British Journal of Haematology, 1988, 70, 403–409

The mixed epidermal cell lymphocyte-reaction is the most predictive factor of acute graft-versus-host disease in bone marrow graft recipients

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Received 27 May 1988; accepted for publication 6 July 1988

Summary. Risk factors for acute graft-versus-host disease (GvHD) remain controversial. We performed uni- and multivariate statistical analyses on a series of 37 patients receiving a non-depleted allogeneic bone marrow transplant from an HLA-identical sibling donor for a haematological malignancy, in order to identify risk factors for GvHD. Three factors were associated with development of moderate to severe GvHD: a positive mixed epidermal cell-lymphocyte reaction (MECLR) between donor and recipient, previous pregnancies in female donors and chronic myeloid leukaemia diagnosis. The MECLR was the most important predictive factor, selected in first rank by the stepwise linear discriminant analysis. Combining these three prognostic factors in the jackknifed procedure, we could correctly classify 33/37 patients in two groups: grade 0–I versus grade II–IV acute GvHD. These results should apply to donor selection and to predict donor/recipient pairs at high risk of GvHD who might benefit of bone marrow T-cell depletion and those at low risk for whom depletion could be avoided.

Graft-versus-host disease (GvHD) remains a major cause of morbidity and mortality in patients receiving bone marrow grafts from HLA-identical siblings. Previous experiments in mice have shown that this reaction can be induced by incompatibilities for minor histocompatibility antigens and that T lymphocytes are the effector cells of this disease (Mathé *et al*, 1979; Korngold & Sprent, 1983). In the past few years, several attempts to prevent this reaction have been performed in man by depleting mature T lymphocytes from the donor marrow inoculum. Incidence of GvHD is effectively decreased in patients grafted with T-cell depleted marrow. However, numerous severe complications are seen in these patients, especially graft failures and rejections and increased incidence of leukaemia relapse (Martin *et al*, 1985; Mitsuyasu *et al*, 1986; Maraninchi *et al*, 1987). It would therefore be of major importance to predict donor/recipient pairs at high risk of GvHD who might benefit from bone marrow depletion and those at low risk for whom depletion should be avoided.

We have shown in preliminary studies that mixed epidermal cell–lymphocyte reaction (MECLR) can detect incompatibilities between HLA-identical mixed lymphocyte reaction (MLR) negative donors and recipients and that the intensity of the proliferation observed, before graft, in MECLR is correlated with later incidence of acute and chronic GvHD (Bagot *et al*, 1986a, b). In a series of 37 leukaemia patients grafted with non-depleted marrow from an HLA-identical sibling, we evaluated eight factors for their influence on acute GvHD, in order to assess the value of the MECLR among other possible prognostic factors. Statistical analyses clearly showed that three factors were associated with significantly increased risk of acute GvHD: high MECLR cpm/MLR cpm index values, which appeared as the most important risk factor, previous pregnancies in female donors and chronic myeloid leukaemia diagnosis. Combining these three prognostic factors, with coefficients calculated according to their importance, we could correctly classify 33/37 patients in two groups: absent–mild versus moderate–severe GvHD.

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PATIENTS AND METHODS

Patients. Included in this analysis were 35 patients from the same institution and two additional patients from another institution who received bone marrow transplants between 1 January 1984 and 31 October 1986. All patients were in complete remission from acute leukaemia or in chronic phase of chronic myeloid leukaemia (CML). They received a non-depleted allogeneic bone marrow transplant from an HLA-identical sibling donor for a haematological malignancy and survived more than 30 d with evidence of engraftment. Conditioning regimen consisted of cyclophosphamide, 60 mg/kg of body weight on days 5 and 4 before grafting, and total body irradiation on day 1 before grafting (10 Gy *in toto* with lung shielding above 8 Gy). In order to minimize GvHD, patients received post-transplantation methotrexate, 15 mg per square metre, on day 1 after transplantation and 10 mg per square metre on days 3, 6 and 11, and then every week until day 102. The grade of severity of acute GvHD was evaluated according to usual criteria by clinicians not aware of the results of the MECLR (Thomas *et al*, 1975). During this interval, 82 additional patients were grafted but excluded from this study for several reasons: patients younger than 12 years, patients grafted with syngeneic bone marrow, recipients of T-cell depleted bone marrow, patients with acute phase of CML or acute leukaemia not in complete remission and patients dying less than 30 d post-transplant who could not be evaluated for acute GvHD. Patients grafted after October 1986 were not included in this study because they received a combination of methotrexate and cyclosporin. Data were analysed as at 31 March 1988.

Mixed epidermal cell–lymphocyte reactions. Epidermal cells (EC) were obtained from the recipients a few weeks before grafting by trypsin disaggregation of suction blister tops as already described (Bagot *et al*, 1985). Peripheral blood lymphocytes (PBL) from recipients and donors were isolated from heparinized blood samples by Ficoll–Hypaque density gradient centrifugation. Cultures were conducted before grafting in 0·2 ml round-bottomed microtitre plates by mixing 10^5 donor PBL and 10^5 recipient stimulator cells (either EC for MECLR or 2600 rads irradiated PBL for mixed lymphocyte reactions (MLR)). Medium consisted of RPMI 1640 (Eurobio, Paris, France) supplemented with 2 mM L-glutamine, penicillin (400 U/ml), streptomycin (500 μg/ml) and 5% pooled heat-inactivated human AB serum. All cultures were set up in sextuplicate and maintained at 37°C in a humid 5% CO_2 atmosphere. Proliferation was assessed by 18 h tritiated thymidine incorporation after 6 d. For each donor/recipient pair, the index of the mean counts per minute (cpm) in MECLR divided by the mean cpm in MLR was calculated.

Statistical methods. To analyse the risk of GvHD occurrence, we compared each independent variable distribution between the two groups with absent–mild (grade 0–I) or moderate–severe (grade II–IV) acute GvHD by non-parametric methods (Siegel, 1956). This comparison was performed for qualitative independent variables using a chi-squared test (with Yates correction when one calculated frequency was lower than 5 and Fisher exact test when one observed frequency was lower than or equal to 2). For quantitative independent variables, the comparison was performed using Wilcoxon rank test. Results were presented in terms of relative risk of disease occurrence, that is the ratio of the probability to develop the disease in one group, divided by the probability to develop it in the other group (Jenicek & Cleroux, 1982). For that purpose, quantitative variables, such as the MECLR/MLR index, were dichotomized. Limits were chosen either on logical grounds or in order to separate the whole sample in groups of approximately equal size. To take into account possible relationships between these parameters, multivariate statistical analysis was performed to derive which combination of variables could predict the probability of incidence of GvHD. Stepwise multiple linear discrimination (Morrison, 1976) was used to determine the best linear combination of independent variables which allows the correct classification of the patients in their own group. Weights to apply to each selected variable were determined on the whole sample. To classify one patient, coefficients were recalculated from the data of the initial sample, omitting the data of this individual. This jackknifed procedure allows classification without bias, since data of the subject to be classified are not used to determine the linear combination of variables (Lachenbruch & Mickey, 1968). Multiple logistic regression, which allows the derivation of significant risk factors associated with the probability of GvHD occurrence through a model different from multiple linear discrimination, was also used to check the stability of risk factors according to the model associated with this probability of GvHD occurrence. All these analyses were performed using programs from the BMDP software (Dixon, 1983).

RESULTS

Clinical characteristics

Characteristics of patient and donor populations, and clinical outcome in the 37 patients are given in Table I. The median age of the patients was 23 (13–43) years; there were 23 males and 14 females; 16 had acute lymphocytic leukaemia (ALL), 11 acute myeloid leukaemia (AML) and 10 chronic myeloid leukaemia (CML); 25 patients received sex-mismatched grafts (16 males and nine females); 20 had grade 0–I acute GvHD and 17 had grade II–IV acute GvHD. The median age of the donors was 23 (14–43). There were 16 males and 21 female donors; 10 of the female donors had been pregnant.

Risk factors in univariate analysis

Univariate analysis of the influence of eight factors on the occurrence of acute GvHD is presented in Table II. Recipient and donor sex, and sex-mismatch between donor and recipient were not related to the incidence of GvHD. Increasing donor and recipient ages, donor previous pregnancies and CML diagnosis were correlated with GvHD. The most predictive factor was the MECLR cpm/MLR cpm index, which was significantly higher in the group of patients with grade II–IV GvHD. Relative risks associated with evidenced prognostic factors are shown in Table III. High index values

Table I. Characteristics of the study population and clinical outcome of the recipients

UPN*	Diagnosis†	Patient age/sex	Donor age/sex (no. of previous pregnancies)	MECLR cpm / MLR cpm	Acute GvHD clinical stage: Skin	Acute GvHD clinical stage: Overall	Chronic GvHD clinical stage‡	Clinical outcome§
125	AML	22/F	20/M	3·70	+++	II	1	Death on day 190; relapse
127	ALL	18/F	20/M	2·64	0	0	0	Alive, day 1504
128	ALL	28/M	38/M	0·49	0	0	0	Alive, day 1497
131	ALL	27/M	23/F(0)	0·27	0	0	NE	Death on day 95; CMV pneumonitis
132	ALL	22/F	20/F(0)	0·50	0	0	0	Death on day 1181; relapse
136	AML	35/M	39/F(2)	3·73	++	II	NE	Death on day 100; GvHD, CMV pneumonitis
138	CML	28/M	25/F(1)	4·52	+	II	2	Death on day 142; GvHD, intestinal bleeding
140	AML	33/F	36/M	0·86	0	0	1	Death on day 266; relapse
143	ALL	13/M	18/F(0)	0·31	0	0	0	Alive, day 1385
145	AML	28/M	25/M	2·45	0	0	2	Alive, day 1378
147	ALL	17/M	15/F(0)	2·01	++	I	2	Alive, day 1364
148	CML	33/M	43/M	1·50	+	III	1	Death on day 112; viral colitis
151	ALL	21/M	22/F(0)	0·48	0	0	2	Death on day 810; HIV infection
156	AML	24/F	25/F(1)	3·78	0	0	0	Death on day 849; relapse
157	CML	20/F	24/M	4·72	+	I	2	Alive, day 1266
160	ALL	21/M	35/F(5)	1·16	++	II	NE	Death on day 41; GvHD, aspergillosis
162	AML	19/M	20/M	10·11	++	II	2	Death on day 114; toxoplasmosis
165	CML	31/F	21/M	1·52	+	I	2	Alive, day 1182
170	CML	33/M	34/M	9·00	++	II	1	Alive, day 1126
180	AML	16/M	14/F(0)	4·13	++	II	2	Death on day 177; GvHD, aspergillosis
181	CML	27/F	31/M	2·34	++	II	2	Alive, day 1019
186	ALL	19/M	19/F(0)	0·81	0	0	0	Alive, day 993
189	AML	23/M	35/F(3)	1·10	++	III	2	Death on day 186; relapse, pneumocystosis
197	ALL	39/M	35/F(6)	4·52	+++	IV	NE	Death on day 80; GvHD
204	AML	19/M	19/F(0)	0·39	0	0	0	Death on day 465; infectious pneumonitis
205	ALL	23/F	38/M	1·88	++	I	1	Alive, day 841
206	ALL	20/M	23/F(0)	0·82	+	I	0	Death on day 138; relapse
207	CML	23/F	19/F(0)	5·07	++	II	1	Alive, day 803
216	ALL	26/F	16/F(0)	2·40	0	0	0	Alive, day 695
218	ALL	42/M	35/F(2)	2·30	+++	III	NE	Death on day 45; GvHD, CMV pneumonitis
221	CML	21/F	23/M	1·45	++	II	2	Alive, day 667
231	ALL	17/M	20/M	1·10	0	0	0	Alive, day 600
234	AML	18/F	19/M	0·92	+	I	0	Alive, day 579
235	CML	29/M	24/F(1)	1·85	++	II	0	Alive, day 572
240	ALL	43/F	29/F(1)	2·44	++	II	0	Death on day 479; relapse
a	CML	29/M	32/M	4·71	++	II	2	Alive, day 1105
b	AML	24/M	21/F(1)	2·60	0	0	0	Alive, day 736

* UPN denotes Unique Patient Number (Bone Marrow Transplantation Unit, Hôpital Henri Mondor); a, b: two additional patients from another institution.

† ALL, acute lymphocytic leukaemia; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia.

‡ NE: not evaluable; 1: limited chronic GvHD; 2: extensive chronic GvHD.

§ CMV denotes cytomegalovirus; HIV, human immunodeficiency virus.

increased the risk of acute GvHD whereas low index values appeared to be a factor of better prognosis. In spite of the relatively low number of female donors, a high relative risk of severe GvHD was observed when they had experienced at least one pregnancy. 80% of the patients who received bone marrow from an HLA-identical sister with previous pregnancies developed severe acute GvHD, compared to 18% of the patients with a female donor with no previous pregnancy.

Risk factors in multivariate analysis

The eight factors were re-examined in a stepwise multiple linear discrimination analysis. This method determined first rank of selection for the MECLR cpm/MLR cpm value, second rank for donor previous pregnancies, and third for CML diagnosis. The same selection was found when using either the MECLR index values or classes of this variable according to the limits 1 and 3. Table IV shows the *F* statistics used to

Table II. Univariate analysis of the influence of eight factors on the occurrence of acute GvHD

	Acute GvHD		
	Grade 0–I ($n=20$)	Grade II–IV ($n=17$)	
Recipient age (mean ± SD)	22·4 ± 5·2	28·4 ± 8·1	$P<0·02$
Donor age (mean ± SD)	23·1 ± 6·7	29·0 ± 8·1	$P<0·04$
$\frac{\text{MECLR cpm}}{\text{MLR cpm}}$ (mean ± SD)	1·55 ± 1·24	3·74 ± 2·57	$P<0·003$
Recipient sex			
Male (%)	55	71	
Female (%)	45	29	$P>0·50$
Donor sex			
Male (%)	45	41	
Female (%)	55	59	$P>0·90$
Sex mismatch			
Yes (%)	70	65	
No (%)	30	35	$P>0·90$
Diagnosis			
AML (%)	30	29	
ALL (%)	60	24	$P<0·03$
CML (%)	10	47	
	($n=11$)	($n=10$)	
Donor previous pregnancies			
Yes (%)	18	80	
No (%)	82	20	$P<0·02$

select the most important factors in a stepwise manner, using either interval or categorical variables for the MECLR/MLR index. Jackknifed procedure allowed correct classification of 16/20 patients in the group with grade 0–I GvHD and 17/17 patients in the group with grade II–IV GvHD. Table V gives the *F* statistics levels for unselected factors after the last step as compared to the same statistics at the first step when no variable was yet selected. It clearly shows that donor and patient ages no longer remained significant when donor parity was taken into account. Their influence was therefore related to the correlation of increasing donor and recipient ages with higher probability of previous pregnancy experience in female donors. Stepwise logistic regression selected the same three factors when using either categorical or interval factors for quantitative variables, except that donor age was entered in one analysis and removed as soon as donor previous pregnancy was selected.

Clinical implications

Taking into account the linear function selected by the stepwise multiple discriminant analysis, individuals could be classified into 12 groups (three for MECLR/MLR values according to the limits 1 and 3, two for donor pregnancy experience and two for CML or acute leukaemia diagnosis). The model allowed calculation of the theoretical probability of severe acute GvHD occurrence in each group of patients. These probabilities could be compared to the observed proportions of severe acute GvHD in our sample of 37 subjects (Table VI). As expected, a good correlation was observed between these probabilities and the corresponding proportions. A MECLR/MLR index lower than 1 was of good prognosis in acute leukaemia patients grafted with bone marrow from a male donor or a female donor with no previous pregnancy (0/10 acute GvHD). However, no individual of our sample with a MECLR/MLR index lower than 1 was observed in the three other groups (Table VI). Therefore, the predictive value of the model cannot be assessed with any confidence in these conditions. In acute leukaemia patients with an index within the range 1–3, a graft from a male donor or a female donor with no previous pregnancy was of good prognosis (0/6 GvHD), whereas a graft from a female donor with at least one pregnancy was at high risk (4/5 GvHD). In acute leukaemia patients, an MECLR/MLR index higher than 3 was of poor prognosis whatever the donor parity (5/6 GvHD). Finally, CML patients with an index greater than 1 were at high risk of GvHD, whatever the donor parity (8/10 GvHD).

DISCUSSION

Uni- and multivariate analyses of our group of 37 patients grafted with non-depleted marrow from an HLA-identical sibling show that three factors are associated with increased risk of GvHD: MECLR/MLR value, previous pregnancies of female donors and diagnosis of the haematological malignancy. It has been shown that epidermal cells are more potent than PBL for the induction of allogeneic and viral-specific proliferative responses (Bagot *et al*, 1985) and that the MECLR can detect incompatibilities between HLA-identical MLR negative siblings (Bagot *et al*, 1986b). Moreover, the intensity of the proliferation observed in MECLR is correlated with later incidence of acute and chronic GvHD (Bagot *et al*, 1986a). In this previous study, no precise criteria for patient selection had been defined, so that 19 patients from the present study were mixed with patients treated with T-cell depleted bone marrow and only univariate analysis of the MECLR/MLR index had been performed. The aim of the present study was to evaluate the influence of several risk factors (especially the MECLR/MLR index) on GvHD occurrence on a series of patients treated in strictly standardized conditions, resulting in a relatively small number of cases. Anyway, the small sample size, which might result in a lack of power for risk factor selection, could not jeopardize the validity of selected factors. The MECLR/MLR value thus appears to be the factor selected in first rank. The ability of the MECLR to detect incompatibilities not seen in MLR may be related either to more efficient antigen presentation by dendritic epidermal Langerhans cells and/or to the existence of skin-specific minor histocompatibility antigens, already demonstrated in mice (Steinmuller, 1984). Indeed, preliminary results suggest that the MECLR can be strongly positive between non-apparented HLA-identical MLR negative individuals. Another *in vitro* predictive test has been described, using culture of a skin explant from the recipient with donor PBL previously sensitized by co-culture with irradiated recipient PBL (Vogelsang *et al*, 1985). Histologic changes in the skin-explant are correlated with later incidence of acute

Table III. Relative risk of acute GvHD

Independent variables	Group 1/Group 2	Relative risk (1/2)* mean (95% confidence interval)
Donor previous pregnancies	Yes/No	4·4(1·3–14·8)
MECLR cpm	≧1/<1	Not evaluable
MLR cpm	≧3/<3	2·7(1·2–5·8)
Diagnosis	CML/ALL	3·2(1·2–8·5)
	CML/ALL+AML	2·4(1·1–5·3)
Donor age	>25/≦25	2·6(1·2–5·8)
Recipient age	>28/≦28	2·4(1·1–5·3)

* Relative risk of acute GvHD is the ratio of the probability to develop grade II–IV GvHD in one group, divided by the probability to develop it in the other group.

Table IV. Stepwise selection factors by multiple linear discriminant analysis

Factor	Type	Step	*F* to enter*	Global *F*†
MECLR/MLR	Interval	1	11·51	11·51
Donor previous pregnancies	Categorical	2	8·24	11·06
CML diagnosis	Categorical	3	7·02	11·02

* *F* to enter: Statistics measuring the intensity of the relationship between the selected factor at a given step and acute GvHD occurrence taking into account previously selected factors.

† Global *F*: Statistics measuring the intensity of the relationship between all selected factors at a given step and acute GvHD (model adequacy).

Table V. Unselected factors by stepwise multiple linear discriminant analysis

		F to enter*	
Factor	Type	At the first step	After the last step
Donor age	Interval	5·92	2·38
Patient age	Interval	7·83	0·87
Donor sex	Categorical	0·05	0·00
Patient sex	Categorical	0·92	0·76
Sex-mismatch	Categorical	0·11	0·07
ALL diagnosis	Categorical	5·44	0·02

* *F* to enter: Statistics measuring the intensity of the relationship between unselected factors and acute GvHD occurrence, either before any factor selection (at the first step) or when the stepwise selection procedure stops (after the last step).

Table VI. Multivariate analysis: clinical implications

			Acute GvHD (grade II–IV)	
MECLR cpm / MLR cpm	Diagnosis	Donor previous pregnancies	Predicted probability	Observed proportion (sample size)
<1	AML+ALL	No	0·024	0·00(10)
	CML	No	0·231	—
	AML+ALL	Yes	0·289	—
	CML	Yes	0·832	—
≧1 and <3	AML+ALL	No	0·155	0·00(6)
	CML	No	0·690	0·75(4)
	AML+ALL	Yes	0·750	0·80(5)
	CML	Yes	0·973	1·00(1)
≧3	AML+ALL	No	0·599	1·00(3)
	CML	No	0·948	0·75(4)
	AML+ALL	Yes	0·961	0·67(3)
	CML	Yes	0·997	1·00(1)

GvHD. The MECLR is, however, easier to perform and does not require histological examination.

Risk factors for acute GvHD remain controversial. Previous studies have found several predictive factors: female donor sex (Bortin *et al*, 1981; Gluckman *et al*, 1981; Storb *et al*, 1977), increasing recipient age (Bortin *et al*, 1981; Gluckman *et al*, 1981; Bross *et al*, 1984), and sex-mismatch between donor and recipient (Bortin *et al*, 1981; Bross *et al*, 1984; Storb *et al*, 1977). All these studies, except Bross *et al*, referred to series of patients grafted for aplastic anaemia. Moreover, when using multivariate analysis, sex-mismatch influence disappeared (Bortin *et al*, 1981; Storb *et al*, 1983) as well as female donor sex (Storb *et al*, 1977) or increasing recipient age (Bortin *et al*, 1981). Influence of sex-mismatch was not observed in several other studies (Gluckman *et al*, 1981; Storb *et al*, 1983; Atkinson *et al*, 1986). In the study of Atkinson *et al* (1986), increasing patient and donor ages and

female donor sex were found to be risk factors in univariate analysis. However, multivariate analysis demonstrated that influence of patient age was due to its relationship with donor age, that the effect of donor age was restricted to female donors and that a strong correlation could be found between increasing female donor age and donor parity. A significant relationship was found between the severity of acute GvHD and the number of pregnancies (including abortions and miscarriages). In a recent study, the strongest predictive factor of GvHD was the use of previously pregnant or transfused female donors for male recipients (Gale *et al*, 1987). In this latter study, the influence of patient age disappeared in multivariate analysis. Very similar results were observed in our series of patients, since donor previous pregnancies was the risk factor selected in second rank. Moreover, we also found that influence of recipient and donor ages completely disappeared when donor parity was entered. The influence of female to male graft was not found in our sample, which might be due to the small sample size. Influence of donor parity may be explained by the fact that previous pregnancies can sensitize female donors against minor histocompatibility antigens, as already known (Tekolf & Shaw, 1983). Acute GvHD is thought to be induced by donor T-cell cytotoxic activity directed against recipient minor antigens (Korngold & Sprent, 1983). In this regard, it is interesting to demonstrate that the most significantly predictive factors for this reaction are, on the one hand, the MECLR/MLR index, which may be related to the degree of non-HLA histocompatibility antigen disparity between HLA-identical siblings, and, on the other hand, donor previous pregnancies which are known to prime female donors *in vivo* against minor antigens.

Influence of the underlying disease, with increased risk of acute GvHD in patients with CML, compared to the patients with acute leukaemia has recently been reported (Beelen *et al*, 1987). In this series, 9/9 male patients with CML receiving transplants from female donors developed GvHD, which is also true for our two patients in this situation. It is, however, remarkable to notice that in our series of CML patients, none had a MECLR/MLR index lower than 1, which was proved to be of good prognosis in acute leukaemia patients. The influence of the diagnosis was not found in a large study recently published (Gale *et al*, 1987). Nevertheless, this factor was clearly in interaction with the MECLR values, since, as shown in Table VI, none of our CML patients had an index lower than 1. According to our small sample size, this could be due to random selection.

Clinical implications of these analyses should be useful for donor selection and patient management. When possible, male donors or non-parous female donors should be chosen rather than female donors with previous pregnancies, especially for acute leukaemia patients with a MECLR/MLR index lower than 3. Incidence of GvHD following bone marrow transplantation with T-cell depleted marrow inoculum is considerably decreased. However, graft rejections and leukaemia relapses are very frequent in these patients, leading to severe prognosis (Martin *et al*, 1985; Mitsuyasu *et al*, 1986; Maraninchi *et al*, 1987). These observations suggest that T-cell depletion should actually be used only for patients with a high risk of GvHD. It is therefore of major importance to develop a model predicting the probability of severe GvHD occurrence. The most interesting point of our data arises from the demonstration of the great predictive value of the MECLR/MLR index, which appears to be the most important predictive factor, selected in first rank by the stepwise linear discriminant analysis. Our analyses also confirm the higher incidence of GvHD associated with donor previous pregnancies and CML diagnosis reported in other recent studies (Atkinson *et al*, 1986; Beelen *et al*, 1987; Gale *et al*, 1987). It is also of interest to note that jackknifed procedure, using the three risk factors, allowed us to correctly classify 33/37 patients in the two groups absent–mild versus moderate–severe GvHD. The possible use of this method, allowing one to precisely evaluate the risk for a recipient to develop GvHD, however deserves further evaluation of its applicability on greater series of patients receiving association of cyclosporin and methotrexate as GvHD prophylaxis. Moreover, a multicentre study is currently in progress in order to test the possible application of these findings in larger series as well as in other bone marrow graft units.

ACKNOWLEDGMENT

This work was supported by grants from the Fonds d'Etudes du Corps Médical des Hôpitaux de Paris.

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