Prognostic factors identifiable at the time of onset of acute graft-versus-host disease after allogeneic hematopoietic cell transplantation

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Background and Objectives. Current grading systems of acute graft-versus-host disease (GVHD) cannot effectively identify patients with poor prognosis at the onset of acute GVHD after allogeneic hematopoietic cell transplantation.

Design and Methods. In a retrospective analysis, we evaluated the prognostic value of various clinical parameters at the initiation of treatment in 83 patients who developed systemic treatment-requiring acute GVHD after allogeneic hematopoietic cell transplantation.

Results. Forty-three of 83 patients (52%) experienced initial treatment failure (40 required secondary treatment due to lack of response and 3 died) and 43 (52%) experienced treatment success, defined as completion of treatment (initial and, if given, secondary) within 100 days. The GVHD-specific survival rate was 65.5%, with 27 deaths due to GVHD-related complications without relapse of underlying malignancies within 1 year. HLA-mismatched transplantation, visceral initiation, and peripheral blood lymphocytopenia (≤100/μL) were independent variables predicting higher initial treatment failure (Odd ratios (OR)=12.225, 12.036, and 7.481, respectively). The above variables and initial acute GVHD grade III-IV vs. II were independent variables predicting shorter GVHD-specific survival (OR=0.322, 0.247, 0.340, and 0.385, respectively). High-risk disease status, visceral initiation, and hypoalbuminemia (≤2.8 g/dL) were independent variables predicting lower treatment success (OR=0.221, 0.162, and 0.270, respectively). The predictive value of visceral initiation and lymphocytopenia for GVHD-specific survival was verified in an independent cohort of 58 patients.

Interpretation and Conclusions. Lymphocytopenia and hypoalbuminemia may be useful baseline prognostic factors for acute GVHD after allogeneic hematopoietic cell transplantation.

Key words: acute graft-versus-host disease, allogeneic hematopoietic cell transplantation, visceral organ involvement, hypoalbuminemia, lymphocytopenia.

Haematologica 2005; 90:939-948

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Allogeneic hematopoietic cell transplantation is a well-established curative treatment for a significant proportions of patients with hematologic malignancies and bone marrow failure syndromes. Wider application of allogeneic hematopoietic cell transplantation is, however, impeded by treatment-related complications, most notably, acute graft-versus-host disease (GVHD). Acute GVHD is an untoward effect of an alloimmune response initiated by donor T cells that recognize the recipient's minor or major histocompatibility antigens.[1-3] Following allogeneic hematopoietic cell transplantation, 30% to 60% of patients develop clinically evident acute GVHD depending on the degree of HLA-match between donor and recipient,[4] age of the patient,[5] intensity of conditioning (especially, the dose of total body irradiation),[6] and the intensity of post-transplant immunosuppression.[7-9] Depletion of T cells from the donor hematopoietic cell graft, which can effectively prevent acute GVHD, is not performed routinely because of increased graft failure and leukemia relapse.[10]

The severity of acute GVHD is heterogenous among patients. The most benign form of acute GVHD manifests as a localized skin rash and requires no systemic treatment. Patients with generalized skin rash or visceral manifestations are treated with systemic corticosteroids (usually methylprednisolone or prednisone 2 mg/kg/day), which reduce disease manifestations significantly in about 50% of patients.[11-13] In randomized trials, increasing the dose of methylprednisolone,[14] and

the addition of antithymocyte globulin,[15] CD5-specific monoclonal immunotoxin (XomaZyme CD5-Plus),[16] or interleukin-2 receptor monoclonal antibody (daclizumab)[17] did not improve the response rate or patient survival achieved by standard dose methylprednisolone or prednisone alone. For patients who fail to respond to the initial treatment, there are no standard salvage regimens. The mortality rate of patients with treatment-refractory acute GVHD exceeds 70%, with a median survival of only 3 to 12 months. Infections and organ failure are the main causes of death.[18-24] One effective strategy to improve the outcome of patients with acute GVHD after allogeneic hematopoietic cell transplantation may be the early administration of innovative or intensive immunosuppressive treatment to a selected group of patients who are likely to have poor responses to the initial treatment and adverse outcomes subsequently.

Prognostic factors that can be identified at the onset of acute GVHD after allogeneic hematopoietic cell transplantation have not been defined. Existing grading systems of acute GVHD utilize the stages of involvement of the skin (extent of rash), liver (serum bilirubin level), and gut (daily amount of diarrhea and presence of persistent nausea/vomiting) and reflect the overall severity of acute GVHD throughout the course of the disease. The maximum grade in individual patients shows a good correlation with their eventual outcome.[12,25-27] When these grading systems are applied at the onset of acute GVHD, however, they fail to reliably identify patients with poor outcomes.[11-13,15] Furthermore, there is no generally accepted method regarding the application of current grading systems to individual patients at the onset of the disease, particularly the time point as well as the time span of observation.[11,13] In addition to aforementioned clinical manifestations, acute GVHD after allogeneic hematopoietic cell transplantation is known to be associated with elevated levels of hepatic transaminases,[28] elevated serum alkaline phosphatase,[29] hypoalbuminemia,[30] and peripheral blood cytopenia.[31-35] The frequencies and prognostic values of these clinical abnormalities at the onset of acute GVHD have not been studied adequately.

We, therefore, performed a retrospective analysis of 83 patients who developed acute GVHD after allogeneic hematopoietic cell transplantation in order to identify variables that could predict disease course and patient outcome at the onset of acute GVHD. Our results on GVHD-specific survival of patients were tested in a second independent cohort of 58 patients with acute GVHD treated in another hospital.

Design and Methods

Patients

Patients in the first cohort were among 303 adult patients who underwent allogeneic hematopoietic cell transplantation at the Asan Medical Center in Seoul, Korea, between January 1996 and August 2003. Of 94 patients (31.0%) who developed acute GVHD, 11 presented with localized skin GVHD and did not receive systemic treatment. The remaining 83 patients who required systemic immunosuppressive treatment for acute GVHD constituted the first cohort. The 58 patients in the second cohort underwent allogeneic hematopoietic cell transplantation at the Kyungpook National University Hospital in Daegu, Korea, between September 1998 and January 2004 and developed acute GVHD requiring systemic immunosuppressive treatment. Various clinical and laboratory data of the patients were retrieved from the hematopoietic cell transplantation database and medical records of each hospital (Table 1). The median ages of the first and second cohorts were 32 years (range, 15-59 years) and 37 years (range, 17-56 years), respectively, and their respective indications for hematopoietic cell transplantation were acute leukemia/myelodysplastic syndrome (47 and 40), chronic myelogenous leukemia (20 and 11), severe aplastic anemia/paroxysmal nocturnal hemoglobinuria (8 and 4) and others (8 and 3). At the time of hematopoietic cell transplantation, 36% of patients in the first cohort and 34% of the patients in the second cohort had high-risk disease features, including acute leukemia beyond the first remission, high-risk myelodysplastic syndrome (refractory anemia with excess blasts with or without transformation), chronic myelogenous leukemia beyond the first chronic phase, severe aplastic anemia/paroxysmal nocturnal hemoglobinuria with active infection, and chemotherapy-resistant malignancies.

Transplantation procedures

The protocols for specific conditioning regimens were approved by the Institutional Review Board of each hospital. Written informed consent was obtained from each patient and donor. In both patient cohorts, the conditioning regimens for malignant hematologic disorders were busulfan-cyclophosphamide-based chemotherapy and did not include total body irradiation.[36,37] Fludarabine-based reduced intensity conditioning regimens were administered to 8 and 12 patients in the first and second cohorts, respectively; these patients were primarily those with leukemia

Table 1. Characteristics of the patients and transplants.

Characteristics	*First cohort (n=83) (%)*	*Second cohort (n=58) (%)*
Age, years		
≥25	29 (35)	10 (17)
26-45	41 (49)	38 (66)
≤ 46	13 (16)	10 (17)
Sex		
Male	50 (60)	39 (67)
Female	33 (40)	19 (33)
Diagnosis of underlying disease		
Acute leukemia/myelodysplastic syndrome	47 (57)	40 (69)
Chronic myelogenous leukemia	20 (24)	11 (19)
Severe aplastic anemia/paroxysmal nocturnal hemoglobinuria	8 (10)	4 (7)
Others	8 (10)	3 (5)
Disease status at transplantation		
Standard risk	53 (64)	38 (66)
High risk	30 (36)	20 (34)
Type of hematopoietic cell donor		
Sibling	48 (58)	46 (79)
Unrelated	35 (42)	12 (21)
Source of hematopoietic cell graft		
Bone marrow	75 (90)	19 (33)
Peripheral blood	8 (10)	39 (67)
Sex pair (donor-recipient)		
Female-male	15 (18)	13 (22)
Others	68 (82)	45 (78)
HLA match		
Full match	74 (89)	54 (93)
1-antigen mismatch	9 (11)	3 (5)
2-antigen mismatch		1 (2)
Conditioning regimen		
Busulfan-cyclophosphamide or variants	67 (81)	38 (66)
Cyclophosphamide-antithymocyte globulin or variants	8 (10)	5 (9)
Busulfan-fludarabine-antithymocyte globulin or variants	8 (10)	12 (21)
Others		3 (5)
GVHD prophylaxis		
Cyclosporine plus methotrexate	58 (70)	56 (97)
Cyclosporine plus dexamethasone		1 (2)
Cyclosporine alone	25 (30)	1 (2)

High risk was defined as patients with acute leukemia in relapse or in second or subsequent remission, chronic myelogenous leukemia in accelerated or blastic phase, chemotherapy resistant or relapsed lymphoma, myeloma, or solid tumors, advanced myelodysplastic syndrome (chronic myelomonocytic leukemia, refractory anemia with excess of blasts, refractory anemia with excess of blasts in transformation), and non-malignant hematologic disorder with active infection or bleeding.

who were elderly or who had co-morbid diseases, and patients with low-risk myelodysplastic syndrome, paroxysmal nocturnal hemoglobinuria or chemotherapy-resistant solid tumors. Patients with severe aplastic anemia and paroxysmal nocturnal hemoglobinuria with cytopenia received mainly a cyclophosphamide-antithymocyte globulin regimen. HLA matching for donor selection was based on serologic typing for HLA-A, -B, and -C antigens and molecular typing for HLA-DRB1 antigen. Granulocyte colony-stimulating factor and/or granulocyte-macrophage colony-stimulating factor-mobilized peripheral blood hematopoietic cells were administered to 8 and 39 patients in the first and second cohorts, respectively. None of the hematopoietic cell grafts was T-cell-depleted. The regimen for the prophylaxis of GVHD consisted of cyclosporine 1.5 mg/kg by intravenous infusion twice daily starting on day -1, switching to oral cyclosporine when feasible. In addition, the majority of patients (70% and 97% in the first and second cohorts, respectively) received a short course of methotrexate.[8] All patients in the first cohort received a daily dose of granulocyte colony-stimulating factor 450 μg, administered intravenously, starting on day 0 or day 5 of infusion of donor cells until the peripheral blood absolute neutrophil count was over 3,000/μL. The same dose of granulocyte colony-stimulating factor was administered daily when the absolute neutrophil count decreased below 1,000/μL and continued until the absolute neutrophil count recovered to 3,000/μL.

Diagnosis of acute GVHD

Acute GVHD involving the skin and oral mucosa was diagnosed on the basis of the presence of a maculopapular skin rash and oral mucosal inflammation, respectively, with an appropriate histopathologic examination whenever necessary. Liver involvement was determined to be present when the serum bilirubin level was elevated to 2.0 mg/dL or higher and this could not be explained by other causes, such as veno-occlusive disease of the liver, drugs, sepsis, hepatitis, hemolysis, or biliary tract obstruction. Lower gastrointestinal tract involvement was determined to be present when the patient experienced diarrhea over 500 mL/day and stool or colonoscopic examination showed inflammatory changes with no documented pathogens. Upper gastrointestinal tract involvement was determined to be present when the patient experienced persistent nausea and gastroscopic examination showed mucosal changes consistent with GVHD without evidence of viral infections. Complete blood counts, as determined by automated counters, were performed daily in all patients. Chemistry profiles were evaluated at least twice weekly, or more often if necessary. In the first cohort of patients, peripheral blood hematopoietic chimerism, assayed by a polymerase chain reaction-based procedure analyzing short tandem repeats of DNA, was analyzed monthly for at least for 3 months after allogeneic hematopoietic cell transplantation.[38]

Assessment of prognostic factors

The day of onset of acute GVHD was defined as the date of initiation of systemic immunosuppressive treatment. In the first cohort of patients, the following clinical parameters were assessed; patient's age (≤25 years vs. 26-45 years vs. ≥46 years), sex, disease status at transplantation, type of hematopoietic cell donor, source of hematopoietic cell graft, donor-recipient sex pair, HLA match, GVHD prophylaxis, onset of acute GVHD (≤day 15 vs. day 16-45 vs. ≥ day 46 after transplantation), and involved organs at the onset (skin, liver, and lower gastrointestinal tract). The following laboratory parameters were obtained from the day (77 patients), within 1 day (4 patients), or within 2 days (2 patients) of initiation of systemic immunosuppressive treatment for acute GVHD; serum concentrations of alanine aminotransferase, alkaline phosphatase, and albumin, peripheral blood absolute lymphocyte count (obtained by multiplying the white blood cell count by lymphocyte fraction, as determined by manual counting), and platelet count. Initial stage and grade of acute GVHD, as defined by the Consensus criteria,[26] were also assessed from the clinical data available at the time of initiation of systemic immunosuppressive treatment. Initial organs of involvement were also compared as skin (with or without mouth involvement) vs. visceral (with or without skin/mouth involvement).

Statistical analysis

Three statistical end-points were used for the outcome analysis in the first cohort: initial treatment failure, treatment success, and GVHD-specific survival. Initial treatment failure was defined as the necessity for secondary treatment for acute GVHD due to a lack of response or progression following initial treatment or the patient's death due to acute GVHD while receiving initial treatment. Treatment success was defined as the successful control of acute GVHD and discontinuation of immunosuppressive treatment, whether initial or secondary, except for the prophylactic doses of cyclosporine or tacrolimus within 100 days after the onset of acute GVHD. GVHD-specific survival was defined as the interval from the onset of acute GVHD to death due to acute or chronic GVHD-related complications without relapse of underlying malignancies within 1 year after the onset of acute GVHD. Patients who relapsed with their underlying malignancies and who died due to causes other than GVHD-related complications were censored at the time of relapse and at the time of death, respectively. Overall survival was measured from the onset of acute GVHD to death due to any cause. Potential prognostic factors were analyzed using the χ^2 test for the prediction of initial treatment failure and treatment success. Curves for the GVHD-specific and overall survivals were plotted according to the Kaplan-Meier method and compared using the log-rank test. For multivariate analysis, variables with a p value < 0.05, as determined by univariate analysis, were considered for entry into the model selection procedure based on a multiple logistic regression model (initial treatment failure, treatment success) or the Cox proportional hazards model (GVHD-specific survival). Initial organ involvement was considered as skin only vs. visceral initiation rather than considering each organ involvement separately. The final models were selected using the backward elimination method with a predetermined risk of 0.1.

Results

First cohort

Of the 83 patients in this cohort, 23 (28%) were diagnosed with acute GVHD on the basis of clinical findings alone, and 60 (72%) were diagnosed on the basis of clinical findings and by histological examination. The median day of onset of acute GVHD after hematopoietic cell transplantation was day 32 (range, day 7-85). Hematopoietic chimerism analysis was performed in 75 patients within 23 days of onset of acute GVHD, of whom 65 patients showed complete donor chimerism and 10 showed a low degree of mixed chimerism, with recipient DNA ranging from 5-20%. The patients' clinical characteristics at the onset of acute GVHD are summarized in Table 2. Sixty-four patients (77%) had skin involvement, 19 (23%) had liver involvement, and 22 (27%) had lower gastrointestinal tract involvement. The initial manifestation of acute GVHD was confined to the skin or mouth in 45 patients (54%), while 38 (46%) had involvement of at least one visceral organ. Initial acute GVHD grading, according to the data available on the day of onset of acute GVHD, was grade II in 62 patients and grade III or IV in 21. The maximum acute GVHD stage and grade for the cohort are presented in Table 2. At the onset of acute GVHD, 19 patients (23%) each had serum alanine aminotransferase ≥120 IU/L and serum alkaline phosphatase ≥160 IU/L and 22 (27%) had serum albumin ≤2.8 g/dL. Forty-three patients (52%) had a platelet count ≤20,000/μL and were dependent on platelet transfusions. The median absolute lymphocyte count was 360/μL (range, 0-2,484/μL) with 21 patients (25%) having lymphocyte counts ≤100/μL.

Initially, 79 patients were treated with methylprednisolone or prednisone 1-2 mg/kg/day; two were treated with methylprednisolone ≥500 mg/day for 3 days, then the dose was rapidly tapered; and two were treated with mycophenolate mofetil 1 g/day.

Table 2. Clinical characteristics at the onset of acute GVHD (first cohort).

Characteristics	*Number of patients (%)*
Onset, day of transplantation	
≤15	23 (28)
16-45	42 (51)
≥46	18 (22)
Initial organ of involvement	
Skin	64 (77)
Liver	19 (23)
Lower gastrointestinal tract	22 (27)
Upper gastrointestinal tract	8 (10)
Mouth	8 (10)
Skin only vs. visceral initiation	
Skin (with or without mouth) only	45 (54)
Visceral (with or without skin)	38 (46)
Liver + gut + skin involvement	5
Liver + skin involvement	4
Gut + skin involvement	10
Liver + gut involvement	2
Liver involvement only	8
Gut involvement only	9
Acute GVHD stage, initial/maximum*	
Skin 1	1/2
Skin 2	3/1
Skin 3	59/56
Skin 4	1/6
Liver 1	4/8
Liver 2	9/7
Liver 3	5/14
Liver 4	1/10
Gut 1	21/8
Gut 2	3/5
Gut 3	0/3
Gut 4	2/22
Acute GVHD grade, initial/maximum*	
II	62/38
III	19/27
IV	2/18
Serum alanine aminotransferase (IU/L)	
< 120	64 (77)
≥ 120	19 (23)
Serum alkaline phosphatase (IU/L)	
< 160	64 (77)
≥ 160	19 (23)
Serum albumin (g/dL)	
> 2.8	61 (73)
≤2.8	22 (27)
Absolute lymphocyte count (/μL)	
> 100	62 (75)
≤100	21 (25)
Platelet count (/μL)	
> 20,000	40 (48)
≤20,000	43 (52)

**by the Consensus criteria.*[26]

Table 3. Prognostic factor analysis based on a multivariate model (first cohort).

Variables	*p value**	*Odd ratio*	*95% CI*
Initial treatment failure			
HLA match			
Full match		1	
1 Ag mismatch	0.050	12.225	1.002-149.869
Skin vs. visceral initiation			
Skin only		1	
Visceral	0.000	12.036	3.517-41.191
Absolute lymphocyte count (/μL)			
> 100		1	
≤ 100	0.008	7.481	1.672-33.470
Treatment success			
Disease status at transplantation			
Standard risk		1	
High risk	0.015	0.221	0.065-0.748
Skin vs. visceral initiation			
Skin only		1	
Visceral	0.002	0.162	0.050-0.525
Serum albumin (g/dL)			
> 2.8		1	
≤ 2.8	0.044	0.270	0.076-0.963
GVHD-specific survival			
HLA match			
Full match		1	
1 Ag mismatch	0.020	0.322	0.124-0.835
Initial acute GVHD grade			
Grade II		1	
Grade III-IV	0.035	0.385	0.159-0.933
Skin vs. visceral initiation			
Skin only		1	
Visceral	0.014	0.247	0.081-0.756
Absolute lymphocyte count (/μL)			
> 100		1	
≤100	0.008	0.340	0.153-0.753

*CI, confidence interval. * Multivariate analysis using the multiple logistic regression models (initial treatment failure and treatment success) or Cox proportional hazards models (GVHD-specific survival) with the backward elimination method. For initial treatment failure, GVHD prophylaxis, type of donor, HLA match, initial acute GVHD grade, skin vs. visceral initiation, serum albumin level at onset, and absolute lymphocyte count at onset were entered into the model selection procedure. For treatment success, disease status at transplantation, type of donor, HLA match, initial acute GVHD grade, skin vs. visceral initiation, serum albumin level at onset, and absolute lymphocyte count at onset were entered. For GVHD-specific survival, type of donor, HLA match, initial acute GVHD grade, skin vs. visceral initiation, serum albumin level at onset, and absolute lymphocyte and platelet counts at onset were entered.*

Of these 83 patients, 43 (52%) experienced initial treatment failure, with 40 patients requiring additional immunosuppressive agents (secondary treatment) at a median of 10 days (range, 3 to 48 days) after initial treatment and three died from acute GVHD while receiving initial treatment. Of the 40

patients who required secondary treatment, 16 were treated with mycophenolate mofetil, 10 with antithymocyte globulin/antilymphocyte globulin, 9 with higher doses of corticosteroids, and 5 with tacrolimus. Forty-three patients (52%) experienced successful control of acute GVHD at a median of 39 days (range, 7-96 days) after initiation of systemic treatment and discontinued immunosuppressive treatment (initial and, when necessary, secondary) except for prophylactic doses of cyclosporine or tacrolimus.

The median follow-up period of surviving patients was 805 days (range, 370-2299 days) after the onset of acute GVHD. Forty-two patients died, resulting in a 3-year projected overall survival rate of 44.4%. Twenty-seven patients died due to acute or chronic GVHD-related complications without relapse of underlying malignancies within 1 year after the onset of acute GVHD (GVHD-specific survival rate of 65.5%). Six patients died due to relapse of underlying malignancies, occurring between 52 and 988 days after the onset of acute GVHD. Other causes of death were idiopathic interstitial pneumonitis and fulminant hepatic failure in two patients each, suicide and thrombotic thrombocytopenic purpura-like syndrome in one each, and sepsis with chronic GVHD beyond 1 year in three.

Analysis of prognostic factors

Univariate analysis showed that statistically significant prognostic factors for initial treatment failure included GVHD prophylaxis (p=0.017), type of donor (p=0.002), HLA match (p=0.012), initial acute GVHD grade II vs. III-IV (p=0.009), skin vs. visceral initiation (p=0.000), liver involvement (p=0.027), lower gastrointestinal tract involvement (p=0.000), serum albumin level (p=0.004), and absolute lymphocyte count (p=0.001). Multivariate analysis showed that the one-antigen mismatched transplantation (p=0.050), visceral initiation as opposed to skin initiation (p=0.000), and low absolute lymphocyte count (p=0.008) were significant independent factors predicting higher initial treatment failure (Table 3).

Univariate analysis showed that the statistically significant predictors of treatment success were disease status at hematopoietic cell transplantation (p=0.037), type of donor (p=0.006), HLA match (p=0.006), initial acute GVHD grade (p=0.013), skin vs. visceral initiation (p=0.003), liver involvement (p=0.043), lower gastrointestinal tract involvement (p=0.001), serum albumin level (p=0.007), and absolute lymphocyte count (p=0.013). Multivariate analysis showed that high risk disease status (p=0.015), visceral initiation (p=0.002), and hypoalbuminemia (p=0.044) were significant independent predictors of reduced treatment success (Table 3).

For GVHD-specific survival, univariate analysis showed that type of donor (p=0.008), HLA match (p=0.009), initial acute GVHD grade (p=0.000), skin vs. visceral initiation (p=0.000, Figure 1A), liver involvement (p=0.000), lower gastrointestinal tract involvement (p=0.002), serum albumin level (p=0.001), absolute lymphocyte count (p=0.004, Figure 1A), and platelet count (p=0.024) were statistically significant prognostic factors. Multivariate analysis showed that one-antigen mismatched transplantation (p=0.020), initial acute GVHD grade III-IV vs. II (p=0.035), visceral initiation (p=0.014), and low absolute lymphocyte count (p=0.008) were significant independent predictors of shorter GVHD-specific survival (Table 3).

Second cohort

The predictive value of visceral initiation and lymphocytopenia at the onset of acute GVHD on GVHD-specific survival was tested in an independent cohort of 58 patients treated at the Kyungpook National University Hospital. The median day of onset of acute GVHD, defined as the date of initiation of systemic immunosuppressive treatment after transplantation, was day 20 (range, day 9 to 80). At GVHD onset, 50 patients (86%) showed skin involvement, 13 (22%) showed lower gastrointestinal tract involvement, 4 (7%) showed liver involvement, and 3 (5%) showed upper gastrointestinal tract involvement, with 39 patients (67%) having acute GVHD confined to the skin and 19 (33%) having involvement of at least one visceral organ. The median peripheral blood absolute lymphocyte count was 385/μL (range, 6-5,546/μL) with 14 patients (24%) having ≤100/μL. The median follow-up of surviving patients was 636 days (range, 280-1924 days). Seven of 39 patients (18%) with skin initiation of acute GVHD versus 8 of 19 (42%) with visceral initiation died within 1 year of the onset of acute GVHD due to acute or chronic GVHD-related complications without relapse of underlying malignancies (p=0.026, Figure 1B). Eight of 44 patients (18%) with absolute lymphocyte counts >100/μL versus 7 of 14 (50%) with absolute lymphocyte counts ≤100/μL died within 1 year (p=0.005, Figure 1B).

When we combined these two risk factors, skin versus visceral initiation and presence of severe lymphocytopenia, and divided the combined cohort of 141 patients into four groups, we were able to identify three distinct groups of patients having good (skin initiation without lymphocytopenia), intermediate (skin initiation with lymphocytopenia and visceral initiation without lymphocytopenia), and poor (visceral initiation with lymphocytopenia) prognosis, regarding GVHD-related survival (Figure 2A) as well as overall survival (Figure 2B).

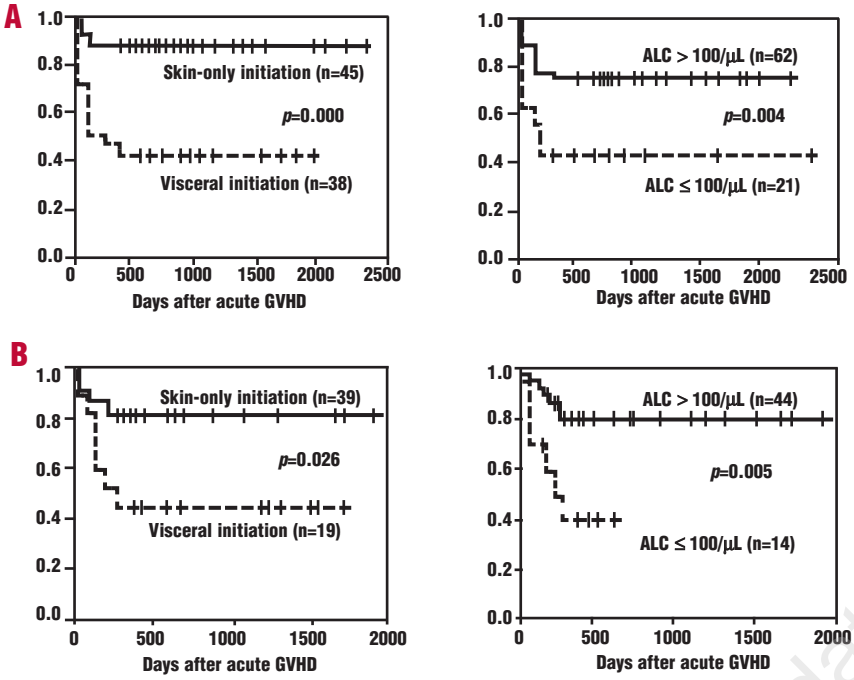

Figure 1. Kaplan-Meier plots of GVHD-specific survival in the first (A) and second (B) cohorts of patients relative to organ involvement and peripheral blood absolute lymphocyte count. In the first cohort, GVHD-related death within 1 year of onset of acute GVHD was observed in 5/45 (11%) patients with skin-only involvement vs. 22/38 (58%) with visceral involvement (p=0.000), and, according to absolute lymphocyte count, in 15/62 (24%) patients with lymphocyte count >100/μL vs. 12/21 (57%) with lymphocyte count ≤ 100/μL (p=0.004). In the second cohort, GVHD-related death was observed in 7/39 (18%) patients with skin-only involvement vs. 8/19 (42%) with visceral involvement (p=0.026), and in 8/44 (18%) patients with lymphocyte count > 100/μL vs. 7/14 (50%) with lymphocyte count ≤100/μL (p=0.005).

Discussion

Application of existing grading systems for acute GVHD as prognostic factors at the onset of the disease has not been satisfactory.[39] For example, determination of the extent of skin GVHD is subject to each examiner's interpretation, and assessment of the stage of lower gastrointestinal tract involvement requires daily measurements of the amount of diarrhea. In situations in which a patient's clinical features change rapidly, there is no consensus regarding how to apply current grading systems to the individual patient. For example, when the volume of diarrhea was measured as the average for the day of evaluation and the two preceding days, initial acute GVHD grading could not predict response to initial treatment.[11] In addition, when the initial stage of

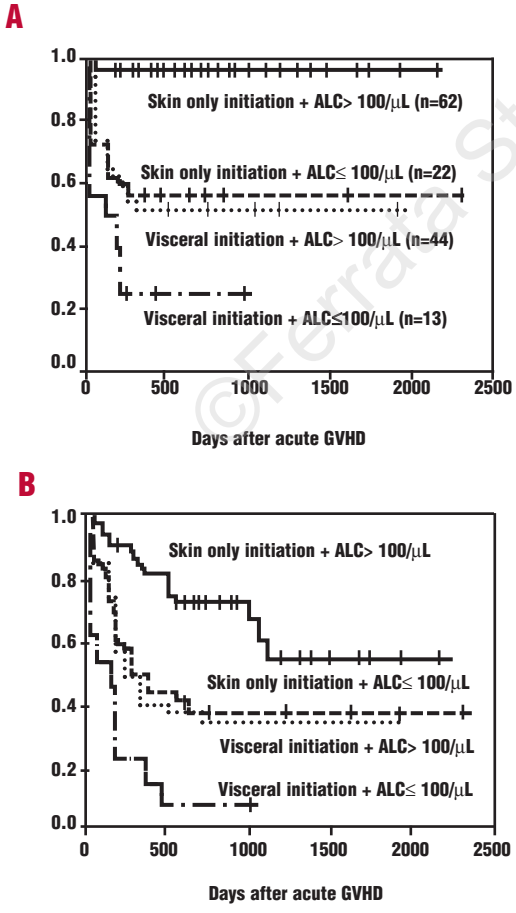

Figure 2 [left]. Kaplan-Meier plots of GVHD-specific survival (A) and overall survival (B) in the combined cohort of 141 patients relative to organ involvement and peripheral blood absolute lymphocyte count at the onset of acute GVHD. Sixty-two patients with skin-only involvement and absolute lymphocyte count >100/μL had good outcomes, with 2-year GVHD-specific and overall survival rates of 95% and 72%, respectively. Thirteen patients with visceral organ involvement and lymphocyte count ≤100/μL had a poor outcome, with 2-year GVHD-specific and overall survival rates of 23% and 8%, respectively. Twenty-two patients with skin-only involvement and lymphocyte count ≤100/μL and 44 patients with visceral involvement and lymphocyte count >100/μL had an intermediate outcome, with 2-year GVHD-specific survival rates of 54% and 50% and overall survival rates of 38% and 35%, respectively.

acute GVHD was defined as the maximum stages within 5 days of initiation of glucocorticoid treatment, overall initial acute GVHD grading (grades I-II vs. III-IV) by Minnesota[13] and Consensus criteria,[26] were not predictive of response to treatment but showed a borderline significance for 1-year mortality (p=0.05).[13] In contrast, the type of donor (mismatched unrelated vs. matched related) and GVHD prophylaxis regimens (single agent methotrexate vs. others) were independent variables that predicted poor response to initial treatment, and patient's age, type of donor, and GVHD prophylaxis were independent variables that predicted 1-year mortality.[13] In a study of HLA-compatible sibling hematopoietic cell transplant recipients, initial acute GVHD grade was a statistically significant predictor of response to initial treatment on univariate analysis (grade II vs. grades III-IV, p=0.002) but lost significance upon multivariate analysis, and complete response to treatment and maximum but not initial acute GVHD grade were significant predictors for the patients' survival.[12] Furthermore, in 96 patients randomly assigned to treatment with prednisone or prednisone plus antithymocyte globulin, initial acute GVHD grading, scored as overall grade or as the sum of scores of skin, liver, and gut involvement, did not correlate with response to initial treatment.[15]

Our study showed that, at the time of initiation of systemic immunosuppressive treatment for acute GVHD, about a quarter of patients each had elevated serum alanine transferase level ≥120 IU/L, elevated serum alkaline phosphatase level ≥160 IU/L, hypoalbuminemia ≤2.8 g/dL, and lymphocytopenia ≤100/μL. About half of the patients had thrombocytopenia ≤20,000/μL. When these abnormalities were correlated with the clinical courses of the patients, upon univariate analysis, hypoalbuminemia and lymphocytopenia showed significant correlations along with the type of donor, HLA mismatch, initial acute GVHD grade (III-IV vs. II), and visceral organ involvement. Multivariate analysis showed that lymphocytopenia, HLA mismatch, and visceral organ involvement were independent variables that predicted higher initial treatment failure while the aforementioned variables plus initial acute GVHD grade were independent variables that predicted shorter GVHD-specific survival. Hypoalbuminemia, disease status, and visceral organ involvement were independent variables that predicted lower treatment success.

The prognostic significance of peripheral blood cytopenia and hypoalbuminemia in patients with acute GVHD after allogeneic hematopoietic cell transplantation has not been studied well in the past. On the other hand, post-transplant cytopenia and hypoalbuminemia have been described to occur in association with severe acute GVHD.[30-35,37] Grade II-IV acute GVHD was a significant independent predictor of secondary thrombocytopenia (< 20,000/μL), which occurred in 285 of 1401 patients (20%) after allogeneic hematopoietic cell transplantation, and secondary thrombocytopenia itself was associated with increased patient mortality, with a hazard ratio of 2.6.[33] Thrombocytopenia on days 50, 100, 180, and 365 after allogeneic hematopoietic cell transplantation could be correlated with the presence and grade of acute GVHD in a retrospective study of 342 patients and a platelet count of ≤50,000/μL 50 days after transplantation was a significant predictive factor for transplantation-related mortality in a subset of patients with grade II acute GVHD.[35] In addition, an absolute lymphocyte count of less than 200μL on day 29[32] and less than 350/μL on day 21[37] following allogeneic hematopoietic cell transplantation were found to be associated with an increased incidence of severe acute GVHD and decreased patient survival. Although the mechanisms underlying cytopenia and hypoalbuminemia in acute GVHD may be multifactorial, inflammatory cytokines are likely to play significant roles. Pro-inflammatory cytokines, such as interferon-γ and tumor necrosis factor-α were shown to impair lymphocyte expansion upon mitogenic stimulation[40,41] and cause bone marrow dysfunction.[41,42] Hypoalbuminemia in acute GVHD is likely due to impaired synthesis and increased catabolism, which are known to be associated with increased pro-inflammatory cytokines.[43] Excessive intestinal loss may be a contributing factor in patients with acute GVHD involving the lower gastrointestinal tract.[30] It may be that, in patients who develop acute GVHD after allogeneic hematopoietic cell transplantation, lymphocytopenia and hypoalbuminemia are related to a more extensive alloimmune response, wide-spread pro-inflammatory cytokine response, and severe tissue damage, which contribute to the eventual poor outcomes of patients. If so, they may serve as objective and easily measurable prognostic factors at the onset of acute GVHD after allogeneic hematopoietic cell transplantation. Furthermore, early blockage of pro-inflammatory cytokines may prove to be a beneficial treatment strategy in these subsets of patients.

There are no generally accepted criteria regarding how to define response to treatment in acute GVHD. As measurements of disease response in our study, we used frequencies of failure of initial treatment and success of initial and, if given, secondary treatment. These intermediate end-points may vary widely according to the treatment policies of individual attending physicians and hospitals, especially when studies are conducted retrospectively as was ours. On the other hand, GVHD-specific and overall sur-

vivals are more objective and reliable. When visceral organ involvement and lymphocytopenia, the two most significant predictors of GVHD-specific survival in the first cohort of patients in our study, were applied as prognostic factors to an independent cohort of 58 patients, both showed statistically significant correlations with GVHD-specific survival. In addition, when these two prognostic factors were combined and applied to both cohorts of patients, we were able to identify three distinct groups of patients having good, intermediate, and poor prognosis, as measured by GVHD-specific and overall survivals. Our findings do, however, need to be confirmed in larger numbers of patients, preferably with prospectively collected data sets.

Acute GVHD is a dynamic and heterogenous disease process. Clinical characteristics of acute GVHD at initiation of systemic treatment may change depending on the threshold of abnormality that triggers treatment by the individual physicians. Other factors, including the ethnicity of patients,[44,45] the nature of the underlying disease, and the source of donor hematopoietic cells, may also influence the clinical characteristics of acute GVHD. In our study, more patients in the second cohort received growth factor-mobilized peripheral blood hematopoietic cells (68% vs. 10%, p=0.000). While similar proportions of patients (25% and 24%) had lymphocytopenia of 100/μL or less, initial liver involvement was higher in the first cohort (23% vs. 7%, p=0.007). The influence of various patient- and transplant-related factors on the clinical characteristics of acute GVHD requires further investigation. When we used different cut-off points for lymphocytopenia (i.e., 200/μL, 300/μL, and 500/μL) in our study, the statistical significance was lost for all three statistical end-points (*data not shown*), which suggested that only a severe degree of lymphocytopenia had clinical significance. Likewise, hypoalbuminemia, using the criterion of an albumin concentration below 2.8 g/dL, showed most statistical power in univariate analyses. Thromobocytopenia showed a significant correlation with GVHD-specific survival on univariate analysis but lost its significance on multivariate analysis. Although neutropenia is known to be associated with acute GVHD after allogeneic hematopoietic cell transplantation,[34] we did not use total white blood and absolute neutrophil counts as parameters, since granulocyte colony-stimulating factor was administered to all patients when they became neutropenic, resulting in wide daily variations of these counts. The optimal prognostic criteria for cytopenia and hypoalbuminemia in patients with acute GVHD need to be defined in the future.

In conclusion, our study suggests that, in patients who develop acute GVHD after allogeneic hematopoietic cell transplantation and require systemic immunosuppressive treatment, severe lymphocytopenia and hypoalbuminemia may be utilized as part of baseline prognostic factors for disease response and patient survival. Further studies involving larger numbers of patients as well as different populations of patients are warranted.

K-HL and J-HL contributed equally to the work and assume primary responsibility for it. K-H L was responsible for the design of study, supervision of data collection, data analysis, and writing the manuscript. J-HL was responsible for the supervision of data collection, data analysis, and critical revision of the manuscript. S-JC, J-HL, S-KS, J-GK, and D-HK were involved, to varying degrees, in the interpretation of data and critical revision of the manuscript. J-S L, W-KK, and K-BL were involved in critical revision of the manuscript. MS and Y-SL contributed primarily to the data collection and analysis.

Manuscript received February 16, 2005. Accepted May 15, 2005.

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