Dynamic International Prognostic Scoring System scores, pre-transplant therapy and chronic graft-*versus*-host disease determine outcome after allogeneic hematopoietic stem cell transplantation for myelofibrosis

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ABSTRACT

Background
Myelofibrosis is a myeloproliferative stem cell disorder curable exclusively by allogeneic hematopoietic stem cell transplantation and is associated with substantial mortality and morbidity. The aim of this study was to assess disease-specific and transplant-related risk factors that influence post-transplant outcome in patients with myelofibrosis.

Design and Methods
We retrospectively assessed 76 consecutive patients with primary (n=47) or secondary (n=29) myelofibrosis who underwent bone marrow (n=6) or peripheral blood stem cell (n=70) transplantation from sibling (n=30) or unrelated (n=46) donors between January 1994 and December 2010. The median follow-up of surviving patients was 55±7.5 months.

Results
Primary graft failure occurred in 5% and the non-relapse mortality rate at 1 year was 28%. The relapse-free survival rate was 50% with a relapse rate of 19% at 5 years. The use of pharmacological pre-treatment and the post-transplant occurrence of chronic graft-*versus*-host disease were significant independent unfavourable risk factors for post-transplant survival in multivariate analysis. Using the Dynamic International Prognostic Scoring System for risk stratification, low-risk patients had significantly better overall survival (P=0.014, hazard ratio 1.4) and relapse-free survival (P=0.02, hazard ratio 1.3) compared to the other risk groups of patients. The additional inclusion of thrombocytopenia, abnormal karyotype and transfusion need (Dynamic International Prognostic Scoring System Plus) resulted in a predicted 5-year overall survival of 100%, 51%, 54% and 30% for low, intermediate-1, intermediate-2 and high-risk groups, respectively. The relapse incidence was significantly higher in the absence of chronic graft-*versus*-host disease (P=0.006), and pharmacological pre-treatment (n=43) was associated with reduced relapse-free survival (P=0.001).

Conclusions
The data corroborate a strong correlation between alloreactivity and long-term post-transplant disease control and confirm an inverse relationship between disease stage, pharmacotherapy and outcome after allogeneic hematopoietic stem cell transplantation for myelofibrosis. The Dynamic International Prognostic Scoring System was demonstrated to be useful for risk stratification of patients with myelofibrosis who are to undergo hematopoietic stem cell transplantation.

Key words: myelofibrosis, allogeneic, graft-*versus*-host disease, DIPSS, stem cell transplantation.

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Introduction

Myelofibrosis is a clonal proliferative disorder of the hematopoietic stem cells unconnected with the *BCR-ABL* translocation, and clinically characterized by bone marrow fibrosis, splenomegaly, leukoerythroblastosis, extramedullary hematopoiesis and a constellation of debilitating symptoms.[1] The affected hematopoietic clone harbours the V617F mutation in Janus Kinase 2 (JAK2) in approximately 50% of patients with myelofibrosis.[2] Other mutations in the *JAK2* gene, for instance in exon 12, or in the myeloproliferative leukemia virus oncogene, *MPL*, have also been shown to result in exaggerated JAK2 signaling.[3] Myelofibrosis encompasses primary myelofibrosis and secondary forms, which include post-polycythemia vera and post-essential thrombocythemia myelofibrosis and blast-phase primary myelofibrosis.[4] The presentation and course of this myeloproliferative neoplasm, affecting mainly the elderly, is heterogeneous. Survival ranges between 2 and 15 years and is linked to a number of risk factors.[5] Scoring systems have been developed based on these risk factors,[6-8] but are only applicable for stratification of patients at diagnosis. The International Working Group for Myeloproliferative Neoplasms Research and Treatment has established the Dynamic International Prognostic Scoring System (DIPSS) to classify patients any time during their disease course; more recently the additional incorporation of the poor prognostic factors thrombocytopenia, unfavorable karyotype and transfusion need resulted in the development of DIPSS Plus.[9,10] Accurate risk stratification is of critical importance because treatment decisions, in particular with regard to the timing of allogeneic hematopoietic stem cell transplantation (HSCT), are often challenging. Allogeneic HSCT offers the only potential for cure for myelofibrosis, with the overall survival rate being 40% to 65% after myeloablative conditioning. However, this procedure was largely restricted to younger individuals with poor prognostic factors because of the substantial rate of transplant-related mortality (approximately 30%).[11-14] Transplant-related mortality was lower in small series of patients treated with reduced intensity conditioning, suggesting a wider applicability of transplantation, especially for older patients.[15-16] Recent evaluations have, however, revealed comparable long-term disease-free and overall survival rates of patients, regardless of whether they were treated with reduced intensity or myeloablative conditioning.[17,18] Steward *et al*. reported a trend towards a higher relapse incidence in patients who received reduced intensity conditioning than in patients who underwent myeloablative conditioning.[19] However, some studies demonstrated a certain impact of conditioning regimen on overall survival or overall mortality after transplantation.[12,16] There are currently no accepted guidelines on how to choose the best time to conduct allogeneic HSCT in patients with myelofibrosis. The DIPSS, as a dynamic time-dependent prognostic model, may provide useful information given that it is applicable to the transplant setting. Data for assessing the influence of chronic graft-*versus*-host disease (GVHD) on patients' outcome after allogeneic HSCT are rare to date. To provide a basis to assess the impact of GVHD and dynamic time-dependent risk stratification on patients' survival, we reanalyzed data from 76 patients with myelofibrosis who received transplants from sibling or unrelated donors. Analyses focused on the impact on post-transplant survival of transplant-related factors, including donor, graft and HLA characteristics and time-dependent occurrence of chronic GVHD in addition to pre-transplant characteristics such as DIPSS, DIPSS-Plus, *JAK2* mutation status, time-interval between diagnosis and HSCT and whether pharmacotherapy or splenectomy was carried out.

Design and Methods

This study included 76 consecutive patients undergoing HSCT from genotypic HLA-identical (n=27) or HLA-mismatched (n=3) siblings and matched (n=33) or mismatched (n=13) unrelated donors between January 1994 and December 2010. All patients gave their written informed consent to all aspects of the stem cell transplantation procedure and family donors to the donation process in accordance with the institutional standards of our department, which comply with the standards of Good Clinical Practice and the Declaration of Helsinki. Permission to conduct the study was given by the institutional review board.

The patients' clinical profiles and transplant characteristics are summarized in Table 1. The DIPSS with age-adjustment for patients younger than 65 years old,[9] the European Bone Marrow Transplantation (EBMT) risk score[20] and the DIPSS Plus[10] at time of HSCT were calculated for each patient wherever possible (Table 1). Grafts consisted of unmanipulated peripheral blood stem cells, bone marrow and highly purified $CD34^+$ cells produced using the CliniMACS device (Milteny Biotech, Bergisch Gladbach, Germany), as described previously.[21] Conditioning in 45 patients was conducted over 4-5 days and consisted of total body irradiation in four daily 2.5 Gy fractions in combination with 120 mg cyclophosphamide/kg body weight or 30 mg fludarabine/m^2; in the other 31 patients who were not given total body irradiation, the treatment consisted of 12-14 g treosulfan (Medac, Hamburg, Germany)/kg body weight for 3 days. GVHD prophylaxis consisted of a short course of methotrexate on days 1, 3, 6, and 11 in combination with continuous intravenous cyclosporine (n=46). Patients given purified $CD34^+$ cells received no further GVHD prophylaxis, but 13 patients were given 10-20 mg alemtuzumab (MabCampath, Genzyme, Neu-Isenburg, Germany) for 5 days followed by continuous intravenous cyclosporine, and 17 patients were given 10-20 mg additional anti-thymocyte globulin (ATG-S, Fresenius, Bad Homburg, Germany)/kg body weight for 3 days.

The *JAK2* V617F mutation status could be examined in 67 patients prior to transplantation using real-time polymerase chain reaction analysis of whole blood, as previously described.[21] At the time of HSCT, 47 patients (62%) had been diagnosed as having primary myelofibrosis, of whom 41 exhibited advanced disease stages, previously defined by the presence of at least two poor prognostic factors, including circulating blast cells, osteosclerosis and blood hemoglobin levels ≤10 g/dL.[13] Acute and chronic GVHD was classified according to standard criteria.[23,24] Every long-term survivor participated in continuous outpatient follow-ups at our center, during which GVHD characteristics were documented. Relapse was defined as reappearance of expression of the *JAK2* V617F mutation or other pre-transplant disease-specific molecular, cytogenetic or morphological markers accompanied by a concomitant decline of donor chimerism.

A total 43 patients (57%) had a history of prior treatment with different cytoreductive and/or immunemodulatory treatment regimens, including hydroxyurea (n=29), anagrelide (n=10), interferon-α (n=11), polychemotherapy (n=6), corticosteroids (n=2), danazol (n=1), imatinib (n=1), busulfan (n=1) and thalidomide (n=1). At the time of HSCT pharmacotherapy dated back several months or

Table 1. Summary of characteristics describing the patients' profile, disease classification, treatments and transplantation modalities

Variable	Type of value	Value
Male/ female	patients in entire cohort	40 / 36
Age at diagnosis	median years (range)	45 (7-65)
Age at transplantation	median years (range)	50.5 (22-67)
Time from diagnosis to transplantation	median months (range)	28 (3-244)
Diagnosis		
Primary myelofibrosis	number of patients (percentage)	47 (62%)
Secondary myelofibrosis	number of patients (percentage)	29 (38%)
Advanced/ non-advanced disease	patients in entire cohort	41 / 35
Cytogenetic abnormalities before transplantation	number of patients (percentage)	11 (15%)
DIPSS at transplantation		
Low	number of patients (percentage)	18 (24%)
Intermediate-1	number of patients (percentage)	39 (51%)
Intermediate-2	number of patients (percentage)	13 (17%)
High	number of patients (percentage)	6 (8%)
DIPSS plus at transplantation		
Low	number of patients (percentage)	7 (10%)
Intermediate-1	number of patients (percentage)	19 (28%)
Intermediate-2	number of patients (percentage)	33 (49%)
High	number of patients (percentage)	9 (13%)
EBMT risk score		
2	number of patients (percentage)	2 (3%)
3	number of patients (percentage)	7 (9%)
4	number of patients (percentage)	11 (14%)
5	number of patients (percentage)	56 (73%)
Dupriez score at transplantation		
Low	number of patients (percentage)	19 (25%)
Intermediate	number of patients (percentage)	31 (41%)
High	number of patients (percentage)	26 (34%)
JAK2 V617F mutation status		
Positive	number of patients (percentage)	37 (55%)
Negative	number of patients (percentage)	30 (45%)
Unknown	patients in entire cohort	9
Treatment before transplant		
Splenectomy	number of patients (percentage)	16 (21%)
Chemotherapy/ no cytoreductive therapy	patients in entire cohort	35/ 33
Immune modulating therapy (interferon-α, thalidomide)	patients in entire cohort	12
Androgens	patients in entire cohort	1
Steroids	patients in entire cohort	2
Donor		
HLA-identical sibling	number of patients (percentage)	27 (36%)
Mismatched sibling	number of patients (percentage)	3 (4%)
HLA-identical unrelated	number of patients (percentage)	33 (43%)
Mismatched unrelated	number of patients (percentage)	13 (17%)
Graft source		
Bone marrow	number of patients (percentage)	6 (8%)
Peripheral blood stem cells	number of patients (percentage)	68 (89%)
CD 34 purified stem cells	number of patients (percentage)	2 (3%)
Conditioning regimen		
TBI + Cy/fludarabine	number of patients (percentage)	45 (59%)
Treosulfan/busulfan + fludarabine/ Cy	number of patients (percentage)	31 (41%)
GVHD prophylaxis		
CSA + MTX	number of patients (percentage)	46 (61%)
CSA + ATG	number of patients (percentage)	17 (22%)
CSA + alemtuzumab	number of patients (percentage)	13 (17%)
Engraftment		
Leukocytes > $1x10^9$/L	median days post-transplant (range)	18 (9-32)
Platelets > $20x10^9$/L	median days post-transplant (range)	17 (8-57)
Acute GVHD		
Grade 0-I	number of patients (percentage)	52 (69%)
Grade II-IV	number of patients (percentage)	24 (31%)
Grade III or IV	number of patients (percentage)	9 (12%)
Chronic GVHD	patients in entire cohort	41
Limited	patients in entire cohort	23
Extended	patients in entire cohort	18
Graft failure	number of patients (percentage)	4 (5%)
Relapse	number of patients (percentage)	12 (16%)

TBI: total body irradiation, Cy: cyclophosphamide, CSA: cyclosporine A; ATG: anti-thymocyte globuline.

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years in all of these patients. The characteristics of patients divided according to whether they had or had not been treated with drug therapy prior to transplantation are presented in Table 2.

Statistics

Differences in the frequencies of discrete variables were tested using a two-sided Fisher's exact test or the χ^2 test. Wilcoxon's rank-sum test was used to test differences in continuous variables. In cases in which no competing event needed to be considered, the probabilities of events over time were calculated by the product-limit method, and heterogeneity of time-to-event distribution functions was compared using log-rank scores.[25] To determine whether possibly competing events were independent (i.e. relapse and death without relapse) the probabilities of events over time were estimated by cause-specific cumulative incidence rates.[26] The proportional hazards general linear model was used to compare cumulative incidence rates between subsets of patients, by comparing time-to-events with the cause-specific hazard functions using the two-sided Wald test.[27] Multivariate proportional hazards general linear model analysis was also performed for relapse, treatment-related mortality, overall survival and relapse-free survival as endpoints.[28] In all multivariate analyses of these endpoints, dichotomous variables were included as categorical covariates: pre-transplant pharmacotherapy (0=no, 1=yes), splenectomy (0=no, 1=yes), cytogenetic abnormalities (0=no, 1=yes), *JAK2* mutation status (0=wild type, 1=*JAK2* V617F mutation), disease stage (0=non-advanced, 1=advanced), categorized disease stratification according to age-adjusted DIPSS score (0=low, 1=higher than low), stem cell source (0=bone marrow cells, 1=blood stem cells), donor type (0=identical sibling, 1=matched unrelated donor), age group (0=below 50 years, 1=older than 50 years) and European Bone Marrow Transplantation (EBMT) risk score. Acute GVHD (0=grades 0-I, 1=grades II-IV) and chronic GVHD (0=absent, 1=present) were included in model building as time-dependent covariates with the time interval from allogeneic HSCT (day 0) until occurrence of GVHD. All proportional hazards general linear model analyses were performed using stepwise forward and backward selection procedures, and only covariates with a significance level below 1% were included in the model building. Only covariates attaining a significance level below 1% after adjustment for the other significant covariates selected in the forward and backward model building procedure were regarded as significant in the final models. Univariate and multivariate day-100 landmark analyses were performed on the 67 patients (88% of the cohort) who survived for 100 days after allogeneic HSCT without relapse to account for potential interactions of grades II-IV acute GVHD and chronic GVHD on relapse. Hazard ratios (HR)

Table 2. Comparative summary of patient characteristics in the untreated and pre-treated cohort

Variable	Pharmacotherapy N	Untreated N	*P* value
Advanced disease	21	14	n.s.
Non-advanced disease	22	19	n.s.
Cytogenetic abnormalities pre-transplant	7	4	n.s.
Primary myelofibrosis	20	27	
Secondary myelofibrosis	23	6	< 0.01
Dupriez score at transplantation			
Low	12	7	n.s.
Intermediate	17	14	n.s.
High	14	12	n.s.
DIPSS			
Low	12	6	n.s.
Interleukin-1	22	17	n.s.
Interleukin-2	6	7	n.s.
High	3	3	n.s.
Age > 50 years	19	21	n.s.
HLA non-identical donor	12	4	n.s.
Splenectomy	10	6	n.s.
Circulating blasts at HSCT	14	8	n.s.
Chronic GVHD	24	17	0.04
Hemoglobin ≤ 10 g/dL	20	21	n.s.
JAK2 V617F mutation positive	19	18	n.s.

n.s.: not significant.

and 95% confidence intervals (CI) were derived for each significant covariate included in the final proportional hazards general linear models. Statistical analysis and presentation was performed using the 9.22 release of Statistical Analysis Software™ procedures and macros (SAS, Cary, NC, USA).

Results

Patients and transplant-related characteristics

Follow-up data were retrospectively analyzed for the 76 consecutive patients with myelofibrosis who underwent HSCT at Essen University Hospital between January 1994 and December 2010 (Table 1). The median interval between diagnosis and HSCT in patients who were pharmacologically pre-treated was not significantly different from that of patients who received no pharmacological pre-treatment. Significantly more secondary myelofibrosis was observed among pre-treated patients (P=0.012) (Table 2). White blood cell engraftment was observed in 73 patients, and occurred at a median of 18 days post-transplantation. The cumulative incidence of successful engraftment at day 30 after transplantation was calculated to be 94% (95% CI: 89 - 100%). Primary graft failure occurred in three patients and secondary graft loss in one patient (Table 1). The cumulative incidence of white blood cell engraftment failure at 30 days after transplantation in these patients was calculated to be 3.7% (95% CI: 0.9 - 15%). Platelet engraftment occurred at a median of 17 days (Table 1). Three stem cell recipients with pre-transplant splenomegaly (one with primary, two with secondary myelofibrosis) underwent successful splenectomy because of persistent pancytopenia after HSCT. The time interval between diagnosis and HSCT was 34 months among patients with primary myelofibrosis compared to 96 months among patients with secondary myelofibrosis (P<0.001).

After HSCT, 24 patients developed acute GVHD grades II to IV (Table 1). The cumulative incidence of GVHD at day 100 for this cohort of patients was calculated to be 32% (95% CI: 19-44%). Chronic GVHD developed in 41 patients (Table 1), with a median onset at 6 months post-transplantation (range, 3.8-8.2 months). The 5-year cumulative incidence for chronic GVHD was calculated to be 77% (95% CI: 66-91%) using day 100 landmark analysis. The occurrence of chronic GVHD was significantly reduced in patients who had received pre-transplant pharmacotherapy (P=0.004) or antibodies for immunoprophylaxis (P=0.015).

Several factors were equally distributed between patients regardless of whether they developed acute or chronic GVHD. These included age, graft source, the type of conditioning and immunoprophylaxis, HLA-match, donor type, donor-recipient gender pairing, Lille-score, DIPSS score, *JAK2* mutation status, disease stage and whether the patient also underwent splenectomy.

Patients' outcome: non-relapse mortality, relapse and survival

The cumulative incidences for non-relapse mortality at 1, 3 and 5 years after HSCT were calculated to be 26% (95% CI: 17-38%), 33% (95% CI: 22-35%) and 36% (95% CI: 35-50%), respectively. In our cohort, 22 patients died after HSCT (median, 5 months; range, 1-5 months) of treatment-related causes: more precisely, 14 died of infections and eight died of GVHD. Acute GVHD caused a significant increase of non-relapse mortality (P=0.006) in the univariate model. Relapse occurred in 12 patients (16%) at a median time of 5.5 months (range, 3-88 months) after HSCT, and nine patients died of relapse. The 5-year cumulative incidence of relapse was calculated to be 19% (95% CI: 11 - 32%). Univariate analysis using relapse as the endpoint identified three decisive predictors. Cytogenetic abnormalities with aberrant karyotype (P=0.004), alemtuzumab treatment for immunoprophylaxis (P=0.009) and absence of chronic GVHD were all correlated with higher relapse rates (P=0.001). Landmark analysis on day 100 showed that the cumulative 5–year relapse incidence was 14% (95% CI: 6-31%) in patients with chronic GVHD compared to 40% (95% CI: 21 - 81%) in patients without chronic GVHD (P=0.001, Figure 1).

The median follow-up was 55 months (range, 5-191 months) for surviving patients and 25 months (range, 1-191 months) for the entire cohort of patients. The median overall survival of the entire cohort was predicted to be 96.2 months (95% CI: 75.2-117.2%), with a predicted 5-year overall survival of 53% (95% CI: 40-85%). The probability of relapse-free survival at 5 years was 50% (95% CI: 38-62%). Overall survival was significantly longer in patients who did not have advanced disease (P=0.008). Correspondingly, patients with low DIPSS scores had the highest predicted 5-year survival rate (76%) compared with patients classified with intermediate-1 scores (48%) or stratified intermediate-2 and high scores (38%, Figure 2). The follow-up period was not long enough for patients with low DIPSS scores to predict median survival. Predicted median survival was calculated to be 38 months for patients with intermediate-1 scores and 35 months for patients with intermediate-2 and high scores. Considering DIPSS-Plus, the follow up was again not long enough to

assess the median survival for low-risk patients. The predicted median survival was 100 (95% CI: 60-140), 61 (95% CI: 44-79) and 22 (95% CI: 6-38) months for patients with intermediate-1, intermediate-2 and high-risk DIPSS-Plus scores, respectively. Correspondingly, the 5-year overall survival was calculated to be 100%, 51%, 54% and 30% for DIPSS-Plus low, intermediate-1, intermediate-2 and high scores, respectively.

Overall survival was significantly reduced in patients who did not suffer chronic GVHD ($P<0.001$) or who received pharmacological pre-treatment ($P=0.007$, Figure 3). Overall but not relapse-free survival was significantly ($P=0.029$) increased in patients with primary myelofibrosis compared to those with secondary myelofibrosis (65% *versus* 33% after 5 years). However, this difference was abrogated by stratification for pharmacological pre-treatment. Both predicted overall and relapse-free survival were significantly lower ($P=0.013$ and $P=0.046$, respectively) in patients receiving HLA-mismatched transplants.

Advanced disease stage ($P=0.006$), medical pre-treatment ($P=0.003$), circulating blasts at the time of HSCT ($P=0.02$), presence of cytogenetic abnormalities ($P=0.019$) and absence of chronic GVHD ($P<0.001$, Figure 4) were identified as risk factors adversely influencing relapse-free survival in the univariate model. Multiple model analysis for relapse-free survival identified low DIPSS score (HR 1.3, 95% CI: 1.1 to 1.7, $P=0.02$) and abnormal karyotype (HR 2.2, 95% CI: 1.0 to 5.0, $P=0.049$) as independent factors increasing the risk of relapse or death whereas chronic GVHD significantly reduced it (HR 0.2, 95% CI 0.08 to 0.49, $P=0.0004$).

Multiple model analysis with stepwise pre-transplant variable selection identified non-advanced disease stage (HR 2.5, 95% CI: 1.2 to 4.9; $P=0.01$), low DIPSS score (HR 1.4, 95% CI: 1.1 to 1.7; $P=0.014$), and no pharmacotherapy prior to HSCT (HR 2.7, 95% CI: 1.3 to 5.7; $P=0.009$), as being independently associated with prolonged overall survival. The strong association between absence of chronic GVHD (HR 0.07, 95% CI: 0.02 to 0.3; $P=0.0009$) and reduced overall survival was confirmed in multivariate analysis (proportional hazards general linear model analysis) which included transplant-related variables (Table 3).

Discussion

This retrospective evaluation of HSCT in patients with primary or secondary myelofibrosis corroborates the potential of allogeneic transplantation to achieve long-term remission. With predicted 5-year overall and event-free survival rates of 53% and 50%, respectively, our results are in line with reports from national registries or other single-center studies.[11,12,14-19,29-32] On the whole, our observed cumulative incidence rates of non-relapse-mortality corresponded to those found in evaluations of more extensive registry data.[17,29-30] However, our evaluation identified chronic GVHD and pre-transplant pharmacotherapy as independent factors influencing outcome after HSCT for the first time. Additionally, by testing the applicability of the DIPSS score in the transplant setting, we demonstrated that in our cohort of patients, this score, unlike the Dupriez and EBMT scores, was able to predict different risks of transplantation and refine the prognostic accuracy of HSCT outcome. Overall and event-free survival after HSCT were significantly improved in patients with low DIPSS scores compared to those classified as intermediate-1, intermediate-2 or high risk by the DIPSS. Analysis of overall survival stratified by DIPSS-Plus scores demonstrated similar and even better results for each risk group. Furthermore, a comparison of the results obtained in the present study and those described by Gangat *et al.*[10] showed that the median survival for each risk group was,

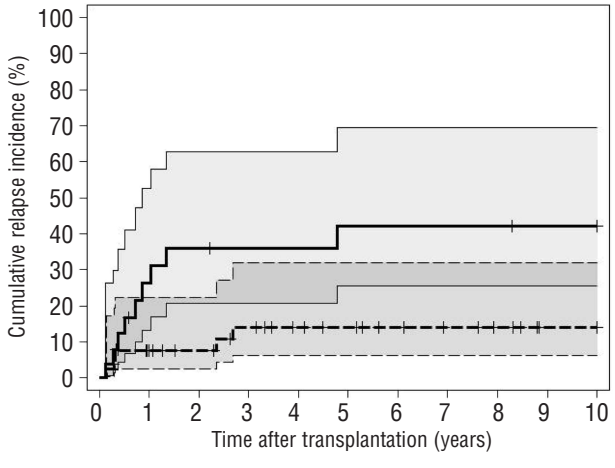

Figure 1. Cumulative incidence of relapse in patients surviving more than 100 days after HSCT stratified by chronic GVHD. Kaplan-Meier analysis was retrospectively conducted on 76 patients who underwent HSCT between 1994 and 2010. ($P=0.001$). Cumulative incidence of relapse in patients who did not develop chronic GVHD after HSCT (solid line). Cumulative incidence of relapse for patients developing chronic GVHD after HSCT (dashed line). Tick marks indicate patients surviving free of relapse or competing events. The 95% confidence intervals for all values are indicated by shaded zones around the lines.

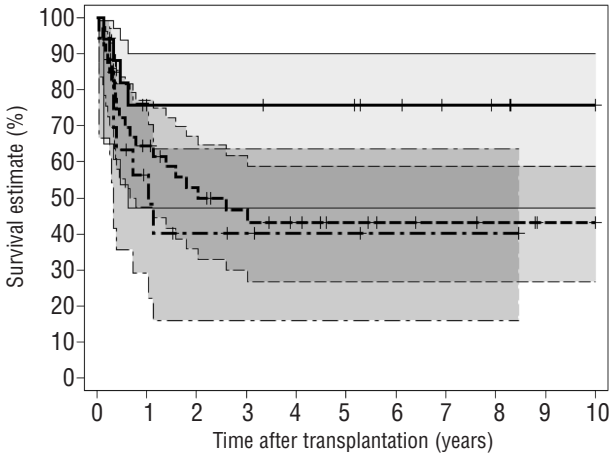

Figure 2. Predicted overall survival of patients stratified by pre-transplant DIPSS score. Kaplan-Meier analysis was used to predict overall survival in all 76 patients. $P>0.05$. Overall survival for patients with low DIPSS scores (n=18; solid line), patients with intermediate-1 DIPSS scores (n=39; dashed line) and patients with intermediate-2 or high DIPSS scores (n=19; dotted line) are shown. Tick marks indicate surviving patients. The 95% confidence intervals for all values are indicated by shaded zones around the lines.

in contrast to the natural course of disease, superior after allogeneic HSCT. The unfavorable effect of advanced disease stages on relapse-free survival was demonstrated in univariate analysis, in line with the results of previous studies identifying the predictive value of myelofibrosis disease stage for post-transplant survival.[12,13] Several publications have reported that a high Lille score is a major risk factor for reduced post-transplant survival, suggesting a distinct association between disease stage and HSCT outcome.[11,29,32,33] Recently, Robin *et al.*[29] reported that non-chronic phase disease was the worse prognostic factor for overall survival after HSCT, while the Dupriez score had no impact, in accordance with our results.

Mismatched transplants have been reported to have an adverse impact on post-transplant survival[33] and engraftment.[29] Our calculated estimates of survival after mismatched HSCT were significantly decreased, but only in univariate analysis. In contrast to other reports, we identified no influence of splenectomy,[31] *JAK2* V617F mutation,[33] time interval between diagnosis and HSCT,[30] donor type,[29-30,33] or patient's age[31-33] on post-transplant outcome in our cohort of patients. Our findings contradict the reported significance of *JAK2* expression regarding an improved outcome after allogeneic HSCT,[33,35] and emphasize the usefulness of the V617F mutation as a marker for minimal residual disease in patients initially positive for this mutation.[22] We did, however, verify that the presence of cytogenetic abnormalities in general increased the risk of relapse and reduced relapse-free survival.[12] The role of splenectomy prior to transplantation remains controversial and our findings support the position of not recommending splenectomy prior to HSCT because there was no significant impact of splenectomy on clinical endpoints or outcome. Although we observed three cases of persistent pancytopenia after transplantation, which may have been due to massive splenomegaly, hematopoietic recovery was achieved by subsequent post-transplant splenectomy in these cases.

The fact that this study showed that chronic GVHD had an influence on relapse and relapse-free survival might be related to the higher incidences of chronic GVHD observed in our cohort and the comparatively long follow-up of surviving patients. In published reports on patients with myelofibrosis, follow-up periods ranged from 33[12,29,33] to 64[14] months after HSCT. Our evaluation indicates that chronic GVHD may play an essential role in reducing the risk of relapse, which is additionally corroborated by the finding that intensified immunosuppression using alem-

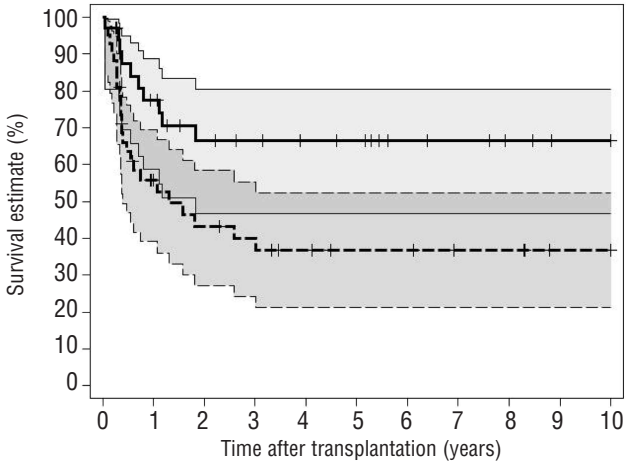

Figure 3. Predicted overall survival of patients stratified by pre-transplant pharmacotherapy. Kaplan-Meier analysis was used to predict overall survival in all 76 patients. *P*=0.007. Overall survival for patients not given pharmacological pre-treatment (n=33; solid line) and patients previously given pharmacotherapy (n=43; dashed line) is shown. Tick marks indicate surviving patients. The 95% confidence intervals for all values are indicated by shaded zones around the lines.

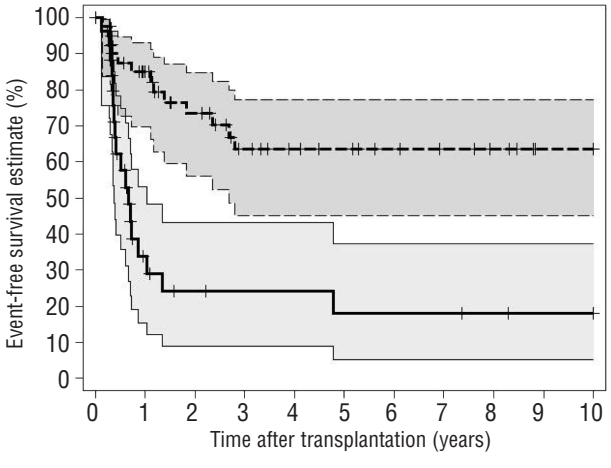

Figure 4. Predicted relapse-free survival for patients stratified by chronic GVHD in patients surviving more than 100 days after HSCT. Kaplan-Meier analysis was used to predict event-free survival in all 76 patients. *P*<0.001. Relapse-free survival for patients without chronic GVHD (n=26; solid line) and patients with chronic GVHD (n=41; dashed line). Tick marks indicate surviving patients. The 95% confidence intervals for all values are indicated by shaded zones around the lines.

Table 3. Proportional hazards general linear model analysis of overall and event-free survival.

Overall survival improvement			Event-free survival impairment		
Covariates*	Value	*P*	Covariates*	Value	*P*
Non-advanced disease†	2.5 (1.2-4.9)	0.01	Abnormal karyotype†	2.2 (1.0-5.0)	0.049
Low age-adjusted DIPSS score†	1.4 (1.1-1.7)	0.014	aaDIPSS > low†	1.3 (1.1-1.7)	0.02
No pharmacological pre-treatment†	2.7 (1.3-5.7)	0.009	cGVHD†	0.2 (0.08-0.49)	0.0004

**Proportional hazards general linear models (PHGLM) with forward and backward selection of covariates; time-dependent covariates: time intervals to grades II-IV acute GVHD, chronic GVHD; further covariates included in all PHGLM analyses: stratified patient age; patient/donor sex match; pre-transplant risk scores; donor type; graft source;* JAK2 *mutation status; degree of bone marrow fibrosis; anemia; circulating blasts. †Hazard ratio (HR) and 95% confidence interval (95% CI) after adjustment for all significant (*P*< 0.05) covariates in the final models.*

tuzumab significantly increased relapse rates. Overall survival rates for patients with various other hematologic malignancies were reported to double in patients who developed chronic GVHD after HSCT,[35] suggesting a basic allo-immune reaction in terms of a chronic graft-versus-neoplasm effect after allogeneic transplantation.

Stem cell recipients who received pharmacotherapy prior to HSCT had substantially reduced relapse-free survival, even though such patients were equally distributed within demographic subgroups and subgroups based on disease characteristics or risk stratification. When pharmacologically pre-treated and untreated patients were considered separately, there were no differences in the overall survival of patients with primary compared to secondary myelofibrosis. This is notable because the proportion of patients with secondary myelofibrosis was higher among the pre-treated patients. An inferior post-transplant survival among patients with secondary myelofibrosis could be related to longer disease duration or the significantly longer interval between diagnosis and HSCT. Differences in post-transplant outcomes observed between patients pre-treated pharmacologically and those who did not receive any drug therapy may be related to more aggressive forms of disease and more rapid disease progression for which therapy was thought to be indicated.

Only one observation about the influence of pre-transplant pharmacotherapy has been published to date, and concerns the myeloproliferative neoplasm, chronic myeloid leukemia. An association was reported between interferon-α therapy prior to bone marrow transplantation and inferior post-transplant outcome, for which the causative pathomechanism remains unclear.[36] In our cohort of patients, pre-transplant therapy was associated with inferior outcome just as was the absence of chronic GVHD. The fact that pre-treated stem cell recipients developed less chronic GVHD might be responsible for the poorer survival after transplantation. It is possible that the lack of allo-immune reactivity resulting from pharmacotherapy may suppress the development of chronic GVHD and, therefore, contribute to reduced survival after HSCT.

Our findings demonstrate a distinct impact of disease-specific features as well as transplant-related factors on outcome after allogeneic HSCT. It should be noted that patients with primary myelofibrosis with intermediate-1 to high DIPSS scores had a median survival between 2.3 and 9.8 years if they remain untreated, using a wait-and-see strategy.[9] The 3-year survival rate for transplantation-eligible, high- or intermediate-risk patients (<60 years of age) with primary myelofibrosis who did not undergo HSCT has been reported to range between 55% and 77%.[37] However, by applying the DIPSS-Plus model for the first time the beneficial effect of allogeneic HSCT becomes apparent for each risk group, when compared to the median survival rates reported by Gangat *et al.*[10] Probably the difficulty in comparing relevant clinical endpoints for different cohorts of patients, which is basically caused by the heterogeneity of patients and their selection, can be overcome by using the DIPSS- Plus categorization. The overall reported safety and efficacy of HSCT supports the concept that this treatment option should not be unnecessarily delayed, particularly if an HLA-identical donor is available and the risk of disease begins to increase. To assess the risk of disease better, dynamic risk stratification using the DIPSS or DIPSS-Plus should be carried out periodically. Disease-specific pharmacological treatment should be carefully considered if the patient is to undergo HSCT. Immunosuppressive GVHD prophylaxis in transplanted patients with high-risk characteristics should also be considered carefully, and reduced where possible. The choice of conditioning regimen should be adapted to the clinical status and comorbidities of each patient in order to minimize transplant-related mortality. In consideration of all disease-specific and transplantation-related adverse factors, Barbui *et al.* concluded that the risk of allogeneic HSCT for myelofibrosis can be expected to be justifiable in patients with a predicted median survival of less than 5 years.[38] Treatment algorithms derived from individual prognostic factors should be established and verified in prospective clinical trials in order to improve the selection of patients eligible for transplantation and the appropriate transplant scheduling in patients with myelofibrosis.

Authorship and Disclosures

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