4. Selby PJ, Powles RL, Easton D, et al. The prophylactic role of intravenous and long-term oral acyclovir after allogeneic bone marrow transplantation. Br J Cancer 1989; 59: 434–438.

5. Steer CB, Szer J, Sasadeusz J, Matthews JP, Beresford JA, Grigg A. Varicella-zoster infection after allogeneic bone marrow transplantation: incidence, risk factors and prevention with low-dose aciclovir and ganciclovir. Bone Marrow Transplant 2000; 25: 657–664.

6. Boeckh M, Kim HW, Flowers ME, Meyers JD, Bowden RA. Long-term acyclovir for prevention of varicella zoster virus disease after allogeneic hematopoietic cell transplantation – a randomized double-blind placebo-controlled study. Blood 2006; 107: 1800–1805.

7. Thomson KJ, Hart DP, Banerjee L, Ward KN, Peggs KS, Mackinnon S. The effect of low-dose aciclovir on reactivation of varicella zoster virus after allogeneic haemopoietic stem cell transplantation. Bone Marrow Transplant 2005; 35: 1065–1069.

8. Kanda Y, Mineishi S, Saito T, et al. Long-term low-dose acyclovir against varicella-zoster virus reactivation after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2001; 28: 689–692.

9. Asano-Mori Y, Kanda Y, Oshima K, et al. Long-term ultra-low-dose acyclovir against varicella-zoster virus reactivation after allogeneic hematopoietic stem cell transplantation. Am J Hematol 2008; 83: 472–476.

10. Erard V, Guthrie KA, Varley C, et al. One-year acyclovir prophylaxis for preventing varicella-zoster virus disease after hematopoietic cell transplantation: no evidence of rebound varicella-zoster virus disease after drug discontinuation. Blood 2007; 110: 3071–3077.

11. Weller S, Blum MR, Doucette M, et al. Pharmacokinetics of the acyclovir pro-drug valaciclovir after escalating single- and multiple-dose administration to normal volunteers. Clin Pharmacol Ther 1993; 54: 595–605.

12. Beutner KR, Friedman DJ, Forszpaniak C, Andersen PL, Wood MJ. Valaciclovir compared with acyclovir for improved therapy for herpes zoster in immunocompetent adults. Antimicrob Agents Chemother 1995; 39: 1546–1553.

13. 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.

14. Hata A, Asanuma H, Rinki M, et al. Use of an inactivated varicella vaccine in recipients of hematopoietic-cell transplants. N Engl J Med 2002; 347: 26–34.

CASE REPORT

Dasatinib followed by second allogeneic hematopoietic stem cell transplantation for relapse of Philadelphia chromosome-positive acute lymphoblastic leukemia after the first transplantation

Yoko Ishida · Kiriko Terasako · Kumi Oshima · Kana Sakamoto · Masahiro Ashizawa ·
Miki Sato · Misato Kikuchi · Shun-ichi Kimura · Hideki Nakasone · Shinya Okuda ·
Shinichi Kako · Rie Yamazaki · Junji Nishida · Yoshinobu Kanda

Abstract Although allogeneic hematopoietic stem cell transplantation (HSCT) is an established treatment for Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL), the prognosis of patients who relapse after allogeneic HSCT has been extremely poor. Dasatinib, a second-generation tyrosine kinase inhibitor, is a promising agent for the treatment of Ph-ALL. We report on a Ph-ALL patient who relapsed early after the first allogeneic HSCT, but achieved complete molecular remission with dasatinib alone. She remains in molecular remission 12 months after the second allogeneic HSCT. Dasatinib was generally well tolerated, but she developed myalgia, nausea and positive cytomegalovirus antigenemia. In addition, sudden-onset bloody diarrhea was observed 10 days after the second HSCT, which was possibly associated with the use of dasatinib in addition to the effect of the conditioning regimen and graft-versus-host disease. In conclusion, dasatinib is an effective agent for Ph-ALL with a poor prognosis, but may be associated with specific adverse events including opportunistic infection and gastrointestinal bleeding.

Keywords Dasatinib · Philadelphia chromosome-positive acute lymphoblastic leukemia · Hematopoietic stem cell transplantation

Y. Ishida · K. Terasako · K. Oshima · K. Sakamoto ·
M. Ashizawa · M. Sato · M. Kikuchi · S. Kimura ·
H. Nakasone · S. Okuda · S. Kako · R. Yamazaki ·
J. Nishida · Y. Kanda (✉)
Division of Hematology, Saitama Medical Center,
Jichi Medical University, 1-847 Amanuma, Omiya-ku,
Saitama, Saitama 330-8503, Japan
e-mail: ycanda-tky@umin.ac.jp

1 Introduction

Although Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph-ALL) has been associated with the most unfavorable prognosis among leukemias, the introduction of tyrosine kinase inhibitor (TKI) has led to a marked improvement in the treatment outcome of Ph-ALL. Recently, overall survival for this disease has been reported to range from 40 to 50% [1]. However, the observation duration is still not long enough and allogeneic hematopoietic stem cell transplantation (HSCT) still has an important role in the management of Ph-ALL. The use of imatinib in remission induction therapy and post-remission therapy has increased the number of patients who can undergo allogeneic HSCT for Ph-ALL in first remission [2]. Especially, the best prognosis has been observed in patients who received allogeneic HSCT in molecular remission. However, some patients are imatinib resistant or intolerant and experience an undesirable clinical course even after allogeneic HSCT. The prognosis of patients who relapse after allogeneic HSCT has been extremely poor.

Dasatinib is a second-generation TKIs that inhibits a wider range of tyrosine kinases than imatinib, including BCR–ABL and SRC family kinases. Dasatinib has also been predicted to bind to multiple conformations of the ABL kinase and is 300-fold more potent than imatinib [3]. A phase 2 study of dasatinib at 140 mg in patients with imatinib-resistant or -intolerant Ph-ALL, including 15 post-HSCT patients, was reported [4]. With a minimum follow-up of 8 months, major hematologic response and complete cytogenetic response were achieved in 42 and 58% of the 36 enrolled patients, respectively. However, various adverse events have been observed with the use of dasatinib, including myelosuppression, bleeding-related events and fluid retention [3].

We report on a patient who had a relapse of Ph-ALL early after the first allogeneic HSCT. She achieved a complete molecular response after salvage therapy with dasatinib alone and is still in complete molecular response 1 year after the second HSCT. Here, we describe the clinical course and discuss the efficacy and toxicity of dasatinib.

2 Case report

A 15-year-old female developed persistent fever and headache, and blood examination revealed hyperleukocytosis with a white blood cell (WBC) count of 610×10^9/L with 96% blast cells. An immunophenotypic analysis showed that bone marrow blast cells were positive for CD10, CD19, CD79a and HLA-DR. She was diagnosed to have Ph-ALL based on the detection of t(9;22)(q34;q11.2) translocation by a chromosomal analysis and e1a2 minor BCR–ABL fusion transcript by reverse transcriptase-polymerase chain reaction (RT-PCR). She received imatinib-combined induction therapy and achieved complete hematological and cytogenetic remission [5]. After consolidation therapies containing imatinib, the BCR–ABL fusion transcript was still positive by qualitative nested PCR, although quantitative PCR became negative. The patient was scheduled for her first allogeneic HSCT from an HLA-matched sibling donor in September 2008. During these treatments, she was taking imatinib with a reduced dose of 300 or 400 mg/day due to severe nausea, but sometimes vomited the tablets within 30–60 min of taking imatinib. Therefore, the actual average bioavailable daily dose may have been lower.

The conditioning regimen consisted of total body irradiation with a total dose of 12 Gy divided into 6 fractions, and cyclophosphamide at a total dose of 120 mg/kg divided over 2 days. Bone marrow cells collected from the HLA-matched sibling donor were infused without ex vivo manipulation. The number of infused nucleated cells was 3.17×10^8 cells/kg of recipient body weight. Prophylaxis against graft-versus-host disease (GVHD) was performed with the continuous infusion of cyclosporine at 3 mg/kg/day with a target concentration of 500 ng/ml and short-term methotrexate (MTX) at 10 mg/m² on day 1 and 7 mg/m² on days 3 and 6 [6]. Neutrophil engraftment, defined as the first of 3 consecutive days with an absolute neutrophil count of at least 0.5×10^9/L, was documented on day 14. Grade I acute GVHD of the skin only was observed, but did not require treatment. Cytomegalovirus (CMV) antigenemia was detected with a maximum of 13 positive cells per 2 slides by C10/11 on day 49, but became negative 3 weeks later without antiviral treatment.

Qualitative nested PCR for BCR–ABL fusion transcript was persistently positive even after the first transplantation, and furthermore, quantitative PCR became positive at 2.5×10^2 copies/μg RNA on day 112. Immunosuppressive agent was rapidly reduced to harness graft-versus-leukemia, but hematological relapse was confirmed on day 159 with a blast cell count of 12% in the peripheral blood and 37.6% in the bone marrow.

The clinical course of the patient after relapse is shown in Fig. 1. Imatinib at 800 mg/day was started again as soon as hematological relapse was observed, but she could not tolerate imatinib due to nausea and vomiting. Therefore, dasatinib at 140 mg/day was substituted for imatinib on day 167. She noted general myalgia, nausea, headache and eruption 2 days after starting dasatinib. However, these symptoms quickly disappeared upon the addition of prednisolone at 0.5 mg/kg/day, except for persistent low-grade fever, which improved thereafter upon administration of cefepime. CMV antigenemia became positive with a maximum count of 222 positive cells per 2 slides, and ganciclovir at 10 mg/kg/day was required [7]. Otherwise, she tolerated dasatinib well and a complete molecular response was achieved on day 258.

We performed the second transplantation from the same donor 266 days after the first HSCT following a conditioning regimen that consisted of fludarabine (25 mg/m²/day for 5 days), etoposide (200 mg/m²/day for 4 days), cytarabine (140 mg/m²/day for 4 days) and melphalan (140 mg/m²/day for 1 day). Dasatinib was stopped 1 day before the start of the conditioning regimen. A total of 4.01×10^8 nucleated cells/kg of recipient body weight were infused. GVHD prophylaxis was performed by the continuous infusion of cyclosporine at 3 mg/kg/day. Neutrophil engraftment was observed on day 11. She developed sudden-onset bloody diarrhea of 1500 ml per day on day 10. Total colonoscopy on day 15 revealed markedly edematous mucosa with diffuse redness and bleeding (Fig. 2). Pathological examination showed lymphocyte infiltration and apoptosis in the mucous membrane. We made a diagnosis of gastrointestinal acute GVHD and started methyl-prednisolone at 2 mg/kg/day. The gastrointestinal symptoms mostly disappeared within 2 weeks. CMV antigenemia of 21 positive cells per 2 slides was detected on day 18 and ganciclovir was started on day 20. The number of CMV antigenemia-positive cells increased to 820 per 2 slides, but no CMV disease was observed. CMV antigenemia became negative several weeks later. On day 139, the patient complained of a lower abdominal pain, pollakiuria, pain with urination and macroscopic hematuria. PCR of a urine sample revealed adenovirus infection, and thus a diagnosis of hemorrhagic cystitis caused by adenovirus was made. The symptoms gradually improved with supportive care and by decreasing the dose of immunosuppressants.

Fig. 1 Clinical course of the patient after relapse of leukemia and until the second allogeneic hematopoietic stem cell transplantation (HSCT). X-axis shows days after the first transplantation. PSL prednisolone, FLU fludarabine, AraC cytarabine, ETP etoposide, MEL melphalan, CMV cytomegalovirus
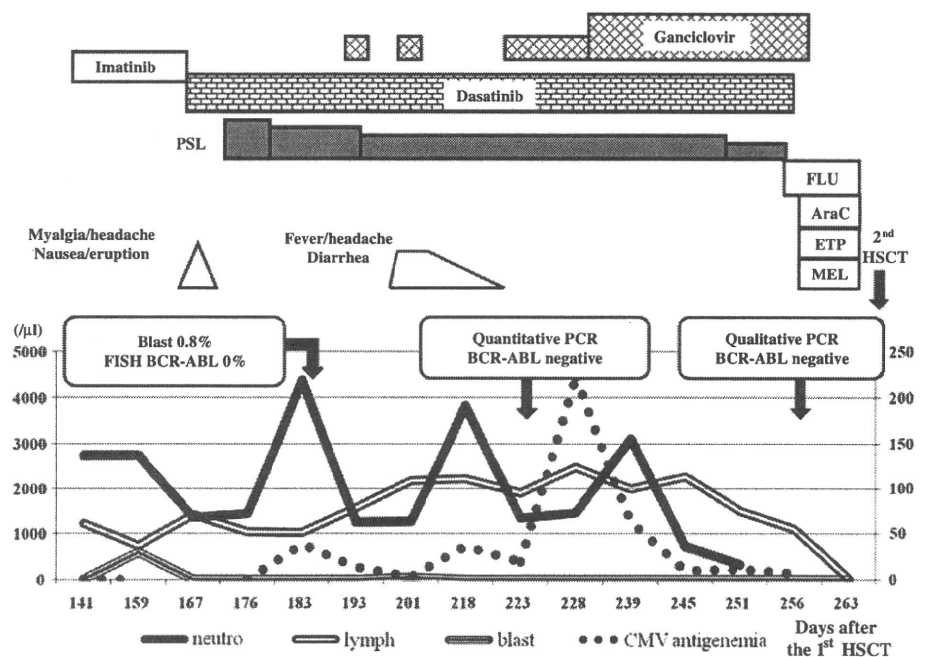
Fig. 2 Endoscopic findings included markedly edematous mucosa and diffuse spotty bleeding, which were compatible with gastrointestinal acute GVHD
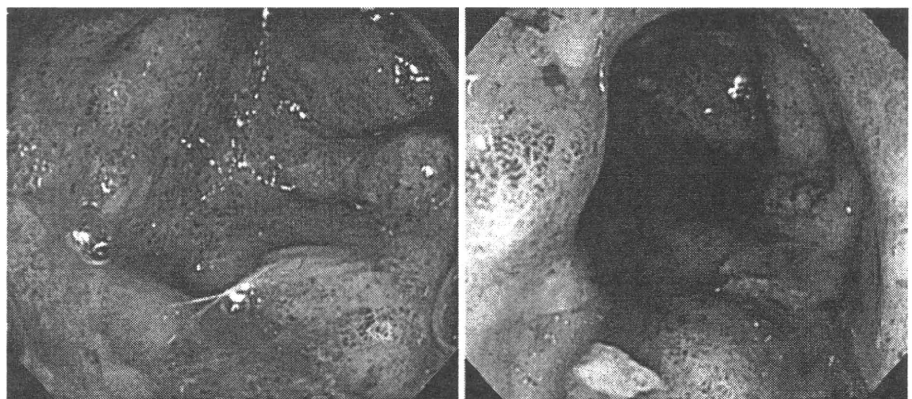
We started imatinib at 400 mg/day on day 57 as prophylaxis for relapse of Ph-ALL and, unexpectedly, this time she tolerated imatinib. We did not choose dasatinib, since she had high-grade CMV antigenemia. Complete molecular remission was confirmed 12 months after the second HSCT.

3 Discussion

In the present case, minimal residual disease was detected by qualitative nested PCR after chemotherapy with imatinib and even after the first allogeneic HSCT, but thereafter complete molecular remission was obtained with dasatinib alone, which was started after the hematological relapse. Sustained complete molecular remission was observed for more than 12 months after the second HSCT. The clinical course strongly suggested that dasatinib showed much stronger anti-leukemic activity than imatinib in this patient, although the actual dose of imatinib might have been insufficient due to adverse events. Surprisingly, she was able to tolerate imatinib at 400 mg/day after the second HSCT and this may partly contribute to her ability to maintain molecular response.

Dasatinib was well tolerated in this patient and could be continued until the second HSCT. While myalgia, nausea, headache and eruption were observed just after dasatinib was started, these symptoms soon disappeared on the addition of prednisolone. Myelosuppression was mild and dose reduction was not required. However, CMV antigenemia after dasatinib was started at a higher level than that after the first HSCT, even though all immunosuppressants

had been discontinued. This suggested that dasatinib might have an immunosuppressive effect, although the addition of steroid was partly responsible for the development of CMV antigenemia. Recently, Fei et al. [8] showed that dasatinib inhibits the proliferation of CD8+ T cells in a dose-dependent manner with a decreased secretion of interferon-gamma and granzyme B. The inhibitory effect of dasatinib on SRC and T-cell receptor signaling transduction was >100-fold greater than that of imatinib.

In addition, the patient experienced severe gastrointestinal bleeding after the second HSCT. Although the effects of the conditioning regimen and gastrointestinal acute GVHD might play a major role in the gastrointestinal damage, the sudden-onset bloody diarrhea was atypical, since gastrointestinal symptoms due to the conditioning regimen and acute GVHD usually begin with watery diarrhea that gradually becomes bloody. Therefore, we considered that the use of dasatinib before the conditioning regimen might have adversely affected the gastrointestinal damage just after the second HSCT. Although several studies have shown that the use of second-generation TKIs before HSCT did not increase transplant-related toxicity, most of the patients in these studies had chronic myelogenous leukemia (CML) in the chronic phase [9–11]. The adverse effects of dasatinib may vary depending on the phase of the underlying leukemia. During treatment with dasatinib for CML, for example, bleeding occurred in 12% of patients in the chronic phase, whereas 31 and 35% of patients in the accelerated phase and blast phase, respectively, developed bleeding [12]. Furthermore, 81% of the bleeding episodes affected the gastrointestinal tract. With regard to Ph-ALL, Revandi et al. [13] reported the incidence of adverse events during treatment with dasatinib plus HyperCVAD for Ph-ALL. Four of 35 and 11 of 31 patients in induction therapy and subsequent cycles, respectively, developed grade 3–4 hemorrhage, and 11 of these episodes were gastrointestinal bleeding. Therefore, the risk of gastrointestinal bleeding due to dasatinib may be higher in patients with Ph-ALL or CML in an advanced phase compared to those with CML in the chronic phase.

Bleeding episodes were often observed in patients with a platelet count of >30 × 10^9/L [12]. In fact, the platelet count of the current patient at the onset of gastrointestinal bleeding was greater than 30 × 10^9/L. Recently, the effect of dasatinib on platelet function was studied in detail. Platelet aggregation upon stimulation with arachidonic acid, epinephrine or both was impaired in 70, 85 and 59% of patients who were receiving dasatinib, respectively, whereas 85% of patients on bosutinib, 100% of those on nilotinib and 33% of those on imatinib had normal platelet aggregation [14]. It is not clear why most episodes of bleeding were observed in the gastrointestinal tract. Possible explanations include the oral administration of dasatinib

and a major route of elimination in the feces [15]. The expansion of clonal large granular lymphocytes may also be associated with the development of colitis during dasatinib treatment [16].

In conclusion, dasatinib induced complete molecular remission in a patient who had a relapse of Ph-ALL early after the first allogeneic HSCT. This suggested that dasatinib has a strong anti-leukemic activity against Ph-ALL and, therefore, the use of dasatinib not only as salvage therapy but also as prophylaxis against relapse of Ph-ALL may be beneficial for patients after allogeneic HSCT for Ph-ALL. However, caution should be paid to the possibility of adverse effects of dasatinib, especially infectious complications and gastrointestinal bleeding. Prospective studies on dasatinib in these settings are warranted.

References

1. Ottmann OG, Pfeifer H. Management of Philadelphia chromosome-positive acute lymphoblastic leukemia (ph+ all). Hematol Am Soc Hematol Educ Program. 2009;371–81.
2. Yanada M, Naoe T, Iida H, Sakamaki H, Sakura T, Kanamori H, et al. Myeloablative allogeneic hematopoietic stem cell transplantation for Philadelphia chromosome-positive acute lymphoblastic leukemia in adults: significant roles of total body irradiation and chronic graft-versus-host disease. Bone Marrow Transplant. 2005;36:867–72.
3. Khoury HJ, Guilhot F, Hughes TP, Kim DW, Cortes JE. Dasatinib treatment for Philadelphia chromosome-positive leukemias: practical considerations. Cancer. 2009;115:1381–94.
4. Ottmann O, Dombret H, Martinelli G, Simonsson B, Guilhot F, Larson RA, et al. Dasatinib induces rapid hematologic and cytogenetic responses in adult patients with Philadelphia chromosome positive acute lymphoblastic leukemia with resistance or intolerance to imatinib: interim results of a phase 2 study. Blood. 2007;110:2309–15.
5. Yanada M, Takeuchi J, Sugiura I, Akiyama H, Usui N, Yagasaki F, et al. High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed BCR–ABL-positive acute lymphoblastic leukemia: a phase II study by the Japan Adult Leukemia Study Group. J Clin Oncol. 2006;24:460–6.
6. Oshima K, Kanda Y, Nakasone H, Arai S, Nishimoto N, Sato H, et al. Decreased incidence of acute graft-versus-host disease by continuous infusion of cyclosporine with a higher target blood level. Am J Hematol. 2008;83:226–32.
7. Kanda Y, Mineishi S, Saito T, Saito A, Ohnishi M, Niiya H, et al. Response-oriented preemptive therapy against cytomegalovirus disease with low-dose ganciclovir: a prospective evaluation. Transplantation. 2002;73:568–72.
8. Fei F, Yu Y, Schmitt A, Rojewski MT, Chen B, Greiner J, et al. Dasatinib exerts an immunosuppressive effect on CD8+ T cells specific for viral and leukemia antigens. Exp Hematol. 2008;36:1297–308.
9. Jabbour E, Cortes J, Kantarjian H, Giralt S, Andersson BS, Giles F, et al. Novel tyrosine kinase inhibitor therapy before allogeneic stem cell transplantation in patients with chronic myeloid leukemia: no evidence for increased transplant-related toxicity. Cancer. 2007;110:340–4.

10. Breccia M, Palandri F, Iori AP, Colaci E, Latagliata R, Castagnetti F, et al. Second-generation tyrosine kinase inhibitors before allogeneic stem cell transplantation in patients with chronic myeloid leukemia resistant to imatinib. Leuk Res. 2010; 34:143–7.
11. Shimoni A, Leiba M, Schleuning M, Martineau G, Renaud M, Koren-Michowitz M, et al. Prior treatment with the tyrosine kinase inhibitors dasatinib and nilotinib allows stem cell transplantation (SCT) in a less advanced disease phase and does not increase SCT toxicity in patients with chronic myelogenous leukemia and Philadelphia positive acute lymphoblastic leukemia. Leukemia. 2009;23:190–4.
12. Quintas-Cardama A, Kantarjian H, Ravandi F, O'Brien S, Thomas D, Vidal-Senmache G, et al. Bleeding diathesis in patients with chronic myelogenous leukemia receiving dasatinib therapy. Cancer. 2009;115:2482–90.

13. Ravandi F, O'Brien S, Thomas D, Faderl S, Jones D, Garris R, et al.: First report of phase II study of dasatinib with hyperCVAD for the frontline treatment of patients with Philadelphia chromosome positive (ph+) acute lymphoblastic leukemia. Blood (in press).
14. Quintas-Cardama A, Han X, Kantarjian H, Cortes J. Tyrosine kinase inhibitor-induced platelet dysfunction in patients with chronic myeloid leukemia. Blood. 2009;114:261–3.
15. Christopher LJ, Cui D, Wu C, Luo R, Manning JA, Bonacorsi SJ, et al. Metabolism and disposition of dasatinib after oral administration to humans. Drug Metab Dispos. 2008;36:1357–64.
16. Mustjoki S, Ekblom M, Arstila TP, Dybedal I, Epling-Burnette PK, Guilhot F, et al. Clonal expansion of T/NK-cells during tyrosine kinase inhibitor dasatinib therapy. Leukemia. 2009;23: 1398–405.

Retrospective Evaluation of the Area Over the Neutrophil Curve Index to Predict Early Infection in Hematopoietic Stem Cell Transplantation Recipients

Shun-ichi Kimura, Kumi Oshima, Ken Sato, Miki Sato, Kiriko Terasako, Hideki Nakasone, Misato Kikuchi, Shinya Okuda, Shinichi Kako, Rie Yamazaki, Yukie Tanaka, Aki Tanihara, Junji Nishida, Yoshinobu Kanda

We investigated the impact of neutropenia on the development of early bloodstream and pulmonary infections in hematopoietic stem cell transplantation (HSCT) recipients, and evaluated the utility of an index (D-index) that reflects both the intensity and duration of neutropenia. Fifty-eight patients (23 autologous, 35 allogeneic HSCT recipients) were enrolled in this retrospective study. The D-index was defined as the area over the neutrophil curve during neutropenia. We also evaluated the utility of the cumulative D-index from the start of neutropenia until the development of infection (c-D-index), which may enable real-time assessment of the risk for infection. The patients showed 12 and 7 episodes of bloodstream and pulmonary infection, respectively. The D-index, days of neutropenia ($<500/\mu L$) and days of profound neutropenia ($<100/\mu L$) had at least a nearly significant impact on the development of both bloodstream and pulmonary infections. On the other hand, the c-D-index, cumulative days of neutropenia, and cumulative days of profound neutropenia significantly affected pulmonary infection, but not bloodstream infection. The c-D-index had a high negative predictive value of 97.4% for pulmonary infection with a cutoff of 5500, but the area under the receiver operating characteristic curve was similar to that of the cumulative days of neutropenia and profound neutropenia. Our results showed that although the c-D-index may be useful for identifying patients who are at low risk for early pulmonary infection after HSCT, its performance was similar to that of the simple duration of neutropenia.

INTRODUCTION

Infection remains the leading cause of morbidity and mortality in the early period after hematopoietic stem cell transplantation (HSCT) [1-6]. Common sites of infection include the bloodstream and the lungs [7]. During the neutropenic period before engraftment, both autologous and allogeneic HSCT recipients have 2 critical risk factors for infection: prolonged severe neutropenia, and breaks in the mucocutaneous barrier resulting from preparative regimens [8,9]. The latter increase the risk of infection caused by oral, gastrointestinal, and skin flora [10], which results in bloodstream infections through bacterial translocation [11]. Although neutropenia is a well-recognized risk factor for documented infections in the early period of HSCT [1], it is still unclear whether it has similar or different effects on the development of bloodstream and pulmonary infections. In addition, there is no useful index that reflects both the intensity and duration of neutropenia.

In this study, we retrospectively investigated the impact of neutropenia on the development of early bloodstream and pulmonary infections in HSCT recipients. As indexes of the severity of neutropenia, we used the D-index and c-D-index, which were recently proposed by Portugal et al. [12]. The D-index was based on a graph that showed the absolute neutrophil count during neutropenia and was calculated as the area over the neutrophil curve (Figure 1).
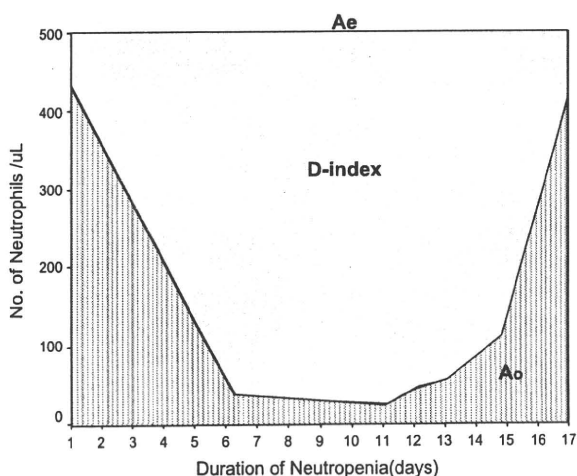
Figure 1. Area over the neutrophil curve (D-index) of a hypothetical neutropenic patient. If the duration of neutropenia is 16 days, the expected neutrophil area (shaded area, A_e) is $16 \times 500 = 8000$. If the area under the neutrophil curve calculated by the trapezoidal method (striped area, A_o) is 2062, the D-index $= 8000 - 2062 = 5938$.

Thus, it could be used to evaluate the dynamics of neutropenia, and combined both intensity and duration. However, the neutrophil counts for the whole duration of neutropenia are required to calculate the D-index. Therefore, the D-index becomes available only after the recovery of neutropenia and is not useful as a predictor of infectious complications. To resolve this problem, a cumulative D-index (c-D-index), defined as the cumulative D-index from the start of neutropenia, was also investigated. According to their study, the D-index and c-D-index had high negative predictive values for invasive mold infections in acute myelogenous leukemia patients undergoing chemotherapy. This study was performed to identify the utility of these indexes for predicting early bloodstream and pulmonary infections in HSCT recipients.

PATIENTS AND METHODS

Patients

The Transplantation Unit of Saitama Medical Center, Jichi Medical University has 3 individual rooms and 2 quad rooms (11 beds in total) that are equipped with a laminar air-flow (LAF) system with high-efficiency particulate air (HEPA) filters. In principle, allogeneic and autologous HSCTs are performed in these individual and quad rooms, respectively. We retrospectively reviewed the charts of consecutive patients who underwent autologous or allogeneic HSCT, between April 2005 and March 2009. Patients who had already developed documented infections before HSCT were excluded. Twenty-three autologous and

35 allogeneic HSCT recipients were finally included in this study.

Transplantation Procedure

The conditioning regimen in autologous HSCT was mainly a combination of ranimustine, etoposide, cytarabine, and melphalan (M-BEAM) for lymphoma (n = 9) and high-dose melphalan (Mel) for multiple myeloma (n = 13) [13]. One patient with acute promyelocytic leukemia received a combination of busulfan (Bu) and Mel [14]. In allogeneic HSCT, the combination of cyclophosphamide (Cy) and either total body irradiation (TBI) (n = 16) or Bu (n = 2) was used as a myeloablative regimen [15]. High-dose cytarabine was added to Cy and TBI in 2 patients. Fludarabine (Flu)-based reduced-intensity regimens, such as Flu combined with Bu [16] or Mel [17], were used in elderly or clinically infirm patients (n = 10). Patients with severe aplastic anemia were prepared with Flu, Cy, antithymoglobulin (ATG) and low-dose TBI at 2 Gy (n = 2) [18]. Alemutuzumab-containing regimens were used in HSCT from a 2- or 3-antigen-mismatched donor (n = 3) [19]. Regimen-related toxicity was graded according to Bearman's criteria [20].

Graft-versus-host disease (GVHD) prophylaxis in allogeneic HSCT consisted of the continuous infusion of cyclosporine A with a starting dose of 3 mg/kg/day and short-term methotrexate (10-15 mg/m^2 on day 1, 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) [21] with the exception of 1 patient who received a continuous infusion of tacrolimus with a starting dose of 0.03 mg/kg/day and short-term methotrexate. Acute GVHD (aGVHD) was graded as previously described [22].

Prophylaxis against bacterial infections consisted of levofloxacin in all autologous and most of the allogeneic HSCTs, except that 7 allogeneic recipients had been receiving fourth-generation cephalosporine or carbapenem for fever of unknown origin at HSCT. Prophylaxis against fungal infections consisted of fluconazole (n = 17), itraconazole (n = 31), micafungin (n = 7), or other antimold agents (n = 3). As prophylaxis against *Pneumocystis jiroveci* infection, sulfamethoxazole/trimethoprim or inhalation of pentamidine was used after engraftment. As prophylaxis against herpes simplex virus infection, acyclovir was given from days −7 to 35. In allogeneic HSCT, this was followed by the long-term low-dose administration of acyclovir for varicella zoster reactivation [23]. Preemptive therapy with ganciclovir for cytomegalovirus infection was performed by monitoring cytomegalovirus antigenemia [24].

D-Index and c-D-Index Calculation

The D-index was calculated based on a graph that plotted the absolute neutrophil counts over the

course of the episode of neutropenia (Figure 1) [12]. The D-index ($A_e - A_o$) was calculated as the difference between the observed area under the curve (A_o), which was calculated by the trapezoidal method, and the expected neutrophil area (A_e; 500/μL × days with neutropenia) if the patient did not develop neutropenia. A cumulative D-index (c-D-index) was calculated as the cumulative D-index from the start of neutropenia until the development of infections in patients with early pulmonary or bloodstream infections, whereas the c-D-index was equal to the D-index in patients without these infections. The cumulative duration of neutropenia or profound neutropenia was defined as the duration of neutropenia until the development of infections in patients with early pulmonary or bloodstream infections, respectively, whereas it was equal to the entire duration of neutropenia or profound neutropenia in patients without these infections.

Definition of Early Bloodstream and Pulmonary Infections

Early infection was defined as that which developed between the start of the conditioning regimens and 1 week after engraftment. Bloodstream infection was diagnosed by culturing bacteria from the blood. To distinguish between true bloodstream infections and contamination, common skin contaminants such as diphtheroids, *Bacillus* species, *Propionibacterium* species, coagulase-negative staphylococci, and micrococci had to have been cultured in at least 2 consecutive blood cultures drawn on separate occasions. Pulmonary infection was defined as new pulmonary infiltrate observed in a chest X-ray or chest computed tomography (CT) regardless of microbiological evidence. Clinically apparent noninfectious pulmonary infiltrates, including those caused by cardiogenic pulmonary edema or engraftment syndrome, were excluded.

Statistical Considerations

We evaluated the impact of the D-index, total duration of neutropenia (<500/μL), and total duration of profound neutropenia (<100/μL) as indexes of the severity of neutropenia over the entire duration of neutropenia, whereas we evaluated the c-D-index, cumulative duration of neutropenia, and cumulative duration of profound neutropenia as indexes of the cumulative severity of neutropenia from the start of neutropenia. We assessed the impact of these indexes along with other epidemiologic and clinical factors, separately for bloodstream and pulmonary infections.

Dichotomous variables were compared using Fisher's exact test, and continuous variables were compared using the Mann-Whitney U test. A P-value of <.05 was considered to be significant. To assess the ability of the D-index, c-D-index, and duration of neu-

tropenia to predict infections we performed a receiver operating characteristic (ROC) curve analysis and calculated the positive and negative predictive values in this patient population.

RESULTS

Patients

The clinical and epidemiologic characteristics of the patients are shown in Table 1. Among the 58 patients, 1 autologous and 11 allogeneic HSCT recipients developed bloodstream infections and 7 allogeneic HSCT recipients developed pulmonary infections. The median number of days between HSCT and the development of bloodstream and pulmonary infections was 9.5 days (range: 1-24) and 14.5 days (range: 4-27), respectively. Eleven of the 12 patients developed bloodstream infections before engraftment and 1 patient did so within 1 week after engraftment. Four of the 7 patients developed pulmonary infections before engraftment and the other 3 patients did so within 1 week after engraftment. The pathogens that caused bloodstream infections included coagulase-negative staphylococci (n = 11), *Enterococcus faecium* (n = 1), *Pseudomonas* species (n = 2), *Acinetobacter* (n = 1), and *Candida parapsilosis* (n = 1). Two patients developed bacteremia by multiple pathogens. Although the causes of pulmonary infections were not proven in all 7 patients, 1 and 3 cases were classified as probable and possible invasive mold infection, respectively, according to the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) revised criteria for invasive fungal infections [25]. Another case did not fulfill the criteria on chest CT, but mold infection was suspected because of the elevation of serum β-D glucan. The other case was also suspected to have mold infection based on the clinical course and chest X-ray, but chest CT was not performed because of the patient's poor general condition. The remaining 1 patient developed a unilateral interstitial pulmonary infiltrate. In 6 of the 7 cases of pulmonary infection, antifungal treatment with voriconazole or liposomal amphotericin B was started.

Epidemiologic and Clinical Factors

The incidence of both bloodstream and pulmonary infections was higher in allogeneic HSCT recipients than in autologous patients (P = .013 and .022, respectively). However, age, sex, underlying diseases, conditioning regimens, and antifungal prophylaxis did not show a statistically significant impact on the development of either infection. Furthermore, regimen-related toxicity of the oral mucosa and gastrointestine, the duration of central venous catheter insertion, and

Table I. Clinical and Epidemiological Characteristics of the Study Patients

	Total Cases (n = 58)	Bloodstream Infection (n = 12)	P-value*	Pulmonary Infection (n = 7)	P-value†
Age, years median (range)	50.5 (15-64)	43.5 (15-61)	.274	41 (21-54)	.312
Sex male / female	29 / 29	7 / 5	.517	4 / 3	.687
Autologous / allogeneic HSCTs	23 / 35	1 / 11	.013	0 / 7	.022
Underlying disease			.068		.075
Acute myelogenous leukemia	14 (24.1%)	4 (33.3%)		4 (57.1%)	
Acute lymphoblastic leukemia	6 (10.3%)	2 (16.7%)		1 (14.3%)	
Lymphoma	17 (29.3%)	0		0	
Myelodysplastic syndrome	3 (5.2%)	1 (8.3%)		0	
Aplastic anemia	4 (6.9%)	2 (16.7%)		1 (14.3%)	
Multiple myeloma	11 (19.0%)	1 (8.3%)		0	
Others	3 (5.2%)	2 (16.7%)		1 (14.3%)	
Conditioning regimen			0.699		.656
Myeloablative regimen	46 (79.3%)	10 (83.3%)		6 (85.7%)	
Reduced-intensity regimen	12 (20.7%)	2 (16.7%)		1 (14.3%)	
Prophylactic antifungal agent			.190		.096
FLCZ	17 (29.3%)	6 (50.0%)		2 (28.6%)	
ITCZ	31 (53.4%)	4 (33.3%)		2 (28.6%)	
Other antimold agents	10 (17.2%)	2 (16.7%)		3 (42.9%)	
Days of neutropenia (<500/μL) median (range)	11.5 (3-40)	17.5 (5-27)	.072	24 (13-29)	.003
Days of profound neutropenia (<100/μL) median (range)	8 (0-35)	15 (3-35)	.031	18 (8-29)	.008
D-index median (range)	4553.5 (942-17,800)	7102.5 (1653.5-13445.5)	.055	9816.5 (4599.5-13973)	.007
c-D-index median (range)		3374.75 (1378-10,086)	.443	7589 (4599.5-11159)	.028
Regimen-related toxicity (Bearman's grade)					
Oral mucosa > Grade II	34 (58.6%)	7 (58.3%)	.982	6 (85.7%)	.121
Gastrointestine > Grade I	21 (36.2%)	3 (25%)	.364	3 (42.9%)	.696
Days of central venous catheter insertion, days median (range)	32.5 (0-85)	39 (17-50)	1.000	32 (24-56)	.674
Acute GVHD > Grade II‡	8 (22.9%)	2 (18.1%)	.656	1 (14.3%)	.546

GVHD indicates graft-versus-host disease; HSCT, hematopoietic stem cell transplantation.
*Compared to cases without bloodstream infection.
†Compared to cases without pulmonary infection.
‡Analyzed only among allogeneic HSCT recipients.

the development of grade II-IV aGVHD were not statistically significant risk factors for the development of either early bloodstream or pulmonary infections.

Evaluation of Indexes for the Severity of Neutropenia: D-Index, c-D-Index, and Duration of Neutropenia

Among the indexes of the severity of neutropenia over the whole duration of neutropenia, days of profound neutropenia (<100/μL) significantly affected the development of bloodstream infections (median 15 versus 7 days, $P = .031$). The D-index and days of neutropenia (<500/μL) tended to be higher or longer in patients with bloodstream infections, with borderline significance (median 7102.5 versus 3963.5 and 17.5 versus 10.5 days, $P = .055$ and .072, respectively). As indexes of the cumulative severity of neutropenia from the start of neutropenia, neither the c-D-index, cumulative duration of neutropenia, nor cumulative duration of profound neutropenia significantly affected bloodstream infections (median 3375 versus 3963.5, 8.5 versus 10.5 days, and 7 versus 7 days, $P = .443, .397,$ and .900, respectively). On the other hand, both the indexes of the severity of the whole duration of neutropenia, including the D-index, days of neutropenia and days of

profound neutropenia (median 9816.5 versus 3999.5, 24 versus 11 days, and 18 versus 7 days, $P = .007,$ .003, and .008, respectively), and the indexes for the cumulative severity of neutropenia, including the c-D-index, cumulative duration of neutropenia, and cumulative duration of profound neutropenia, significantly affected pulmonary infections (median 7589 versus 3999.5, 20 versus 11 days, and 15 versus 7 days, $P = .028, .020,$ and .024, respectively). When we focused on the 6 cases with probable, possible, or suspected pulmonary mold infections, the indexes of the severity of neutropenia over the whole duration of neutropenia significantly affected the development of invasive mold infections (median 8702 versus 4059, 23 versus 11 days, 17 versus 7 days, $P = .027, .020,$ and .027, respectively), whereas the indexes for the cumulative severity of neutropenia affected it with borderline significance (median 6678 versus 4059, 18 versus 11 days, and 15 versus 7 days, $P = .081, .090,$ and .077, respectively).

ROC analysis revealed that the D-index, c-D-index, and duration of neutropenia were equally useful for predicting early pulmonary infections. The area under the ROC curves were 0.810, 0.801, and 0.832 for the D-index, days of neutropenia and days of profound neutropenia, respectively (Figure 2A). These
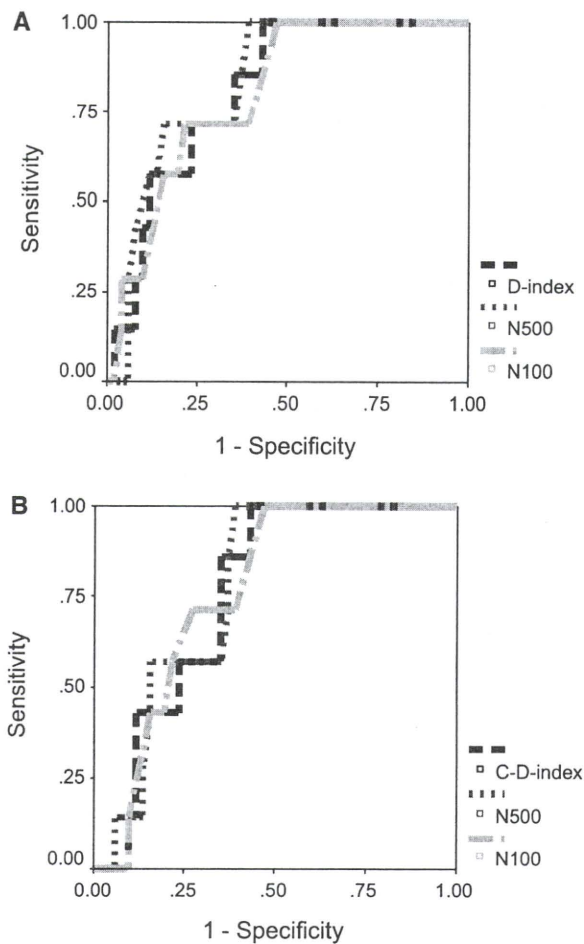
Figure 2. Receiver operating characteristic curves comparing the D-index with the days of neutropenia (<500/μL, N500) and profound neutropenia (<100 /μL, N100) (A), and comparing the cumulative D-index (c-D-index) with the cumulative durations of neutropenia (<500/μL, N500) and profound neutropenia (<100/μL, N100) (B) as predictors of pulmonary infection.

positive and negative predictive values were 29.4% and 95.1%, 38.7% and 95.6%, and 31.3% and 95.2%, respectively. Similarly, the ROC curve was closest to the left corner of the plot when the thresholds for the c-D-index, cumulative duration of neutropenia, and cumulative duration of profound neutropenia were 5500, 13, and 14, respectively. With the use of these cutoff values, the sensitivity and specificity for predicting pulmonary infections were 85.7% and 74.7%, 100% and 60.8%, and 71.4% and 72.5%, respectively. The positive and negative predictive values were 31.6% and 97.4%, 25.9% and 100.0%, and 35.7% and 94.9%, respectively (Table 2).

We did not perform ROC analyses for bloodstream infections, because none of the indexes for the severity of neutropenia, except for the total days of profound neutropenia (<100/μL), significantly affected early bloodstream infections.

DISCUSSION

Bloodstream and pulmonary infections are the main types of documented infection [7] and are sometimes fatal in the early period after HSCT [26,27]. In this study, 12 and 7 of the 58 patients developed bloodstream infections and pneumonia within 1 week after engraftment, and these incidences were similar to those in previous reports [6,7,26]. With regard to the causative pathogens, Gram-positive organisms, most of which were coagulase-negative staphylococci, were the predominant cause of bloodstream infection. As reported previously, Gram-positive bacteria became the predominant microorganism that caused bloodstream infections after the introduction of prophylaxis with fluoroquinolones [1,2,7]. Among 7 cases of pulmonary infections, 1 and 3 cases were classified as probable and possible invasive pulmonary mold infection, respectively, according to the EORTC/MSG revised criteria [25]. Invasive fungal infection, especially invasive aspergillosis, is also a life-threatening infectious complication in the early period after HSCT [28,29].

Neutropenia is considered to be a critical risk factor for infectious complications in the preengraftment phase of HSCT [8-10]. Engels et al. [1] reported that the logarithm10 of the neutrophil count was significantly associated with the risk of infection in bone marrow

values were .756, .769, and .762 for the c-D-index, cumulative duration of neutropenia, and cumulative duration of profound neutropenia, respectively (Figure 2B). The ROC curve was closest to the left corner of the plot when the thresholds for the D-index, days of neutropenia, and days of profound neutropenia were 7600, 20, and 15, respectively. With the use of these cutoff values, the sensitivity and specificity for predicting pulmonary infections were 71.4% and 84.3%, 71.4% and 78.4%, and 71.4% and 76.5%, respectively. The

Table 2. Predictive Values of Each Parameter for Early Pulmonary Infection

	CO value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
D-index	7600	71.4	84.3	29.4	95.1
Days of neutropenia (<500/μL)	20	71.4	78.4	38.7	95.6
Days of profound neutropenia (<100/μL)	15	71.4	76.5	31.3	95.2
c-D-index	5500	85.7	74.7	31.6	97.4
Cumulative duration of neutropenia	13	100	60.8	25.9	100
Cumulative duration of profound neutropenia	14	71.4	72.5	35.7	94.9

CO indicates cutoff; PPV, positive predictive value; NPV, negative predictive value.

transplant recipients, with a hazard ratio of 0.49. According to the study by Bonadio et al. [4], most infectious episodes in HSCT recipients occurred during the leukopenic period, especially in patients with a deeper (white blood cell count <200/μL) and prolonged leukopenia. Offidani et al. [5] reported that >5 days of an absolute neutrophil count <100/μL was associated with a higher risk of early infection in autologous HSCT recipients. Marr et al. [30] considered neutropenia as a time-dependent covariate, and reported that delayed neutrophil engraftment was associated with an increased risk for early invasive aspergillosis in allogeneic HSCT recipients. However, there has been no tool to assess the severity of neutropenia that combined both the intensity and the duration until Portugal et al. [12] developed the D-index and c-D-index, which are calculated from the neutrophil count curve.

Our present study showed that the cumulative severity of neutropenia significantly affected early pulmonary infections in HSCT recipients. In contrast, although bloodstream infections tended to occur in patients with a higher D-index and a longer total duration of neutropenia, the c-D-index and cumulative duration of neutropenia had no predictive value for bloodstream infections. This difference may reflect the fact that bloodstream infections tended to occur earlier after HSCT than pulmonary infections (median 9.5 versus 14.5 days). Bloodstream infections often occur soon after HSCT as a result of bacterial translocation through oral and gastrointestinal mucosa damaged by the conditioning regimen [11] or in association with the central venous catheter [31], and therefore are not strongly related to the duration of neutropenia. With regard to pulmonary infections, the significant influence of neutropenia on pulmonary infections might be at least partly because of pulmonary mold infections, for which prolonged neutropenia is a strong risk factor [30].

The negative predictive value of the c-D-index for early pulmonary infections after HSCT was 97.4% using a cutoff value of 5500. This means that patients with a c-D-index less than 5500 have little probability of developing pulmonary infections and are less likely to require the empiric or preemptive administration of intensive antimold treatment, if they have received treatment in a clean room equipped with a LAF system. However, it can be difficult to calculate the c-D-index compared to the simple duration of neutropenia. Although Portugal et al. [12] reported that the D-index and c-D-index were superior to the duration of neutropenia for predicting invasive mold infections in acute myelogenous leukemia patients with chemotherapy, there seemed to be no great difference according to their ROC curves. The current study showed that the D-index and c-D-index were as effective as the duration of neutropenia for predicting early pulmonary infection in HSCT recipients, probably because neutropenia was more severe and uniform in

HSCT recipients than in patients with standard chemotherapy. In fact, the D-index and c-D-index were strongly correlated with the total days of neutropenia and the cumulative duration of neutropenia, respectively, in this study (correlation coefficients 0.974 and 0.968, $P < .001$ and $< .001$, respectively).

We analyzed both autologous and allogeneic HSCT recipients together, because neutropenia and mucocutaneous damage are the strongest risk factors for infections during the first month after HSCT regardless of the type of HSCT. The difference between autologous and allogeneic HSCT in terms of the risk of infectious events becomes apparent after engraftment, for example, because of the use of steroid for the treatment of GVHD. Therefore, the significant difference in the incidence of infections between allogeneic and autologous HSCT recipients in this study was because of the difference in the duration of neutropenia, as reported in a previous study [1]. The duration of neutropenia in autologous HSCT was significantly shorter than that in allogeneic HSCT in this study (median 18.5 versus 6.5 days, $P < .001$).

There are some limitations in this study. The first is the small number of patients evaluated. The D-index for bloodstream infection and c-D-index for pulmonary mold infection might have attained significance if the study had been larger. The second limitation is that the day of the development of pulmonary infections was considered as the time when pulmonary infiltrate was detected by imaging tests in this study. The true occurrence of pulmonary infections might have been earlier. Third, the predictive value of c-D-index might vary depending on the anitfungal prophylaxis. In this study, patients who received fluconazole and antimold agents as antifungal prophylaxis were evaluated together. In addition, because the positive predictive value of c-D-index for pulmonary infection was only 31.5%, it may not be useful as a trigger to start empiric antifungal therapy.

In conclusion, both bloodstream and pulmonary infections tended to occur more frequently in patients with a higher D-index and a longer total duration of neutropenia early after HSCT. On the other hand, the c-D-index was helpful for predicting the risk of pulmonary infections, with a high negative predictive value, but not for predicting bloodstream infections. In HSCT recipients, the c-D-index was as useful as the simple duration of neutropenia and therefore may add little value to the daily practice of autologous and allogeneic HSCT.

ACKNOWLEDGMENTS

REFERENCES

1. Engels EA, Ellis CA, Supran SE, et al. Early infection in bone marrow transplantation: quantitative study of clinical factors that affect risk. *Clin Infect Dis*. 1999;28:256-266.
2. Ketterer N, Espinouse D, Chomarat M, et al. Infections following peripheral blood progenitor cell transplantation for lymphoproliferative malignancies: etiology and potential risk factors. *Am J Med*. 1999;106:191-197.
3. Saavedra S, Jarque I, Sanz GF, et al. Infectious complications in patients undergoing unrelated donor bone marrow transplantation: experience from a single institution. *Clin Microbiol Infect*. 2002;8:725-733.
4. Bonadio M, Morelli G, Mori S, Riccioni R, Papineschi F, Petrini M. Fluoroquinolone resistance in hematopoietic stem cell transplant recipients with infectious complications. *Biomed Pharmacother*. 2005;59:511-516.
5. Offidani M, Corvatta L, Olivieri A, et al. Infectious complications after autologous peripheral blood progenitor cell transplantation followed by G-CSF. *Bone Marrow Transplant*. 1999; 24:1079-1087.
6. Kolbe K, Domkin D, Derigs HG, Bhakdi S, Huber C, Aulitzky WE. Infectious complications during neutropenia subsequent to peripheral blood stem cell transplantation. *Bone Marrow Transplant*. 1997;19:143-147.
7. Krüger W, Rüssmann B, Kröger N, et al. Early infections in patients undergoing bone marrow or blood stem cell transplantation—a 7 year single centre investigation of 409 cases. *Bone Marrow Transplant*. 1999;23:589-597.
8. Dykewicz CA. Summary of the guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. *Clin Infect Dis*. 2001;33:139-144.
9. Wingard JR. Opportunistic infections after blood and marrow transplantation. *Transpl Infect Dis*. 1999;1:3-20.
10. CDC, Infectious. Disease Society of America, the American Society of Blood and Marrow Transplantation. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. Recommendations of CDC, the Infectious Disease Society of America, and the American Society of Blood and Marrow Transplantation. *Cytotherapy*. 2001;3:41-54.
11. Berg RD, Garlington AW. Translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs in a gnotobiotic mouse model. *Infect Immun*. 1979;23:403-411.
12. Portugal RD, Garnica M, Nucci M. Index to predict invasive mold infection in high-risk neutropenic patients based on the area over the neutrophil curve. *J Clin Oncol*. 2009;27: 3849-3854.
13. Moreau P, Facon T, Attal M, et al. Comparison of 200 mg/m(2) melphalan and 8 Gy total body irradiation plus 140 mg/m(2) melphalan as conditioning regimens for peripheral blood stem cell transplantation in patients with newly diagnosed multiple myeloma: final analysis of the Intergroupe Francophone du Myélome 9502 randomized trial. *Blood*. 2002;99:731-735.
14. Srivastava A, Bradstock KF, Szer J, de Bortoli, Gottlieb DJ. Busulphan and melphalan prior to autologous bone marrow transplantation. *Bone Marrow Transplant*. 1993;12:323-329.
15. Blaise D, Maraninchi D, Archimbaud E, et al. Allogeneic bone marrow transplantation for acute myeloid leukemia in first remission: a randomized trial of a busulfan-Cytoxan versus Cytoxan-total body irradiation as preparative regimen: a report from the Group d'Etudes de la Greffe de Moelle Osseuse. *Blood*. 1992;79:2578-2582.
16. Kusumi E, Kami M, Yuji K, et al. Feasibility of reduced intensity hematopoietic stem cell transplantation from an HLA-matched unrelated donor. *Bone Marrow Transplant*. 2004;33:697-702.
17. Giralt S, Thall PF, Khouri I, et al. Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. *Blood*. 2001;97:631-637.
18. Okuda S, Terasako K, Oshima K, et al. Fludarabine, cyclophosphamide, anti-thymocyte globulin, and low-dose total body irradiation conditioning enables 1-HLA-locus-mismatched hematopoietic stem cell transplantation for very severe aplastic anemia without affecting ovarian function. *Am J Hematol*. 2009;84:167-169.
19. Kanda Y, Oshima K, Asano-Mori Y, et al. In vivo alemtuzumab enables haploidentical human leukocyte antigen-mismatched hematopoietic stem-cell transplantation without ex vivo graft manipulation. *Transplantation*. 2005;79:1351-1357.
20. Bearman SI, Appelbaum FR, Buckner CD, et al. Regimen-related toxicity in patients undergoing bone marrow transplantation. *J Clin Oncol*. 1988;6:1562-1568.
21. Oshima K, Kanda Y, Nakasone H, et al. Decreased incidence of acute graft-versus-host disease by continuous infusion of cyclosporine with a higher target blood level. *Am J Hematol*. 2008;83: 226-232.
22. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus conference on acute GVHD grading. *Bone Marrow Transplant*. 1995;15:825-828.
23. Asano-Mori Y, Kanda Y, Oshima K, et al. Long-term ultra-low-dose acyclovir against varicella-zoster virus reactivation after allogeneic hematopoietic stem cell transplantation. *Am J Hematol*. 2008;83:472-476.
24. Kanda Y, Mineishi S, Saito T, et al. Pre-emptive therapy against cytomegalovirus (CMV) disease guided by CMV antigenemia assay after allogeneic hematopoietic stem cell transplantation: a single-center experience in Japan. *Bone Marrow Transplant*. 2001;27:437-444.
25. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis*. 2008;46:1813-1821.
26. Sparrelid E, Hägglund H, Remberger M, et al. Bacteraemia during the aplastic phase after allogeneic bone marrow transplantation is associated with early death from invasive fungal infection. *Bone Marrow Transplant*. 1998;22:795-800.
27. Krowka MJ, Rosenow EC 3rd, Hoagland HC. Pulmonary complications of bone marrow transplantation. *Chest*. 1985;87: 237-246.
28. Wald A, Leisenring W, van Burik JA, Bowden RA. Epidemiology of Aspergillus infections in a large cohort of patients undergoing bone marrow transplantation. *J Infect Dis*. 1997; 175:1459-1466.
29. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis*. 2002;34:909-917.
30. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. *Blood*. 2002;100:4358-4366.
31. Engelhard D, Elishoov H, Strauss N, et al. Nosocomial coagulase-negative staphylococcal infections in bone marrow transplantation recipients with central vein catheter. A 5-year prospective study. *Transplantation*. 1996;61:430-434.

Letter to the Editor

Complete molecular remission in refractory acute myeloid leukemia with MLL/AF9 treated with gemtuzumab ozogamicin

1. Introduction

Gemtuzumab ozogamicin (GO) is a calicheamicin-conjugated humanized anti-CD33 monoclonal antibody developed as new agent for targeted chemotherapy. It is used as drug for monotherapy or combination chemotherapy of CD33-positive acute myeloid leukemia (AML). We report a case of refractory AML with MLL/AF9 who achieved complete molecular remission with GO therapy.

2. Case report

A 72-year old man was diagnosed as AML (French-American-British (FAB) classification M5), and his leukemic blasts were positive for CD33 antigen. Cytogenetic investigation found 50, XY, +8, t(9;11)(p22;q23), +der(9)t(9;11), +14, +19 karyotype in 20/20 metaphase. Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) analysis showed 5×10^4 copies μg RNA^{-1} of MLL/AF9 fusion mRNA. We treated him with idarubicin (12 mg m^{-2} day^{-1} for 2 days) and cytarabin (100 mg m^{-2} day^{-1}; continuous infusion for 5 days) as induction chemotherapy. He achieved hematological complete remission (CR), but minimal residual disease (MRD) remained to be detectable with quantitative RT-PCR. After three-course of consolidation therapy (idarubicine and cytarabin, the same dose as induction therapy), he maintained hematological CR, but MRD was still positive. One month later from consolidation therapy, the relapse of AML was diagnosed with bone marrow aspiration. We administered a single dose of idarubicin (12 mg m^{-2}) as his bone marrow was hypercellular. One week later, we started re-induction therapy with fractionated doses of GO (3 mg m^{-2}; days 1, 4, 7) according to previous report [1]. He achieved hematological CR again and MRD was not detectable by RT-PCR (Fig. 1). No serious adverse effect occurred except for

grade 4 hematological toxicity and grade 3 febrile neutropenia. His leukemia has not relapsed for 4 months after re-induction therapy.

3. Discussion

Richard et al. reported the efficacy of GO in CD33-positive AML in first relapse; 26% of patients treated with GO achieved hematological CR and median recurrence free survival was 6.4 months for patients who achieved hematological CR [2]. We attempted to improve the efficacy and safety of GO therapy using two strategies. Firstly, we administered a single dose of idarubicin for cytoreduction prior to GO. Effectiveness of sequential or combinational administration of GO with conventional chemotherapy was reported [3]. Secondly, we administered fractionated doses of GO, which is recommended in some reports to avoid severe side effects [1]. In our case, although the patient was rather elderly, severe side effects (liver dysfunction, infusion reaction, etc.) did not occur.

MLL/AF9-positive AML is known to show poorer prognosis, compared to AML with wild type MLL [4]. Relapsed AML with MLL/AF9 in elderly shows especially poor prognosis. To our knowledge, this is the first report of relapsed MLL/AF9-positive AML treated with GO resulted in molecular CR. It was reported that the negativity of RT-PCR in hematological CR indicates lower cumulative incidence of relapse and better overall survival in AML with MLL/AF9 [4]. We believe GO therapy should be attempted for the cases with MRD as well as hematological relapse, especially in elderly patients.

Further studies are required to evaluate the efficacy and safety of our GO regimen; fractionated doses of GO following a single dose of idarubicin.

Conflict of interest

There is no conflict of interest to disclose.

Acknowledgments

References

[1] Taksin AL, Legrand O, Raffoux E, de Revel T, Thomas X, Contentin N, et al. High efficacy and safety profile of fractionated doses of Mylotarg as induction therapy in patients with relapsed acute myeloblastic leukemia: a prospective study of the alfa group. Leukemia 2007;21:66–71.
[2] Richard AL, Eric LS, Edward AS, et al. Final report of the efficacy and safety of Gemtuzumab Ozogamicin(Mylotarg) in patients with CD33-positive acute myeloid leukemia in first recurrence. Cancer 2005;104:1442–52.
[3] Sergio A, Stefan S, Roel W, et al. Sequential administration of gemtuzumab ozogamicin and conventional chemotherapy as first line therapy in elderly patients with acute myeloid leukemia: a phase II study(AML-15) of the EORTC and GIMEMA leukemia groups. Haematologica 2004;89:950–5.
[4] Claudia S, Richard FS, Karina E, et al. The prognostic value or MLL-AF9 detection in patients with t(9;11)(p22;q23)-positive acute myeloid leukemia. Haematologica 2005;90:1626–34.
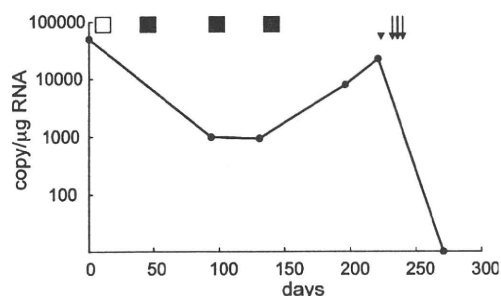
Fig. 1. Clinical course of the patient and copy number of MLL/AF9 fusion mRNA of bone marrow aspirates. Open square denotes induction therapy; closed square: consolidation therapy; arrowhead: idarubicin; arrow: GO. On day 271, MLL/AF9 fusion mRNA was not detected by RT-PCR. Minimal detection level was 50 copies μg RNA^{-1}.

Hiroki Asano
Go Yamamoto
Masataka Hosoi
Tsuyoshi Takahashi
Akira Hangaishi
Mineo Kurokawa *
Department of Hematology and Oncology,
Graduate School of Medicine, University of Tokyo,
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

* Corresponding author. Tel.: +81 3 5800 9092;
fax: +81 3 5840 8667.
E-mail address: kurokawa-tky@umin.ac.jp
(M. Kurokawa)

15 October 2009
Available online 12 January 2010

higher in SLE patients with antiphospholipid antibodies. We anticipated that the T-max would simply reflect the AUC and we were surprised to observe discordant results between the AUC and T-max. We do not have an explanation for this. Because a relatively low amount of phospholipid is used to initiate thrombin formation, it is conceivable that the prolonged lag time reflects the effect of antiphospholipid antibody in blocking the effect of phospholipid. While the increased T-max may reflect accelerated formation of thrombin, the prolonged T-lag may actually contribute toward the finding. In the calculation of T-max both the lag time and slope of the thrombin generation curve determine T-max. If indeed there is a higher rate of thrombin formation, the significance of this is unclear. It is conceivable that this may reflect an initial burst of thrombin formation that is dampened by anti-thrombin or other natural thrombin inhibitors. Regardless of this, overall our findings do not indicate that under the conditions we employed could we demonstrate increased thrombin potential. The limitation of our in vitro studies to reflect in vivo phenomenon, is that we are not using whole blood which includes the contribution of platelets and platelet microparticles.

Clearly the pathogenesis of thrombosis associated with antiphospholipid antibodies in SLE is multifactorial, involving not just the procoagulant proteins, the cellular constituents involving hemostasis, the anticoagulant mechanisms and fibrinolytic pathway. We know that the thrombotic complications are ameliorated by anti-thrombin agents including heparin and warfarin. This clearly argues for a key role of thrombin in the pathogenesis of the complications associated with antiphospholipid antibodies.

As we begin to correlate the results of thrombin generation tests with a variety of clotting and bleeding disorders, we are likely to gain a better understanding of the meaning of different parts of the thrombogram. The analysis of wave forms, of which the thrombogram is an example, is a new way to dissect out the complex interactions of coagulation proteins. The challenge is to correlate these parameters with clinical events or other biochemical or functional measurements of coagulation.

Our studies show that there are changes in the thrombogram associated with antiphospholipid antibodies and history of thrombosis in SLE. Future prospective study is now warranted to determine the predictive value of ETP measures for future thrombosis.

¹Cleveland Clinic, Department of Rheumatic and Immunologic Diseases, Baltimore, Maryland
²Division of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, Maryland
³Pathology Department, Special Coagulation Laboratory, Johns Hopkins University School of Medicine, Baltimore, Maryland
*Correspondence to: Michelle Petri; 1830 East Monument Street, Suite 7500, Baltimore MD 21205, USA., Telephone: 410-955-3823, Fax no: 410-614-0498.
E-mail: mpetri@jhmi.edu

References

1. Petri M. Systemic lupus erythematosus: 2006 update. J Clin Rheumatol 2006;12:37–40.
2. Cervera R, Khamashta MA, Font J, et al. Morbidity and mortality in systemic lupus erythematosus during a 5-year period. A multicenter prospective study of 1,000 patients. European Working Party on Systemic Lupus Erythematosus. Medicine (Baltimore) 1999;78:167–175.
3. Petri M, Rheinschmidt M, Whiting-O'Keefe Q, et al. The frequency of lupus anticoagulant in systemic lupus erythematosus. A study of sixty consecutive patients by activated partial thromboplastin time, Russell viper venom time, and anticardiolipin antibody level. Ann Intern Med 1987;106:524–531.
4. Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. Ann Intern Med 1990;112:682–698.
5. Martinez F, Forner MJ, Ruano M, et al. Factors related to the risk of thrombosis in patients with lupus and antiphospholipid antibodies. Med Clin (Barc) 2006;127:405–408.
6. Afeltra A, Vadacca M, Conti L, et al. Thrombosis in systemic lupus erythematosus: Congenital and acquired risk factors. Arthritis Rheum 2005;53:452–459.
7. Cines DB, Lyss AP, Reeber M, et al. Presence of complement-fixing anti-endothelial cell antibodies in systemic lupus erythematosus. J Clin Invest 1984;73:611–625.
8. Machin SJ. Platelets and antiphospholipid antibodies. Lupus 1996;5:386–387.
9. Ekdahl KN, Bengtsson AA, Andersson J, et al. Thrombotic disease in systemic lupus erythematosus is associated with a maintained systemic platelet activation. Br J Haematol 2004;125:74–78.
10. Hunt BJ, Khamashta MA. Antiphospholipid antibodies and the endothelium. Curr Rheumatol Rep 2000;2:252–255.
11. Nojima J, Kuratsune H, Suehisa E, et al. Acquired activated protein C resistance associated with anti-protein S antibody as a strong risk factor for DVT in non-SLE patients. Thromb Haemost 2002;88:716–722.
12. Gardiner C, Cohen H, Jenkins A, et al. Detection of acquired resistance to activated protein C associated with antiphospholipid antibodies using a novel clotting assay. Blood Coagul Fibrinolysis 2006;17:477–483.
13. Lopez-Pedrera C, Buendia P, Aguirre MA, et al. Antiphospholipid syndrome and tissue factor: a thrombotic couple. Lupus 2006;15:161–166.
14. Hemker HC, Al Dieri R, De Smedt E, et al. Thrombin generation, a function test of the haemostatic-thrombotic system. Thromb Haemost 2006;96:553–561.
15. Barrowcliffe TW, Cattaneo M, Podda GM, et al. New approaches for measuring coagulation. Haemophilia 2006;12 (Suppl 3):76–81.
16. Dargaud Y, Luddington R, Gray E, et al. Effect of standardization and normalization on imprecision of calibrated automated thrombography: An international multicentre study. Br J Haematol 2007;139:303–309.
17. Hemker HC, Giesen P, Al Dieri R, et al. Calibrated automated thrombin generation measurement in clotting plasma. Pathophysiol Haemost Thromb 2003;33:4–15.
18. Hemker HC, Al Dieri R, Beguin S. Thrombin generation assays: Accruing clinical relevance. Curr Opin Hematol 2004;11:170–175.
19. Petri M, Kim MY, Kalunian KC, et al. Combined oral contraceptives in women with systemic lupus erythematosus. N Engl J Med 2005;353:2550–2558.
20. Gladman D, Ginzler E, Goldsmith C, et al. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. Arthritis Rheum 1996;39:363–369.
21. Rugeri L, Beguin S, Hemker C, et al. Thrombin-generating capacity in patients with von Willebrand's disease. Haematologica 2007;92:1639–1646.
22. Brummel-Ziedins KE, Vossen CY, Butenas S, et al. Thrombin generation profiles in deep venous thrombosis. J Thromb Haemost 2005;3:2497–2505.
23. Dargaud Y, Trzeciak MC, Bordet JC, et al. Use of calibrated automated thrombinography +/- thrombomodulin to recognise the prothrombotic phenotype. Thromb Haemost 2006;96:562–567.
24. Tripodi A, Martinelli I, Chantarangkul V, et al. The endogenous thrombin potential and the risk of venous thromboembolism. Thromb Res 2007;121:353–359.
25. Tappenden KA, Gallimore MJ, Evans G, Mackie IJ, Jones DW. Thrombin generation: A comparison of assays using platelet-poor and -rich plasma and whole blood samples from healthy controls and patients with a history of venous thromboembolism. Br J Haematol 2007;139:106–112.
26. Pereira J, Alfaro G, Goycoolea M, et al. Circulating platelet-derived microparticles in systemic lupus erythematosus. Association with increased thrombin generation and procoagulant state. Thromb Haemost 2006;95:94–99.

HIV-negative, HHV-8-unrelated primary effusion lymphoma-like lymphoma: report of two cases

Tsuyoshi Takahashi, Akira Hangaishi, Go Yamamoto, Motoshi Ichikawa, Yoichi Imai, and Mineo Kurokawa*

Primary effusion lymphoma (PEL) is a rare type of lymphoma confined to the body cavities, such as pleural, pericardial, and peritoneal cavities. PEL is usually associated with human herpes virus 8 (HHV-8) and human immunodeficiency virus (HIV) infection, however, there are some reports of HIV-negative and HHV-8-unrelated cases. Recently, these cases are described as HHV-8-unrelated PEL-like lymphoma. Here, we report two
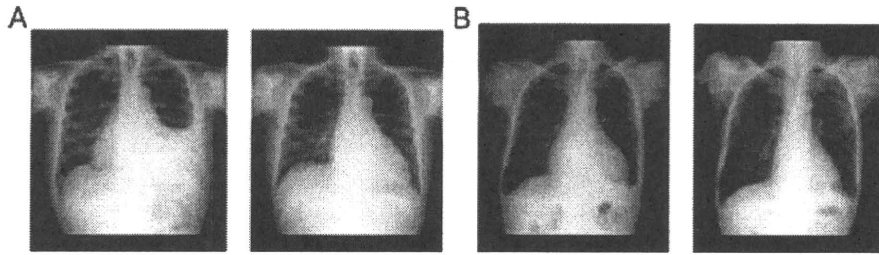
Figure 1. Chest X-rays of the cases. A: Chest X-rays when case 1 was diagnosed (left panel) and now (right panel). B: Chest X-rays when case 2 was diagnosed (left panel) and now (right panel).

such cases. In both cases, no lymphadenopathy or organ involvement with lymphoma was found. Surface marker revealed that they were both CD20 positive lymphoma. Systemic chemotherapy with CHOP regimen with rituximab was effective and gradually led to disappearance of the lymphoma. HHV-8-unrelated PEL-like lymphoma is truly a distinct clinical entity and the prognosis of it seems to be better than PEL.

PEL is a very rare type of non-Hodgkin lymphoma that involves only body cavities [1]. According to the World Health Organization (WHO) classification of hematological malignancies, PEL is classified as a subtype of diffuse large B-cell lymphoma that is closely associated with human herpes virus-8 (HHV-8) and HIV [2]. On the other hand, it has been reported that there are some patients with HHV8-negative and HIV-negative PEL that highly expresses B-cell markers, which are described as HHV-8-unrelated PEL-like lymphoma [2]. The reports of HHV-8-unrelated PEL-like lymphoma are anecdotal and the character of the lymphoma is not well known yet. Here, we report two cases of HHV-8-unrelated PEL-like lymphoma who were successfully treated with R-CHOP and review of the literature.

Case 1

A 82-year-old man went to an outpatient clinic because of edema of his lower extremities in January, 2008. He was found to have massive pericardial effusion, left pleural effusion, and sign of cardiac decompensation. Soon after admission, the patient was treated with drainage of the pericardial and pleural effusion. On cytological examination of the pleural and pericardial effusion, middle to large-sized atypical lymphoid cells were observed. The cells were positive for CD20 and CD79a, but negative for CD3. The immunoglobulin light chain restriction was also observed. He was suspected to have PEL and introduced to our hospital. When he was admitted, he had massive left pleural effusion and moderate pericardial effusion. The serum lactose dehydrogenase (LDH) level was 214 IU/L. Tests for hepatitis C virus (HCV) and HIV antibody were negative. Cytological evaluation of the pleural effusion demonstrated middle to large-sized atypical lymphoid cells with prominent nucleoli. The cell block preparation of pleural effusion revealed that atypical lymphoid cells were negative for HHV-8, but positive for EBER-ISH and EBNA2. The pleural effusion test for HHV-8 using polymerase chain reaction (PCR) method was also negative. No mass or lymphoma cells were detected on whole body CT scan, FDG-PET, and bone marrow biopsy. He was diagnosed as HIV-negative HHV-8-unrelated PEL-like lymphoma. The patient was treated with six courses of chemotherapy consisting of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP). After six courses of R-CHOP, the pleural effusion and pericardial effusion became little left. Now, 12 months passed since the last chemotherapy, and although the slight effusion is still left, disease status has continued to be stable without further treatment. Chest X-rays when the patient was diagnosed and now are shown in Fig. 1A.

Case 2

A 73-year-old man had edema of his lower thighs and he was diagnosed as having pericardial effusion, pleural effusion, and ascites on whole body CT scan. In January 2009, he had shortness of breath and came to the outpatient clinic of our hospital. He was diagnosed as cardiac decompensation with massive pericardial effusion and treated with drainage of it. On the cytological examination of the pericardial effusion, large atypical lymphoid cells with prominent nucleoli were observed. The cell block preparation of the pericardial effusion revealed that the cells were positive for CD20, but negative for CD3, HHV-8, and EBER-ISH. No masses or lymphoma cells were detected on whole body CT scan, FDG-PET, and bone marrow biopsy. Tests for HCV and HIV antibody were negative. He was diagnosed as HIV-negative HHV-8-unrelated PEL-like lymphoma. The patient was treated with six courses of R-CHOP therapy. After repeated courses of R-CHOP, the pericardial effusion and pleural effusion gradually decreased. However, after five courses of R-CHOP, liver dysfunction appeared. The ultrasonographic examination revealed that he had congestion of the liver due to recurred pericardial effusion. Aspiration of fluid from pericardium was performed twice. However, invasion of lymphoma cells were not detected in evaluation of cytology and flow cytometric analysis at this time. After that, liver dysfunction resolved and pericardial effusion was stable with slight pleural effusion. He was performed six round of R-CHOP treatment then discharged. Chest X-rays when the patient was diagnosed and now are shown in Fig. 1B.

PEL was originally described in 1989 as B-cell lymphomatous effusion in a body cavity without detectable tumor masses and associated with HHV-8 and HIV infection, mostly occurs in immunodeficiency status [1–3]. However, this entity has been reported in a small number of cases associated with HIV-negative HHV-8-unrelated PEL-like lymphoma [4–6]. The PEL lymphoma cells are usually negative for pan-B-cell markers, such as CD19, CD20, and CD79a. On the other hand, HIV-negative HHV-8-unrelated PEL-like lymphoma cells highly express B-cell markers. In our cases, the lymphoma cells also expressed CD20 and CD79a. As for the pathogenesis of PEL-like lymphoma, Tanaka et al. reported that some of these were EBV positive [7]. HCV had also been suggested to be an etiological agent [8]. Both of the present cases were HCV negative, although case 1 was EBV positive and case 2 was negative.

As to treatment, there is no standard chemotherapeutic regimen recommended for HIV-negative HHV-8-unrelated PEL-like lymphoma because of small numbers of reports. CHOP-like regimen had been frequently given in these cases. Recently, rituximab, an anti-CD20 monoclonal antibody, has been incorporated into the standard chemotherapy for many B-cell NHLs showing CD20 positivity. In both of our cases, we used rituximab containing regimen because the lymphoma cells were CD20 positive and it was effective in both cases.

The prognosis of PEL is poor and the median survival of PEL is less than 6 months, whereas the prognosis of HIV-negative HHV-8-unrelated PEL-like lymphoma may be better than that [9,10]. In our cases, one is alive for 21 months and another is alive for 9 months after their diagnoses. Thus prognosis of PEL-like lymphoma seems to be better than that of PEL as reported previously. In light of the cases from literature and our present ones, PEL and HIV-negative HHV-8-unrelated PEL-like lymphoma may have different pathogenesis, immunophenotypic features, and prognosis.

Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
*Correspondence to: Mineo Kurokawa, Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail: kurokawa-tky@umin.ac.jp

References

1. Nador RG, Cesarman E, Chadburn A, et al. Primary effusion lymphoma: A distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus. Blood 1996;88:645–656.

2. Carbone A, Gloghini A. PEL and HHV8-unrelated effusion lymphomas: Classification and diagnosis. Cancer 2008;114:225–227.
3. Knowles DM, Inghirami G, Ubriaco A, Dalla-Favera R. Molecular genetic analysis of three AIDS-associated neoplasms of uncertain lineage demonstrates their B-cell derivation and the possible pathogenetic role of the Epstein-Barr virus. Blood 1989;73:792–799.
4. Carbone A, Gloghini A, Vaccher E, et al. Kaposi's sarcoma-associated herpesvirus DNA sequences in AIDS-related and AIDS-unrelated lymphomatous effusions. Br J Haematol 1996;94:533–543.
5. Hermine O, Michel M, Buzyn-Veil A, Gessain A. Body-cavity-based lymphoma in an HIV-seronegative patient without Kaposi's sarcoma-associated herpesvirus-like DNA sequences. N Engl J Med 1996;334:272–273.
6. Ashihara E, Shimazaki C, Hirai H, et al. Human herpes virus 8-negative primary effusion lymphoma in a patient with a ventriculoperitoneal shunt tube. Int J Hematol 2001;74:327–332.
7. Tanaka S, Katano H, Tsukamoto K, et al. HHV8-negative primary effusion lymphoma of the peritoneal cavity presenting with a distinct immunohisto-chemical phenotype. Pathol Int 2001;51:293–300.
8. Paner GP, Jensen J, Foreman KE, Reyes CV. HIV and HHV-8 negative primary effusion lymphoma in a patient with hepatitis C virus-related liver cirrhosis. Leuk Lymphoma 2003;44:1811–1814.
9. Adiguzel C, Bozkurt SU, Kaygusuz I, et al. Human herpes virus 8-unrelated primary effusion lymphoma-like lymphoma: Report of a rare case and review of the literature. APMIS 2009;117:222–229.
10. Kobayashi Y, Kamitsuji Y, Kuroda J, et al. Comparison of human herpes virus 8 related primary effusion lymphoma with human herpes virus 8 unrelated primary effusion lymphoma-like lymphoma on the basis of HIV: Report of 2 cases and review of 212 cases in the literature. Acta Haematol 2007;117: 132–144.

—80—

Contents lists available at ScienceDirect

Leukemia Research

Letter to the Editor

A novel MLL-AF1p/Eps15 fusion variant in therapy-related acute lymphoblastic leukemia, lacking the EH-domains

Chromosomal translocation involving the *MLL* gene, located at 11q23, is the most frequent abnormality in hematological malignancies and more than 50 genes have been identified as MLL fusion partners. However, unifying leukemogenic properties of partner genes remain unclear. Here, we describe a novel MLL-AF1p/Eps15 (epidermal growth factor receptor pathway substrate 15) fusion variant in previously reported therapy-related acute lymphoblastic leukemia (ALL) patient [1] and argue for the function of AF1p as MLL fusion partner gene.

A 63-year-old female received high-dose dexamethasone and high-dose melphalan therapy followed by autologous peripheral blood stem cell transplantation for multiple myeloma. One year later, the patient was affected with ALL. The cytogenetic analysis revealed a reciprocal chromosomal translocation involving 1p32–34 and 11q23, and fluorescence *in situ* hybridization analysis showed split signals of the *MLL* gene.

In order to elucidate the break point between the *MLL* gene and the partner gene, we performed 3'-rapid amplification of cDNA ends (RACE) with the total RNA extracted from her bone marrow cells. We designed the first specific primer on *MLL* exon 6, the 5'-outer side of the break point cluster region (BCR) [2] of the *MLL* gene (5'-TCC AAA GCC TAC CTG CAG AAG C-3') and used the second specific primer on *MLL* exon 7, within the BCR (5'-TCA TCC CGC CTC AGC CAC CTA CTA CAG GAC CGC-3') [3]. Using these primers and the adaptor primer (5'-CTG ATC TAG AGG TAC CGG ATC C-3', TAKARA Bio Inc., Japan), we performed semi-nested polymerase chain reaction (PCR) and obtained a 2 kbp fragment as an incomplete fusion cDNA. Dye terminator sequencing of the fragment revealed a fusion mRNA with an in-frame junction between *MLL* exon 10 and *AF1p* exon 12 (Fig. 1A).

MLL N-terminus is reported not to be sufficient to immortalize cells, thus each partner gene must play an indispensable role in leukemogenesis. Recent studies proposed mainly two types of mechanisms in MLL-related leukemogenesis [4]. Firstly, some MLL fusion proteins directly alter transcriptional regulation of target genes with dependence on the function of their fusion partners (e.g. MLL-ENL and MLL-ELL/MEN [5]), most of which are nuclear proteins with transcriptional activity. In contrast, some MLL fusion partners do not have their own transcriptional activity, but bear self-association motifs or protein–protein interaction domains. Some of such fusion partners are cytoplasmic proteins (e.g. MLL-AF6 and MLL-Septin6). They alter the structure or the complex of MLL fusions, consequently modulating the transcriptional activities or the interaction between proteins.

AF1p is reported to be the latter type. Normal AF1p is localized to the plasma membrane clathrin-coated pits and vesicles and is involved in endocytosis. Through homophilic interaction with its coiled-coil (CC) domain, AF1p is constitutively dimerized or

oligomerized in normal cells [6]. Previously reported MLL-AF1p conserves almost all functional domains of AF1p (Fig. 1B) and is also dimerized with the CC-domain of AF1p [7,8]. In a colony replating assay using deletion mutants of MLL-AF1p, the deletion mutants lacking the EH-domain preserved colony replating capacity, while the mutants lacking the CC-domain did not [7]. It indicates the indispensability of the CC-domain and the dispensability of the EH-domain in murine leukemia. This MLL-AF1p variant found in our case lacks the EH-domain, but conserves the CC-domain (Fig. 1B). Therefore, the EH-domain is dispensable in actual human leukemogenesis similar to the murine model.

Furthermore, while the known MLL-AF1p fusion was identified primarily in cases with acute myeloid leukemia, this MLL-AF1p fusion variant is associated with ALL. Therefore, missing EH-domain
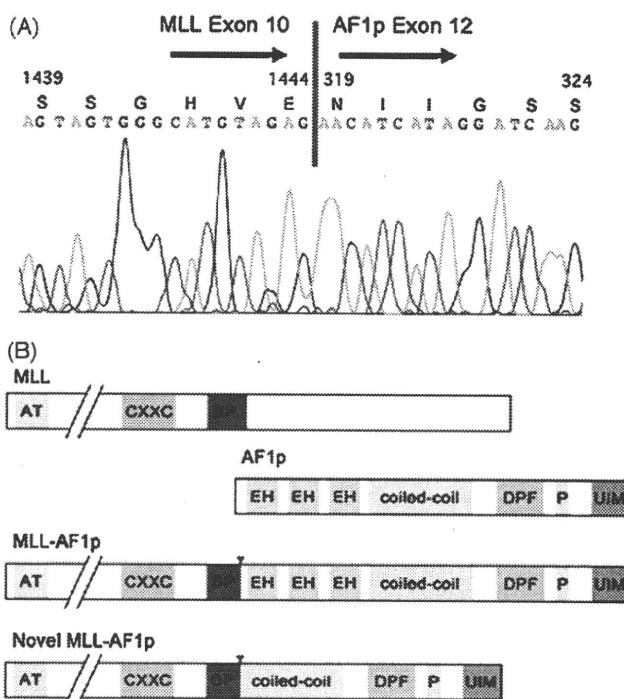
Fig. 1. The structure of the novel MLL-AF1p transcript and the amino acid sequence. (A) Sequencing analysis of the novel MLL-AF1p transcript from the product of 3'-RACE/semi-nested PCR. A break point of the fusion mRNA lies between *MLL* exon 10 and *AF1p* exon 12. (B) The schematic structure of the MLL-AF1p fusion proteins. MLL-AF1p: reported MLL-AF1p fusion, Novel MLL-AF1p: novel MLL-AF1p fusion lacking EH-domain, AT: AT-hook, CXXC: CXXC domain, BP: break point region, EH: Eps15 homology domain, coiled-coil: coiled-coil region, DPF: region rich in asparate-proline-phenylalanine repeats, P: proline rich region and UIM: ubiquitin-interacting motif.

might be involved in leukemic cell phenotype determination. In normal cells, the EH-domain of AF1p interacts with some proteins containing Asn-Pro-Phe (NPF) motifs [9]. The interaction with these proteins containing NPF motifs may modify the leukemogenic property of the fusion protein. In summary, this case suggests that each domain of MLL fusion partners play different roles in leukemogenesis.

Conflict of interest

The authors declare no conflicts of interest and no financial support.

References

[1] Ueda K, Yamamoto G, Shinohara A, Hangaishi A, Kurokawa M. Early onset of acute lymphoblastic leukemia with MLL rearrangement after autologous stem cell transplantation for multiple myeloma. Ann Hematol 2009;88:813–4.
[2] Gu Y, Alder H, Nakamura T, Schichman SA, Prasad R, Canaani O, et al. Sequence analysis of the breakpoint cluster region in the ALL-1 gene involved in acute leukemia. Cancer Res 1994;54:2327–30.
[3] Borkhardt A, Bojesen S, Haas OA, Fuchs U, Bartelheimer D, Loncarevic IF, et al. The human GRAF gene is fused to MLL in a unique t(5;11)(q31;q23) and both alleles are disrupted in three cases of myelodysplastic syndrome/acute myeloid leukemia with a deletion 5q. Proc Natl Acad Sci USA 2000;97:9168–73.
[4] Li ZY, Liu DP, Liang CC. New insight into the molecular mechanisms of MLL-associated leukemia. Leukemia 2005;19:183–90.
[5] Mitani K, Kanda Y, Ogawa S, Tanaka T, Inazawa J, Yazaki Y, et al. Cloning of several species of MLL/MEN chimeric cDNAs in myeloid leukemia with t(11;19)(q23;p13.1) translocation. Blood 1995;85:2017–24.
[6] Tebar F, Confalonieri S, Carter RE, Di Fiore PP, Sorkin A. Eps15 is constitutively oligomerized due to homophilic interaction of its coiled-coil region. J Biol Chem 1997;272:15413–8.
[7] So CW, Lin M, Ayton PM, Chen EH, Cleary ML. Dimerization contributes to oncogenic activation of MLL chimeras in acute leukemias. Cancer Cell 2003;4:99–110.
[8] Bernard OA, Mauchauffe M, Mecucci C, Van den Berghe H, Berger R. A novel gene, AF-1p, fused to HRX in t(1;11)(p32;q23), is not related to AF-4, AF-9 nor ENL. Oncogene 1994;9:1039–45.
[9] Rumpf J, Simon B, Jung N, Maritzen T, Haucke V, Sattler M, et al. Structure of the Eps15-stonin2 complex provides a molecular explanation for EH-domain ligand specificity. EMBO J 2008;27:558–69.

Akihito Shinohara [a], Motoshi Ichikawa [a], Koki Ueda [a], Tsuyoshi Takahashi [a], Akira Hangaishi [a], Mineo Kurokawa [a,b,*]
[a] *Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan*
[b] *Department of Cell Therapy and Transplantation Medicine, University of Tokyo, Tokyo, Japan*

·* Corresponding author at: Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Tel.: +81 3 5800 9092; fax: +81 3 5840 8667.
E-mail address: kurokawa-tky@umin.ac.jp (M. Kurokawa)

13 July 2009
Available online 8 August 2009