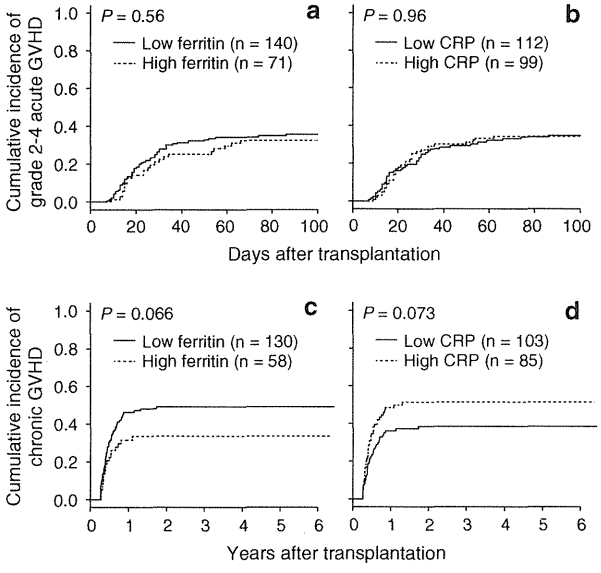

Fig. 1 Cumulative incidence of grade 2–4 acute GVHD according to pretransplant **a** ferritin and **b** CRP levels, and chronic GVHD according to pretransplant **c** ferritin and **d** CRP levels. **a**, **c** *Solid line* low-ferritin group (<880 ng/mL), *dotted line* high-ferritin group (≥880 ng/mL). **b**, **d** *Solid line* low-CRP group (<2 mg/L), *dotted line* high-CRP group (≥2 mg/L). The statistical significance between the 2 groups was calculated using the Gray test

respectively. There was no significant difference in the incidence rates of grade 2–4 and 3 or 4 acute GVHD between the low- and high-ferritin groups or between the low- and high-CRP groups.

The cumulative incidence rates of chronic GVHD at 3 years after transplantation were 49.3 % (95 % CI 40.1–57.8 %) and 33.5 % (95 % CI 21.6–45.8 %) in the low- and high-ferritin groups, respectively, and 38.6 % (95 % CI 29.0–48.2 %) and 51.4 % (95 % CI 40.0–61.7 %) in the low- and high-CRP groups, respectively (Fig. 1, panels c, d). The patients in the high-ferritin group tended to have a low incidence of chronic GVHD, although this association was not significant in the multivariate analysis [≥880 vs. <880 ng/mL; hazard ratio (95 % CI), 0.64 (0.38–1.09); $P = 0.099$; Table 2]. A subgroup analysis showed that the negative effect of the ferritin levels on the incidence of chronic GVHD was significant only in patients with myeloid malignancies ($n = 103$; hazard ratio, 0.46; $P = 0.040$), but not in patients with lymphoid malignancies ($n = 78$; hazard ratio, 1.11; $P = 0.79$). The multivariate analysis showed that the elevated serum CRP levels (≥2 vs. <2 mg/L) were significantly associated with the increased incidence of chronic GVHD [hazard ratio (95 % CI), 1.71 (1.07–2.74); $P = 0.024$; Table 2]. The effect of the CRP levels on the incidence of chronic GVHD was similar regardless of the primary disease (myeloid or lymphoid malignancies). There was no significant difference

Table 2 Multivariate analysis of acute and chronic GVHD

	Hazard ratio (95 % CI)	*P* value
Grade 2–4 acute GVHD		
Conditioning regimen		
Myeloablative intensity	1.00	Reference
Reduced intensity	0.60 (0.37–0.97)	0.037
Serum ferritin level		
<880 ng/mL	1.00	Reference
≥880 ng/mL	0.86 (0.52–1.43)	0.57
Serum CRP level		
<2 mg/L	1.00	Reference
≥2 mg/L	1.13 (0.70–1.81)	0.62
Grade 3–4 acute GVHD		
Risk of disease		
Standard	1.00	Reference
High	3.93 (1.52–10.14)	0.005
Conditioning regimen		
Myeloablative intensity	1.00	Reference
Reduced intensity	0.39 (0.15–1.02)	0.055
Serum ferritin level		
<880 ng/mL	1.00	Reference
≥880 ng/mL	2.10 (0.87–5.09)	0.10
Serum CRP level		
<2 mg/L	1.00	Reference
≥2 mg/L	0.57 (0.22–1.43)	0.23
Chronic GVHD[a]		
Risk of disease		
Standard	1.00	Reference
High	1.48 (0.93–2.37)	0.098
GVHD prophylaxis		
Tacrolimus-based	1.00	Reference
Cyclosporine based	0.52 (0.27–1.01)	0.052
Serum ferritin level		
<880 ng/mL	1.00	Reference
≥880 ng/mL	0.64 (0.38–1.09)	0.099
Serum CRP level		
<2 mg/L	1.00	Reference
≥2 mg/L	1.71 (1.07–2.74)	0.024

[a] Patients who survived for at least 100 days after transplantation were included in the analysis

in the incidence of extensive chronic GVHD between the low- and high-ferritin groups ($P = 0.43$) or between the low- and high-CRP groups ($P = 0.52$).

A history of acute GVHD is known to be an important risk factor for developing chronic GVHD. Therefore, we also conducted an analysis of the association between the incidence of chronic GVHD and the incidence of prior acute GVHD (none or grade 1 vs. grade 2–4) stratified by the pretransplant ferritin and CRP levels. The cumulative incidence rates of chronic GVHD at 3 years were as

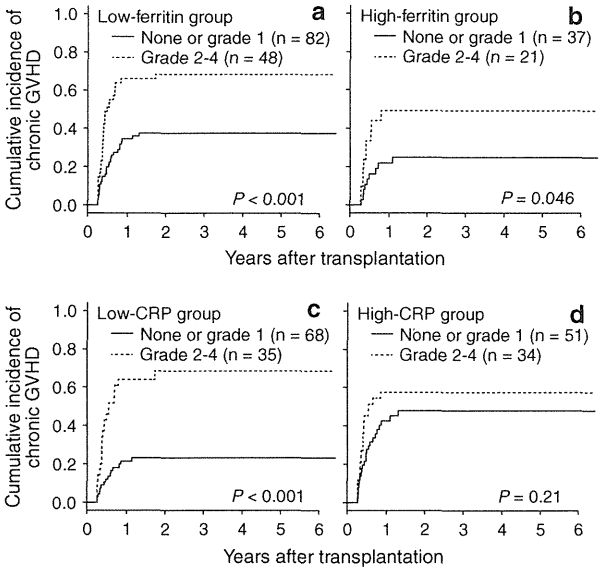

Fig. 2 Cumulative incidence of chronic GVHD according to prior acute GVHD stratified by pretransplant ferritin and CRP levels. **a** Low-ferritin group (<880 ng/mL, $n = 130$), **b** high-ferritin group (≥880 ng/mL, $n = 58$), **c** low-CRP group (<2 mg/L, $n = 103$), and **d** high-CRP group (≥2 mg/L, $n = 85$). *Solid line* no or grade 1 acute GVHD, *dotted line* grade 2–4 acute GVHD. The statistical significance between the 2 groups was calculated using the Gray test

follows: low-ferritin group, 37.8 versus 68.4 %, $P < 0.001$ (Fig. 2, panel a); high-ferritin group, 24.9 versus 49.2 %, $P = 0.046$ (Fig. 2, panel b); low-CRP group, 23.2 versus 68.5 %, $P < 0.001$ (Fig. 2, panel c); and high-CRP group, 47.8 versus 57.5 %, $P = 0.21$ (Fig. 2, panel d). Notably, the patients in the high-CRP group demonstrated a high incidence of chronic GVHD, both in the absence and presence of prior acute GVHD.

TRM, relapse, and OS

At 3 years, the patients in the high-ferritin group had a significantly higher TRM than those in the low-ferritin group (21.5 vs. 9.7 %, $P = 0.026$; Fig. 3, panel a), with the main cause of TRM being infection in both groups [5 of 14 TRM cases in the high-ferritin group ($n = 71$) and 5 of 13 TRM cases in the low-ferritin group ($n = 140$)]. The patients in the high-CRP group had a significantly higher TRM than those in the low-CRP group (20.0 vs. 7.5 %, $P = 0.035$; Fig. 3, panel b). The impact of ferritin on TRM was significant ($P = 0.046$) according to the multivariate analysis, whereas the impact of CRP was not (Table 3).

The cumulative incidence of relapse was similar between the low- and high-ferritin groups and between the low- and high-CRP groups (Fig. 3, panels c, d). However, in the multivariate analysis, the high ferritin levels were significantly associated with the high relapse rate ($P = 0.018$; Table 3).

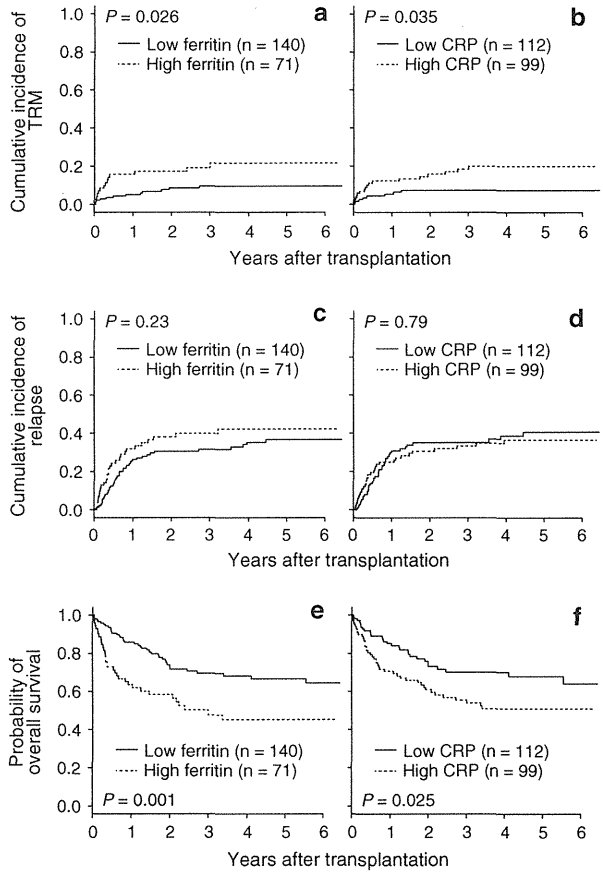

Fig. 3 Cumulative incidence of TRM according to pretransplant **a** ferritin and **b** CRP levels, and relapse according to pretransplant **c** ferritin and **d** CRP levels. The Kaplan–Meier estimate of overall survival according to pretransplant **e** ferritin and **f** CRP levels is also shown. **a**, **c**, **e** *Solid line* low-ferritin group (<880 ng/mL), *dotted line*, high-ferritin group (≥880 ng/mL). **b**, **d**, **f** *Solid line* low-CRP group (<2 mg/L), *dotted line* high-CRP group (≥2 mg/L). The statistical significance between the 2 groups was calculated using the Gray test or the log-rank test. **e**, **f** Note that pretransplant high ferritin levels were significantly associated with inferior overall survival in both univariate ($P = 0.001$) and multivariate ($P = 0.002$) analyses, while high CRP levels were significantly associated with inferior overall survival in only univariate analysis ($P = 0.025$) but not in multivariate analysis ($P = 0.79$; see Table 3)

In the 211 cases, early death within 100 days after SCT occurred in 17 cases, with the cause being TRM in 11 cases and disease relapse in 6 cases. Fifty-seven patients died after day 100, with the cause of death being TRM in 16 cases and disease relapse in 41 cases. At 3 years, the patients in the high-ferritin group had a significantly inferior OS than those in the low-ferritin group (47.7 % vs. 69.5 %, $P = 0.001$; Fig. 3, panel e); the patients in the high-CRP group had a significantly inferior OS than those in the low-CRP group (54.0 % vs. 70.1 %, $P = 0.025$; Fig. 3f). In the multivariate analysis, high ferritin levels ($P = 0.002$), older age ($P = 0.014$), male sex ($P = 0.031$),

Table 3 Multivariate analysis of TRM, relapse, and overall mortality

	Hazard ratio (95 % CI)	*P* value
TRM		
Sex		
Male	1.00	Reference
Female	0.37 (0.15–0.92)	0.034
Serum ferritin level		
<880 ng/mL	1.00	Reference
≥880 ng/mL	2.19 (1.01–4.75)	0.046
Serum CRP level		
<2 mg/L	1.00	Reference
≥2 mg/L	1.67 (0.73–3.82)	0.23
Relapse		
Risk of disease		
Standard	1.00	Reference
High	2.83 (1.74–4.59)	< 0.001
Serum ferritin level		
<880 ng/mL	1.00	Reference
≥880 ng/mL	1.81 (1.11–2.95)	0.018
Serum CRP level		
<2 mg/L	1.00	Reference
≥2 mg/L	0.73 (0.45–1.19)	0.21
Overall mortality		
Age at transplant		
<50 years	1.00	Reference
≥50 years	1.80 (1.13–2.87)	0.014
Sex		
Male	1.00	Reference
Female	0.58 (0.36–0.95)	0.031
Risk of disease		
Standard	1.00	Reference
High	2.51 (1.55–4.07)	<0.001
Serum ferritin level		
<880 ng/mL	1.00	Reference
≥880 ng/mL	2.16 (1.34–3.48)	0.002
Serum CRP level		
<2 mg/L	1.00	Reference
≥2 mg/L	1.07 (0.66–1.73)	0.79

and high-risk disease ($P < 0.001$) were significantly associated with a higher mortality rate (Table 3). The high ferritin levels remained to be associated with a high mortality rate among the patients who survived for 100 days or longer after transplantation ($P = 0.032$).

Discussion

In our cohort of 211 patients with hematologic diseases who underwent allogeneic HSCT, we found that an elevated CRP level before transplantation was a significant risk factor for the development of chronic GVHD. We also observed a tendency toward a lower incidence of chronic GVHD in the high-ferritin group, although a significant association was found only in patients with myeloid malignancies. In contrast, the ferritin and CRP levels were not associated with the development of acute GVHD. Consistent with previous reports, we also confirmed that an elevated ferritin level was an adverse prognostic factor for survival [2–8, 14].

Chronic GVHD is the primary cause of late morbidity and nonrelapse mortality after allogeneic HSCT. Although a history of acute GVHD is one of the strongest predictors for the development of chronic GVHD, successful strategies for reducing acute GVHD with combinations of immunosuppressive agents have not resulted in reduced incidence of chronic GVHD [19–21]. In the present study, we demonstrated that an elevated pretransplant CRP level was significantly associated with a high incidence rate of chronic GVHD, without affecting the incidence of acute GVHD (Fig. 1, panels b, d). In addition to the current study, we have analyzed the association between post-transplant (between day +50 and +99) serum CRP levels and the incidence of chronic GVHD and found no significant association between them. When we divided the 186 cases into low- and high-CRP groups based on the post-transplant serum CRP levels (<2 vs. ≥2 mg/L), the incidence of chronic GVHD was 49.4 % in the low-CRP group ($n = 96$) and 40.0 % in the high-CRP group ($n = 90$, $P = 0.43$). Chronic GVHD is considered an immune-mediated syndrome, and its clinical manifestation often resembles autoimmune and other immunological disorders. However, the pathophysiological mechanism underlying chronic GVHD remains poorly understood and there is no reliable marker for predicting and monitoring chronic GVHD. On the other hand, the pathophysiological mechanism underlying acute GVHD is thought to involve the release of proinflammatory cytokines and chemokines from damaged host tissues that activate host antigen-presenting cells and the infused donor T lymphocytes that proliferate and differentiate in response to the host antigen-presenting cells, resulting in target tissue destruction [22]. The difference between the pathophysiological mechanisms of acute and chronic GVHDs may explain our results, which showed that ferritin or CRP levels had no significant influence on the development of acute GVHD. CRP, a surrogate marker of systemic inflammation, has been shown to be a risk factor for the progression of atherosclerosis and future cardiovascular events [23, 24]. In addition, several studies have suggested that CRP itself has a proatherogenic effect [25, 26]. An elevated pretransplant CRP level may be associated with vascular endothelial damage or immune activation in transplant recipients as a

result of minute inflammation. However, whether such pretransplant status can cause an increased incidence of chronic GVHD remains unclear and requires clarification in future studies.

In contrast to the positive association between elevated CRP levels and the development of chronic GVHD, we observed an inverse association trend between high ferritin levels and the development of chronic GVHD, as previously reported by others, though it was not statistically significant ($P = 0.099$), [6, 7]. In a subgroup analysis, we observed a significant association between the high ferritin levels and the development of chronic GVHD in patients with myeloid malignancies, but not in those with lymphoid malignancies. The reason why this association was observed only in those with myeloid malignancies is uncertain. Further well-designed studies are needed to confirm this result.

Ferritin, a heteropolymer comprising 24 H- and L-type subunits, has been suggested to play a role as an immune regulator [27]. Several lines of evidence have demonstrated that ferritin can suppress the proliferation of T cells in response to mitogens, impair the maturation of B cells, and inhibit the proliferation of myeloid cells [28–30]. H-ferritin has been suggested to induce the production of interleukin 10 from regulatory T cells and suppress immune responses [31, 32]. In the light of these findings, among the patients with myeloid malignancies, the decreased incidence of chronic GVHD and significantly higher relapse rate, as determined by the multivariate analysis, in the high-ferritin group (Table 3) may be related to the suppressive effect of ferritin on adaptive immune responses. Given that ferritin levels are increased not only by iron overload but also by other factors such as inflammation, the objective measurement of liver iron content using magnetic resonance imaging [33] will be helpful in providing a more accurate analysis of the association between pretransplant iron overload and the incidence of chronic GVHD.

Several limitations of this study should be mentioned. First, the retrospective study design and heterogeneous background of the diseases and transplantation procedures might have biased the results. Second, posttransplant serum ferritin levels were not evaluated in this cohort owing to the lack of adequate information. We could obtain posttransplant (between day +50 and +99) serum ferritin data from only 28 cases among our cohort. Third, although we included CRP in the multivariate analysis, it was difficult to adjust for the effect of factors other than iron overload on ferritin levels. In a future study, measuring serum ferritin levels and liver iron content using magnetic resonance imaging before and after transplantation, and reanalyzing the effect of iron overload on the outcome may be worthwhile.

In conclusion, our results suggest that the pretransplant serum ferritin levels influenced the incidences of TRM and relapse rate after allogeneic HSCT and that, overall, an elevated pretransplant serum ferritin level is a strong adverse prognostic factor for survival. Furthermore, our results also suggest that a pretransplant serum CRP level may be a useful biomarker for predicting the risk of chronic GVHD.

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Conflict of interest The authors declare that they have no conflict of interest.

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LETTER TO THE EDITOR

Successful reduced-intensity umbilical cord blood transplant for fulminant hemophagocytic syndrome in an adult with pre-existing rheumatoid arthritis and autoimmune hemolytic anemia

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Hemophagocytic syndrome (HPS) is a potentially fatal syndrome of dysregulated immune activation characterized by severe clinical manifestations such as pancytopenia, high fever and hepatosplenomegaly. In adults, it usually occurs in association with a variety of underlying immune abnormalities including lymphoproliferative disorders (LPDs), viral infections and collagen vascular diseases. Most cases of mild to moderate severity HPS respond to high-dose steroid therapy or immunochemotherapy, but the treatment of severe HPS with pharmacologic therapy alone is often difficult. A few previous studies have reported that allogeneic hematopoietic cell transplant (HCT) is an effective therapy for refractory HPS, especially in inherited cases in children [1]. However, the benefit of allogeneic HCT in sporadic HPS in adults has not yet been established [2].

A 48-year-old woman with rheumatoid arthritis was admitted to our hospital with anemia and mild leukocytopenia. She had received oral methotrexate (MTX) for 1 year before admission. Direct and indirect Coombs tests were strongly positive, and marked splenomegaly was revealed on computed tomography (CT). She was diagnosed as having autoimmune hemolytic anemia (AIHA). MTX was discontinued and prednisolone was started. Her anemia gradually improved, but thereafter she began to show intermittent fever. Four months after the diagnosis of AIHA, she suddenly developed HPS with disseminated intravascular coagulation (DIC) and *Staphylococcus aureus* bacteremia. She was treated with high-dose methylprednisolone (mPSL), recombinant thrombomodulin and glycopeptide antibiotics. Her HPS transiently improved after the treatment but relapsed after 3 weeks. Positron emission tomography (PET) showed diffusely intense fluorodeoxyglucose (FDG) uptake in her liver, spleen and bone marrow. Bone marrow examination revealed numerous hemophagocytic cells and a few atypical lymphoid cells expressing cytoplasmic CD3, granzyme B and Epstein-Barr virus (EBV)-LMP antigens. EBV-DNA load in the patient's peripheral blood was elevated to 6.5×10^2 copies/10^6 cells, although the clonality of these cells was not evident by Southern hybridization analysis of T-cell receptor rearrangement and EBV-DNA tandem repeats. Subsequent administration of other immunochemotherapeutic agents failed to induce durable responses (Figure 1), and the patient's clinical condition showed further deterioration. We therefore decided to perform allogeneic HCT as the curative treatment option. Due to the lack of an immediately available related donor, we chose to use cord blood as a stem-cell source. Although the patient had human leukocyte antigen (HLA) antibodies against multiple HLA-class I antigens, a cord blood unit that contained sufficient cell dose and did not express HLA antigens reactive to her HLA antibodies was available (HLA type of the cord blood unit, A*02:06/A*11:01, B*40:06/B*67:01, DRB1*09:01/DRB1*16:02; recipient, A*11:01/A*26:02, B*40:06/B*67:01, DRB1*09:01/DRB1*16:02). Four months after the onset of HPS, she received a cord blood transplant following a conditioning regimen of melphalan (40 mg/m^2/day $\times$ 2 days), fludarabine (25 mg/m^2/day $\times$ 5 days) and total-body irradiation (2 Gy $\times$ 2). Tacrolimus was used for the prophylaxis of graft-versus-host disease (GVHD). Engraftment of neutrophils (>500/mm^3) and platelets ($>2 \times 10^4$/mm^3) occurred on days 14 and 32, respectively. Bone marrow examination on day 60 showed complete donor-type chimerism by short tandem repeat-polymerase chain reaction and no evidence of HPS. EBV-DNA load was under the detectable limit.

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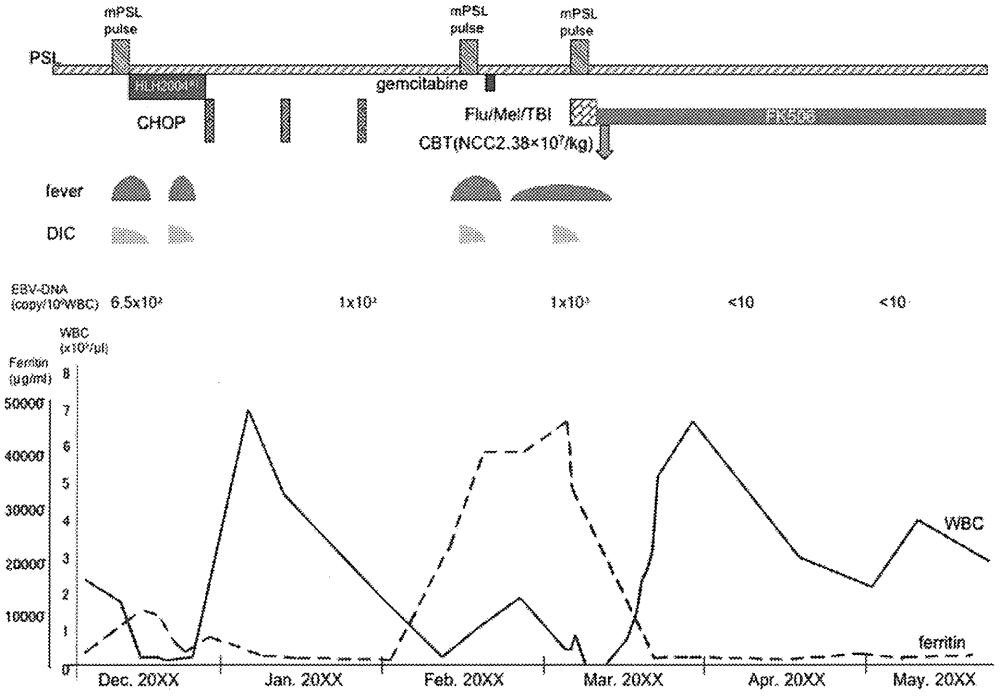

Figure 1. Clinical course from second onset of HPS to 3 months' progression after cord blood transplant (CBT). After CBT, the serum ferritin level was decreased and the general condition, including leukocytopenia, improved immediately. HLH 2004 is cited by ref. 8.

Autoimuune antibodies such as Coombs tests and rheumatoid factor became negative, and the levels of HLA antibody, evaluated by Luminex analysis, also became very low (Table I). Neither acute nor chronic GVHD developed, and the patient's pre-existing AIHA was improved. Tacrolimus was gradually tapered and discontinued on day 120. The patient had no signs of active HPS relapse 12 months after transplant. Her clinical course is summarized in Figure 1.

Allogeneic HCT following a reduced-intensity conditioning regimen is an effective therapy for severe HPS or hemophagocytic lymphohistiocytosis (HLH) in children [1]. In contrast, reports of allogeneic stem cell transplant in adult HPS are rare, probably because there is less chance of undertaking transplant due to rapid clinical deterioration and uncontrollable disease. Severe HPS in adults frequently occurs in association with underlying LPDs and viral infections. Some researchers suggested that the presence of a graft-versus-lymphoma effect might improve LPDs complicated with HPS [2,3].

We speculated that the background of HPS in our case was a T-cell LPD associated with EBV infection. However, the clonality of abnormal CD3 + cells was not detected in bone marrow samples, and biopsies of the most affected organs, such as the liver and spleen, could not be performed due to the presence of DIC. This patient might be most appropriately placed in the category of EBV-HLH, or EBV associated with lymphoma, which includes iatrogenic immunodeficiency-associated LPDs according to the World Health Organization (WHO) classification [4]. Approximately 40% of patients with rheumatoid arthritis treated with MTX who develop iatrogenic immunodeficiency-associated LPDs are EBV-positive; our patient also had a prior history of receiving MTX [5]. In general, EBV-HLH is a disease of children or adolescents, but there are reports of middle-aged patients [2]. In a Japanese nationwide survey for HLH, approximately 10% of all patients reported were more than 30 years old [5].

Table I. Titer of autoimmune antibodies pre- and post-transplant.

	Before transplant	6 Months after transplant
RF (IU/mL)	164	< 3
ANA	× 160	< 40
Cold agglutinin	× 512	< 40
Direct Coombs	4 +	Negative
Indirect Coombs	2 +	Negative
HLA antibody		
Class I	Positive	Negative
Class II	Negative	Negative

RF, rheumatoid factor; ANA, anti-nuclear antibody; HLA, human leukocyte antigen.

Our patient suffered from rheumatoid arthritis and AIHA before transplant, and it was intriguing to observe that the symptoms associated with these autoimmune disorders were almost completely resolved after transplant, concomitantly with the disappearance of the related autoantibodies. Such clinical improvement appears to be durable, and was maintained after the discontinuation of post-transplant immune-suppressive agents. Some reports have shown the efficacy not only of autologous but also of allogeneic HCT for refractory autoimmune disease [6,7], presumably because allogeneic HCT can eradicate autoreactive effector cells by conditioning and can also replace the autoreactive host immune system with a new donor immune system. Therefore, when patients with pre-existing autoimmune diseases also develop intractable hematologic disorders, allogeneic HCT for these patients may confer a "double-cure" of both conditions.

In conclusion, our experience suggests that reduced-intensity umbilical cord blood transplant may be an effective therapy for adult patients with refractory HPS, at least when they maintain an adequate organ function despite the presence of HPS-associated poor performance status. Future studies are warranted to determine optimal timing and safer strategies to perform allogeneic HCT for this devastating disorder.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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Splenic Irradiation as a Component of a Reduced-Intensity Conditioning Regimen for Hematopoietic Stem Cell Transplantation in Myelofibrosis with Massive Splenomegaly

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Primary myelofibrosis is a hematologic neoplasm characterized by bone marrow fibrosis and extramedullary hematopoiesis. A similar clinical condition can occur at late stage of myeloproliferative neoplasms such as polycythemia vera and essential thrombocythemia. Although allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only curative strategy for both conditions, massive splenomegaly frequently observed in patients with myelofibrosis is considered to be a risk factor for graft failure or engraftment delay after transplantation. A proportion of patients can benefit from splenectomy before transplantation but such procedures have been associated with substantial surgical morbidity. Here, we report two elderly patients with myelofibrosis who received scheduled splenic irradiation for massive splenomegaly immediately prior to allogeneic HSCT instead of undergoing splenectomy. The first patient was a 60-year-old woman who received peripheral blood stem cell transplantation for post-essential thrombocythemia myelofibrosis from an HLA-identical sibling; the second patient was a 60-year-old man who received unrelated bone marrow transplantation for primary myelofibrosis. After receiving fractionated splenic irradiation and fludarabine-based reduced-intensity conditioning regimens, these patients showed remarkable reduction of their splenomegaly at the time of transplantation. They attained successful donor cell engraftment without severe complications related to splenic irradiation, while improvement in splenomegaly was durable. Our experience suggests that splenic irradiation before allogeneic HSCT might be a safe and effective alternative to splenectomy for myelofibrosis patients with massive splenomegaly in terms of reducing the risk of surgical morbidity.

Keywords: allogeneic hematopoietic stem cell transplantation; myelofibrosis; myeloproliferative neoplasms; reduced-intensity conditioning regimen; splenic irradiation
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Primary myelofibrosis is a clonal hematologic neoplasm characterized by excessive proliferation of megakaryocytes and granulocytes, bone marrow fibrosis, and extramedullary hematopoiesis. Clinical manifestations include leukoerythroblastic anemia, splenomegaly, and constitutional symptoms, such as fatigue, weight loss, night sweat, and fever. Myelofibrosis can also occur at an advanced stage of other hematologic neoplasms, especially during the course of polycythemia vera and essential thrombocythemia. The median survival time is about six years with wide variation (Cervantes et al. 2009). A similar clinical condition can occur at late stage of polycythemia vera and essential thrombocythemia and also is associated with a poor prognosis.

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative strategy for primary myelofibrosis or post-polycythemia vera/essential thrombocythemia myelofibrosis, although novel Janus kinase (JAK) inhibitors have recently been introduced in clinical practice. However, in myelofibrosis patients undergoing reduced intensity conditioning HSCT, the engraftment failure is reported between 8% and 17% (Patriarca et al. 2008; Stewart et al. 2010; Robin et al. 2011). In addition, the presence of massive splenomegaly is considered to be a risk factor for graft failure or delayed engraftment (Stewart et al. 2010). In some patients, splenectomy is performed prior

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to allogeneic HSCT in order to improve the likelihood of engraftment. Splenectomy before HSCT results in the faster leukocyte engraftment and the better engraftment probability, but the effect on treatment-related mortality, relapse rate, and overall survival is still debatable (Kroger et al. 2009; Bacigalupo et al. 2010; Ballen et al. 2010; Stewart et al. 2010; Robin et al. 2011). Moreover, splenectomy can cause undesired complications (Bacigalupo et al. 2010). Therefore, splenectomy prior to HSCT is not routinely recommended strategy.

Splenic irradiation is one of the methods employed for the palliative management of patients with myelofibrosis and massive splenomegaly. Splenic irradiation in the palliative setting immediately relieves the patient's symptoms, but a short duration of effectiveness and severe pancytopenia were found to be major concerns (Elliott et al. 1998; Bouabdallah et al. 2000). Because such cytopenia after irradiation can be rescued by the infusion of allogeneic stem cell grafts, we assumed that splenic irradiation as a component of a reduced-intensity conditioning regimen could favorably influence on donor cell engraftment by decreasing the size of the spleen. Here, we report two myelofibrosis patients who received splenic irradiation immediately prior to allogeneic HSCT with a reduced-intensity conditioning regimen.

Patient 1

A 60-year-old woman, who had been diagnosed with essential thrombocythemia 10 years previously, presented with progressive splenomegaly, which resulted in her spleen being located 6 cm below the left costal margin; leukocytosis; and transfusion-dependent anemia. She was diagnosed with post-essential thrombocythemia myelofibrosis. After the written informed consent was obtained, she was treated with splenic irradiation (10 Gy in 1 Gy fraction) on days -22 through -9, followed by reduced-intensity allogeneic HSCT according to the protocol approved by the ethics committee of Kyoto University Graduate School of Medicine. The conditioning regimen consisted of fludarabine (days -6 through -2, total dose 125 mg/m^2), oral busulfan (days -3 and -2, total dose of 8 mg/kg), and 2 Gy total body irradiation on day-1. Her donor was an HLA-A, -B, -C, -DRB1 allele matched sibling male donor. She was infused with a peripheral blood stem cell graft containing 2.72×10^6 CD34+ cells/kg on day 0. The graft-versus-host disease (GVHD) prophylaxis involved the continuous injection of tacrolimus and methotrexate (Fig. 1).

During splenic irradiation, her spleen gradually reduced in size until it was located 3 cm below the left costal margin, and her abdominal pain was immediately relieved. Although further reduction was not evident after HSCT, improvement of splenomegaly was durable. Neutrophil engraftment, which was defined as the first consecutive days on which an absolute neutrophil count of at least 500/μl was detected, occurred on day 13. Prednisolone was added on day 28 because acute GVHD of the intestine was suspected. She was discharged 44 days after the transplant without any other severe complications. Acute GVHD of the skin occurred on day 71, and chronic GVHD of the liver, skin, and oral mucosa requiring additional immunosuppressive therapy occurred 5 months after the transplant. No increase in spleen size was observed until she relapsed. Unfortunately, 3 years and 2 months after the transplant her disease relapsed and progressed to acute

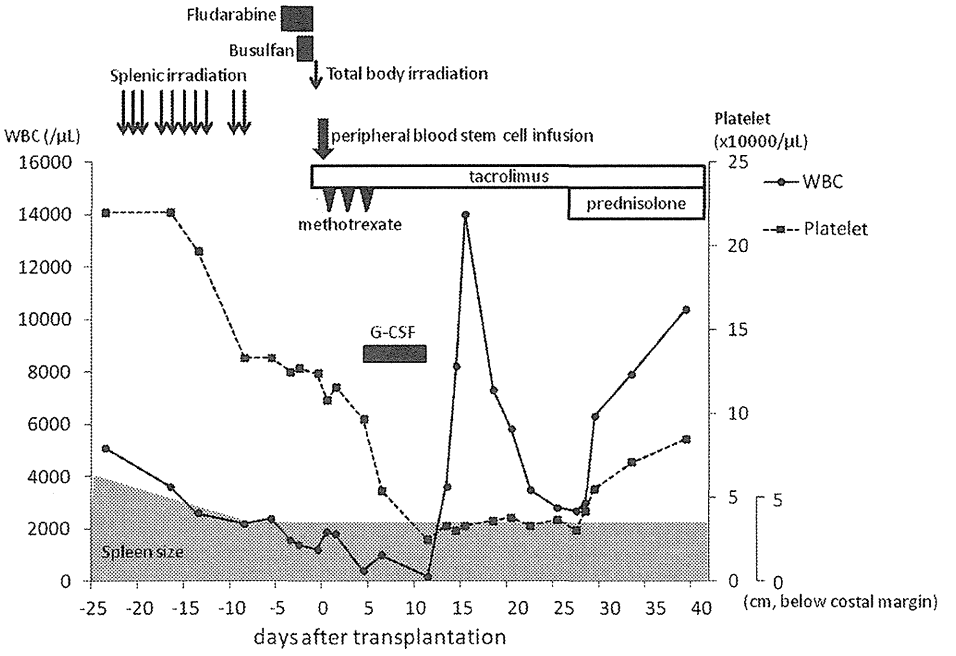

Fig. 1. Clinical course of patient 1.

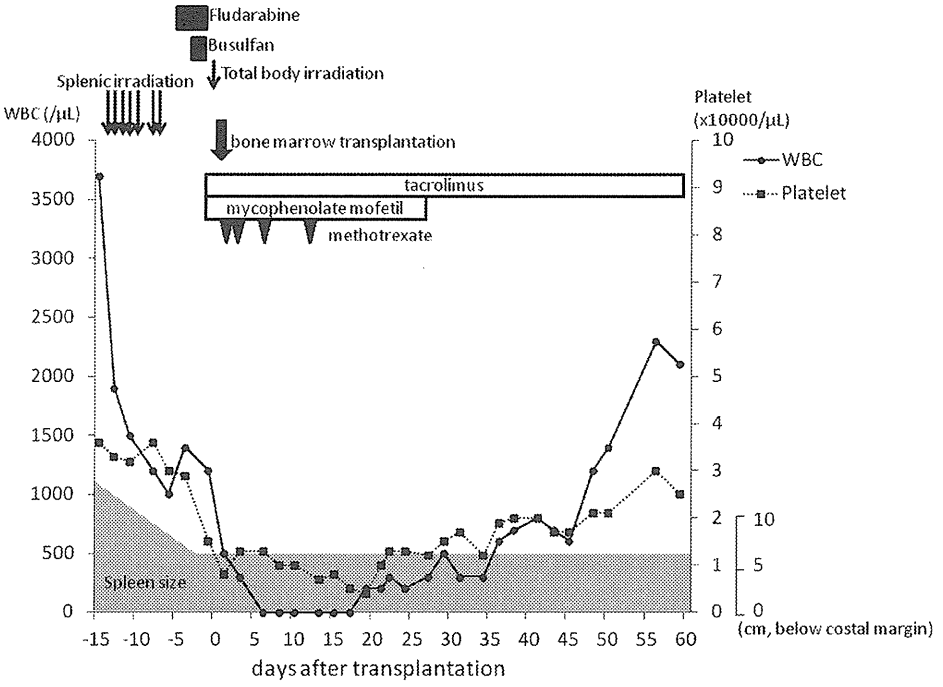

Fig. 2. Clinical course of patient 2.

myeloid leukemia. She died of the relapsed disease 5 years after the first transplant, even though a second transplant was performed.

Patient 2

A 60-year-old man suffering from progressive fatigue; abdominal pain combined with massive splenomegaly, which had reached his pelvis; transfusion-dependent anemia; thrombocytopenia; and leukoerythroblastosis was diagnosed with JAK2 V617F-associated primary myelofibrosis. After the written informed consent was obtained, he was treated with splenic irradiation (7 Gy in 1 Gy fraction) on days -15 through -7 followed by reduced-intensity allogeneic HSCT. The conditioning regimen consisted of fludarabine (total dose 125 mg/m^2), intravenous busulfan (total dose 6.4 mg/kg), and total body irradiation (2 Gy). On day 0, the patient was infused with 2.76×10^8 cells/kg bone marrow nucleated cells from an HLA-A, -B, -C, and –DRB1 allele matched unrelated male donor. The graft-versus-host disease (GVHD) prophylaxis consisted of the continuous injection of tacrolimus, methotrexate, and mycophenolate mofetil (Fig. 2).

During the splenic irradiation, his spleen gradually decreased in size until it was located 6 cm below the left costal margin. No other splenic irradiation-related complications occurred except for mild nausea. Although the pace of neutrophil recovery was relatively slow without the post-transplant administration of granulocyte colony stimulating factor, the absolute neutrophil count constantly exceeded 500/μl after day 48. No severe complications were observed until the patient was discharged (day 60). Acute GVHD of the skin was observed on day 41, but improved without additional immunosuppressive therapy. Six months after the transplantation he suffered chronic GVHD of the liver and gut, but it improved after the administration of steroids. His spleen did not subsequently increase in size, and he is still alive without any sign of relapse at one year and eight months after the transplant.

Discussion

In reduced-intensity HSCT for myelofibrosis, engraftment delay or failure is the primary concern. In a large prospective study from European Group for Blood and Marrow Transplantation, engraftment failure occurs only 2 in 103 myelofibrosis patients (Kroger et al. 2009). But in this study, 11% received an additional stem cell boost after transplantation because of poor graft function. In other recent studies, engraftment failure was reported to occur in 8% to 17% patients in reduced-intensity conditioning (Patriarca et al. 2008; Stewart et al. 2010; Robin et al. 2011). The massive splenomegaly which is sometimes seen in myelofibrosis can lead to delayed engraftment and graft failure. To improve the likelihood of engraftment, splenectomy prior to HSCT is performed. Splenectomy results in the faster leukocyte engraftment (Kroger et al. 2009; Stewart et al. 2010) and the better engraftment probability (Robin et al. 2011). Robin et al. (2011) reported the improvement of overall survival by splenectomy before HSCT, but other reports did not show the favorable influence of splenectomy on non-relapse mortality and overall survival (Patriarca et al. 2008; Kroger et al. 2009; Bacigalupo et al. 2010; Ballen et al. 2010; Stewart et al.

2010). Concerning relapse, the effect of splenectomy differs between reports. Kroger et al. (2009) reported the increase of relapse rate in splenectomized patients, but Bacigalupo et al. (2010) reported the decrease of relapse related death of splenecomized patients with large splenomegaly. Moreover, splenectomy is associated with perioperative complications, such as bleeding, infection, and thrombosis. Although Bacigalupo et al. (2010) reported that splenectomy before HSCT was not associated with increased mortality, six and five in 28 patients experienced deep vein thrombosis and febrile left pleural effusion, respectively. Among a total of 223 patients who underwent splenectomy in the palliative care setting, 68 patients suffered perioperative complications and 20 patients experienced fatal complications (Tefferi et al. 2000). Taken together, performing splenectomy prior to allogeneic HSCT for patients with massive splenomegaly is not routinely recommended. Novel JAK inhibitor therapy immediately induces reduction of splenomegaly and amelioration of constitutional symptoms (Harrison et al. 2012; Verstovsek et al. 2012). Although the effect of JAK inhibitors on transplanted hematopoietic stem cells and the withdrawal response after transplantation is not fully understood, the use of JAK inhibitors in combination with HSCT should be the area of further research.

Among chronic myeloid leukemia patients, splenic irradiation involving total doses of between 2.5 Gy and 10 Gy was safely performed in an attempt to enhance tumor cytoreduction, and it contributed to improvements in survival in some situations (Gratwohl et al. 1996; Jabro et al. 1999). To our knowledge, there have been no published studies investigating the role of splenic irradiation prior to allogeneic HSCT in myelofibrosis. In our two patients, splenic irradiation immediately relieved the patients' symptoms and successfully reduced the size of their spleens before the allograft infusion. In Patient 2, although neutrophil engraftment was delayed, severe leukocytopenia less than 200 /μl was not observed after day 19 and eventually successful engraftment was confirmed. In both patients, no severe splenic irradiation-related complications were observed.

In conclusion, our present experience suggests that splenic irradiation prior to allogeneic HSCT is feasible and might facilitate engraftment without having adverse effects on the outcome of allogeneic HSCT for selected patients with primary or post-polycythemia vera/essential thrombocythemia myelofibrosis. Future studies are warranted to evaluate the role of splenic irradiation as a component of a reduced-intensity conditioning regimen in allogeneic HSCT for myelofibrosis with massive splenomegaly.

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ORIGINAL ARTICLE

Excellent outcome of allogeneic bone marrow transplantation for Fanconi anemia using fludarabine-based reduced-intensity conditioning regimen

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Abstract Fanconi anemia (FA) is a disorder characterized by developmental anomalies, bone marrow failure and a predisposition to malignancy. It has recently been shown that hematopoietic stem cell transplantation using fludarabine (FLU)-based reduced-intensity conditioning is an efficient and quite safe therapeutic modality. We retrospectively analyzed the outcome of bone marrow transplantation (BMT) in eight patients with FA performed in two institutes between 2001 and 2011. There were seven females and one male with a median age at diagnosis = 4.5 years (range 2–12 years). The constitutional characteristics associated with FA, such as developmental anomalies, short stature and skin pigmentation, were absent in three of the patients. One patient showed myelodysplastic features at the time of BMT. All patients received BMT using FLU, cyclophosphamide (CY) and rabbit antithymocyte globulin (ATG) either from a related donor ($n = 4$) or an unrelated donor ($n = 4$). Acute graft-versus-host disease (GVHD) of grade I developed in one patient, while chronic GVHD was not observed in any patient. All patients are alive and achieved hematopoietic recovery at a median follow-up of 72 months (range 4–117 months). BMT using FLU/low-dose CY/ATG -based regimens regardless to the donor is a beneficial therapeutic approach for FA patients.

Keywords Fanconi anemia · Hematopoietic stem cell transplantation · Fludarabine

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Introduction

Fanconi anemia (FA) is a complex disorder characterized by developmental anomalies, early onset progressive bone marrow failure (BMF) and a tendency to develop hematological and non-hematological malignancies. The risk of FA patients to develop BMF and malignancies increases with progression of age, and the cumulative incidence by age of 40 years was 90 % for BMF and 30 % for hematologic and nonhematologic neoplasms [1–4]. Short stature and abnormal skin pigmentation are particularly common features found in patients with FA and a wide variety of congenital malformations has been described in 60–75 % of FA patients [3–5]. The incidence of FA in Japan was approximately 5–10 new cases diagnosed annually and hematological abnormalities usually manifested early in childhood at a median age of 7 years (range 0–31 years). Owing to the complexity of the disease and multisystem involvement, FA has a high mortality rate with a median age of death of 30 years [3–5]. Therefore, the study of FA holds a great promise for elucidation of the heterogeneity of this disorder in the future. Hematopoietic stem cell transplantation (HSCT) is the only curative therapy known so far for correcting the hematological manifestations in

Table 1 Clinical characteristics of 8 Fanconi anemia patients

No.	Male/ female	Disease status at HSCT	Age at diagnosis (year)	Age at HSCT (year)	Karyotype	Chromosomal fragility test (gap and break/100 cells)	Short stature (<−1SD)	Skin pigmentation	Other anomalies
1	F	SAA	7	8	46,XX	114	No	No	No
2	F	SAA	3	5	46,XX	514	−2SD	Yes	Shortness of first finger
3	F	SAA	8	8	46,XX	193	No	No	No
4	F	SAA	12	13	46,XX	299	−1SD	Yes	No
5	M	SAA	2	7	46,XY	152	−3SD	No	Hyperdactylia
6	F	MDS (RAEB)	3	6	Other[a]	148	−1.5SD	No	Hyperdactylia
7	F	SAA	6	11	46,XX	159	No	No	No
8	F	SAA	3	5	46,XX	169	−2SD	Yes	Radius defect, esophageal atresia

SAA severe aplastic anemia, *MDS* myelodysplastic syndrome, *HSCT* hematopoietic stem cell transplantation, *RAEB* refractory anemia with excess blasts

[a] others; 46,XX,add(3)(q26),der(7)add(7)(p22)add(7)(q11)add(8)(q22)

FA patients. Initially, potential problems in designing HSCT conditioning regimens for patients with FA appeared due to the acquired hypersensitivity to DNA cross-linking or oxidative agents such as alkylating agents or ionizing radiation [6], but recently a significant progress has been achieved by using FLU-based reduced-intensity conditioning regimens that markedly improved the efficiency and safety of this procedure [7, 8]. Specifically, FLU-based reduced-intensity conditioning allogeneic HSCT resulted in reduction of regimen-related toxicity (RRT), superior engraftment and less graft-versus-host disease (GVHD), which in turn led to improvement of patients' survival. In this study, we aimed to investigate the effectiveness and safety profile of bone marrow transplantation (BMT) using FLU-based reduced-intensity conditioning regimens in eight patients with FA, who were transplanted from either a related donor (3 HLA-genetically matched and 1 HLA-A locus mismatched) or an unrelated donor (2 HLA-matched and 2 HLA-DRB1 mismatched).

Patients and methods

We retrospectively analyzed eight patients, who were diagnosed as having FA and received BMT at Nagoya University and Nagoya First Red Cross Hospital between 2001 and 2011. There were seven females and one male with a median age at diagnosis of 4.5 years (range 2–12 years) (Table 1). Clinical features suggestive of FA including low birth weight, short stature, hyperpigmented skin, radial abnormality and duplicated thumbs were defined in five out of eight patients, while three patients were asymptomatic. Diagnosis of FA was confirmed in all patients by a reliable cellular marker for FA cells and all of them showed a high incidence of chromosomal breaks and gaps, which indicated chromosomal instability by adding mitomycin C at a final concentration of 0.5 μM (Table 1). Seven patients suffered from severe aplastic anemia and one patient evolved to refractory anemia with excess of blasts (RAEB) with the emergence of cytogenetic abnormalities in the form of add(3)(q26) at the time of HSCT. All patients underwent allogeneic BMT at a median age of 7.5 years (range 5–13 years), and they were transplanted from either a related donor (3 HLA-genetically matched and 1 HLA-A locus mismatched) or an unrelated donor (2 HLA- matched and 2 HLA-DRB1 mismatched). All patients received a preparative regimen including a combination of fludarabine (FLU 120–180 mg/m^2), cyclophosphamide (CY 40 mg/kg) and rabbit anti-thymocyte globulin (ATG, Thymoglobulin, Genzyme, 5–10 mg/kg). Patients transplanted from unrelated donor received a total body irradiation (TBI)/total lymphoid irradiation (TLI) of 4–4.5 Gy, and patients transplanted from HLA-A locus mismatched related donor received 2 Gy. As GVHD prophylaxis, BM recipients from related donor received cyclosporine A (CyA) plus short-term methotrexate (MTX), while BM recipients from unrelated donor received tacrolimus (FK506) plus short-term MTX. Details on the donors' characteristics, conditioning regimen and GVHD prophylaxis are listed in Table 2.

Table 2 Hematopoietic stem cell transplantation for 8 Fanconi anemia patients

No.	Performance status	Transfusion before SCT	Conditioning	Donor	Concordance of HLA-serological typing	Transfused cell number ($\times 10^8$/kg)	GVHD prophylaxis
1	0	MAP 2u, PC10u	FLU (120 mg/m^2) + CY (40 mg/kg) + ATG (10 mg/kg)	Mother	6/6	3.9	CyA + short MTX
2	0	MAP 2u, PC10u	FLU (150 mg/m^2) + CY (40 mg/kg) + ATG (6 mg/kg)	Sister	6/6	7.4	CyA + short MTX
3	0	MAP 4u, PC 20u	FLU (180 mg/m^2) + CY (40 mg/kg) + ATG (10 mg/kg) + TBI (2 Gy)	Father	5/6 (mismatched A 1 locus)	3.0	CyA + short MTX
4	0	MAP 4u, PC 20u	FLU (150 mg/m^2) + CY (40 mg/kg) + ATG (5 mg/kg)	Brother	6/6	2.2	CyA + short MTX
5	1	MAP 21u, PC 120u	FLU(180 mg/m^2) + CY(40 mg/kg) + ATG(10 mg/kg) + TLI (4 Gy)	Unrelated	6/6	3.0	FK506 + short MTX
6	0	MAP 16u, PC100u	FLU (150 mg/m^2) + CY (40 mg/kg) + ATG (10 mg/kg) + TBI (4.5 Gy)	Unrelated	5/6 (mismatched DR 1 locus)	4.5	FK506 + short MTX
7	0	MAP 12u, PC120u	FLU (120 mg/m^2) + CY (40 mg/kg) + ATG (10 mg/kg) + TLI (4 Gy)	Unrelated	5/6 (mismatched DR 1 locus)	3.8	FK506 + short MTX
8	0	MAP 4u, PC 20u	FLU (150 mg/m^2) + CY (40 mg/kg) + ATG (10 mg/kg) + TBI (4.5 Gy)	Unrelated	6/6	4.6	FK506 + short MTX

SCT stem cell transplantation, *FLU* fludarabine, *CY* cyclophosphamide, *ATG* anti-thymocyte globulin (rabbit ATG, Thymoglobulin), *TBI* total body irradiation, *TLI* total lymphoid irradiation, *CyA + short MTX* cyclosporine plus short-term methotrexate

Results

The median transfused nucleated cell number was 3.8 × 10^8/kg (range 2.2 to 7.4 × 10^8/kg) and all patients achieved sustained engraftment; the median time to neutrophil (>500), platelet (>50,000/ml) and reticulocyte (>10‰) recovery was 15.5, 20.5 and 21.5 days, respectively. Among eight patients enrolled in this study with a median survival period of 72 months (range 4–117 months), acute GVHD grade I was detected in one patient, whereas chronic GVHD was not found in any patient. Two patients experienced hepatic dysfunction and one patient had gastric hemorrhage as regimen-related toxicities (grade 1 according to the National Cancer Institution-Common Toxicity Criteria, NCI-CTC Version 4.0). Three patients exhibited febrile neutropenia and one of them showed disseminated fungal infection (grade 4 according to NCI-CTC) complicated by development of a renal abscess that showed complete remission after amphotericin B treatment. Cytomegalovirus (CMV)-polymerase chain reaction (PCR) and CMV-pp65 antigen detection were performed on a weekly basis for identification of CMV infection. We found four out of eight patients showed CMV reactivation without clinical symptom and they were treated with ganciclovir and foscavir. One patient suffered from hemorrhagic cystitis and lymphoproliferative disorder (LPD) due to BK virus and Epstein–Barr virus (EBV) reactivation, respectively. This patient was completely cured from the EBV–LPD after successful treatment with rituximab. Otherwise, no patients developed veno-occlusive disease (VOD) or thrombotic microangiopathy (TMA) (Table 3). Over the total length of the follow-up period, no patients showed secondary bone marrow failure and/or malignancies with a median follow-up of 72 months (range 4–117 months).

Discussion

Through a retrospective analysis of the medical records of eight pediatric patients with FA, who received FLU-based reduced-intensity conditioning allogeneic HSCT to evaluate their outcome, we found that all patients achieved favorable outcome using this procedure and the type of the donor did not significantly influence the clinical outcome. In previous studies, it has been proved that the use of alkylating agents and radiation therapy for FA patients was harmful. The impact of lower irradiation dose on immune recovery and risk of malignancy remains a matter of debate and a longer follow-up period is needed. However, it was reported that TBI 300 cGy was the lowest possible irradiation dose in the context of FLU/CY-based regimen; other

Table 3 Results of hematopoietic stem cell transplantation for 8 Fanconi anemia patients

No.	Days of engraftment	Acute GVHD	Chronic GVHD	RRT	VOD	TMA	CMV Ag positivity and treatment	Other virus related disease	FN	Fungal infection	Overall survival (Mo)
1	19	0	0	No	No	No	Day 35, GCV	No	No	No	114
2	15	I	0	No	No	No	Day 13, GCV + FCV	No	Yes	No	41
3	19	0	0	Liver dysfunction (grade 1)	No	No	Day 30, GCV	No	Yes	No	17
4	14	0	0	Gastric hemorrhage (grade 1)	No	No	No	No	No	No	4
5	17	0	0	No	No	No	Day 48, GCV	No	No	No	117
6	19	0	0	No	No	No	No	No	No	Disseminated fungal infection, renal abscess (grade 4)	99
7	15	0	0	Diarrhea (grade 1), liver dysfunction (grade 1)	No	No	No	Hemorrhagic cystitis due to BKV (grade 2) and LPD due to EBV	Yes	No	72
8	19	0	0	No	No	No	No	No	No	No	26

RRT grade was determined by National Cancer Institute-Common Toxicity Criteria (NCI-CTC Ver 4.0)

GVHD graft-versus-host disease, *RRT* regimen-related toxicity, *VOD* veno-occlusive disease, *TMA* thrombotic microangiopathy, *CMV Ag* AMV antigenemia (pp65), *GCV* ganciclovir, *FCV* foscavir, *BKV* BK virus, *EBV*, Epstein–Barr virus, *FN* febrile neutropenia

successful trials of eliminating irradiation in the conditioning regimens even in recipients of BMT from unrelated donor have been achieved [8–11]. Subsequently, alternative regimens have been developed to reduce the potential risk of irradiation and GVHD using a non-irradiation based preparative therapy including a low-dose CY, FLU and ATG [6–8, 10]. Here, we showed that BMT using FLU-based reduced-intensity conditioning regimens led to improvement of the outcome for FA patients regardless of the donor type. In line with our findings, other investigators reported that HSCT using FLU-containing regimens was associated with a better outcome even for recipients of allograft from unrelated donor with stable engraftment and minimal toxicity, whereas adding ATG to the conditioning regimen contributed to decreasing the incidence of GVHD [7, 8, 11, 12]. On the other hand, it was reported that bone marrow recipients from unrelated donor showed less successful transfusion rate with high percentage of graft failure and RRT compared to bone marrow recipients from related donor [13]. In our cohort, FLU/ATG/low-dose CY-based conditioning regimen was tolerable and efficient for FA patients even for BM recipient from unrelated (HLA-1 locus mismatched) donor, but some patients developed virus reactivation such as CMV, BKV and EBV. To overcome virus reactivation that might occur as an adverse event following the use of ATG-containing regimen, it is better to decrease the ATG dose from 10 to 5 mg/kg. FA patients are more prone to cancer development such as squamous cell carcinomas with special predilection sites (esophagus, head and neck) [4, 14, 15]. FLU-containing regimens were employed in a limited number of FA patients, making it difficult to speculate on its implications on cancer progression. Three out of four patients received HSCT from HLA-matched related donor without irradiation and they showed successful and excellent outcomes. Therefore, it is important to consider the use of the conditioning regimen without irradiation for this group of patients. Several studies have demonstrated that TBI with 300–450 cGY is needed for consistent engraftment in recipients from unrelated donor [12, 16]. We would like to emphasize that reduction of irradiation dose in the conditioning regimens even for recipients of HSCT from unrelated donor is an important target that will improve the patient's quality of life by reducing late effects, particularly the risk of malignancy. Noteworthy, in our series three out of eight patients were asymptomatic. Although our findings were consistent with other investigators' results [3–5], early diagnosis and optimal timing of transplant in

asymptomatic FA patients is challenging and it is of utmost importance to confirm FA diagnosis in those patients who might be misdiagnosed with acquired aplastic anemia. In conclusion, the identification of asymptomatic FA patients requires careful consideration by testing for cross-linker hypersensitivity that provides a reliable cellular marker for FA diagnosis. HSCT using FLU/low-dose CY/ATG-based regimen is beneficial and could be a promising therapeutic approach for FA patients regardless of the donor type with favorable clinical outcome.

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Conflict of interest The authors declare no conflict of interest.

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LETTER TO THE EDITOR

Autoimmune-like hepatitis following unrelated BMT successfully treated with rituximab

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We report a case of autoimmune-like hepatitis, as defined by the modified criteria of the International Autoimmune Hepatitis Group,[1] that developed in a patient following unrelated BMT and was successfully treated using rituximab.

A 1-year-old girl was diagnosed with AML in August 2007. She achieved CR with multi-drug chemotherapy. However, relapse occurred in the BM in May 2009. Following reinduction chemotherapy, she was transferred to our hospital, where she underwent allogeneic BMT from an HLA 8/8-matched unrelated donor in March 2010 during second CR. The conditioning regimen consisted of BU 1.2 mg/kg/dose × 4 doses a day for 4 days from day −9 to −6, melphalan 90 mg/m^2 for 2 days from day −5 to −4 and fludarabine 30 mg/m^2 for 4 days from day −5 to −2. On day 0, 4.5×10^8/kg nucleated marrow cells (0.5×10^6/kg CD34+ cells) were infused. Tacrolimus (0.02 mg/kg/day) and short-term MTX (15 mg/m^2 on day 1, 10 mg/m^2 on days 3, 6 and 11) were employed as prophylaxis against GVHD. Engraftment of neutrophils was achieved on day 14. Complete chimerism of blood cells was confirmed by STR analysis on day 80. Acute GVHD was not observed. Tacrolimus was discontinued on day 85 and the patient was discharged on day 99 without any complications.

She was re-admitted to our hospital on day 135 with elevated liver enzymes (aspartate aminotransferase, 495 IU/L; alanine aminotransferase, 748 IU/L). She exhibited no signs of intestinal or skin GVHD. Serological tests for hepatitis A, B and C yielded negative results. No CMV, EBV or human herpesvirus 6 genomic DNA was detected from PBMCs by real-time PCR. Although her Ig level was relatively high (IgG, 1878 mg/dL), negative results were obtained for all available autoimmune markers (antinuclear Ab, anti-double-strand DNA Ab, anti-single-strand DNA Ab, anti-smooth muscle Ab, anti-mitochondrial Ab and anti-liver kidney microsomal-1 Ab). The patient was treated with tacrolimus based on a tentative diagnosis of hepatic GVHD. After 15 days of treatment, serum transaminases had decreased, whereas total bilirubin level was elevated to 7.5 mg/dL. I.v. methyl prednisolone (mPSL) therapy (2.0 mg/kg/day) was started soon after liver biopsy on day 151 (Figure 1a).

The histological picture of the liver specimen, including immunostaining, revealed that eosinophil, CD138+ plasmacytes and CD20+ B-lymphocyte infiltration predominated in portal areas. Interlobular bile ducts were preserved and CD8+ T-cells were less than in GVHD or viral infections. Portal mononuclear cells invaded the limiting plates surrounding lobules, leading to interface hepatitis in the periportal area. Lobular inflammation was modest and marked hepatocyte swelling (ballooning degeneration) was observed (Figures 1b–h). These findings were rather more consistent with autoimmune hepatitis (AIH) than with classical hepatic GVHD. Drug-induced hepatitis was excluded because drug administration was not changed before and after the onset of disease, although eosinophilic infiltration might suggest it. As no Epstein–Barr-encoded RNA-positive cells were found in any of the cells analyzed, the possibility of post-transplant lymphoproliferative disorder was excluded. According to the modified criteria of the International Autoimmune Hepatitis Group, our patient was diagnosed with definite AIH, defined by a score >15 (the patient's score was 17).[1]

Hepatic dysfunction improved with mPSL treatment and the dosage was gradually tapered. On day 178, mPSL was converted to oral prednisolone (PSL). When the dosage of PSL was tapered to a dose of 1.0 mg/kg/day, serum transaminases again began to increase. Even though we increased the dosage of PSL to 1.3 mg/kg/day and added oral mycophenolate mofetil at a dose of 30 mg/kg/day on day 191, hyperbilirubinemia could not be fully controlled.

The patient was then treated using rituximab (375 mg/m^2 every 2 weeks for six consecutive weeks), as liver dysfunction was steroid-dependent and the histological picture demonstrated infiltration of CD20+ B-lymphocytes. After rituximab treatment, serum transaminases gradually decreased and PSL was tapered to 0.1 mg/kg/day.

On day 313, the serum transaminases increased and she was again treated using rituximab. Ten days after the last rituximab treatment, another liver biopsy was performed. CD20+ B-lymphocytes were not observed in the histological picture (Figure 1e), and serum transaminases gradually improved and normalized. Although the steroid dose was tapered and stopped, her liver dysfunction did not recur.

AIH is an immune-mediated necroinflammatory condition of the liver. Presentation can vary from the asymptomatic individual with abnormal liver function tests to fulminant liver failure. The diagnosis is based on the combination of biochemical, autoimmune and histological parameters, and the exclusion of other causes of liver diseases. We summarized occasional examples of *de novo* autoimmune-like hepatitis following allo-SCT in which the target liver cells and effector cells are different in origin (Table 1).[2–5,8,9] Habib *et al.* used the term 'alloimmune hepatitis' to describe this entity. The laboratory and histological

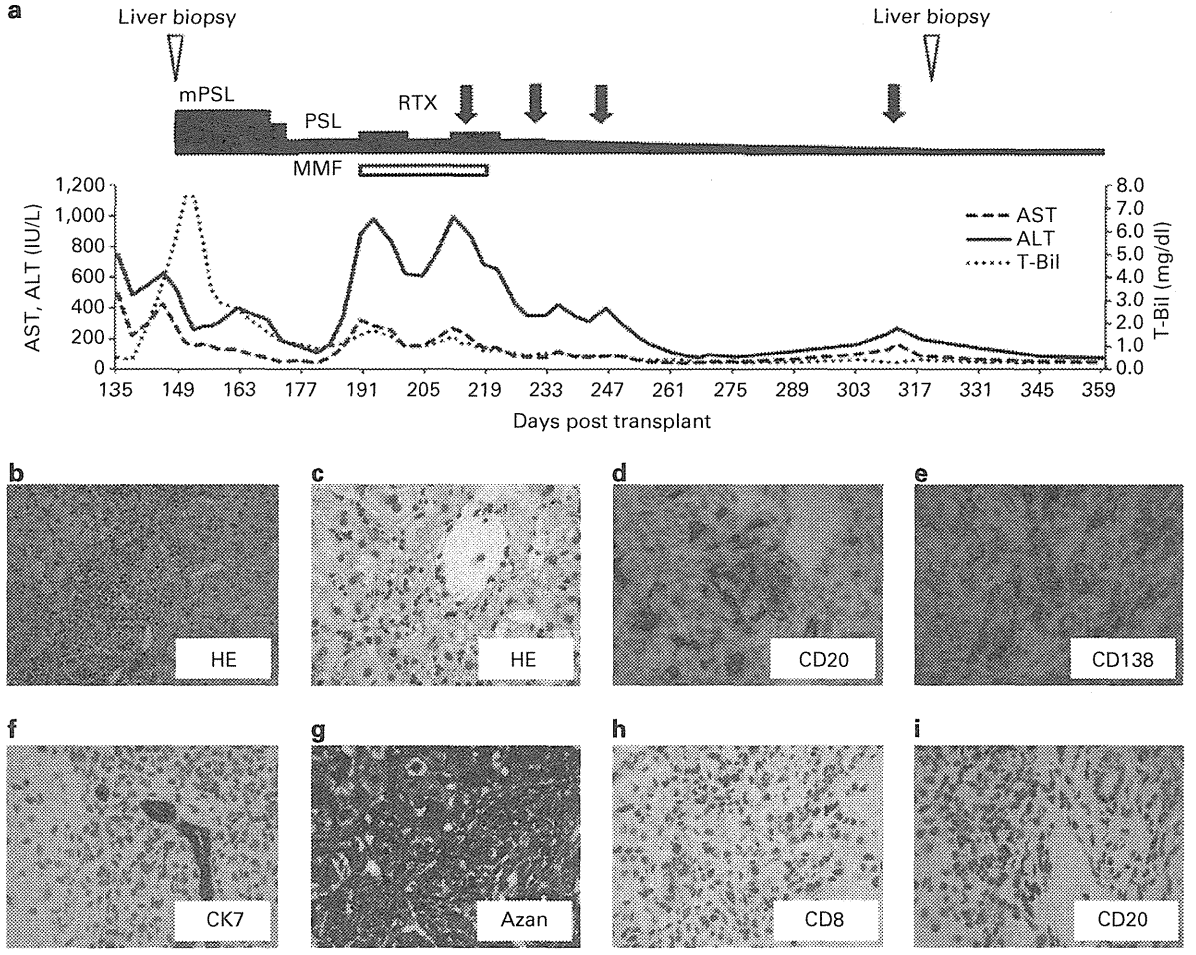

Figure 1 Clinical course of liver dysfunction (**a**), and pathological findings from liver biopsies at diagnosis (**b–h**) and after rituximab administration (**i**). (**a**) After four doses of rituximab (375 mg/m^2/dose) from day 214, serum transaminases gradually decreased and steroid dose was tapered and stopped. (**b**) Low magnification (× 100, HE staining). Portal mononuclear cells invading the limiting plates surrounding lobules, leading to interface hepatitis. Moderate number of eosinophils were observed. (**c**) High magnification (× 400, HE staining). Lobular inflammation was modest and marked hepatocyte swelling (ballooning degeneration) was observed. Immunostaining revealed that cells infiltrating portal areas were predominantly CD20 + lymphocytes (**d**) and CD138 + plasma cells (**e**), and interlobular bile ducts are rather preserved (**f, g**). CD8 + T-cells are much less than GVHD or viral infections (**h**). These findings were rather more consistent with that of autoimmune-like hepatitis than with classical hepatic GVHD. CD20 + B- lymphocytes were not observed at all after rituximab treatment (**i**). ALT, alanine transaminase; AST, aspartate aminotransferase; MMF, mycophenolate mofetil; mPSL, methyl predonisolone; PSL, predonisolone; RTX, rituximab.

Table 1 Clinical characteristics of patients diagnosed with autoimmune-like hepatitis after allo-SCT in the literature

Age (years)	*Sex*	*Original disease*	*Donor*	*Stem cell source*	*Response to steroid*	*Other treatment*	*Prognosis*	*Reference*
30	F	ALL	MSD	BM	Poor	Liver transplant	Alive	[8]
7	M	ALL	MSD	PBSC	Poor	CSA, Tacrolimus	Alive	[3]
47	M	AML	MSD	ND	Good	—	Alive	[9]
30	M	CML	MSD	BM	Good	—	Alive	[4]
43	F	CML	MSD	PBSC	Good	Azathioprine	Alive	[2]
44	M	NHL	MSD	PBSC	Poor	—	Dead	[5]
1	F	AML	MUD	BM	Poor	Rituximab	Alive	Present case

Abbreviations: MSD = matched sibling donor; MUD = matched unrelated donor; ND = not described; NHL = non-Hodgkin's lymphoma.

findings and clinical course of our patient were very similar to these cases and she may be included in the same disease entity. As our patient and her donor were sex matched, we could not confirm the donor origin of immune-reactive cells, which was demonstrated by *in situ* hybridization in the patient reported by Habib *et al.*, Mullighan *et al.* report