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Acute Graft-Versus-Host Disease: Analysis of Risk Factors After Allogeneic Marrow Transplantation and Prophylaxis With Cyclosporine and Methotrexate

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Previous studies of risk factors for acute graft-versus-host disease (GVHD) involved patients receiving predominantly single-agent prophylaxis. Therefore, a retrospective analysis was performed on 446 patients, from a single institution, who received transplants of marrow from HLA-identical siblings and the combination of cyclosporine (CSP) and methotrexate (MTX) to determine risk factors for acute GVHD associated with this more effective form of GVHD prophylaxis. The incidences of Grades II-IV and Grades III-IV (severe) acute GVHD were 35% and 16%, respectively. Increased clinical grades of acute GVHD in patients without advanced malignant disease were associated with a decreased survival. In a multivariate Cox regression analysis, risk factors associated with the onset of Grades II-IV acute GVHD were sex mismatch and donor parity ($P = .001$), increased dose of total body irradiation (TBI) ($P = .001$), and reduction to less than 80% of the scheduled dose of MTX ($P = .02$) or CSP ($P = .02$). The multivariate analysis indicated a relative risk of 1.37 for acute GVHD in a group defined as having advanced malignant disease at transplant; however, this difference failed to reach conventional levels of statistical significance ($P = .07$). Reduction of MTX and CSP occurred in up to 36% and 44% of patients, respectively, primarily because of renal or hepatic dysfunction. The periods of increased risk for the onset of acute GVHD were up to 1 week after a reduction of MTX and 2 weeks after a reduction in CSP. When only patients who developed Grades II-IV acute GVHD were considered, the more severe acute GVHD of Grades III-IV was associated with increased patient age of 40 years or greater ($P = .05$) and dose reductions of CSP ($P = .008$). Serologic status of patient and donor for cytomegalovirus (CMV), HLA antigens in the A and B loci, and isolation in a laminar air flow room during marrow transplantation, all previously identified as risk factors for acute GVHD, were not confirmed as risk factors in this study population. The toxicity of MTX and CSP and the development of acute GVHD from inadequate immunosuppression because of dose reduction warrants further trials with potentially less toxic immunosuppressive agents. Risk factors for acute GVHD should be considered in clinical management and in the design of clinical trials.

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ACUTE GRAFT-VERSUS-HOST disease (GVHD) is a complication of allogeneic marrow transplantation that results in significant morbidity and mortality. To further the understanding of disease mechanisms and to assist in clinical management, risk factors for the development of acute GVHD after marrow transplantation from an HLA-identical sibling have been identified in previous studies.[1-7] These studies have included primarily patients who received single agent prophylaxis. The combination of methotrexate (MTX) and cyclosporine (CSP) has reduced the incidence and severity of acute GVHD when compared with single agent prophylaxis[8,9] and is especially effective in children.[10] Because the combination of MTX and CSP is significantly more effective than single agent therapy, risk factors for the development of acute GVHD may be modified in a way not predictable from studies using single-agent prophylaxis.

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Even with the combination of MTX and CSP for GVHD prophylaxis, acute GVHD persists as a significant complication after marrow transplantation. Certain disparate non-HLA antigens or other biologic factors that we cannot currently identify may preclude effective GVHD prophylaxis. Dose reductions of MTX or CSP frequently are required because of drug-related toxicity and may jeopardize the effectiveness of GVHD prophylaxis. Moreover, the combination of MTX and CSP has recently been used in treating patients who have had more intensive conditioning regimens than were used previously when GVHD prophylaxis consisted of a single agent.[11-13] Dose reductions of MTX or CSP may occur not only because of toxicity related to these agents themselves,[14] but also because of conditioning-related organ toxicity that may attenuate drug administration.

The present study was performed to identify risk factors for acute GVHD in patients administered a standardized regimen of GVHD prophylaxis with MTX and CSP after marrow transplantation from HLA-identical siblings.

MATERIALS AND METHODS

Patients. A retrospective analysis was conducted on data from 446 patients administered CSP and MTX after a first marrow transplant from an HLA-identical sibling at the Fred Hutchinson Cancer Research Center between August 1981 and August 1988. Data from all patients surviving more than 14 days and showing evidence of engraftment were analyzed. Characteristics of the study population are shown in Table 1. Methods for histocompatibility typing of marrow donors and patients have been described.[15] Patients who received total body irradiation (TBI) were also administered intravenous (IV) cyclophosphamide (60 mg/kg of body weight) on each of 2 successive days. In 26 patients, additional chemotherapeutic agents were administered with a modified dose of cyclophosphamide and 4 patients were treated with high-dose

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Table 1. Patient Characteristics and Univariate Analyses of Potential Risk Factors for Acute GVHD

Factor	Total No. of Patients	Patients With Grade II-IV (% of total)	*P* Value*
Patient age (yr)			.04
<20	83	24 (29%)	
20–39	282	93 (33%)	
≥40	81	37 (46%)	
Donor age (yr)			.04
<20	93	30 (33%)	
20–39	267	85 (32%)	
≥40	86	39 (45%)	
Donor-patient sex match			<.001
match	241	66 (28%)	
mismatch	199	88 (44%)	
Donor parity-sex/patient sex			<.001†
Nonparous female/female	41	6 (15%)	
Parous female/female	47	15 (32%)	
Male/female	89	42 (47%)	
Nonparous female/male	51	14 (28%)	
Parous female/male	59	32 (54%)	
Male/male	153	45 (29%)	
Diagnosis—bone marrow status			
Lymphoma: remission	31	8 (26%)	.16
relapse	18	8 (44%)	
AML/ALL: remission	104	35 (33%)	.31
relapse	69	27 (39%)	
CML: chronic phase	119	40 (34%)	.03
accl/blast crisis	30	15 (50%)	
Nonmalignant hematologic disease (eg, aplastic anemia)	52	9 (18%)	
Myelodysplasia	14	8 (57%)	
Other	9		
Advanced malignant disease‡			
No	325	102 (31%)	.006
Yes	121	52 (43%)	
Total body irradiation (cGy)			
0-1,200	274	77 (28%)	<.001
>1,200	172	77 (44%)	
CMV/HSV serology pretransplant			
Patient CMV +	207	72 (35%)	.75
−	175	60 (35%)	
Donor CMV +	164	61 (37%)	.53
−	209	71 (34%)	
Patient HSV +	226	75 (33%)	.63
−	160	56 (36%)	
IgG prophylaxis			
Randomized to IgG	71	19 (27%)	.31
Randomized to no IgG	61	23 (38%)	
Not randomized (no IgG)	314	112 (35%)	
Laminar Airflow Room (LAF)			
No	270	91 (34%)	.60
Yes	176	63 (36%)	
Patient HLA antigens§			
B8 +	89	28 (31%)	.60
−	357	126 (35%)	
B18 +	43	13 (31%)	.74
−	403	141 (35%)	
B35 +	77	22 (28%)	.16
−	369	132 (36%)	
B21 (49, 50) +	24	5 (21%)	.13
−	422	149 (35%)	
Aw19 A29 Aw30-33 +	124	46 (37%)	.55
−	322	108 (33%)	
Transplant year			
≤1985	114	29 (25%)	<.001
1986	85	22 (26%)	
1987	124	40 (32%)	
1988	123	63 (51%)	

*Based on a logrank test for time to Grade II-IV acute GVHD.

†Based on a likelihood ratio test.

‡Disease in relapse, accelerated phase, or blast crisis at the time of marrow transplantation.

§References 3 and 7.

cytosine arabinoside instead of cyclophosphamide. In 67 patients TBI was hyperfractionated, and all but one of these received a total exposure greater than 1,200 cGy. Fractionated TBI was administered to 197 patients in increments of 200 cGy on each of 6 successive days for a total of 1,200 cGy, and to 110 patients in increments of 225 cGy on each of 7 successive days for a total of 1,575 cGy. One patient received 1,000 cGy of TBI in a single exposure. There were 21 patients who received only chemotherapy as a conditioning regimen for hematologic malignancy. This consisted of either busulfan (16 mg/kg) and cyclophosphamide (120 mg/kg), or etoposide (2,400 mg/m^2), carmustine (300 mg/m^2), and cyclophosphamide (7,200 mg/m^2). Patients with aplastic anemia received cyclophosphamide 50 mg/kg body weight on each of 4 successive days. No in vitro T-cell depletion was performed on donor marrow. The day of marrow infusion was designated as day 0.

GVHD management. Acute GVHD prophylaxis consisted of CSP beginning on day −1 before transplantation at a dose of 1.5 mg/kg every 12 hours IV and continued until gastrointestinal toxicity from the preparative regimen had resolved. Patients then received 6.25 mg/kg of oral CSP every 12 hours. From day 50 or 60 (different protocols) the dose was tapered 5% per week and discontinued by day 180 unless otherwise clinically indicated. MTX was to be administered IV at a dose of 15 mg/m^2 of body surface area on day 1 and then 10 mg/m^2 on days 3, 6, and 11. Dose modification of MTX and CSP was a clinical decision based on general guidelines. Reduction of MTX occurred if significant renal dysfunction, severe mucositis with the potential for airway obstruction, or significant third-spaced fluids (ascites, pleural effusion) were present. Reduction of CSP was advised if serum creatinine doubled over pretransplant levels, if serum creatinine increased to > 2 mg/dL, or if neurotoxicity or significant hepatic dysfunction were present. The total dose of MTX and CSP received was determined by review of pharmacy records on patient charts. The total dose of CSP received for each week up to and including 5 weeks after marrow transplant (day −1 to day +34) was calculated and recorded as a percent of the dose prescribed per protocol. The percentage dose of MTX administered on each of days 1, 3, 6, and 11 was also determined.

The assessment and grading of acute GVHD has been reported.[15] Initial therapy of established Grades II to IV acute GVHD for patients receiving prophylaxis with the combination of MTX and CSP consisted initially of prednisone 2 mg/kg/d (IV or orally) for 7 to 14 days after which the patient's status was reevaluated.[8,16] Progressive acute GVHD was treated with antithymocyte globulin or investigational agents such as monoclonal T-cell–specific antibodies.[17]

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Risk factors. To evaluate the risk of acute GVHD in recipients of marrow grafts from alloimmunized donors, female donors 16 years of age or older had their charts reviewed or were contacted to obtain histories of pregnancy and spontaneous or therapeutic abortion. Female donors less than 16 years of age were considered to be nonparous. Because of the expected low incidence of blood transfusion in an otherwise healthy donor population, this was not considered as a potential cause of alloimmunization.

To assess the effects of disease status at the time of marrow transplantation on the development of acute GVHD, a category of "advanced malignancy" was established. Advanced malignancy included patients with lymphoma or acute leukemia in relapse or chronic myelogenous leukemia in accelerate phase or blast crisis. Patients with other diagnoses or different disease status were included in the comparison group.

Assignment of patients to laminar airflow (LAF) isolation was based on protocol and availability. Patients were otherwise treated in reverse-isolation rooms. Patients in LAF rooms were given sterile food, oral nonabsorbable antibiotics, and skin cleansing. Since 1983, patients transplanted for severe aplastic anemia were assigned to LAF rooms.[2] For the purpose of this analysis, patients were considered in LAF if they entered before marrow transplantation and remained until at least day 8 after marrow transplant.

Seventy-one patients were randomized to receive prophylactic IV immunoglobulin (IVIg) from days 7 to 360 as described.[18] Because a decreased risk of acute GVHD had been noted in adult patients receiving IVIg, this variable was included in the present analysis. Herpes viruses, such as cytomegalovirus (CMV) and herpes simplex have previously been noted by others as a risk factor for acute GVHD.[19,20] Serologic testing of donors and recipients for CMV and recipients for herpes simplex was conducted routinely before marrow transplantation.

Statistical analysis. The probability of survival was estimated using the method of Kaplan and Meier.[21] Median follow-up amongst surviving patients was 4.6 years (range 0.7 to 10.0). The probability of acute GVHD was estimated using a cumulative incidence curve.[22] Differences in the risk of acute GVHD were determined by logrank test. Factors that appeared predictive in univariate analysis ($P \leq .20$) were included in the multivariate Cox regression analysis of acute GVHD (Grades II-IV) using a stepwise technique. In addition to baseline prognostic factors known at the time of transplant, dose modification of MTX and CSP were considered as time-dependent covariates. Dose reduction was defined as receiving $\leq 80\%$ of the protocol-recommended dose. The independent influences of the day 6 and day 11 MTX doses on the risk of acute GVHD were evaluated. The effects of the drug during different time periods were also studied independently, with a view to determining the duration of the influence on acute GVHD. Cyclosporine doses were calculated as weekly averages for 5 weeks posttransplant, starting with day −1. The influence of the CSP dose received during the ensuing 2 weeks was investigated using a Cox regression analysis. Because of the high correlation of the doses received in 2 consecutive weeks, independent effects of the dose during a single week could not be assessed.

Factors that might influence dose reductions were also investigated using stepwise Cox regression analyses for time to dose reduction. Candidate risk factors included all those listed in Table 1. Deaths, relapses, and incidence of acute GVHD were censored in these analyses. A logistic regression analysis was used to determine factors associated with severe (Grades III-IV) acute GVHD among patients who developed Grades II-IV acute GVHD. All factors listed in Table 1 were considered. In addition, dose reductions of MTX and CSP were included as continuous variables. Analyses were performed using the STATA statistical package on an IBM-286 personal computer. All P values were two-sided.

RESULTS

Incidence of acute GVHD and survival. The incidence of moderate to severe (Grades II-IV) acute GVHD was 35% (Fig 1). The incidence of Grades III-IV acute GVHD was 16%, and only seven patients (2%) developed Grade IV disease. Median times to onset of GVHD in patients who eventually developed Grade II and Grades III-IV acute GVHD were days 16 and 18, respectively. Earlier onset of acute GVHD was not associated with higher grades of disease ($P = .46$, Wilcoxon test).

Because GVHD is associated with increased mortality, but also with a potentially beneficial graft-versus-leukemia effect,[23] we analyzed the data in regards to survival. There was no association between grade of acute GVHD and survival in patients with advanced malignancy because of the high relapse rate. In the absence of advanced malignancy, survival decreased with increased severity of acute GVHD (Grade 0-I v II, $P < .01$; Grade II v III-IV, $P < .01$) (Fig 2).

Risk factors for the development of Grades II-IV acute GVHD. In a univariate analysis, increasing patient age, increasing donor age, and patient-donor sex mismatch were significantly associated with the risk of developing Grades II-IV acute GVHD (Table 1). A history of pregnancy in female donors was also a predictive factor. In a multivariate analysis, the recipient-donor sex combination and the parity of female donors were the most significant predictive factors (Table 2) ($P = .001$). Patient and donor age had no additional predictive value ($P = .60$).

Disease status before marrow transplant and intensity of the TBI in the conditioning regimen significantly affected the risk for acute GVHD. Patients with advanced malignant hematologic disorders were at higher risk for acute GVHD than were all other patients combined (Table 1) ($P = .006$). Moreover, patients with advanced malignancy had a signifi-

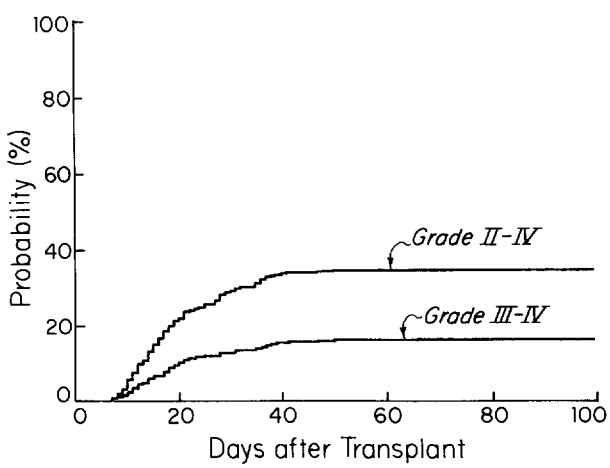

Fig 1. Incidence of Grades II-IV and Grades III-IV acute GVHD in a group of 446 patients who received an allogeneic marrow transplant from an HLA-identical sibling and a combination of MTX and CSP for GVHD prophylaxis.

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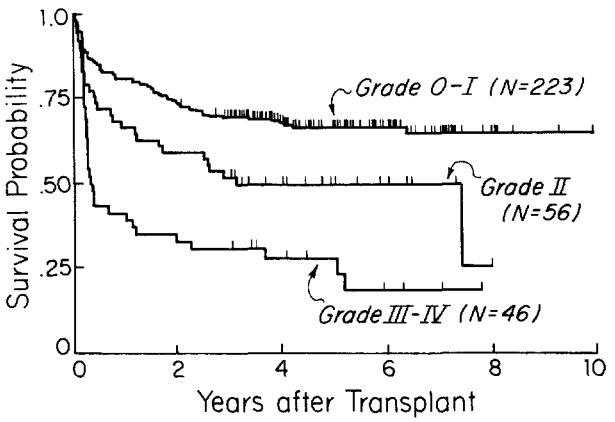

Fig 2. Kaplan-Meier estimates of survival based on the clinical grade of acute GVHD. For this analysis, 325 patients without advanced malignancy were studied. Tic marks indicate surviving patients. Median last contact date was September 1991.

cantly increased risk of acute GVHD when compared only with those in remission or chronic phase ($P = .03$). Higher doses of TBI also predicted the onset of acute GVHD with 44% of patients who received more than 1,200 cGy developing Grade II-IV acute GVHD compared with 28% of patients who received chemotherapy only, or who received ≤1,200 cGy. In a multivariate analysis, higher doses of TBI remained predictive for acute GVHD ($P = .001$); moreover, there was a trend suggesting that advanced malignancy had an independent effect on acute GVHD (Table 2).

Neither patient nor donor seropositivity for CMV were risk factors for acute GVHD (Table 1). Similarly, patient seropositivity for herpes simplex virus was not associated with an increased risk of acute GVHD. Patients treated in LAF rooms had the same incidence of acute GVHD as those not treated in LAF rooms (Table 1). Nor was there an association evident when LAF was treated as a time-dependent covariate, which adjusted for removal from the LAF isolation during the risk period for acute GVHD ($P = .32$). Among patients entered as part of a randomized trial of Ig prophylaxis, those who received IVIg were at decreased risk of acute GVHD relative to those randomized not to receive Ig.[18] However, this difference was not statistically significant, perhaps because of the smaller number of the randomized patients in the present study.

The data did not confirm any of the previously reported associations between patient HLA alleles and risk of acute GVHD in aplastic anemia patients receiving MTX only (Table 1). There was an increased incidence of acute GVHD among patients treated during 1988 when compared with those before 1988. Multivariate analysis suggested that this trend was not explained by more intensive conditioning regimens or being transplanted with later stages of disease during 1988 than previously (Table 2).

Drug dose modification. Among patients at risk for acute GVHD, 6% (29 of 446) received less than 80% of the protocol dose of MTX on day 1 after transplant, 7% (32 of 446) on day 3, 16% (70 of 445) on day 6, and 36% (147 of 413) on day 11, respectively; 26% (108 of 413) received no MTX at all on day 11. In a multivariate analysis there was a trend suggesting that reductions in MTX doses were associated with increasing patient age ($P = .08$), advanced disease at transplant ($P = .09$), and higher doses of TBI ($P = .14$). Dose reduction was less frequent in patients transplanted in 1988 than in previous years of the study (Table 3).

The percentages of patients receiving less than 80% of prescribed CSP dose in weeks 1 through 5 were 14% (65 of 446), 29% (116 of 403), 44% (150 of 344), 41% (131 of 316), and 41% (115 of 281), respectively. Dose reductions were associated with increased patient age ($P < .001$) and male gender ($P < .001$). As was the case with MTX, dose modification was less prevalent during 1988 than previously (Table 3).

Almost all patients received full doses of MTX on days 1 and 3, so the effect of dose reductions on those days could not be evaluated. Among patients receiving a reduced dose of MTX on day 6, 16% developed acute GVHD with the onset between days 7 and 11, whereas only 6% (21 of 375) of those administered ≥80% of the protocol dose developed acute GVHD during that time period ($P = .003$). Beyond day 11, the risk was similar in both cohorts.

Table 2. Multivariate Analysis of Risk Factors for Grade II-IV Acute GVHD

Factor	Relative Risk (95% C.I.)	*P* Value*
Donor parity/sex–patient sex		.001†
nonparous female-female	1.00	
nonparous female-male	1.88 (0.72, 4.89)	
male-female	3.76 (1.59, 8.88)	
male-male	2.07 (0.88, 4.90)	
parous female-female	2.41 (0.93, 6.22)	
parous female-male	3.85 (1.58, 9.37)	
Advanced malignancy		.07
no	1.00	
yes	1.37 (0.97,1.92)	
Total body irradiation		.001
≤1200 cGy	1.00	
>1200 cGy	1.74 (1.26, 2.41)	
Transplant year		<.001
≤1987	1.00	
1988	2.37 (1.69, 3.32)	
Methotrexate (>80% *v* <80%)		.02†
Effect of day 6 full-dose for		
days 7–11	0.32 (0.15, 0.67)	(.002)
days 12–19	1.19 (0.56, 2.51)	(.67)
days ≥20	0.78 (0.34,1.79)	(.56)
Effect of day 11 full-dose for		
days 12–19	0.57 (0.32, 1.00)	(.05)
days ≥20	1.45 (0.77, 2.72)	(.24)
Cyclosporine (>80% *v* <80%)		0.02†
Effect of full-dose in the previous week	0.86 (0.57, 1.30)	(.49)
Effect of full-dose two weeks before	0.57 (0.35, 0.92)	(.02)

*P values in parentheses are based on estimates and their standard errors.

†Based on a likelihood ratio test for the addition of this variable to the model.

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Table 3. Cox Regression Analysis of Factors Predictive for Dose Reduction

Factor	Methotrexate		Cyclosporine	
	Relative Risk	*P* Value	Relative Risk	*P* Value
Patient age (yr)				
<20	1.00		1.00	
20-39	1.16	.46	1.93	.001
≥40	1.54	.08	2.55	<.001
Patient sex				
M	1.00		1.00	
F	0.89	.43	0.61	<.001
Advanced malignant disease				
No	1.00		1.00	
Yes	1.32	.09	0.94	.71
TBI (cGy)				
≤1200	1.00		1.00	
>1200	1.25	.14	1.22	.16
Transplant year				
≤1987	1.00		1.00	
1988	0.75	.11	0.72	.04

Dose reduction of immunosuppression is defined as receiving <80% of protocol dose.

Similarly, among patients who received less than 80% of the protocol dose on day 11, 20% (30 of 147) developed acute GVHD during the following week compared with 12% (31 of 266) of those receiving a full dose of MTX ($P = .02$). This association was independent of the day 6 dose. After day 19, the day 11 MTX dose was not associated with the risk of onset of acute GVHD. The association of full-dose MTX and the lower acute GVHD risk in the most immediate time intervals after the administration appeared independent of other risk factors (Table 2).

Because development of acute GVHD after day 40 was infrequent, the association of the days 6 and 11 MTX doses on acute GVHD in patients surviving to day 40 was determined. Data from 29 patients who died before day 40 from toxicity related to the conditioning regimen, infection, or residual leukemia were excluded. Among those patients administered a full dose of MTX on day 6, 124 of 360 (34%) developed acute GVHD compared with 29 of 56 (52%) of those receiving a reduced dose ($P = .01$). Similarly 73 of 261 (28%) of patients administered a full dose on day 11 developed acute GVHD compared with 48 of 123 (39%) administered a reduced dose ($P = .03$). The relative risk of the development of acute GVHD was decreased primarily in those time periods immediately after the administration of MTX and this resulted in an overall lower incidence of acute GVHD in the group receiving full-dose MTX.

Cyclosporine dose reduction was also associated with an increased risk of acute GVHD. Because there was little variability in compliance during week 1, the influence of the week 1 dose on risk of acute GVHD could not be analyzed. In weeks 2 to 5, reductions in cyclosporine dose were more frequent among patients who subsequently developed the onset of acute GVHD (Fig 3). By multivariate analysis the effect appeared independent of other risk factors and of reductions in MTX doses (Table 2).

Risk factors for the development of Grades III-IV acute GVHD. In a multivariate logistic regression analysis involving only patients with Grades II-IV acute GVHD, higher doses of CSP were associated with a decreased risk of Grades III-IV acute GVHD in the following week (Table 4). There was no significant effect of day 6 and 11 MTX doses. Patients more than 40 years of age were more likely to develop Grades III-IV acute GVHD. Disease status, conditioning regimen, parity of female donors, and donor-recipient sex mismatch did not increase the risk of Grades III-IV acute GVHD, beyond that of Grade II acute GVHD.

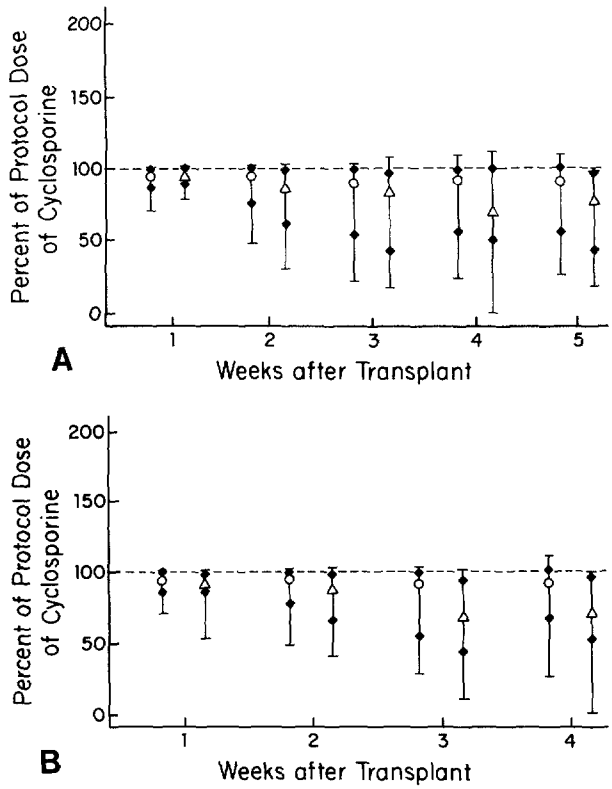

***Fig 3.* Percent of protocol CSP dose received by week after marrow transplant among patients who did or did not develop acute GVHD (A) 1 week later and (B) 2 weeks later. Median doses are indicated for those with acute GVHD (△) and for those without evidence of acute GVHD (○) but still at risk for GVHD in the relevant time period. Twenty-fifth and 75th percentiles are indicated by ◆. Tenth and 90th percentiles are indicated by —.**

Table 4. Multivariate Analysis of Risk Factors for Grade III-IV Acute GVHD

	Odds Ratio*	*P* Value
Cyclosporine prophylaxis received during previous week (per 10% increase in percent of recommended dose)	0.84	.008
Patient age (yr)		
<20	(1.0)	
29-39		.52
≥40	0.74	.05
	3.2	

*Risk of developing severe (Grade III-IV) acute GVHD among all patients with Grade II-IV GVHD.

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DISCUSSION

This study of patients receiving MTX and CSP confirms previous reports showing an increased risk of acute GVHD after marrow transplantation from (1) a donor of the opposite sex and (2) a parous female donor.[1,4,6,24] The biologic basis for the increased risk of acute GVHD may be the existence of sex-specific minor histocompatibility antigens. The H-Y antigen on the Y chromosome is a well characterized, minor histocompatibility antigen expressed on male cells. The presence of the H-Y antigen can be recognized as a target by female cytotoxic T cells.[25,26] Therefore, this antigen alone may explain the increased risk for acute GVHD in male recipients of a female marrow graft. However, an increased risk for acute GVHD in female recipients with a male donor was also suggested in this study.

This observation may be explained by a polymorphic X-chromosome–encoded minor histocompatibility antigen or one that may not be fully expressed on male cells.[27,28] Parity of female donors was also a strong risk factor that might be explained by maternal alloimmunization to minor histocompatibility antigens of fetal tissue not shared by the mother.[4,6,24] Marrow grafts from previously alloimmunized donors may be primed to react to antigens that are expressed in the transplant recipient. The strongest risk factor for Grades II-IV acute GVHD in this setting of sex mismatch is for male recipients of a graft from a parous female donor. However, female recipients of marrow grafts from parous female donors also have a slightly increased risk of acute GVHD. Therefore, alloimmunization may possibly develop against both sex-specific and polymorphic autosomal non-HLA antigens.

The intensity of the conditioning regimen and disease status before marrow transplantation were also found to be significant risk factors for acute GVHD. The observation has been made in animals that the incidence of acute GVHD increases with dose of irradiation.[29,30] Clinical studies have also suggested a relationship between the intensity of the conditioning regimen and the development of acute GVHD.[5,11-13] In some of these clinical reports the increased incidence of acute GVHD was considered to be related to the lower doses of CSP or MTX that could be tolerated.[11,12] The present analysis suggests that both the intensity of the conditioning regimen and the disease status have an independent effect in addition to the risk associated with a reduced dose of CSP. A possible common mechanism to explain the effects of intensive conditioning regimen and disease status is damage to host tissue, especially in the gastrointestinal tract. Tissue damage may facilitate priming of the alloimmune response possibly because of a change in presentation of antigens derived from infectious agents or host cells to immunocompetent donor cells.[31,32] Also, changes in the production of cytokines, such as tumor necrosis factor and interferon, can affect the manifestations of the disease process.[33,34] Finally, a decreased degree or incidence of mixed chimerism from a more intensive conditioning regimen ($\geq$1,200 cGy) may increase the likelihood of acute GVHD.[35,36]

Full-doses of MTX and CSP were associated with a decreased relative risk of acute GVHD when compared with a group who received reduced doses. There may be unidentified factors that may be important in the occurrence of this association. However, the observation of a reduced relative risk of acute GVHD in the present study is compatible with the expected biologic activity of these immunosuppressive agents. Because the effect of full-dose MTX on days 6 and 11 was a reduced relative risk in the time period immediately after the dose, improved control of acute GVHD in high-risk patients might be achieved by continuing MTX beyond day 11. The effectiveness of MTX beyond day 11 has been shown, but not when administered concomitantly with CSP.[37,38]

The decreased relative risk for acute GVHD associated with full-dose CSP ($\geq$80%) was observed up to 2 weeks after the dosing period. Moreover, full-dose CSP was associated with less severe GVHD among patients who developed acute GVHD. The maintenance of full-dose CSP in this study population was more predictive of the onset of acute GVHD than serum CSP levels, although an association between CSP levels and acute GVHD has been described previously.[39] Trough serum CSP levels as determined by the polyclonal radioimmunoassay (Sandoz, Basel, Switzerland) did not correlate with the oral dose of CSP received and were not predictive of the onset of acute GVHD. Because dose reductions of CSP were necessitated by clinical events, it is unknown whether more aggressive administration of CSP would have been indicated or beneficial. Recently, it has been reported in small studies that a reduction of MTX or CSP dose early after marrow transplantation may reduce organ toxicity and allow the administration of full dose CSP in subsequent weeks.[13,40] An increase in the incidence or severity of acute GVHD in these studies was not observed. In this study dose reductions of CSP after marrow transplantation occurred after the first week. Further controlled studies of dose modification on toxicity and efficacy early after marrow grafting appear warranted.

Patient age had a significant influence on the development of Grades II-IV acute GVHD but this was primarily the result of an increased frequency of parous female donors in the older age group. Other studies have also observed the increased frequency of parous female donors for older patients and the association with an increased risk of Grades II-IV acute GVHD.[4,6,24] In the present study, of those patients $\geq$40 years of age who did develop Grades II-IV acute GVHD, many had Grades III-IV acute GVHD. Recently, a different analysis of haploidentical related marrow transplants also showed a significant correlation between the development of Grades III-IV acute GVHD and patient age.[41] Therefore, although donor parity is associated with the development of acute GVHD, an older patient age contributes to severity.

Measures to prevent infection in marrow transplant recipients, such as administration of IVIg and isolation in a LAF room, have been reported to reduce the incidence of acute GVHD.[2,18,42] The present study included patients already described[18] and the earlier observation that IVIg can reduce the risk of acute GVHD was again suggested. However, this beneficial effect may result from an immuno-

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modulatory effect of the infused Ig and not necessarily a decreased infection rate. Isolation in LAF was studied in several ways in this analysis, but an association with a reduced risk of acute GVHD could not be shown. Decontamination of the gastrointestinal tract decreased the risk of acute GVHD in animals.[43] However, the success of gut decontamination, shown in animal models to be the factor most relevant to the success of a sterile environment in preventing acute GVHD, was not evaluated in this analysis of LAF isolation.

This study attempted to confirm previous observations of an association of HLA[3,7] and CMV serologic status[17,18,44,45] to the risk of acute GVHD. Those HLA antigens noted in previous studies as significant were reanalyzed, and no correlation was seen. A comprehensive analysis of the association of other HLA antigens to the risk of acute GVHD was outside the scope of this study. Serologic status for CMV before marrow transplant of both patient and donor was also analyzed, and no association found with acute GVHD. This analysis also included a comparison of CMV seronegative donor and patient pairs with CMV seropositive donor and patient pairs, and no differences in the incidence of acute GVHD were observed. Although in the present study pretransplant seropositivity for CMV was not confirmed as a risk factor for acute GVHD, a higher risk of developing CMV infection in patients with acute GVHD has previously been shown.[46,47]

It is unclear why the risk of acute GVHD increased in 1988. Because of this recent observation, a similar cohort of patients from the years 1989 and 1990 was analyzed. The incidence of acute GVHD in both these years was similar to the incidence in the years before 1988 (R.A.N., unpublished observations, 1992). Because the risk factor of a marrow transplant in 1988 was included in all multivariate analyses, the other observations in this study were not affected.

Patients included in this analysis were all treated with the combination of MTX and CSP, which may account for some of the differences in risk factors for acute GVHD between the present study and previous studies. Risk factors for acute GVHD identified in this analysis will be important to consider in future clinical trials. Dose reductions of MTX and CSP are necessary in a large number of patients because of toxicity and, therefore, modified dose schedules early after marrow transplant or regimens incorporating potentially less toxic agents need to be studied. Because the treatment of established acute GVHD is less than optimal, the risks and benefits of dose reduction for both CSP and MTX, which may result in the development of acute GVHD, should be carefully considered.

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