local and temporary effect at best, and do not prolong survival (3,4).

Hematopoietic stem cell transplantation (HSCT) is a treatment established principally for hematologic malignancies. This treatment exerts not only a cytotoxic effect generated by antineoplastic agents, but also an alloimmune graft-versus-leukemia effect (5). Some studies have shown that the therapeutic effects of HSCT may also be effective against solid tumors (6–8). After the promising report by Childs et al. (10) in 1999, several studies confirming these results were reported (9). However, only ~100 total cases have been reported so far. Also, few studies have histologically shown the anti-tumor effect elicited by the alloimmune reaction (11). We report here the results of an institutional clinical study of HSCT for refractory RCC with reduced-intensity conditioning (RIC), and the histological findings that suggest a graft-versus-tumor (GVT) effect *in vivo*.

PATIENTS AND METHODS

This prospective study was conducted between July 2000 and March 2005 at Kanazawa University Hospital, Japan. RCC patients aged 50 years of age and older with multiple metastases refractory to therapy, and remaining good performance status ($\leq$2) with no severe co-morbid conditions, were eligible for the study. Primary endpoint was defined as the survival at day 100 after HSCT with complete donor chimerism, and secondary endpoint was the effectiveness of HSCT. Since all patients were in their 50s and 60s, we chose an RIC regimen regarded as feasible for elderly patients because it involves a lower dose of chemoradiotherapy leading to less toxicity (12). Transplants were performed between July 2000 and March 2005 at Kanazawa University Hospital, Japan. Our institutional review board approved this study, and all patients and donors provided with written informed consent to participate in the study. Treatment response after HSCT was evaluated monthly by computed tomography according to the Response Criteria in Solid Tumors (RECIST). Patients with progressive disease after HSCT were first treated by withdrawal of immunosuppressive agents. Additional treatments were at the discretion of the attending physician. The probability of OS was estimated by the Kaplan–Meier method. If a patient suffered graft failure after the first transplant, we performed a second transplant, and used only the second transplant for analysis.

RESULTS

Patients Characteristics

Table 1 shows the characteristics of the patients enrolled in this study, which includes seven patients (six males and one female, median patient age: 61 years old). RCC in these patients was refractory to treatment, including interferon-α (IFN-α) and/or IL-2. All patients underwent nephrectomy for debulking of the tumor prior to HSCT, except for Patient #2 who was unable to undergo removal of the primary focus. Histology showed a clear cell type in six patients and adenocarcinoma in Patient #2. Five of seven patients were positive for one or more poor prognostic factors (C-reactive protein, hemoglobin and lactase dehydrogenase).

Response to HSCT

Table 2 shows the results of the HSCT treatment. Five patients received peripheral blood stem cells from a matched sibling donor, and two received umbilical cord blood from an unrelated donor, one of which was serologically HLA matched and the other which had one HLA locus mismatched. The latter patient suffered graft failure and underwent a second transplant with unrelated cord blood with two HLA loci mismatched. Neutrophils were engrafted in seven of eight HSCT (88%); the neutrophil count exceeded 5 ×

Table 1. Patients characteristics

Patient	Age/ gender	Histology	Site(s) of metastases	Previous systemic therapies	PS	CRP (mg/dl; 0.0–0.3)[a]	Hb (g/dl; 13.5–17.0)[a]	LDH (IU/l; 119–229)[a]
1	64/M	Clear cell	Bone, lung, pleura	IFN-α	2	0.6	15.2	170
2	59/F	Papillary	Pleura, liver, adrenal gland, LNs	IFN-α, IL-2	2	6.7	8.3	423
3	56/M	Clear cell	Bone, lung, pleura, adrenal gland, LNs	IFN-α, IL-2	2	0.2	9.2	161
4	61/M	Clear cell	Bone, lung, adrenal gland, brain	IFN-α, IL-2	2	5	11.7	325
5	69/M	Clear cell	Bone, lung, LNs	IFN-α, IL-2	2	7.8	8.2	94
6	68/M	Clear cell	Lung, pluera, adrenal gland, maxillary sinus, pancreas, retroperitoneum, subcutaneous, contralateral kidney	IFN-α, IL-2, 5-FU	1	0.3	13.7	179
7	60/M	Clear cell	Lung	IFN-α, IL-2	0	0.0	15.4	126

PS, performance status; CRP, C-reactive protein; Hb, hemoglobin; LDH, lactase dehydrogenase; LNs, lymphnodes; IFN-α, interferon-α; IL-2, interleukin-2; 5-FU, 5-fluorouracil.
[a]Institutional normal range is described.

Table 2. Preparative regimen and outcomes after transplantation

Patient	Graft	Conditioning regimen[a]	GVHD prophylaxis	Number of transplanted $CD34^+$ cells ($\times 10^6$/kg)	Neutrophil $>5.0 \times 10^8$/l (days)	Acute GVHD grade	Chronic GVHD	Response to therapy	Outcome	Accumulation of $CD8^+$ lymphocytes at metastases
1	PBSC	Flu 125 mg/m^2 + CY 120 mg/kg	CsA	5.6	13	0	skin, oral	PR→SD	Dead (pneumonia), day 534	Yes
2	PBSC	Flu 125 mg/m^2 + CY 120 mg/kg	CsA	6.3	13	III	none	PD	Dead (RCC), day 68	No
3	PBSC	Flu 125 mg/m^2 + CY 120 mg/kg	CsA	4.9	10	II	skin, oral, liver	PR→SD	Dead (interstitial pneumonia), day 709	Yes
4	PBSC	Flu 180 mg/m^2 + BU 8 mg/body + ATG 5 mg/kg	CsA	3.5	11	0	none	PD	Dead (RCC), day 262	No
5	CB	Flu 200 mg/m^2 + CY 50 mg/kg + TBI 2 Gy	CsA	1.4[b]	11	II	none	SD	Dead (pneumocystis pneumonia), day 145	No
6-1	CB	Flu 200 mg/m^2 + CY 50 mg/kg + TBI 2 Gy	CsA	1.4[b]	NE	NE	NE	NE	Graft failure, re-registered as patient 6–2	NE
6-2	CB	Flu 125 mg/m^2 + CY 80 mg/kg + TBI 2 Gy	FK506	2.2[b]	15	I	none	PR→SD→PD	Dead (RCC), day 720	Yes
7	PBSC	Flu 125 mg/m^2 + CY 80 mg/kg + TBI 2 Gy	FK506	2.2	11	II	skin, oral, gut	PR→SD→PD	Alive, day 1350	ND

GVHD, graft-versus-host disease; PBSC, peripheral blood stem cells; Flu, fludarabine; CY, cyclophosphamide; CsA, cyclosporine; PR, partial response; SD, stable disease; PD, progressive disease; RCC, renal cell carcinoma; BU, busulfan; ATG, antithymocyte globuline; CB, cord blood; TBI, total body irradiation; NE, not evaluable; Mel, melphalan; FK506, tacrolimus; ND, not done.
[a]Total dosage of each drugs was described.
[b]Total number of transplanted cells ($\times 10^7$/kg).

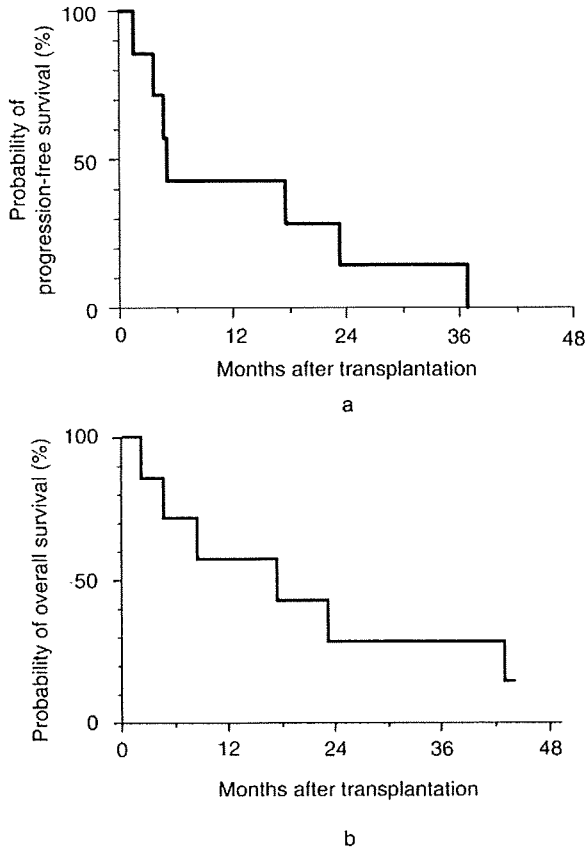

Figure 1. Outcomes after hematopoietic stem cell transplantation. (a) Probability of progression-free survival of seven patients. (b) Probability of overall survival of seven patients.

10^8/l at a median of day 11 (range, 10–15 days), and four of the seven patients had Grade II or greater acute graft-versus-host disease (GVHD). All seven patients showed >95% of donor-type cells in both of peripheral blood and bone marrow mononuclear cells in chimerism study at day 30 after HSCT, and these donor-type hematopoiesis were consistently maintained. Chronic GVHD developed in three of the seven patients, all of whom developed limited-type GVHD. Four patients achieved a partial response (PR) and stable disease was observed in another patient (total response rate, 71%). All of the responders were diagnosed with clear cell carcinoma. However, these clinical responses have lasted only a short time. The estimated probability of progression-free survival is shown in Fig. 1a; one patient is still alive at day 1350, and three of the remaining six patients died due to underlying disease. The estimated probability of 3-year OS was 29% (Fig. 1b).

Autopsy Findings

We performed an autopsy on all six deceased patients. In the three patients who responded to HSCT, we observed an accumulation of $CD8^+$ lymphocytes and degenerative changes of the local lesion at the sites of metastases. In the meanwhile, few lymphocytes were accumulated in tumors or in the sites of metastases in patients who did not show clinical response. In Patient #6, who achieved PR for 6 months but then suffered from systemic metastases to the left kidney, adrenal glands, lung, pancreas, liver, heart, mesentery, right arm and thoracic wall, most of which showed necrosis. We observed small necrotic foci and local lymphocyte involvement in the kidney, mesentery (Fig. 2a) and lung (Fig. 2c) with hematoxylin and eosin staining. Immunohistochemistry revealed cells that were positive for CD56 (data not shown) and granzyme B (Fig. 2b and d) that may have therefore been cytotoxic T cells (CTLs).

DISCUSSION

The response rates reported by Childs et al. and in subsequent studies varied between 0% and 57%. Our result, a 43% response rate, is in line with these reports (13,14) and is slightly superior to that of conventional treatment for Stage IV RCC in terms of the estimated 3-year OS (1). Two patients did not respond to HSCT, which might be related to non-clear cell histology in Patient #2 and usage of antithymocyte globulin as conditioning in Patient #4 as suggested in previous reports (9,15,16). Recently, molecularly targeted therapy using tyrosine-kinase inhibitors such as sorafenib and sunitinib has become available for the treatment of advanced RCC and is being adopted as a first-line therapy (17–20). Sorafenib confers significantly prolonged OS relative to placebo in patients with metastatic RCC, and sunitinib confers significantly better progression-free survival than IFN-α treatment. Since molecularly targeted agents have a low incidence of adverse effects, they are indicated for most patients with advanced RCC. However, they are not curative. On the other hand, a proportion of patients treated with allogeneic HSCT reach a plateau of prospective survival probability (15). Allogeneic HSCT provides a 'GVT effect' that develops ~4 months after HSCT, and is strongly correlated with the appearance of GVHD (13). In the present study, the clinical response was substantially associated with development of GVHD as seen in previous reports (9,15), suggesting that the beneficial GVT effect may be inducible at the expense of complications by GVHD. The separation of GVHD and GVT remains the ultimate goal of HSCT, and improvements are urgently needed to undergo HSCT for RCC more effectively. In contrast, most cases of early death after HSCT caused by progression of the underlying disease were observed within 100 days after HSCT (6,21). Therefore, combination of molecularly targeted therapy and HSCT may be a promising treatment strategy. The short-term benefits of molecularly targeted therapy for suppression of tumor growth could buy time until the development of an alloimmune reaction provided by HSCT occurs.

Currently, transplantation-related adverse effects such as drug toxicity, GVHD and opportunistic infection negate the benefit of HSCT in many patients. One strategy for

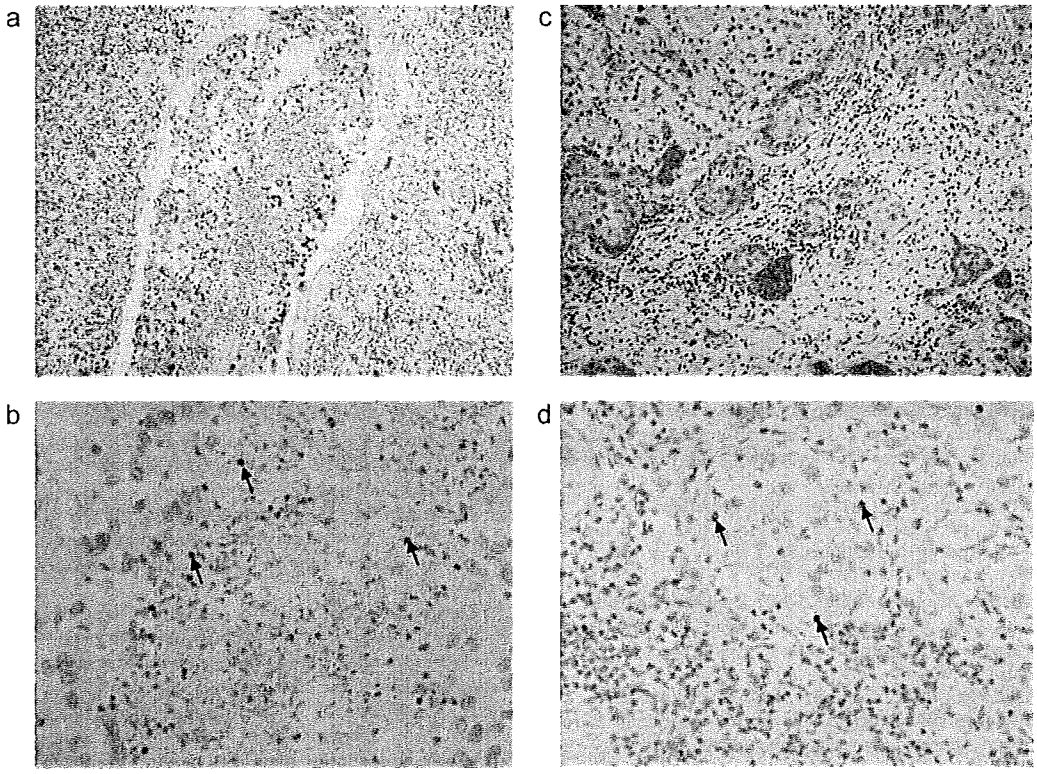

Figure 2. Hematoxylin and eosin staining and immunohistochemistry at the sites of metastasis. (a and b) Metastases to mesentery. (c and d) Metastases to right lung, lower lobe. (a and c) Hematoxylin and eosin staining. We observed small necrotic foci, consistent with metastases of renal cell carcinoma. (b and d) Immunohistochemical staining with granzyme B antibody. We observed a small number of granzyme B-positive cells (arrows).

reducing these adverse effects is infusion of donor leukocytes at the time of transplantation or after cyclophosphamide treatment. This regimen enables to dissociate the effects of GVT from development of GVHD in a mouse model (22,23). In addition, Takahashi et al. (24) have recently reported that RCC-specific CTLs that recognize HERV-E antigen were elicited in patients who responded to HSCT. Another group has started a clinical trial for RCC using Wilms' Tumor 1 peptide, obtained clinical response in two of three patients (25). These results suggest that the use of tumor-specific CTLs that distinguish between the host and the tumor could preferentially promote the effect of GVT.

The anti-tumor activity of tumor-infiltrating lymphocytes derived from RCC has been reported (26,27). However, few studies have pathologically and immunohistochemically detected such CTLs elicited after HSCT resulting in an alloimmune response. We observed T lymphocytes infiltrating into the metastatic foci in partial responders for HSCT, although it remains unclear whether these lymphocytes could have functioned as CTLs *in vivo*. The number of CTLs infiltrating the tumor was relatively small, but it is possible that they would affect GVT if their affinity for the tumor was high (28). This patient achieved transient PR, but then developed progressive disease leading to death in 20 months (29). We hypothesize that in a patient such as this, tumor growth eventually overcame the cytotoxicity of the tumor-specific CTLs, leading to death. We suggest that if the immune response generated by the CTLs could be maintained in the absence of aggressive tumor growth, prolonged survival of the patient might result.

Most recent clinical trials of allogeneic HSCT for RCC have targeted patients who were refractory to conventional therapy, and HSCT was regarded as a last resort. However, it has also been shown that patients with a smaller primary lesion had a better clinical outcome (24). Therefore, it is likely that allogeneic HSCT as a first-line therapy for patients with early-stage RCC would be superior to conventional therapy. The efficacy and safety of this method should be confirmed in a larger number of patients.

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Conflict of interest statement

None declared.

References

1. Motzer RJ, Mazumdar M, Bacik J, Berg W, Amsterdam A, Ferrara J. Survival and prognostic stratification of 670 patients with advanced renal cell carcinoma. *J Clin Oncol* 1999;17:2530–40.
2. Rosenberg SA, Yang JC, Topalian SL, Schwartzentruber DJ, Weber JS, Parkinson DR, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin 2. *JAMA* 1994;271:907–13.
3. Holtl L, Zelle-Rieser C, Gander H, Papesh C, Ramoner R, Bartsch G, et al. Immunotherapy of metastatic renal cell carcinoma with tumor lysate-pulsed autologous dendritic cells. *Clin Cancer Res* 2002;8: 3369–76.
4. Marten A, Flieger D, Renoth S, Weineck S, Albers P, Compes M, et al. Therapeutic vaccination against metastatic renal cell carcinoma by autologous dendritic cells: preclinical results and outcome of a first clinical phase I/II trial. *Cancer Immunol Immunother* 2002;51:637–44.
5. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. *N Engl J Med* 1981;304:1529–33.
6. Pedrazzoli P, Da Prada GA, Giorgiani G, Schiavo R, Zambelli A, Giraldi E, et al. Allogeneic blood stem cell transplantation after a reduced-intensity, preparative regimen: a pilot study in patients with refractory malignancies. *Cancer* 2002;94:2409–15.
7. Aschan J. Allogeneic haematopoietic stem cell transplantation: current status and future outlook. *Br Med Bull* 2006;77–78:23–36.
8. Ueno NT, Cheng YC, Rondon G, Tannir NM, Gajewski JL, Couriel DR, et al. Rapid induction of complete donor chimerism by the use of a reduced-intensity conditioning regimen composed of fludarabine and melphalan in allogeneic stem cell transplantation for metastatic solid tumors. *Blood* 2003;102:3829–36.
9. Nakagawa T, Kami M, Hori A, Kim SW, Murashige N, Hamaki T, et al. Allogeneic hematopoietic stem cell transplantation with a reduced-intensity conditioning regimen for treatment of metastatic renal cell carcinoma: single institution experience with a minimum 1-year follow-up. *Exp Hematol* 2004;32:599–606.
10. Childs RW, Clave E, Tisdale J, Plante M, Hensel N, Barrett J. Successful treatment of metastatic renal cell carcinoma with a nonmyeloablative allogeneic peripheral-blood progenitor-cell transplant: evidence for a graft-versus-tumor effect. *J Clin Oncol* 1999;17:2044–9.
11. Takami A, Asakura H, Koshida K, Namiki M, Nakao S. Reduced-intensity allogeneic stem cell transplantation for renal cell carcinoma: in vivo evidence of a graft-versus-tumor effect. *Haematologica* 2004;89:375–6.
12. Giralt S, Estey E, Albitar M, van Besien K, Rondon G, Anderlini P, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood* 1997; 89:4531–6.
13. Yang JC, Childs R. Immunotherapy for renal cell cancer. *J Clin Oncol* 2006;24:5576–83.
14. Peres E, Abidi MH, Mellon-Reppen S, Klein J, Braun T, Abella E, et al. Reduced intensity transplantation for metastatic renal cell cancer with 2-year follow-up. *J Immunother* 2007;30:562–6.
15. Childs R, Chernoff A, Contentin N, Bahceci E, Schrump D, Leitman S, et al. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med* 2000;343:750–8.
16. Bregni M, Dodero A, Peccatori J, Pescarollo A, Bernardi M, Sassi I, et al. Nonmyeloablative conditioning followed by hematopoietic cell allografting and donor lymphocyte infusions for patients with metastatic renal and breast cancer. *Blood* 2002;99:4234–6.
17. Motzer RJ, Rini BI, Bukowski RM, Curti BD, George DJ, Hudes GR, et al. Sunitinib in patients with metastatic renal cell carcinoma. *JAMA* 2006;295:2516–24.
18. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med* 2007;356:115–24.
19. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med* 2007;356:125–34.
20. Ratain MJ, Eisen T, Stadler WM, Flaherty KT, Kaye SB, Rosner GL, et al. Phase II placebo-controlled randomized discontinuation trial of sorafenib in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2006;24:2505–12.
21. Peccatori J, Barkholt L, Demirer T, Sormani MP, Bruzzi P, Ciceri F, et al. Prognostic factors for survival in patients with advanced renal cell carcinoma undergoing nonmyeloablative allogeneic stem cell transplantation. *Cancer* 2005;104:2099–103.
22. Eto M, Harano M, Tatsugami K, Harada M, Kamiryo Y, Kiyoshima K, et al. Cyclophosphamide-using nonmyeloablative allogeneic cell therapy against renal cancer with a reduced risk of graft-versus-host disease. *Clin Cancer Res* 2007;13:1029–35.
23. Ramirez-Montagut T, Chow A, Kochman AA, Smith OM, Suh D, Sindhi H, et al. IFN-gamma and Fas ligand are required for graft-versus-tumor activity against renal cell carcinoma in the absence of lethal graft-versus-host disease. *J Immunol* 2007;179:1669–80.
24. Takahashi Y, Harashima N, Kajigaya S, Yokoyama H, Cherkasova E, McCoy JP, et al. Regression of human kidney cancer following allogeneic stem cell transplantation is associated with recognition of an HERV-E antigen by T cells. *J Clin Invest* 2008;118:1099–109.
25. Iiyama T, Udaka K, Takeda S, Takeuchi T, Adachi YC, Ohtsuki Y, et al. WT1 (Wilms' tumor 1) peptide immunotherapy for renal cell carcinoma. *Microbiol Immunol* 2007;51:519–30.
26. Goedegebuure PS, Douville LM, Li H, Richmond GC, Schoof DD, Scavone M, et al. Adoptive immunotherapy with tumor-infiltrating lymphocytes and interleukin-2 in patients with metastatic malignant melanoma and renal cell carcinoma: a pilot study. *J Clin Oncol* 1995;13:1939–49.
27. Hayakawa K, Salmeron MA, Parkinson DR, Markowitz AB, von Eschenbach AC, Legha SS, et al. Study of tumor-infiltrating lymphocytes for adoptive therapy of renal cell carcinoma (RCC) and metastatic melanoma: sequential proliferation of cytotoxic natural killer and noncytotoxic T cells in RCC. *J Immunother* 1991;10:313–25.
28. Molldrem JJ, Lee PP, Kant S, Wieder E, Jiang W, Lu S, et al. Chronic myelogenous leukemia shapes host immunity by selective deletion of high-avidity leukemia-specific T cells. *J Clin Invest* 2003;111:639–47.
29. Takami A, Takamatsu H, Yamazaki H, Ishiyama K, Okumura H, Ohata K, et al. Reduced-intensity unrelated cord blood transplantation for treatment of metastatic renal cell carcinoma: first evidence of cord-blood-versus-solid-tumor effect. *Bone Marrow Transplant* 2006;38:729–32.

NKG2D gene polymorphism has a significant impact on transplant outcomes after HLA-fully-matched unrelated bone marrow transplantation for standard risk hematologic malignancies

J. Luis Espinoza,[1] Akiyoshi Takami,[1] Makoto Onizuka,[2] Hiroshi Sao,[3] Hideki Akiyama,[4] Koichi Miyamura,[5] Shinichiro Okamoto,[6] Masami Inoue,[7] Yoshinobu Kanda,[8] Shigeki Ohtake,[1] Takahiro Fukuda,[9] Yasuo Morishima,[10] Yoshihisa Kodera,[11] and Shinji Nakao,[1] for the Japan Marrow Donor Program

[1]Department of Hematology and Oncology, Kanazawa University Hospital, Kanazawa; [2]Department of Hematology and Oncology, Tokai University School of Medicine, Isehara; [3]Department of Hematology, Meitetsu Hospital, Nagoya; [4]Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo; [5]Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya; [6]Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo; [7]Department of Hematology and Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka; [8]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama; [9]Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo; [10]Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, Nagoya; [11]Department of Promotion for Blood and Marrow Transplantation, Aichi Medical University, Nagoya, Japan

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Correspondence: Akiyoshi Takami, M.D., Ph.D., Department of Hematology & Oncology, Kanazawa University Hospital, 13-1 Takaramachi, Kanazawa, 920-8641, Japan. E-mail: takami@med3.m.kanazawa-u.ac.jp

ABSTRACT

Background

NKG2D, an activating and co-stimulatory receptor expressed on natural killer cells and T cells, plays pivotal roles in immunity to microbial infections as well as in cancer immunosurveillance. This study examined the impact of donor and recipient polymorphisms in the *NKG2D* gene on the clinical outcomes of patients undergoing allogeneic T-cell-replete myeloablative bone marrow transplantation using an HLA-matched unrelated donor.

Design and Methods

The *NKG2D* polymorphism was retrospectively analyzed in a total 145 recipients with hematologic malignancies and their unrelated donors. The patients underwent transplantation following myeloablative conditioning; the recipients and donors were matched through the Japan Marrow Donor Program.

Results

In patients with standard-risk disease, the donor *NKG2D-HNK1* haplotype, a haplotype expected to induce greater natural killer cell activity, was associated with significantly improved overall survival (adjusted hazard ratio, 0.44; 95% confidence interval, 0.23 to 0.85; p=0.01) as well as transplant related mortality (adjusted hazard ratio, 0.42; 95% confidence interval, 0.21 to 0.86; p=0.02), but had no impact on disease relapse or the development of grade II-IV acute graft-versus-host disease or chronic graft-versus-host disease. The *NKG2D* polymorphism did not significantly influence the transplant outcomes in patients with high-risk disease.

Conclusions

These data suggest an association between the donor *HNK1* haplotype and better clinical outcome among recipients, with standard-risk disease, of bone marrow transplants from HLA-matched unrelated donors.

Key words: *NKG2D*, *HNK1*, *LNK1*, unrelated donor; bone marrow transplantation, single nucleotide polymorphism.

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Introduction

Hematopoietic stem cell transplantation (SCT) is a potentially curative treatment for a range of hematologic malignancies. Although the use of an HLA-matched unrelated donor is well accepted when an HLA-identical sibling donor is unavailable, the risk of transplantation- related complications may be increased.[1] Despite improvements in clinical and supportive care, transplant- related life-threatening complications, including graft-versus-host disease (GVHD), infections and disease relapse, remain an enormous obstacle to overcome.[2] Although HLA matching is the major genetic determinant of clinical outcome after allogeneic SCT, recent evidence suggests that non-HLA immune-associated genes are also implicated.[3] Previous investigations have revealed that several single nucleotide polymorphisms (SNP) which affect individual immune response to infections and inflammatory reactions are associated with the risk of GVHD and transplant outcomes.[4-15]

NKG2D is an activating and co-stimulatory receptor belonging to the C-type lectin-like family of transmembrane proteins and is expressed as a homodimer on natural killer (NK) cells, $CD8^+$ $\alpha\beta^+$ T cells, $\gamma\delta^+$ T cells and activated macrophages.[16-18] The ligands for NKG2D, such as MHC class I-chain related proteins (MICA and MICB), UL16 binding proteins are usually absent or expressed at very low levels in normal cells but are up-regulated by cellular stress including heat shock and microbial infections and are frequently expressed in epithelial tumor cells.[19] Ligand engagement of NKG2D triggers cell-mediated cytotoxicity and co-stimulates cytokine production through a DAP10-phosphoinositol 3-kinase dependent pathway and plays an important role in the elimination of tumors and infected cells.[16-18,20]

Recently, SNP were identified between *LNK1* and *HNK1* haplotypes of the *NKG2D* gene.[21] In Japanese individuals, the *HNK1* haplotype is associated with greater activity of NK cells in the peripheral blood[21,22] and a lower prevalence of cancers originating from epithelial cells.[21,23,24] The present study investigates the impact of donor and recipient polymorphisms in the *NKG2D* gene on the clinical outcomes of patients undergoing allogeneic myeloablative bone marrow transplantation using an HLA allele-matched unrelated donor.

Design and Methods

Patients

NKG2D genotyping was performed on a total 145 recipients with hematologic malignancies and their unrelated donors who were part of the Japan Marrow Donor Program (JMDP). The recipients underwent transplantation, following myeloablative conditioning, with T-cell-replete marrow from an HLA-A, -B, -C, -DRB1 allele-matched donor between November 1995 and March 2000. HLA genotypes of the HLA-A, -B, -C, and -DRB1 alleles of the patients and donors were determined by the Luminex microbead method described previously. (Luminex 100 System; Luminex, Austin, TX, USA).[25,26] No patient had a history of prior transplantation. The final clinical survey of these patients was completed by November 1, 2007. Diagnoses were acute myeloid leukemia (n=49; 34%), acute lymphoblastic leukemia (n=37; 26%), chronic myeloid leukemia (n=41; 28%), myelodysplastic syndrome (n=11; 8%) and malignant lymphoma (n=7; 5%), (Table 1). The recipients were defined as having standard risk disease if they had acute myeloid or lymphoblastic leukemia in first complete remission, malignant lymphoma in complete remission, chronic myeloid leukemia in any chronic phase or myelodysplastic syndrome. All other patients were designated as having high-risk disease. Myeloid malignancies included acute myeloid leukemia, chronic myeloid leukemia and myelodysplastic syndrome, whereas lymphoid malignancies included acute lymphoblastic leukemia and malignant lymphomas. Cyclosporine or tacrolimus- based regimens were used in all patients for GVHD prophylaxis whereas anti-T-cell therapy, such as anti-thymocyte globulin and *ex vivo* T-cell depletion, was not. All patients and donors gave their written informed consent to molecular studies, according to the declaration of Helsinki, at the time of transplantation. The project was approved by the Institutional Review Board of Kanazawa University Graduate School of Medicine and the JMDP.

NKG2D genotyping

NKG2D was genotyped using the TaqMan-Allelic discrimination method[27] with a 9700-HT real time polymerase chain reaction (PCR) system (Applied Biosystems, Foster City, CA, USA) and results were analyzed using allelic discrimination software (Applied Biosystems). The genotyping assay was conducted in 96-well PCR plates. The amplification reaction contained template DNA, TaqMan universal master mix and a specific probe (product No. C_9345347_10; Applied Biosystems) for rs1049174, a single locus featuring a G-C substitution to distinguish between the *HNK1* (G) and *LNK1* (C) haplotypes of the *NKG2D* gene.[21,23,24]

Data management and statistical analysis

Data were collected by the JMDP using a standardized report form. Follow-up reports were submitted at 100 days, 1 year and annually after transplantation. Pre-transplant cytomegalovirus serostatus was routinely tested only in patients but not in their donors. Engraftment was confirmed by an absolute neutrophil count of more than 0.5×10^9/L for at least 3 consecutive days. Acute and chronic GVHD were diagnosed and graded using established criteria.[28,29] Overall survival was defined as the number of days from transplantation to death from any cause. Disease relapse was defined as the number of days from transplantation to disease relapse. Transplant-related mortality was defined as death without relapse. Any patients who were alive at the last-follow-up date were censored. When collecting data, only the main cause of death was recorded if two or more causes were combined. Data on etiological agents of infections, post-mortem changes and supportive care (including prophylaxis of infections and therapy of GVHD, which were given on an institutional basis), were not available for this

cohort of patients. The analysis was performed using Excel 2007 (Microsoft Corp, Redmond, WA, USA), OriginPro version 8.0J (Lightstone Inc, Tokyo, Japan), and R (The R Foundation for Statistical Computing, Perugia, Italy).[30] The probability of overall survival was calculated using the Kaplan-Meier method and compared using the log-rank test. The probabilities of transplant-related mortality, disease relapse, acute GVHD, chronic GVHD, and each cause of death were compared using the Grey test[31] and analyzed using cumulative incidence analysis,[30] considering relapse, death without disease relapse, death without acute GVHD, death without chronic GVHD, and death without each cause as respective competing risks. The analysis was stratified for patients with standard-risk disease and high-risk disease to take into account the already recognized prognostic differences. The variables considered were recipient age at time of transplantation, sex, recipient cytomegalovirus serosta-

Table 1. Characteristics of the donors and recipients.

	Standard-risk disease (n=93, 64%)					High-risk disease (n=52, 36%)				
	Donor *NKG2D* haplotype					Donor *NKG2D* haplotype				
	HNK1 positive n=55, 59%		*HNK1* negative n=38, 41%			*HNK1* positive n=28, 54%		*HNK1* negative n=24, 46%		
Variable	N.	Ratio	N.	Ratio	p	N.	Ratio	N.	Ratio	p
Age, years										
Recipient					0.08					0.39
Median	31		23			23		22		
Range	1-50		1-50			7-46		2-48		
Donor					0.54					
Median	33		28			34		29		0.02
Range	22-49		21-50			21-47		21-50		
Recipient *NKG2D* haplotype					0.17				0.48	
HNK1 positive	33	60%	28	74%		19	68%	14	58%	
HNK1 negative	22	40%	10	26%		9	32%	10	42%	
Sex, male					0.37					0.77
Recipient	30	55%	23	61%		19	68%	15	63%	
Donor	42	76%	23	61%		19	68%	13	54%	
Recipient/donor sex					0.23					0.86
Sex matched	31	56%	20	53%		18	64%	16	67%	
Male/female	6	11%	9	24%		5	18%	5	21%	
Female/male	18	33%	9	24%		5	18%	3	13%	
Disease					0.86					0.99
Acute myeloid leukemia	14	25%	9	24%		14	50%	12	50%	
Acute lymphoblastic leukemia	10	18%	8	21%		10	36%	9	38%	
Myelodysplastic syndrome	6	11%	5	13%		0	0%	0	0%	
Malignant lymphoma	2	4%	3	8%		1	4%	1	4%	
Chronic myeloid leukemia	23	42%	13	34%		3	11%	2	8%	
ABO matching					0.37					0.18
Matched	35	64%	19	50%		14	50%	17	71%	
Major mismatch	11	20%	10	26%		6	21%	5	21%	
Minor mismatch	9	16%	9	24%		8	29%	2	8%	
Bi-directional	0	0%	1	3%		0	0%	1	4%	
Conditioning regimen					0.93					0.51
With total body irradiation	43	78%	30	79%		26	93%	21	88%	
Without total body irradiation	12	22%	8	21%		2	7%	3	13%	
Pretransplant CMV serostatus				0.30					0.99	
CMV-negative recipient	14	25%	5	13%		6	27%	5	21%	
Missing data	4	7%	2	5%		5	18%	4	17%	
GVHD prophylaxis					0.58					0.11
With cyclosporine	51	93%	34	89%		27	96%	20	83%	
With tacrolimus	4	7%	4	11%		1	4%	4	17%	
TNC, $\times 10^8$/kg					0.40					0.04
Median	5.4		5.8			5.8		8.2		
Range	2.3-14.6		2.3-57.6			2.9-20.0		2.4-42.8		
Engraftment	53	96%	38	100%	0.23	28	100%	23	96%	0.28

CMV: cytomegalovirus; TNC: total nucleated cell count harvested.

tus before transplantation, disease characteristics (disease type and disease lineage), donor characteristics (age, sex, sex compatibility, and ABO compatibility), transplant characteristics (total body irradiation-containing regimen, tacrolimus versus cyclosporine, and total nucleated cell count harvested per recipient weight). The median was used as the cut-off point for continuous variables. The χ^2 test and Mann-Whitney test were used to compare results of two groups. The Hardy-Weinberg equilibrium for the *NKG2D* gene polymorphism was tested using the Haploview program.[32] Multivariate Cox models were used to evaluate the hazard ratio associated with the NKG2D polymorphism. Co-variates found to be statistically significant in univariate analyses ($p \leq 0.10$) were included in the models. For both the univariate and multivariate analyses, p values were two-sided and outcomes were considered to be statistically significant with $p \leq 0.05$.

Results

Frequencies of NKG2D haplotype

The *NKG2D* gene polymorphism was analyzed in 145 pairs of unrelated donors-recipients of bone marrow following myeloablative conditioning (Table 1). The haplotype frequencies of *LNK1/LNK1*, *HNK1/LNK1* and *HNK1/HNK1* were 43%, 42% and 15%, respectively in donors and 35%, 45% and 20%, respectively in recipients. These frequencies were similar to those reported in previous studies in Japanese populations[21,24] and were in accordance with the Hardy-Weinberg equilibrium (p=0.80).

Transplant outcomes according to NKG2D haplotype

With a median follow-up of 115 months among survivors (range, 74 to 140 months), 30 recipients (21%) had relapsed or progressed and 62 (47%) had died. Three patients (2%) died before engraftment. The analysis of the influence of the *NKG2D* genotype on clinical outcomes after transplantation was stratified according to whether the recipients had standard-risk disease or high-risk disease to account for the already recognized prognostic difference. The overall survival at 5 years in patients with standard-risk disease was 63% while that of patients with high-risk disease was 44% (p=0.06). The 5-year cumulative incidences of trasplant-related mortality were 32% and 27%, respectively (p=0.33) and those of disease relapse were 10% and 31%, respectively (p=0.0006).

The transplant outcomes according to *NKG2D* genotype are summarized in Table 2. Patients with standard-risk disease receiving transplants from donors with the *HNK1* haplotype had a significantly better 5-year overall survival (73% *vs.* 49%, p=0.01; Figure 1A) and lower transplant-related mortality rate (22% *vs.* 45%, p=0.02; Figure 1B) than those receiving transplants from donors without the *HNK1* haplotype. No difference was noted in disease relapse in relation to the donors' polymorphism (9% *vs.* 11%, p=0.81; Figure 1C) or in the development of grades II to IV acute GVHD (28% *vs.* 41%, p=0.25) or chronic GVHD (37% *vs.* 41%, p=0.83). When patients with acute myeloid leukemia or myelodysplastic syndrome were separately analyzed, there was still no difference in disease relapse in relation to NKG2D polymorphisms (*data not shown*). In patients with high-risk disease, the donor *HNK1* haplotype had no significant effects on transplant outcomes (Table 2).

Multivariate analysis

Any factors found to be significant in univariate analyses were included in the multivariate analysis. When patients with standard-risk disease were analyzed, the *HNK1* haplotype in donors remained statistically significant in multivariate analyses for both overall survival and transplant-related mortality (Table 3). The presence of the *HNK1* haplotype in the donor resulted in better overall survival (hazard ratio, 0.44; 95% confidence interval, 0.23 to 0.85; p=0.01) and transplant-related mortality (hazard ratio, 0.42; 95% confidence interval, 0.21 to 0.86; p=0.02).

Table 2. Univariate analysis of the association of *NKG2D* polymorphisms with clinical outcomes after transplantation.

	N	5-year OS	p	5-year TRM	p	5-year relapse	p	Acute II-IV acute GVHD	p	Chronic GVHD	p
Standard-risk disease											
Donor *NKG2D* haplotype			0.01		0.02		0.81		0.25		0.83
HNK1-positive	55	73%		22%		9%		28%		37%	
HNK1-negative	38	49%		45%		11%		41%		41%	
Recipient *NKG2D* haplotype			0.39		0.31		0.93		0.48		0.98
HNK1-positive	61	62%		33%		10%		37%		39%	
HNK1-negative	32	66%		28%		9%		25%		38%	
High-risk disease											
Donor *NKG2D* haplotype			0.91		0.77		0.93		0.08		0.47
HNK1-positive	28	43%		26%		33%		54%		44%	
HNK1-negative	24	46%		29%		29%		30%		35%	
Recipient *NKG2D* haplotype			0.41		0.43		0.10		0.40		0.68
HNK1-positive	33	42%		23%		39%		39%		37%	
HNK1-negative	19	47%		35%		18%		50%		47%	

OS: overall survival; TRM: transplant-related mortality.

The donor and recipient *HNK1* haplotype did not significantly influence the transplant outcomes in patients with high-risk disease.

Main causes of death

The main causes of death according to the *HNK1* haplotype of the donors and recipients are illustrated in Figure 2A for patients with standard-risk disease, and in Figure 2B for those with high-risk disease. In patients with standard-risk disease receiving transplants from *HNK1*-negative donors, the most frequent cause of death was acute GVHD, followed by interstitial pneumonia. Transplants from *HNK1*-positive donors resulted in a statistically significantly reduced incidence of death attributed to acute GVHD (Figure 3A; p=0.006) as well as a trend toward a lower incidence of death attributed to interstitial pneumonia (Figure 3B; p=0.09). Other causes of death did not differ according to the *HNK1* haplotype.

Discussion

The current study showed an association between the *NKG2D-HNK1* haplotype in unrelated donors of HLA-matched myeloablative bone marrow transplants (haplotype frequency, 61%) and a significantly reduced transplant-related mortality and better overall survival for their recipients with standard-risk disease. The polymorphism of the donor *NKG2D* gene did not influence disease relapse or the development of grades II to IV acute GVHD or chronic GVHD in the patients. One possible explanation for the absence of the beneficial effects of the *HNK1* haplotype in patients with high-risk disease may be that the number of cases in the study was insufficient for a meaningful assessment of the effect. Alternatively, disease progression may precede the emergence of the potential advantageous effects of the *HNK1* donor haplotype that could protect the recipient from severe transplant-related complications. There was a larger difference in disease relapse between patients with standard-risk disease and those with high-risk disease: 10% and 31% at 3 years after transplantation, respectively.

NKG2D plays important roles in immunity to microbial infections and is especially prominent in controlling viral and bacterial infections.[16] Therefore, the reduced transplant-related mortality in patients with standard-risk disease receiving grafts from donors with the *HNK1* haplotype in this study might be a consequence of increased resistance to infections in the recipients. However, the hypothesis is too speculative because of the unavailability of data on causes of infections in this cohort. Further studies will be needed to clarify whether the *HNK1* haplotype in donors can effectively protect patients against infections.

Several studies have shown that NK cell activity has an important role in the outcomes of patients undergoing allogeneic transplantation.[35,36] Alloreactive NK cells reduced the risk of relapse of acute myeloid leukemia without increasing the incidence of GVHD, resulting in a marked improvement of event-free survival in a series of haploidentical transplant recipients.[35,36] In HLA-identical sibling transplants, the absence of HLA-C and HLA-B ligand for donor-inhibitory killer immunoglobulin-like receptors (KIR) provided benefits in terms of survival and relapse of patients with acute myeloid leukemia and myelodysplastic syndrome in recipients of T-cell-depleted SCT.[37] On the other hand, the JMDP found that KIR ligand mismatch was unfavorably correlated with relapse of leukemia and survival in patients undergoing T-cell-replete unrelated bone marrow transplants.[38] All patients in the present study received grafts from an HLA-A, -B, and -C allele-matched donor, implying KIR ligand match between each patient and donor. It is an open question whether the *NKG2D* polymorphism could affect the outcomes of patients undergoing transplantation with KIR-mismatched grafts.

In this study, major and minor ABO incompatibilities between the donor and recipient tended to be associated with poorer transplant outcomes, regardless of the risk

Table 3. **Multivariate analysis of the association of *NKG2D* polymorphisms with clinical outcomes after transplantation.**

Variable	Overall survival			Transplant-related mortality			Relapse			Grades II-IV acute GVHD			Chronic GVHD		
	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
Standard-risk disease															
HNK1-positive donor	0.44	0.23-0.85	0.01	0.42	0.21-0.86	0.02	0.71	0.19-2.67	0.61	0.83	0.39-1.75	0.63	0.83	0.39-1.75	0.62
HNK1-positive recipient	1.22	0.60-2.50	0.58	1.32	0.61-2.87	0.48	1.11	0.28-4.48	0.88	1.54	0.66-3.57	0.32	1.06	0.49-2.31	0.88
Donor age, >31 years	-	-	-	-	-	-	-	-	-	2.17	0.95-4.96	0.07	-	-	-
Major ABO incompatibility	-	-	-	-	-	-	-	-	-	3.12	1.49-6.56	0.003	0.50	0.17-1.45	0.20
Minor ABO incompatibility	2.42	1.17-5.03	0.02	-	-	-	-	-	-	-	-	-	0.29	0.07-1.24	0.10
High-risk disease															
HNK1-positive donor	0.68	0.30-1.51	0.34	0.62	0.20-1.91	0.40	1.25	0.41-3.80	0.69	1.87	0.69-5.07	0.22	1.55	0.60-4.01	0.37
HNK1-positive recipient	1.41	0.65-3.07	0.39	0.76	0.25-2.29	0.63	2.35	0.66-8.44	0.19	0.47	0.18-1.22	0.12	0.92	0.35-2.38	0.86
Age, >26 years	1.95	0.93-4.09	0.08	6.30	1.86-21.32	0.003	-	-	-	-	-	-	-	-	-
Donor age, >31 years	-	-	-	-	-	-	0.53	0.17-1.65	0.27	-	-	-	-	-	-
Minor ABO incompatibility	2.94	1.19-7.25	0.02	-	-	-	-	-	-	5.10	2.08-12.52	0.004	-	-	-

category of the disease. These findings are compatible with those of a previous study by the JMDP,[39] although the impact of ABO incompatibilities on SCT outcomes is controversial.

This study also identified age as a significant predictive factor for transplant-related mortality in the patients with standard-risk disease. This is consistent with the results of a previous study[40] showing that age over 35 years increased the risk of transplant-related mortality after allogeneic myeloablative SCT in high-risk patients.

A possible limitation of this study is the fact that no direct evidence is yet available regarding the ability of *NKG2D* polymorphisms to protect against microbial infections. The association observed between the *NKG2D* haplotype and transplant outcome might be due to another genetic polymorphism in linkage disequilibrium responsible for a better transplant outcome. One candidate gene is *NKG2F* (*KLRC4*), which is located in the NK complex region adjacent to the *NKG2D* gene, because an intrinsic SNP (rs2617171) in the gene has been reported to be in complete linkage with the *NKG2D* genotype.[24] Alternatively, polymorphisms may not be directly associated with controlling infection, but rather may be associated with other factors, such as sensitivity to treatment against GVHD or protection against organ toxicities related to transplants, which also influence the transplant outcome. These hypotheses have yet to be verified give the insufficient evidence.

Polymorphisms in genes encoding for nucleotide-binding oligomerization domain 2 (NOD2)/caspase recruitment domain 15 (CARD15),[9] heme oxygenase-1 (HO-1) promoter,[6] the Toll-like receptor 4,[4] CC chemokine ligand (CCL) 5 promoter,[52] transforming growth factor (TGF) β1,[11] interleukin (IL) 12, tumor necrosis factor (TNF) α,[15] IL-23,[5] mannose-binding lectin (MBL),[10] Fcγ receptor IIa (FcγRIIa), myeloperoxidase (MPO), FcγRIIIb, IL-1Ra, IL-10,[12] Fc receptor-like 3 (FCRL3), peptidylarginine deimi-

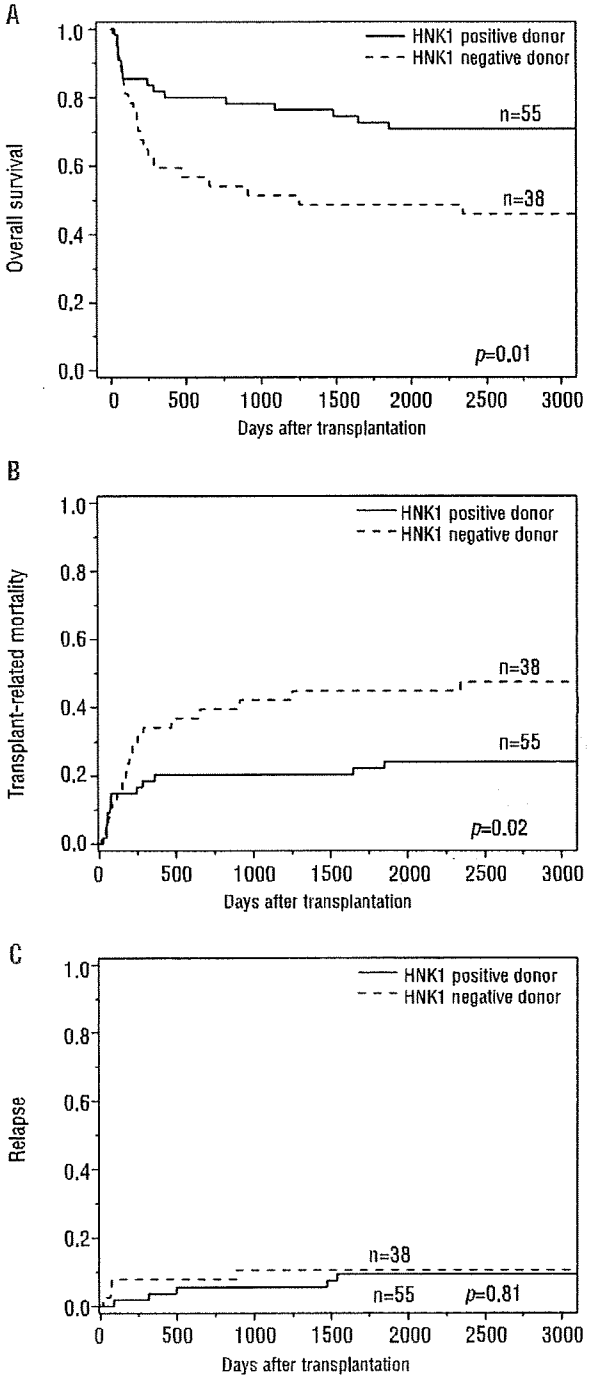

Figure 1. Kaplan-Meier analysis of (A) overall survival, (B) cumulative incidence of transplant-related mortality and (C) disease relapse after transplantation according to the donor *NKG2D* polymorphism in patients with standard-risk disease. Patients with donors with the *HNK1* haplotype had better overall survival and lower transplant-related mortality. Donor haplotype had no significant impact on disease relapse.

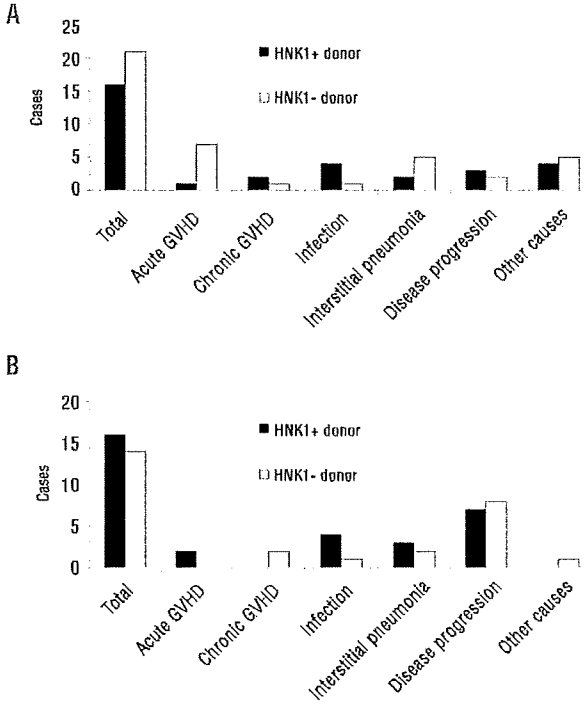

Figure 2. Main causes of death after transplantation according to the *NKG2D* polymorphism in patients with (A) standard-risk disease (B) high-risk disease.

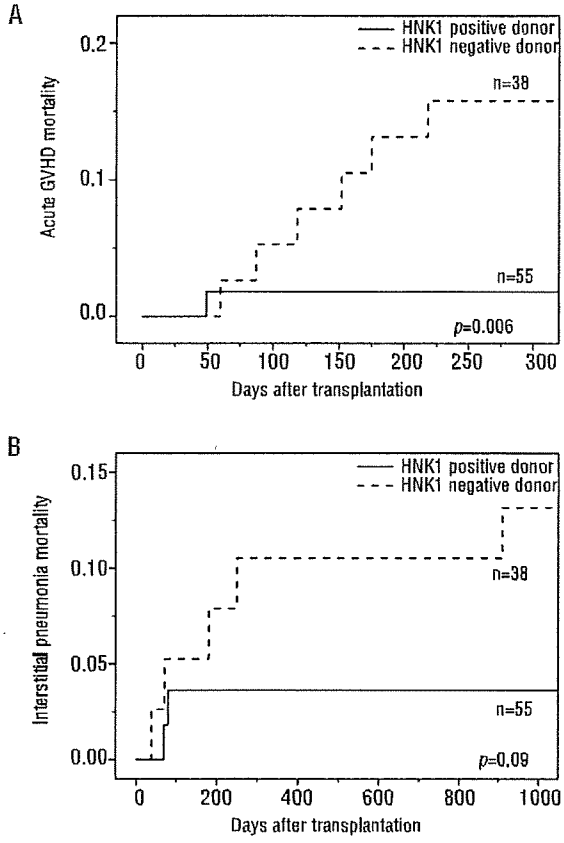

Figure 3. Cumulative incidence of deaths due to (A) acute GVHD and (B) interstitial pneumonia after transplantation in patients with standard-risk disease. The *HNK1* haplotype in donors was associated with a significantly lower incidence of deaths due to acute GVHD (p=0.006) as well as a trend toward a lower incidence of deaths due to interstitial pneumonia (p=0.09).

nase citullinating enzymes 4 (PADI4)[15] and methylenetetrahydrofolate reductase (MTHFR)[14] have been shown to influence the outcome after allogeneic SCT. Most of them are associated with the development of GVHD. Only the *NOD2/CARD15* and *HO-1* promoter polymorphisms have a significant impact on overall survival after SCT. Furthermore, the impact of the *HO-1* promoter polymorphisms depends on donor cells but not on recipient cells, as observed with the *NKG2D* polymorphism which, in the donor, was shown to be significantly associated with overall survival in the present study. This may prompt the determination of the donor *NKG2D* polymorphism prior to SCT in order to choose the best donor, expected to minimize transplant-related mortality after SCT, when multiple donors for a patient are available. Otherwise, prior information on the donor *NKG2D* polymorphism may be helpful in selecting risk-specific appropriate precautions following transplantation.

In conclusion, the present data suggest that the *NKG2D* polymorphism, in addition to HLA disparity between recipients and donors, affects prognosis after a bone marrow transplant from an unrelated donor. However, care should be made in drawing conclusions because the number of patients in the present study was small. The finding of a gene polymorphism may not be equivalent to differences in gene expression, which may be influenced by multiple factors because the NKG2D receptor is found on many tissues and cells.[41] Experimental evidence is required to substantiate the effect of the *NKG2D* polymorphism on immune function. We next plan to conduct a prospective study to confirm these results and to extend this investigation to other transplantation settings, such as related donor SCT, reduced-intensity SCT, HLA-mismatched SCT and SCT for patients with non-hematologic malignancies.

Authorship and Disclosures

JLE and AT designed and performed the research, and contributed to the same aspects of the work; AT, JLE and SN wrote the paper; AT, YKa, and SOh performed the statistical analyses; MO, HS, HA, KM, SOk, MI, TF, YM, and YKo contributed to data collection.

The authors reported no potential conflicts of interest.

References

1. Weisdorf DJ, Anasetti C, Antin JH, Kernan NA, Kollman C, Snyder D, et al. Allogeneic bone marrow transplantation for chronic myelogenous leukemia: comparative analysis of unrelated versus matched sibling donor transplantation. Blood 2002; 99:1971-7.
2. Gratwohl A, Brand R, Frassoni F, Rocha V, Niederwieser D, Reusser P, et al. Cause of death after allogeneic haematopoietic stem cell transplantation (HSCT) in early leukaemias: an EBMT analysis of lethal infectious complications and changes over calendar time. Bone Marrow Transplant 2005;36:757-69.
3. Dickinson AM, Middleton PG, Rocha V, Gluckman E, Holler E. Genetic polymorphisms predicting the outcome of bone marrow transplants. Br J Haematol 2004; 127:479-90.
4. Bochud PY, Chien JW, Marr KA, Leisenring WM, Upton A, Janer M, et al. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. N Engl J Med 2008; 359:1766-77.
5. Elmaagacli AH, Koldehoff M, Landt O, Beelen DW. Relation of an interleukin-23 receptor gene polymorphism to graft-versus-host disease after hematopoietic-cell transplantation. Bone Marrow Transplant 2008; 41:821-6.
6. Gerbitz A, Hillemanns P, Schmid C, Wilke A, Jayaraman R, Kolb HJ, et al. Influence of polymorphism within the heme oxygenase-1 promoter on overall survival and transplantation-related mortality after allogeneic stem cell transplantation. Biol Blood Marrow Transplant 2008;14:1180-9.
7. Holler E, Rogler G, Brenmoehl J, Hahn J, Herfarth H, Greinix H, et al. Prognostic significance of NOD2/CARD15 variants in HLA-identical sibling hematopoietic stem cell transplantation: effect on long-term outcome is confirmed in 2 independent cohorts and may be modulated by the type of gastrointestinal decontamination. Blood 2006;107: 4189-93.
8. Holler E, Rogler G, Herfarth H, Brenmoehl J, Wild PJ, Hahn J, et al. Both donor and recipient

NOD2/CARD15 mutations associate with transplant-related mortality and GvHD following allogeneic stem cell transplantation. Blood 2004;104:889-94.
9. Mayor NP, Shaw BE, Hughes DA, Maldonado-Torres H, Madrigal JA, Keshav S, et al. Single nucleotide polymorphisms in the NOD2/CARD15 gene are associated with an increased risk of relapse and death for patients with acute leukemia after hematopoietic stem-cell transplantation with unrelated donors. J Clin Oncol 2007;25:4262-9.
10. Mullighan CG, Heatley S, Doherty K, Szabo F, Grigg A, Hughes TP, et al. Mannose-binding lectin gene polymorphisms are associated with major infection following allogeneic hemopoietic stem cell transplantation. Blood 2002;99:3524-9.
11. Noori-Daloii MR, Rashidi-Nezhad A, Izadi P, Hossein-Nezhad A, Sobhani M, Derakhshandeh-Peykar P, et al. Transforming growth factor-β1 codon 10 polymorphism is associated with acute GVHD after allogenic BMT in Iranian population. Ann Transplant 2007;12:5-10.
12. Rocha V, Franco RF, Porcher R, Bittencourt H, Silva WA Jr, Latouche A, et al. Host defense and inflammatory gene polymorphisms are associated with outcomes after HLA-identical sibling bone marrow transplantation. Blood 2002;100:3908-18.
13. Shimada M, Onizuka M, Machida S, Suzuki R, Kojima M, Miyamura K, et al. Association of autoimmune disease-related gene polymorphisms with chronic graft-versus-host disease. Br J Haematol 2007;139:458-63.
14. Soydan E, Topcuoglu P, Dalva K, Arat M. The impact of methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism on transplant-related variables after allogeneic hematopoietic cell transplantation in patients receiving MTX as GVHD prophylaxis. Bone Marrow Transplant 2008;42:429-30.
15. Viel DO, Tsuneto LT, Sossai CR, Lieber SR, Marques SB, Vigorito AC, et al. IL2 and TNFA gene polymorphisms and the risk of graft-versus-host disease after allogeneic haematopoietic stem cell transplantation. Scand J Immunol 2007;66: 703-10.
16. Burgess SJ, Maasho K, Masilamani M, Narayanan S, Borrego F, Coligan JE. The NKG2D receptor: immunobiology and clinical implications. Immunol Res 2008;40:18-34.
17. Hyka-Nouspikel N, Phillips JH. Physiological roles of murine DAP10 adapter protein in tumor immunity and autoimmunity. Immunol Rev 2006;214:106-17.
18. Jamieson AM, Diefenbach A, McMahon CW, Xiong N, Carlyle JR, Raulet DH. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. Immunity 2002;17:19-29.
19. Raulet DH. Roles of the NKG2D immunoreceptor and its ligands. Nat Rev Immunol 2003;3:781-90.
20. Groh V, Wu J, Yee C, Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature 2002;419: 734-8.
21. Hayashi T, Imai K, Morishita Y, Hayashi I, Kusunoki Y, Nakachi K. Identification of the NKG2D haplotypes associated with natural cytotoxic activity of peripheral blood lymphocytes and cancer immunosurveillance. Cancer Res 2006;66: 563-70.
22. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet 2000;356:1795-9.
23. Furue H, Kumimoto H, Matsuo K, Suzuki T, Hasegawa Y, Shinoda M, et al. Opposite impact of NKG2D genotype by lifestyle exposure to risk of aerodigestive tract cancer among Japanese. Int J Cancer 2008; 123:181-6.
24. Furue H, Matsuo K, Kumimoto H, Hiraki A, Suzuki T, Yatabe Y, et al. Decreased risk of colorectal cancer with the high natural killer cell activity NKG2D genotype in Japanese. Carcinogenesis 2008; 29:316-20.
25. Kawase T, Morishima Y, Matsuo K, Kashiwase K, Inoko H, Saji H, et al. High-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease and implication for its molecular mechanism. Blood 2007;110:2235-41.
26. Sasazuki T, Juji T, Morishima Y, Kinukawa N, Kashiwabara H, Inoko H, et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. Japan Marrow Donor Program. N Engl J Med 1998;339:1177-85.
27. Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. Genet Anal 1999;14:143-9.
28. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995;15:825-8.
29. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med 1980;69:204-17.
30. Scrucca L, Santucci A, Aversa F. Competing risk analysis using R: an easy guide for clinicians. Bone Marrow Transplant 2007;40:381-7.
31. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med 1999;18: 695-706.
32. Kim DH, Jung HD, Lee NY, Sohn SK. Single nucleotide polymorphism of CC chemokine ligand 5 promoter gene in recipients may predict the risk of chronic graft-versus-host disease and its severity after allogeneic transplantation. Transplantation 2007;84:917-25.
33. Dulphy N, Haas P, Busson M, Belhadj S, Peffault de Latour R, Robin M, et al. An unusual CD56(bright) CD16(low) NK cell subset dominates the early posttransplant period following HLA-matched hematopoietic stem cell transplantation. J Immunol 2008; 181:2227-37.
34. Hamby K, Trexler A, Pearson T, Larsen C, Rigby M, Kean L. NK cells rapidly reject allogeneic bone marrow in the spleen through a perforin- and Ly49D-dependent, but NKG2D-independent mechanism. Am J Transplant 2007;7:1884-96.
35. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik W, Tosti A, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 2002;295:2097-100.
36. Ruggeri L, Mancusi A, Burchielli E, Capanni M, Carotti A, Aloisi T, et al. NK cell alloreactivity and allogeneic hematopoietic stem cell transplantation. Blood Cells Mol Dis 2008;40: 84-90.
37. Hsu K, Keever-Taylor C, Wilton A, Pinto C, Heller G, Arkun K, et al. Improved outcome in HLA-identical sibling hematopoietic stem-cell transplantation for acute myelogenous leukemia predicted by KIR and HLA genotypes. Blood 2005;105: 4878-84.
38. Morishima Y, Yabe T, Matsuo K, Kashiwase K, Inoko H, Saji H, et al. Effects of HLA allele and killer immunoglobulin-like receptor ligand matching on clinical outcome in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor. Biol Blood Marrow Transplant 2007;13: 315-28.
39. Kimura F, Sato K, Kobayashi S, Ikeda T, Sao H, Okamoto S, et al. Impact of ABO-blood group incompatibility on the outcome of recipients of bone marrow transplants from unrelated donors in the Japan Marrow Donor Program. Haematologica 2008;93: 1686-93.
40. Goldstone AH, Richards SM, Lazarus HM, Tallman MS, Buck G, Fielding AK, et al. In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). Blood 2008;111: 1827-33.
41. Collins RW. Human MHC class I chain related (MIC) genes: their biological function and relevance to disease and transplantation. Eur J Immunogenet 2004;31:105-14.

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ORIGINAL ARTICLE

Prediction of infectious events by high-sensitivity C-reactive protein level before undergoing chemotherapy for acute myeloid leukaemia

MIKI SATO, SHINICHI KAKO, KUMI OSHIMA, KEN SATO, KIRIKO TERASAKO, SHUN-ICHI KIMURA, HIDEKI NAKASONE, SHINYA OKUDA, RIE YAMAZAKI, TAKAKAZU HIGUCHI, JUNJI NISHIDA & YOSHINOBU KANDA

From the Division of Haematology, Saitama Medical Centre, Jichi Medical University, Saitama, Japan

Abstract

We retrospectively evaluated the serum high-sensitivity C-reactive protein (CRP) level before chemotherapy for the prediction of infectious events during neutropenia in patients with acute myeloid leukaemia. Thirty-eight patients who underwent first induction chemotherapy and 37 patients who underwent first consolidation chemotherapy were analyzed separately. A receiver-operating characteristic (ROC) curve revealed that the serum CRP level just before the first consolidation chemotherapy, but not just before the induction chemotherapy, had a significant predictive value for febrile neutropenia (FN) at a cut-off value of 0.19 mg/dl and documented infection (DI) at a cut-off value of 0.26 mg/dl. The high-sensitivity CRP measurement enabled the detection of slight increases in the serum CRP level, which might reflect a minute inflammation by occult infection, and discriminated high-risk patients for infectious events.

Introduction

Infection is the most common complication in neutropenic patients undergoing chemotherapy for haematological malignancies. Several measures, such as the use of high efficiency particulate air (HEPA) filters and the prophylactic administration of antibiotics, have been shown to be effective in preventing infectious events. However, considering the cost and the emergence of resistant bacteria, these interventions should not be applied to low-risk patients. Therefore, predictive factors before starting chemotherapy have been investigated to discriminate high-risk patients for infectious events during neutropenia.

C-reactive protein (CRP) is an acute phase reactant that is mainly produced in the liver. Serum CRP levels rapidly rise within 24 h in response to infection or tissue injuries [1]. Several reports have demonstrated that the prognoses of infectious events can be predicted by the serum CRP level measured at their onset in patients with neutropenia [2,3]. Other studies have demonstrated that changes in serum CRP levels reflect the response to antibiotic therapy [4,5]. However, it has been difficult to predict infectious events by the serial measurement of serum CRP levels [4]. High-sensitivity quantitation of serum CRP levels has recently become available; this can determine quantities of CRP of <0.3 mg/dl in sera, which is the detection limit in the conventional measurement of CRP, and enables the detection of slight inflammation. It has been reported that a slight increase in the serum CRP level in patients with atherosclerosis is associated with the risk of ischaemic heart disease, suggesting the clinical usefulness of the sensitive measurement of CRP [6]. Therefore, in this study, we examined the relationship between the high-sensitivity serum CRP level before chemotherapy and infectious events during neutropenia in patients with acute myeloid leukaemia (AML), to evaluate its predictive value for such events.

Patients and methods

High-sensitivity measurement of CRP became available in routine practice at our centre in October 2003. Therefore, we retrospectively analyzed consecutive patients

Correspondence: Y. Kanda, Division of Haematology, Saitama Medical Centre, Jichi Medical University, 1-847 Amanuma, Omiya-ku, Saitama City, Saitama 330-8503, Japan. Tel: +81 48 647 2111 ext. 5416. Fax: +81 48 648 5188. E-mail: ycanda-tky@umin.ac.jp

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with AML treated at our institution from October 2003 to January 2008. Patients who underwent first induction chemotherapy or first consolidation chemotherapy for AML and who had neutropenia (neutrophil count <500/μl) for 7 days or longer were included in the study. We excluded patients who had had fever or definite infection and those who had received intravenous antibiotics at the start of chemotherapy. All the included patients received oral antibacterial and antifungal agents prophylactically. Antibacterial prophylaxis was performed with levofloxacin or polymyxin B. Oral fluconazole, itraconazole, or amphotericin B was given for antifungal prophylaxis. Antibacterial agents were changed to intravenous antibiotics when the neutrophil count became <500/μl, at the discretion of the attending physician (in 8 patients in the induction group and 12 patients in the consolidation group).

The serum high-sensitivity CRP level was measured at least twice a week as routine practice by latex immunoagglutination assay (Nanopia CRP, Sekisui Medical, Tokyo, Japan; minimum detection level 0.01 mg/dl). We collected data on serum CRP levels just before the start of chemotherapy. Infectious episodes were categorized into 2 groups: febrile neutropenia (FN) defined as fever during neutropenia with axillary temperature ≥37.5°C, and documented infection (DI), which included microbiologically documented infection and presumed infection based on clinical and/or radiological findings [7].

Patients who received induction chemotherapy and those who received consolidation chemotherapy were analyzed separately. The predictive value of the serum CRP level was evaluated using a receiver-operating characteristic (ROC) curve. ROC curves were drawn by plotting the sensitivity (y-axis) against (1 − specificity) (x-axis). The points on the ROC curves closest to the left upper corner were considered to be the best cut-off values, with which the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. In addition, the predictive value of the following factors on the incidence of infectious events was also evaluated: sex, age, type of AML, performance status, history of recent infection, use of central venous catheter, prophylactic use of intravenous antibiotics after the start of chemotherapy, and duration of neutropenia. The incidence of FN was calculated using the Kaplan–Meier method. Univariate comparisons for dichotomous, continuous, and time-to-event variables between groups were performed with the Fisher's exact test, t-test, and the log-rank test, respectively. Factors associated with at least borderline significance ($p < 0.15$) in the univariate analysis were subjected to a multivariate analysis using proportional hazards modelling.

Results

Patient characteristics and infectious events during hneutropenia

Patient characteristics are summarized in Table I. Thirty-eight patients who underwent first induction chemotherapy and 37 patients who underwent first consolidation chemotherapy were analyzed separately. Seventeen patients were included in both analyses. In the induction group there were 26 males and 12 females with a median age of 54 y (range 16–78) and in the consolidation group there were 22 males and 15 females with a median age of 50 y (range 16–72). The median serum CRP level was 0.3 mg/dl (range 0.02–5.69) just before the first induction chemotherapy and 0.18 mg/dl (range 0.02–2.19) just before the first consolidation chemotherapy. The median durations of neutropenia <500/μl and <100/μl were 22 and 14 days, respectively, in the induction group and 18 and 10 days, respectively, in the consolidation group.

During the induction chemotherapy, 31 patients developed FN and 18 patients developed DI. During the consolidation chemotherapy, 26 patients developed FN and 11 patients developed DI. All patients who developed DI in both groups had experienced FN. Therefore, 18 patients in the induction group and 11 patients in the consolidation group had neither FN nor DI. The details of DI are summarized in Table II. In most of these patients, DI was documented by clinical or radiological evidence and the

Table I. Patient characteristics

	Induction (n = 38)	Consolidation (n = 37)
Age (range), y	54 (16–78)	50 (16–72)
Sex (M/F)	26/12	22/15
Performance status (0/1/2/3/4)	29/7/2/0/0	33/4/0/0/0
Antibiotic prophylaxis	38	37
HEPA filter	21	20
Central venous catheter	21	20
CRP level (range), mg/dl	0.3 (0.02–5.69)	0.18 (0.02–2.19)
Duration of neutropenia <500/μl (range), days	22 (7–69)	18 (9–51)
Duration of neutropenia <100/μl (range), days	14 (0–37)	10 (4–18)

HEPA filter, high efficiency particulate air filter; CRP, C-reactive protein.

Table II. Documented infections (DI)

	Induction chemotherapy	Consolidation chemotherapy
DI	18	11
Blood stream infection	5	4
Anorectal infection	4	2
Oral infection	3	0
Cutaneous infection	2	1
Upper respiratory tract infection	1	1
Lower respiratory tract infection	1	3
Peripheral venous catheter-related infection	1	2
Central venous catheter-related infection	0	1
Others	1	2

Some patients in the consolidation group developed 2 or more episodes of DI.

causative pathogen was not identified. However, in patients with blood stream infections, the most frequent causative pathogens were Staphylococcus epidermidis ($n = 4$), followed by Staphylococcus aureus, Staphylococcus capitis, Streptococcus mitis, and Enterococcus faecalis ($n = 1$ each).

Statistical analyses

The area-under-the ROC curve (AUC) plot, based on the serum CRP level just before induction chemotherapy was 0.48 for FN and 0.56 for DI, suggesting that the serum CRP level before induction therapy had poor predictive value for FN and DI. On the other hand, the serum CRP level just before consolidation chemotherapy had a better predictive value for FN and DI, with AUCs of 0.77 and 0.67, respectively (Figure 1). The best cut-off value of serum CRP level for FN was 0.19, which gave sensitivity, specificity, PPV, and NPV of 0.64, 0.82, 0.89, and 0.50, respectively. The best cut-off value of serum CRP level for DI was 0.26, which gave sensitivity, specificity, PPV, and NPV of 0.64, 0.80, 0.58, and 0.83, respectively. With these cut-off values, the likelihood ratio for a positive result of CRP for FN was 3.6 and for DI was 3.2. The likelihood ratio for a negative result of CRP for FN was 0.44 and for DI was 0.45.

In a univariate analysis, the prophylactic use of intravenous antibiotics after the start of chemotherapy and serum CRP level less than 0.2 mg/dl were associated with a lower incidence of FN during consolidation chemotherapy with at least borderline significance ($p = 0.12$ and $p = 0.07$, respectively; Table III). In a multivariate analysis, the adjusted p-value of the serum CRP level for the prediction of FN was 0.057. Cumulative incidences of FN calculated by the Kaplan–Meier method were 88% and 59% in patients with serum CRP levels of <0.2 mg/dl or in those with higher CRP levels ($p = 0.05$; Figure 2A). However, no difference in the incidence of FN was observed when the patients in the induction group were grouped according to the serum CRP level using the same cut-off value (Figure 2B).

Discussion

High-sensitivity measurement of the serum CRP level has enabled the detection of slight inflammatory change. We examined the relationship between the serum CRP level measured just before chemotherapy and the incidence of infectious events during neutropenia in patients undergoing first remission induction therapy or first consolidation therapy for AML. The serum CRP level before the first consolidation chemotherapy demonstrated a high predictive value for infectious events. High-risk patients for FN and DI could be discriminated by the serum CRP level, with cut-offs of 0.19 mg/dl and 0.26 mg/dl, respectively. This means that high-risk patients could not be discriminated by

Table III. Predictive factors for febrile neutropenia during consolidation chemotherapy

	Univariate analysis		Multivariate analysis	
	Incidence	p-Value	OR (95% CI)	p-Value
Sex (M/F)	68%/73%	>0.99		
Age (<50 y/≥50 y)	68%/72%	>0.99		
FAB (M3/other than M3)	63%/72%	0.67		
ECOG-PS (0/1)	68%/83%	0.65		
History of recent infection (+/−)	82%/60%	0.17		
Central venous catheter (+/−)	70%/71%	>0.99		
Prophylactic intravenous antibiotics (+/−)	50%/98%	0.12	0.26 (0.05–1.37)	0.11
Serum CRP level (<0.2 mg/dl/≥0.2 mg/dl)	57%/88%	0.07	5.70 (0.95–34.3)	0.057
Duration of neutropenia, <500/μl (<18 days/≥18 days)	63%/78%	0.48		
Duration of neutropenia, <100/μl (<11 days/≥11 days)	67%/75%	0.72		

OR, odds ratio; CI, confidence interval; FAB, French–American–British Cooperative Group criteria for the classification of acute myeloid leukaemia; ECOG-PS, performance status according to the Eastern Cooperative Oncology Group scale; CRP, C-reactive protein.

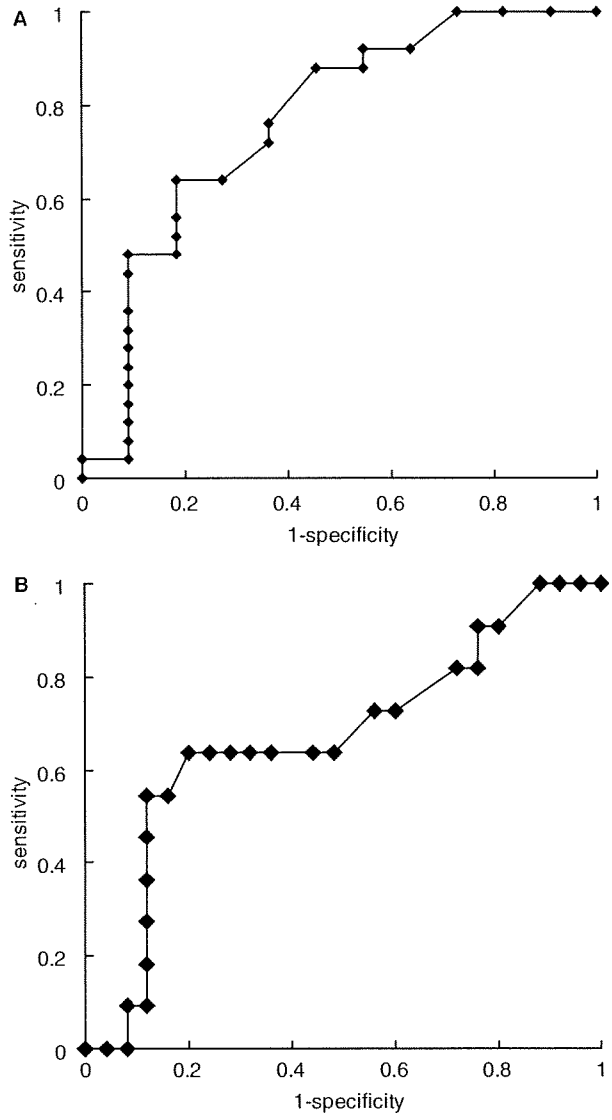

Figure 1. Receiver-operating characteristic (ROC) curve plot based on the serum C-reactive protein level before first consolidation therapy and the incidence of febrile neutropenia (A) and documented infection (B).

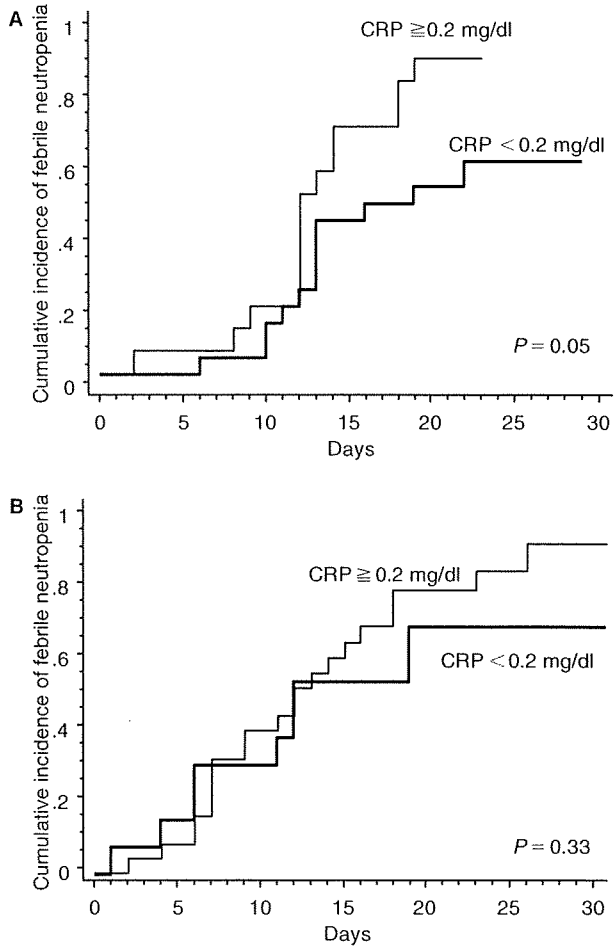

Figure 2. Cumulative incidence of febrile neutropenia grouped according to the serum C-reactive protein (CRP) level before chemotherapy in patients who underwent first consolidation therapy (A) and induction therapy (B).

the conventional measurement of the serum CRP level. The slight increase in the serum CRP level might reflect a minute inflammation by occult infection, leading to the overt infectious events during neutropenia.

On the other hand, serum CRP level before the first induction chemotherapy did not have a significant predictive value for infectious events. Serum CRP levels rise in response not only to inflammation, but also to tumour cells [8,9]. Patients before the first induction chemotherapy had a huge number of tumour cells in their bodies, which might have affected the serum CRP level and made it difficult to detect the slight inflammation. In fact, the median serum CRP level just before the first induction chemotherapy was significantly higher than that just before the first consolidation chemotherapy (median 0.3 mg/dl vs 0.18 mg/dl, $p = 0.031$). This might have reflected the difference in the number of leukaemic cells (average white cell count 22,202/μl vs 4488/μl, $p = 0.014$ and average blast cell count 15,587/μl vs 14/μl, $p = 0.0016$), although the difference in the CRP level was surprisingly small if we consider the huge difference in the number of leukaemic cells. Another possible explanation is the longer neutropenic duration after induction therapy than consolidation therapy, which increases the possibility of infectious events acquired after starting chemotherapy.

There have been many reports regarding other markers for infectious events. Interleukin-6 (IL-6) is a cytokine that is produced by T-cells or macrophages and regulates the humoral immune response. Procalcitonin (PCT) is a precursor of calcitonin, which is usually produced by thyroid c-cells, but is also produced markedly by monocytes or hepatocytes in response to proinflammatory cytokines. Both IL-6 and PCT have been reported to be useful markers of infection [10,11], especially as markers of bacterial infection [10].

Von Lilienfeld-Toal et al. reported that PCT and IL-6 were more reliable markers than CRP for bacteraemia in patients with FN [12], although Hambach et al. did not confirm the superiority of PCT in allogeneic hematopoietic stem cell transplantation recipients [13]. However, whether IL-6 or PCT has predictive value for infectious events has not been evaluated. If IL-6 and PCT are more specific to inflammation than CRP, the serum levels of IL-6 and PCT, or these factors in combination with CRP, would be more suitable to predict infectious events even during the first induction chemotherapy. This hypothesis should be tested in a future study.

Prophylactic antibiotics are widely administered in patients undergoing chemotherapy. A recent meta-analysis of randomized controlled trials revealed that antibiotic prophylaxis for neutropenic patients, especially with fluoroquinolones, reduced mortality [14]. However, such prophylaxis may lead to the emergence of antibiotic-resistant pathogens. Therefore, it is still important to limit the use of prophylactic antibiotics as much as possible. Identifying low-risk patients by serum inflammatory markers might prevent the excessive use of antibiotics.

In conclusion, serum high-sensitivity CRP levels measured just before consolidation chemotherapy for AML show significant correlation with the development of FN and DI during neutropenia. Therefore, it may become possible to discriminate high-risk patients for infectious events during neutropenia by the CRP level. Further studies are required to establish the appropriate prophylactic methods during neutropenia based on risk stratification according to baseline CRP level.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Pepys MB, Baltz ML. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. Adv Immunol 1983;34: 141–212.

[2] Ortega M, Rovira M, Almela M, de la Bellacasa JP, Carreras E, Mensa J. Measurement of C-reactive protein in adults with febrile neutropenia after hematopoietic cell transplantation. Bone Marrow Transplant 2004;33:741–4.

[3] Arber C, Passweg JR, Fluckiger U, Pless M, Gregor M, Tichelli A, et al. C-reactive protein and fever in neutropenic patients. Scand J Infect Dis 2000;32:515–20.

[4] Yonemori K, Kanda Y, Yamamoto R, Hamaki T, Suguro M, Chizuka A, et al. Clinical value of serial measurement of serum C-reactive protein level in neutropenic patients. Leuk Lymphoma 2001;41:607–14.

[5] Schofield KP, Voulgari F, Gozzard DI, Leyland MJ, Beeching NJ, Stuart J. C-reactive protein concentration as a guide to antibiotic therapy in acute leukaemia. J Clin Pathol 1982;35:866–9.

[6] Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997;336:973–9.

[7] Masaoka T. Evidence-based recommendations for antimicrobial use in febrile neutropenia in Japan: executive summary. Clin Infect Dis 2004;39(Suppl 1):S49–52.

[8] Drahovsky D, Dunzendorfer U, Ziegenhagen G, Drahovsky M, Kellen JA. Reevaluation of C-reactive protein in cancer sera by radioimmunoassay and radial immunodiffusion. I. Diagnostic value and use in battery of conventional tumor markers. Oncology 1981;38:286–91.

[9] Koukourakis MI, Kambouromiti G, Pitsiava D, Tsousou P, Tsiarkatsi M, Kartalis G. Serum C-reactive protein (CRP) levels in cancer patients are linked with tumor burden and are reduced by anti-hypertensive medication. Inflammation 2009;32:169–75.

[10] Sandri MT, Passerini R, Leon ME, Peccatori FA, Zorzino L, Salvatici M, et al. Procalcitonin as a useful marker of infection in hemato-oncological patients with fever. Anticancer Res 2008;28:3061–5.

[11] Prat C, Sancho JM, Dominguez J, Xicoy B, Gimenez M, Ferra C, et al. Evaluation of procalcitonin, neopterin, C-reactive protein, IL-6 and IL-8 as a diagnostic marker of infection in patients with febrile neutropenia. Leuk Lymphoma 2008;49:1752–61.

[12] von Lilienfeld-Toal M, Dietrich MP, Glasmacher A, Lehmann L, Breig P, Hahn C, et al. Markers of bacteremia in febrile neutropenic patients with hematological malignancies: procalcitonin and IL-6 are more reliable than C-reactive protein. Eur J Clin Microbiol Infect Dis 2004;23:539–44.

[13] Hambach L, Eder M, Dammann E, Schrauder A, Sykora KW, Dieterich C, et al. Diagnostic value of procalcitonin serum levels in comparison with C-reactive protein in allogeneic stem cell transplantation. Haematologica 2002;87:643–51.

[14] Gafter-Gvili A, Fraser A, Paul M, Leibovici L. Meta-analysis: antibiotic prophylaxis reduces mortality in neutropenic patients. Ann Intern Med 2005;142:979–95.

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ORIGINAL ARTICLE

Pharmacokinetics of CsA during the switch from continuous intravenous infusion to oral administration after allogeneic hematopoietic stem cell transplantation

S Kimura[1], K Oshima[1], S Okuda[1], K Sato, M Sato, K Terasako, H Nakasone, S Kako, R Yamazaki, Y Tanaka, A Tanihara, T Higuchi, J Nishida and Y Kanda

Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan

We investigated the serial changes in the blood CsA concentration during the switch from continuous intravenous infusion to twice-daily oral administration in allogeneic hematopoietic stem cell transplant recipients ($n=12$). The microemulsion form of CsA, Neoral, was started at twice the last dose in intravenous infusion in two equally divided doses. The area under the concentration–time curve during oral administration (AUC_{PO}) was significantly higher than the AUC during intravenous infusion (AUC_{IV}) (median 7508 vs 6705 ng/ml × h, $P=0.050$). The median bioavailability of Neoral, defined as ($AUC_{PO}/DOSE_{PO}$) divided by ($AUC_{IV}/DOSE_{IV}$), was 0.685 (range, 0.45–1.04). Concomitant administration of oral voriconazole ($n=4$) significantly increased the bioavailability of Neoral (median 0.87 vs 0.54, $P=0.017$), probably due to the inhibition of gut CYP3A4 by voriconazole. Although the conversion from intravenous to oral administration of CsA at a ratio of 1:2 seemed to be appropriate in most patients, a lower conversion ratio may be better in patients taking oral voriconazole. To obtain a similar AUC, the target trough concentrations during twice-daily oral administration should be halved compared with the target concentration during continuous infusion.

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Keywords: CsA; pharmacokinetics; bioavailability; drug interaction

Introduction

CsA is the most widely used immunosuppressive agent for the prophylaxis of GVHD after allogeneic hematopoietic stem cell transplantation (HSCT). It is usually administered by intravenous infusion for at least several weeks after allogeneic HSCT because of the damage done to the oral and gastrointestinal mucosa by the conditioning regimen. However, the dose, target blood level, and schedule of administration vary among protocols and have not been optimized.[1] It has been shown that the blood concentration of CsA affects the incidences of acute GVHD and adverse events,[2] and an increase in the target blood concentration from 300 to 500 ng/ml in the continuous infusion of CsA significantly decreased the incidence of acute GVHD.[3] On the basis of these results, we are currently administering CsA by continuous infusion with target concentrations of 500 ng/ml for standard-risk patients and 300 ng/ml in high-risk patients. When patients can tolerate oral intake, CsA is switched from intravenous to oral administration at a dose ratio of 1:2. Neoral, a microemulsion formulation of CsA, has improved bioavailability and is the most commonly used oral product.[4] However, the appropriateness of this conversion rate has been inconsistent among earlier studies.[5,6] Parquet *et al.* reported that doubling the last intravenous dose provided the best therapeutic range concentration, whereas the concentration/dose ratio was similar in intravenous administration and oral administration and thus, 1:1 conversion seemed appropriate in the McGuire's study. In addition, no data are available regarding the detailed pharmacokinetics in allogeneic HSCT recipients. Therefore, in this study, we investigated the serial changes in the CsA blood concentration during the switch from intravenous to oral administration and assessed the bioavailability of Neoral.

Patients and methods

Patients

Patients who underwent allogeneic HSCT with GVHD prophylaxis consisting of the continuous infusion of CsA and short-term MTX were included. This single-center prospective study was approved by the Institutional Review Board of Jichi Medical University, and each patient provided their written informed consent to be enrolled in the study.

Correspondence: Dr Y Kanda, Division of Hematology, Saitama Medical Center, Jichi Medical University, 1-847 Amanuma, Omiya-ku, Saitama-city, Saitama 330-8503, Japan.
E-mail: ycanda-tky@umin.ac.jp
[1]These authors contributed equally to this work.
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Transplantation procedure

The conditioning regimen was mainly a combination of cyclophosphamide (60 mg/kg for 2 days) and TBI (2 Gy twice daily for 3 days) ($n=8$). Patients with severe aplastic anemia ($n=3$) were prepared with fludarabine, cyclophosphamide, and anti-thymoglobulin with or without a low dose of TBI at 2 Gy.[7] A reduced-intensity regimen with fludarabine and melphalan was used for a 58-year-old patient with acute lymphoblastic leukemia ($n=1$). GVHD prophylaxis consisted of the continuous infusion of CsA with a starting dose of 3 mg/kg/day and short-term MTX (10–15 mg/m^2 on day 1 and 7–10 mg/m^2 on days 3 and 6, and optionally on day 11 in HSCT from a donor other than an HLA-matched sibling). The dose of CsA was adjusted to maintain the blood CsA concentration between 450 and 550 ng/ml in standard-risk patients ($n=9$) or 250 and 350 ng/ml in high-risk patients ($n=3$) according to the disease status.[3] Acute GVHD was graded as described earlier.[8] Prophylaxis against bacterial, fungal, and Pneumocystis jiroveci infection consisted of levofloxacin, fluconazole (FLCZ), and sulfamethoxazole/trimethoprim (ST) or inhalation of pentamidine. In three patients, micafungin (MCFG) was used instead of FLCZ because of persistent fever despite broad-spectrum antibiotic therapy, development of Candidemia, and high risk for invasive aspergillosis, respectively. As prophylaxis against herpes simplex virus infection, acyclovir (ACV) was given from days −7 to 35, followed by a long-term low-dose administration of ACV for varicella zoster reactivation.[9] Pre-emptive therapy with ganciclovir for cytomegalovirus infection was performed by monitoring cytomegalovirus antigenemia.[10]

Study schedule

When patients were able to tolerate oral intake, CsA was switched from continuous infusion to oral administration. Intravenous infusion was stopped just before the first oral administration. The initial dose of Neoral was twice the last daily dose of continuous infusion, and was given in two equally divided doses based on the reported bioavailability of Neoral of about 0.4 (40%) in allogeneic HSCT recipients.[5] On the last day of the continuous infusion of CsA (day −1), the serum CsA concentration was measured at 9:00, 15:00, and 21:00. After the patient was switched to Neoral, the CsA concentration was measured just before (C_0), and 1 (C_1), 2 (C_2), 3 (C_3), 4 (C_4), 6 (C_6), and 12 (C_{12}) hours after the oral administration of Neoral on the first day (day 0) and between day 3 and day 5. The CsA concentration was measured using the CYCLO-Trac SP-whole blood kit (DiaSorin, Inc., Stillwater, MN, USA).[11] In brief, 200 µl of whole blood sample was mixed with 800 µl of methanol and centrifuged at 1600 *g* for 5 min. The methanolic supernatant (50 µl in duplicate) was mixed with 100 µl of ^{125}I-ligand and 1 ml of anti-CYCLO-Trac Immune Sep (pre-mixed mouse monoclonal antibody, donkey anti-mouse serum, and normal mouse serum). After centrifuging, the ligand was discarded by decanting and the amount of radioactivity of the pellet was determined. Data were analyzed by logit-log reduction. The standard curve was obtained using the CsA standard sera provided in the kit. The intra-assay coefficient of variance was <15%. The inter-assay coefficient of variance was <14%. The limit of detection was 4.0 ng/ml. The results of this assay showed good correlation with those obtained by high-performance liquid chromatography ($r=0.98$).

During the study, the dose of CsA could be modified at the discretion of each physician. Vital signs and laboratory variables including renal and liver function tests were evaluated on days 0, 3, 7, and 14. Concomitant medications that could potentially interact with CsA were recorded.

Statistical considerations

The area under the concentration–time curve (AUC) (0–12 h) of CsA was calculated by the trapezoidal method. We estimated the bioavailability of Neoral by dividing ($AUC_{PO}/DOSE_{PO}$) by ($AUC_{IV}/DOSE_{IV}$). Toxicities after switching from intravenous to oral administration were evaluated compared with the baseline data on day 0. Renal toxicity was defined as an elevation of the creatinine (Cr) level above ×1.5 the baseline value. Liver dysfunction was defined as an elevation of alanine aminotransferase (ALT) above ×2 the baseline value, or elevation of the total bilirubin (T-bil) level by 2 mg per 100 ml compared with the baseline value. Comparisons were made using the Wilcoxon signed-rank test for continuous variables. The Pearson correlation coefficient was used to analyze the correlation between AUC and the CsA concentration at each measurement point after logarithmic transformation. The effect of concomitant medications on CsA pharmacokinetics was first analyzed by a univariate analysis with the Mann–Whitney *U*-test, and then those with at least borderline significance ($P<0.10$) were subjected to a multivariate analysis using multiple regression modeling. A *P*-value of <0.05 was considered to be significant.

Results

Patients

Between January 2008 and April 2009, 12 patients were enrolled in the study. There were 7 males and 5 females with a median age of 34.5 years (range, 16–58). Underlying diseases included acute myeloblastic leukemia ($n=4$), acute lymphoblastic leukemia ($n=3$), severe aplastic anemia ($n=3$), chronic myelogenous leukemia ($n=1$), and myelodysplastic syndrome ($n=1$). Five patients received bone marrow graft from an unrelated donor, whereas 1 and 6 patients, respectively, received bone marrow and peripheral blood stem cell graft from a related donor. There was an HLA mismatch in three donor-recipient pairs.

Pharmacokinetic analysis

The median duration from transplantation to the switch from intravenous to oral administration was 40 days (range, 27–60). The dose of CsA and the pharmacokinetic parameters during intravenous and oral administration are shown in Table 1. Neoral was started at approximately twice the last dose of intravenous infusion, except that 1 patient (No. 8) received Neoral at the same dose as in intravenous infusion, as the mean CsA concentration on the last day of intravenous infusion was >700 ng/ml.