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Outcomes of Patients with Early Hyperbilirubinemia after Allogeneic Hematopoietic Stem Cell Transplantation

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Background: Because the cause of liver dysfunction after allogeneic hematopoietic stem cell transplantation (HSCT) is difficult to identify in the early stages, treatment may be delayed. Therefore, early factors associated with unfavorable outcomes of liver dysfunction must be identified. The objective of this study was to identify unfavorable prognostic factors for liver dysfunction during the early period after transplantation.

Methods: We defined liver dysfunction as elevated liver or biliary enzyme levels (corresponding to Grade 2 in the Common Terminology Criteria for Adverse Events version 4.0) within 30 days of transplantation and retrospectively investigated data from 82 patients who had undergone allogeneic HSCT at our center.

Results: Elevated liver or biliary enzyme levels were observed in almost half of the patients studied (n= 40, 48.7%). Elevated total bilirubin (T-Bil) level was the most frequently observed unfavorable prognostic factor and had the greatest effect on overall survival (OS), progression-free survival (PFS), and non-relapse mortality (NRM) (probability of unfavorable outcome in patients without and with elevated T-Bil level: OS, 58.9% vs. 15.4%, $p < 0.001$; PFS, 46.4% vs. 15.4%, $p < 0.001$; NRM, 10.7% vs. 53.8%, $p < 0.001$). Moreover, the probability of an unfavorable outcome increased in relation to the degree of T-Bil elevation and absence of improvement over time in T-Bil level.

Conclusion: Elevated T-Bil level was an important marker of outcomes for liver dysfunction after allogeneic HSCT. (J Nippon Med Sch 2020; 87: 142–152)

Key words: liver dysfunction, allogeneic hematopoietic stem cell transplantation, total bilirubin

Introduction

Hematopoietic stem-cell transplantation (HSCT) is one of the most effective treatments for hematological diseases. Recently, the number of transplants has been increasing because of the less burdensome conditioning treatments and easier access to donor sources, eg, through umbilical cord blood transplants and transplant from haploidentical related donors. However, the high mortality rate attributable to transplant-associated complications remains a concern.

Liver dysfunction is a frequent complication of transplantation. Causes of liver dysfunction include infectious disease and drug-related factors such as use of immunosuppressants, graft-versus-host disease (GVHD), and sinusoidal obstruction syndrome (SOS)/veno-occlusive disease (VOD)[1,2]. In the early post-transplant period, liver dysfunction follows a particularly complex course in which a variety of overlapping pathologies are present. During this stage, platelet count is low and liver biopsy is difficult. Therefore, diagnosis is often not possible and appropriate treatment is thus delayed.

Prognosis was reported to be poor after onset of SOS/

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VOD and acute GVHD[3,4]. There are no effective therapies for SOS/VOD, but defibrotide was developed as a new therapeutic drug in recent years and is likely to improve outcomes[4,5]. Additionally, the European Society for Blood and Marrow Transplantation has proposed a new diagnostic standard intended to diagnose SOS/VOD at an early stage and assess disease severity[6]. In addition, the combination of calcineurin inhibitors and methotrexate (MTX) has reduced GVHD incidence, while preventive administration of ursodeoxycholic acid has mitigated the extent of liver dysfunction[7]. Nevertheless, once GVHD has become manifest, the only established treatment is steroid. Research on treatments for GVHD refractory to steroid treatment is continuing.

Differential diagnosis of early post-transplant liver dysfunction is difficult, and available therapies are limited. Therefore, it is important to accurately identify liver dysfunction and its outcomes and to quickly develop treatments. Numerous studies have investigated early post-transplant liver dysfunction and its outcomes, but nearly all have focused on liver dysfunction as defined by elevated total bilirubin value (T-Bil). The present study, which retrospectively analyzed data from 82 transplant patients treated at our center, defined liver dysfunction as elevation of any one of five test values, including T-Bil and other liver enzymes and biliary enzymes. We then sought to identify associated risk factors and outcomes.

Materials and Methods

Patient Selection

We retrospectively analyzed data from 82 patients who had undergone allogeneic HSCT for certain hematological diseases at Nippon Medical School Hospital during the period from March 2006 through October 2015. The background characteristics of the patients are shown in **Table 1**. The study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients, and the analysis and treatments were conducted while respecting the welfare and free will of the patients. This protocol was approved by our institutional review board (30-09-991).

Transplantation Procedure

HBs antigen, HBc antibody, and HBs antibody were measured for HBV, HCV antibody was measured for HCV, and the respective antibody titers of EBV, CMV, VZV, and HSV were also measured. Patients with test results indicating active infection by any of these were excluded. Patients with positive test results for HBc antibody or HBs antibody received oral nucleic acid analogue treatment, to prevent reactivation. Tests, including an ocular fundus test, full body CT, and serum diagnosis of β-D glucan and aspergillus antigen, were performed to exclude active fungal infection, and antifungal drugs such as micafungin were administered prophylactically.

Calcineurin inhibitors and short-term MTX were used for prevention of GVHD. The calcineurin inhibitors used were cyclosporine (CsA), for cases involving related donors, and tacrolimus (Tac), for cases involving unrelated donors. CsA was administered by intravenous infusion for 10 h; the target trough level was 250 to 300 ng/mL. Tac was administered over 24 h, and the target blood concentration was 12 to 15 ng/mL. The MTX regimen was 15 mg/m^2 on Day 1 and 10 mg/m^2 on Days 3 and 6, for cases involving related HLA-matched donors, and 15 mg/m^2 on Day 1 and 10 mg/m^2 on Days 3, 6, and 11, for cases involving unrelated donors and non-HLA-matched donors. However, when renal dysfunction or a similar complication was noted, the dose was reduced to 10 mg/m^2 on Day 1 and to 7 mg/m^2 on Days 3, 6, and 11. When mucosal damage was severe, appropriate adjustments were made, such as omitting administration on Day 11.

For conditioning treatment, a choice was made between myeloablative conditioning (MAC) and reduced-intensity conditioning (RIC), in accordance with the status of the underlying disease and the age and general condition of the patient. The sources used for stem cells were, in descending order of preference, related donors, bone marrow bank donors, and umbilical cord blood.

Definition of Liver Dysfunction

Liver dysfunction was defined as elevation of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), γ-glutamyltransferase (γ-GTP), or T-Bil (Grade 2 or higher according to the CTCAE version 4.0) within 30 days of transplantation. Such values are hereafter referred to as AST 2, ALT 2, ALP 2, GTP 2, and Bil 2, respectively.

Statistical Analysis

The chi-square test was used to analyze nominal variables. When a value of less than 5 was present in any field of the 2 × 2 table, Fisher's exact test was used for analysis. The nonparametric Mann-Whitney U test was used to determine the statistical significance of differences in median values. All statistical tests were two-sided. The Kaplan-Meier method and log-rank test were used to analyze overall survival (OS) and progression-free survival (PFS). Cumulative incidences of relapse and non-relapse mortality (NRM) were compared with the stratified Gray test. Multivariate analyses for survival

Table 1 Patient background

		Number of cases
Age		
	mean	42.9 (Range 14-64)
	median	45.5 (Range 14-64)
Sex		
	Male	54 (65.9%)
	Female	28 (34.1%)
Diseases		
	AML	35 (42.7%)
	ALL	12 (14.6%)
	other leukemias	5 (6.1%)
	MDS	8 (9.8%)
	CML	3 (3.7%)
	lymphoid malignancy	12 (14.6%)
	AA	4 (4.9%)
	plasma cell neoplasms	1 (1.2%)
	MPD	1 (1.2%)
	granulocytic sarcoma	1 (1.2%)
Disease Risk		
	standard	47 (57.3%)
	advanced	35 (42.7%)
PS		
	0	46 (56.1%)
	≥1	36 (43.9%)
HCT-CI		
	0	68 (82.9%)
	≥1	14 (17.1%)
CMV antibody		
	negative	14 (17.3%)
	positive	67 (82.7%)
Blood Relationship		
	Related	23 (28.0%)
	Unrelated	59 (72.0%)
HLA disparity		
	Matched	44 (59.8%)
	Mismatched	38 (37.8%)
ABO disparity		
	Matched	44 (59.8%)
	Mismatched	38 (37.8%)
GVHD prophylaxis		
	CsA + MTX	21 (24.4%)
	Tac + MTX	61 (72.0%)
Conditioning		
	MAC	59 (72.0%)
	RIC	23 (28.0%)
TBI		
	<6 Gy	39 (47.6%)
	≥6 Gy	43 (52.4%)
Donor source		
	non-CB	49 (59.8%)
	CB	33 (40.2%)
Number of SCT		
	1	74 (90.2%)
	≥2	8 (9.8%)

※AML: acute myeloid leukemia, ALL: acute lymphoid leukemia, MDS: myelodysplastic syndromes, CML: chronic myeloid leukemia, AA: aplastic anemia, MPD: myeloproliferative neoplasms, PS: performance status, HCT-CI: Hematopoietic Cell Transplantation-specific Comorbidity Index, CMV: cytomegalovirus, GVHD: graft versus host disease, CsA: cyclosporin, Tac: tacrolimus, MTX: methotrexate, MAC: myeloablative conditioning, RIC: reduced-intensity conditioning, TBI: total body irradiation, CB: cord blood, SCT: stem cell transplantation.

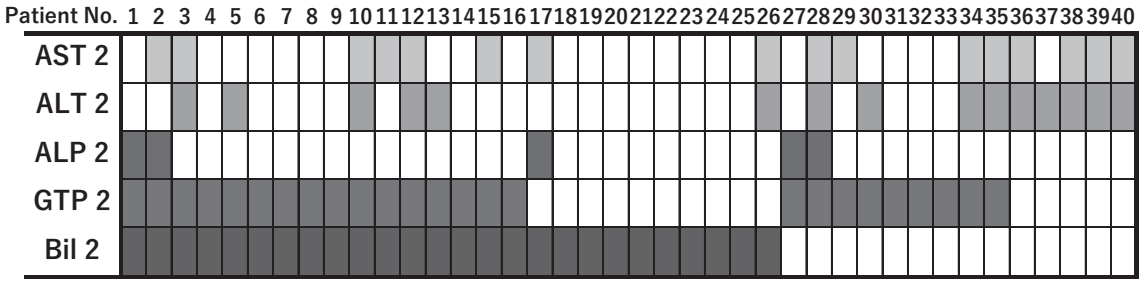

Fig. 1 Profile of patients who developed early-onset liver dysfunction (Day 0 to 30) after hematopoietic stem-cell transplantation
The type of liver dysfunction in each of the 40 patients is shown. Each row represents one type of liver dysfunction and each column represents a patient. Colored cells indicate the presence of liver dysfunction.

Table 2 Frequency and severity of early-onset liver dysfunction

	AST 2 (n= 16)	ALT 2 (n= 15)	ALP 2 (n= 5)	GTP 2 (n= 25)	Bil 2 (n= 26)
Onset [day]	17.6 (3-27)	15.4 (0-27)	17.4 (0-27)	6.6 (0-27)	11.6 (0-29)
Maximum (range) [IU/L]	326 (95-2,105)	429 (116-2,509)	1,503 (915-2,499)	286 (140-1,143)	7.0 (2.0-32.2)
Improvement	4 (25%)	4 (26.7%)	1 (20.0%)	12 (48.0%)	12 (46.1%)

were performed by using a Cox proportional hazards regression model, whereas multivariate analyses for NRM and relapse were performed by using competing risk regression based on the Fine and Gray model. All statistical analyses were performed with EZR software (Version 2.4-0, Saitama Medical Center, Jichi Medical University, Saitama).

Results

Patient Characteristics

The characteristics of the patients are shown in **Table 1**. The average age of the 82 patients was 42.9 years (range, 14-64 years), and the underlying disease in almost all cases was hematological malignancy. Regarding disease stage, leukemia and lymphoma patients in a non-remission state accounted for 42.7% of cases. Umbilical cord blood was a frequent source of transplanted stem cells (40.2% of cases). Most patients were in good general condition and did not have a poor performance status (PS) or systemic complications; scores on the hematopoietic cell transplantation-specific comorbidity index were low.

Overall Transplant Outcome

Engraftment was achieved in 74 (90.2%) patients, and the median was at 19.7 days (9-56) after HSCT. Acute GVHD was observed in 31 (39.2%) patients, and GVHD progressed to Grade 2 or higher in 18 (22.7%) patients. Thirty-six (43.9%) patients experienced relapse after transplantation, and relapse developed after an average of 205.2 (32-659) days after HSCT. After 1 year of HSCT, OS was 49.8%, PFS was 38.9%, and NRM was 22.0%.

Clinical Significance of Early-Onset Liver Dysfunction (Day 0 to 30) after HSCT

Frequency and severity of liver dysfunction

Liver dysfunction occurred in 40 (48.7%) of the 82 patients (**Fig. 1**). The frequency and severity of liver dysfunction are shown in **Table 2**. Many patients had GTP 2 and Bil 2, and onset was soon after HSCT. In addition, numerous patients showed improvement in GTP 2 and Bil 2 within 30 days, but it is unclear if this was attributable to early onset.

Analysis of Risk Factors for Liver Dysfunction

The risk factors for AST 2, ALT 2, ALP 2, GTP 2, and Bil 2 are shown in **Table 3**. For AST 2, ALP 2, and GTP 2, univariate analysis revealed no significant risk factors. For ALT 2, univariate analysis showed that total body irradiation ≥6 Gy tended to be a risk factor (p = 0.082). For GTP 2, univariate analysis showed that a PS of ≥1 tended to be a risk factor (p = 0.055). For Bil 2, univariate analysis indicated that an age of ≥50 years (p = 0.030), a PS of ≥1 (p = 0.031), and number of transplants (p = 0.027) were risk factors, and disease risk (p = 0.091) and hematopoietic cell transplantation-specific comorbidity index score of ≥1 (p = 0.053) were likely risk factors. However, multivariate analysis showed no significant risk factors for Bil 2.

Table 3 Risk factors for early-onset liver dysfunction

Risk Factor	AST 2		ALT 2		ALP 2		GTP 2		Bil 2			
	Univariate		Univariate		Univariate		Univariate		Univariate		Multivariate	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age												
<50	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-
≥50	0.51 (0.15-1.76)	0.289	0.00 (0.00-Inf)	0.992	1.17 (0.18-7.41)	0.870	1.57 (0.60-4.12)	0.357	2.92 (1.11-7.65)	0.030	2.17 (0.74-6.40)	0.160
Sex												
Male	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
Female	0.56 (0.18-1.71)	0.308	0.69 (0.22-2.17)	0.520	0.72 (0.11-4.59)	0.729	1.39 (0.50-3.89)	0.530	1.16 (0.43-3.14)	0.777	-	-
Disease risk												
standard	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-
advanced	0.79 (0.26-2.44)	0.686	0.64 (0.20-2.07)	0.455	2.18 (0.34-13.8)	0.409	1.59 (0.61-4.16)	0.344	2.29 (0.88-5.99)	0.091	1.62 (0.50-5.23)	0.418
PS												
0	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-
≥1	0.51 (0.16-1.64)	0.261	0.40 (0.12-1.38)	0.145	2.00 (0.32-12.7)	0.462	2.57 (0.98-6.74)	0.055	2.88 (1.10-7.53)	0.031	1.40 (0.42-4.71)	0.582
HCT-CI												
0	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-
≥1	0.00 (0.00-Inf)	0.992	0.64 (0.13-3.19)	0.585	3.28 (0.50-21.6)	0.217	2.38 (0.76-7.52)	0.139	3.11 (0.99-9.82)	0.053	2.44 (0.71-8.45)	0.159
CMV antibody												
negative	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
positive	0.35 (0.09-1.26)	0.109	1.44 (0.29-7.26)	0.655	0.83 (0.09-8.00)	0.868	1.14 (0.32-4.06)	0.838	1.22 (0.34-4.34)	0.756	-	-
Blood relationship												
Related	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
Unrelated	3.04 (0.63-14.7)	0.165	1.58 (0.40-6.24)	0.512	0.00 (0.00-Inf)	0.994	1.70 (0.55-5.28)	0.358	0.57 (0.21-1.58)	0.281	-	-
ABO disparity												
matched	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
mismatched	0.64 (0.21-1.96)	0.431	0.73 (0.23-2.28)	0.587	0.27 (0.03-2.53)	0.252	0.87 (0.40-2.25)	0.778	1.24 (0.49-3.15)	0.651	-	-
HLA disparity												
matched	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
mismatched	1.64 (0.55-4.92)	0.378	1.38 (0.45-4.26)	0.576	2.35 (0.37-14.9)	0.365	2.00 (0.77-5.21)	0.154	1.80 (0.70-4.62)	0.222	-	-
GVHD prophylaxis												
CsA-based	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
Tac-based	3.11 (0.65-15.0)	0.157	1.62 (0.41-6.38)	0.493	0.00 (0.00-Inf)	0.994	1.74 (0.56-5.42)	0.336	0.59 (0.21-1.62)	0.302	-	-
Conditioning												
RIC	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
MAC	0.91 (0.28-2.97)	0.874	1.87 (0.48-7.32)	0.371	0.61 (0.10-3.91)	0.603	0.65 (0.24-1.78)	0.403	0.92 (0.33-2.55)	0.877	-	-
TBI												
<6 Gy	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
≥6 Gy	1.67 (0.54-5.11)	0.372	3.01 (0.87-10.4)	0.082	0.21 (0.02-1.95)	0.169	0.61 (0.24-1.58)	0.313	0.55 (0.22-1.41)	0.213	-	-
Donor source												
non-CB	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
CB	1.50 (0.48-4.71)	0.487	1.30 (0.42-4.00)	0.651	2.23 (0.35-14.1)	0.396	1.47 (0.57-3.79)	0.427	2.10 (0.82-5.40)	0.124	-	-
Number of SCT												
1	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
≥2	1.20 (0.23-6.44)	0.828	0.00 (0.00-Inf)	0.990	2.16 (0.21-21.7)	0.515	1.98 (0.48-8.11)	0.342	5.30 (1.21-23.2)	0.027	2.62 (0.52-13.3)	0.244

※OR: odds ratio, CI: confidence interval, PS: performance status, HCT-CI: Hematopoietic Cell Transplantation-specific Comorbidity Index, CMV: cytomegalovirs, GVHD: graft versus host disease, CsA: cyclosporin, Tac: tacrolimus, MTX: methotrexate, MAC: myeloablative conditioning, RIC: reduced-intensity conditioning, TBI: total body irradiation, CB: cord blood, SCT: stem cell transplantation.

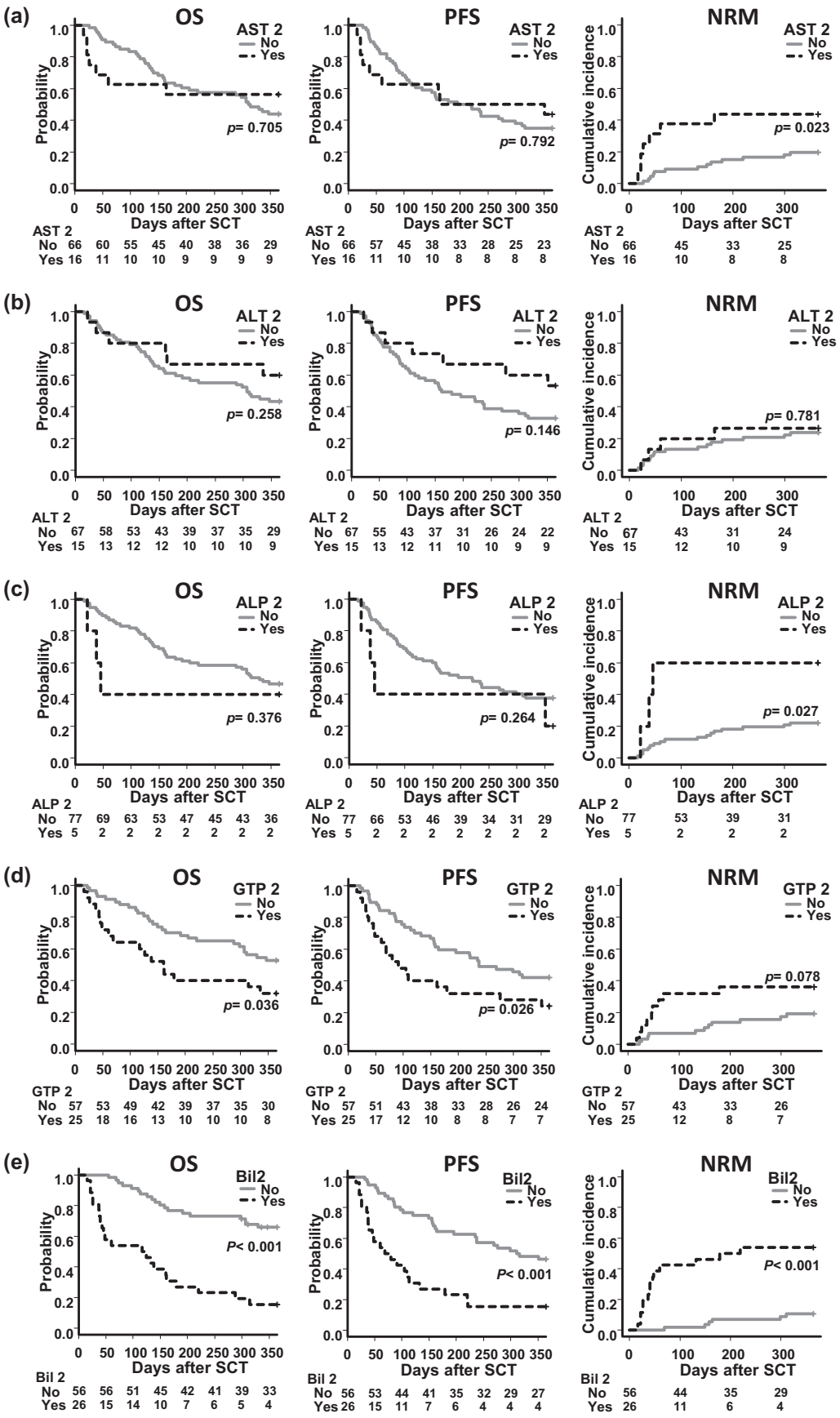

Fig. 2 Prognostic impact of liver dysfunction

Overall survival rate, progression-free survival, and non-relapse mortality rate in patients with five types of liver dysfunction and those without liver dysfunction: (a) AST 2, (b) ALT 2, (c) ALP 2, (d) GTP 2, (e) Bil 2.

Table 4 Prognostic factor analysis of early-onset liver dysfunction

Risk Factor	OS				PFS				NRM			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age												
<50	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
≥50	2.47 (1.37-4.46)	0.002	1.25 (0.62-2.54)	0.534	2.55 (1.47-4.43)	< 0.001	1.38 (0.69-2.75)	0.360	1.53 (0.64-3.66)	0.340	-	-
Sex												
Female	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
Male	1.89 (0.93-3.82)	0.077	1.44 (0.68-3.03)	0.337	1.73 (0.92-3.25)	0.088	1.38 (0.69-2.76)	0.356	0.87 (0.35-2.16)	0.760	-	-
Disease risk												
standard	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
advanced	2.52 (1.39-4.58)	0.002	0.89 (0.42-1.89)	0.952	3.16 (1.80-5.55)	< 0.001	2.12 (1.01-4.46)	0.048	1.20 (0.50-2.87)	0.690	-	-
PS												
0	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-
≥1	6.55 (3.38-12.7)	< 0.001	7.71 (3.20-18.6)	< 0.001	5.27 (2.92-9.51)	< 0.001	2.95 (1.40-6.23)	0.005	2.91 (1.19-7.11)	0.019	2.53 (0.91-7.06)	0.076
HCT-CI												
0	1.00	-	-	-	1.00	-	-	-	1.00	-	-	-
≥1	1.74 (0.88-3.44)	0.112	-	-	1.50 (0.77-2.93)	0.234	-	-	1.16 (0.39-3.46)	0.790	-	-
CMV antibody												
negative	1.00	-	-	-	1.00	-	-	-	1.00	-	-	-
positive	0.79 (0.37-1.70)	0.548	-	-	0.75 (0.37-1.49)	0.406	-	-	0.78 (0.26-2.36)	0.660	-	-
Blood relationship												
Related	1.00	-	-	-	1.00	-	-	-	1.00	-	1.00	-
Unrelated	1.03 (0.53-2.00)	0.930	-	-	0.93 (0.50-1.72)	0.824	-	-	7.94 (1.03-61.2)	0.047	11.0 (0.82-146.6)	0.070
ABO disparity												
matched	1.00	-	-	-	1.00	-	-	-	1.00	-	-	-
mismatched	0.79 (0.44-1.43)	0.441	-	-	0.64 (0.37-1.12)	0.121	-	-	1.21 (0.51-2.87)	0.670	-	-
HLA disparity												
matched	1.00	-	-	-	1.00	-	-	-	1.00	-	-	-
mismatched	1.33 (0.74-2.40)	0.342	-	-	1.06 (0.61-1.84)	0.843	-	-	1.98 (0.83-4.73)	0.120	-	-
GVHD prophylaxis												
CsA-based	1.00	-	-	-	1.00	-	-	-	1.00	-	1.00	-
Tac-based	0.86 (0.45-1.65)	0.651	-	-	0.80 (0.44-1.47)	0.471	-	-	3.50 (0.81-15.2)	0.095	1.08 (0.43-2.74)	0.870
Conditioning												
RIC	1.00	-	-	-	1.00	-	1.00	-	1.00	-	-	-
MAC	0.69 (0.37-1.29)	0.247	-	-	0.55 (0.31-0.98)	0.043	0.61 (0.27-1.37)	0.231	0.69 (0.28-1.73)	0.430	-	-
TBI												
<6 Gy	1.00	-	-	-	1.00	-	1.00	-	1.00	-	-	-
≥6 Gy	0.61 (0.34-1.11)	0.105	-	-	0.51 (0.29-0.89)	0.017	0.98 (0.47-2.05)	0.949	0.72 (0.30-1.72)	0.460	-	-
Donor source												
non-CB	1.00	-	-	-	1.00	-	-	-	1.00	-	1.00	-
CB	1.70 (0.95-3.05)	0.077	-	-	1.22 (0.70-2.11)	0.480	-	-	3.10 (1.26-7.64)	0.014	0.94 (0.29-3.01)	0.910
Number of SCT												
1	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
≥2	2.59 (1.15-5.84)	0.021	0.92 (0.34-2.48)	0.864	3.00 (1.45-6.24)	0.003	1.18 (0.50-2.80)	0.708	2.57 (0.83-7.94)	0.100	-	-

Table 4 Prognostic factor analysis of early-onset liver dysfunction (Continued)

Risk Factor	OS				PFS				NRM			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
AST 2												
No	1.00	-	-	-	1.00	-	-	-	1.00	-	1.00	-
Yes	0.86 (0.38-1.92)	0.705	-	-	0.91 (0.44-1.87)	0.792	-	-	2.96 (1.15-7.57)	0.024	3.38 (1.07-10.7)	0.038
ALT 2												
No	1.00	-	-	-	1.00	-	-	-	1.00	-	-	-
Yes	0.61 (0.26-1.45)	0.264	-	-	0.56 (0.25-1.24)	0.153	-	-	1.17 (0.39-3.51)	0.780	-	-
ALP 2												
No	1.00	-	-	-	1.00	-	-	-	1.00	-	-	-
Yes	1.69 (0.52-5.46)	0.382	-	-	1.77 (0.64-4.93)	0.272	-	-	4.31 (1.11-16.7)	0.034	1.40 (0.29-6.83)	0.680
GTP 2												
No	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
Yes	1.89 (1.03-3.46)	0.039	0.72 (0.32-1.63)	0.434	1.88 (1.07-3.32)	0.029	1.13 (0.53-2.41)	0.756	2.22 (0.92-5.33)	0.075	0.53 (0.19-1.49)	0.230
Bil 2												
No	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-
Yes	4.23 (2.33-7.68)	< 0.001	5.14 (2.33-11.3)	< 0.001	3.10 (1.77-5.43)	< 0.001	2.43 (1.18-5.02)	0.017	7.49 (3.00-18.7)	< 0.001	14.3 (4.87-41.9)	< 0.001

※OS: overall survival, PFS: progression free survival, NRM: non-relapse mortality, HR: hazard ratio, CI: confidence interval, PS: performance status, HCT-CI: Hematopoietic Cell Transplantation-specific Comorbidity Index, CMV: cytomegalovirus, GVHD: graft versus host disease, CsA: cyclosporin, Tac: tacrolimus, MAC: myeloablative conditioning, RIC: reduced-intensity conditioning, TBI: total body irradiation, CB: cord blood, SCT: stem cell transplantation.

Liver Dysfunction and Prognosis

We analyzed the effect of liver dysfunction on outcomes (**Fig. 2**). Patients with GTP 2 (OS: $p = 0.036$, PFS: $p = 0.026$) and Bil 2 (OS: $p < 0.001$, PFS: $p < 0.001$) had significantly worse OS and PFS. Patients with GTP 2 ($p = 0.078$) tended to have higher NRM, while patients with AST 2 ($p = 0.023$), ALP 2 ($p = 0.027$), and Bil 2 ($p < 0.001$) had significantly higher NRM. Multivariate analysis of prognostic factors identified Bil 2 as an unfavorable independent prognostic factor for OS and PFS (OS: $p < 0.001$, PFS: $p = 0.017$), while AST 2 ($p = 0.038$) and Bil 2 ($p < 0.001$) were unfavorable independent prognostic factors for NRM (**Table 4**).

Stratified Prognostic Analysis of T-Bil Elevation

As shown in **Figure 2** and **Table 4**, T-Bil elevation was significantly associated with worse outcomes. Stratified analysis of the prognostic impact of T-Bil elevation was therefore performed in order to analyze maximum T-Bil value, presence or absence of improvement, and time of onset.

Prognostic Impact of Maximum T-Bil

The 26 patients with Bil 2 were divided into three groups according to maximum T-Bil-max T-Bil, low: 1.9-2.9 mg/dL (n = 11); intermediate: 3.0-9.9 mg/dL (n = 11); and high: ≥10 mg/dL (n = 4). Further analysis showed that higher maximum values were associated with worse survival rates (OS: max T-Bil at 100 days—low: 81.8%; intermediate: 45.5%; high: 0%; $p = 0.002$; PFS: max T-Bil at 100 days—low: 63.6%; intermediate: 36.4%; high: 0%; $p = 0.004$, **Fig. 3a**). Similarly, higher maximum values were associated with higher NRM values (NRM: max T-Bil at 100 days-low: 9.1%; intermediate: 54.5%; high: 100%; $p = 0.001$, **Fig. 3a**).

Prognostic Impact of Bil 2 Onset Time

The 26 patients with Bil 2 were divided into three groups according to onset time, as follows: Day 0-5 (n = 8), Day 6-15 (n = 11), and Day 16-30 (n = 8). OS tended to be low in the groups with early and late onset and high in the group with intermediate onset (OS at Day 100—Day 0-5: 37.5%; Day 6-15: 81.8%; Day 16-30: 37.5%; $p = 0.100$, **Fig. 3b**). PFS showed no significant difference in relation to onset time (PFS at Day 100—Day 0-5: 37.5%; Day 6-15: 45.5%; Day 16-30: 37.5%; $p = 0.721$, **Fig. 3b**). Although NRM did not significantly differ in relation to onset time, it was highest in the group with early onset, followed by the groups with late onset and intermediate onset (NRM at Day 100—Day 0-5: 62.5%; Day 6-15: 18.2%; Day 16-30: 50.0%; $p = 0.129$, **Fig. 3b**).

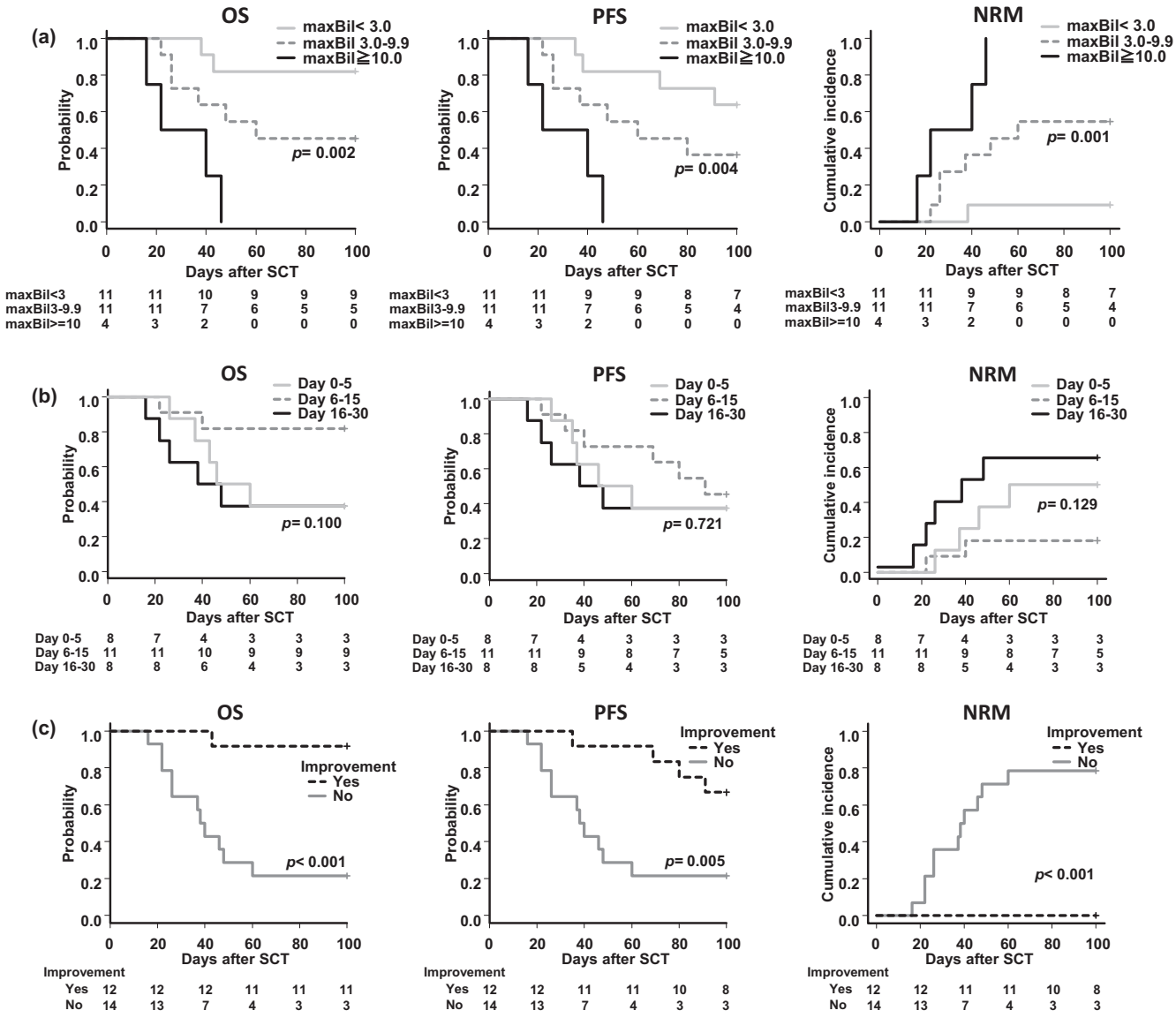

Fig. 3 Stratified prognostic analysis of T-Bil elevation

(a) Prognostic impact of maximum T-Bil value: Comparison of overall survival rate, progression-free survival, and non-relapse mortality rate in 26 patients with Bil 2, divided into three groups according to maximum T-Bil value-max T-Bil low: 1.9-2.9 mg/dL (n = 11); intermediate: 3.0-9.9 mg/dL (n = 11); high: ≥10 mg/dL (n = 4).

(b) Prognostic impact of Bil 2 onset time: Comparison of overall survival rate, progression-free survival, and non-relapse mortality rate in 26 patients with Bil 2, divided into three groups according to onset time-Day 0-5 (n = 8); Day 6-15 (n = 11); Day 16-30 (n = 8).

(c) Prognostic impact of presence or absence of Bil 2 improvement: Comparison of overall survival rate, progression-free survival, and non-relapse mortality rate in 26 patients with Bil 2, divided into those that improved (n = 12) by Day 30 after transplantation and those that did not (n = 14).

Prognostic Impact of Bil 2 Improvement

To compare outcomes, the 26 patients with Bil 2 were divided into those that improved by Day 30 after HSCT (n = 12) and those that did not (n = 14). OS and PFS were significantly better in the improved group than in the unimproved group (OS at Day 100: 91.7% vs. 21.4%; $p < 0.001$; PFS at Day 100: 66.7% vs. 21.4%; $p = 0.005$, **Fig. 3c**). NRM was significantly lower in the improved group than in the unimproved group (0% vs. 78.6%; $p = <$ 0.001, **Fig. 3c**).

Discussion

Early elevation of liver and biliary enzymes after allogeneic HSCT has numerous causes. The present study focused on identifying prognostic factors while damage is manageable. Liver dysfunction was defined as Grade 2 or higher elevation of liver or biliary enzymes (according to CTCAE version 4.0) within 30 days of transplantation.

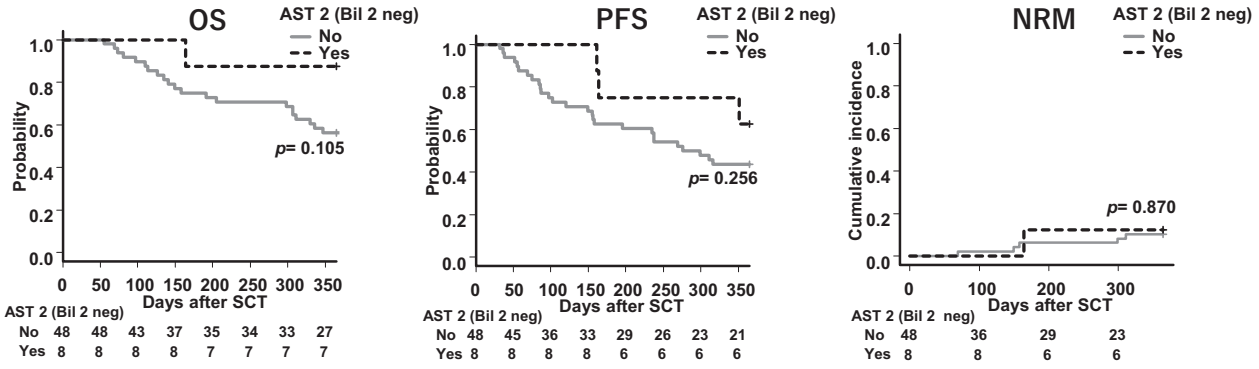

Fig. 4 Prognostic impact of AST 2 on patients without Bil 2

To accurately assess the prognostic impact of AST 2, stratified analysis was carried out by excluding cases of Bil 2, which has a major prognostic impact. Excluding the 26 cases of Bil 2 from the total of 82 patients, the remaining 56 were divided into those with (n = 8) and without AST 2 (n = 48) for comparison of overall survival rate, progression-free survival, and non-relapse mortality rate.

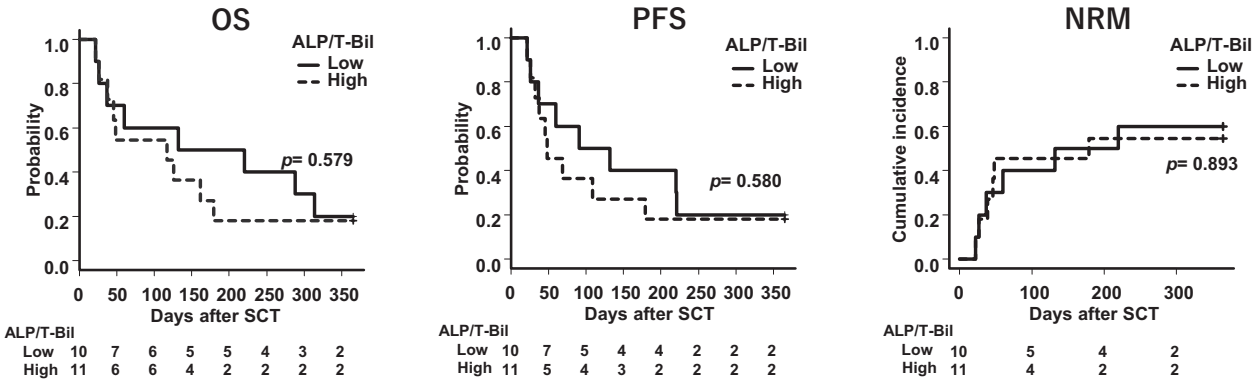

Fig. 5 Prognostic impact of ALP/T-Bil ratio

Excluding the five cases in which ALP and T-Bil were not simultaneously measured, the 21 patients with Bil 2 were divided into those with a low ALP/T-Bil ratio (<124; n = 10) and those with a high ALP/T-Bil ratio (≥124; n = 11) for comparison of overall survival rate, progression-free survival, and non-relapse mortality rate.

In the early period after HSCT, liver or biliary enzymes were elevated in almost half of the present patients. In other words, AST, ALT, ALP, γ-GTP, or T-Bil reached CTCAE Grade 2 or higher. T-Bil elevation was most frequent and most strongly associated with worse OS, PFS, and NRM. The extent of T-Bil elevation and the presence or absence of improvement allowed for significant prognostic stratification.

Numerous studies have investigated liver dysfunction after allogeneic HSCT, and many have focused on hyperbilirubinemia, which was reported to be more frequently associated with MAC than with RIC[8]. Moreover, hyperbilirubinemia patients with a T-Bil level of 4 mg/dL or higher[8,9] and patients with liver dysfunction onset at 28 days or more after HSCT were reported to have unfavorable outcomes[8]. Other factors associated with unfavorable prognosis are AST elevation and high ALP/T-Bil ratio. AST elevation is thought to be caused by reduced oxygen supply to hepatocytes and can be described as hypoxic hepatitis due to SOS, hypoxemia, shock, or related causes. Marked AST elevation (≥30 fold) was associated with high mortality (77%)[10]. In patients with a low ALP/T-Bil ratio at the onset of T-Bil elevation, the most frequent cause of liver dysfunction was drug-induced damage related to CyA. Outcomes were better for those patients than for those with a high ALP/T-Bil ratio (≥124)[11].

In agreement with previous research, the results of our study indicate that NRM was significantly increased and OS significantly reduced in patients with hyperbilirubinemia. The extent of T-Bil elevation and the presence or absence of improvement also allowed for useful prognostic stratification. In addition to hyperbilirubinemia, AST elevation was found to be an unfavorable prognostic factor associated with high NRM, but when patients with hyperbilirubinemia were excluded, stratified analysis showed no difference in outcomes in relation to presence

of AST elevation (**Fig. 4**). In addition, we found no difference between groups with a low and high ALP/TB ratio in relation to improvement in T-Bil, or OS or NRM (**Fig. 5**). Our results indicate that among variables of early liver and biliary enzyme elevation after allogeneic HSCT, T-Bil elevation is the most important prognostic factor. An intermediate group, with a T-Bil value of 4 mg/dL to <7 mg/dL, and a high-value group, with T-Bil values of ≥7 mg/dL, had unfavorable outcomes, but the difference was not significant. In the present study, however, it was possible to use maximum T-Bil value to stratify patients into three groups. This indicates that, at values of 3 mg/dL or higher, a higher T-Bil level indicates worse prognosis. Moreover, patients with improvement in T-Bil by Day 30 after HSCT had better outcomes, which suggests that early treatment improves outcomes.

Liver dysfunction within 30 days of HSCT has a range of causes, but differential diagnosis is not usually possible because the cases since liver biopsy is difficult owing to the risks of infection and hemorrhage. Thus, treatment may be delayed. Because of this, we defined liver dysfunction solely in terms of blood test variables and analyzed their prognostic impact. T-Bil elevation was an important unfavorable prognostic factor, and greater elevation was associated with worse outcomes. Future studies should investigate risk factors of T-Bil elevation and develop effective preventive and treatment methods. As it is likely that the cases of liver dysfunction described in this study included a fair number of undiagnosed SOS/VOD cases, we would like to actively consider the usefulness of FFP, recombinant human soluble thrombomodulin, defibrotide, and similar agents in future studies.

Author Contributions: IO and HY were the principal investigators and take primary responsibility for the manuscript. KI, DO, AM, SY, MS, YF, MO, SW, MO, HT, KN, SY, and KI recruited the study patients. IO, HY, and TH analyzed the data and wrote the manuscript. IO and HY contributed equally to the study.

Conflict of Interest: The authors declare no conflicts of interest to declare.

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