Table 1 Clinical findings in 53 reported patients with nephrotic syndrome after hematopoietic stem cell transplantation

	No. of patients (%)
At onset of NS	
Microscopic hematuria	13/17 (76)
Normal serum creatinine	34/38 (89)
Chronic GVHD	41/53 (77)
Positive ANA	16/37 (43)
Renal pathology	
MN	35
MCNS	13
FSGS	1
Unknown	4
Treatment for NS	
Steroids	50
Cyclosporine A	29
Relapse of NS	4

NS nephrotic syndrome, *ANA* anti-nuclear antibody, *GVHD* graft-versus-host disease, *MN* membranous nephropathy, *MCNS* minimal change nephrotic syndrome, *FSGS* focal segmental glomerulosclerosis

Fifty-three patients with NS after HSCT are summarized in Table 1 [1–23]. NS due to rapidly progressive glomerulonephritis was excluded. Primary hematological diseases, types of HSCT (bone marrow or peripheral blood, related or unrelated donor), the conditioning regimen for HSCT and prophylaxis regimen for GVHD varied. NS occurred at a mean of 17.6 ± 10.1 months (3–56 months) after HSCT. Most patients had microscopic hematuria, but their renal function was normal. These patients had chronic GVHD. In 43% of the patients, anti-nuclear antibody was positive. The CD4/CD8 ratio examined in six adult patients with MN, five of whom had chronic GVHD, was normal [7, 11, 20, 22, 23]. Our patient did not have B2 of HLA, which was detected in two Japanese patients who developed MN after HSCT [15, 23]. MN was observed in 35 patients, MCNS in 13 and focal segmental glomerulosclerosis (FSGS) in 1. Proteinuria decreased after the treatment with steroids and/or CyA in most patients, and disappeared in 20 patients within 4.0 ± 4.5 months (0.3–12 months). Even if pathological findings show MCNS, the steroid response seems to be different from that of idiopathic MCNS. Only three patients with MCNS achieved complete remission after an 8-week course of PSL [8, 19]. Relapse of NS occurred in four patients, two with MN [3, 9] and two with MCNS [8, 19], when PSL and/or CyA were reduced or withdrawn. One MN patient had three relapses [9], and three patients had a relapse only once [3, 8, 19].

Renal changes of mouse models of GVHD support the association of GVHD and MN [26]. Two weeks after the injection of lymphocytes from donor mice, deposits of IgG and C3 were found along the glomerular basement membrane of the kidney in a linear pattern, which changed to a granular pattern 6–8 weeks later. At this stage, large electron-dense deposits were present in the subepithelial area, and massive proteinuria developed. Of the 13 MCNS patients, 8 had chronic GVHD of other organs. Subepithelial electron-dense deposits without immunofluorescent staining were observed in one child [6] and two adults [12, 16]. Immunofluorescent microscopy showed deposits of IgG and C1q along capillary walls in one child, but her electron microscopic examination was not available [1]. Fine granular deposits of IgG and C3 along the capillary walls were observed in one adult, but electron-dense deposits were not observed [8]. These five patients with chronic GVHD and MCNS may have had early stage MN. T-cell dysfunction in patients with malignancies, including leukemia and Hodgkin's lymphoma, may have been involved in causing MN or MCNS. In two adult patients without chronic GVHD, relapse of acute myeloid leukemia was associated with the development of NS. One patient had MN, and the other had MCNS [19]. In one adult patient without other manifestations of GVHD, tumor necrosis factor-α released from donor T-cells may have increased permeability of glomeruli and induced MCNS [17].

MN after HSCT occurred mainly in adults, and MCNS occurred in children [10]. Pathological diagnoses of reported patients under 15 years of age are listed in Table 2. Our patient is the youngest among patients with NS due to MN after HSCT. Idiopathic MN is rare in childhood and becomes more frequent with age. Chronic GVHD in children is less frequent than in adults. Immature T-cell function in children may be related to these findings. The percentage of CD4 positive (helper/inducer) T-cells decreases until 7–10 years of age, and that of CD8 positive (suppressor/cytotoxic) T-cells increases until 3–7 years of age. The CD4/CD8 ratio decreases with age and reaches the adult level at 10 years [27]. Immaturity or dysfunction of T-cells in this child with chronic GVHD and MN after

Table 2 Children under 15 years of age with nephrotic syndrome after hematopoietic stem cell transplantation

Age at onset of NS (years)	Chronic GVHD	Renal pathology	References
3	–	MCNS	[10]
5	–	FSGS	[4]
10	–	MCNS	[21]
13	+	MN	[3]
15	+	MCNS	[6]
15	+	MCNS	[1]

NS nephrotic syndrome, *GVHD* graft-versus-host disease, *MCNS* minimal change nephrotic syndrome, *FSGS* focal segmental glomerulosclerosis, *MN* membranous nephropathy

HSCT was not detected by the CD4/CD8 ratio. Reconstitution of T-cell subpopulations continues 2 years after HSCT. T-cell function varies in children 5 months after HSCT. Chronic GVHD and the treatment also affect T-cell function. In our patient with chronic GVHD, his T-cell function caused MN.

References

1. Akar H, Keven K, Celebi H, Orhan D, Nergizoglu G, Erbay B, et al. Nephrotic syndrome after allogeneic peripheral blood stem cell transplantation. J Nephrol. 2002;15:79–82.
2. Bernis C, Camara R, Sanchez AG, Martinez MA, Selgas R. Nephrotic syndrome in a bone marrow transplant recipient after cyclosporine withdrawal. Nephrol Dial Transplant. 2003;18:2457.
3. Chan GS, Chim S, Fan YS, Chan KW. Focal segmental glomerulosclerosis after membranous glomerulonephritis in remission: temporal diversity of glomerulopathy after bone marrow transplantation. Human Pathol. 2006;37:1607–10.
4. Chien YH, Lin KH, Lee TY, Lu MY, Tsau YK. Nephrotic syndrome in a bone marrow transplant recipient without chronic graft-versus-host disease. J Formos Med Assoc. 2000;99:503–6.
5. Colombo AA, Rusconi C, Esposito C, Bernasconi P, Caldera D, Lazzarino M, et al. Nephrotic syndrome after allogeneic hematopoietic stem cell transplantation as a late complication of chronic graft-versus-host disease. Transplantation. 2006;81:1087–92.
6. Gomez-Garcia P, Herrera-Arroyo C, Torres-Gomez A, Gomez-Carrasco J, Aljama-Garcia P, Lopez-Rubio F, et al. Renal involvement in chronic graft-versus-host disease: a report of two cases. Bone Marrow Transplant. 1988;3:357–62.
7. Haseyama K, Watanabe J, Oda T, Katoh S, Suzuki N, Kudoh T, et al. Nephrotic syndrome related to chronic graft versus host disease after allogeneic bone marrow transplantation in a patient with malignant lymphoma (in Japanese with English abstract). Jpn J Clin Hematol. 1996;37:1383–8.
8. Ikee R, Yamamoto K, Kushiyama T, Imakiire T, Suzuki S, Miura S. Recurrent nephrotic syndrome associated with graft-versus-host disease. Bone Marrow Transplant. 2004;34:1005–6.
9. Miyazaki Y, Mori Y, Kishimoto N, Matsumoto N, Zen K, Amakawa R, et al. Membranous nephropathy associated with donor lymphocyte infusion following allogeneic bone marrow transplantation. Int J Hematol. 2003;78:262–5.
10. Morisada N, Okita S, Sato T, Miyaji R, Kamizono J, Shimono M, et al. Girl with nephrotic syndrome after peripheral blood stem cell transplantation. Pediatr Int. 2004;46:480–3.
11. Ohsawa I, Ohi H, Fujita T, Endo M, Ito T, Sawada U, et al. Glomerular and extraglomerular immune complex deposits in a bone marrow transplant recipient. Am J Kidney Dis. 2000;36(E3):1–5.
12. Oliveira JSR, Bahia D, Franco M, et al. Nephrotic syndrome as a clinical recipient after cyclosporin withdrawal. Bone Marrow Transplant. 1999;23:99–101.
13. Reddy P, Johnson K, Uberti JP, Reynolds C, Silver S, Ayash L, et al. Nephrotic syndrome associated with chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2006;38:351–7.
14. Sakoda K, Shibuya A, Suzuki H, Saito H, Nemoto T, Tabei K, et al. Nephrotic syndrome in patients after successful myeloablative allogeneic hematopoietic stem cell transplantation: Clinical findings obtained from four transplanted patients (in Japanese with English abstract). Jpn J Nephrol. 2007;49:999–1006.
15. Sato N, Kishi K, Yagisawa K, Kasama J, Karasawa R, Shimada H, et al. Nephrotic syndrome in a bone marrow transplant recipient with chronic graft-versus-host disease. Bone Marrow Transplant. 1995;16:303–5.
16. Sato Y, Hara S, Fujimoto S, Yamada K, Sakamaki H, Eto T. Minimal change nephrotic syndrome after allogenic hematopoietic stem cell transplantation. Intern Med. 2004;43:512–5.
17. Seconi J, Watt V, Ritchie DS. Nephrotic syndrome following allogenic stem cell transplantation associated with increased production of TNF-alpha and interferon-gamma by donor T cells. Bone Marrow transplant. 2003;32:447–50.
18. Shimokawa T, Sakai M, Kojima Y, Takeyama H. A case of nephrotic syndrome after allogeneic bone marrow transplantation for myelodysplastic syndrome (in Japanese). Med Postgrad. 2003;41:78–82.
19. Stevenson WS, Nankivell BJ, Hertzberg MS. Nephrotic syndrome after stem cell transplantation. Clin Transplant. 2005;19:141–4.
20. Tsutsumi C, Miyazaki Y, Fukshima T, Yoshida S, Taguchi J, Miyake C, et al. Membranous nephropathy after allogeneic stem cell transplantation. Int J Hematol. 2004;79:193–7.
21. Walker JV, Morich D, Anasetti C. Minimal-change nephrotic syndrome after cyclosporine withdrawal in a marrow transplant recipient. Am J Kidney Dis. 1995;26:532–4.
22. Yabana M, Takagi N, Kihara M, Muto R, Tamura K, Maemoto S, et al. A case of nephrotic syndrome after bone marrow transplantation (in Japanese with English abstract). Jpn J Nephrol. 1997;39:414–20.
23. Yorioka N, Taniguchi Y, Shimote K, Komo T, Yamakido M, Hyodo H, et al. Membranous nephropathy with chronic graft-versus-host disease in a bone marrow transplant recipient. Nephron. 1998;80:371–2.
24. Sullivan KM, Shulman HM, Storb R, Weiden PL, Witherspoon RP, McDonald GB, et al. Chronic graft-versus-host disease in 52 patients: adverse natural course and successful treatment with combination immunosuppression. Blood. 1981;57:267–76.
25. Churg J, Sobin LH. Diffuse membranous glomerulonephritis. In: Churg J, Sobin LH, editors. Renal disease. Classification and atlas of glomerular diseases. Tokyo: Igaku-shoin; 1982. p. 54–65.
26. Bruijn JA, Hogendoorn PCW, Corver WE, van den Broek LJCM, Hoedemaeker PJ, Fleuren GJ. Pathogenesis of experimental lupus nephritis: a role for anti-basement membrane and anti-tubular brush border antibodies in murine chronic graft-versus-host disease. Clin Exp Immunol. 1990;79:115–22.
27. Neubert R, Delgado I, Abraham K, Schuster C, Helge H. Evaluation of the age-dependent development of lymphocyte surface receptors in children. Life Sci. 1998;62:1099–110.

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Treatment of Children With Refractory Anemia: The Japanese Childhood MDS Study Group Trial (MDS99)

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Background. Although hematopoietic stem cell transplantation (HSCT) is offered as a curative therapy for pediatric myelodysplastic syndrome (MDS), it may cause severe complications and mortality. Several reports have shown the efficacy of immunosuppressive therapy (IST) in adult patients with refractory anemia (RA), but its safety and efficacy remains to be fully elucidated in childhood RA. ***Procedure.*** Eleven children diagnosed with RA and enrolled on a prospective multicenter trial conducted by the Japanese Childhood MDS Study Group were eligible for analysis. If patients showed transfusion dependent or suffered from infection due to neutropenia, they received IST consisting of antithymocyte globulin (ATG), cyclosporine (CyA), and methylprednisolone (mPSL). ***Results.*** Eight children received IST, 2 received only supportive therapy, and one underwent HSCT without IST. Five (63%) of eight children who received IST showed hematological response. Of note, one patient showed the disappearance of monosomy 7 after IST. Responders were significantly younger than non-responders (29 months vs. 140 months; $P = 0.03$). No severe adverse events related to IST were reported in this study. Of 6 children with chromosomal abnormalities who received IST, four showed hematological response. The probability of failure-free and overall survival at 5 years was $63 \pm 17\%$ and $90 \pm 9\%$ respectively. ***Conclusion.*** IST is likely to be a safe and effective modality for childhood RA. Pediatr Blood Cancer 2009;53:1011–1015. © 2009 Wiley-Liss, Inc.

Key words: myelodysplastic syndrome; refractory anemia; children; immunosuppressive therapy

INTRODUCTION

Myelodysplastic syndrome (MDS) is a hematopoietic stem cell disorder and rarely occurs in childhood [1,2]. Refractory anemia (RA) is a subgroup of MDS with less than 5% of blasts in the bone marrow (BM) and little is known about childhood RA because of its rarity. European Working Group of MDS in Childhood (EWOG-MDS) retrospectively analyzed the clinical characteristics of children with RA [3]. They found that neutropenia and thrombocytopenia were more prominent than anemia [3,4] and karyotype had a strong impact on prognosis in children with RA [3]. Children with monosomy 7 were significantly more likely to progress to advanced disease and they recommended hematopoietic stem cell transplantation (HSCT) for this unfavorable group as early as possible, whereas, appropriate treatment for children with chromosomal abnormalities other than monosomy 7 and those with normal karyotypes remained to be determined.

Disturbance of the immune system may play a role in pathogenesis in some adults and children with RA [5–7]. Several reports have shown positive effects of immunosuppressive therapy (IST) in adult patients with RA [8–12]. The hematological response rate of IST was reported as 30–80% but IST could not restore the cytogenetic abnormalities or dysplastic features. Recently, EWOG-MDS reported the results of IST consisting of antithymocyte globulin (ATG) and cyclosporine A (CyA) in children with hypoplastic refractory cytopenia (RC) and normal karyotype or trisomy 8 who were thought as being at low risk of progression to advanced MDS [13]. However, the role of IST in children with RA has not been fully elucidated because the above study selected children with favorable predictive factors for a positive response to IST.

This study reports the outcome of 11 children with RA enrolled on a prospective multicenter trial (MDS99) conducted by the Japanese Childhood MDS Study Group, which applied IST with ATG and CyA to unselected patients who needed intervention.

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PATIENTS AND METHODS

Patients

Eleven children younger than 16 years of age were enrolled onto MDS99 from September 1999 to March 2004. They were diagnosed as having RA according to the French-American-British (FAB) classification [14] and diagnosis was confirmed by the central review of morphology by two independent investigators [15]. Cytogenetic analysis of the bone marrow cells was performed in each institution. There were no patients who had undergone previous chemotherapy or radiotherapy, nor patients with a history of congenital bone marrow failure syndrome or aplastic anemia in the analysis. The study was approved by the Steering Committee of the Japanese Childhood MDS Study Group and the institutional review boards of the participating institutions or the equivalent organization. Informed consent was obtained from the guardians of the patients.

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The authors report no potential conflicts of interest.

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Treatment Protocol

Each patient with RA required repetitive bone marrow aspiration at 6–8 weeks intervals in order to confirm the diagnosis. If the disease was stable and blood transfusion was not required, patients were observed closely without any therapy. If patients showed transfusion dependent or suffered from infection due to neutropenia, IST was administered as follows: horse ATG (15 mg/kg/day) for 5 days as a slow intravenous infusion over 12 hr, CyA (6 mg/kg/day given orally as an initial dose, and the dose was adjusted to achieve a whole blood trough level of 100–200 ng/ml) was started on day 1 and continued until day 180, and methylprednisolone (mPSL; 2 mg/kg/day) was administered intravenously on days 1–7, then mPSL was administered orally and slowly tapered from day 8 to end on day 29. In this study, the use of G-CSF was not restricted. HSCT was recommended when a patient showed no response to IST and required further intervention because of cytopenia or progression to more advanced disease.

Evaluation and Statistical Analysis

Response to IST was evaluated at 6 months. Complete response (CR) was defined as a neutrophil count $>1.5 \times 10^9$/L, platelet count $>100 \times 10^9$/L, and hemoglobin (Hb) level of >11.0 g/dl. Partial response (PR) was defined as a neutrophil count $>0.5 \times 10^9$/L, platelet count $>20 \times 10^9$/L, and Hb level of >8.0 g/dl. When neither the CR nor the PR criteria were met, a patient was considered as no response (NR) to IST.

Mann–Whitney test and Fisher's exact test were applied to evaluate the differences between patients that responded to IST and those who did not. Failure-free survival (FFS) was calculated from the date of initiating IST to the date of treatment failure as follows; death, no response to IST at 6 months, HSCT, a second course of IST, acquisition of chromosomal abnormality, progression to advanced disease, or relapse. Overall survival (OS) was calculated from the date of diagnosis to the date of death or last follow-up. Both FFS and OS were estimated by the Kaplan–Meier method.

RESULTS

Patient Characteristics

Eleven children, 6 males and 5 females, were analyzed in this study (Table I). The median age at diagnosis was 67 months (range, 9 months to 15 years). Eight of 11 children had neutrophil counts of less than 1.5×10^9/L. All except 1 patient had Hb levels below 10 g/dl. Eight patients had platelet counts below 50×10^9/L. In total, one patient had anemia only, five had bi-cytopenia (anemia and neutropenia 2, anemia and thrombocytopenia 2, and neutropenia and thrombocytopenia 1), and five had pancytopenia at diagnosis. Since bone marrow biopsy specimen was available in only 6 of 11 cases, we determined cellularity by central pathological review from bone marrow smear rather than biopsy specimens and used a more suitable term, cell content, instead of cellularity in this report. Overall, there were only three patients in whom BM cell content was low. All patients showed dysplasia in multilineage series, which was compatible with the definition of refractory cytopenias with multilineage dysplasia (RCMD) in the World Health Organization (WHO) classification [16]. Data on the cytogenetic analyses at diagnosis were available for all patients. Karyotype was normal in three patients, and of the remaining eight patients, two had monosomy 7, two had trisomy 8, and four had other abnormalities; del (7)(q11), i(8)(q10), 20q-, and +der(1;19)(q10;q10). Of four patients in whom presence of paroxysmal nocturnal hemoglobinuria (PNH) cells was assessed by flow cytometry, none showed an expansion of PNH clone. Of five patients in whom data on HLA-DR was available, only one patient showed DR2 antigen, which is a broad antigen of DR15 and DR16.

TABLE I. Patients Characteristics

	Median (range)
Age	5y7m (9m to 15y5m)
Gender	M/F = 6:5
WBC ($\times 10^9$/L)	3.8 (1.1–12.5)
Neutrophil ($\times 10^9$/L)	0.94 (0.16–8.1)
PB blast (%)	0 (0)
Hb (g/dl)	6.2 (3.6–11.7)
Reticulocyte (%)	2 (1–44)
Reticulocyte ($\times 10^9$/L)	41.7 (12.3–572.0)
MCV (fl)	104 (84–123)
Plt ($\times 10^9$/L)	23.0 (3.0–117.0)
BM blast (%)	1.0 (0–4.8)
BM cell content	Low 3, normal 5, high 3
Chromosome	Normal/abnormal = 3:8
Cytopenia[a]	Anemia only 1, bi-cytopenia 5, pancytopenia 5

[a]Cut-off; neutrophils <1,500/μl, Hb < 10.0 g/dl, Plt < 50,000/μl.

Observation Without Intervention

Figure 1 shows the outcome of the 11 patients analyzed. Of 3 patients who initially received only supportive therapy, one with normal karyotype was still stable without therapy, one with trisomy 8 showed spontaneous improvement of anemia but the chromosomal abnormality remained. One with 20q- (UPN 046) showed stable disease for 2 years, but cytopenia deteriorated and IST was initiated at 968 days after diagnosis.

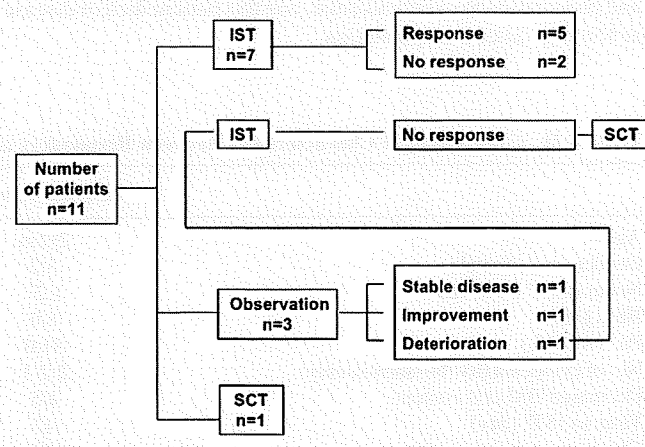

Fig. 1. Outcome of 11 patients with refractory anemia. SCT, stem cell transplantation; IST, immunosuppressive therapy.

Immunosuppressive Therapy

Seven patients received IST as the first-line treatment and one (UPN 046) received IST because of recurrence of cytopenia after 2-year observation. IST was given at a median of 42 (range 0–968) days after the diagnosis of RA. Five of eight patients showed response to IST at 6 months after the initiation of treatment (response rate was 63%; CR 2, PR3). Of five responders, three were able to successfully discontinue IST and remained disease-free, and the remaining two patients have been continuing therapy. Of note, the disappearance of a monosomy 7 clone after IST was observed in UPN 035 [17] and the patient is still in remission after 63 months. Of three non-responders, one was lost to follow up, one responded to a second course of IST, and one (UPN 046) underwent HSCT 3 months after initiating IST.

To address predictive factors for response to IST, the characteristics were compared between children who responded to IST and those who did not (Table II). The age at diagnosis was significantly younger in responders than in non-responders (median 29 months vs. 140 months; $P = 0.03$), whereas there was no statistically significant associations between response to IST and sex, neutrophil count, Hb level, platelet count, interval from diagnosis to IST, chromosomal abnormality, BM cell content, or number of cytopenia. Serious adverse events related to IST were not observed, including the progression to advanced disease. The most frequent adverse event in this study was pyrexia.

Hematopoietic Stem Cell Transplantation

Two children underwent HSCT in this series. One patient with 20q- (UPN 046) received bone marrow transplantation (BMT) from her human leukocyte antigen (HLA) 1-locus-mismatched father at 1,088 days after diagnosis because of non-response to IST. This patient suffered from adenoviral colitis, salmonella colitis, herpes zoster, and grade III acute GVHD of the skin, however, she is still alive without disease 23 months after BMT. One other patient with monosomy 7 (UPN 053) received BMT from a matched unrelated donor on 537 days after diagnosis without IST by physician's decision. His post-transplant course was uneventful, but disease relapsed 151 days after transplantation. A BM specimen at relapse showed severe fibrosis and progression to overt leukemia, and this patient died of disease at 656 days after transplantation.

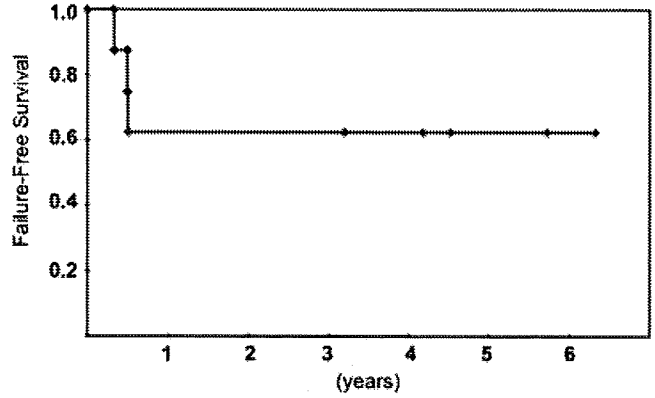

Fig. 2. Kaplan–Meier estimate of failure-free survival of patients who received immunosuppressive therapy. Failure-free survival was calculated from the date of initiating IST to the date of treatment failure as follows; death, no response to IST at 6 months, HSCT, a second course of IST, acquisition of additional chromosomal abnormality, progression to advanced disease, or relapse. The 5-year failure-free survival was $63 \pm 17\%$ (n = 8). Median follow-up was 1,346 days.

Chromosomal Abnormality

There were eight children with chromosomal abnormality in this study. Of those, six received IST and four showed responses to IST, including one with cytogenetic response (UPN 35).

Survival

Of eight children who received IST, three non-responders were considered as treatment failure. No patient died with IST after a median follow-up of 1,346 days; the 5-year FFS was $63 \pm 17\%$ (Fig. 2). Of total, 10 patients are alive after a median follow-up of 1,685 days; the 5-year OS was $90 \pm 9\%$ (Fig. 3).

DISCUSSION

Although HSCT is the curative modality for children with MDS, it may cause severe complications, mortality, and late sequelae. Several reports have shown encouraging results from the use of IST in adults with RA, and the hematological response rate to IST was 30–80% [8–12]. Yoshimi et al. [13] reported on 31 children with hypoplastic RC and normal karyotype or trisomy 8 treated with IST, which resulted in a response rate at 6 months of 71%, 3-year OS of

TABLE II. Comparison of Characteristics Between Responders and Non-Responders to IST

	Responder (n = 5)	Non-responder (n = 3)	*P*-value
Age[a]	2y5m	11y8m	0.03
Gender (male/female)	3:2	1:2	n.s.
Neutrophils[a] ($\times 10^9$/L)	1.27	0.63	n.s.
Hb[a] (g/dl)	8.0	6.2	n.s.
Plt[a] ($\times 10^9$/L)	31.0	20.0	n.s.
No. of cytopenia (tri-/bi-/anemia only)	2:2:1	2:1:0	n.s.
Decreased BM cell content	1/5	2/3	n.s.[b]
Time to IST[a] (day)	42	42	n.s.[b]
Chromosomal abnormality	4/5	2/3	n.s.[b]

[a]Median; [b]Evaluated by Mann–Whitney test and Chi-square test.

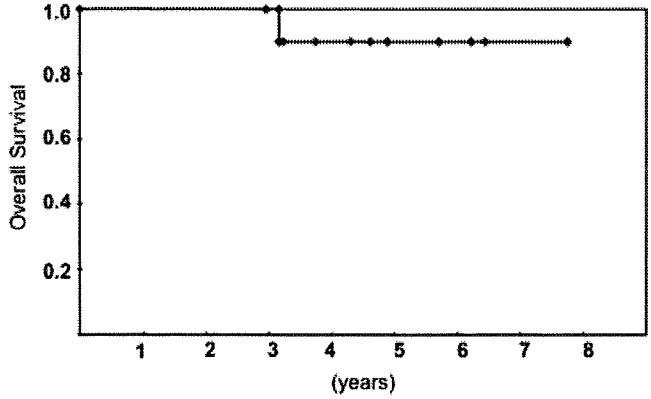

Fig. 3. Kaplan–Meier estimate of overall survival of all evaluable patients. Overall survival was calculated from the date of diagnosis to the date of death or last follow-up. The 5-year overall survival was $90 \pm 9\%$ ($n = 11$). Median follow-up was 1,685 days.

88%, and 3-year FFS of 57%. In contrast to the larger series by Yoshimi et al. children with RA and karyotypic abnormalities or non-hypoplastic marrow were also enrolled in this study. Overall, 5 of 8 patients (63%) responded to IST, and similar responses were observed in two-thirds of patients with chromosomal abnormalities. Patients whose BM cell content was not low also responded to IST (responder 4, non-responder 1); however, the significance of cellularity in pediatric RA still needs further study. No severe adverse events, disease progression, or death due to any cause after IST was reported. Only one death in this study was due to disease progression after HSCT, which was not related with IST. As a whole, the 5-year OS and FFS were 90% and 63%, which were comparable with the previous study in adult MDS and superior to our previous retrospective analysis of children with RA (4-year OS was 79%) [2]. Therefore, although the number of subjects was limited, we infer from these results that the IST is effective and safe for children with MDS.

The rationale for IST used as treatment of RA is based on previous studies, which suggested that alterations in the immune system might contribute to the pathogenesis in some subgroups of RA [5–7]. Dysregulated T cells are thought to destroy normal hematopoietic cells as bystanders as well as MDS clones [6]. IST can reduce MDS clone-specific T cells and improve normal hematopoiesis, but cytogenetic abnormalities and dysplastic features often persist [9,11,12]. However, in this study one patient showed the disappearance of karyotypic abnormalities. In addition, three of the responders were able to successfully discontinue IST. These results might be explained by the findings that the residual healthy stem cells can compensate for the loss of stem cells after the immune-mediated destruction is interrupted by IST in the setting of aplastic anemia [18,19]. Recovery of healthy hematopoiesis might outstrip MDS clones in these patients. In the patient with monosomy 7 who experienced cytogenetic response another mechanism could be speculated. The investigators from the EWOG-MDS reported that almost half of children with RA had monosomy 7 and they were likely to experience disease progression [3]. In contrast, anecdotal case reports described a decline or disappearance of a monosomy 7 clone [20]. Sloand et al. [21] reported paradoxical responses of monosomy 7 cells to G-CSF. Namely, high concentrations of G-CSF induced significant proliferation of monosomy 7 cells, but survival and proliferation of monosomy 7 cells were inferior to those of diploid cells at lower G-CSF levels. Thus, there is a possibility that the recovery of normal hematopoiesis after the administration of IST might affect the intrinsic level of G-CSF and survival of monosomy 7 cells. However, the interpretation of the present results still needs caution because most patients with RA and monosomy 7, including another case in this study, showed poor prognosis.

Previous studies on IST in adult RA found some factors that could predict good responders to IST, such as younger age, shorter duration of transfusion dependence, HLA-DR15, and presence of an expanded clone of PNH cells [8,10–12]. In this study, age was the only factor that showed a statistically significant difference between responders and non-responders to IST. The European study published by Yoshimi et al. [13] also contained older patients, but the proportion and treatment responses of older patients were not shown. Therefore, the effects of patient age on pathophysiology of pediatric RA and treatment response remain to be elucidated. Of the limited cases who were examined, no patient showed an expansion of PNH clone and only one patient had HLA-DR2 antigen, who responded to IST well. We did not systemically examine the immunological status such as TCR Vbeta repertoire [7] in this study. Clinical trials, including systematic studies on immunological status, are required to investigate prognostic factors more precisely in childhood RA because the sample size in this study was small.

Thus, a significant drawback of our study was small size of registered patients. We assumed that considerable number of patients with RA did not enter this study and might have received HSCT without IST. In fact, retrospective analysis of pediatric MDS in Japan showed that 52 patients with RA were diagnosed by the central morphological review between 1999 and 2006 [22]. Consecutive enrollment on both diagnostic and therapeutic trials would be essential for a future trial. It might allow the determination of biologic parameters that correlated with clinical characteristics.

In conclusion, the present results suggest the efficacy and safety of IST for children with RA. Disease-free status might be expected with IST in a subset of patients. Chromosomal aberration was not an absolute contraindication for IST, whereas using this approach for patients with monosomy 7 has not been substantiated. A larger prospective study including biological surrogate markers for therapeutic interventions would be important to elucidate the clinical characteristics of this rare disease as well as the prognostic factors and mechanism of IST.

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REFERENCES

1. Luna-Fineman S, Shannon KM, Atwater SK, et al. Myelodysplastic and myeloproliferative disorders of childhood: A study of 167 patients. Blood 1999;93:459–466.
2. Sasaki H, Manabe A, Kojima S, et al. MDS Committee of the Japanese Society of Pediatric Hematology, Japan. Myelodysplastic syndrome in childhood: A retrospective study of 189 patients in Japan. Leukemia 2001;15:1713–1720.

3. Kardos G, Baumann I, Passmore SJ, et al. Refractory anemia in childhood: A retrospective analysis of 67 patients with particular reference to monosomy 7. Blood 2003;102:1997–2003.
4. Hasle H, Niemeyer CM, Chessells JM, et al. A pediatric approach to the WHO classification of myelodysplastic and myeloproliferative diseases. Leukemia 2003;17:277–282.
5. Selleri C, Maciejewski JP, Catalano L, et al. Effects of cyclosporine on hematopoietic and immune functions in patients with hypoplastic myelodysplasia: In vitro and in vivo studies. Cancer 2002;95:1911–1922.
6. Sloand EM, Mainwaring L, Fuhrer M, et al. Preferential suppression of trisomy 8 compared with normal hematopoietic cell growth by autologous lymphocytes in patients with trisomy 8 myelodysplastic syndrome. Blood 2005;106:841–851.
7. de Vries AC, Langerak AW, Verhaaf B, et al. T-cell receptor Vbeta CDR3 oligoclonality frequently occurs in childhood refractory cytopenia (MDS-RC) and severe aplastic anemia. Leukemia 2008;22:1170–1174.
8. Molldrem JJ, Caples M, Mavroudis D, et al. Antithymocyte globulin for patients with myelodysplastic syndrome. Br J Haematol 1997;99:699–705.
9. Jonásova A, Neuwirtová R, Cermák J, et al. Cyclosporin A therapy in hypoplastic MDS patients and certain refractory anaemias without hypoplastic bone marrow. Br J Haematol 1998;100:304–309.
10. Sauntharajah Y, Nakamura R, Nam JM, et al. HLA-DR15 (DR2) is overrepresented in myelodysplastic syndrome and aplastic anemia and predicts a response to immunosuppression in myelodysplastic syndrome. Blood 2002;100:1570–1574.
11. Shimamoto T, Tohyama K, Okamoto T, et al. Cyclosporin A therapy for patients with myelodysplastic syndrome: Multicenter pilot studies in Japan. Leuk Res 2003;27:783–788.
12. Stadler M, Germing U, Kliche KO, et al. A prospective, randomised, phase II study of horse antithymocyte globulin vs rabbit antithymocyte globulin as immune-modulating therapy in patients with low-risk myelodysplastic syndromes. Leukemia 2004;18:460–465.
13. Yoshimi A, Baumann I, Führer M, et al. Immunosuppressive therapy with anti-thymocyte globulin and cyclosporine A in selected children with hypoplastic refractory cytopenia. Haematologica 2007;92:397–400.
14. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 1982;51:189–199.
15. Manabe A, Nakahata T. Experiences on MDS and JMML from Japan. In: Lopes LF, Hasle H, editors. Myelodysplastic and myeloproliferative disorders in children. Sao Paulo, Brazil: Lemar Livraria; 2003. pp. 317–324.
16. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002;100:2292–2302.
17. Nagasawa M, Tomizawa D, Tsuji Y, et al. Pancytopenia presenting with monosomy 7 which disappeared after immunosuppressive therapy. Leuk Res 2004;28:315–319.
18. Maciejewski JP, Kim S, Sloand E, et al. Sustained long-term hematologic recovery despite a marked quantitative defect in the stem cell compartment of patients with aplastic anemia after immunosuppressive therapy. Am J Hematol 2000;65:123–131.
19. Führer M, Rampf U, Baumann I, et al. Immunosuppressive therapy for aplastic anemia in children: A more severe disease predicts better survival. Blood 2005;106:2102–2104.
20. Mantadakis E, Shannon KM, Singer DA, et al. Transient monosomy 7: A case series in children and review of the literature. Cancer 1999;85:2655–2661.
21. Sloand EM, Yong AS, Ramkissoon S, et al. Granulocyte colony-stimulating factor preferentially stimulates proliferation of monosomy 7 cells bearing the isoform IV receptor. Proc Natl Acad Sci USA 2006;103:14483–14488.
22. Hirabayashi S, Manabe A, Watanabe S, et al. Myelodysplastic syndrome (MDS) and myeloproliferative disease (MPD) in children: A prospective registration of 222 cases. Blood (ASH Annu Meet Abstr) 2008;112:2678.

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CASE REPORT

A male patient with malignant lymphoma and thyroid papillary carcinoma after pediatric renal transplantation

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Abstract A 6-year-old boy received renal transplantation and was treated with methylprednisolone, cyclosporine A and mizoribine. He developed Epstein–Barr virus-associated malignant lymphoma at 10 years and thyroid papillary carcinoma at 20 years of age. Chemotherapy for the malignant lymphoma was done after withdrawal of cyclosporine A and mizoribine, and thyroidectomy was performed for thyroid carcinoma. He was well and his serum creatinine was 1.0 mg/dl at 22 years of age. To our knowledge, no pediatric renal transplant recipient who had thyroid carcinoma or two different types of tumor has been reported in Japan.

Keywords Renal transplantation · Child · Epstein–Barr virus · Malignant lymphoma · Thyroid papillary carcinoma

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Introduction

The frequency of malignancies is higher in patients on dialysis and after renal transplantation than that in the general population. Immunosuppression after renal transplantation increases the risk of cancer as well as infection [1–3]. Patient and graft survival rates of pediatric renal transplantation have improved after use of calcineurin inhibitors, mizoribine, basiliximab, etc. The occurrence of malignancies increases with follow-up time after transplantation and with age.

Case report

A 6-year-old boy presented with convulsions. His blood pressure was 150/100 mmHg and serum creatinine was 7.2 mg/dl. Chronic renal failure due to multicystic dysplastic kidney was diagnosed. He had left internal strabismus, delayed development (intelligence quotient of 62) and chromosomal abnormality with 46, XY, add (13) (p13). His parents and younger brother were healthy. He received renal transplantation from his father after 6 months of continuous ambulatory peritoneal dialysis. At the time of transplantation, the serum IgG titer against Epstein–Barr virus (EBV) viral capsid antigen (VCA) of 1:640 and EBV nuclear antigen (EBNA) of 1:10 were positive, and serum IgG against cytomegalovirus (CMV) was negative. Serum CMV-IgG of the donor was positive and that of the recipient became positive 1 year after transplantation. His serum intact parathyroid hormone level was normal. He was treated with methylprednisolone, cyclosporine A (CyA) and mizoribine after transplantation. There was no rejection episode. Methylprednisolone was withdrawn 1 year after

transplantation, when he had a traumatic right forearm bone fracture.

He developed fever and hematochezia 3 years after transplantation when he was 10 years old. Abdominal tumor, anemia (hemoglobin 7.4 g/dl) and elevated serum level of lactate dehydrogenase (1,441 U/l: normal 216–383) were noted. Serum EBV-VCA IgG titer was 1:5,120 and EBNA was 1:10. Computed tomography of the abdomen showed a node of 10-cm diameter in the left hepatic lobe and two nodules posterior to the gastro-duodenum. Endoscopy showed sub-mucosal tumors in the gastro-duodenum, and gastric biopsy was done. Diffuse monoclonal immunoglobulin lambda chain was stained, but the size of B-cells was not evaluated because of diffuse necrosis of the specimen. The presence of EBV was documented by the EBV encoded small RNA (EBER-1 gene) using the in situ hybridization method. EBV latent membrane proteins were negative. EBV-associated malignant lymphoma (diffuse B-cell type) was diagnosed. There was no central nervous system or bone marrow involvement, and the stage of the lymphoma was 3 according to the Murphy staging system [4]. CyA of 250 mg/day (trough level 80 ng/ml) and mizoribine of 50 mg/day (41.7 mg/m^2) were withdrawn. The tumor reduced in size after 4 months of chemotherapy with prednisolone (60 mg/m^2/day for 6 weeks), cyclophosphamide (1.2 g/m^2, once), vincristine (1.5 mg/m^2, 10 times), etoposide (100 mg/m^2, 4 times) and doxorubicin (30 mg/m^2, once). Radiation therapy was not performed.

When he was 20 years old, struma was noted. Ultrasound examination of the thyroid showed low echoic masses in both lobes. Intranuclear cytoplasmic inclusions and nuclear grooves were detected by fine-needle aspiration of the struma. Total thyroidectomy and resection of regional lymph nodes was performed at another institution. The tumors were 2 cm in diameter in the right lobe and 1 cm in diameter in the left lobe. There was no metastasis to regional lymph nodes or invasion outside the thyroid gland. He was diagnosed as having low-risk papillary thyroid carcinoma.

The immunosuppressive therapy for the graft with methylprednisolone of 20 mg/day was restarted after cessation of prednisolone for the lymphoma, and the methylprednisolone was gradually reduced. Methylprednisolone of 4 mg/day was continued after chemotherapy for the lymphoma, and azathioprine (1 mg/kg/day) was added 6 months after onset of the lymphoma. Azathioprine was discontinued because of gout, and methylprednisolone alone at 8 mg/day was continued after 20 years of age. At 22 years, his serum creatinine was 1.0 mg/dl. The course of the immunosuppressive therapy and graft function is summarized in Table 1. The serum creatinine level rose with growth. His body height was 125 cm at the renal transplantation and 170 cm at 22 years of age. There were no signs of recurrence of lymphoma or thyroid tumor. Serum EBV-VCA IgG titer was 1:320, and EBNA became negative after the chemotherapy for the lymphoma. EBV-DNA in the peripheral blood leukocytes was normal (<2.0 × 10/10^6 leukocytes).

Table 1 Course of immunosuppressive therapy and graft function in a patient with malignant lymphoma and thyroid carcinoma after renal transplantation

Age (years)	Immunosuppressive therapy for the graft	Serum creatinine (mg/dl)
Renal transplantation		
6	MP, CyA, MZ	0.5
7–9	CyA, MZ	0.8
Malignant lymphoma		
10	MP	0.8
11–19	MP, AZA	1.0
Thyroid carcinoma		
20	MP	1.0
22	MP	1.0

MP Methylprednisolone, *CyA* cyclosporine A, *MZ* mizoribine, *AZA* azathioprine

Discussion

The incidence of malignancies in patients after renal transplantation is higher than that in the general population [1–3]. Sixty-three children under 15 years of age received renal transplantation between 1986 and 2006 at Toho University Omori Hospital. Transplantation was performed after dialysis for 1.9 ± 2.5 (0.2–14.9) years in 53 patients (peritoneal dialysis 41, hemodialysis 12) and preemptively in 10. They received transplantation at a mean age of 8.5 ± 4.2 (0.8–15.3) years and were followed up for 8.6 ± 5.2 (0.1–22.5) years after transplantation. Forty-eight patients were treated with a CyA-based regimen and 15 with a tacrolimus-based regimen [5]. Basiliximab was administered at the time of transplantation in 22 patients, and OKT3 monoclonal antibody was given for severe steroid-resistant rejections in 2. There were no other patients with malignancy occurring after renal transplantation. Some have reported that malignancies occurred in 0.8–1.3% of children after renal transplantation [3].

Posttransplant lymphoproliferative disorder (PTLD) is an unregulated expansion of lymphoid cells in patients receiving immunosuppressive therapy after solid organ and hematopoietic stem cell transplantation. Its spectrum ranges from polyclonal hyperplasia to monoclonal malignant lymphoma. It occurs in 2% of renal transplant recipients. The incidence increases according to the strength of the

immunosuppressive therapy [2]. In most cases, the disorder results from EBV-induced B-cell proliferation because of suppression of the T-cell system. PTLD is more common in children than in adults, especially in EBV seronegative recipients receiving grafts from EBV seropositive donors [6].

Of 200 recipients who underwent renal transplantation under 18 years of age in Cincinnati between 1968 and 1993 and developed malignancies, carcinomas of the thyroid gland occurred in 7 patients (3%) [1]. The tumors were diagnosed at an average age of 23 years (range 16–35 years) and 10 years (range 4–18 years) after transplantation. The tumors were papillary carcinoma in six patients and an unclassified carcinoma in one. No one died of the tumor during an average of 3 years (range 0.5–7 years) of follow-up. The association of tumors and their incidence after renal transplantation varies geographically. It may reflect environmental factors and genetic susceptibility. According to the survey of 149 institutions in Japan, 13 of 204 patients with malignancies after renal transplantation (6%) had thyroid carcinoma [2]. The incidence of thyroid carcinoma in Japan may be higher than in other countries.

The relationship among chromosomal abnormality, chronic renal failure, malignant lymphoma and thyroid carcinoma was discussed. Genetic factors including genetic mutations are known to be related to malignancies, but a chromosomal abnormality of the patient has not been reported to correlate with malignancies. Papillary microcarcinoma of the thyroid of less than 1 cm in size associated with secondary hyperparathyroidism due to chronic renal failure has been reported. The incidence was 4 of 124 patients (3.2%) who underwent parathyroidectomy [7]. Serum intact parathyroid hormone levels were normal before and after transplantation in our patient. Thyroid cancer accounts for about 10% of second malignancies among survivors of cancer because of treatment with radiation and alkylating agents, such as cyclophosphamide [8]. Mike et al. [9] reported that 2 of 1,051 children with non-Hodgkin lymphoma (0.2%) who had been followed up for at least 10 years developed second malignant neoplasms of the thyroid. According to a report by Olsen et al. [10], 11 of 3,711 patients with lymphoma (0.3%) who had been diagnosed before the age of 20 years between 1943 and 1987 had a second thyroid carcinoma. Chemotherapy including cyclophosphamide was used in our patient. Second malignancy after PTLD has not been reported.

The incidence of malignancies may increase when the number of pediatric transplantations increases and the patient and graft survival improves. Malignancies after renal transplantation are often progressive, and reduction of immunosuppressive drugs results in subsequent rejection and loss of the graft. Prompt diagnosis and appropriate treatment improve the outcome. The thyroid gland should be checked regularly in renal transplant recipients, especially after radiotherapy and chemotherapy for malignancies. In the report by Penn, 6 of 200 pediatric renal transplant recipients with malignancies had two different types of tumor and 1 had three types [1].

References

1. Penn I. Malignancy in children. In: Tejani AH, Fine RN, editors. Pediatric renal transplantation. New York: Wiley; 1994. p. 461–70.
2. Sugitani A. Malignant tumor after renal transplantation (in Japanese). Jpn J Nephrol. 2005;47:430–6.
3. Wingen AM, Wiesel M, Möhring K, Schärer K, Mehls O. Malignancies in children with renal replacement therapy. Transplant Proc. 1994;26:5–6.
4. Dror Y, Greenberg M, Taylor G, Superina R, Hebert D, West L, et al. Lymphoproliferative disorders after organ transplantation in children. Transplantation. 1999;67:990–8.
5. Motoyama O, Hasegawa A, Aikawa A, Arai K, Ohara T. Clinical management and outcome of preemptive renal transplantation in children and adolescents. Clin Exp Nephrol. 2002;6:237–41.
6. Shroff R, Rees L. The post-transplant lymphoproliferative disorder, a literature review. Pediatr Nephrol. 2004;19:369–77.
7. Burmeister LA, Sandberg M, Carty SE, Watson CG. Thyroid carcinoma found at parathyroidectomy. Association with primary, secondary, and tertiary hyperparathyroidism. Cancer. 1997;79:1611–6.
8. Herzog CE. Rare tumors. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF, editors. Nelson textbook of pediatrics. 18th ed. Philadelphia: Saunders; 2007. p. 2157–9.
9. Mike V, Meadows AT, D'Angio GJ. Incidence of second malignant neoplasms in children: results of an international study. Lancet. 1982;2:1326–31.
10. Olsen JH, Garwicz S, Hertz H, Jonmundsson G, Langmark F, Lanning M, et al. Second malignant neoplasms after cancer in childhood or adolescence. Br Med J. 1993;307:1030–6.

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ORIGINAL ARTICLE

The ex vivo production of ammonia predicts L-asparaginase biological activity in children with acute lymphoblastic leukemia

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Abstract Patients with acute lymphoblastic leukemia (ALL), who develop antiasparaginase antibodies without clinical allergic reactions ("silent inactivation") during L-asparaginase (L-Asp) treatment, have poor outcomes. Ammonia is produced by hydrolysis of asparagine by L-Asp. We postulated that plasma ammonia level might reflect the biological activity of L-Asp. Five children with ALL treated according to the Tokyo Children's Cancer Study Group (TCCSG) protocol were enrolled. Plasma ammonia levels were analyzed immediately and 1 h after incubation at room temperature and "ex vivo ammonia production" was defined as increase in ammonia concentration. Ex vivo ammonia production well correlated with L-Asp activity ($r = 0.882$, $P < 0.01$, $n = 23$). It always exceeded 170 μg/dL (170–345 μg/dL) in induction therapy. We found 3 patients whose ammonia production was negligible during later phases of therapy. Antiasparaginase antibody was detected and L-Asp activity decreased in these patients. Ex vivo ammonia production is a surrogate marker of L-Asp biological activity.

Keywords Asparaginase · Ammonia · Acute lymphoblastic leukemia · Children · Silent inactivation

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1 Introduction

L-Asparaginase (L-Asp) is a key drug in the treatment of childhood acute lymphoblastic leukemia (ALL). Leukemic blast cells are unable to synthesize sufficient amounts of asparagine, and the resulting asparaginase-induced deficiency impairs cellular function and eventually causes cellular death of ALL cells [1].

Because L-Asp is produced by bacteria, antiasparaginase antibodies are frequently induced, which not only causes hypersensitivity reactions (IgE class) but also leads to the inactivation of L-Asp (IgG glass). The frequency of clinical hypersensitivity reactions to *Escherichia coli* (*E. coli*) L-Asp is above 50% [2, 3]. The antiasparaginase antibodies development varied from 30 to 70% of children after *E. coli* L-Asp administration [4, 5], and the appearance of antiasparaginase antibodies correlated with the occurrence of allergic reactions [2, 6]. However, inactivation of L-Asp by asparaginase-specific IgG antibody has not been well understood [7].

Recently, Panosyan et al. [8] showed that patients that produce neutralizing antiasparaginase antibodies but who do not suffer from clinical allergic reactions during native *E. coli* L-Asp treatment have a poorer outcome. This phenomenon is called "silent inactivation". Although

consecutive measurement of antiasparaginase antibody levels and L-Asp activity in real time is necessary to detect the occurrence of silent inactivation, it is not easy to perform in all patients.

Ammonia, which is produced by L-Asp biochemical reactions, can be easily measured in a routine laboratory setting. It is possible that the plasma level of ammonia is closely related to the biological activity of L-Asp. Since ammonia is immediately removed from the liver via its incorporation into the urea cycle, the elevation of ammonia in serum should reflect either the effects of L-Asp administration or hepatic dysfunction. Recently, there was a report that showed that the ammonia concentration increased when the serum of patients receiving L-Asp was incubated ex vivo [9]. In our preliminary experiment, we added L-Asp to the plasma samples of healthy adults and measured the ammonia concentration before and 1 h after incubation. The concentration of L-Asp used ranged from 0.01 to 5 U/mL. The ammonia concentration increased in a dose-dependent manner. In addition, Asn was completely depleted at all doses of L-Asp (Miyake et al., unpublished).

In this study, we investigated the ammonia increase in children with ALL receiving L-Asp and compared it with other parameters such as L-Asp activity and antiasparaginase antibody production. We prospectively assessed the clinical significance of ammonia increases in children with ALL.

2 Patients and methods

2.1 Patients

Five children who had been newly diagnosed with ALL in our institution between May and December of 2007 were enrolled in this study. The patients were treated according to protocol L04-16 of the Tokyo Children's Cancer Study Group (TCCSG). The median age at initial diagnosis was 4 years (range 2–7 years). The study was approved by the institutional review board, and written informed consent was obtained from the patients' guardians. Three patients were placed into the standard-risk group, and 2 patients were placed into the intermediate-risk group based on their age, leukocyte counts at diagnosis, immunophenotype, prednisolone response, and cytogenetics (Table 1). Figure 1 shows an outline of the induction and reinduction therapies given to the two groups. In induction therapy, 6,000 U/m^2 native *E. coli* L-Asp was given as a 1-h intravenous infusion. In reinduction therapy, 10,000 U/m^2 L-Asp was given by intramuscular injection. The chemotherapy regimens were basically identical to those described in the TCCSG L99-15 [10]. The native *E. coli* L-Asp preparation used in this study was Leunase® (Kyowa Hakko Kogyo, Tokyo, Japan). It is known that the L-Asp activity is comparable when L-Asp is administered either by intravenous or by intramuscular [11]. Therefore, we analyzed the data obtained by both administration routes together.

2.2 Blood sampling points

First, we collected blood sample at various time points after the administration of L-Asp to elucidate the relation between L-Asp activity and ammonia production in vitro.

Next, we collected blood samples before and 24 h after L-Asp to demonstrate the fluctuation of L-Asp activity and ammonia production in each patient.

L-Asp activity was measured 24 h after L-Asp administration. The antiasparaginase antibody level was assessed before each phase of therapy.

2.3 Measurement of ammonia concentrations

Plasma ammonia concentrations were analyzed by a standard photometric assay, immediately after the blood collection and, an hour later, were incubated at room temperature (20°C). We defined "ex vivo ammonia production" as the following:

Ex vivo ammonia production = ammonia concentration at 1 h after incubation − ammonia concentration before incubation.

2.4 Measurement of asparagine

The plasma was immediately deproteinized by adding an equal volume of 10% sulfosalicylic acid (SSA) and was

Table 1 Patient characteristics

No.	Sex	Age (years)	WBC/μL of diagnosis	Immunophenotype	Prednisolone response[a]	Risk group
1	F	4	6,100	B-precursor	Good	SR
2	F	3	8,000	B-precursor	Good	SR
3	M	6	5,900	B-precursor	Good	SR
4	M	2	40,700	B-precursor	Good	IR
5	F	7	33,500	B-precursor	Good	IR

WBC white blood cell count

[a] Day 8 blast cells. The cut off is 1,000/μL

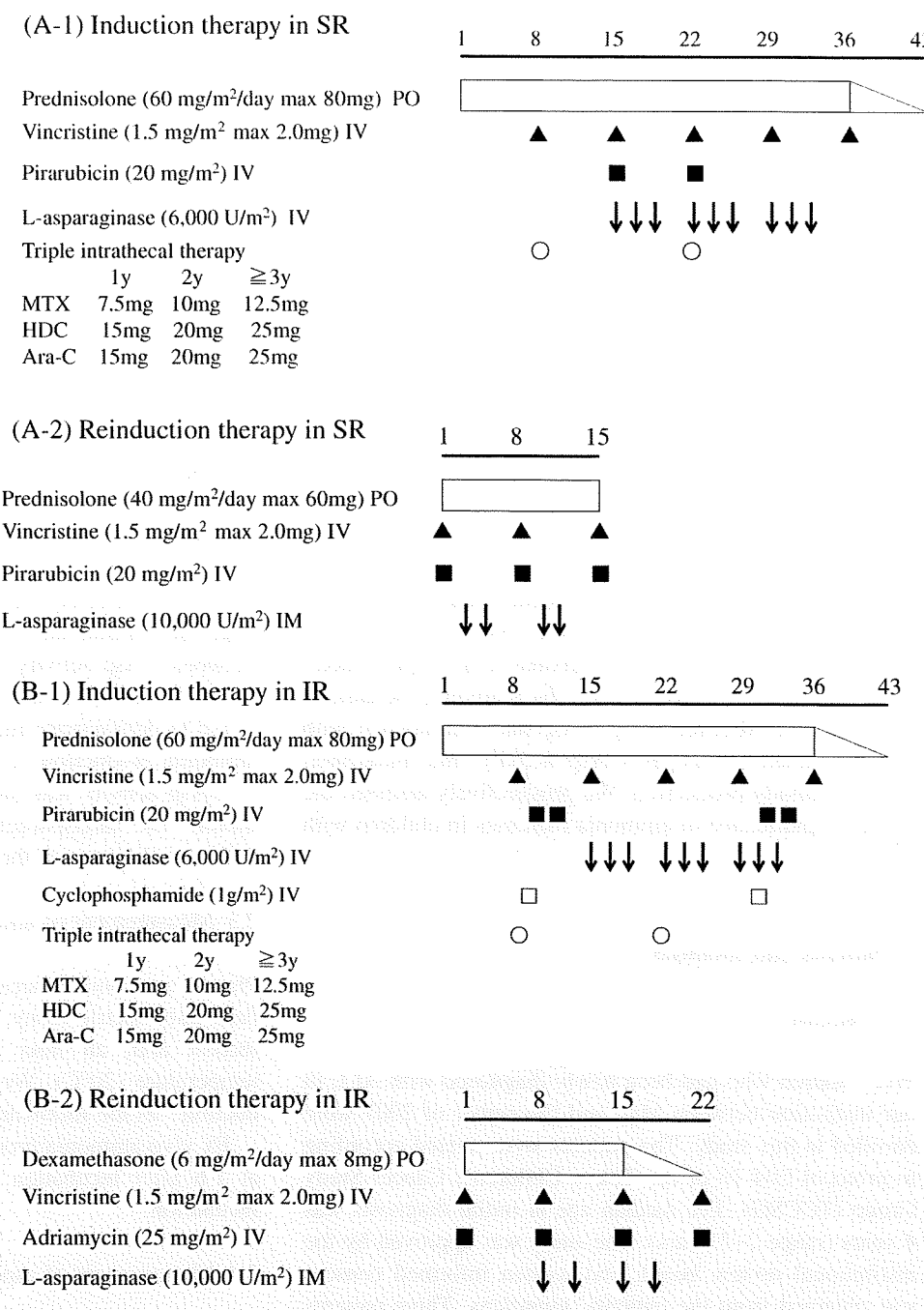

Fig. 1 Outline of induction therapy and reinduction therapy in SR and IR groups

then stored at −80°C until the measurements were performed [12]. The asparagine levels were measured by high-performance liquid chromatography (HPLC) using pre-column derivatization with *o*-phthalaldehyde. The lower detection limit of this method was 0.2 μmol/L.

2.5 Measurement of L-Asp activity

L-Asp activity was measured by the enzyme coupling methods described by Tsurusawa et al. [13]. The detection limit of this method was 0.002 U/mL.

2.6 Measurement of antiasparaginase IgG antibody

Antiasparaginase IgG antibody was measured by an enzyme-linked immunoadsorbent assay (ELISA), according to the method described by Tsukimoto et al. [7]. The lower detection limit of this method was 4 U/mL.

2.7 Statistical analysis

The correlation between ex vivo ammonia production and L-Asp activity was measured by Spearman's rank

correlation test. SPSS statistical analysis software (SPSS 11.5 J) was used to do this.

3 Results

3.1 Ex vivo ammonia production correlates with L-Asp biological activity

We prospectively enrolled 5 children with ALL in our study and tested 23 samples at various time points after L-Asp administration. Ex vivo ammonia production correlated well with L-Asp activity according to Spearman's rank correlation test ($r = 0.882$, $P < 0.01$, $n = 23$) (Fig. 2).

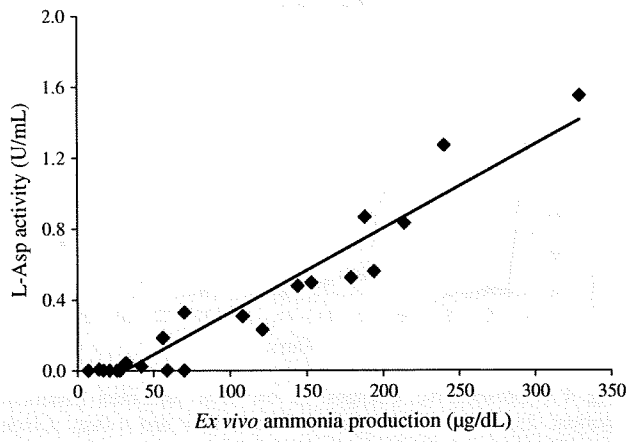

Fig. 2 The correlation between ex vivo ammonia production and L-Asp activity. Ex vivo ammonia production correlated with activity according to Spearman's rank correlation test ($r = 0.882$, $P < 0.01$, 23 samples)

3.2 Longitudinal changes of ex vivo ammonia production and L-Asp activity in individual patients

The ex vivo ammonia production in the 5 patients always exceeded 170 μg/dL (median 230 μg/dL, range 170–345 μg/dL) after 24 h of native *E. coli* L-Asp administration in the induction phase of treatment (Table 2). The ammonia production level of 170 μg/dL correlates well the L-Asp activity of 0.7 U/mL, which exceeds the reported effective dose of 0.4 U/mL (Fig. 2) [14].

Figure 3 shows representative changes in ex vivo ammonia production, L-Asp activity, and Asn levels during the reinduction phase of treatment (patient 1). The patient did not have measurable antibody levels before the reinduction phase of treatment, and no allergic reactions were observed. Ex vivo ammonia production correlated with L-Asp activity and was inversely related to Asn concentration after each administration of L-Asp. The parameter changes in patient 4 were similar to those in patient 1 (data not shown).

Figure 4 shows the changes in ex vivo ammonia production, L-Asp activity, Asn level, and antiasparaginase antibody level in patient 2. No ex vivo ammonia production was observed after the first and third doses of L-Asp. The antiasparaginase antibody levels increased during L-Asp treatment. There were no hypersensitivity reactions in this patient. Interestingly, ex vivo ammonia production was reinstated after the second and fourth doses of L-Asp. This suggests that the antiasparaginase antibodies had become saturated with L-Asp. The clinical course in patient 3 was similar to that in patient 2. He also produced antibodies but did not suffer from allergic reactions. Ex vivo ammonia production was also reduced in this patient. We hypothesized that "silent inactivation" had occurred in patients 2 and 3. In patient 5, ex vivo ammonia production was suboptimal after the third and fourth doses of L-Asp,

Table 2 The ex vivo ammonia production after 24 h of L-Asp treatment and antiasparaginase antibody during induction and reinduction therapy

No.	Induction therapy[a]			Reinduction therapy[b]				Antiasparaginase antibody[c]
	1	2	3	1	2	3	4	
Ex vivo ammonia production (μg/dL)								
1	252	216	170	194	329	188	240	<1
2	211	345	252	21	144	32	179	188
3	197	320	230	14	121	70	216	130
4	212	172	262	260	197	160	229	<1
5	225	239	261	165	204	35	85	27

[a] The samples of ex vivo ammonia production in induction therapy were collected after every third dose of L-Asp

[b] The samples of ex vivo ammonia production in reinduction therapy were collected after each administration of L-Asp

[c] Antiasparaginase antibody was examined before the initiation of reinduction therapy

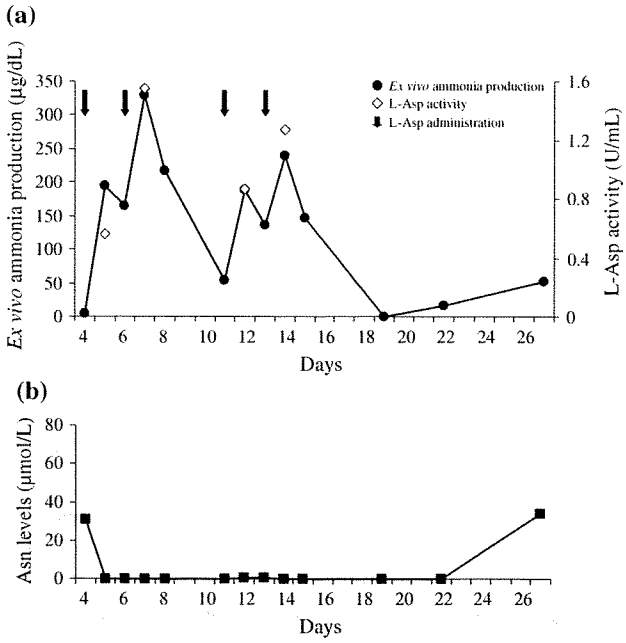

Fig. 3 Representative changes in ex vivo ammonia production, L-Asp activity, and Asn levels (patient 1). **a** Ex vivo ammonia production and L-Asp activity. **b** Asn levels

while L-Asp biological activity was undetectable after the third dose and was not available after the fourth dose. In fact, this patient had an intermediate level of antibody before the reinduction phase. The patient did not develop an allergic reaction (data not shown).

4 Discussion

L-Asp is an essential component of the standard treatment regimens for ALL. The unique mechanism of L-Asp is that the drug depletes the Asn in the serum. Its mechanism is different from that of any other cytotoxic drug. Unfortunately, one of the side effects of L-Asp treatment is the development of antiasparaginase antibodies, which often means that therapy cannot be completed. It was reported that patients that produce antiasparaginase antibodies have lower event-free survival (EFS) as well as overall survival (OS) in comparison with children that do not produce the antibodies [15]. However, patients that suffer from allergic reactions could still have a comparable prognosis, if another preparation of asparaginase was used instead [5].

Recent reports suggested that patients that produce antiasparaginase antibodies but do not suffer clinical allergic reactions have poor outcomes [8]. Silent inactivation may be detected by serial monitoring of the biological activity of L-Asp; however, this method is cumbersome. During the induction phase of treatment, the ex vivo ammonia production of all 5 patients exceeded 100 μg/dL.

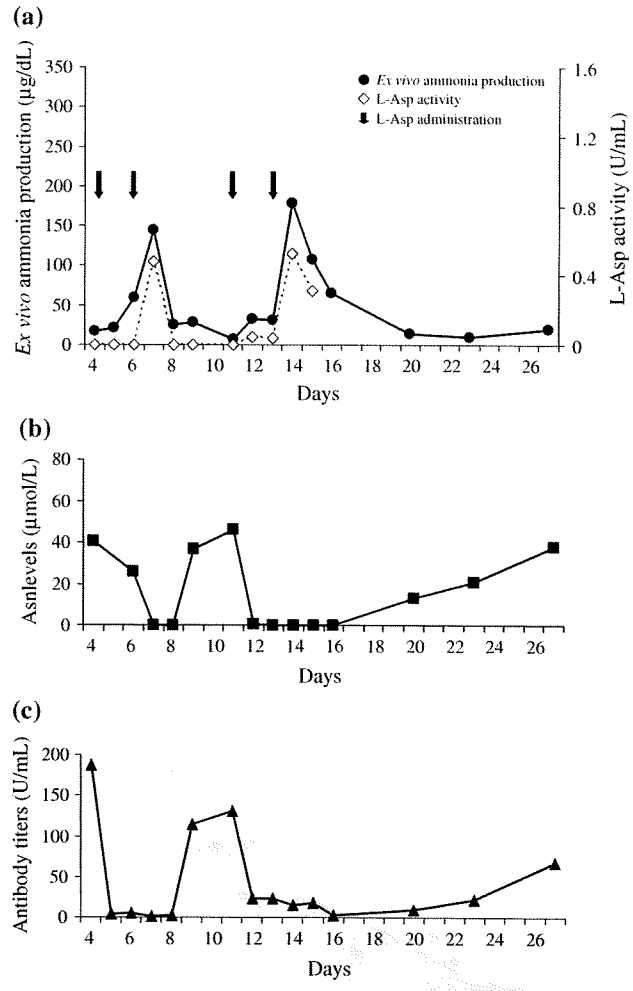

Fig. 4 Representative changes in ex vivo ammonia production (patient 2). **a** Ex vivo ammonia production and L-Asp activity. **b** Asn levels. **c** Antibody titers

However, in the reinduction phase of treatment, the ex vivo ammonia production of 3 patients was below 100 μg/dL, and antiasparaginase antibodies were detected in these patients. Recently, Patel et al. reported that a proportion of patients with ALL possess blasts with a high asparaginyl endopeptidase (AEP) expression level. AEP is known to cleave *E. coli* L-Asp [16]. Therefore, it is necessary to prospectively monitor the efficacy of L-Asp treatment.

Steiner et al. [17] reported that ammonia levels were elevated 1 day after intravenous native *E. coli* L-Asp administration. Their observation was in our line but they did not study other important parameters such as the activity of the drug or antibody production. We systematically examined the ex vivo ammonia production, Asn level, L-Asp activity, and antiasparaginase antibody level during treatment for ALL. We defined "ex vivo ammonia production" as the increase in the ammonia concentration 1 h after incubation. In this study, we could distinguish the

increase in ammonia by L-Asp administration from hepatic failure.

Ex vivo ammonia production was correlated with L-Asp activity ($r = 0.882$, $P < 0.01$; Fig. 2). On the other hand, the ammonia concentration immediately after blood sampling and L-Asp activity were more weakly correlated ($r = 0.627$, $P < 0.01$, data not shown). In fact, the longitudinal change of ex vivo ammonia production was correlated with L-Asp biological activity and was inversely correlated with Asn level in patients with ALL.

We decided that it would be interesting to observe ex vivo ammonia production after L-Asp administration using *Erwinia chrysanthemi* (*Erwinia*) instead of native *E. coli* L-Asp, and so we investigated it in two patients who were given 10,000 U/m^2 of *Erwinia* L-Asp. The result was that ex vivo ammonia production was observed in response to *Erwinia* L-Asp treatment always exceeded 361 μg/dL (median 395 μg/dL, range 361–480 μg/dL, 7 samples). Thus, ex vivo ammonia production can also be used to monitor the biological effects of *Erwinia* L-Asp.

In patients 2 and 3, additional administration of native *E. coli* L-Asp reinstated the increase in ammonia production, suggesting that saturation of the antiasparaginase antibodies with excessive L-Asp had occurred. Currently, utilizing another preparation of asparaginase, that is, *Erwinia* L-Asp, may be the only way to manage silent inactivation. One might introduce an additional dose of native *E. coli* L-Asp to overcome the neutralization of asparaginase by antibodies. Ex vivo ammonia production should be measured before considering a switch to *Erwinia* L-Asp.

In conclusion, measurement of ex vivo ammonia production is a simple method that predicts in vivo L-Asp activity. It can be used to easily identify patients that are suffering from silent inactivation of L-Asp and suggest a suitable treatment.

References

1. Muller HJ, Boos J. Use f L-asparaginase in childhood ALL. Crit Rev Oncol Hematol. 1998;28:97–113.
2. Woo MH, Hak LJ, Storm MC, Evans WE, SandLund JT, Rivera GK, et al. Anti-asparaginase antibodies following *E. coli* asparaginase therapy in pediatric acute lymphoblastic leukemia. Leukemia. 1998;12:1527–33.
3. Muller HJ, Beier R, Loning L, Bulteers-Sawatzki R, Dorffel W, Maass E, et al. Pharmacokinetics of native *Escherichia coli* asparaginase (Asparaginase medac) and hypersensitivity reactions in ALL-BFM 95 reinduction treatment. Br J Haematol. 2001;114:794–9.
4. Oettgen HF, Stephenson PA, Schwartz MK, et al. Toxicity of *E. coli* L-asparaginase in man. Cancer. 1970;25:253–78.
5. Woo MH, Hak LJ, Storm MC, SandLund JT, Ribeiro RC, Rivera GK, et al. Hypersensitivity or development of antibodies to asparagines does not impact treatment outcome of childhood acute lymphoblastic leukemia. J Clin Oncol. 2000;18:1525–32.
6. Cheung N-KV, Chau IY, Coccia PF. Antibody response to *Escherichia coli* L-asparaginase: prognostic significance and clinical utility of antibody measurement. Am J Pediatr Hematol Oncol. 1986;8:99–104.
7. Tsukimoto I, Matsui J, Iwashita H, Shigeta K, Suzuki H, Hashimoto T. Improved measurements of anti-L-asparaginase IgG antibody and its clinical applications. Jpn J Clin Hematol. 1992;33:9–24. (in Japanese).
8. Panosyan EH, et al. Asparaginase antibody and asparaginase activity in children with high-risk acute lymphoblastic leukemia children's cancer group study CCG-1961. J Pediatr Hematol Oncol. 2004;26:217–26.
9. Laterza OF, Gerhardt G, Sokoll LJ. Measurement of plasma ammonia is affected in patients receiving asparaginase therapy. Clin Chem. 2003;49:1710–1.
10. Manabe A, Ohara A, Hasegawa D, Koh K, Saito T, Kiyokawa N, et al. Significance of the complete clearance of peripheral blast after 7 days of prednisolone treatment in children with acute lymphoblastic leukemia: the Tokyo Children's Cancer Study Group Study L99-15. Haematologica. 2008;93:1155–60.
11. Rizzari C, Zucchetti M, Conter V, Diomede L, Bruno A, Gavazzi L, et al. L-Asparagine depletion and L-asparaginase activity in children with acute lymphoblastic leukemia receiving i.m. or i.v. *Erwinia* C. or *E. coli* L-asparaginase as first exposure. Ann Oncol. 2000;11:189–93.
12. Gentili D, Zucchetti M, Conter V, Masera G, D'Incalci M. Determination of L-asparagine in biological samples in the presence of L-asparaginase. J Chromatogr B Biomed Appl. 1994;657:47–52.
13. Tsurusawa M, Chin M, Iwai A, Nomura K, Maeda H, Taga T, et al. L-Asparagine depletion levels and L-asparaginase activity in plasma of children with acute lymphoblastic leukemia under asparaginase treatment. Cancer Chemother Pharmacol. 2004;53:204–8.
14. Avrramis VI, Panosyan EH. Pharmacokinetic/pharmacodynamic relationships of asparaginase formulations. Clin Pharmacokinet. 2005;44:367–93.
15. Zalewska-szewczyk B, Andrzejewski W, Mlynarski W, Jedrychowska-danska K, Witas H, Bodalski J. The anti-asparagines antibodies correlate with L-asparaginase activity and may affect outcome of childhood acute lymphoblastic leukemia. Leukemia Lymphoma. 2007;48:931–6.
16. Patel N, Krishnan S, Offman MN, Krol M, Moss CX, Leighton C, et al. A dyad of lymphoblastic lysosomal cysteine proteases degrades the antileukemic drug L-asparaginase. J Clin Invest. 2009;119:1964–73.
17. Steiner M, Attarbaschi A, Kastner U, Dworzak M, Haas OA, Gadner H, et al. Distinct fluctuations of ammonia levels during asparaginase therapy for childhood acute leukemia. Pediatr Blood Cancer. 2007;15:640–2.

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GUIDELINE

Guidelines for safety management of granulocyte transfusion in Japan

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Abstract Granulocyte transfusion (GTX) has recently been revived by the ability to stimulate granulocyte donors with granulocyte colony-stimulating factor (G-CSF), resulting in a greatly increased number of cells that can be collected. However, there is a paucity of guidelines for assessing the appropriateness and safety management of GTX. The objective of this study was to establish guidelines for the safety management of GTX appropriate for the clinical situation in Japan. The Japan Society of Transfusion Medicine and Cell Therapy, Granulocyte Transfusion Task Force issued the first version of guidelines for GTX considering the safety management of both granulocyte donors and patients who receive GTX therapy. The current guidelines cover issues concerning: (1) the appropriateness of medical institutions, (2) management of granulocyte donors, (3) quality assurance of granulocyte concentrates, (4) administration of granulocyte concentrates, (5) evaluation of the effectiveness of GTX therapy, and (6) complications of GTX therapy. The simple 'bag separation method' without apheresis may be recommended for granulocyte collection in pediatric patients. The first version of guidelines for GTX therapy has been

The Japan Society of Transfusion Medicine and Cell Therapy, Granulocyte Transfusion Task Force.

Although the recommendation and information are believed to be true and accurate at the time of preparation of the guidelines, neither the authors nor the Japan Society of Transfusion Medicine and Cell Therapy accept any legal responsibility for the content of current guidelines.

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established, which may be appropriate for the clinical situation in Japan. Care should be taken to perform the safety management of both granulocyte donors and patients who receive GTX therapy.

Keywords Granulocyte transfusion · Guidelines · Granulocyte colony-stimulating factor · Safety management

1 Introduction

Neutrophils play an essential role in the body's first line of defense against bacterial and fungal infections, and severe neutropenia, defined as an absolute neutrophil count (ANC) of less than 500/μl, is a well-recognized factor predisposing patients to these infections [1]. A direct correlation between the depth and duration of neutropenia and the risk of infection was demonstrated [2]. Because febrile neutropenia (FN), defined as a fever ≥38.3°C (101°F) with severe neutropenia, is associated with potentially life-threatening infection, patients with FN require treatment with broad-spectrum antibiotics as soon as possible without waiting for the results of blood cultures or other studies [3]. In spite of modern antimicrobials and supportive therapy, infections associated with severe neutropenia have been a major cause of morbidity and mortality in patients undergoing aggressive cancer chemotherapy and hematopoietic stem cell transplantation (HSCT) [4]. Granulocyte colony-stimulating factor (G-CSF) stimulates the proliferation of granulocytic precursors, reduces the transit time through the granulocytic compartment, and potently stimulates neutrophil release from the bone marrow [5]. G-CSF also activates neutrophils to enhance their phagocytic function, including respiratory burst activity and surface CD11b/CD18 antigen expression in vitro and in vivo [6, 7]. G-CSF is widely employed in the clinical setting to treat or prevent neutropenia attributable to hematological disorders, myelosuppressive chemotherapy, or HSCT. In addition, the use of G-CSF for the mobilization of peripheral blood progenitor cells (PBSC) has been adopted as an international standard of care [8].

When infections occur in severe neutropenic patients who do not respond to G-CSF therapy, providing the patient with normally functioning neutrophils seems to be logical. Traditional granulocyte transfusion (GTX) therapy showed marginal efficacy, mainly attributable to the inadequacy of the cell dose ordinarily provided [9]. In the G-CSF era, G-CSF stimulation with or without corticosteroids of healthy individuals is well tolerated and allows the collection of large numbers of neutrophils [10, 11]. Although the evidence for the clinical efficacy of GTX therapy is less clear, many single case reports and small cohort studies have been published. The objective of this study was to establish guidelines for GTX therapy considering the safety management of both granulocyte donors and patients, being appropriate for the clinical situation in Japan.

2 Text of the guidelines

2.1 Purpose of the guidelines

This document sets out guidelines specifically addressing the issues regarding GTX therapy, especially the safety management of both granulocyte donors and patients who receive GTX therapy. These guidelines include: (1) the appropriateness of medical institutions, (2) management of granulocyte donors, (3) quality assurance of granulocyte concentrates, (4) administration of granulocyte concentrates, (5) evaluation of the effectiveness of GTX therapy, and (6) complications of GTX therapy.

2.2 Indications for GTX therapy

A good indication for GTX therapy is prolonged 'reversible' neutropenia with an ANC of less than 500/μl, which is refractory to G-CSF therapy and is associated with severe uncontrolled infection (e.g., sepsis including suspicious cases, abscess in the liver or spleen, cellulites, and marrow myelitis). The cause of neutropenia is typically HSCT or aggressive cancer chemotherapy-induced bone marrow failure that is expected to recover. Because the underlying disease process is the main determinant of the outcome in neutropenic patients, the indication for GTX therapy in hematologic disorders may be limited to patients who have received HSCT or aggressive cancer chemotherapy. Patients with congenital neutrophil dysfunction, such as chronic granulomatous disease and leukocyte adhesion deficiency, may also be indicated for GTX therapy when severe uncontrolled infection is accompanied.

2.3 Appropriateness of medical institutions

2.3.1 Transfusion service

Although blood components are administered to patients in most large-scale community and university hospitals in Japan, some hospitals neither have transfusion services nor employ laboratory technologists licensed by the Japan Society of Transfusion Medicine and Cell Therapy. Because granulocyte concentrates are not supplied from branches of the Japanese Red Cross Blood Center (JRCBC), unlike other allogeneic blood components, they need to be collected from granulocyte donors in hospitals. Thus, the hospital where GTX therapy is performed should have a transfusion service or appropriate system, approved

by the hospital transfusion committee, as described below. In particular, the hospital should appoint a professional medical doctor(s) responsible for managing the overall safety of GTX therapy. In the case of granulocyte collection by employing the apheresis method, the hospital is encouraged to employ a professional medical technologist(s) practicing apheresis therapy.

2.3.2 Role of the hospital transfusion committee

Every hospital where GTX therapy is performed should have a multidisciplinary hospital transfusion committee to oversee the provision of safe and appropriate transfusion support. The hospital transfusion committee may comprise doctors and nurses from clinical departments where blood administrations are frequently required, pharmacists, laboratory technologists, as well as representatives of the hospital. The practice of GTX therapy should be approved by the committee.

2.3.3 Area for collection of granulocyte concentrates

Blood collection from granulocyte donors should be carried out in a well-cleaned room, and it is recommended to use a reclining phlebotomy seat. In addition, there should be emergency kits including oxygen inhalation for resuscitation if the conditions of donors deteriorate. As described below, granulocyte concentrates should be irradiated before administration to the patient to prevent transfusion-associated graft-versus-host disease (TA-GVHD). Thus, the hospital should have an exclusive irradiation apparatus or an alternative way to irradiate blood components.

2.4 Management of granulocyte donors

2.4.1 Selection of granulocyte donors

A phase I/II trial of GTX therapy employing donors selected from pools of community apheresis donors has been reported [12]. Because the JRCBC does not participate in the collection of granulocyte concentrates for GTX therapy, granulocyte donors may be selected from family members or friends of the patient undergoing GTX therapy. The current guidelines do not positively recommend non-family members for granulocyte donors at present, unlike allogeneic HSCT.

2.4.2 Age of granulocyte donors

The criteria for granulocyte donor selection should be broadly inline with those used for other blood donations. The age of granulocyte donors should be from 19 to 54 years old, in accordance with the standard for platelet apheresis donors of the JRCBC.

2.4.3 Blood group of granulocyte donors

Granulocyte donors should be ABO- and Rh(D)-compatible with the patient, because a relatively large number of red blood cells (RBCs) are contained in a typical granulocyte concentrate. If the hospital has a transfusion service, where the plasma fraction can be removed from granulocyte concentrates in the case of 'the bag separation method' as described below, granulocyte donors with minor incompatibility may also be selected.

2.4.4 Collection from the same donor

Granulocyte concentrates may be collected from the same donor in the case of a limited number of available granulocyte donors. Granulocyte collection from the same donor should be conducted on two consecutive days in the case of apheresis donation, but repeated collections from the same donor are not prohibited in the presence of an intermission.

2.4.5 Cytomegalovirus (CMV) serology

If the patient is CMV-seronegative, granulocyte donors should also be CMV-seronegative except for life-threatening situations, because most patients who receive GTX therapy are in a patient population that requires CMV-safe components.

2.4.6 Alloimmunization

In the case of alloimmunized patients, granulocyte concentrates may be collected from either HLA-matched donors or donors who are selected by leukoagglutination crossmatching, although the best method to accurately assess donor and leukocyte compatibility has yet to be determined [13]. Considering life-threatening situations, granulocyte concentrates may also be collected from an HLA-mismatched donor for GTX therapy for the patient with anti-HLA antibody.

2.4.7 Medical examinations and laboratory testing

A doctor responsible for GTX therapy should fully interview granulocyte donors regarding episodes of suspected infectious disease transmission and conduct physical examinations before granulocyte collection. The timing of medical examinations may be optimal at the time of G-CSF administration 12–18 h before granulocyte collection. Laboratory tests for granulocyte donors should be as consistent as possible with those for any allogeneic blood

components supplied from branches of the JRCBC, including blood group ABO and Rh(D); serum antibody screening; infectious disease screening of hepatitis B virus (HBs-Ag and Hbc-Ab), hepatitis C virus (HCV-Ab), human immunodeficiency virus (HIV-1/-2-Ab), human T cell lymphotropic virus type I (HTLV-I-Ab), and syphilis (TPHA); complete blood count; and biochemical analysis (e.g., alanine aminotransferase). In the case of infectious disease screening, the current guidelines recommend performing the tests as many as possible in the hospital, although the results of tests will not immediately be obtained.

2.4.8 Informed consent

Informed consent should always be obtained from the granulocyte donor for: (a) granulocyte collection, (b) collection procedures, (c) the administration of G-CSF with or without corticosteroids, (d) use of RBC-sedimenting agents (when employed), and (e) any possible short- and long-term consequences of granulocyte collection. There should always be an opportunity for the donor to reconsider granulocyte donation in the light of a response or lack or response.

2.4.9 Post-donation care

Considering the administration of G-CSF to healthy individuals and its potential long-term adverse effects, as described below, a record of granulocyte donors regarding any post-donation complications should be made. Care of granulocyte donors should include observations in the immediate post-apheresis period to minimize the occurrence of delayed complications (e.g., thrombocytopenia). The current guidelines recommend the establishment of a donor registry to collect the necessary data on short- and long-term side effects of G-CSF administration to normal donors [14, 15]. Comprehensive, prospectively obtained registration data are needed to fully evaluate long-term safety concerns among healthy individuals who receive G-CSF.

2.5 Quality assurance of granulocyte concentrates

2.5.1 Collection of granulocyte concentrates

2.5.1.1 G-CSF For granulocyte mobilization, donors may receive recombinant human G-CSF (non-glycosylated G-CSF [Filgrastim] or glycosylated G-CSF [Lenograstim]) with or without corticosteroid administration. It has been reported that optimal granulocyte mobilization can be achieved in normal donors with a combined regimen of subcutaneous G-CSF at 450 μg and oral dexamethasone (DEX) at 8 mg in a single-dose format designed for clinical GTX therapy [10]. Although the daily administration of G-CSF (e.g., 5 consecutive days) results in higher yields of granulocytes, the current guidelines recommend a single subcutaneous dose of G-CSF (5–10 μg/kg) 12–18 h before each granulocyte collection. As described above, granulocyte collection from the same donor on consecutive days is recommended over 2 days, but repeated collections from the same donor are not prohibited in the presence of an intermission.

2.5.1.2 Corticosteroids To maximize the number of granulocytes obtained, corticosteroids have been administered to mobilize granulocytes from the marrow storage pool and to increase circulating granulocyte counts [10, 11]. Usually, DEX at 8 mg is orally administered once 12 h before granulocyte collection. On frequent collection from the same donor, the medical doctor in charge should monitor the donor regarding corticosteroid-induced adverse events, as discussed below.

2.5.1.3 RBC-sedimenting agent The RBC-sedimenting agent, traditionally hydroxyethyl starch (HES), may be continuously added to the donor's blood during an apheresis procedure to achieve an adequate separation of granulocytes from RBCs. It has been shown that high-molecular weight (MW) HES resulted in a significantly higher yield compared with low-MW HES [16]. However, high-MW HES products have, at present, not been approved in Japan. In the case of using a high-MW HES for granulocyte collection, it should be approved by the Ethics Committee of the hospital.

2.5.2 Methods of granulocyte collection

2.5.2.1 Bag separation method The simple 'bag separation method' without apheresis may be recommended for granulocyte collection in pediatric patients [17]. In brief, whole blood (200 or 400 ml) is drawn into the main bag of a triple-collection bag [200- or 400-ml capacity containing 34 or 68 ml, respectively, of citrate–phosphate–dextrose (CPD) solution] employing the gravity-flow principle. After centrifugation at 640*g* for 15 min at 20°C, the plasma layer is separated into the first sub-bag. The buffy-coat layer and the upper one-third of the RBC layer, both of which are rich in granulocytes, are collected into the second sub-bag by applying pressure on the main bag. The remaining RBC and plasma components are returned to the donor using a sterile-connecting device. This process is repeated two or three times, when necessary. It is noteworthy that the bag separation method does not require the use of an RBC-sedimenting agent, such as high-MW HES, which reduces the burden on the donor [17, 18].