Value of Day 100 Screening Studies for Predicting the Development of Chronic Graft-Versus-Host Disease After Allogeneic Bone Marrow Transplantation

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We prospectively evaluated 169 patients with a number of screening studies performed between 71 to 121 days after allogeneic marrow transplantation to detect the development of chronic graft-versus-host disease (GVHD). Group 1 patients (n = 78) were asymptomatic and had normal physical examinations at the time of screening and, with a minimum of 8 years follow-up, have not developed chronic GVHD. Group 2 patients (n = 38) had signs and symptoms of chronic GVHD at time of testing. Group 3 patients (n = 53) were similar to those in group 1 in having no clinically evident GVHD at the time of testing, but later developed clinical chronic GVHD. Using time to an event analysis, we compared patients in groups 1 and 3 to determine which of 17 clinical and laboratory factors evaluated at screening accurately predicted the development of subsequent chronic GVHD. Multivariate analyses showed several factors to have independent predictive value. In the first model, results of oral biopsies were excluded since these were done only in one half of the patients. Predictive factors in this analysis included: (1) histologic findings of GVHD on skin biopsy, relative risk 3.23 (95% confidence interval 1.75 to 5.94), $P = .0002$; and (2) history of grade II through IV acute GVHD, relative risk 3.12 (95% confidence interval 1.72 to 5.64), $P = .0002$. When oral biopsy results were included in the second model, independent risk factors included: (1) histologic findings of GVHD on skin biopsy, relative risk 5.96 (95% confidence interval 1.95 to 18.19), $P = .0017$; and (2) low numbers of immunoglobulin A (IgA)-bearing plasma cells detected by direct immunofluorescence in salivary gland areas on oral biopsy, relative risk 11.53 (95% confidence interval 2.51 to 52.03), $P = .0017$. Our study demonstrates the value of day 100 screening studies for predicting subsequent development of clinical chronic GVHD.

A MAJOR LATE complication of allogeneic marrow transplantation is the development of chronic graft-versus-host disease (GVHD). The clinical manifestations of chronic GVHD are protean and resemble the features of several autoimmune diseases.[1,2] Untreated, less than 20% of patients with extensive chronic GVHD survive with Karnofsky scores ≥70%.[3] Because immunosuppressive therapy has greatly decreased morbidity and mortality,[3-5] it would be valuable to detect the onset of chronic GVHD before clinical deterioration. We evaluated prospectively 169 allogeneic marrow transplant recipients for chronic GVHD with screening studies performed at 70 to 121 days posttransplant. This report describes the value of certain screening tests for predicting the subsequent development of clinical chronic GVHD.

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MATERIALS AND METHODS

One hundred sixty-nine patients receiving allogeneic marrow grafts entered the study from 1977 to 1980. All patients received intravenous methotrexate administered intermittently for 102 days as prophylaxis for acute GVHD.[6] The preparatory regimen for patients with aplastic anemia consisted of cyclophosphamide, 50 mg/kg on each of 4 successive days.[7] Patients with hematologic malignancies received high-dose cyclophosphamide or other chemotherapy regimens, followed by total body irradiation given as a single 10-Gy dose or in fractions to a total of 12.0 to 17.5 Gy.[8,9] Studies to screen for chronic GVHD were performed at a median of 89 (range 71 to 121) days posttransplant. Screening studies included physical examination, complete blood counts, liver function tests, skin and lip biopsies, and Schirmer's testing for lacrimal function. In general, children less than 6 years old did not have oral biopsies or Schirmer's tests performed. Each skin and oral labial biopsy contained two portions. The portion fixed in formalin was evaluated by light microscopy with multiple hematoxylin and eosin stained serial sections.[3,10] Histologic criteria for GVHD were lymphocytic infiltration of epithelium (epidermis, mucosa, or salivary gland duct) with individual epithelial cell necrosis (apoptosis).[10,11] Biopsies with these features were scored as positive regardless of whether the overall patterns were more consistent with acute or chronic GVHD. Direct immunofluorescent microscopic examination of coded skin biopsies was done independently by two observers (K.M.S. and C.B.) and evaluated the degree of immunoglobulin G (IgG), IgM, or complement deposition in the basement membranes, blood vessels, and cytoid bodies.[12,13] Immunofluorescent staining was graded as ± (0.5, equivocal), + (1.0, mild), ++ (2.0, moderate), and +++ (3.0, strong) intensity of immunofluorescence. A mean score from both observers of ≥1.5 was regarded as a positive finding. Direct immunofluorescent microscopic examination of coded oral biopsies was performed by two observers (K.M.S. and T.M.) who evaluated the degree of IgG, IgM, IgA complement, or fibrinogen deposition in basement membrane, intercellular area, cytoid bodies, periductal, and immunocompetent areas. Additionally, numbers of IgG-, IgM-, and IgA-bearing plasma cells in salivary gland areas were enumerated since we previously showed disordered salivary Ig secretion in patients with chronic GVHD.[14] For these studies, the number of

immunocompetent cells (surface Ig-bearing plasma cells in salivary gland areas) was graded as follows: score 0 = mean of ≤1 immunocompetent cell per high power field; score 1 = mean of 2 to 5 immunocompetent cells per high power field; score 2 = mean of 6 to 10 immunocompetent cells per high power field; score 3 = mean of 11 to 20 immunocompetent cells per high power field; and score 4 = mean of >20 immunocompetent cells per high power field. Forty high power fields were counted in each specimen. If the specimen contained minor salivary glands in less than 10 high power fields, then it was considered as being insufficient for analysis. For statistical analyses, a score of 0 or 1 was considered a negative test, while a score of ≥2 was considered a positive finding.

The predictive value of the screening studies for the subsequent development of chronic GVHD was examined using univariate and multivariate time to an event analyses. Age as a predictive factor was analyzed as a continuous variable. Chi-squared comparisons and log rank tests for censored observations were used in univariate analyses. Cause-specific hazards were analyzed by proportional hazards regression models.[15] The step-up proportional hazards technique was used to construct the multivariate models. The relative risk of chronic GVHD in the proportional hazards model was the instantaneous relative risk of developing chronic GVHD for those with the trait under consideration compared with those without the trait adjusted for other variables in the model. Tests of significance of the relative risks were given as two-sided *P* values.

RESULTS

Demographic data are listed in Table 1. Three groups of patients were defined. Group 1 patients (n = 78) were asymptomatic, had normal physical examinations at time of screening, and never developed subsequent chronic GVHD (minimum follow-up 8 years). Group 2 patients (n = 38) had symptoms and signs of chronic GVHD at the time of testing. Group 3 patients (n = 53) were asymptomatic and had normal physical examinations at time of screening, similar to group 1 patients, but later developed signs of clinical chronic GVHD, at a median of 218 days posttransplant (range 109 to 2,313 days).

Table 2 shows results of 17 individual screening studies, arranged according to patient group. Positive screening tests were noted not only in group 2 patients, but also in apparently normal (by physical examination) patients who subsequently developed chronic GVHD (group 3). The value of these screening tests for predicting subsequent development of chronic GVHD in the group 3 patients was studied using univariate and multivariate analyses.

In the univariate analysis (Table 3), nine screening factors that had a significant predictive value included: histologic findings of GVHD on skin or oral biopsy; complement deposition in the dermal basement zone; positive IgM staining of cytoid bodies on oral biopsy; low numbers of IgA-bearing plasma cells in salivary gland areas of labial tissue; bilirubin values greater than 1.0 mg/dL; previous grade II through IV acute GVHD; prednisone therapy after day 50 posttransplant; and older patient age, analyzed as a continuous variable. There was no correlation between finding GVHD on oral biopsy and the finding of low numbers of IgA-bearing plasma cells in salivary gland areas of labial tissue. Because our previous study had indicated that buffy coat administration is a risk factor for subsequent development of chronic GVHD,[16] we also examined this issue in the 37 patients transplanted for aplastic anemia who had normal screening studies. Of these 37 patients, 17 received buffy coat infusions. There was a suggestion that buffy coat administration was a risk factor for subsequent development of chronic GVHD, although not statistically significant ($P = .065$). However, the sample size of the study may have been too small to fully evaluate this question, since our most recent study of 165 patients transplanted for aplastic anemia continues to confirm the association of buffy coat administration and subsequent development of chronic GVHD.[17]

Table 1. Patient Characteristics

	Group 1 (n = 78)	Group 2 (n = 38)	Group 3 (n = 53)
Diagnosis			
ANL	28	16	19
ALL	31	10	11
CML	1	1	3
Lymphoma	0	1	1
Aplastic anemia	18	10	19
No. of patients			
<10 years old	15	1	5
10-30 years old	53	32	39
>30 years old	10	5	9
Median (range) age	16 (2-42)	20 (9-39)	24 (3-52)
Mean age	18	22	23
Sex (male/female)	44/34	23/15	31/22
HLA-nonidentical donor (%)	9 (12)	4 (11)	6 (11)
Grade of acute GVHD			
0 (none)	47	5	20
I (mild)	14	10	11
II (moderate)	11	8	7
III (severe)	6	14	15
IV (life-threatening)	0	1	0
Total grade II-IV (%)	17 (22)	23 (61)	22 (42)

Abbreviations: ANL, acute nonlymphocytic leukemia; ALL, acute lymphocytic leukemia; CML, chronic myelogenous leukemia.

In the multivariate analysis (Table 4), significant factors from the univariate analysis were considered to determine independent risk factors for developing chronic GVHD. In the first model examined, oral biopsy results were not included since these biopsies were obtained only in approximately half of the group 1 and 3 patients due to patient age considerations. The independent risk factors determined from the analysis were: (1) histologic findings of GVHD on skin biopsy; and (2) previous history of grade II through IV acute GVHD. When results of examination of oral biopsies were added in the second model, independent risk factors included: (1) histologic findings of GVHD on skin biopsy; and (2) direct immunofluorescent microscopic findings of low numbers of IgA-bearing plasma cells in salivary gland areas on oral biopsy (Table 4). A history of grade II through IV acute GVHD was not an independently significant risk factor in this second proportional hazards model.

Figures 1 and 2 present Kaplan-Meier estimates of the

Table 2. Day 100 Screening Studies Performed

Screening Test	Group 1 (n = 78) Studied	Group 1 (n = 78) Positive (%)	Group 2 (n = 38) Studied	Group 2 (n = 38) Positive (%)	Group 3 (n = 53) Studied	Group 3 (n = 53) Positive (%)
1. Skin biopsy–LM	71	7 (10)	32	26 (81)	46	17 (37)
2. Skin biopsy–DIF						
Basement membrane						
IgG	69	7 (10)	32	3 (9)	46	6 (13)
IgM	71	13 (18)	32	8 (25)	45	3 (7)
C'3	68	9 (13)	33	12 (36)	43	11 (26)
Any positive	71	20 (28)	33	16 (48)	46	16 (35)
Any except C'3	71	17 (24)	33	11 (33)	46	9 (20)
3. Skin biopsy–DIF						
Cytoid bodies						
IgG	69	3 (4)	32	5 (16)	46	2 (4)
IgM	71	7 (10)	32	5 (16)	45	4 (9)
C'3	68	2 (3)	33	5 (15)	43	2 (5)
Any positive	71	9 (13)	33	10 (30)	46	6 (13)
Any except C'3	71	9 (13)	33	8 (24)	46	6 (13)
4. Skin biopsy–DIF						
Vasculitis						
IgG	69	0 (0)	32	0 (0)	46	1 (2)
IgM	71	0 (0)	32	0 (0)	45	0 (0)
C'3	68	2 (3)	33	1 (3)	43	0 (0)
Any positive	71	2 (3)	33	1 (3)	46	1 (2)
Any except C'3	71	0 (0)	33	0 (0)	46	1 (2)
5. Oral biopsy–LM	41	19 (46)	24	18 (75)	21	16 (76)
6. Oral biopsy–DIF						
Basement membrane						
IgG	38	3 (8)	24	1 (4)	20	0 (0)
IgM	37	0 (0)	25	3 (12)	21	0 (0)
IgA	38	3 (8)	25	0 (0)	21	1 (5)
C'3	39	5 (13)	25	2 (8)	21	1 (5)
Fibrinogen	37	8 (22)	25	10 (40)	20	7 (35)
Any positive	39	15 (39)	25	12 (48)	21	8 (38)
Any except C'3	39	11 (28)	25	12 (48)	21	7 (33)
7. Oral biopsy–DIF						
Intercellular area						
IgG	39	2 (5)	24	5 (21)	20	0 (0)
IgM	37	2 (5)	25	0 (0)	21	0 (0)
IgA	38	1 (3)	25	2 (8)	21	1 (5)
C'3	39	4 (10)	25	2 (8)	21	0 (0)
Fibrinogen	36	0 (0)	25	0 (0)	20	2 (10)
Any positive	39	7 (18)	25	7 (28)	21	2 (10)
Any except C'3	39	5 (13)	25	7 (28)	21	2 (10)
8. Oral biopsy–DIF						
Cytoid bodies						
IgG	39	1 (3)	24	5 (21)	20	1 (5)
IgM	37	2 (5)	25	3 (12)	21	4 (19)
IgA	38	1 (3)	25	2 (8)	21	2 (10)
C'3	40	4 (10)	25	2 (8)	21	1 (5)
Fibrinogen	36	1 (3)	25	2 (8)	20	2 (10)
Any positive	40	6 (15)	25	8 (32)	21	4 (19)
Any except C'3	39	3 (8)	25	8 (32)	21	4 (19)
9. Oral biopsy–DIF						
Periductal area						
IgG	40	7 (18)	22	5 (23)	19	1 (5)
IgM	37	1 (3)	22	0 (0)	20	0 (0)
IgA	39	2 (5)	22	0 (0)	20	1 (5)
C'3	39	3 (8)	22	2 (9)	20	1 (5)
Fibrinogen	37	18 (49)	22	13 (59)	19	5 (26)
Any positive	40	21 (53)	22	14 (64)	20	7 (35)
Any except C'3	40	21 (53)	22	14 (64)	20	6 (30)

(Continued on following page)

Table 2. Day 100 Screening Studies Performed (Cont'd)

Screening Test	Group 1 (n = 78) Studied	Group 1 (n = 78) Positive (%)	Group 2 (n = 38) Studied	Group 2 (n = 38) Positive (%)	Group 3 (n = 53) Studied	Group 3 (n = 53) Positive (%)
10. Oral biopsy–DIF						
Immunocompetence area						
IgG	40	8 (20)	22	4 (18)	19	3 (16)
IgM	37	8 (22)	22	6 (27)	20	2 (10)
IgA	39	23 (59)	22	11 (50)	20	3 (15)
C'3	39	7 (18)	22	3 (14)	21	1 (5)
Fibrinogen	37	0 (0)	22	0 (0)	19	0 (0)
Any positive	40	26 (65)	22	12 (55)	21	8 (38)
Any except C'3	40	25 (63)	22	12 (55)	20	7 (35)
11. Schirmer's ≤10 mm	46	9 (20)	33	21 (64)	36	10 (28)
12. Bilirubin >1.0 mg/dL	78	5 (6)	38	17 (45)	52	13 (25)
13. SGOT >50 IU	78	54 (69)	38	34 (89)	53	36 (68)
14. Alkaline phosphatase >120 IU	78	49 (63)	38	31 (82)	53	33 (62)
15. Prednisone after day 50	78	21 (27)	38	26 (68)	53	27 (51)
16. Acute GVHD ≥ grade II	78	17 (22)	38	23 (61)	53	22 (42)
17. Patient age ≥20 years*	78	33 (42)	38	20 (53)	53	34 (64)

Abbreviations: LM, light microscopy; DIF, direct immunofluorescent microscopy; C'3, complement.

*Age was evaluated as a continuous variable, but for purposes of illustration results are shown as age ≥20 years.

Table 3. Significant Predictive Factors for Chronic GVHD: Univariate Analyses

Predictive Factor	No. of Patients	Probability of Developing Chronic GVHD Within 4 Years (%)	95% Confidence Interval (%)	Significance (*P*)
1. Skin biopsy–LM				
Positive	24	85	67-100	.0001
Negative	93	36	25-47	
2. Skin biopsy–DIF				
Basement membrane				
Positive for C'3	20	62	38-86	.0153
Negative for C'3	91	40	29-51	
3. Oral biopsy–LM				
Positive	35	53	34-71	.0110
Negative	27	20	5-30	
4. Oral biopsy–DIF				
Cytoid bodies				
Positive for IgM	6	78	52-100	.0456
Negative for IgM	52	37	22-51	
5. Oral biopsy–DIF				
Periductal area				
IgA plasma cell score* of <2	33	56	38-74	.0006
IgA plasma cell score of ≥2	26	14	2-22	
6. Total bilirubin ≥1.0 mg/dL				
Yes	18	92	77-100	.0004
No	112	38	29-48	
7. Prednisone after day 50				
Yes	48	78	52-83	.0014
No	83	34	23-45	
8. Acute GVHD grade II-IV				
Yes	39	67	50-84	.0005
No	92	37	26-48	
9. Patient age ≥20 years†				
Yes	67	58	45-71	.0059
No	64	35	20-45	

Patients with normal physical examinations at time of screening (groups 1 and 3) were analyzed for factors predicting subsequent chronic GVHD. Significant risk factors by log rank test are listed; all other day 100 screening studies included in Table 2 were not statistically significant predictive factors. Confidence intervals of 95% were constructed using the Greenwood SEs.

Abbreviations: LM, light microscopy; DIF, direct immunofluorescent microscopy; C'3, complement.

*For an IgA plasma cell score of less than 2, the number of IgA-bearing plasma cells per high power field was ≤5; for a score of ≥2, the number of IgA-bearing plasma cells per high power field was greater than 6 (see Materials and Methods for details).

†Age was analyzed as a continuous variable, but for purposes of illustration results are shown for patients less than 20 years or ≥20 years of age.

Table 4. Significant Predictive Factors for Chronic GVHD: Multivariate Analysis

Predictive Factor	Relative Risk	95% Confidence Interval	Significance (P)
Model 1*			
Positive skin biopsy (LM)	3.23	1.76-5.95	.0002
Grade II-IV acute GVHD	3.12	1.72-5.64	.0002
Model 2*			
Positive skin biopsy (LM)	5.96	1.95-18.19	.0017
Low numbers of IgA plasma cells by DIF on oral biopsy	11.52	2.51-52.03	.0017

Patients with normal physical examinations at the time of screening (groups 1 and 3) were analyzed for factors predicting subsequent chronic GVHD.
Abbreviations: DIF, direct immunofluorescent microscopy; LM, light microscopy.
*Model 1 includes all factors from Table 3 as potential predictors except oral biopsies, whereas Model 2 includes this factor.

probability of development of chronic GVHD in the combined group 1 and 3 patients subgrouped by the predictive factors identified in multivariate analysis. In each model the probability of developing chronic GVHD was high if both risk factors were present at time of screening, although it should be acknowledged that the number of patients studied who were positive for both risk factors was small. Conversely, if both risk factors were absent at time of screening, the probability of developing chronic GVHD was low, particularly if both skin and oral biopsy results were negative (Figure 2).

DISCUSSION

The natural history of clinical extensive chronic GVHD is unfavorable.[3] Pilot clinical trials initially suggested that early institution of combination immunosuppressive therapy was effective in improving disability-free survival.[3] Therefore, in 1977 we began to evaluate prospectively allogeneic marrow transplant patients to determine if screening studies performed around day 100 posttransplant would detect the onset of chronic GVHD before clinical deterioration.

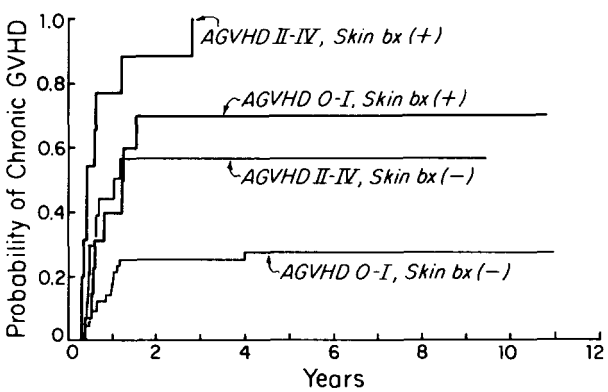

Fig 1. Model 1. Kaplan-Meier product limit estimates of the probability of developing chronic GVHD among patients who were asymptomatic and had normal physical examinations at time of day 100 screening (ie, group 1 and 3 patients). This model excludes results of oral biopsies. The numbers of patients in each group are: (1) acute GVHD (AGVHD) II through IV, skin bx+, n = 10; (2) AGVHD 0 through I, skin bx+, n = 14; (3) AGVHD II through IV, skin bx−, n = 25; (4) AGVHD 0 through I, skin bx−, n = 67.

Univariate analyses in this study identified several risk factors for predicting subsequent development of chronic GVHD in patients who had normal physical examinations at the time of screening. Earlier histologic studies demonstrated the predictive value of oral biopsies[10,18]; however, these studies did not simultaneously evaluate skin biopsies from the patients. Other studies have identified older patient age and a history of acute GVHD as significant risk factors for developing chronic GVHD.[3,19] We conducted multivariate analyses to determine which variables acted as independent risk factors. Our initial analysis showed that a positive skin biopsy using light microscopy and previous history of grade II through IV acute GVHD were significant independent risk factors for developing subsequent chronic GVHD (model 1). When results of oral biopsies were included in this analysis (model 2), we found that a positive oral biopsy using light microscopy was not significant as an independent risk factor. However, the finding of low numbers of IgA-bearing plasma cells in salivary gland areas of labial tissue examined using direct immunofluorescent microscopy was a significant independent risk factor. Moreover, when these results were included in the analysis, a history of grade II through IV acute GVHD was no longer a significant independent predictive factor. The low number of IgA-bearing plasma cells in these patients may contribute to the decreased or absent levels of salivary IgA described in patients with chronic GVHD.[14]

A recent multivariate analysis of risk factors at time of onset of chronic GVHD identified three as independent predictors of death: progressive presentation, histologic findings of GVHD on skin biopsy, and elevated serum bilirubin.[20] Our study was aimed at determining risk factors that would predict subsequent development of chronic GVHD in patients who were clinically normal at time of screening; thus, patients with the progressive onset form of GVHD were not included in these analyses. In a univariate analysis we found that an elevated serum bilirubin at time of screening was a significant risk factor; however, in the multivariate analysis it was not an independent risk factor for subsequent development of chronic GVHD. Our data and that from Johns Hopkins University (Baltimore, MD)[20] would suggest that a

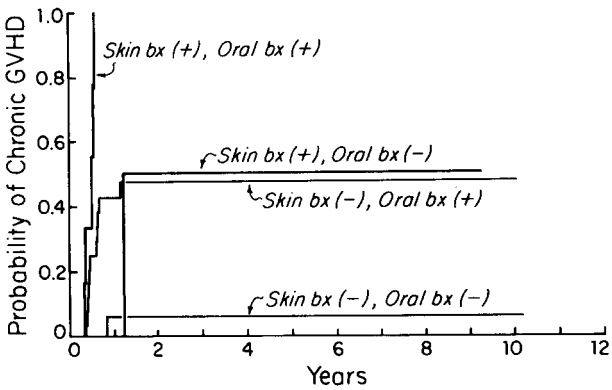

Fig 2. Model 2. Kaplan-Meier product limit estimates of the probability of developing chronic GVHD among group 1 and 3 patients. This model includes results of oral biopsies. Positive results for oral biopsies indicate the finding of decreased numbers of IgA-bearing plasma cells in salivary gland areas. The numbers of patients in each group are: (1) skin bx+, oral bx+, n = 3; (2) skin bx+, oral bx−, n = 6; (3) skin bx−, oral bx+, n = 20; and (4) skin bx−, oral bx−, n = 25.

positive skin biopsy may be used to identify both patients at risk of developing GVHD and of having a poor clinical outcome once clinical GVHD has ensued.

The current study entered patients for prospective evaluation from 1977 to 1980. The long follow-up of these patients allowed for a complete assessment, since the time to onset of development of chronic GVHD was as late as 6.4 years posttransplant. However, a potential drawback to the study is that all of these patients received methotrexate as GVHD prophylaxis. It is conceivable that the findings of our study may not be valid for patients receiving other types of GVHD prophylaxis, such as cyclosporine. However, this is an unlikely possibility since randomized, controlled studies from our institution have shown repeatedly that the combination of methotrexate and cyclosporine, or cyclosporine alone, has not changed the incidence of chronic GVHD compared with the incidence observed in patients receiving only methotrexate.[21-23]

A prospective randomized clinical trial begun in 1980 demonstrated the efficacy of early treatment of patients with extensive chronic GVHD using prednisone therapy.[4] Some patients entering that trial had positive screening tests (skin and lip biopsies both showing chronic GVHD by light microscopy), without symptoms or signs of clinical disease, ie, subclinical extensive chronic GVHD. It was anticipated that treatment of these patients might decrease morbidity and mortality by preventing the clinical progression of extensive chronic GVHD. However, greater than 70% of patients with subclinical disease progressed to clinical chronic GVHD despite immunosuppressive therapy.[4] Furthermore, in the patients in whom chronic GVHD remained subclinical throughout treatment, there was a statistically significant increased probability of relapse of malignant disease.[4] Overall, there was no survival benefit in treating patients with subclinical disease, and we no longer recommend preemptive treatment for patients with hematologic malignancies transplanted in relapse, since a graft-versus-leukemia effect associated with clinical chronic GVHD appears to be an important contribution to long-term disease-free survival.[24,25]

Our data show that extensive evaluation of patients who have normal physical examinations at time of screening cannot be justified. The most cost-effective screening test as a predictor for subsequent development of chronic GVHD is histologic examination of a skin biopsy specimen. Patients with skin biopsies showing histologic evidence of GVHD should be closely monitored for development of clinical GVHD as posttransplant immunosuppression is tapered so that effective therapy can be instituted early in the course of the disease evolution. Additionally, a skin biopsy to screen for subclinical GVHD may also be valuable in patients with nonmalignant diseases (such as aplastic anemia and thalassemia) or for patients with malignant diseases with a low probability of relapse (such as acute nonlymphocytic leukemia in first remission, chronic myelogenous leukemia in chronic phase, and preleukemia). It is possible that these patients identified as having subclinical extensive chronic GVHD might benefit from continuing rather than tapering immunosuppression after day 100.

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REFERENCES

1. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, Hackman R, Tsoi MS, Storb R, Thomas ED: Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med 69:204, 1980

2. Sullivan KM: Acute and chronic graft-versus-host disease in man. Int J Cell Cloning 4:42, 1986

3. Sullivan KM, Shulman HM, Storb R, Weiden PL, Witherspoon RP, McDonald GB, Schubert MM, Atkinson K, Thomas ED: Chronic graft-versus-host disease in 52 patients: Adverse natural course and successful treatment with combination immunosuppression. Blood 57:267, 1981

4. Sullivan KM, Witherspoon RP, Storb R, Weiden P, Flournoy N, Dahlberg S, Deeg HJ, Sanders JE, Doney KC, Appelbaum FR, McGuffin R, McDonald GB, Meyers J, Schubert MM, Gauvreau J, Shulman HM, Sale GE, Anasetti C, Loughran TP, Strom S, Nims J, Thomas ED: 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 72:546, 1988

5. Sullivan KM, Witherspoon RP, Storb R, Deeg HJ, Dahlberg S, Sanders JE, Appelbaum FR, Doney KC, Weiden P, Anasetti C, Loughran TP, Hill R, Shields A, Yee G, Shulman H, Nims J, Strom S, Thomas ED: Alternating-day cyclosporine and prednisone for treatment of high-risk chronic graft-v-host disease. Blood 72:555, 1988

6. Thomas ED, Storb R, Clift RA, Fefer A, Johnson FL, Neiman PE, Lerner KG, Glucksberg H, Buckner CD: Bone-marrow transplantation. N Engl J Med 292:832, 895, 1975

7. Storb R, Thomas ED, Buckner CD, Appelbaum FR, Clift RA, Deeg HJ, Doney K, Hansen JA, Prentice RL, Sanders JE, Stewart

P, Sullivan KM, Witherspoon RP: Marrow transplantation for aplastic anemia. Semin Hematol 21:27, 1984

8. Thomas ED, Buckner CD, Banaji M, Clift RA, Fefer A, Flournoy N, Goodell BW, Hickman RO, Lerner KG, Neiman PE, Sale GE, Sanders JE, Singer J, Stevens M, Storb R, Weiden PL: One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. Blood 49:511, 1977

9. Thomas ED: Marrow transplantation for malignant diseases (Karnofsky Memorial Lecture). J Clin Oncol 1:517, 1983

10. Sale GE, Shulman HM, Schubert MM, Sullivan KM, Kopecky KJ, Hackman RC, Morton TH, Storb R, Thomas ED: Oral and ophthalmic pathology of graft versus host disease in man: Predictive value of the lip biopsy. Hum Pathol 12:1022, 1981

11. Sale GE, Lerner KG, Barker EA, Shulman HM, Thomas ED: The skin biopsy in the diagnosis of acute graft-versus-host disease in man. Am J Pathol 89:621, 1977

12. Saurat JH, Bonnetblanc JM, Gluckman E, DidierJean L, Bussel A, Puissant A: Skin antibodies in bone marrow transplanted patients. Clin Exp Dermatol 1:377, 1976

13. Tsoi M-S, Storb R, Jones E, Weiden PL, Shulman H, Witherspoon R, Atkinson K, Thomas ED: Deposition of IgM and complement at the dermo-epidermal junction in acute and chronic cutaneous graft-vs-host disease in man. J Immunol 120:1485, 1978

14. Izutsu KT, Sullivan KM, Schubert MM, Truelove EL, Shulman HM, Sale GE, Morton TH, Rice JC, Witherspoon RP, Storb R, Thomas ED: Disordered salivary immunoglobulin secretion and sodium transport in human chronic graft-versus-host disease. Transplantation 35:441, 1983

15. Cox DR: Regression models and life-tables (with discussions), Series B. J R Stat Soc 34:187, 1972

16. Storb R, Prentice RL, Sullivan KM, Shulman HM, Deeg HJ, Doney KC, Buckner CD, Clift RA, Witherspoon RP, Appelbaum FR, Sanders JE, Stewart PS, Thomas ED: Predictive factors in chronic graft-versus-host disease in patients with aplastic anemia treated by marrow transplantation from HLA-identical siblings. Ann Intern Med 98:461, 1983

17. Niederwieser D, Pepe M, Storb R, Witherspoon R, Longton G, Sullivan K: Factors predicting chronic graft-versus-host disease and survival after marrow transplantation for aplastic anemia. Bone Marrow Transplant 4:151, 1989

18. Janin-Mercier A, Devergie A, Arrago JP, Brocheriou C, Lemarchand-Venencie F, Rain JD, Gluckman E: Systemic evaluation of Sjogren-like syndrome after bone marrow transplantation in man. Transplantation 43:677, 1987

19. Storb R, Prentice RL, Sullivan KM, Shulman HM, Deeg HJ, Doney KC, Buckner CD, Clift RA, Witherspoon RP, Appelbaum FR, Sanders JE, Stewart PS, Thomas ED: Predictive factors in chronic graft-versus-host disease in patients with aplastic anemia treated by marrow transplantation from HLA-identical siblings. Ann Intern Med 98:461, 1983

20. Wingard JR, Piantadosi S, Vogelsang GB, Farmer ER, Jabs DA, Levin LS, Beschorner WE, Cahill RA, Miller DF, Harrison D, Saral R, Santos GW: Predictors of death from chronic graft versus host disease after bone marrow transplantation. Blood 74:1428, 1989

21. Storb R, Deeg HJ, Thomas ED, Appelbaum FR, Buckner CD, Cheever MA, Clift RA, Doney KC, Flournoy N, Kennedy MS, Loughran TP, McGuffin RW, Sale GE, Sanders JE, Singer JW, Stewart PS, Sullivan KM, Witherspoon RP: Marrow transplantation for chronic myelocytic leukemia: A controlled trial of cyclosporine versus methotrexate for prophylaxis of graft-versus-host disease. Blood 66:698, 1985

22. Storb R, Deeg HJ, Farewell V, Doney K, Appelbaum F, Beatty P, Bensinger W, Buckner CD, Clift R, Hansen J, Hill R, Longton G, Lum L, Martin P, McGuffin R, Sanders J, Singer J, Stewart P, Sullivan K, Witherspoon R, Thomas ED: Marrow transplantation for severe aplastic anemia: Methotrexate alone compared with a combination of methotrexate and cyclosporine for prevention of acute graft-versus-host disease. Blood 68:119, 1986

23. Storb R, Deeg HJ, Pepe M, Doney K, Appelbaum F, Beatty P, Bensinger W, Buckner CD, Clift R, Hansen J, Hill R, Longton G, Anasetti C, Martin P, Loughran TP, Sanders J, Singer J, Stewart P, Sullivan KM, Witherspoon R, Thomas ED: Graft-versus-host disease prevention by methotrexate combined with cyclosporine compared to methotrexate alone in patients given marrow grafts for severe aplastic anaemia: Long-term follow-up of a controlled trial. Br J Haematol 72:567, 1989

24. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED, Seattle Marrow Transplant Team: Antileukemic effect of chronic graft-versus-host disease. Contribution to improved survival after allogeneic marrow transplantation. N Engl J Med 304:1529, 1981

25. Sullivan KM, Weiden PL, Storb R, Witherspoon RP, Fefer A, Fisher L, Buckner CD, Anasetti C, Appelbaum FR, Badger C, Beatty P, Bensinger W, Berenson R, Bigelow C, Cheever MA, Clift R, Deeg HJ, Doney K, Greenberg P, Hansen JA, Hill R, Loughran T, Martin P, Neiman P, Petersen FB, Sanders J, Singer J, Stewart P, Thomas ED: Influence of acute and chronic graft-versus-host disease on relapse and survival after bone marrow transplantation from HLA-identical siblings as treatment of acute and chronic leukemia. Blood 73:1720, 1989