall survival

Using a number of risk factors [age (> 30 years), time from diagnosis to allo-HSCT (> 12 months) and HLA matching (9/10)] we created a score to predict OS. We attributed the same weight to these three variables since the hazard ratios produced by the Cox model were similar (i.e. close to 2) (Table 3). No risk factors were seen in 35 patients (25%), one risk factor was seen in 59 patients (42%), two risk factors in 41 patients (30%), and three risk factors in 4 patients (3%). We stratified patients into a low-risk group (zero or one risk factors, based on the fact that we found no significant difference in OS between patients with zero and one risk factor (*Online Supplementary Table S2*) and a high-risk group (two or three risk factors, because the number of patients with 3 risk factors is too low for a separate analysis). Four-year OS was 74% in the low-risk group and 49% in the high-risk group (*P*<0.001) (Table 3 and Figure 1A). After adjustment for the transplantation period in a multivariate Cox model, patients in the high-risk group had significantly shorter survival [HR=3.04 (1.64-5.62); *P*<0.001] (Table 3). In both low-risk and high-risk groups, main causes of death were GvHD (39% and 36%, respectively), infections (39% and 23%, respectively) and graft failure (10% and 27%, respectively).

Independent validation set (EBMT database)

Two-hundred and ninety patients matched inclusion criteria for the validation set. Validation cohort characteristics are shown in *Online Supplementary Table S3*. Age was over 30 years in 63 patients (21%), 175 patients (59%) were transplanted later than 12 months after diagnosis of SAA, and 44 patients (15%) received grafts from a mismatched unrelated donor (MUD). Based on these 3 factors, 232 (78%) and 64 (22%) were categorized as low-risk (zero or one risk factor) and high-risk (2 or 3 risk fac-

Table 1. Patients', disease and transplantation characteristics in the study population.

	All patients (N = 139) N.	%
Median age (years)	23	[1-66]
Time from diagnosis to allo-HSCT (months)		
≤ 12 months	58	42%
> 12 months	81	58%
CMV serostatus		
D-/R-	53	39%
Others	83	61%
Unknown	3	
Conditioning regimen		
Cy +/- Flu	100	72%
Bu-Cy +/- Flu	24	17%
Other	15	11%
Use of TBI	64	46%
In vivo T-cell depletion	112	81%
GvHD prophylaxis		
CSA	11	8%
CSA + MTX	94	68%
CSA + MMF	17	12%
Others	17	12%
Unrelated donor		
10/10 MUD	113	81%
9/10 MUD	26	19%
Median donor age (years)	35	[3-60]
Graft source		
BM	118	85%
PBSC	21	15%

BM: bone marrow; Bu: busulfan; CSA: cyclosporine A; Cy: cyclophosphamide; D-/R-: seronegative donor and recipient; Flu: fludarabine; GvHD: graft-versus-host disease; MMF: mycophenolate mofetil; MTX: methotrexate; MUD: matched unrelated donor; PBSC: peripheral blood stem cell; TBI: total body irradiation.

tors), respectively. Low-risk patients had significantly better 4-year OS compared to patients of the high-risk group (low-risk *vs.* high-risk: 76% *vs.* 60%; *P*=0.003) (Figure 1B). In adjusted multivariate analyses, we confirmed the prognostic impact of the score, with a shorter OS in the high-risk group [HR=2.13 (1.26-3.59); *P*=0.005].

Discussion

This paper provides details of the French experience of UD allo-HSCT from 2000 to 2012 for idiopathic SAA, with a reasonable follow-up period (89% of patients for at least 24 months). Four-year OS was 66%, and importantly, results continued to improve over time, with the best results from 2006 onwards. *Online Supplementary Table S4* reviews major series of UD allo-HSCT for SAA, showing overall improvement of OS over the last 20 years.[3-5,16-18] Due to an improvement in transplantation procedures, recent survival after matched UD allo-HSCT has approached that of post HLA-identical sibling donor allo-HSCT. This is supported by the recent analysis of our group showing that, once stratified using risk factors, OS was roughly similar using a related or matched unrelated donor.[7] In this paper, we focused on the impact of age, timing between diagnosis and allo-HSCT as well as HLA matching (excluded from the recent EBMT analysis[7]) on outcome after transplantation for patients with refractory aplastic anemia. In addition, in order to provide physicians with a more complete tool to make therapeutic decisions when patients are considered for UD allo-HSCT, we tested the combined effect of these factors to predict outcomes in our cohort. We acknowledge that other variables may influence outcome. However, most of them are related to transplantation procedure (e.g. conditioning regimen, *in vivo* T-cell depletion, stem cell source

Table 2. Cumulative incidences of acute and chronic graft-*versus*-host disease in the study population.

	Day-100 aGvHD	(95%CI)	*P*	4-years cGvHD	(95%CI)	*P*
All patients (n = 139)	35%	(26-42)		24%	(17-31)	
Transplantation period						
2000-05 (n = 46)	44%	(27-56)	0.073	22%	(9-33)	0.704
2006-12 (n = 93)	30%	(20-39)		26%	(16-34)	
In vivo T-cell depletion						
No (n = 27)	44%	(22-60)	0.137	22%	(5-36)	0.789
Yes (n =112)	32%	(23-40)		25%	(16-33)	
Age at allo-HSCT						
≤ 30 years (n = 93)	38%	(27-47)	0.330	25%	(16-34)	0.813
> 30 years (n = 46)	28%	(14-40)		22%	(9-34)	
Time from diagnosis to allo-HSCT						
≤ 12 months (n = 58)	33%	(20-44)	0.575	26%	(14-37)	0.609
> 12 months (n = 81)	36%	(25-45)		23%	(13-31)	
Graft source						
BM (n = 118)	31%	(23-39)	0.044	24%	(16-32)	0.946
PBSC (n = 21)	52%	(25-70)		24%	(3-40)	
HLA matching						
10/10 MUD (n = 113)	35%	(14-51)	0.949	25%	(16-32)	0.816
9/10 MUD (n = 26)	35%	(25-43)		23%	(5-38)	

*95%CI: 95% confidence interval; aGvHD: acute graft-*versus*-host disease; BM: bone marrow; cGvHD: chronic graft-*versus*-host disease; HSCT: allogeneic hematopoietic stem cell transplantation; MUD: matched unrelated donor; PBSC: peripheral blood stem cell.*

Table 3. Univariate and multivariate analyses of overall survival in the study population.

	Univariate analysis			Multivariate analysis*		
	4-y	[95%CI]	*P*	HR	[95%CI]	*P*
Age at allo-HSCT						
≤ 30 years (n = 93)	70%	[61-81]	0.037	1		
> 30 years (n = 46)	57%	[44-74]		2.39	[1.23-4.66]	0.011
Time from diagnosis to allo-HSCT						
≤ 12 months (n = 58)	77%	[65-90]	0.017	1		
> 12 months (n = 81)	58%	[48-70]		2.18	[1.09-4.37]	0.027
HLA matching						
10/10 (n = 113)	68%	[59-78]	0.196	1		
9/10 (n = 26)	57%	[40-80]		2.14	[1.05-4.38]	0.036
Predictive score						
0 or 1 risk factor (n = 94)	74%	[65-84]	< 0.001	1		
2 or 3 risk factors (n = 45)	49%	[36-67]		3.04	[1.64-5.62]	<0.001

**Adjustment with cytomegalovirus serostatus,* in vivo *T-cell depletion and transplantation period; 4-y: 4-year; HR: Hazard ratio; 95%CI: 95% confidence interval.*

or GvHD prophylaxis) and have been optimized over the years to continuously improve overall outcome. Although these parameters are determining factors in how to proceed, they are not usually key factors in deciding whether or not to transplant. Variables not available at the time of decision (e.g. the number of CD34 cells) were purposely excluded from the analysis. Finally, we validated the impact of our key factors as a composite score in an independent population provided by the EBMT SAAWP (Severe Aplastic Anemia Working Party), including patients with idiopathic SAA who underwent first allo-HSCT from a UD during the same period of time.

Patients over 30 years of age experienced shorter survival (57%) compared with younger patients (70%). The finding of younger age as associated with a better outcome is in line with two recent large EBMT studies that showed OS rates of 83% and of 78%, respectively, in children and adolescents undergoing UD HSCT after failed IST.[19,20] Moreover, it is of interest that previous reports identified a significant age cut off of 20 years for UD allo-HSCT.[16,21] In the EBMT series,[3] Bacigalupo *et al.* showed that, in the context of global improvement of transplantation procedures and outcomes, an age cut off of 27 years could not predict OS, underlining the need to reassess the age limit in this context. We, therefore, suggest that UD allo-HSCT should be safely considered up to 30 years of age, approaching the clinically relevant age cut off of 40 years in the setting of HLA-identical sibling allo-HSCT.[22,23] For older patients, UD allo-HSCT should be performed with caution in highly selected patients, especially in the presence of additional poor risk factors such as comorbidities.[5] Regarding the timing of allo-HSCT, we found better OS when allo-HSCT was performed earlier, within the first year following diagnosis. This is in line with previous reports showing better results with early allo-HSCT for SAA in different clinical settings.[3,22,24,25] Because of the absence of a non-transplanted control group, we were not able to directly assess the optimal timing for UD allo-HSCT after first IST failure. However, we suggest that patients should be transplanted as early as possible in this situation, which implies starting the search for an unrelated donor soon after diagnosis in younger patients without a sibling donor. Lastly, we confirmed the unfavorable impact of HLA mismatches, which underlines the importance of HLA matching in a cohort exclusively comprising allele-level HLA-matched patients, which is in agreement with previous reports suggesting better outcomes with matched UD.[4,17,18]

Accordingly, and in order to provide a more comprehensive evaluation tool, we built a prognostic OS score taking into account age (≤ 30 *vs.* > 30 years), timing of allo-HSCT (≤ 12 *vs.* > 12 months) and HLA matching (9/10 *vs.* 10/10). In the same low-risk group, we decided to combine patients with zero and one risk factor because we found no statistical difference in OS between these 2 groups in the study population or in the validation cohort (*Online Supplementary Table S2*). However, we cannot exclude the possiblity that OS might be better for patients who present zero risk factors in comparison with patients with one risk factor (hazard ratio of 1.41 and 1.87, respectively, in the study population and validation cohort). That said, a dramatic impairment of survival was observed in patients with more than one risk factor (*Online Supplementary Table S2*). Therefore, we separated patients into a low-risk (0 or 1 risk factor) and a high-risk (2 or 3 risk factors) group. We found that patients with more than one of these risk factors experienced lower survival (49%), while those with zero or one risk factor obtained a 4-year OS of 74%. As some of these patients were transplanted in the early 2000s, we might expect lower mortality rates in more recent times following the systematic introduction of procedures that have since been described to provide better results (*in vivo* T-cell depletion, bone marrow as graft source, fludarabine-based conditioning regimens).[3] Moreover, supportive care has improved over time, partly contributing to the lower mortality rate in the most recent period.[26] We then evaluated the impact of our prognostic score on the independent validation set and confirmed that it is easy to use and reproducible. Indeed, it turned out to have strongly predictive value in both univariate and multivariate analyses, although the high number of participating centers leads to a high heterogeneity in the baseline characteristics of patients and transplantation

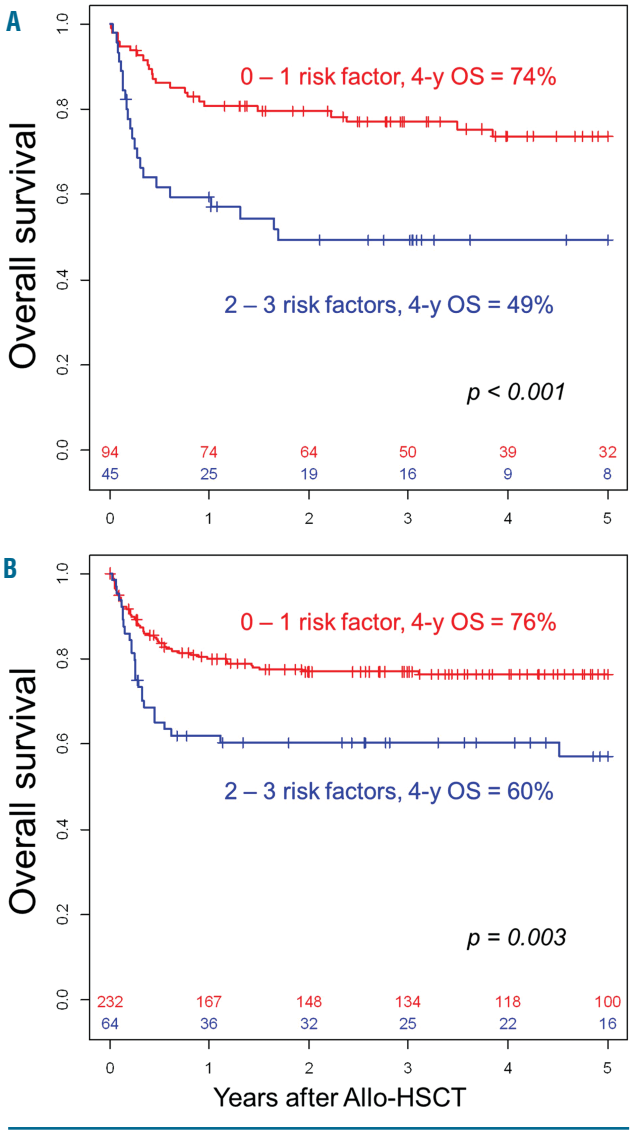

Figure 1. Overall survival by the presence of selected risk factors: age > 30 years; time from diagnosis to allogeneic hematopoietic stem cell transplantation (allo-HSCT) > 12 months; presence of an HLA mismatch. (A) Study population. (B) Validation cohort.

procedures. Stratifying risk in this manner may prove to be an easy way to select low-risk patients for UD allo-HSCT, while care should be exercised when considering those with more than one risk factor, and for whom an additional course of IST could be debated. As recently published, there has been a significant decrease in invasive fungal infections, infection-related mortality, and overall mortality in patients with SAA unresponsive to initial IST, with a 5-year OS among non-responders of approximately 57%, similar to that expected in patients with more than one risk factor in our study.[26]

Our work has both strengths and limitations. Its strengths include the large enrollment of all consecutive patients with idiopathic SAA undergoing UD allo-HSCT in France over the most recent 12-year period. This provides not only a clear picture of our current practice in this setting, but also shows how transplantation strategies have improved over time. Moreover, our analyses focus on patients with available allele-level HLA matching at 10 loci. This allows for a clear assessment of the impact of HLA matching, which is already known to have contributed to some degree to the overall improvement in transplantation strategies. Moreover, the presence of a validation set of patients represents a major strength to evaluate the power and the reproducibility of a new prognostic score. Our study also has a number of limitations that are mostly due to its retrospective nature. It is likely that only the healthiest patients over 30 years of age were considered for UD allo-HSCT, especially in the presence of an HLA mismatch. We do not have data for similar patients who were referred for a UD allo-HSCT but were considered unfit. It would have been interesting to evaluate their outcomes after further non-allogeneic treatment in comparison with our cohort. Data concerning previous treatment were also lacking for a significant number of patients. We acknowledge that these data would have been helpful in order to make an accurate evaluation of the disease risk at the time of allo-HSCT. As a surrogate marker, we used the time from diagnosis to allo-HSCT, but the number, type and response duration to previous IST would have been more informative to better identify the optimal timing of UD allo-HSCT in the treatment strategy of SAA.

Despite these obvious limitations, our results have led us to consider UD Allo-HSCT soon after IST failure and to start the search for an UD at time of diagnosis in younger patients, although age and HLA matching should be key factors in the decision-making process. Integrating these 3 simple parameters in our proposed prognostic score would appear useful in selecting patients for UD allo-HSCT. Although it seems to be reproducible in an independent cohort, a prospective evaluation is needed to better define the overall treatment strategy of idiopathic SAA in IST failure.

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