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ORIGINAL ARTICLE

Phase II study of tacrolimus and methotrexate for prophylaxis of acute graft-versus-host disease after HLA-A, B, and DRB1 genotypically mismatched unrelated bone marrow transplantation among Japanese patients

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Abstract Bone marrow transplantation from unrelated donors (UR-BMT) has been considered to be effective for patients with hematological malignancies who have no suitable related donor. However, disparities of HLA between a recipient and a donor increase the risk of severe acute graft-versus-host disease (GVHD). We evaluated GVHD prophylaxis using tacrolimus and methotrexate for HLA-A, B, or DRB1 genotypically mismatched UR-BMT. Fifty-five patients were enrolled in this study. The incidence of grade III to IV acute GVHD was 23.6% for all patients. No significant difference in the incidence of grade III to IV acute GVHD was observed between HLA-A or B 1 locus mismatch transplantation (18.8%) and HLA-DRB1 1 locus mismatch transplantation (16.7%) ($P = 0.96$). The incidence of chronic GVHD was 71.7%. Disease-free survival at 5 years was 53.2% for patients with standard risk disease and 24.5% for patients with high-risk disease. Patients with chronic GVHD exhibited better disease-free survival than those without chronic GVHD (53.2 vs. 30.9%, $P = 0.011$). Twenty patients (36.4%) had a relapse of leukemia and 14 of them died of recurrent leukemia. This study indicates tacrolimus and methotrexate can lower the risk of severe acute GVHD after HLA-A, B, or DRB1 genotypically 1 locus mismatched UR-BMT.

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Tacrolimus · GVHD prophylaxis · HLA mismatched UR-BMT

Acute graft-versus-host disease (GVHD) is one of the most common causes of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT) [1, 2]. Although bone marrow transplantation from unrelated donors (UR-BMT) has been established as an effective treatment for patients with hematological malignancies who have no suitable related donor, the control of GVHD is indispensable to successful outcome after UR-BMT [3–5].

Tacrolimus has been shown to be more potent as an immunosuppressant than cyclosporine since its discovery in 1984 [6]. It was initially demonstrated to be effective for the treatment and prevention of rejection in solid-organ transplantations [7, 8]. Tacrolimus was also reported to be effective in the treatment and prevention of GVHD after allogeneic BMT [9–12].

In several clinical studies containing UR-BMT, the incidence of grade II to IV acute GVHD was 21–56% in patients who received tacrolimus and methotrexate for the prevention of acute GVHD[13–17]. Multicenter randomized studies have shown that tacrolimus is more effective in preventing GVHD than cyclosporine, which has been used commonly for GVHD prophylaxis [16, 17]. Despite the lower rate of GVHD, tacrolimus has exhibited no survival benefits, compared with cyclosporine [16, 17]. In these clinical studies, most patients received stem cells from an HLA matched unrelated donor. The risk of GVHD after HLA-mismatched UR-BMT is higher than after HLA-matched UR-BMT because immunological events such as GVHD are affected by the HLA disparity between a patient and a donor [18–21]. We conducted a phase II study to evaluate tacrolimus and methotrexate for the prevention of GVHD in recipients of marrow transplants from an HLA-A, B, or DRB1 genotypically mismatched unrelated donors.

2.1 Patients

From August 1999 to August 2001, 55 patients were enrolled in this study at 27 transplantation centers in Japan. The study was approved by the institutional review board at each center and all patients gave written informed consent for participation in the study. Eligible patients had leukemia or myelodysplastic syndrome (MDS), and had an HLA-A, B, or DRB1 genotypically mismatched unrelated donor. Patients were required to be between the ages of 16 and 50 years old, to have an Eastern Cooperative Oncology Group performance status of 0, 1 or 2, and to have adequate hepatic function (total bilirubin less than 1.5 mg/dl and transaminase ≤ 2 upper normal limit), renal function (serum creatinine less than 1.2 mg/dl and estimated creatinine clearance greater than 60 ml/min), cardiac function (ejection fraction greater than 50%) and pulmonary function (arterial oxygen pressure greater than 70 mmHg). Patients were ineligible if they fulfilled one of the following exclusion criteria: (1) a previous transplantation, (2) use of T cell-depleted marrow, (3) use of anti-thymocyte globulin (ATG) for preparative regimen, (4) any history of severe cardiac disease, pancreatitis, diabetes mellitus, hyperkalemia or hypertension necessitating medication, (5) any infection including hepatitis B and hepatitis C, human immunodeficiency virus or syphilis, and (6) pregnant or lactating women.

2.2 Study design

The primary endpoint of this study was the incidence of grade III to IV acute GVHD. The secondary endpoints were the incidence of chronic GVHD, survival, disease-free survival, relapse and complications. Sample size was derived to ensure that the 95% confidential interval (CI) of the point estimation of the incidence of grade III–IV acute GVHD, which was set to be 15%, did not exceed 10%. The historical incidence in case of cyclosporine was 30% [18].

2.3 Treatment protocol

Patients received bone marrow from an HLA-A, B, or DRB1 genotypically mismatched unrelated donor on day 0, following myeloablative chemoradiotherapy. Preparative regimens and supportive care were assigned according to each institutional protocol at the clinical sites.

All patients received tacrolimus and methotrexate for GVHD prophylaxis. Tacrolimus was administered by 24-h continuous intravenous infusion at a dose of 0.03 mg/kg beginning on day-1. When patients were able to tolerate oral intake, the route of administration was switched from intravenous to oral at the ratio of 1:3 in two divided doses per day based on the intravenous dose at the time of conversion.

Methotrexate was given at 10 mg/m^2 intravenously on day 1 and 7 mg/m^2 on days 3 and 6.

Whole blood level of tacrolimus was measured twice a week during the first 4 weeks posttransplantation and weekly thereafter by EIA method. The target blood level was determined as 15 ng/ml and the dose was adjusted so as not to exceed 20 ng/ml. Unless patients had evidence of GVHD after day 50, the tacrolimus dose was tapered by 10% every 2 weeks. If the serum creatinine increased to

levels above 1.5 upper normal limit, tacrolimus was temporarily withheld. When the serum creatinine lowered to levels below 1.5 upper normal limit, tacrolimus was resumed at the dose of 25% reduction.

In principle, patients who developed grade II to IV acute GVHD were treated initially with prednisolone or methylprednisolone at the dose of 1–2 mg/kg. When acute GVHD was not controlled, secondary treatment was assigned according to treatment protocols at each site.

2.4 Assessment of GVHD

Acute GVHD was diagnosed and graded by clinicians at each institution according to the consensus criteria [22]. The clinical and laboratory parameters used to assess the grade of acute GVHD included the percentage of body surface area with skin rash, the volume of diarrhea, total bilirubin, and Karnofsky's performance status. Chronic GVHD was categorized as limited type or extensive type [23]. Tissue biopsy samples were obtained to confirm the diagnosis of GVHD as much as possible. The response of treatment for acute GVHD was evaluated based on previously described criteria [24].

2.5 Statistics

Standard risk disease was defined as acute leukemia in first complete remission, chronic myelogeneous leukemia (CML) in first chronic phase and MDS classified as refractory anemia, whereas high-risk disease was defined as a more advanced status than standard risk disease. The time to develop acute GVHD was determined from the date of transplantation. Patients who did not develop acute GVHD were censored at the date of their last contact, death or relapse, whichever occurred first. Overall survival was calculated from the date of transplantation to the date of death. Disease-free survival was calculated from the date of transplantation to the date of death or relapse, whichever occurred first. Patients alive at the date of last contact were censored. The Kaplan–Meier method was used to estimate the incidences of GVHD, survival, and relapse. values of less than 0.05 were regarded as statistically significant. The grade of adverse events was assessed according to National Cancer Institute Common Toxicity Criteria version 2.0.

3.1 Patient characteristics

Fifty-five patients were enrolled in the study between August 1999 and August 2001. Patients characteristics are summarized in Table 1. All patients received bone marrow from an HLA-A, B, or DRB1 genotypically mismatched unrelated donor. In 50 patients, there were no HLA-A, B or DR serological disparities. Five patients underwent an HLA-DR serological 1 locus mismatched transplantation. Four of them received bone marrow matched for broad specificities at HLA-DR loci, and the other one received bone marrow matched in GVHD direction and mismatched in rejection direction. The median follow-up period for surviving patients was 1,629 days.

Patients characteristics	
No. of patients	55
Median patient age, years (range)	33 (16–50)
Median donor age, years (range)	33 (20–51)
Sex (donor/recipient), (%)	
Male/male	21
Male/female	14
Female/male	15
Female/female	5
HLA disparity, (%)	
Class I DNA 1 locus mismatch	16 (29.1%)
Class II DNA 1 locus mismatch	24 (43.6%)
≧DNA 2 loci mismatch	10 (18.2%)
Class II serological 1 locus mismatch	5 (9.1%)
Diagnosis, (%)	
Acute myelogeneous leukemia	19 (34.5%)
Acute lymphoblastic leukemia	10 (18.2%)
Chronic myelogeneous leukemia	20 (36.4%)
Myelodysplastic syndrome	6 (10.9%)
Disease status, (%)	
Standard risk disease[a]	24 (43.6%)
High-risk disease[b]	31 (56.4%)
Preconditioning, (%)	
TBI regimen	52
Non-TBI regimen	3

total body irradiation

[a] Acute leukemia in first complete remission, chronic myelogenous leukemia in first chronic phase and myeloid displastic syndrome in refractory anemia

[b] More advanced status than standard risk leukemia

3.2 Adverse events

Nephrotoxicity occurred in 20 patients (36.4%) within 100 days post-transplantion. Most of them developed grade 2 or less, and only two patients developed grade 3 or 4 nephrotoxicity. Grade 3 or 4 hepatotoxicity developed in six patients (10.9%). Veno-occlusive disease occurred in three (5.5%) patients. Eight patients (14.5%) had a neurological adverse event. Twenty-nine patients had at least one documented infection during the first 100 days.

Tacrolimus was discontinued in 14 patients (25.5%) due to adverse effects within 100 days post-transplantion. The causes of discontinuance are shown in Table 2.

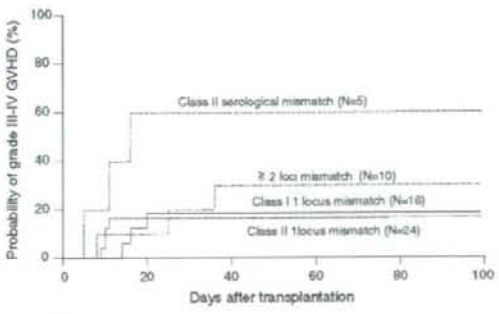
The mean peak of the tacrolimus blood level in patients who discontinued the administration was 20.8 ng/ml, against 23.7 ng/ml in patients who continued tacrolimus.

3.3 Acute GVHD

The incidence of grade III to IV acute GVHD was 23.6% for all patients. Seven (17.5%) of 40 patients who received bone marrow from an HLA-A, B or DRB1 1 locus genotypically mismatched donor developed grade III to IV acute GVHD. There was no significant difference between the incidence of grade III to IV acute GVHD for patients who underwent an HLA-A or B 1 locus mismatch transplant (18.8%) and that for patients who underwent an HLA-DRB1 mismatch transplant (16.7%) (0.96) (Fig. 1). Of ten recipients from an HLA two or more loci mismatched donor, three (30%) developed grade III to IV acute GVHD. Three (60%) of five recipients who underwent an HLA-DR serological 1 locus mismatched transplantation developed grade III to IV acute GVHD.

Thirty-three patients received prednisolone or methylprednisolone to treat grade II to IV acute GVHD initially. Twenty-one (64%) and six (18%) of them had a complete response and a partial response, respectively. Secondary treatment such as ATG and steroid pulse therapy was needed for ten patients.

Cause of tacrolimus discontinuance	
Thrombotic microangiopathy	4
Neurological disorder	3
CMV infection	3
Nephrotoxicity	2
Veno-occlusive disease	1
Hepatotoxicity	1



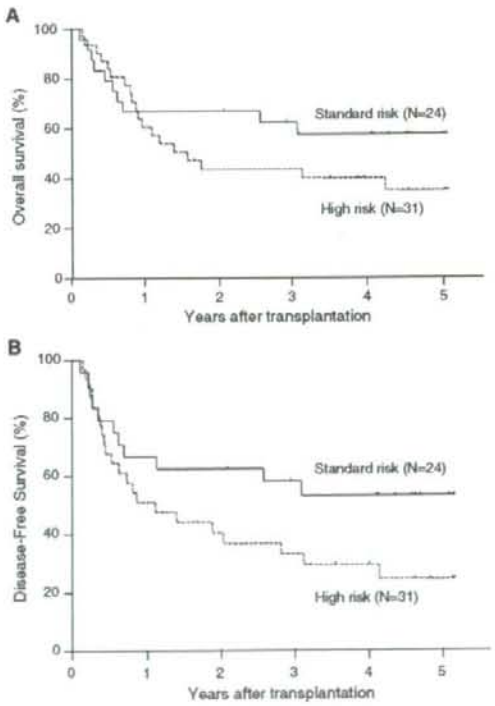
Proportion of patients developing grade III–IV acute GVHD

3.4 Chronic GVHD

Of 55 patients, 46 were evaluable for chronic GVHD. Ten and 18 patients developed limited and extensive chronic GVHD, respectively. Based on Kaplan–Meier estimates, the overall incidence of chronic GVHD was 71.7%.

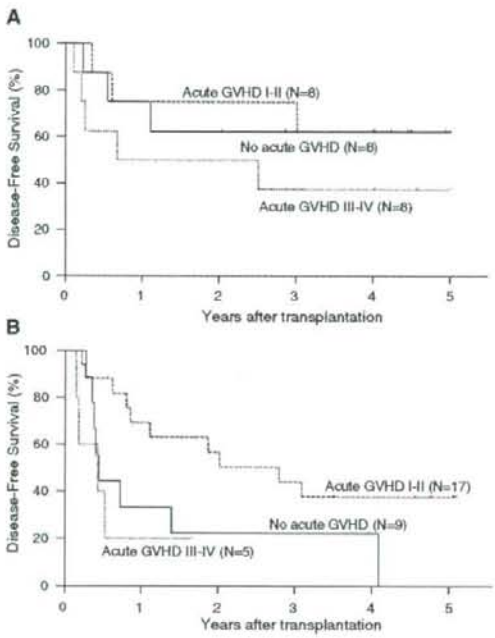
3.5 Relapse and survival

Early death within 100 days after transplant occurred in five patients (9.1%), all of who developed grade III or IV acute GVHD. Overall survival at 5 years was 57.4% for patients with standard risk disease and 34.8% for patients with high-risk disease (Fig. 2a). The primary causes of death are listed in Table 3. Overall, 14 (48%) out of 29 patients died of relapse. Twenty patients (36.4%) had a relapse of leukemia and the median period for relapse was 239 days (77–1,469 days). Leukemia recurred in 11 out of 33 patients who received prednisolone or methylprednisolone for the treatment of acute GVHD. Disease-free survival at 5 years was 53.2% for patients with standard



Probability of overall survival ( ) and disease-free survival ( ) based on disease status at transplantation

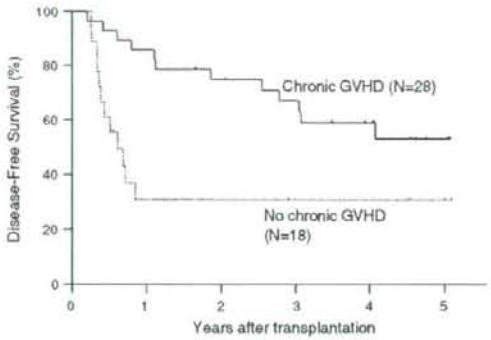
Primary causes of death	
High risk disease	19
Relapse (%)	12 (63)
GVHD (%)	1 (5)
Infection (%)	3 (16)
Others (%)	3 (16)
Standard risk disease	10
Relapse (%)	2 (20)
GVHD (%)	3 (30)
Infection (%)	2 (20)
Others (%)	3 (30)



Probability of disease-free survival based on the grade of acute GVHD in patients with standard risk disease ( ) and in patients with high-risk disease ( )

risk disease and 24.5% for patients with high-risk disease (Fig. 2b).

The effects of acute and chronic GVHD on disease-free survival were examined. No association between acute GVHD and disease-free survival was observed in patients with standard risk disease (Fig. 3a). In patients with high-risk disease, disease-free survival was higher for patients with grade I-II acute GVHD, compared with that for patients without acute GVHD (37.8 vs. 22.2%, 0.055; Fig. 3b). Disease-free survival was significantly higher for



Disease-free survival in patients with or without chronic GVHD

patients who developed chronic GVHD than for those who did not (53.2 vs. 30.9%, 0.011; Fig. 4).

3.6 Retrospective comparison with historical control

To compare tacrolimus with cyclosporine regarding GVHD prophylaxis, we analyzed 60 patients who received cyclosporine and short term MTX for GVHD prophylaxis and fulfilled the following criteria: (1) HLA-A, B, or DRB1 genotypically mismatched unrelated BMT, (2) first transplantation conducted between 1997 when HLA DNA typing started for a donor selection, and 2001 when the enrollment of patients in our study finished, (3) disease: leukemia or MDS, (4) age: 16–50, (5) not use of T cell-depleted marrow, (6) not use of ATG for preparative regimen. There were 35 males and 25 females with a median age of 32 years (range 16–50 years). The underlying disease was acute leukemia in 34 (56.7%), CML in 20 (33.3%), and MDS in 6 (20%). The disease status was standard risk disease in 26 (43.3%) and high-risk disease in 34 (56.7%). Forty-six patients (76.7%) received conditioning regimens with total body irradiation. Twelve (20%) and 35 (58.3%) patients underwent an HLA-A or B and HLA-DRB1 genotypically 1 locus mismatched transplantation, respectively. Thirteen patients (21.7%) received bone marrow from an HLA two or more loci mismatched donor.

The incidence of grade III to IV acute GVHD was 31.3% for all patients in the historical control. Cyclosporine exhibited significantly higher incidence of grade III to IV acute GVHD was 58.3% for patients who underwent an HLA-A or B 1 locus mismatch transplant than tacrolimus (58.3 vs. 18.8%, 0.034). However, Cyclosporine and tacrolimus showed similar incidence of grade III to IV acute GVHD for patients who underwent an HLA-DRB1 1 locus mismatch transplant (16.6 vs. 16.7%, 0.93).

Since GVHD is one of the most common and life-threatening complications after allogeneic HSCT, the control of GVHD is indispensable to successful outcome after HSCT. Although the combination of cyclosporine and methotrexate is most commonly used for the prevention of acute GVHD [25], tacrolimus has been noted to be highly immunosuppressive and a potent alternative to cyclosporine [9–17, 26]. Nephrotoxicity is one of the most common problems related to tacrolimus. In previous studies, the incidence of peak serum creatinine levels greater than 2 mg/dL was 50–60% [14, 17, 27]. Meanwhile, 20 patients (36.4%) had nephrotoxicity and only two developed grade 3 or 4 nephrotoxicity in this study. The association between an increased incidence of nephrotoxicity and an increasing tacrolimus blood level that exceeded 20 ng/ml was observed in both HLA-matched sibling BMT and UR-BMT [28, 29]. The lower incidence of nephrotoxicity in this study may result from the lower target blood level of tacrolimus. Two previous reports did not show a relationship between the blood concentration of tacrolimus and the occurrence of acute GVHD [28, 29]. In a prospective randomized study, however, the use of a lower blood level (4–6 ng/ml) of tacrolimus was associated with a higher rate of acute GVHD, compared with that of a standard blood level (8–12 ng/ml) [30]. Although we determined the starting dose and the target blood level of tacrolimus as 0.03 mg/kg and 15 ng/ml, respectively, the mean peak of the tacrolimus blood level was over 20 ng/ml, suggesting that the starting dose should be reduced to 0.025–0.02 mg/kg. The peak of tacrolimus blood level did not differ between patients who discontinued or continued administration of tacrolimus. Thus, careful observations of other than tacrolimus blood level will be required for early detection of toxicities related to tacrolimus.

UR-BMT has been established as an effective treatment for patients with hematological malignancies who have no suitable related donor [3–5], and HLA mismatched UR-BMT has been considered acceptable for patients lacking an HLA matched unrelated donor. However, the disparity of HLA between the patient and the donor increases the risk of GVHD [18–21]. The present study also indicated that HLA multiple disparities induced a higher incidence of severe acute GVHD, compared with HLA 1 locus mismatch. Despite the small number of HLA-DR serological 1 locus mismatched transplantation cases, they showed a high incidence of severe acute GVHD, as previously reported [21]. The Japan Marrow Donor Program (JMDP) demonstrated that HLA-DRB1 disparity was less responsible for acute GVHD than HLA class I disparity from analysis of 1,298 recipients, most of whom received cyclosporine-based GVHD prophylaxis [19]. Our study did not detect a significant difference in the incidence of grade III to IV acute GVHD between an HLA-A or B mismatch and HLA-DRB1 mismatch (Fig. 1) suggesting that tacrolimus can reduce the risk of severe acute GVHD after an HLA-A or B 1 locus mismatch transplant as much as after an HLA-DRB1 1 locus mismatch transplant. Furthermore, we analyzed historical control used cyclosporine instead of tacrolimus for GVHD prophylaxis to compare the effect of tacrolimus on GVHD prophylaxis with that of cyclosporine. Although it was not the matched case control, the incidence of grade III to IV acute GVHD after HLA-A or B 1 locus mismatch transplant was much higher. After HLA-DRB1 1 locus mismatch transplantation, however, cyclosporine showed the similar incidence of grade III to IV acute GVHD (16.6%) to tacrolimus in our study (16.7%), suggesting that a randomized study to compare tacrolimus with cyclosporine for GVHD prophylaxis after HLA-DRB1 1 locus mismatch transplants is worthy to be conducted.

Although tacrolimus has shown to be a more effective immunosuppressant than cyclosporine, about 20% of patients who underwent HLA genotypically mismatched UR-BMT suffered from severe acute GVHD. New agents such as mycophenolate mofetil [31] and sirolimus [32] have been used for GVHD prophylaxis after UR-HSCT. More efficient GVHD prophylaxis should be established to lower the incidence of severe acute GVHD.

Relapse of leukemia was most often the primary cause of death in this study. A lesser probability of leukemia relapse in recipients with GVHD has been demonstrated than in those without GVHD, indicating that GVHD is associated with graft-versus-leukemia (GVL) effect [33–35]. We did not find any association between acute GVHD and disease-free survival in patients with standard risk disease. In patients with high-risk disease, however, disease-free survival was higher in patients with grade I-II acute GVHD than in patients without acute GVHD (Fig. 3b). Furthermore, patients who developed chronic GVHD showed significantly higher disease-free survival than patients without it (Fig. 4). In our study, 11 patients had a relapse of leukemia after the treatment of acute GVHD, suggesting that more intensive immunosuppressive therapies for GVHD wipe out the GVL effect and augment the risk of recurrent leukemia, as previously reported [35]. It has been shown that the GVL effect could be separable from GVHD [36, 37]. Alemtuzumab has shown the ability to prevent GVHD without inhibition of the GVL effect [38]. Furthermore, HLA allele mismatch combinations are being analyzed in JMDP, and high-risk combinations for severe acute GVHD have been clarified [39]. More detailed analysis may lead to better donor selection with low-risk HLA allele for severe GVHD and HLA allele expected the GVL effect.

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CLINICAL STUDY

Tear function and lipid layer alterations in dry eye patients with chronic graft-*vs*-host disease

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Abstract

Purpose **To investigate the changes in the tear film lipid layer in haematopoietic stem cell transplantation (HSCT) patients with dry eye (DE) associated with chronic graft-*vs*-host disease (cGVHD) and compare with HSCT recipients without DE.**

Methods **We performed a prospective study in 10 HSCT patients with DE associated with cGVHD and 11 HSCT recipients without DE. We performed Schirmer's test, tear film break up time examinations, ocular surface dye staining and meibum expressibility test and DR-1 tear film lipid layer interferometry. DR-1 interferometry images of the tear film surface were assigned a 'DR-1 grade' according to the Yokoi severity grading system. The DR-1 grades were analysed according to the presence or absence of DE, conjunctival fibrosis and systemic cGVHD.**

Results **The mean DR-1 severity grade in patients with DE related to cGVHD (DE/cGVHD group; 3.9 ± 0.9) was significantly higher than in patients without DE after HSCT (non-DE/non-cGVHD group; 1.3 ± 0.6; $P < 0.05$). The DR-1 grade for HSCT recipients with conjunctival fibrosis was significantly higher than in patients without conjunctival fibrosis ($P < 0.05$). When DE severity was graded according to the recommendation of the 2007 Dry Eye Workshop Report, our results showed a correlation between the severity of DE and DR-1 grades ($r = 0.8812$, $P < 0.0001$).**

Conclusion **DR-1 interferometry may be applicable to diagnosing DE and evaluating its progression subsequent to HSCT.**

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Keywords: tear interference; tear lipid layer; dry eye; conjunctival fibrosis; chronic GVHD; bone marrow transplantation

Introduction

Dry eye (DE) is a major complication of chronic graft-*vs*-host disease (cGVHD) and has a significant impact on the quality of life.[1–6] The precorneal tear lipid layer controls tear evaporation, and DE after haematopoietic stem cell transplantation (HSCT) may be associated with changes in the tear lipid layer, like other types of DE or other ocular surface disorders.[7,8] The Schirmer's test is the standard method for diagnosing DE associated with cGVHD, but is invasive, may cause irritation and reflex tearing, and may produce false-negative or false-positive results.[9] Noninvasive tear lipid layer interferometry is a useful method for evaluating the severity of DE.[7–12] However, no report on the tear lipid layer changes with cases of DE associated with cGVHD has been published to date.

In this study, we used DR-1 tear lipid layer interferometry to investigate and classify the tear film lipid layer interference patterns in patients with DE because of cGVHD, and compared the results with those from patients who did not develop DE after HSCT.

Materials and methods

In this prospective and comparative study, we analysed 18 eyes from 10 patients who had DE associated with cGVHD (DE/cGVHD group; median age: 48.0 years; range: 30–63 years; three men, seven women) and 19 eyes from 11 age- and gender-matched patients who did not develop DE after HSCT (non-DE/non-cGVHD

group; median age: 43.8 years; range: 24–64 years; three men, eight women). We excluded two eyes from patients in the DE/cGVHD group that did not fulfil the criteria for DE and three eyes with blepharitis from patients in the non-DE/non-cGVHD group. The median follow-up time after HSCT was 31.9 months for the DE/cGVHD group, and 26.8 months for the non-DE/non-cGVHD group. Topical eye drops including artificial tears, vitamin A, and autologous sera eye drops were instilled five times a day immediately after the diagnosis of DE following HSCT. The median follow-up time from the diagnosis of DE to the first examination was 15.2 ± 12.0 months (range: 3–38 months) for the DE/cGVHD group. Primary diseases and disease stages were also well balanced between the two groups. We used the global diagnostic criteria for DE, which is based on the recommendation of the 2007 International Dry Eye Workshop Report.[13] Patients who had a history of surgical or spontaneous lacrimal punctal occlusion, allergies, simple meibomian gland dysfunction (MGD), glaucoma medications, contact lens use, or other ocular surgery including refractive surgery or radiation to the eyes were excluded, as were patients with infectious blepharitis, blink disorders, disorders of the lid aperture or lid/globe congruity, or other ocular surface disorders. In addition, patients with trachoma and ocular cicatricial pemphigoid were also excluded. The research followed the tenets of the Declaration of Helsinki Principles, and informed consent was obtained from all subjects. IRB/Ethics Committee approval for the examination procedure was obtained for this study.

Ocular surface vital staining

The fluorescein and Rose Bengal stain scores for the ocular surface were obtained using the double vital staining method[14] Both stains were scored on a scale of 0–9.[14,15] The van Bijsterveld scoring system was used for the Rose Bengal staining. Briefly, the ocular surface was divided into three zones: nasal conjunctival, corneal, and temporal. A score of 0–3 points was used for each zone, with a minimum possible score of 0 and a maximum total score of 9 points. Scarce punctuate staining was given 1 point. Denser staining not covering the entire zone was given 2 points. Rose Bengal staining over the entire zone was given 3 points. For the fluorescein staining, the cornea was divided into three equal upper, middle, and lower zones. Each zone had a staining score ranging from 0 to 3 points, as with the Rose Bengal stain, and the minimum and maximum total staining scores were 0 and 9 points, respectively. The presence of scarce staining in a zone was scored as 1 point; frequent puncta not covering the entire zone was scored as 2 points; and punctuate staining covering the entire zone was scored as 3 points.

Tear function test

Tear film break up time (TBUT) was measured three times and the median value was calculated.[14] The Schirmer's test was performed using standard strips (Alcon, Fort Worth, TX, USA) placed in the lower conjunctival sac for 5 min without anaesthesia.

Meibomian gland secretions

MGD was assessed by careful slit-lamp examination of the glandular orifices, mucocutaneous junction changes, and digital expression of the meibomian lipids. The same physician (YO) pressed gently on the lower eyelids to express the meibomian lipids. Meibum viscosity was graded as described by Shimazaki *et al*.[16] Briefly, to assess obstruction of the meibomian gland orifice, digital pressure was applied on the lower tarsus, and the expression of meibomian secretion (meibum) was scored as follows: grade 0, clear meibum is easily expressed; grade 1, cloudy meibum is expressed with mild pressure; grade 2, cloudy meibum is expressed with more than moderate pressure; and grade 3, meibum cannot be expressed even with the hard pressure.

Diagnosis of dry eye

The diagnosis and classification of DE disease based on the severity was carried out according to the recommendation of the 2007 International Dry Eye Workshop Report.[13]

Diagnosis of cGVHD

All the patients in our study fulfilled the revised consensus criteria for cGVHD.[17] Briefly, diagnosis of cGVHD requires the following: (1) a distinction from acute GVHD, (2) the presence of at least one distinctive manifestation (eg, keratoconjunctive sicca) confirmed by pertinent biopsy or other relevant tests (eg, Schirmer's test) in the same or other organs, and (3) the exclusion of other possible diagnoses.

DR-1® tear film lipid layer interferometry

Noncontact interferometry micrographs of the surface of the tear film were recorded using the DR-1® tear film lipid layer interferometry system (Kowa, Tokyo, Japan). DR-1® interferometry records the specular light from the tear surface. Light from a white-light source is reflected by a half mirror, focussed by a lens, and used to illuminate the tear surface. The specular light from the tear surface returns through the half mirror to a charge-coupled device camera that produces an image on the

device monitor. Two polarizers and a quarter-wave plate help eliminate any unnecessary reflected light from the lens and detect only the specular light reflected from the tear fluid. The camera is focussed on a 2.2 × 3.0-mm area of the central cornea such that a circular area 8-mm in diameter is observable. Lipid layer interference images were recorded immediately after a complete blink and were printed out using a colour video printer. The lipid layer grading classification was as reported previously: grade 1, somewhat grey colour, uniform distribution; grade 2, somewhat grey colour, nonuniform distribution; grade 3, a few colours, nonuniform distribution; grade 4, many colours, nonuniform distribution; grade 5, corneal surface partially exposed.[7] This grading scale has been demonstrated to correlate well with the degree of DE.[7] The DR-1 images were analysed by four independent investigators (YB, NT, EG, and DM) who did not collect the interference pattern data (MS, MN, and MS) or perform the DE examination (YO). The clinical status of the patients was masked for the analysis. When three or more of the four physicians agreed on the grade classification, we analysed the relationship between grade and score from the DE examination.[7]

Conjunctival fibrosis

We diagnosed conjunctival fibrosis in patients who had subconjunctival fibrosis, fornix shortening, symblepharon, and/or ankyloblepharon.[18,19] We evaluated these findings by using slit-lamp microscopy during a routine examination.

Statistical analyses

The nonparametric Mann–Whitney test was used to compare the two groups. Spearman's rank sum test was performed for analysis of the correlation between DR-1 grades and DE severity in patients who received HSCT and developed cGVHD with DE disease as well as patients receiving HSCT who did not develop cGVHD or DE disease. A P-value of <0.05 was considered statistically significant.

GraphPad Instat 3 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis.

Results

Tables 1 and 2 summarized the demographic characteristics of DE patients with cGVHD and non-DE subjects without cGVHD. In the DE/cGVHD group, the mean severity grade of the DR-1 images was 3.9 ± 0.9, which was significantly higher than that of the non-DE/non-cGVHD group (1.3 ± 0.6; $P<0.05$; Table 3). All DE patients in this study had cGVHD, and none of the non-DE HSCT recipients did. In some DE patients, with cGVHD the severity score was as high as grade 5, and DR-1 examination showed an irregular tear film, exposed areas of the corneal surface, and dry spots (Figure 1). Figure 2 shows a representative interferometry print from a dry eye with cGVHD patient with grade 3 DR-1 lipid layer change. Figure 3 shows a representative interferometry print from a normal subject. When DE severity was graded according to the recommendation of the 2007 Dry Eye Workshop Report,[13] our results showed a strong correlation between the severity of DE disease and the DR-1 grades ($r=0.8812$, $P<0.0001$; Figure 4).

We also investigated whether the DR-1 score correlated with the severity of cGVHD. Conjunctival involvement in GVHD is a distinct marker for severe systemic GVHD.[19] In HSCT recipients with conjunctival fibrosis, the mean DR-1 grade was 4.7 ± 0.7 ($n=9$ eyes). In contrast, the score was 1.9 ± 1.0 in HSCT recipients without conjunctival fibrosis ($n=28$ eyes). The difference was statistically significant ($P<0.05$; Table 4).

Conclusion

In this study, we found that the grades of the tear film lipid layer interference patterns measured by DR-1 interferometry in the DE/cGVHD group (3.9 ± 0.9) were significantly higher than those of the non-DE/non-cGVHD group (1.3 ± 0.6). In addition, the mean DR-1 grades for age- and gender-matched normal control subjects was 1.2 ± 0.5 (median age: 45.1 years; range 30–69; three men, eight women; Figure 3). Statistically significant differences in the DR-1 grade for patients with conjunctival fibrosis *vs* without it were also observed. Moreover, there was a strong correlation between the severity of DE disease and the DR-1 grades (Figure 4).

DE is a major complication after HSCT.[1,3,18] It has been shown that tear lipid layer interference patterns are highly correlated with DE severity, and the lipid layer becomes thick between grades 2 and 4 in cases of DE.[9,11] Here, grades 3, 4, and 5 were observed only in the DE/cGVHD group, and grades 1 and 2 were noted in the non-DE/non-cGVHD group, as shown by Yokoi *et al* previously. When DE severity was graded according to the recommendation of the 2007 Dry Eye Workshop Report, our results showed a strong correlation between the severity of the DE disease and the DR-1 grade.

Goto and Tseng *et al*[10,11] reported that the thick lipid layer in eyes with aqueous tear deficiency results from the retardation of lipid spread, which leads to an uneven distribution of the lipid film. Yokoi *et al*[7] previously reported that aqueous tear deficiency is associated with higher DR-1 grades because of the movement of tear lipids into areas lacking the aqueous component of the

Table 1 Demographic data for patients with dry eye associated with chronic GVHD (DE/cGVHD group)

Case no.	Sex	Age	Diag	HSCT	VA	F	RB	TBUT	Sch	MGD	DES	DR-1	Term	CF	cGVHD lesions other than eyes
1	F	60	ALL	PBSCT									12 mo		+
Rt					6/6	6	0	5	0	2	4	3		−	
Lt					6/4.8	6	0	5	5	3	3	3		−	
2	F	30	AML	PBSCT									40 mo		+
Rt					6/4.8	3	5	2	1	3	4	3		−	
Lt					6/4.8	4	5	2	1	3	4	3		−	
3	M	44	ALL	BMT									40 mo		+
Rt					6/4.8	4	2	3	1	3	4	5		+	
Lt					6/4.8	4	3	4	0	3	4	5		+	
4	F	33	ABL	BMT									6 mo		+
Rt					6/4.8	5	5	3	1	1	3	3		−	
5	M	49	ALL	BMT									24 mo		+
Rt					6/4.8	5	5	4	3	3	3	4		−	
Lt					6/4.8	5	5	4	2	3	3	4		−	
6	F	45	AML	BMT									57 mo		+
Rt					6/4.8	4	7	2		2	4	5		+	
Lt					6/4.8	5	7	2		2	4	5		+	
7	M	63	MDS	RIST									64 mo		+
Rt					6/12	9	8	3	3	3	3	5		+	
Lt					6/4.8	3	4	2	2	3	4	4		+	
8	F	31	CML	BMT									8 mo		+
Lt					6/4.8	4	1	5	6	2	2	3		−	
9	F	59	ML	BMT									24 mo		+
Rt					6/60	6	0	2	2	3	4	5		+	
Lt					6/120	6	0	2	3	3	3	5		+	
10	F	50	AML	BMT									19 mo		+
Rt					6/4.8	1	0	5	2	2	3	3		−	
Lt					6/6	3	5	5	1	2	3	3		+	

ABL, acute biophenotypic leukaemia; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BMT, bone marrow transplantation; CF, conjunctival fibrosis; CML, chronic myeloid leukaemia; DES, dry eye severity; Diag, diagnosis; DR-1, tear film lipid layer interference patterns; F, fluorescein staining score; HSCT, haematopoietic stem cell transplantation; Lt, left; MDS, myelodysplastic syndrome; MGD, meibomian gland dysfunction; ML, malignant lymphoma; mo, month; PBSCT, peripheral blood stem cell transplantation; RB, Rose Bengal staining score; RIST, reduced intensity stem cell transplantation; Rt, right; Sch, Schirmer's test; TBUT, tear film break up time; Term, the interval between HSCT and the first DR-1 examination; VA, visual acuity.

tear film, which results in a higher interferometry grade and a thicker lipid layer. In our study, 38.9% of the eyes were grade 5 by DR-1 lipid layer interferometry. All but one of the eyes had grade 2 or 3 meibomian gland expressibility with severe aqueous deficiency, which resulted in extensive grade 5 changes with corneal exposure in DR-1 examinations. In DE associated with cGVHD, we noted severe MGD at the time of disease onset in addition to aqueous tear deficiency.

We believe that both aqueous tear deficiency and evaporative type DE coexist in cGVHD. All the subjects had relatively higher ocular surface epithelial damage with higher fluorescein and Rose Bengal scores. Higher DR-1 grades in the severe aqueous tear deficiency DE subjects despite presence of nonexpressible meibomian gland secretions may be explained by the release of cell membrane lipids into the tear film because of the extensive epithelial damage. There were, however, three eyes (16.7%) with moderate grade 3 DR-1 score with low meibum expressibility grade 3 and low Schirmer's test scores. In another case, DR-1 grade was 5, although MGD score was 2. Further studies are necessary to clarify these discrepancies.

All DEs in this study had tear instability with low TBUT score, which might be because of the interaction of the several pathophysiological processes. One possibility is the excessive tear evaporation resulting from lipid deficiency, because 61.1% of the meibomian glands could not be expressed in our patients. Although the pathogenesis of MGD in cGVHD is controversial, meibomian gland function was severely damaged in patients with severe DE and cGVHD, leading to tear evaporation and low TBUT.[3] High DR-1 grades in the seven eyes of four cGVHD patients (grade 5 changes) probably resulted because of total obstruction of meibomian ducts by cGVHD fibrosis around the meibomian ducts. As MGD is a risk factor of DE, the severity of DE is more serious in the pathophysiology of cGVHD related DE, which is different from simple aqueous deficiency type of DE. The other explanation for

Table 2 Demographic data for HSCT recipients without dry eye (non-DE/non-cGVHD group)

Case no.	*Sex*	*Age*	*Diag*	*HSCT*	*VA*	*MGD*	*DES*	*DR-1*	*Term*	*CF*	*cGVHD*
1	M	33	NHL	BMT					10 mo		+
Rt					6/4.8	1	0	2		–	
Lt					6/4.8	1	0	1		–	
2	F	36	MDS	BMT					3 mo		+
Rt					6/4.8		0	2		–	
Lt					6/7.5		0	1		–	
3	F	60	MF	BMT					12 mo		–
Rt					6/4.8	2	0	2		–	
4	F	49	Mantle	BMT					23 mo		–
Rt			Lymphoma		6/4.8	0	0	1		–	
Lt					6/4.8	0	0	1		–	
5	F	46	CML	CBT					14 mo		–
Rt					6/4.8	2	0	1		–	
Lt					6/4.8	2	0	1		–	
6	F	42	MDS	BMT					25 mo		–
Rt					6/4.8	0	0	1		–	
Lt					6/4.8	0	0	1		–	
7	M	50	AML	BMT					37 mo		–
Lt					6/4.8	1	0	2		–	
8	F	24	AML	BMT					49 mo		–
Rt					6/4.8	0	0	2		–	
Lt					6/4.8	1	0	2		–	
9	F	38	ML	BMT					46 mo		–
Rt					6/4.8	0	0	1		–	
Lt					6/4.8	1	0	1		–	
10	M	64	ML	PBSCT					25 mo		–
Rt					6/4.8	0	0	1		–	
Lt					6/4.8	0	0	1		–	
11	F	58	MDS	BMT					70 mo		–
Rt					6/4.8	2	0	1		–	

AML, acute myeloid leukaemia; BMT, bone marrow transplantation; CBT, cord blood transplantation; CF, conjunctival fibrosis; CML, chronic myeloid leukaemia; DES, dry eye severity; Diag, diagnosis; DR-1, tear film lipid layer interference patterns; F, fluorescein staining score; HSCT, haematopoietic stem cell transplantation; Lt, left; mo, Month; MDS, myelodysplastic syndrome; MF, myeloid fibrosis; MGD, meibomian gland dysfunction; ML, malignant lymphoma; mo, month; NHL, non-Hodgkin's lymphoma; PBSCT, peripheral blood stem cell transplantation; RB, Rose Bengal staining score; Rt, right; Sch, Schirmer's test; TBUT, tear film break up time; Term, the interval between HSCT and the first DR-1 examination; VA, visual acuity.

Table 3 Comparison of DR-1 grade between DE/cGVHD group and non-DE/non-cGVHD group

	DE/cGVHD group	*Non-DE/ non-cGVHD group*	*P-value*
F (points)	4.6 ± 1.7*	0.05 ± 0.2	$P<0.0001$
RB (points)	3.4 ± 2.7*	0.05 ± 0.2	$P=0.0002$
TBUT (seconds)	3.3 ± 1.3*	9.0 ± 1.9	$P<0.0001$
Schirmer (mm)	2.1 ± 1.7*	9.8 ± 7.4	$P=0.0017$
MGD (points)	2.6 ± 0.6*	0.8 ± 0.8	$P<0.0001$
DR-1 grade (1–5)	3.9 ± 0.9*	1.3 ± 0.6	$P<0.0001$

*$P<0.05$.
F, fluorescein staining score; MGD, meibomian gland dysfunction; RB, Rose Bengal staining score; TBUT, tear film break up time.
Points of MGD indicate the meibum expressibility grade.

the low TBUT scores can be associated with a mucin deficient DE state. Indeed, conjunctival goblet cells and MUC5AC mRNA expression have been reported to decrease in cGVHD previously by us.[20] Problems of interaction between the tear film and ocular surface in cGVHD resulting from irregularities of the cornea and conjunctiva as evidenced by the high fluorescein and Rose Bengal score may explain the perturbation of the tear film stability.

Lipids are potential targets of oxidative radicals.[12] Oxidative stress in blood cells in mice is noticeable 3 weeks after HSCT, and is higher in mice receiving allogeneic spleen cells than in those receiving transplanted syngeneic cells, consistent with an association between oxidative stress and GVHD.[21] Given that radiation damage is associated with the production of activated oxygen species,[22] the total body irradiation performed before HSCT may affect the targets of oxidative radicals, including the tear lipid layer.

We found that the DR-1 tear film lipid layer interference patterns were significantly altered in cGVHD patients with conjunctival fibrosis and cGVHD. Consistent with our observations, Danjo and Hamano previously reported the DR-1 grade in patients with

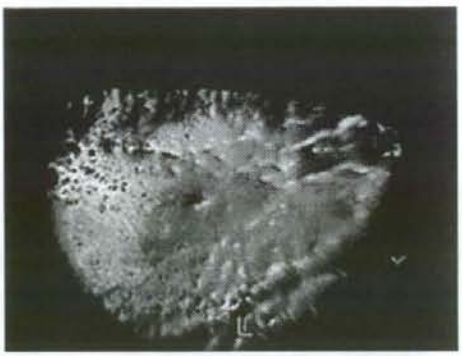

Figure 1 Interferometry pattern from the tear film surface of a 59-year-old woman, 24 months after HSCT. Conjunctival fibrosis (+). Grade 5 DR-1 change with an irregular tear film, areas of corneal surface exposure, and several large dry spots (case 9).

Figure 2 Interferometry pattern from the tear film surface of a 50-year-old woman, 19 months after HSCT. Conjunctival fibrosis (+). DR-1 grade 3 (case 10).

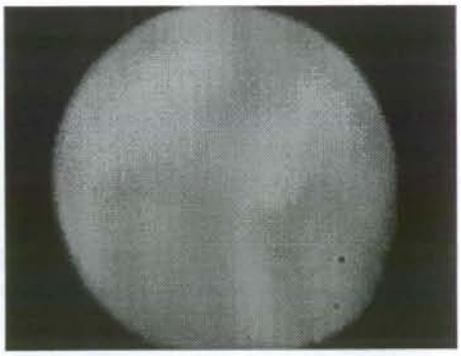

Figure 3 Interferometry pattern from the tear film surface of normal control, a 50-year-old woman. Note the regularity of the tear film and uniform homogenous grey–white lipid interferometry pattern grade 1.

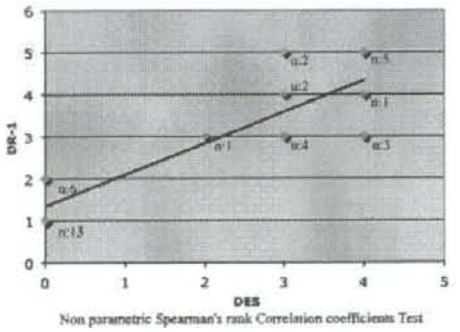

Figure 4 Correlation between DE severity scores and DR-1 tear film lipid layer grades. Nonparametric Spearman's rank test. Correlation coefficients test. $\gamma = 0.8812$, $P < 0.0001$. '*n*' indicates the number of patients.

Sjögren's disease.[23] The reported values of DR-1 grades for Sjogren's syndrome (SS) were much lower than the DR-1 grades in our paper, suggesting that the severity of DE associated with cGVHD. In our study, higher grades of DR-1 lipid layer interferometry were observed in DE patients with cGVHD compared with patients with DE and no history of GVHD such as patients with SS and other simple types of DE disease. We believe that the higher DR-1 grades observed in this study may be representative for severe DE associated with GVHD may be related to severe DEs with cicatrising conjunctivits. It is also our observation that patients with other cicatrising conjunctival diseases such as ocular cicatricial pemphigoid have high DR-1 grades similar to the DE disease in cGVHD (unpublished observation).

Table 4 Comparison of the DR-1 grades for HSCT recipients with or without conjunctival fibrosis and with or without dry eye

	CF (+)	*CF (−)*
DE (+)	4.7 ± 0.7 ($n = 9$)	3.2 ± 0.4 ($n = 9$)
DE (−)	($n = 0$)	1.3 ± 0.6 ($n = 19$)
Total	4.7 ± 0.7 ($n = 9$)	1.9 ± 1.0 ($n = 28$)

CF, conjunctival fibrosis; DE, dry eye; HSCT, haematopoietic stem cell transplantation.

Conjunctival fibrosis occurs subsequent to conjunctival GVHD with pseudomembrane formation, owing to the

loss of the conjunctival epithelium. Conjunctival involvement in GVHD is a marker for severe systemic GVHD.[19] In our study, cGVHD patients with conjunctival fibrosis had systemic complications and a poor prognosis following HSCT. Thus, the DR-1 grade may be a useful tool for predicting the systemic complications as well.

In conclusion, DR-1 lipid layer interferometry grades in cGVHD patients with severe DE disease were markedly higher compared with patients without DE and no history of cGVHD after HSCT. DR-1 tear lipid interferometry is a noninvasive test, which may be a useful tool for monitoring the onset and progression of DE associated with cGVHD. The DR-1 grade also seems to be a promising severity marker for cGVHD. Further studies analysing the potential association between the DR-1 grades and different therapeutic options will definitely enrich our understanding on the pathological changes of the tear lipid film layer in cGVHD.

Acknowledgements

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Disclosure/Conflict of interest

None.

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ORIGINAL ARTICLE

Fasciitis and myositis: an analysis of muscle-related complications caused by chronic GVHD after allo-SCT

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The muscle-related complications of fasciitis and myositis, caused by chronic GVHD after Allo-SCT are relatively rare, but at times will severely impair a patient's quality of life (QOL). We performed a retrospective analysis in Japanese Allo-SCT recipients to identify the incidence, risk factors and clinical features of fasciitis and myositis. In 1967 patients who underwent Allo-SCT between January 1994 and March 2005 and survived beyond 90 days post transplantation, eight patients developed fasciitis and nine patients developed myositis, with a 5-year cumulative incidence of 0.55% and 0.54%, respectively. The median time from SCT to the development of fasciitis and myositis was 991 and 660 days, respectively. PBSCT was a risk factor for developing fasciitis, but no risk factors were found for myositis. The response to immunosuppressive treatment was better in patients with myositis than fasciitis, and the overall survival after developing these symptoms was better in patients with myositis than those with fasciitis. An early diagnosis by a biopsy, which includes fascia and muscle or magnetic resonance imaging (MRI) and prompt treatment may be important to prevent an impairment of the patient's QOL with persistent disability.

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Introduction

Allo-SCT has been an essential part of the treatment of refractory hematopoietic disease such as leukemia and lymphoma.[1] The number of patients who undergo Allo-SCT from various stem cell sources increases every year. GVHD is still a major cause of morbidity and mortality after Allo-SCT and is caused by an immunological reaction against antigens in the SCT recipient by the immunocompetent donor graft. Chronic GVHD occurs in 30–70% of recipients who survived beyond 100 days following transplantation, and it is dependent on the degree of HLA mismatch with the donor and the source of the stem cells.[2–4] The main target organs of chronic GVHD are skin, eyes, mouth, liver, esophagus, bowel, lung and serosa, and the syndrome has features resembling autoimmune and other immunological disorders such as scleroderma, Sjögren's syndrome, primary biliary cirrhosis, wasting syndrome, bronchiolitis obliterans (BO), immune cytopenias and chronic immunodeficiency. Patients with chronic GVHD have decreased performance status, impaired quality of life (QOL) and an increased risk of mortality.[5,6]

Muscle-related complications, fasciitis[7–9] and myositis[10–13] are relatively rare chronic GVHD manifestations, and their clinical features resemble autoimmune eosinophilic fasciitis and idiopathic polymyositis. The new chronic GVHD diagnostic guidelines proposed fasciitis as diagnostic, and myositis as a distinctive sign and symptom of chronic GVHD manifestation.[14] Patients with fasciitis develop skin swelling, and thereafter the skin becomes taut, bound down to the underlying tissue, and irregularly thickened, and thereafter demonstrating multiple small depressed areas, which has been called a 'peau d'orange' (or an orange peel) appearance.[7] Contractures and joint stiffness are also observed. The pathological findings of fasciitis include lymphocytic infiltration in edematous fascia and a subsequent increase of collagen fibers. The infiltration is diffuse and it often extends from the fascia into the interstitium of the muscle. The common clinical symptoms of myositis are

moderate-to-severe proximal muscle weakness, myalgia, fever, contractures and skin indurations over the areas of muscle involvement.[12] The majority of patients present with elevated creatinine phosphokinase and aldolase enzymes. The histopathology of muscle biopsies demonstrates the degeneration, necrosis and regeneration of muscle fibers and infiltrates of inflammatory cells. These two diseases have similar manifestations and it is sometimes difficult to make an early diagnosis. In addition, fasciitis and myositis severely impair a patient's QOL and some patients suffer disability from muscle and joint symptoms. However, scant data are found concerning Allo-SCT recipients, and the accurate incidence of the diseases by stem cell source and HLA disparity, as well as the treatment response and prognosis, remain unclear. Here, we present the results of our retrospective analysis of fasciitis and myositis caused by chronic GVHD among Japanese patients who underwent Allo-SCT, and we also identify the incidence, risk factors and clinical features of these diseases.

Materials and methods

Patients

Between January 1994 and March 2005, 1967 Japanese patients who underwent Allo-SCT at 15 centers of the Kanto Study Group for Cell Therapy (KSGCT) and who survived beyond 90 days after transplantation were retrospectively analyzed. Basic transplantation data were obtained from the KSGCT database, but specific data of fasciitis and myositis were obtained by using data collection forms. This study was not a part of any specific protocol; therefore, the patients were not asked to provide their informed consent for the study at the onset of either fasciitis or myositis. The patient characteristics are summarized in Table 1. The stem cell source included related BMT (R-BMT; $n=645$), related PBSCT (R-PBSCT; $n=414$), unrelated BMT (UR-BMT; $n=732$) and cord blood transplantation ($n=176$). Because PBSC harvest has not been performed through the Japan Marrow Donor Program, none of the patients received UR-PBSCT. The median period of the observation was 958 days.

Definition of fasciitis and myositis

The diagnosis of fasciitis was made based on the pathological findings, magnetic resonance imaging (MRI) findings and clinical symptoms. A fascia or muscle biopsy was not mandatory for making a diagnosis. The typical pathological finding of fasciitis was inflammatory cells infiltrating on the fascia. MRI images suggested the presence of inflammation on the fascia (e.g., thickening of fascia). The clinical symptoms included sclerotic skin changes, restriction of joint movement, myalgia, swelling and edema. The diagnosis of myositis was made similarly and Bohan and Peter[15,16] criteria for polymyositis was also used. The symptoms of myositis included muscle weakness, myalgia, fever and muscle swelling. The increase of serum creatinine phosphokinase or aldolase reflects the destruction of muscle. Other causes of fasciitis or myositis than chronic GVHD were ruled out.

Table 1 Characteristics of patients

Number of patients (survived more than 90 days)	1967	
Male	1189	(60.4%)
Female	778	(39.6%)
Median age (range)	37	(15–69)
Stem cell source		
R-BMT	645	(32.8%)
R-PBSCT	414	(21.0%)
UR-BMT	732	(37.2%)
CBT	176	(8.9%)
Conditioning regimen		
TBI	1310	(66.6%)
Non-TBI	644	(32.7%)
GVHD prophylaxis		
CsA	1527	(77.6%)
FK506	376	(19.1%)
Others	64	(3.3%)
Acute GVHD		
0–I	1119	(56.9%)
II–IV	848	(43.1%)
Chronic GVHD		
Yes	1082	(55.0 %)
	(limited 356, extensive 691, unknown 35)	
No	885	(45.0%)
Primary disease		
AML	647	(32.9%)
ALL	401	(20.4%)
CML	383	(19.5%)
MDS	246	(12.5%)
NHL	115	(5.8%)
MM	40	(2.0%)
SAA	84	(4.3%)
Others	51	(2.6%)

Abbreviations: CBT = cord blood transplantation; MDS = myelodysplastic syndrome; MM = multiple myeloma; NHL = non-Hodgkin lymphoma; R-BMT = related BMT; RPBSCT = related PBSCT; SAA = severe aplastic anemia; UR-BMT = unrelated BMT.

Statistical analysis

To evaluate the potential risk factors for developing fasciitis and myositis, the time-dependent Cox proportional hazard regression model was used for univariate and multivariate analyses. The factors with a P-value of 0.2 or less in the univariate analysis were included in the multivariate analysis. The factors that remained significant were retained in the final model. The factors that correlated with each other were not entered into the model simultaneously.

Survival analyses were performed by the Kaplan–Meier method,[17] and the log-rank test was used for univariate comparisons. The cumulative incidence of fasciitis and myositis was calculated using the Gray[18] method, considering death without fasciitis or myositis as a competing risk, on the basis of information obtained from the patient database. For most of the statistical analyses, SPSS

Table 2 Characteristics of patients with fasciitis and myositis

Patient no.	*Age at transplantation*	*Sex R/D*	*Diagnosis*	*Conditioning regimen*	*Donor source*	*HLA disparity at A, B and DR loci*	*GVHD prophylaxis (CsA or FK506)*	*Acute GVHD (grade)*	*Other chronic GVHD manifestations*
Fasciitis:									
1	57	M/M	MM	Non-TBI	R-PBSCT	Full match	FK506	0	Skin, sicca
2	34	M/F	AML	TBI	UR-BMT	Full match	CsA	0	Sicca, mouth, liver, lung (BO)
3	50	F/F	AML	TBI	UR-BMT	Full match	FK506	I	None
4	39	F/F	MDS	TBI	R-BMT	Single locus mismatch	FK506	0	Mouth, skin, liver (BO), lung, sicca, GI
5	39	F/M	CML	TBI	R-PBSCT	Full match	CsA	0	Sicca, skin, mouth, lung (IP)
6	40	F/F	AML	Non-TBI	R-PBSCT	Full match	CsA	II	Sicca, skin, liver, lung (BO)
7	26	M/M	ALL	TBI	R-PBSCT	Full match	CsA	I	Sicca, mouth, liver, lung (BO), GI
8	20	M/F	CML	TBI	R-PBSCT	Full match	CsA	II	Skin, lung (BOOP)
Median	39								
Myositis:									
1	36	F/M	MM	Non-TBI	R-PBSCT	Full match	CsA	0	Mouth
2	53	F/F	MDS/AML	TBI	R-BMT	Full match	CsA	I	Mouth, sicca
3	50	M/M	AML	TBI	R-PBSCT	Full match	CsA	I	Mouth
4	37	F/F	CML	TBI	R-BMT	Full match	CsA	I	Skin, sicca, mouth, liver
5	39	M/M	CML	Non-TBI	UR-BMT	Full match	CsA	II	Skin, liver
6	21	M/F	ALL	TBI	UR-BMT	Single locus mismatch	CsA	I	GI
7	23	M/M	AML	TBI	R-BMT	Full match	CsA	II	Skin, sicca, liver
8	39	M/M	ALL	TBI	UR-BMT	Full match	CsA	II	Skin
9	56	F/M	ML	Non-TBI	R-PBSCT	Full match	CsA	II	Skin, sicca, mouth, liver
Median	39								

Abbreviations: BO = bronchiolitis obliterans; BOOP = bronchiolitis obliterans organizing pneumonia; GI = gastro-intestinal tract; IP = interstitial pneumonia; NA = not applicable; R/D = recipient/donor.

Table 3 Clinical manifestations of fasciitis

Patient no.	*Symptoms*	*Affected lesion*	*Laboratory data*				*MRI High intensity along the fascia*	*Fascia biopsy Lymphocyte infiltration or fibrosis at fascia*
			CRP (g/100 ml)	*CPK* (IU/l)	*ANA*	*Eosinophil (%)*		
1	Myalgia, edema, swelling, muscle weakness and restriction of movement	All extremities	2.5	25	× 160	13.3	+	+
2	Myalgia and restriction of movement	Forearm and thigh	0.2	69	× 80	0.2	ND	+
3	Edema and swelling	Forearm, leg	1.0	ND	× 20	5.0	+	ND
4	Skin sclerosis, edema and swelling	Forearm, thigh, leg and trunk	0.1	27	× 40	0.0	ND	+
5	Skin sclerosis, myalgia, muscle weakness and restriction of movement	Upper arm and forearm	0.2	171	–	9.0	ND	+
6	Skin sclerosis and restriction of movement	Upper arm and trunk	0.1	64	–	0.1	+	+
7	Skin sclerosis, muscle weakness and restriction of movement	All extremities	1.8	114	ND	2.4	+	+
8	Skin sclerosis	Trunk	0.1	ND	–	1.0	+	ND

Abbreviations: ANA = antinucleus antibody, CPK = creatinine phosphokinase; CRP = C-reactive protein; MRI = magnetic resonance imaging, ND = not done.

software version 11 (SPSS Inc., Chicago, IL, USA) was used. The analyses of cumulative incidences were carried out with package 'cmprsk' of the R statistical software 2.5.1 (the R Foundation for Statistical Computing, Vienna, Austria; available at http://www.r-project.org). All *P*-values were two-sided, and differences were considered to be statistically significant when $P<0.05$. Differences with *P*-values >0.10 are reported as NS.

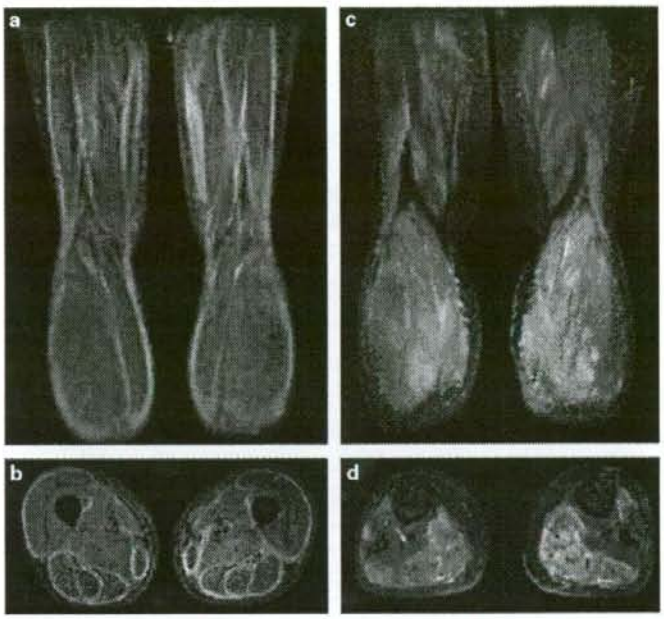

Figure 1 MRI findings of fasciitis and myositis. The MRI images of patients with fasciitis (**a** and **b**) showed a high intensity along the fascia for the short T1 inversion recovery (STIR) method and those of myositis (**c** and **d**) showed a high intensity in muscle in the fat-suppressed T2 weighted images (T2WI). (**a** and **c**) Coronal view of thighs and legs (**a**, STIR; **c**, T2WI, fat suppression). (**b** and **d**) Axial view of thighs and legs (**b**, STIR; **d**, T2WI, fat suppression). MRI, magnetic resonance imaging.

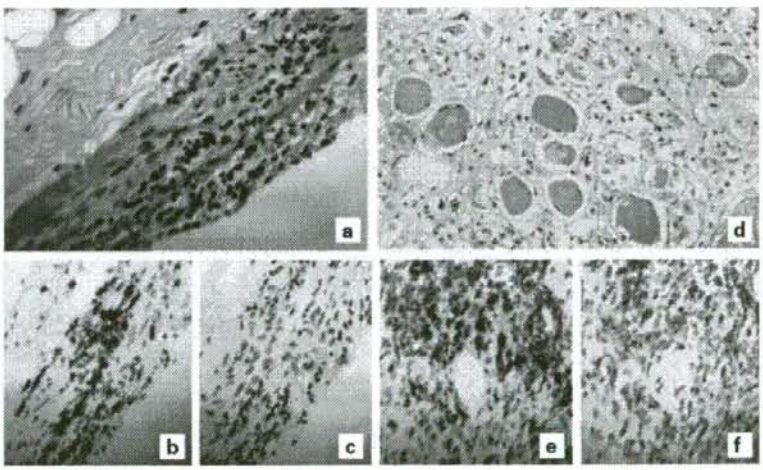

Figure 2 Pathological findings of fasciitis and myositis. (**a–c**) Fasciitis. The fascia were thickened and loosened, and some showed fibrosis. A patchy lymphocyte infiltration was found on the fibrotic fascia or small aggregates were seen around capillaries. The infiltrating lymphocytes on the fascia were predominantly CD8+ T cells. (**d–f**) Myositis. Necrosis and reconstruction of muscle cells, irregularity in muscle fibers, interstitial fibrosis and lymphocytic infiltration surrounding the muscle fibers and interstitium were seen. The lymphocytic infiltration was more predominant in the endomysium than in the perimysium. The CD8+ T cells were dominant, with a CD8:4 ratio of approximately 2:1. Hematoxylin–eosin staining (**a** and **d**), Immunostaining of CD8 (**b** and **e**) and CD4 (**c** and **f**).

Results

Incidence of fasciitis and myositis after Allo-SCT

In 1967, patients who survived over 90 days post transplantation, 1082 patients (55.0%) developed chronic GVHD, including 356 with the limited type (18.1%) and 691 with the extensive type (35.1%). Of these patients, eight patients developed fasciitis and nine patients developed myositis. The 5-year cumulative incidence of fasciitis and myositis was 0.55 and 0.54%, respectively.

The patient characteristics with fasciitis and myositis are shown in Table 2. In the eight patients with fasciitis, there were four men and four women. The median age of the patients was 39 years (range, 20–57 years). The stem cell sources were R-PBSCT in five patients, UR-BMT in two patients and R-BMT from single locus mismatch donor in one patient. Seven patients were matched with their donors for HLA-A, B and DR at the serological level. None of the patients with fasciitis or myositis had HLA DR52, although HLA DR52 is highly associated with idiopathic polymyositis[19] and Couriel *et al.*[12] reported two cases of chronic GVHD-associated myositis with HLA DR52.

In nine patients with myositis, there were five men and four women. The median age of patients was 39 years (range, 21–56 years). The stem cell sources were R-PBSCT in three patients, UR-BMT in three patients and R-BMT in three patients. Eight patients were serologically matched with their donors at the HLA-A, B and DR loci.

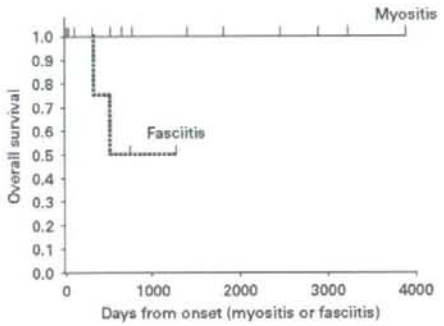

Figure 3 Overall survivals of patients with fasciitis and myositis. Overall survival rates of the patients after developing either fasciitis or myositis according to Kaplan–Meier analysis. The 5-year survival after developing fasciitis or myositis was 50.0 and 100%, respectively.

Clinical manifestations and treatment outcome of the patients with fasciitis

The time of onset of the symptoms of fasciitis ranged from 212 to 1726 days after SCT (mean, 991 days). The initial symptoms were a characteristic sclerodermatous skin change, called peau d'orange appearance, and restriction of joint movement, which were observed in five and four patients, respectively (Table 3). Other symptoms were edema and swelling in three patients and myalgia in two patients. The affected sites were the forearms in five patients, upper arms in four patients, thighs in four patients, legs in four patients and trunk in three patients (Table 3). The affected sites were symmetrical in all patients.

The MRI images of patients with fasciitis showed high intensity along the fascia in short T1 inversion recovery method (Figures 1a and b). MRI seems to be a useful modality to determine the extent of inflammation of the fascia. A fascial biopsy was performed in six patients. All

Table 4 Clinical manifestations of myositis

Patient no.	*Symptoms*	*Affected legion*	*Laboratory data*				*Muscle biopsy: MRI*		*EMG*
			CRP (g/100 ml)	*CPK (IU/l)*	*Aldolase (IU/l)*	*ANA*	*Positive for myositis*	*High intensity*	*Myopathic change in muscle*
1	Swelling, myalgia and weakness	Thigh and leg	10.7	1141	19	×40	ND	+	+
2	Fever, edema, swelling and myalgia	Thigh	21.1	1643	34	×16	+	+	+
3	Edema, swelling, myalgia and weakness	All extremities, trunk	15.9	4072	320	–	+	+	ND
4	Fever, myalgia, weakness, restriction of movement and skin sclerosis	Upper arm, forearm and thigh	6.0	3173	ND	–	+	+	+
5	Myalgia and swelling	Upper arm	15.6	4864	ND	ND	+	ND	+
6	Fever and myalgia	All extremities	ND	3930	ND	ND	+	+	ND
7	Myalgia, weakness and restriction of movement	All extremities, trunk	10.0	2883	25	–	+	ND	+
8	Fever, myalgia and swelling	Trunk	14.5	1790	ND	ND	ND	+	+
9	Myalgia and weakness	Thigh	2.6	358	27	×320	+	+	+

Abbreviations: ANA = antinucleus antibody; CPK = creatinine phosphokinase; CRP = C-reactive protein; EMG = electromyography.