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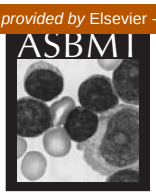

Risk Factors for Moderate-to-Severe Chronic Graft-versus-Host Disease after Allogeneic Hematopoietic Stem Cell Transplantation

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ABSTRACT

Among 810 consecutive hematopoietic stem cell transplantation (HSCT) patients, 679 survived more than 3 months and were evaluated for chronic GVHD. The aim of this study was to find predisposing factors increasing the risk of development of moderate-to-severe chronic GVHD. Many of the donors were HLA-identical siblings or related (n = 435), 185 were HLA-matched unrelated, and 59 were mismatched related or unrelated donors. Most of the patients had a hematological malignancy (n = 568), but 111 patients with a nonmalignant disease were included. Two hundred twenty-three patients (33%) developed mild, 41 (6%) moderate, and 15 (2.2%) severe chronic GVHD. The 5-year probability of development of moderate-to-severe chronic GVHD was 10%. We analyzed 30 potential risk factors for chronic GVHD. In the multivariate analysis, acute GVHD grades II to IV (relative hazard [RH], 2.30; 95% CI, 1.29-4.10; *P* = .005), CML diagnosis (RH, 2.37; CI, 1.38-4.08; *P* = .002) and transplantation from an immunized female donor to a male recipient (RH, 2.16; CI, 1.14-4.11; *P* = .02) were independent risk factors for moderate-to-severe chronic GVHD. Recipient age also was significant (RH, 2.42; CI, 1.23-4.77; *P* = .01) if CML was not included in the analysis. In patients with no risk factors, the 5-year probability of development of moderate-to-severe chronic GVHD was 5%. In patients with 1 risk factor, the probability was 13%; 2 risk factors, 23%; and 3 risk factors, 45%. Among patients who developed chronic GVHD (n = 279), acute GVHD grades II to IV (RH, 2.18; CI, 1.23-3.86; *P* < .01) was the only predictive factor for moderate-to-severe chronic GVHD versus mild disease. Patients with previous acute GVHD grades II to IV may benefit from more aggressive initial treatment. This possibility would have to be examined in clinical trials.

KEY WORDS

Chronic graft-versus-host disease • Stem cell transplantation

INTRODUCTION

The major clinical manifestations of chronic graft-versus-host disease (GVHD) are dermatitis, keratoconjunctivitis, oral mucositis, generalized sicca syndrome, and hepatic dysfunction. The disease has clinicopathological findings resembling those of autoimmune disorders [1,2]. In severe disease, malabsorption, esophageal and vaginal strictures, and pulmonary insufficiency may develop [3]. Chronic GVHD is associated with prolonged immunodeficiency, which is a predisposing factor for recurrent and sometimes fatal infection [4]. Chronic GVHD affects approximately 30% to 70% of long-term survivors and is therefore an important clinical problem after hematopoietic stem cell transplantation (HSCT) [3,5-7].

It is well known that both acute and chronic GVHD are accompanied by a graft-versus-leukemia (GVL) effect [8-10]. This effect is probably greater in chronic than in acute GVHD [9-10]. Many studies have shown that leukemic patients with chronic GVHD have a lower incidence of relapse and better disease-free survival than do those without chronic GVHD [8-10]. However, the presence of moderate-to-severe chronic GVHD also implies a substantially increased immunosuppressive state, which leads to higher risk of infection and reduced function of affected organs. These states lead to higher morbidity and mortality rates among patients with moderate-to-severe chronic GVHD. Patients with mild chronic GVHD need only minimal, if any, immunosuppressive treatment, whereas those with

more severe forms need more aggressive and prolonged treatment. Antithymocyte globulin (ATG), total lymphoid irradiation (TLI), thalidomide [11], and, more recently, extracorporeal psoralen and UV-A radiation (PUVA) [12] are used to treat more severe forms of chronic GVHD.

Chronic GVHD usually is classified with a 2-grade scale: limited or extensive [13]. In new studies a 3-grade scale has been evaluated in which the treating physician, taking the clinical condition of the patient into account, defines the disease as mild, moderate, or severe [14]. This scale has been shown to correlate better with survival of the affected patients. For this reason, we chose to use this 3-grade scale in our study.

In this single-center study, we evaluated predisposing factors for the development of moderate-to-severe chronic GVHD in 679 consecutive patients who underwent allogeneic HSCT at Huddinge University Hospital. The aim was to find patients at increased risk of developing more severe forms of chronic GVHD. These patients may need more aggressive or prolonged immunosuppressive treatment and might be included in studies evaluating new treatments, such as extracorporeal photopheresis [15]. To avoid introducing bias and to increase the statistical power of the analysis, we included all patients who received transplants at Huddinge University Hospital.

MATERIAL AND METHODS

Patients and Donors

Among 810 consecutive patients who underwent allogeneic HSCT between November 1975 and December 2000, 679 survived more than 3 months and were evaluated for chronic GVHD. The source of stem cells was bone marrow (BM) in 561 cases (83%) and granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem cells (PBSC) in 118 cases (17%). Patient and donor characteristics are shown in Table 1.

Table 1. *Patient and Donor Characteristics*

Characteristic	n
Disease	
Acute myeloid leukemia	169 (25%)
Acute lymphoid leukemia	164 (24%)
CML	162 (24%)
Lymphoma	18 (3%)
Myelofibrosis	7 (1%)
Myelodysplastic syndrome	25 (4%)
Chronic lymphoid leukemia	8 (1%)
Myeloma	13 (2%)
Other malignant disease	2
Solid tumors	17 (3%)
SAA	44 (6%)
Fanconi anemia	6 (1%)
Metabolic disorders	44 (6%)
Disease status	
First complete remission/chronic phase	320 (56%)
Second or later remission/chronic phase	176 (31%)
Partial remission	28 (5%)
Not in remission/chronic phase	44 (8%)
Recipient age (range), y	26 (0-77)
Recipient sex, male/female	408/271
Donor age (range), y	33 (0.5-67)
Donor sex, male/female	366/312
Female donor to male recipient	175 (26%)
Nucleated cell dose (range), $\times 10^8$/kg	2.7 (0.1-80)
Donor	
HLA-identical sibling	424 (62%)
HLA-identical parent	11 (2%)
Matched unrelated donor	185 (27%)
Mismatched related donor	28 (4%)
Mismatched unrelated donor	31 (5%)

Conditioning and Supportive Care

The majority (369/568 [65%]) of patients with malignant disease received 10 Gy of total body irradiation (TBI) (lungs shielded so that the patient received a median of 9 Gy) combined with cyclophosphamide (Cy) 60 mg/kg for 2 consecutive days [16]. As an alternative, some patients (109/568 [19%]) received Cy combined with busulfan (Bu) (4 mg/kg on 4 consecutive days) [17] or 12 Gy of fractionated TBI (3 Gy on 4 consecutive days) (54/568 [10%]). In 24 patients given a T-cell–depleted graft, 6 Gy (2 Gy for 3 consecutive days) of TLI was given before TBI of 7.5 Gy (lungs shielded so that the patient received a median of 7 Gy) [18]. Patients with metabolic disorders were given Cy (2 mg/m^2), frequently (36/44 [82%]) in combination with Bu (80 mg/m^2), although other combinations with TBI have been used [19]. Patients with severe aplastic anemia (SAA) received Cy (50 mg/kg on 4 consecutive days). Since 1988, this agent has been given in combination with ATG (2-5 mg/kg) for 2 to 5 days. In SAA patients with an unrelated donor, Cy was frequently combined with TBI, Bu, or thoracoabdominal irradiation (TAI)/TLI. Thirty patients received a nonmyeloablative conditioning regimen with 2 Gy TBI in combination with fludarabine (30 mg/m^2 for 3-5 days) (n = 16), Bu (4 mg/kg for 2 days) in combination with fludarabine (n = 11), or fludarabine plus Cy (30 mg/kg for 2 days) (n = 3).

All patients with an unrelated or mismatched related donor were treated with ATG (2-5 mg/kg per day) or muromonab-CD3 (OKT-3) (5 mg/d) for 2 to 5 days prior to transplantation [20]. Before November 1988, all patients with hematological malignant disease received 8 to 12 mg methotrexate (MTX) or 20 mg cytarabine (Ara-C) intrathecally (IT) twice before HSCT to prevent central nervous system (CNS) leukemia. After transplantation, IT MTX was given from day 32 and every other week until day 102. After 1988, only patients with acute lymphoid leukemia, acute myeloid leukemia M4 or M5, or a history of CNS disease were given this treatment. Patients with previous CNS disease were given IT treatment until 24 months after HSCT.

Since 1995, G-CSF has been routinely given to 258 patients from day +10 until neutrophil engraftment (>0.5 × 10/L).

Ninety-seven patients were given intravenous immune globulin (IVIG), most as part of a randomized study [21].

GVHD Prophylaxis

Monotherapy with MTX according to the Seattle protocol was given to 61 patients [22,23]. Forty-eight patients received cyclosporine (CsA) alone as GVHD prophylaxis [24]. The CsA dose was gradually tapered 6 months after transplantation. One year after HSCT, CsA was discontinued if

no signs of chronic GVHD were found. T-cell–depleted BM was given to 34 patients by previously described techniques [18]. Between August 1985 and April 1989, 274 patients received combination therapy with the first 4 doses of MTX given together with CsA, as described above [18,25,26]. Between May 1989 and January 1995, 77 leukemic patients were given combination therapy on an individual basis [27]. CsA was tapered after 2 months, if possible, and MTX was continued until day 102 after HSCT. Since 1995 (n = 157), a short course of MTX in combination with low-dose CsA has been used [28]. Six patients were given CsA with prednisolone and 22, CsA combined with mycophenolate mofetil.

Diagnosis of GVHD

Both acute and chronic GVHD were diagnosed on the basis of clinical symptoms or verified by biopsy (skin, liver, gastrointestinal tract, or oral mucosa). Acute GVHD was graded on a scale from 0 (absent) to IV (severe) according to published criteria [22]. The severity of chronic GVHD was defined as mild, moderate, or severe. Mild disease included sicca and minor skin and/or liver symptoms with a Karnofsky score of ≥90%. Moderate disease involved symptoms from 1 or more organs that were controlled by immunosuppression but necessitated prolonged or continuous therapy. The Karnofsky score in these patients ranged from 70% to 80%. Severe disease was defined as restricted functions, such as malabsorption, severe bronchiolitis, and sclerosis of the skin with Karnofsky score less than 70% [1,13,14]. The severity of chronic GVHD was classified by the treating physician. Grading was according to what we report to the international bone marrow transplantation registry (IBMTR) as "overall total severity."

Treatment of GVHD

Acute GVHD grade I was treated with prednisolone 2 mg/kg per day. After 1 week, the dose was gradually tapered, if possible. In more severe cases, ATG, methylprednisolone, MTX, and/or PUVA were given. Several strategies were used to treat extensive chronic GVHD, including prednisolone, CsA, prednisone on alternate days [29], azathioprine [30], PUVA [31], thalidomide [11], TLI [32], and, more recently, extracorporeal photopheresis [12].

Cytomegalovirus Infection, Reactivation, and Disease

Before 1988, cytomegalovirus (CMV) infection was diagnosed by a standard viral culture technique. Between 1988 and 1992 a rapid isolation technique was applied. Seroconversion also was accepted as a sign of infection. After 1992, patients in whom CMV DNA was detected in 2 consecutive leukocyte-based semiquantitative polymerase chain reaction (PCR) tests, although not fulfilling criteria for CMV disease, were given the diagnosis of CMV infection/reactivation. In all cases of CMV disease, the diagnosis required relevant clinical symptoms from affected organs and, with the exception of retinitis, proof of CMV from the affected organ [33].

Statistical Analysis

Results were analyzed as of January 21, 2002. Time to development of moderate-to-severe chronic GVHD was used to calculate the cumulative probability curves (Kaplan-Meier method). Death or relapse was a censored observation, unless moderate-to-severe chronic GVHD was present [34]. Comparisons were made with the log-rank (Mantel-Haenszel) test [35]. Mild chronic GVHD was not considered an event. In patients who had relapses, grade of chronic GVHD before the relapse and subsequent donor leukocyte infusion treatment was used. The Cox proportional hazard regression model was used for univariate and multivariate analyses [36]. Only patients surviving at least 90 days after HSCT, and therefore at risk of development of chronic GVHD, were included in the analyses. Thirty potential risk factors for chronic GVHD were studied (Table 2). Only factors at ≤10% levels in the univariate analyses were introduced into the stepwise elimination multivariate analyses. This analytic approach is designed to generate predictive factors for moderate-to-severe chronic GVHD, not causality.

Patients given monotherapy with MTX or CsA were grouped together, because they had the same incidence of acute and chronic GVHD [24]. For the same reason, patients receiving T-cell–depleted grafts were grouped with those receiving combination therapy with CsA and MTX as GVHD prophylaxis [18]. A female donor was considered immunized if she had been pregnant or had undergone blood transfusion. Patients with malignant disease in first complete remission or first chronic phase and all with nonmalignant disorders were considered as having early disease. All others were considered as having late disease. Bidirectional ABO mismatch means blood group A to B or B to A. Administration of G-CSF was analyzed as a potential risk factor because we have found a higher incidence of acute GVHD grades II to IV in patients receiving G-CSF after HSCT (unpublished data). In patients with CMV infection and moderate-to-severe chronic GVHD, the CMV infection always preceded the GVHD. The cumulative probability curves for survival and relapse-free survival were calculated with the Kaplan-Meier method. Censored observations were taken into account.

RESULTS

Incidence of Chronic GVHD

Among the 679 patients, 279 developed chronic GVHD (223 mild, 41 moderate, and 15 severe). The overall cumulative probability of presence of chronic GVHD at 5 years was 50%; the probability was 10% for moderate-to-severe disease (Figure 1).

Risk Factors for Moderate to Severe Chronic GVHD

In Cox regression univariate analysis, 5 factors were significant at the (5% level (Table 2). These factors and 2 additional ones at the 5% to 10% level were included in the subsequent stepwise elimination multivariate analysis. Significant risk factors for moderate-to-severe chronic GVHD are shown in Table 3a. If CML was excluded from the multivariate analysis, recipient age was also statistically significant (Table 3b). The analysis was corrected for year of transplantation. The cumulative probability of moderate-to-severe chronic GVHD in patients with previous acute GVHD grades II to IV was 17%, compared with 8% in those with acute GVHD grades 0 to I (P = .004) (Figure 2). The probability of development of moderate-to-severe

Table 2. *Univariate Analysis of Risk Factors for Moderate-to-Severe Chronic GVHD in 679 HSCT Patients*

Factor	0/1	n	Relative Hazard	*P*
Recipient age	<18 y/≥18 y	248/431	2.31	.01*
Donor age	<18 y/ ≥18 y	149/524	2.41	.04*
Female donor to male recipient	No/yes	501/175	1.49	.17
Immunized female donor to male recipient	No/yes	595/81	2.29	.01*
HLA-identical sibling	No/yes	435/244	0.89	.69
Unrelated donor	No/yes	216/463	1.23	.50
Malignant disease	No/yes	94/585	1.26	.57
CML	No/yes	518/161	2.54	<.001*
Disease stage	Early/late	406/273	1.09	.74
Nucleated cell dose	Continuous	Variable	0.96	.30
Cell source	BM/PBSC	561/118	1.05	.88
Conditioning	TBI/Bu	533/146	1.21	.55
GVHD prophylaxis	Mono/comb	109/570	1.23	.59
Splenectomy	No/yes	653/26	0.49	.48
ATG during conditioning	No/yes	426/253	0.74	.31
ABO minor mismatch	No/yes	543/133	0.85	.66
ABO major mismatch	No/yes	528/148	1.54	.14
ABO bidirectional mismatch	No/yes	645/31	1.27	.39
G-CSF after HSCT	No/yes	421/258	0.61	.10
IVIG after HSCT	No/yes	581/97	0.87	.73
Acute GVHD	0/I-IV	183/496	1.82	.09
Acute GVHD	0-I/II-IV	553/126	2.41	.003*
CMV reactivation	No/yes	371/308	1.26	.40
CMV disease	No/yes	630/49	1.79	.18*
Recipient herpes virus serology†	0-2/3-4	154/496	1.42	.31
Donor herpes virus serology†	0-2/3-4	183/407	1.32	.43
Recipient CMV serology	Neg/pos	185/475	1.25	.51
Donor CMV serology	Neg/pos	256/353	1.09	.78
CMV-positive recipient and donor	No/yes	319/287	1.09	.83
CMV-negative recipient and donor	No/yes	493/106	1.12	.78
HSCT year	Continuous	Variable	0.98	.28

*Factors included in the multivariate analyses.

†Seropositivity for 0 to 2 versus 3 or 4 herpes viruses (CMV, herpes simplex virus, varicella zoster virus, Epstein-Barr virus).

chronic GVHD increased with increasing severity of acute GVHD. The cumulative probability was 6% for those with no previous acute GVHD, 8% for those with grade I GVHD, 16% for those with grade II, and 44% for patients with previous acute GVHD grade III. The cumulative probability of severe chronic GVHD was 1%, 2%, 4%, and 24% for patients without and those with grade I, grade II, and grade III acute GVHD, respectively. Among patients with CML, the probability of moderate-to-severe chronic GVHD was 17% compared with 7% for patients with other diagnoses ($P < .001$) (Figure 2). In male recipients with an immunized female donor the probability of moderate-to-severe

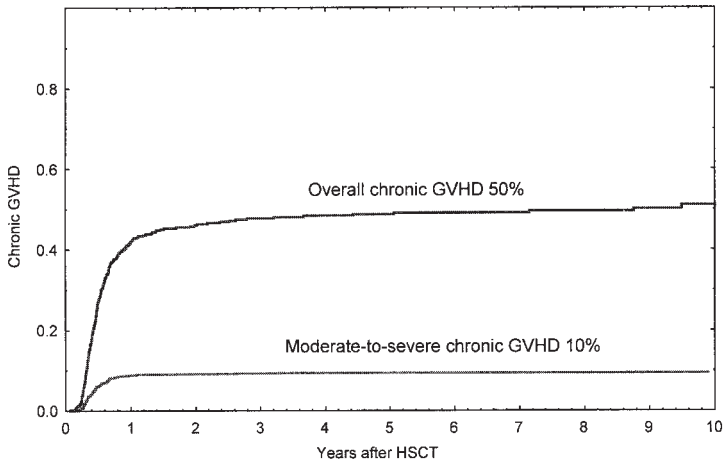

Figure 1. Cumulative probability of overall and moderate-to-severe chronic GVHD in 679 long-term survivors of HSCT.

Table 3a. *Multivariate Analysis of Risk Factors for Moderate-to-Severe Chronic GVHD in 679 HSCT Patients**

Factor	RH	95% CI	P
Acute GVHD II-IV	2.30	1.29-4.10	.005
CML	2.37	1.38-4.08	.002
Immunized female donor to male recipient	2.16	1.14-4.11	.02

Table 3b. *Multivariate Analysis with CML Omitted from the Analysis**

Factor	RH	95% CI	P
Acute GVHD II-IV	2.61	1.46-4.66	.001
Recipient age ≥18 y	2.42	1.23-4.77	.01
Immunized female donor to male recipient	1.95	1.02-3.74	.04

*Adjusted for year of transplantation.

chronic GVHD was 17% compared with 8% in all others (P = .01). Children (<18 years) had a lower probability than adults, 5% and 12%, respectively (P = .01).

The incidence of moderate-to-severe chronic GVHD increased with an increasing number of risk factors (acute GVHD, CML, and immunized female donor to male recipient). If no risk factors were present, the cumulative probability was 5%, with 1 risk factor it was 13%, with 2 it was 23%, and with all 3 risk factors present it was 45% (Figure 3).

To answer who needs more aggressive treatment after the development of chronic GVHD, we performed an analysis of predictive factors for moderate-to-severe chronic GVHD versus mild disease among all patients who developed chronic GVHD (n = 279). We found the presence of grade II to IV acute GVHD (relative hazard [RH], 2.18; 95% CI, 1.23-3.86; P < .01) was the only predictive factor for moderate-to-severe chronic GVHD among patients who developed chronic GVHD.

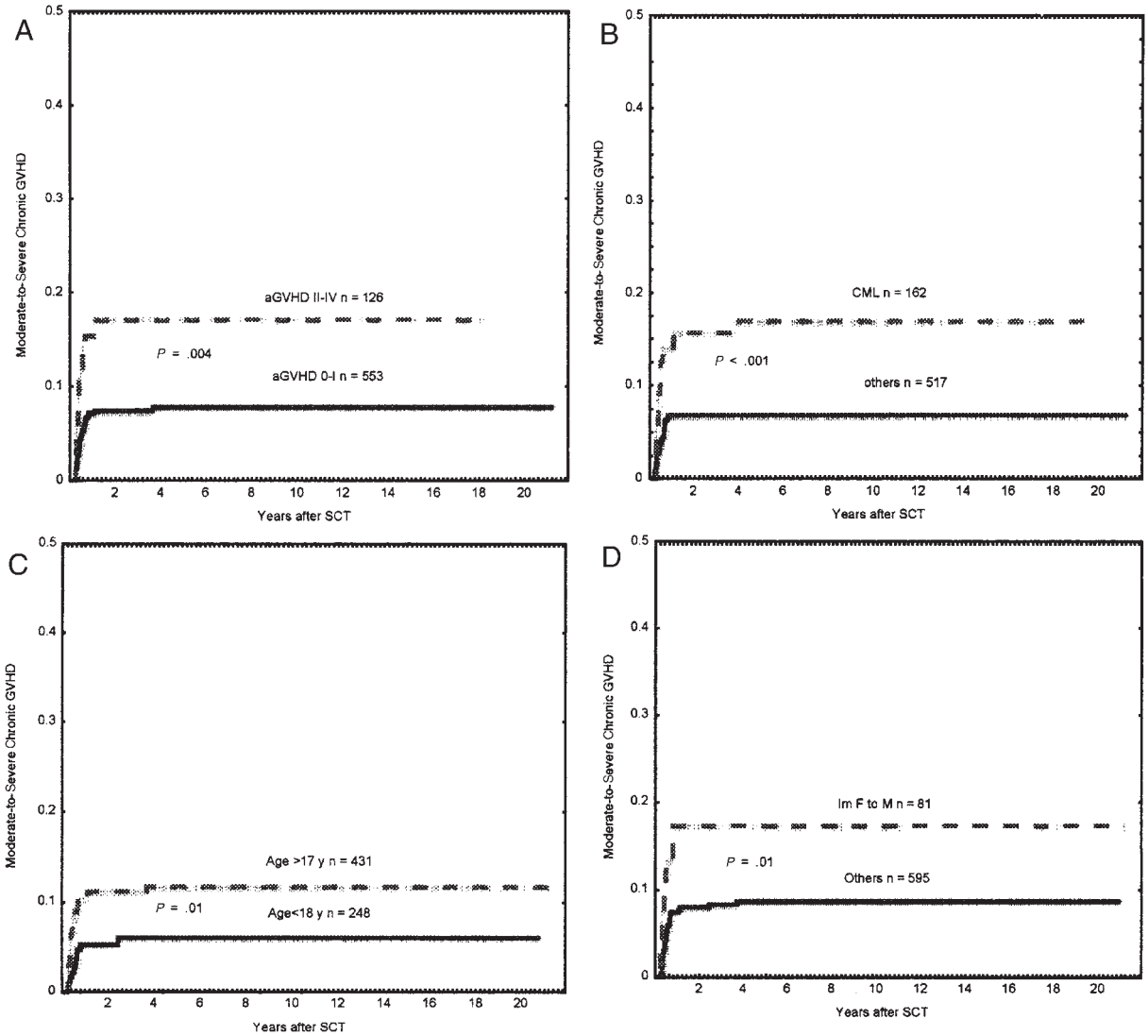

Figure 2. The cumulative probability of moderate-to-severe chronic GVHD in 679 long-term survivors of HSCT in the presence or absence (univariate comparison) of the 4 significant risk-factors. A, Acute GVHD (aGVHD) grades II to IV or grades 0 or I. B, Patients with CML or other disease. C, Recipient age <18 or ≥18 years. D, With or without immunized female donor to male recipient.

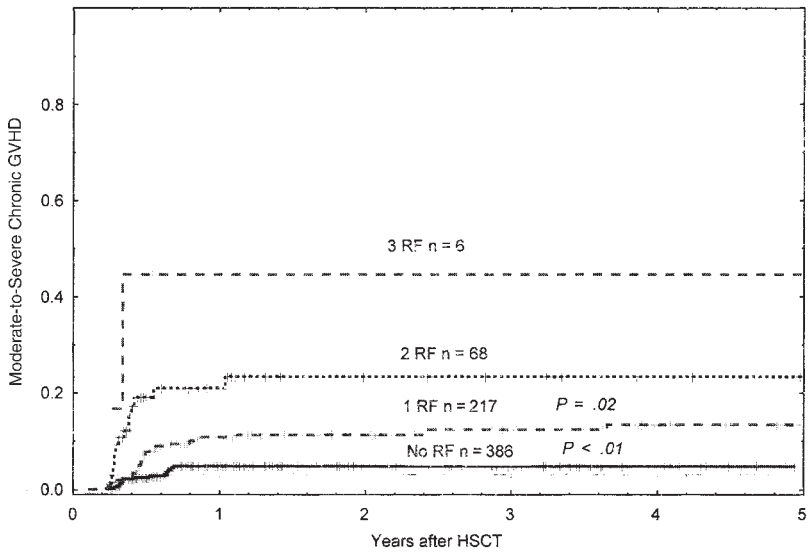

Figure 3. The cumulative probability of moderate-to-severe chronic GVHD in patients with 0 to 3 significant risk-factors (RF) present according to Table 3a.

Survival and Disease-Free Survival

The best overall survival and disease-free survival (DFS) were seen in patients with mild chronic GVHD (Figures 4 and 5). In patients with moderate chronic GVHD, survival and DFS were no better than in patients without chronic GVHD. Patients with severe chronic GVHD, however, had very poor survival and DFS rates (Figures 4 and 5).

DISCUSSION

In this study we used a 3-graded scale for classification of chronic GVHD that was based on mild/moderate/severe clinical impression [14]. This system appears to be the most predictive, but it has some limitations. In the absence of objective criteria, reproducibility, reliability, and validity are questionable. These classifications also may reflect a lengthy period of observation. However, at our center all classifications of chronic GVHD have been made by a very small number of physicians using similar criteria for classification.

All previous studies of risk factors for chronic GVHD analyzed chronic GVHD in general, regardless of severity. However, the severity of chronic GVHD markedly affects outcome. For example, mild chronic GVHD has a beneficial effect on survival in patients with leukemia because of the GVL effect [8-10]. In this study, the best long-term survival and DFS rates were seen among patients with mild chronic GVHD (Figures 4 and 5). In patients with moderate-to-severe chronic GVHD, survival was worse than in patients with mild GVHD or without chronic GVHD. We found no difference in relapse rates between patients with

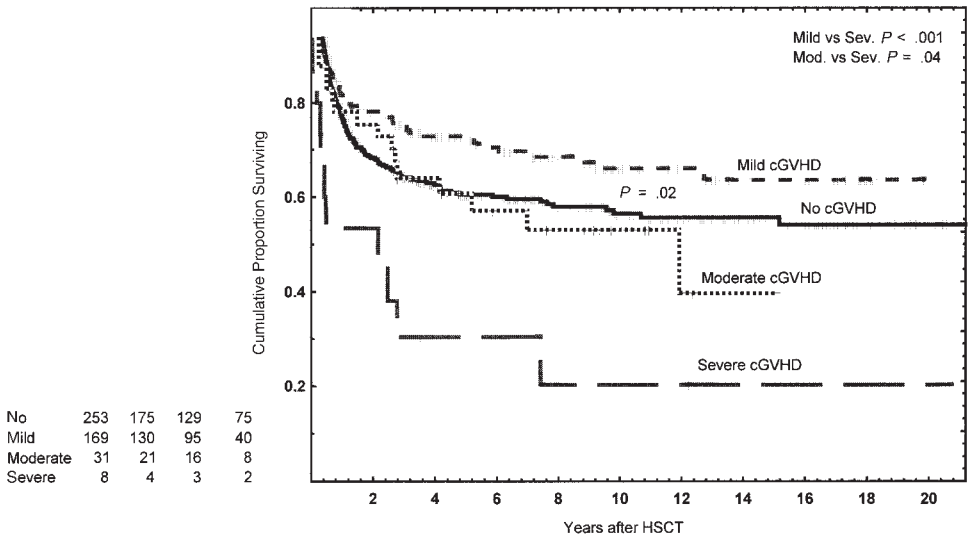

Figure 4. Probability of survival among 679 patients without or with mild, moderate, or severe chronic GVHD after HSCT. Left truncated survival curves are presented. The number of patients at risk at 1, 3, 5, and 10 years in the different groups is shown to the left of the figure.

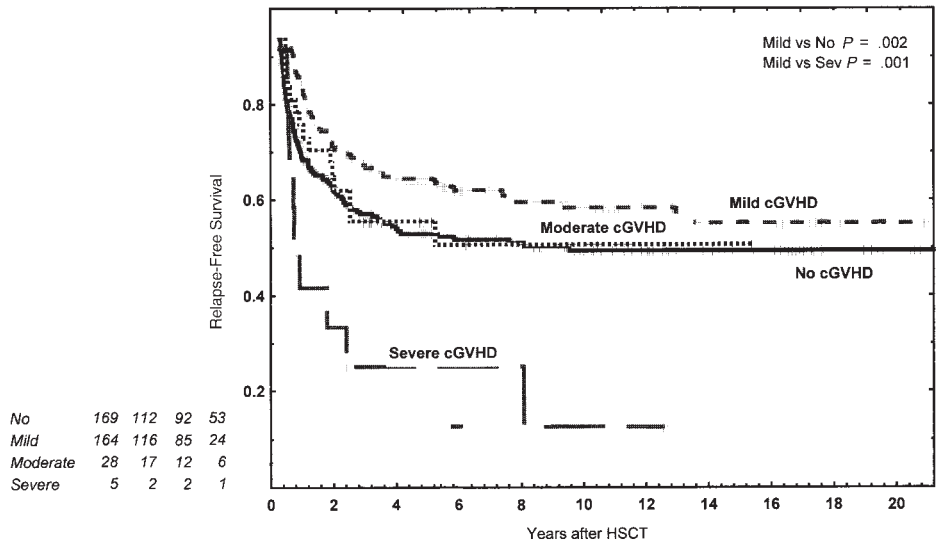

Figure 5. Probability of DFS among 568 patients with malignant disease without or with mild, moderate, or severe chronic GVHD after HSCT. Left truncated DFS curves are presented. The number of patients at risk at 1, 3, 5, and 10 years in the different groups is shown to the left of the figure.

more severe chronic GVHD and those with mild disease, so there is probably not a stronger GVL effect in more severe disease.

Because moderate-to-severe chronic GVHD is disabling for the patient and is not associated with improved survival, it is a complication that should be prevented. One must therefore detect risk factors for moderate-to-severe disease rather than those associated with any degree of GVHD. Whether more aggressive or prolonged immunosuppressive treatment can prevent or modulate severe chronic GVHD is controversial. Results of some studies have shown satisfactory results with extracorporeal photopheresis [12,15,37].

We found that patients with previous acute GVHD grades II to IV run a higher risk of developing more severe chronic GVHD. Among patients who developed chronic GVHD, previous acute GVHD grades II to IV was the only predictive factor for development of moderate-to-severe versus mild disease. Many other studies have shown that acute GVHD is a risk factor for the development of chronic GVHD [5-7,38,39). One could therefore assume that more severe forms of acute GVHD may lead to more severe forms of chronic GVHD. In this study the risk of more severe chronic GVHD increased with increasing grade of acute GVHD, especially in patients with previous acute GVHD grade III. After HSCT, thymus function is negatively affected by various factors, such as conditioning with chemotherapy and/or irradiation, use of corticosteroids, and acute GVHD [40-42]. Conditioning before HSCT leads to decreased stromal production of interleukin-7 and consequent blocks in the maturation of thymocytes. Acute GVHD is mainly caused by activated alloreactive T-cells that produce substantial organ destruction, whereas chronic GVHD clinicopathologically has more autoimmune features [1,2]. Therefore it is possible that a damaged thymus, which has decreased capability of negative T-cell selection, will release more autoreactive T-cells and that this process would lead to more chronic GVHD with autoimmune similarities [43]. In patients with more severe acute GVHD (grades II-IV), the thymus is probably more damaged than in milder forms, both by GVHD itself and by the additional treatment these patients receive [1,40-42].

Another factor that affects thymus function is age. In older patients thymus function deteriorates. The decline in function, with the conditioning therapy and corticosteroid treatment, may put older patients at a higher risk of chronic GVHD. Many other studies have shown age to be associated with all degrees of chronic GVHD [5-7,38]. Because most patients with CML are adults, age and presence of CML may interact with each other in the multivariate analysis. For this reason we performed analysis with and without CML. In this study we also found that if CML was excluded, there was a correlation between older recipient age and the development of moderate-to-severe chronic GVHD. Others investigators have reported a correlation between donor age and the development of chronic GVHD [5,6]. Donor age was significant in the univariate but not in the multivariate analysis. However, donor and recipient age often correlate with each other, at least for sibling donors [6].

The diagnosis of CML was a significant risk factor in the multivariate analysis. This factor previously has been shown to correlate with the presence of chronic GVHD of any grade [38]. Patients with CML usually are above the mean age for HSCT, and in that respect they are at increased risk of chronic GVHD. Many patients with CML have been given interferon as maintenance treatment before transplantation. Interferon has a well-known immune stimulatory effect with up-regulation of HLA class II cell surface molecules [44]. Whether this effect influences the development of both acute and chronic GVHD is still unknown and needs to be further studied. Some studies have found a correlation between interferon treatment before HSCT and development of GVHD, whereas others have not [45-48]. At our center, we have seen a lower risk of leukemia relapse in patients with CML than in those with acute leukemia [49]. This finding may be due to the higher incidence of chronic GVHD among the CML patients.

A previous study from the IBMTR by Gale et al [50] showed that transplantation from an alloimmune female donor to a male recipient was the strongest predictor of development of acute GVHD. This risk factor has been reported significant for de novo chronic GVHD [6], and the finding was confirmed in our study. A graft from a female donor may contain, through previous pregnancies or transfusions, activated alloreactive T-cells against the male minor histocompatibility antigen H-Y [51].

A previous study from our center showed a correlation between CMV infection and development of chronic GVHD [52], but a more recent report showed no such correlation [39]. An experimental study showed a correlation between CMV infection and development of severe chronic GVHD [53]. This finding was not confirmed for moderate-to-severe disease. An explanation of the difference between this finding and previous findings from our center may be that in the current analysis risk factors for moderate-to-severe disease were evaluated, whereas in the other study any grade of disease was analyzed. In addition, the methods of CMV diagnosis have changed with time. In more recent years, diagnosis based on PCR results has been used. This assay detects CMV DNA, but the presence of the DNA does not necessarily mean that protein synthesis occurs.

We found that previous acute GVHD grades II to IV, higher patient age, CML diagnosis, and transplantation from an immunized female donor to a male recipient were independent predisposing factors for the development of moderate-to-severe chronic GVHD. Among patients who developed chronic GVHD, acute GVHD grades II to IV was the only factor associated with moderate-to-severe chronic GVHD versus mild disease. These data may be used to identify patients who may need more aggressive immunosuppressive protocols, possibly new treatments such as extracorporeal photopheresis. Prospective randomized trials of prolonged immunosuppression or additional treatment at the onset of early chronic GVHD may be conducted with patients with 2 or more risk factors for moderate-to-severe chronic GVHD.

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REFERENCES

1. Lister J, Messner H, Keystone E, Miller R, Fritzler MJ. Autoantibody analysis of patients with graft-versus-host disease. *J Clin Lab Immunol.* 1987;24:19-23.
2. Rouquette-Gally AM, Boyeldieu D, Gluckman E, Abuaf N, Combrisson A. Autoimmunity in 28 patients after allogeneic bone marrow transplantation: comparison with Sjögren syndrome and scleroderma. *Br J Haematol.* 1987;66:45-47.
3. Sullivan KM, Shulman HM, Storb R, et al. Chronic graft-versus-host disease in 52 patients: adverse natural course and successful treatment with combination immunosuppression. *Blood.* 1981;57:267-276.
4. Atkinson K, Storb R, Prentice RL, et al. Analysis of late infections in 89 long-term survivors of bone marrow transplantation. *Blood.* 1979;53:720-731.
5. Ringdén O, Paulin T, Lönnqvist B, Nilsson B. An analysis of factors predisposing for chronic graft-versus-host disease. *Exp Hematol.* 1985;13:1062-1067.
6. Atkinson K, Horowitz M, Van Bekkum DW, et al. Risk factors for chronic graft-versus-host disease after HLA-identical sibling bone marrow transplantation. *Blood.* 1990;75:2459-2464.
7. Storb R, Prentice RL, Sullivan KM, et al. Predictive factors in chronic graft-versus-host disease in patients with aplastic anemia treated by marrow transplantation from HLA-identical sibling. *Ann Intern Med.* 1983;98:461-466.
8. Horowitz M, Gale R, Sondel P, et al. Graft-versus-leukemia reactions following bone marrow transplantation in humans. *Blood.* 1989;75:555-562.
9. Ringdén O, Labopin M, Gluckman E, et al. Graft-versus-leukemia effect in allogeneic marrow transplant recipients with acute leukemia is maintained using cyclosporin A combined with methotrexate as prophylaxis. Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant.* 1996;18:921-929.
10. Weiden P, Flournoy N, Thomas E, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogenic-marrow grafts. *N Engl J Med.* 1979;300:1068-1073.
11. Vogelsang G, Santos G, Colvin O, Chen T. Thalidomide for graft-versus-host disease [letter]. *Lancet.* 1988;1:827.
12. Besnier DP, Chabannes D, Mahe B, et al. Treatment of graft-versus-host disease by extracorporeal photochemotherapy: a pilot study. *Transplantation.* 1997;64:49-54.
13. Shulman H, Sullivan K, Weiden P, et al. Chronic graft-versus-host syndrome in man: a clinicopathological study of 20 long-term Seattle patients. *Am J Med.* 1980;69:204-217.
14. Lee S, Klein J, Barret J, et al. Severity of chronic graft-versus-host disease: association with treatment-related mortality and relapse. *Blood.* 2001;98:3205-3211.
15. Salvaneschi L, Perotti C, Zecca M, et al. Extracorporeal photochemotherapy for treatment of acute and chronic GVHD in childhood. *Transfusion.* 2001;41:1299-1305.
16. Ringdén O, Båryd I, Johansson B, et al. Increased mortality by septicemia, interstitial pneumonitis and pulmonary fibrosis among bone marrow transplant recipients receiving an increased mean dose rate of total irradiation. *Acta Radiol Oncol.* 1983;22:423-428.
17. Ringdén O, Ruutu T, Remberger M, et al. A randomized trial comparing busulphan with total body irradiation as conditioning in allogenic marrow transplant recipients with leukemia. *Blood.* 1994;83:2723-2730.
18. Ringdén O, Pihlstedt P, Markling L, et al. Prevention of graft-versus-host disease with T-cell depletion or cyclosporin and methotrexate: a randomized trial in adult leukemic marrow recipients. *Bone Marrow Transplant.* 1991;7:221-226.
19. Ringdén O, Groth C, Aschan J, et al. Bone marrow transplantation for metabolic disorders at Huddinge Hospital. *Transplant Proc.* 1990;22:198-202.
20. Ringdén O, Remberger M, Persson U, et al. Similar incidence of graft-versus-host disease using HLA-A, -B and -DR identical

unrelated bone marrow donors as with HLA-identical siblings. *Bone Marrow Transplant.* 1995;15:619-625.
21. Klaesson S, Ringdén O, Ljungman P, Aschan J, Hägglund H, Winiarski J. Does high-dose intravenous immune globulin treatment after bone marrow transplantation increase mortality in veno-occlusive disease of the liver? *Transplantation.* 1995;60:1225-1230.
22. Thomas E, Storb R, Clift R, et al. Bone-marrow transplantation, I and II. *N Engl J Med.* 1975;292:832-843.
23. Storb R, Epstein R, Graham T, Thomas E. Methotrexate regimens for control of graft-versus-host disease in dogs with allogenic marrow grafts. *Transplantation.* 1970;9:240-246.
24. Ringdén O, Bäckman L, Lönnqvist B, et al. A randomized trial comparing use of cyclosporin and methotrexate for graft-versus-host disease prophylaxis in bone marrow transplant recipients with haematological malignancies. *Bone Marrow Transplant.* 1986; 1:41-51.
25. Deeg H, Storb R, Appelbaum F, et al. Combined immunosuppression with cyclosporine and methotrexate in dogs given bone marrow grafts from DLA-haploidentical littermates. *Transplantation.* 1984;37:62-65.
26. Storb R, Deeg H, Whitehead J, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft-versus-host disease after marrow transplantation for leukemia. *N Engl J Med.* 1986;314:729-735.
27. Aschan J, Ringdén O, Andström E, Ljungman P, Lönnqvist B, Remberger M. Individualized prophylaxis against graft-versus-host disease in leukemic marrow transplant recipients. *Bone Marrow Transplant.* 1994;14:79-87.
28. Carlens S, Aschan J, Remberger M, Dilber MS, Ringdén O. Low-dose cyclosporine of short duration increases the risk of mild and moderate GVHD and reduces the risk of relapse in HLA-identical sibling marrow transplant recipients with leukemia. *Bone Marrow Transplant.* 1999;24:629-635.
29. Sullivan KM, Witherspoon RP, Storb R, et al. Alternating-day cyclosporine and prednisone for treatment of high-risk chronic graft-v-host disease. *Blood.* 1988;72:555-561.
30. Sullivan KM, Witherspoon RP, Storb R, et al. Prednisone and azathioprine compared with prednisone and placebo for treatment of chronic graft-v-host disease: prognostic influence of prolonged thrombocytopenia after allogeneic marrow transplantation. *Blood.* 1988;72:546-554.
31. Hymes SR, Morison WL, Farmer ER, Walters LL, Tutschka PJ, Santos GW. Methoxsalen and ultraviolet A radiation in treatment of chronic cutaneous graft-versus-host reaction. *J Am Acad Dermatol.* 1985;12:30-37.
32. Socie G, Devergie A, Cosset JM, et al. Low-dose (one gray) total-lymphoid irradiation for extensive, drug- resistant chronic graft-versus-host disease. *Transplantation.* 1990;49:657-658.
33. Ljungman P, Plotkin S. Workshop on CMV disease: definition, clinical severity scores, and new syndromes. *Scand J Infect Dis Suppl.* 1995;99:87-89.
34. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc.* 1958;53:457-481.
35. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient, II: analysis and examples. *Br J Cancer.* 1977;35:1-39.
36. Cox D. Regression models and life-tables. *J R Stat Soc [Ser B].* 1972;34:187-220.
37. Greinix HT, Volc-Platzer B, Kalhs P, et al. Extracorporeal photochemotherapy in the treatment of severe steroid- refractory acute graft-versus-host disease: a pilot study. *Blood.* 2000;96:2426-2431.
38. Carlens S, Ringdén O, Remberger M, et al. Risk factors for chronic graft-versus-host disease after bone marrow transplantation: a retrospective single centre analysis. *Bone Marrow Transplant.* 1998;22:755-761.
39. Boström L, Ringdén O, Jacobsen N, Zwaan F, Nilsson B. A European multicenter study of chronic graft-versus-host disease. *Transplantation.* 1990;49:1100-1105.
40. Muller-Hermelink HK, Sale GE, Borisch B, Storb R. Pathology of the thymus after allogeneic bone marrow transplantation in man: a histologic immunohistochemical study of 36 patients. *Am J Pathol.* 1987;129:242-256.
41. Ghayur T, Seemayer TA, Xenocostas A, Lapp WS. Complete sequential regeneration of graft-versus-host-induced severely dysplastic thymuses: implications for the pathogenesis of chronic graft- versus-host disease. *Am J Pathol.* 1988;133:39-46.
42. Weinberg K, Annett G, Kashyap A, Lenarsky C, Forman SJ, Parkman R. The effect of thymic function on immunocompetence following bone marrow transplantation. *Biol Blood Marrow Transplant.* 1995;1:18-23.
43. Hollander GA, Widmer B, Burakoff SJ. Loss of normal thymic repertoire selection and persistence of autoreactive T cells in graft vs host disease. *J Immunol.* 1994;152:1609-1617.
44. Balkwill FR. Interferons. *Lancet.* 1989;1:1060-1063.
45. Giralt SA, Kantarjian HM, Talpaz M, et al. Effect of prior interferon alfa therapy on the outcome of allogeneic bone marrow transplantation for chronic myelogenous leukemia. *J Clin Oncol.* 1993;11:1055-1061.
46. Lee SJ, Klein JP, Anasetti C, et al. The effect of pretransplant interferon therapy on the outcome of unrelated donor hematopoietic stem cell transplantation for patients with chronic myelogenous leukemia in first chronic phase. *Blood.* 2001;98:3205-3211.
47. Beelen DW, Graeven U, Elmaagacli AH, et al. Prolonged administration of interferon-alpha in patients with chronic-phase Philadelphia chromosome-positive chronic myelogenous leukemia before allogeneic bone marrow transplantation may adversely affect transplant outcome. *Blood.* 1995;85:2981-2990.
48. Morton AJ, Gooley T, Hansen JA, et al. Association between pretransplant interferon-alpha and outcome after unrelated donor marrow transplantation for chronic myelogenous leukemia in chronic phase. *Blood.* 1998;92:394-401.
49. Carlens S, Ringdén O, Aschan J, et al. Risk factors in bone marrow transplant recipients with leukaemia: increased relapse risk in patients treated with ciprofloxacin for gut decontamination. *Clin Transplant.* 1998;12:84-92.
50. Gale RP, Bortin MM, van Bekkum DW, et al. Risk factors for acute graft-versus-host disease. *Br J Haematol.* 1987;67:397-406.
51. Goulmy E, Termijtelen A, Bradley BA, van Rood JJ. Alloimmunity to human H-Y. *Lancet.* 1976;2:1206.
52. Lönnqvist B, Ringdén O, Wahren B, Gahrton G, Lundgren G. Cytomegalovirus infection associated with and preceding graft-versus-host disease. *Transplantation.* 1984;38:465-468.
53. Söderberg C, Larsson S, Lundh Rozell B, Sumitran-Karuppan S, Ljungman P, Möller E. Cytomegalovirus-induced CD13-specific autoimmunity: a possible cause of chronic graft-versus-host disease. *Transplantation.* 1996;61:600-609.