

Impact of genomic risk factors on outcome after hematopoietic stem cell transplantation for patients with chronic myeloid leukemia

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ABSTRACT

Background

Non-HLA gene polymorphisms have been shown to influence outcome after allogeneic hematopoietic stem cell transplantation. Results were derived from heterogeneous, small populations and their value remains a matter of debate.

Design and Methods

In this study, we assessed the effect of single nucleotide polymorphisms in genes for interleukin 1 receptor antagonist (*IL1RN*), interleukin 4 (*IL4*), interleukin 6 (*IL6*), interleukin 10 (*IL10*), interferon (*IFNG*), tumor necrosis factor (*TNF*) and the cell surface receptors tumor necrosis factor receptor II (*TNFRSF1B*), vitamin D receptor (*VDR*) and estrogen receptor alpha (*ESR1*) in a homogeneous cohort of 228 HLA identical sibling transplants for chronic myeloid leukemia. Three good predictors of overall survival, identified via statistical methods including Cox regression analysis, were investigated for their effects on transplant-related mortality and relapse. Predictive power was assessed after integration into the established European Group for Blood and Marrow Transplantation (EBMT) risk score.

Results

Absence of patient *TNFRSF1B* 196R, absence of donor *IL10* ATA/ACC and presence of donor *IL1RN* allele 2 genotypes were associated with increased transplantation-related mortality and decreased survival. Application of prediction error and concordance index statistics gave evidence that integration improved the EBMT risk score.

Conclusions

Non-HLA genotypes were associated with survival after allogeneic hematopoietic stem cell transplantation. When three genetic polymorphisms were added into the EBMT risk model they improved the goodness of fit. Non-HLA genotyping could, therefore, be used to improve donor selection algorithms and risk assessment prior to allogeneic hematopoietic stem cell transplantation.

Key words: genetic risk factors, statistical modeling, hematopoietic stem cell transplantation.

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The online version of this article has a Supplementary Appendix.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is the main curative therapy for many congenital or acquired disorders of the hematopoietic system but is still associated with substantial morbidity and mortality.^{1,2} The main clinical factors influencing outcome after HSCT have been determined through analyses of patients undergoing HSCT for chronic myeloid leukemia (CML). Patients' age, stage of the disease, time interval from diagnosis to transplant, histocompatibility and donor and patient gender combination have been identified as key pre-transplant risk factors in the European Group for Blood and Marrow Transplantation (EBMT) clinical risk score³ for all patients undergoing HSCT for a hematologic disorder.⁴ Furthermore, a comorbidity score has been introduced as a major additional co-factor for transplant-related mortality.⁵ Recognition of these factors has led to different decision algorithms and transplant procedures for patients with high-risk disease and low-risk donors or patients with low-risk disease and high-risk donors. In parallel, major progress has been made in the drug treatment of CML, although the best time point for HSCT remains open because of second-generation tyrosine kinase inhibitors.⁶⁻⁸ Even better characterization of the pre-transplant risk profile is, therefore, urgently warranted.

In the last decade several groups, including ours, have demonstrated that polymorphisms of non-HLA genes for components of the innate immune system, pro- and anti-inflammatory cytokines and steroid receptors are predictive of outcome following HSCT.⁹

These results were derived from small series and were not adjusted in a standardized format for the key risk factors for HSCT outcome. We, therefore, investigated the role of non-HLA genetics in a homogenous cohort of CML patients who underwent transplantation from HLA-matched sibling donors and, using the information collected, derived a novel statistical model that integrates genetics into the EBMT clinical risk score.

Design and Methods

Study design

The final study population consisted of 228 HLA-matched sibling adult patient and donor pairs (Table 1) collected prospectively with complete clinical and genotyping data from ten European transplant centers. All clinical data were collected from the EBMT database ProMIsE. At the last date of contact, 37% of patients had died and 24% had relapsed. The majority of patients were in first chronic phase CML (80.3%), 12.3% were in accelerated phase, 4.8% were in second chronic phase and 2.6% were in blast crisis at the time of transplantation. Transplants were conducted between April 1984 and September 2003, all in the pre-imatinib era. HLA genotype was determined by standard technologies using serology or molecular typing and all patients and donors were HLA-matched siblings. All except one patient received standard bone marrow myeloablative conditioning. All patients received standard supportive care which was comparative across centers. The median survival time for those patients who were alive at last contact was 54 months. The median survival time for the patients who had died was 4.5 months. The main causes of death were infection (39%), graft-versus-host disease (GVHD) (24%) and relapse (22%); other causes of death included acute respiratory distress syndrome and veno-occlusive disease. Clinical relapse was defined as hematologic, cytogenetic or molecular relapse.¹⁰

All patients and donors gave informed consent to participation in the study in accordance with EBMT guidelines. The protocol was approved by the Local Research Ethics Committee at the coordinating center (Newcastle upon Tyne, UK).

Genotyping for cytokine gene polymorphisms

DNA from the patients and donors was analyzed at a single coordination center (Newcastle University, UK) for single nucleotide polymorphisms (SNP) or microsatellites. The SNP or microsatellites investigated included those in genes for cytokines or cytokine receptors – interleukin 1 receptor antagonist (*IL1RN*),¹¹ interleukin 4 (*IL4*),¹² interleukin 6 (*IL6*),¹³ interleukin 10 (*IL10*),¹⁴ interferon (*IFNG*),¹⁵ tumor necrosis factor (*TNF*),¹⁶ and tumor necrosis factor receptor II (*TNFRSF1B*),¹⁷ – and the steroid hormone receptors vitamin D receptor (*VDR*)¹⁸ and estrogen receptor alpha (*ESR1*).¹⁹

The *IL10* SNP, *IL10*⁻¹⁰⁸² and *IL10*⁻⁵⁹² were recorded as haplotypes as previously described.²⁰

Statistical analysis

The impact of selected non-HLA genetic factors on survival, transplant-related mortality and relapse was assessed. The methods used included Kaplan-Meier estimates, Cox regression modeling and competing risks analysis.²¹ The predictive accuracy of the models was quantified using the concordance (c) index²² and prediction error (i.e. 0.632+ bootstrap estimator²³). NCSS,²⁴ R (version 2.6.2) and SPSS-14 were used for the computations.

Results

Analysis of factors

After initial univariable analysis, ten genetic variables were identified as being associated with survival. These variables were entered into a variable selection Cox regression modeling procedure alongside the EBMT risk score.²⁵⁻²⁷ The EBMT risk score was entered into the model on an ordinal scale after initial testing for linearity.²⁸

Three genetic variables (Table 2) remained significantly associated with survival together with the EBMT risk score. Absence in the donor of the *IL10* ATA/ACC genotype* (N=200, 87.7%) (HR 0.43, 95% confidence interval: 0.17-1.07), presence in the donor of the *IL1RN* allele 2 genotype (N=45, 19.7%) (HR 2.45, 95% confidence interval: 1.48-4.05) and absence in the patient of the *TNFRSF1B* 196R genotype (N=125, 54.8%) (HR 0.55, 95% confidence interval: 0.35-0.87) decreased survival time – this is further illustrated in *Online Supplementary Figure S1*. The EBMT risk score remained significant for survival in the final model with a hazard ratio of 1.67 (95% confidence interval: 1.38-2.02).

The decrease in survival was primarily due to an increase in transplant-related mortality (*Online Supplementary Table S1*). The three genetic predictors had no impact on relapse as indicated by Gray's test.³⁰

**After stepwise selection, donor IL10 ATA/ACC has a P value of 0.07 in the model – this variable was still found to significantly improve the model when all variables in the final model were assessed via hierarchical likelihood ratio tests (level of significance 0.05).²⁹*

Validity testing of the selected genetic variables

The high-risk group was defined as patients with all three adverse genetic polymorphisms (N=25). The low-risk group comprised all remaining patients. Survival prob-

ability was lower and incidence of transplant-related mortality was higher for the high-risk group (Figure 1). As the EBMT score increased, survival probability decreased and transplant-related mortality incidence increased (Online Supplementary Figure S2 A-B). It was also found that having just one or two of the adverse genotypes conferred an increased risk (hazard) of transplant-related mortality and death as compared to the EBMT score alone (Online Supplementary Table S2). This suggested an additive gene dosage effect.

After rounding and scaling Cox regression model coefficients, the genotypes were scored as 1 or 0 depending on whether their presence or absence was detrimental to transplant outcome i.e. absence of *IL10* ATA/ACC genotype in the donor was scored 1 while its presence was scored 0; presence of *IL1RN* allele 2 genotype in the donor was scored 1 and its absence was scored 0; absence of *TNFRSF1B*-196R genotype in the recipient was scored 1 and its presence was scored 0. This approach enabled up to three score points to be added to the original EBMT score. The resulting scores ranged from 0 to 8. No patients scored "9" i.e. none of the patients whose EBMT score was 6 had all three unfavorable genotypes.

The likelihood ratio test indicated that the genetic predictors, when viewed in addition to the EBMT risk score, improved the goodness of fit ($P < 0.0005$).

Predictive value was quantified using the concordance

index²² and prediction error curves²³ for (i) a model with the single EBMT risk score variable and (ii) a model containing the single EBMT risk score and the three genotypes. The latter always appeared superior. Further details of these statistical analyses are provided in the Online Supplementary Appendix.

Discussion

In this study we screened DNA from patients and donors for SNP or microsatellites including those associated with cytokine genes transcribing high or low levels of cytokines and/or inhibitors. Three genotypes were demonstrated to be associated with impaired survival, *IL10* ATA/ACC (absence in the donor genotype), *IL1RN* (allele 2) (presence in the donor genotype) and *TNFRSF1B* 196R (absence in the patient genotype). For all three genotypes, survival was reduced due to increased transplant-related mortality and their effects were additive with worst survival in the group of 25 patients with all three unfavorable genotypes. These genotypes had no effect on relapse.

These data fit with previous observations and explain some discrepancies. Tumor necrosis factor (TNF) receptor II, interleukin 10 (IL-10) and interleukin 1 (IL-1) all play a role in modulating the "cytokine storm" of GVHD. Absence of the *TNFRSF1B* allele R in the patient is indica-

Table 1. Patients' characteristics: clinical factors.

	High Risk Genotype Group ¹		Low Risk Genotype Group ¹		Total Frequency (%)	P value (2 sided tests) ⁵
	Frequency	% (of 228)	Frequency	% (of 228)		
Patients' gender						
Female	13	5.70	75	32.89	88 (38.6)	0.19*
Male	12	5.26	128	56.14	140 (61.4)	
Stage of disease at transplantation						
First chronic phase	20	8.77	163	71.49	183 (80.3)	1.00†
First accelerated phase	3	1.32	25	10.96	28 (12.3)	
Higher chronic phase or blast crisis	2	0.88	15	6.58	17 (7.5)	
Female donor to male patient						
Yes	5	2.19	60	26.32	65 (28.5)	0.36†
No	20	8.77	143	62.72	163 (71.5)	
Patients' age at transplantation						
Median age	38	38				0.63‡
Range	16-59	17-60				
Patients' age at transplantation						
16-19 years	1	0.44	6	2.63	7 (3.1)	1.00†
20-40 years	14	6.14	115	50.44	129 (56.6)	
Greater than 40 years	10	4.39	82	35.96	92 (40.4)	
Time from diagnosis to transplant						
12 months	17	7.46	134	58.77	151 (66.2)	1.00†
More than 12 months	8	3.51	69	30.26	77 (33.8)	
Year of HSCT						
1984-1997	13	5.70	117	51.32	130 (57.0)	0.67†
1998-2003	12	5.26	86	37.72	98 (43.0)	
EBMT risk score ²						
0-1	9	3.95	51	22.37	60 (26.3)	0.509‡
2	7	3.07	68	29.82	75 (32.9)	
3	6	2.63	65	28.51	71 (31.1)	
4-6	3	1.32	19	8.33	22 (9.7)	

¹High risk/low risk genotype group: For definitions see Statistical Analysis section. ²EBMT risk score. For definition see Gratwohl et al.³ *chi squared test; †linear-by-linear association (Mantel-Haenszel chi-square); ‡sample t test; §Exact significance is reported providing a reliable result regardless of the size, distribution, sparseness, or balance of the data.

tive of increased levels of soluble TNFR2 in the serum and decreased TNF.¹⁷ With regards to IL10, higher IL-10 secretion has been demonstrated in subjects with the -1082G allele or GCC haplotype, lower levels with the -1082A allele and ATA haplotype, and intermediate levels in those with the ACC haplotype. These haplotypes are defined by three SNP within the promoter region of the *IL-10*: -1082 (G/A), -819 (C/T), and -592 (C/A).^{20,31,32} The presence of *IL1RN* (allele 2) in the donor has been associated with a reduced incidence of acute GVHD (grades II-IV) and protection from chronic GVHD and reflects the down-regulation of IL-1 by IL-1 receptor antagonist.¹¹ These biological effects render it likely that the effects identified in this study do indeed reflect activity of these genetic polymorphisms.

Results so far from other groups have been inconsistent, probably reflecting the heterogeneity of the transplant cohorts under study. The *IL10* ATA/ACC haplotype (low to intermediate IL-10 producer) was the least frequent within the present cohort, occurring in 28/228 (12%) of donors tested. The absence of the *IL10* ATA/ACC haplotype (or conversely, the presence of ACC/ACC, ACC/GCC, ATA/ATA, ATA/GCC, or GCC/GCC) in the remaining 88% of donors was always associated with the EBMT high-risk groups and poor survival. The presence of the *IL10* ATA/ACC haplotype in the recipient has been associated in a large cohort of HLA-matched sibling transplants with a moderate risk of both GVHD and lowest risk of death in remission,³³ more severe GVHD occurring in groups without the *IL10* ATA haplotype. The study by Lin *et al.*³³ assessed overall survival, allele frequency and genotypes at each *IL-10* SNP and showed that the risk of death was lowest in recipients who were homozygous for the -592A allele. This pattern was confirmed in our cohort (*results not shown*) in which the presence of the homozygous AA allele at -592 conferred the lowest hazard ratio (0.41 compared to that of the group with the CC reference genotype). With regards to IL-10 production, it is still debatable whether the GCC haplotype is associated with higher or lower IL-10 production in comparison to the ATA haplotype. In this regard, in another study of unrelated donor transplants, transplant-related mortality was associated with the higher IL-10 producer GCC haplotype when present in the donor.³⁴ However the lower producer allele (*IL10*-1082A) has also been associated with poor survival.³⁵ In a study of peripheral blood stem cell HLA-matched sibling transplants, the presence of *IL10* haplotypes (ACC/ACC *versus* ATA/ATC *versus* ATA/ATA *i.e.* lower IL-10 production) increased the incidence of chronic GVHD and in a further study subjects with ATA haplo-

types required more prolonged immunosuppression and were more susceptible to pulmonary aspergillosis,^{36,37} whereas the ACC haplotype reduced the risk 9-fold.

What are the consequences of these data which were primarily derived from patients with CML and HSCT in the pre-imatinib era? The success of the phase III IRIS trial of the tyrosine kinase inhibitor, imatinib, in 2003³⁸ has led to a substantial decrease in the use of HSCT for newly diagnosed CML and HSCT has become a second-line therapy option for patients in whom imatinib treatment has failed. With the advent of second generation tyrosine

Table 2. Details of model containing EBMT risk score and three additional genotypes. (N=228)

	Coefficient	P value	Hazard ratio	Confidence interval (95%)
EBMT risk score	0.513	<0.0005	1.67	1.38-2.02
Cytokine gene polymorphism				
Donor <i>IL10</i> ATA/ACC	-0.842	0.07	0.43	0.17-1.07
Donor <i>IL1RN</i> allele 2	0.894	0.001	2.45	1.48-4.05
Patient <i>TNFRSF1B</i> 196 R	-0.603	0.01	0.55	0.35-0.87

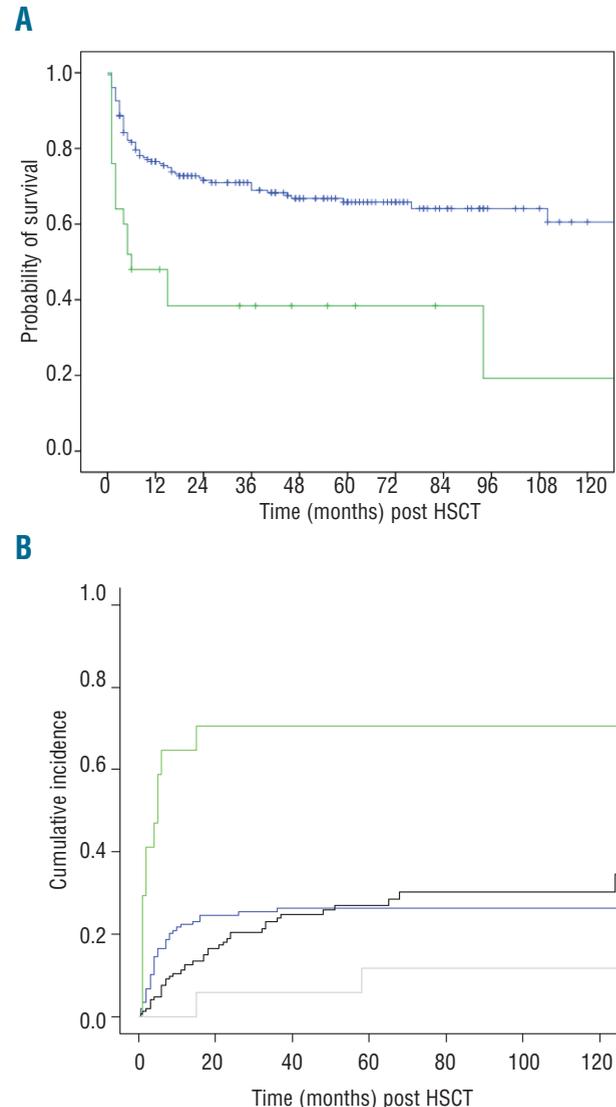


Figure 1. (A) Survival probability of 228 patients undergoing allogeneic HSCT from an HLA identical sibling for CML (Kaplan Meier curves), log rank test, $P < 0.0005$. Green line corresponds to high risk SNP profile, $N = 25$. Blue line corresponds to low risk SNP profile, $N = 203$. Crosses represent censored observations. (B) Cumulative incidence of transplant-related mortality and relapse for 228 patients undergoing allogeneic HSCT from an HLA identical sibling HSCT for CML. Green line corresponds to high risk SNP profile (transplant-related mortality). Blue line corresponds to low risk SNP profile (transplant-related mortality). Gray's test, $P < 0.0005$. Gray line corresponds to high risk SNP profile (relapse). Black line corresponds to low risk SNP profile (relapse). Gray's test, $P = 0.13$.

kinase inhibitors, the timing of HSCT for patients with a donor has become crucial. It will be essential to balance the risks and benefits of a transplant against those of second-line therapies.³⁹ Integration of cytokine polymorphisms into the established EBMT risk score may improve patient counseling in the new era.⁴⁰⁻⁴⁴ A favorable genotype profile may tip the balance towards early HSCT, even in responders, and an unfavorable profile, towards a watch and wait strategy.

The EBMT risk score has recently been confirmed to predict HSCT outcome for all hematologic indications,⁴ and additional SNP testing could be added to the EBMT risk score in general. Furthermore, with the increased use of reduced intensity conditioning regimens,⁴⁵ additional SNP testing could be used to decide between standard conditioning for patients with a low risk for transplant-related mortality, reduced conditioning for patients with a high risk for transplant-related mortality, or for delaying HSCT until progression.⁴⁶

Additional risk factors have also been clearly established, such as cytomegalovirus serostatus of the recipient, performance score or comorbidity index. Further gene polymorphisms will continue to be identified and be assessed for their impact on outcome, such as those detailed in recent studies on polymorphisms in the transglutaminase gene.⁴⁷ It remains difficult to test the impact of genetic variations in such heterogeneous HSCT patient populations with traditional multivariable statistical methods. We propose the novel approach from the present analysis i.e. application of the concordance index, prediction error and the likelihood ratio test to give evidence that genetic predictors do improve the goodness of fit and predictive ability when added to a model consisting of the EBMT risk score alone. Increasing the goodness of fit in other situations, such as in cohorts of matched unrelated donors, would be a strong argument for further utilization of this novel approach. We also recognize the need for validation in a volunteer donor population.

In conclusion, a few well-defined donor or recipient cytokine polymorphisms affect outcome after HSCT, and a statistical technique has been described to verify poten-

tial new genomic risk factors. The approach will refine risk assessment in order to improve future therapeutic protocols on an individual patient basis.

Authorship and Disclosures

AMD designed/performed research, collected, analyzed and interpreted data and wrote the manuscript. KFP performed statistical analyses, analyzed and interpreted data and wrote the manuscript. JN performed research, collected data and wrote the manuscript. SO'B collected data. EH, VR, H-JK, IH, TR, JA, EG, WS, TdW and PS collected data/clinical samples. DN aided in the design of the research, and collected data/clinical samples. RB and RS gave advice on some areas of the statistical analysis. HB and YB carried out the prediction error analysis and AG designed the research, collected, analyzed and interpreted data and, in part, wrote the manuscript.

The authors reported no potential conflicts of interest.

Appendix - Participating teams

Department of Haematology and Haematological Sciences, Institute of Cellular Medicine, Newcastle University and scientific/clinical transplant teams, including Professor G Jackson, Dr A Neylon, Dr A Lennard, Dr G Stark, Dr P Middleton and Dr M Collin; Industrial Statistics Research Unit (ISRU), Stephenson Centre, Newcastle University, UK; Universitätsklinikum Hämatologie/Onkologie, Regensburg, Germany; Hôpital St Louis, especially Professor E Gluckman, Paris, France; Klinikum Grosshadern, Department of Genetics and Epidemiology, University of Göttingen, Ludwig Maximilian Universität, Munich, Germany; 2nd Medical Faculty and 3rd Medical Faculty, Charles University, Prague, Czech Republic; Klinik und Poliklinik, Universitätsklinik Leipzig, Germany; Department of Medical Statistics, University Hospital, Leiden, The Netherlands, Division of Hematology, Department of Medicine, University Central Hospital, Helsinki, Finland; Department of Hematology, Hammersmith Hospital, London, UK including acknowledgment of Dr E Olavarria and team; Immunohematology and Blood Transfusion, University Medical Center, Leiden, The Netherlands; Hämatologie/Onkologie, Virchow Klinikum Charité, Berlin, Germany; Department of Hematology, University Hospital St Radboud, Nijmegen, The Netherlands; Hämatologie, University Hospital, Basel, Switzerland; University of Genova, Department of Internal Medicine, Dr A Bacigalupo and team, Genova, Italy.

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