Increased age-associated mortality risk in HLA-mismatched hematopoietic stem cell transplantation

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ABSTRACT

We investigated a possible interaction between age-associated risk and HLA-mismatch associated risk on prognosis in different age categories of recipients of unrelated hematopoietic stem cell transplants (HSCT) (n=3019). Patients over 55 years of age transplanted with 8/10 donors showed a mortality risk of 2.27 (CI 1.70-3.03, *P*<0.001) and 3.48 (CI 2.49-4.86, *P*<0.001) when compared to 10/10 matched patients in the same age group and to 10/10 matched patients aged 18-35 years, respectively. Compared to 10/10 matched transplantations within each age category, the Hazards Ratio for 8/10 matched transplantation was 1.14, 1.40 and 2.27 in patients aged 18-35 years, 36-55 and above 55 years. Modeling age as continuous variable showed different levels of risk attributed to age at the time of transplantation [OS: 10/10: Hazards Ratio 1.015 (per life year); 9/10: Hazards Ratio: 1.019; 8/10: Hazards Ratio 1.026]. The interaction term was significant for 8/10 transplantations (*P*=0.009). Findings for disease-free survival and transplant-related mortality were similar. Statistical models were stratified for diagnosis and included clinically relevant predictors except cytomegalovirus status and Karnofsky performance status. The risk conferred by age at the time of transplantation varies according to the number of HLA-mismatches and leads to a disproportional increase in risk for elderly patients, particularly with double mismatched donors. Our findings highlight the importance of HLA-matching, especially in patients over 55 years of age, as HLA-mismatches are less well tolerated in these patients. The interaction between age-associated risk and HLA-mismatches should be considered in donor selection and in the risk assessment of elderly HSCT recipients.

Introduction

Unrelated hematopoietic stem cell transplantation is a rapidly evolving field offering a curative therapy for various hematologic diseases. In particular, the proportions of older patients and patients transplanted with unrelated donors have increased over the last decade.[1,2] One prerequisite was the introduction of reduced intensity conditioning regimens (RIC) as an alternative to myeloablative conditioning (MAC) in elderly patients as well as in patients with co-morbidities.[3,4] There is

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Table 1. Patients' characteristics.

	Age 18-35 years	Age 36-55 years	Age >55 years	Total n	*P*
Median age	27	48	62	n.a.	
Number of patients	529	1295	1195	3019	
Number of centers	28	26	25	29	
Diagnosis					
AML	187 (35.3)	393 (30.3)	344 (28.8)	924	<0.001
ALL	178 (33.6)	142 (11.0)	55 (4.6)	375	
AL	13 (2.5)	66 (5.1)	95 (7.9)	174	
CML	59 (11.2)	111 (8.6)	31 (2.6)	201	
CLL	2 (0.4)	60 (4.6)	74 (6.2)	136	
MDS	37 (7.0)	201 (15.5)	342 (28.6)	580	
NHL	49 (9.3)	184 (14.2)	145 (12.1)	378	
MM	4 (0.8)	138 (10.7)	109 (9.1)	251	
HLA-matching status					
10/10	295 (55.8)	774 (59.8)	778 (65.1)	1847	<0.001
9/10	172 (32.5)	397 (30.7)	342 (28.6)	911	
8/10	62 (11.7)	124 (9.6)	75 (6.3)	261	
Ethnicity					
Caucasian	527 (99.6)	1289 (99.5)	1193 (99.8)	3009	n.s.
Asian	1 (0.2)	4 (0.3)	2 (0.2)	7	
African	1 (0.2)	2 (0.2)	0 (0.0)	3	
Disease stage					
Early	250 (47.3)	516 (39.8)	455 (38.1)	1221	<0.001
Intermediate	170 (32.1)	468 (36.1)	403 (33.7)	1041	
Advanced	109 (20.6)	311 (24.0)	337 (28.2)	757	
Karnofsky performance score					
80-100	235 (44.4)	732 (56.5)	856 (71.6)	1823	<0.001
<80	16 (3.0)	31 (2.4)	67 (5.6)	114	
Data missing	278 (52.6)	532 (41.1)	272 (22.8)	1082	
Conditioning regimen					
Myeloablative	460 (87.0)	928 (71.7)	487 (40.8)	1875	<0.001
Reduced intensity	69 (13.0)	367 (28.3)	708 (59.2)	1144	
GvHD prophylaxis					
CsA ± MTX ± other	231 (43.7)	670 (51.7)	720 (60.3)	1621	<0.001
Tacrolimus ± other	22 (4.2)	72 (5.6)	79 (6.6)	173	
MMF ± other	7 (1.3)	10 (0.8)	6 (0.5)	23	
MTX ± other	2 (0.4)	6 (0.5)	7 (0.6)	15	
T-cell depletion	16 (3.0)	22 (1.7)	21 (1.8)	59	
Other	10 (1.9)	11 (0.8)	17 (1.4)	38	
Data missing	241 (45.6)	504 (38.9)	345 (28.9)	1090	
ATG treatment					
Yes	329 (62.2)	810 (62.5)	723 (60.5)	1862	<0.001
No	125 (23.6)	319 (24.6)	416 (34.8)	860	
Data missing	75 (14.2)	166 (12.8)	56 (4.7)	297	
Stem cell source					
BM	109 (20.6)	166 (12.8)	50 (4.2)	325	<0.001
PBSC	420 (79.4)	1129 (87.2)	1145 (95.8)	2694	
Recipient-donor sex match					
male-male	244 (46.1)	575 (44.4)	586 (49.0)	1405	n.s.
male-female	71 (13.4)	174 (13.4)	159 (13.3)	404	
female-male	127 (24.0)	359 (27.7)	297 (24.9)	783	
female-female	87 (16.4)	187 (14.4)	153 (12.8)	427	
Patient HLA-C KIR-ligand status					
C1C1	212 (40.1)	499 (38.5)	480 (40.2)	1191	n.s.
C1C2	240 (45.4)	580 (44.8)	542 (45.4)	1362	
C2C2	77 (14.6)	216 (16.7)	173 (14.5)	466	
CMV status (patient-donor)					
neg-neg	112 (21.2)	312 (24.1)	241 (20.2)	665	<0.001
neg-pos	44 (8.3)	91 (7.0)	82 (6.9)	217	
pos-neg	87 (16.4)	213 (16.4)	301 (25.2)	601	
pos-pos	57 (10.8)	242 (18.7)	373 (31.2)	672	
Data missing	229 (43.3)	437 (33.7)	198 (16.6)	864	
Year of transplantation					
1997-2003	149 (28.2)	222 (17.1)	38 (3.2)	409	<0.001
2004-2007	182 (34.4)	437 (33.7)	320 (26.8)	939	
2008-2011	198 (37.4)	636 (49.1)	837 (70.0)	1671	
Distribution of 8/10 mismatches					
Only HLA-class I MM	39	81	47	167	n.s
HLA-Class I + class II MM	19 (12, 63.2)	32 (18, 56.3)	22 (16, 72.7)	73	
Only HLA-class II MM	4 (4, 100)	11 (11, 100)	6 (6, 100)	21	

AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; AL: unclassified acute leukemia; CML: chronic myeloid leukemia; CLL: chronic lymphocytic leukemia; MDS: myelodysplastic syndrome; NHL: non-Hodgkin lymphoma; MM: multiple myeloma; BM: bone marrow; PBSC: peripheral blood stem cells. Distribution of 8/10 mismatches section - MM: mismatch, for groups including HLA-Class II mismatches, the number and percentage of cases involving HLA-DQB1 mismatches are given in parentheses; n.s.: not significant.

already a wealth of data showing that RIC is a safe and effective treatment form for patients previously not eligible for hematopoietic stem cell transplantation (HSCT).[5,6] As a consequence, therapeutic schemes for elderly patients have been established which now include HSCT as treatment option in some clinical instances.[7] Nevertheless, classical risk factors still apply, and while increasing age did not influence the incidence of acute or chronic graft-*versus*-host disease (GvHD),[8] transplant-associated morbidity and mortality as well as disease relapse still pose challenges in elderly patients.[9,10] One study investigating a significant number of transplanted ALL patients aged over 45 years showed a substantially higher rate for transplant-related mortality (TRM) in MAC-treated patients with HLA-mismatches when compared to the RIC-treated cohort, prompting the authors to discourage MAC conditioning in this patient group altogether.[11] This observation suggests an interaction between transplantation-associated mortality caused by age-associated risk and HLA-mismatching. Age and HLA-matching status are important clinical predictors for the outcome of HSCT and are used among others for risk assessment in HSCT.[12] We analyzed the relationship between age-risk and HLA-risk in a large cohort of patients transplanted with unrelated donors and tested the hypothesis that age-risk varies according to HLA-matching status. Such a differentiation might have an impact on donor search and selection recommendations.

Methods

Patients

A total of 3019 adult patients transplanted for malignant hematologic disorders were included in this analysis. Transplantations were performed at German transplant centers between 1997 and 2011.

All patients received a first allogeneic unrelated transplant from bone marrow (BM) or peripheral blood stem cells (PBSC) with no more than 2 HLA-mismatches on 5-loci (HLA-A, -B, -C, -DRB1 and -DQB1). Disease stage definitions were adopted from a previous study defining the European Group for Blood and Marrow Transplantation (EBMT) risk score.[12] MAC was defined according to the recommendations of the EBMT Central Registry Office (MedAB manual forms).[13] Treatments with busulfan 16 mg/kg + cyclophosphamide 120-200 mg/kg, cyclophosphamide 120 mg/kg fractionated total body irradiation (TBI) 12Gy, etoposide VP-16 30-60 mg/kg + TBI 12Gy fractionated/10Gy single dose, BEAM polychemotherapy, CBV polychemotherapy or TBI 10-14Gy; busulfan 16 mg/kg are considered as myeloablative. Less intense regimens were considered as RIC. Patient and donor consent for HLA typing and for the analysis of clinical data were obtained. The study was approved by the ethical review board of the University of Ulm (project number 263/09).

HLA-typing

All patients and donors were high resolution typed for HLA-A, -B, -C, -DRB1 and -DQB1. Ambiguities within exons 2+3 for HLA-class I and exon 2 for HLA-class II alleles were resolved. Ambiguities involving non-expressed (null) alleles were resolved according to NMDP confirmatory typing requirements. Differences in exon 2 and 3 for HLA-class I alleles and exon 2 for HLA-class II alleles were considered as HLA-mismatch irrespective of the vector of mismatches.[14] Patient HLA-C KIR ligand status was inferred from high resolution HLA-C typing (C1=Asn80; C2=Lys80). Resulting phenotypes were C1C1, C1C2 and C2C2.

Statistical analysis

For univariate analysis of overall survival (OS), the Kaplan-Meier method and logrank testing was applied. Multivariate analysis for OS and disease-free survival (DFS) was performed using extended Cox-proportional hazards models.[15]

For TRM and RI, univariate competing risks analysis and multivariate competing risks regression for stratified data was used.[16] Backward stepwise exclusion was used for multivariate model selection. Evaluated covariates were: patient age, HLA-matching status, disease stage, conditioning regimen intensity, treatment with anti-thymocyte globulin (ATG), year of transplantation, time

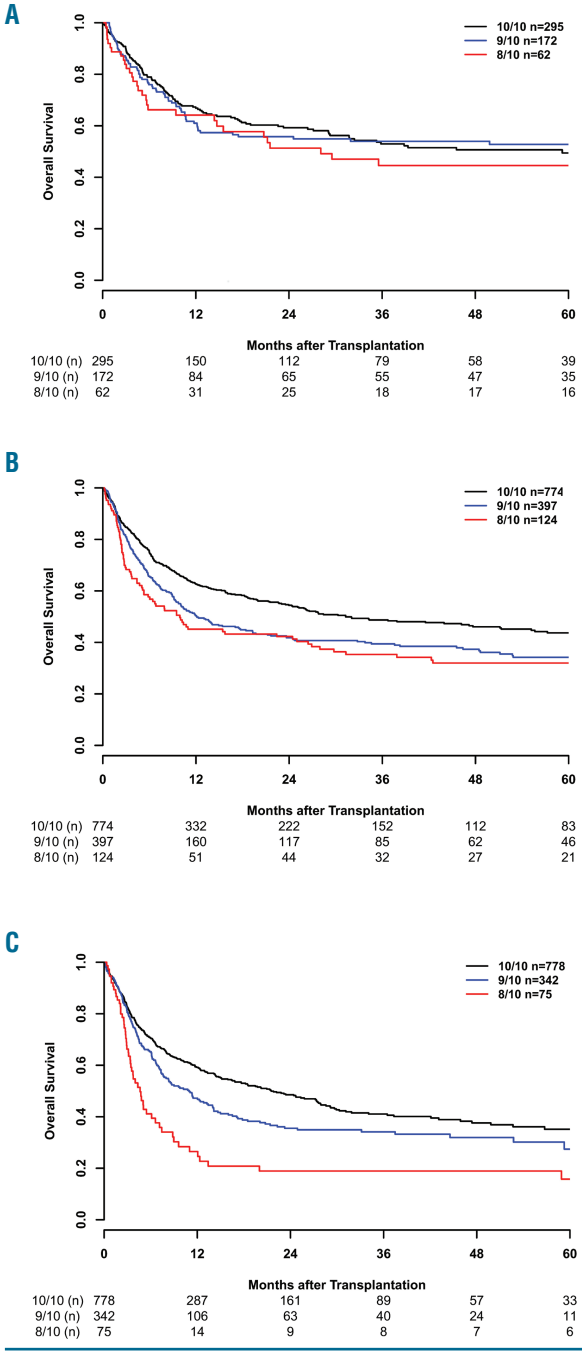

Figure 1. Kaplan-Meier estimates for overall survival. Kaplan-Meier estimates for overall survival according to HLA-matching status (10/10 black lines, 9/10 blue lines, 8/10 red lines) in different age categories. (A) Age 18-35 years, *P*=not significant. (B) age 36-55 years, *P*<0.001. (C) age >55 years, *P*<0.001.

to transplantation, graft source, donor-recipient sex combination, KIR ligand status, and donor origin (national *vs.* international). For antithymocyte globulin (ATG) treatment, some data were missing (Table 1). Models were validated by inclusion of missing values as a separate group and by omission of cases with missing values, and no bias was found.[17]

Stratification was used to account for heterogeneity of diagnosis. Violations of the proportional hazards assumption (PHA) by disease stage, conditioning regimen intensity and transplantation before 2004 were adjusted using time-dependent modeling of these covariates.[15] A significant center effect was adjusted using a frailty term with gamma distribution.[18] To assess the relationship between age and HLA-compatibility, subgroups were formed and analyzed as factors: age group 18-35 years (HLA-match: 10/10, 9/10 and 8/10), 36-55 years (HLA-match: 10/10, 9/10 and 8/10), and over 55 years (HLA-match: 10/10, 9/10 and 8/10). The cut-off value of 55 years for elderly patients has been used in previous studies and the cut-off value of 35 years is close to the arithmetic mean between the age boundaries in the remaining patients.[19]

In addition, an interaction model between age and number of HLA-mismatches was investigated. The relative risk conferred by age was visualized as age-dependent risk in different HLA-match categories relative to an 18-year old patient transplanted with a 10/10 matched donor as baseline. In this model, the covariate age was included as a continuous variable and no violation of the PHA was found. P=0.05 was considered statistically significant.

Results

Patients' characteristics are given in Table 1. Patients over 55 years of age formed the second largest age group (n=1195, 39.6%). The distribution of diagnoses reflects the current spectrum of indications, with acute myeloid leukemia (AML) being the most frequent diagnosis (n=924, 30.6%). Single HLA-mismatches were present in 30.2% (n=911) and double mismatches occurred in 8.7% (n=261) of all patients. Although the proportion of HLA-DQ mismatches among double mismatched transplantations was slightly higher in older patients, there was no statistically significant difference in the distribution of 8/10 mismatches. Ethnicity was almost exclusively Caucasian. MAC was used in 62.1% (n=1875) of the patients, with peripheral blood stem cells (PBSC) being the leading graft source (n=2694, 89.2%). More than half of the transplantations were performed in the years between 2008 and 2011 (n=1671, 55.4%). Median follow-up time was 29 months. Table 2 and Figure 1 show the results of the univariate OS analysis in patients according to their HLA-matching status and age group. Logrank-testing showed no significant difference between 10/10, 9/10 and 8/10 matched transplantations in the youngest age group (aged 18-35 years). In the intermediate age group (36-55 years) a highly significant difference (P<0.001) was found with higher mortality for patients transplanted with single or double mismatches. In patients over 55 years of age, the differences were even more pronounced, showing high mortality, especially in the 8/10 matching group (P<0.001).

In multivariate modeling, these results could be confirmed for OS showing no significant differences between single and double mismatched transplantations in the younger age group (Table 3). Risk sharply increased with age in the respective mismatch groups, reaching the highest relative risk in the age group over 55 years (HR: 3.48, CI 2.49-4.86, P<0.001). Similar patterns were seen for DFS and TRM with hazard ratios spreading with increasing numbers of HLA-mismatches and increasing age, thus conferring highest risk for patients aged over 55 years with double HLA-mismatches [DFS: Hazard Ratio (HR) 2.74, CI 2.00-3.76, P<0.001 and TRM: HR 3.79, CI 2.29-6.30, P<0.001]. No significant differences were observed for relapse incidences.

Modeling an interaction term between age and number of HLA-mismatches allowed estimation of age risk within matched, single-mismatched and double mismatched patient groups. Age risk showed increasing risk estimates with increasing number of HLA-mismatches. In 10/10 matched transplantations, this additional risk per life year at time of transplantation was lowest (HR: 1.015, CI 1.010-1.020; P<0.001). It increased, however, with the decreasing degree of HLA-compatibility between donor and patient (9/10, HR: 1.019, CI 1.014-1.024, P<0.001 and 8/10 HR: 1.026, CI 1.020-1.031, P<0.001). The interaction term for age and 2 HLA-mismatches was significant (P=0.009). The Cox regression model is a multiplicative hazard model. In order to visualize the component of age-risk within the respective HLA-match groups, the change of risk contributed to the prognosis by age at the time of transplantation was plotted relatively to an 18-year-old 'baseline' patient with a 10/10 matched donor. This visualization is based on the different age-associated risk estimates within each HLA-match category as observed in the multivariate model for OS, and it illustrates the change in risk with increasing age (Figure 2).

Discussion

We found a statistically significant interaction between HLA-matching status and age-associated risk. This interaction can be interpreted as different levels of age-associated risk according to the number of HLA-mismatches. Our findings substantiate that transplantation for patients aged over 55 years with two HLA-mismatches are particularly risky with a highly significant hazard ratio of 3.48 (CI 2.49-4.86; P<0.001) when compared to 10/10 matched patients younger than 35 years. If compared to 10/10

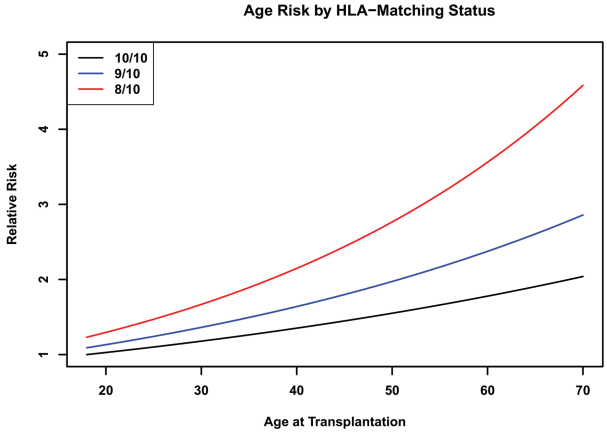

Figure 2. Age risk by HLA-matching status. Relative risk contributed by the continuous covariate age at the time of transplantation according to different levels of HLA-mismatches (completely matched 10/10: black, single mismatched 9/10: blue, double mismatched 8/10: red).

transplantations within each age category, double mismatches increased mortality risk for OS by a factor of 1.14 in the lowest age group, by a factor of 1.40 in the middle age group, and 2.27 in patients aged over 55 years. This disproportional increase and the poor one-year survival rate of only 19% in double mismatched transplantations for elderly patients highlights the importance of HLA-matching especially in this group of patients.

Luckily, donors with 2 HLA-mismatches had to be accepted only in a small fraction of patients aged over 55 years (6.3%). The age cohorts showed expected structural differences in composition with regard to diagnosis and conditioning regimen, as well as graft source. Multivariate analysis adjusted for differences in conditioning treatment, while graft source showed no differential impact on survival end points.

It is known that older patients tolerate conditioning related toxicity less well than younger patients, which is the reason for the development and the use of conditioning regimes with reduced intensity.[6,20,21] Treatment-associated toxicity correlates strongly with transplant-related mortality and therefore it greatly influences OS. HLA-mismatches also associate strongly with treatment-related morbidity and -mortality. This relationship explains our findings from the perspective of transplant biology, suggesting that older patients tolerate HLA-mismatches less well than younger patients as it is also the case for treatment-related toxicity.

On the other hand, it cannot be deduced from this data whether younger patients benefit less from better-matched donors, as life expectancy is higher and HLA-associated risk cumulates over time.

This finding was only made possible because of the relatively high proportion of older patients in our dataset. As most of the transplantations were performed in the years between 2008 and 2011, our dataset reflects the substan-

Table 2. Univariate analysis in different age categories.

	Age group	HLA-compatibility	N	1-year	3-year	P
	18-35 (N=529)	10/10	295	0.67 (0.61-0.73)	0.53 (0.47-0.60)	n.s.
		9/10	172	0.61 (0.54-0.69)	0.54 (0.46-0.63)	
		8/10	62	0.64 (0.53-0.78)	0.45 (0.33-0.61)	
Overall survival	36-55 (N=1295)	10/10	774	0.63 (0.59-0.67)	0.49 (0.45-0.53)	<0.001
		9/10	397	0.50 (0.45-0.56)	0.39 (0.34-0.45)	
		8/10	124	0.45 (0.37-0.55)	0.35 (0.27-0.45)	
	>55 (N=1195)	10/10	778	0.59 (0.55-0.63)	0.41 (0.37-0.46)	<0.001
		9/10	342	0.47 (0.42-0.54)	0.34 (0.29-0.41)	
		8/10	75	0.27 (0.17-0.40)	0.19 (0.11-0.32)	
	18-35 (N=529)	10/10	295	0.59 (0.53-0.65)	0.45 (0.39-0.52)	n.s.
		9/10	172	0.53 (0.46-0.62)	0.50 (0.42-0.59)	
		8/10	62	0.49 (0.38-0.64)	0.37 (0.26-0.52)	
Disease-free survival	36-55 (N=1295)	10/10	774	0.53 (0.49-0.57)	0.38 (0.34-0.42)	0.016
		9/10	397	0.42 (0.37-0.47)	0.32 (0.27-0.37)	
		8/10	124	0.40 (0.32-0.50)	0.32 (0.24-0.42)	
	>55 (N=1195)	10/10	778	0.49 (0.45-0.53)	0.30 (0.26-0.35)	<0.001
		9/10	342	0.40 (0.34-0.46)	0.24 (0.19-0.30)	
		8/10	75	0.20 (0.12-0.33)	0.12 (0.06-0.25)	
	18-35 (N=529)	10/10	295	0.22 (0.17-0.27)	0.28 (0.22-0.34)	n.s.
		9/10	172	0.24 (0.18-0.31)	0.26 (0.19-0.33)	
		8/10	62	0.26 (0.15-0.38)	0.36 (0.24-0.49)	
Relapse incidence	36-55 (N=1295)	10/10	774	0.22 (0.19-0.25)	0.30 (0.26-0.33)	n.s.
		9/10	397	0.24 (0.20-0.28)	0.29 (0.24-0.34)	
		8/10	124	0.23 (0.16-0.31)	0.29 (0.21-0.38)	
	>55 (N=1195)	10/10	778	0.22 (0.19-0.25)	0.32 (0.28-0.36)	n.s.
		9/10	342	0.24 (0.19-0.29)	0.30 (0.25-0.36)	
		8/10	75	0.30 (0.19-0.41)	0.35 (0.23-0.47)	
	18-35 (N=529)	10/10	295	0.15 (0.11-0.20)	–	n.s.
		9/10	172	0.19 (0.13-0.26)	–	
		8/10	62	0.23 (0.13-0.34)	–	
Transplant-related mortality	36-55 (N=1295)	10/10	774	0.19 (0.16-0.22)	–	<0.001
		9/10	397	0.29 (0.25-0.34)	–	
		8/10	124	0.39 (0.30-0.48)	–	
	>55 (N=1195)	10/10	778	0.22 (0.18-0.25)	–	<0.001
		9/10	342	0.30 (0.25-0.36)	–	
		8/10	75	0.40 (0.28-0.52)	–	

N: number within the respective group; 95% confidence interval in parentheses; n.s.: not significant.

tial increase in elderly patients transplanted in Germany in recent years.

Other large studies investigating the impact of risk factors in HSCT contained significantly fewer older patients, which is why this interaction may have remained unnoticed in these studies.[22-24]

Interestingly, in the youngest age group, no significant difference was found between completely 10/10 matched transplantations and single or double mismatched transplantations. However, this age category was the smallest, consisting of only 17.5% of the cases, which limits interpretation of this particular result. Testing for proportional hazards assumption in our models showed no significant violation for the covariate age, which was treated as a continuous variable in the interaction model and in the prediction plot (Figure 2). Thus, the way we chose to visualize the disproportional increase in hazard ratios for age-risk at the time of transplantation is justified.

Our results were obtained from a cohort transplanted with allogeneic unrelated PBSC or bone marrow as a graft source. In our analysis, graft source did not differentially impact outcome, which is why no separate analysis for each graft source was made. Similar findings were reported in other studies.[25,26] Data on the impact of haploidentical transplantation or cord blood transplantations on the outcome of HSCT in elderly patients are very limited, so that a sensible risk-benefit comparison of our data with alternative graft or transplant sources is difficult. However, cord blood transplantation has been reported to result in similar outcomes in a small cohort of single mismatched transplantations in elderly patients treated with RIC.[27]

In multivariate analysis (Table 4), some predictors showed violation of the proportional hazards assumption (PHA). These violations can be explained by a higher early mortality for patients transplanted in advanced disease stage, transplanted before 2004 and treated with MAC. To

Table 3. Risk estimates for HLA mismatches according to age categories.

End point	Age group	HLA	N	HR (95% CI)	Within group	*P*
	18-35 (N=529)	10/10	295	1.00	1.00	
		9/10	172	0.94 (0.70-1.26)	0.94 (0.70-1.26)	n.s.
		8/10	62	1.14 (0.77-1.71)	1.14 (0.77-1.71)	n.s.
Overall survival	36-55 (N=1295)	10/10	774	1.26 (1.01-1.57)	1.00	
		9/10	397	1.57 (1.25-1.98)	1.25 (1.05-1.48)	0.012
		8/10	124	1.76 (1.32-2.36)	1.40 (1.09-1.80)	0.008
	>55 (N=1195)	10/10	778	1.54 (1.22-1.92)	1.00	
		9/10	342	1.93 (1.50-2.47)	1.25 (1.05-1.50)	0.014
		8/10	75	3.48 (2.49-4.86)	2.27 (1.70-3.03)	<0.001
	18-35 (N=529)	10/10	295	1.00	1.00	
		9/10	172	0.87 (0.66-1.14)	0.87 (0.66-1.14)	n.s.
		8/10	62	1.11 (0.77-1.60)	1.11 (0.77-1.60)	n.s.
Disease-free survival	36-55 (N=1295)	10/10	774	1.23 (1.01-1.49)	1.00	
		9/10	397	1.42 (1.15-1.75)	1.16 (0.99-1.35)	n.s.
		8/10	124	1.58 (1.21-2.08)	1.29 (1.02-1.64)	0.033
	>55 (N=1195)	10/10	778	1.45 (1.18-1.77)	1.00	
		9/10	342	1.67 (1.34-2.09)	1.15 (0.98-1-36)	n.s.
		8/10	75	2.74 (2.00-3.76)	1.89 (1.44-2.50)	<0.001
	18-35 (N=529)	10/10	295	1.00	1.00	
		9/10	172	1.04 (0.71-1.51)	1.04 (0.71-1.51)	n.s.
		8/10	62	1.24 (0.77-1.99)	1.24 (0.77-1.99)	n.s.
Relapse incidence	36-55 (N=1295)	10/10	774	1.02 (0.77-1.34)	1.00	
		9/10	397	1.00 (0.74-1.36)	0.99 (0.78-1.25)	n.s.
		8/10	124	1.10 (0.74-1.63)	1.08 (0.76-1.54)	n.s.
	>55 (N=1195)	10/10	778	0.97 (0.73-1.29)	1.00	
		9/10	342	1.00 (0.72-1.39)	1.03 (0.79-1.34)	n.s.
		8/10	75	1.23 (0.76-2.00)	1.27 (0.81-1.98)	n.s.
	18-35 (N=529)	10/10	295	1.00	1.00	
		9/10	172	1.15 (0.71-1.85)	1.15 (0.71-1.85)	n.s.
		8/10	62	1.51 (0.81-2.82)	1.51 (0.81-2.82)	n.s.
Transplant-related mortality	36-55 (N=1295)	10/10	774	1.39 (0.98-1.99)	1.00	
		9/10	397	2.11 (1.47-3.04)	1.51 (1.18-1.95)	0.001
		8/10	124	3.01 (1.96-4.62)	2.16 (1.53-3.05)	<0.001
	>55 (N=1195)	10/10	778	1.70 (1.18-2.45)	1.00	
		9/10	342	2.32 (1.58-3.41)	1.36 (1.05-1.77)	0.022
		8/10	75	3.79 (2.29-6.30)	2.23 (1.47-3.37)	<0.001

N: number within the respective group; HR: Hazard ratio; CI: Confidence Interval; n.s.: not significant. *P*-values are computed for the comparison of 9/10 and 8/10 groups with the 10/10 matched transplantations within each age category. Relative risk computation is also performed for each age group (within group). The *P*-values refer to this comparison. Other covariates included: disease stage, KIR-ligand status, national donor *versus* international donor, conditioning treatment, year of transplantation.

Table 4. Multivariate analysis.

End point	Predictor	HR (95% CI)	P
Overall survival	Age-risk (10/10 HLA)	1.015 (1.010-1.020)	<0.001
	Age-risk (9/10 HLA)	1.019 (1.014-1.024)	<0.001
	Age-risk (8/10 HLA)	1.026 (1.020-1.031)	<0.001
	Intermediate disease stage	1.37 (1.19-1.57)	<0.001
	Advanced disease stage until day 314 post Tx	2.37 (2.04-2.74)	<0.001
	Advanced disease stage after day 314 post Tx	1.03 (0.78-1.36)	n.s.
	Patient C2C2 KIR ligand status	1.25 (1.08-1.43)	0.002
	National donor	0.83 (0.73-0.95)	0.005
	RIC *vs.* MAC until day 96	0.57 (0.46-0.70)	<0.001
	RIC *vs.* MAC after day 96	1.13 (0.98-1.31)	n.s.
	Tx before 2004 until day 198 post Tx	1.43 (1.18-1.72)	<0.001
	Tx before 2004 after day 198 post Tx	1.05 (0.82-1.34)	n.s.
Disease-free survival	Age-risk (10/10 HLA)	1.014 (1.010-1.018)	<0.001
	Age-risk (9/10 HLA)	1.016 (1.012-1.021)	<0.001
	Age-risk (8/10 HLA)	1.023 (1.017-1.028)	<0.001
	Intermediate disease stage	1.51 (1.33-1.71)	<0.001
	Advanced disease stage until day 253 post Tx	2.35 (2.05-2.70)	<0.001
	Advanced disease stage after day 253 post Tx	1.36 (1.08-1.71)	0.009
	Patient C2C2 KIR ligand status	1.17 (1.03-1.33)	0.019
	National donor	0.84 (0.74-0.94)	0.003
	RIC *vs.* MAC until day 81	0.83 (0.70-0.97)	0.021
	RIC *vs.* MAC after day 81	1.01 (0.88-1.16)	n.s.
	Tx before 2004 until day 205 post Tx	1.27 (1.07-1.51)	0.006
	Tx before 2004 after day 205 post Tx	0.80 (0.62-1.03)	n.s.

HR: Hazard Ratio; HLA: Human Leukocyte Antigen; RIC: reduced intensity conditioning; MAC: myeloablative conditioning; Tx: transplantation; n.s.: not significant.

reflect this relationship, an extended Cox regression model was fitted to obtain regression estimates for the respective predictors according to time periods where PHA is satisfied, as we have shown before.[28] In analysis of OS, advanced disease stage showed a substantially higher mortality risk until day 314 but not thereafter. Patients treated with RIC showed a significantly lower early mortality until day 96 and a non-significantly different risk afterwards. In addition, patients transplanted before 2004 showed a higher mortality risk until day 198 after transplantation but not thereafter. Similar findings were present in an analysis of DFS. In our models, also a patient C2C2 KIR-ligand status as well as an international donor status was associated with adverse outcome, which we have reported before.[29] ATG treatment was not included in the final models because it did not reach statistical significance.

Our analysis encompassed some simplifications, namely that any HLA-mismatch was considered equally. HLA-DPB1 mismatches were not included and the vector of mismatches was also not regarded.

We included HLA-DQB1 mismatches in this study, because a previous analysis on the same dataset has shown that these mismatches are associated with higher mortality risk.[29]

HLA-DPB1 mismatches have been shown to influence outcome of HSCT, but due to lower linkage disequilibrium, HLA-DPB1-mismatches in HLA-A, -B, -C, -DRB1 and -DQB1 matched and mismatched transplantations are almost equally distributed.[30] Therefore, we may assume that our results are not biased by not including HLA-DPB1. The vector of mismatches was not considered, because no significant differences in survival outcome have been seen for unidirectional mismatches when compared to bidirectional mismatches for the end points analyzed in our study.[14]

We refrained from including Karnofsky performance status and donor-recipient cytomegalovirus status due to the high proportion of missing data for these variables, which is a limitation of our analysis.

When selecting donors for elderly patients, the additional risk associated with HLA-mismatches in this age group should be considered. Especially when only donors with double HLA-mismatches are available for such a patient, the substantial risk conferred in this situation must be carefully weighed against the benefit of transplantation. Cord blood transplantation might be an alternative in such cases, although data regarding the impact of alternative graft sources for transplantation of elderly patients are still limited.

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