Case Report

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Salvage UCBT with Short-Term Melphalan-based Reduced-Intensity Conditioning for Primary Graft Failure after Upfront UCBT for Fulminant Aplastic Anemia

Mari Morita-Fujita[1,2], Tomohiro Yabushita[1,3], Satoshi Yoshioka[1], Nobuhiro Hiramoto[1], Takayuki Ishikawa[1]

[1]Department of Hematology, Kobe City Medical Center General Hospital, Kobe, Japan, [2]Department of Hematology, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [3]Division of Cellular Therapy, The Institute of Medical Science, University of Tokyo, Tokyo, Japan

Abstract

Umbilical cord blood transplantation (UCBT) is a potential option for patients with very severe aplastic anemia (VSAA) when no suitable related or unrelated donor is available. However, the high incidence of graft failure following UCBT remains a major challenge. The optimal conditioning regimen for UCBT in aplastic anemia (AA), particularly for salvage UCBT after graft failure following an initial transplant, remains undetermined. We report the cases of two adolescent patients with fulminant aplastic anemia who successfully underwent salvage UCBT, conditioned by a short-term melphalan-based regimen for primary graft failure after initial UCBT. The regimen comprised fludarabine (30 mg/m^2) on days -4 to -2, melphalan (40 mg/m^2) on days -3 and -2, and total body irradiation (2 Gy) on day -1. Neutrophil engraftment occurred in both cases approximately three weeks after salvage UCBT. One patient developed grade 1 acute graft-versus-host disease (GVHD) and mild chronic GVHD, while the other experienced no GVHD. Both patients have normal complete blood counts more than two years after salvage UCBT. These cases suggest that a short-term melphalan-based regimen may be a viable conditioning option for salvage UCBT in cases of primary graft failure.

Key words very severe aplastic anemia, cord blood transplantation, primary graft failure

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Correspondence: Tomohiro Yabushita, Department of Hematology, Kobe City Medical Center General Hospital, 2-1-1, Minatojima-Minamimachi Chuo-ku, Kobe 650-0047, Japan, E-mail: tomo527@kuhp.kyoto-u.ac.jp

Introduction

Umbilical cord blood transplantation (UCBT) is a valuable therapeutic option for patients with severe aplastic anemia (SAA) when a suitable human leukocyte antigen (HLA)-matched related or unrelated donor is unavailable or when graft failure occurs after the first transplant[1]. In rare instances, patients with SAA may develop severe neutropenia refractory to granulocyte colony-stimulating factor (G-CSF) treatment at the time of initial diagnosis. This condition, referred to as fulminant aplastic anemia (FAA), often results in poor response to immunosuppressive therapy (IST)[2]. In such cases, upfront UCBT may be a viable option if a suitable donor is not available. A major challenge with UCBT, however, is the high incidence of graft failure. In this case, an allogeneic hematopoietic stem cell transplantation (allo-HSCT) would be the only therapeutic option, except for autologous hematopoietic recovery. Most often, UCBT is chosen as the second transplant due to its availability[3]. However, the optimal conditioning regimen for a second UCBT has not been well established. Here, we report on two cases of FAA with severe infections who developed graft failure after the first UCBT but were successfully rescued by a second UCBT using a short-term, reduced-dose, melphalan-based conditioning regimen.

Case Presentations

A 16-year-old female was admitted with septic shock caused by *Streptococcus equisimilis* and pancytopenia. The patient had a white blood cell (WBC) of 0.3×10^9/L with 2.0% neutrophils, 95.0% lymphocytes, 2.0%

monocytes; hemoglobin (HB) of 5.7g/dL; reticulocytes (RET) of 0.41×10^9/L; and platelets (PLT) of 3.6×10^9/L. Bone marrow (BM) examination showed severe hypocellularity with no megakaryocytes and no morphologic dysplasia in all three lineages. The patient was diagnosed with very severe aplastic anemia (VSAA) based on standard diagnostic criteria. Despite immediate treatment with G-CSF and broad-spectrum antibiotics, her neutrophil count dropped to zero and septic shock became uncontrollable, requiring four granulocyte transfusions from two related donors to manage the septic shock. As no matched sibling donor or partially HLA-matched related donor was available, upfront UCBT was performed 18 days after admission using a 4/6 HLA-matched male donor with 3.35×10^7 cells/kg of total nucleated cells (TNC) and 0.97×10^5 cells/kg of $CD34^+$ cells. The conditioning regimen consisted of fludarabine (Flu, 30 mg/m^2 from day -7 to -2), melphalan (Mel, 40 mg/m^2 on day -3 and -2), and total body irradiation (TBI, 2 Gy on day -1 and 0) (**Figure 1 A**). Graft-versus-host disease (GVHD) prophylaxis included tacrolimus and mycophenolate mofetil (MMF, 30 mg/kg/day). One week post-transplant, the patient developed a fever without signs of engraftment syndrome[4], whereupon antibiotics, antipyretics, and hydrocortisone (50-200 mg/day) were administered. Although transient neutrophil recovery was observed, the count decreased again and never exceeded 0.5×10^9/L (**Figure 1B**). BM examination on day 28 revealed severe hypocellularity with activated macrophages engulfing hematopoietic cells. The donor cell chimerism decreased to 23% in the BM and 0% in peripheral blood, confirming primary graft failure. A second UCBT was performed 36 days after the first UCBT from a 4/6 HLA-matched female donor with 3.64×10^7 cells/kg of TNC and 1.62×10^5 cells/kg of $CD34^+$ cells. The conditioning regimen consisted of Flu (30 mg/m^2 from day -4 to -2), Mel (40 mg/m^2 on day -3 and -2) and TBI (2 Gy on day -1) (**Figure 1A**). GVHD prophylaxis was the same as the first UCBT. Neutrophil ($> 0.5 \times 10^9$/L) and PLT ($> 50 \times 10^9$/L) engraftment were confirmed on days 24 and 49, respectively. A BM examination on day 28 showed complete donor chimerism. Two years after the second UCBT, the patient has no signs of GVHD or late graft failure.

The second patient was a 17-year-old male admitted with pancytopenia. On admission, his WBC count was 1.7×10^9/L, with 7.5% neutrophils and 92.5% lymphocytes; HB was 9.7 g/dL; RET count was 0.96×10^{10}/L; and PLT count was 0.4×10^9/L. He was diagnosed with VSAA according to the standard criteria, and despite daily G-CSF treatment, he developed severe neutropenia ($0.01\text{-}0.11 \times 10^9$/L) and severe pneumonia caused by an unidentified organism. Upfront UCBT was performed 29 days after admission from a 4/6 HLA-matched female donor with 2.81×10^7 TNC/kg and 0.49×10^5 $CD34^+$ cells/kg. The same conditioning regimen and GVHD prophylaxis were used as in the first case. The neutrophil count showed a transient increase to 0.42×10^9/L on day 22 but subsequently dropped to zero (**Figure 1C**). During this period, the patient developed fever, erythroderma, pulmonary edema, and weight gain consistent with engraftment syndrome[4]. In addition to antibiotic and antipyretic treatment, a 50-200 mg daily dose of hydrocortisone was also administered. BM examination on day 35 revealed severe hypocellularity, with hemophagocytic macrophages and low donor cell chimerism (1%). Primary graft failure was diagnosed, and a second UCBT was performed 39 days after the first UCBT, using the same conditioning regimen and GVHD prophylaxis as in the first UCBT. A 4/6 HLA-matched female donor was used, with 2.29×10^7 cells/kg of TNC and 0.78×10^5 cells/kg of $CD34^+$ cells. Neutrophil and PLT engraftment were confirmed on days 20 and 51, respectively. The patient developed acute grade 1 GVHD and mild chronic skin GVHD, which improved only with topical corticosteroids. The patient remains alive two years after the second UCBT with no signs of late graft failure.

Discussion

Graft failure remains a major concern in allo-HSCT for VSAA, particularly when alternative donors are used[5]. Since autologous hematopoietic recovery is rare[6], a second allo-HSCT is often the only viable salvage option. When the first transplant is from an HLA-matched sibling, the same donor is often used[7,8]. Conversely, when the first transplant is from an alternative donor, another alternative donor, such as UCB, should be selected. Although treatment protocols for graft failure after upfront allo-HSCT in adolescents with VSAA have not been well standardized, this report provides valuable insights into managing such cases with a second UCBT.

Onishi et al. reported 22 successful outcomes from second allo-HSCT using UCBT for graft failure after the initial allo-HSCT in adult aplastic anemia (AA) patients[3]. Conditioning regimens involving Flu plus Mel or cyclophosphamide (CY) combined with low-dose TBI and MMF-containing GVHD prophylaxis were associated with successful engraftment. The type of graft failure, whether primary or secondary, did not significantly affect engraftment outcomes. Notably, infectious complications were the leading cause of death (10 of 13 cases, 76.9%), emphasizing the importance of facilitating rapid neutrophil recovery. Monitoring chimerism is essential for early graft failure detection[5], and prompt

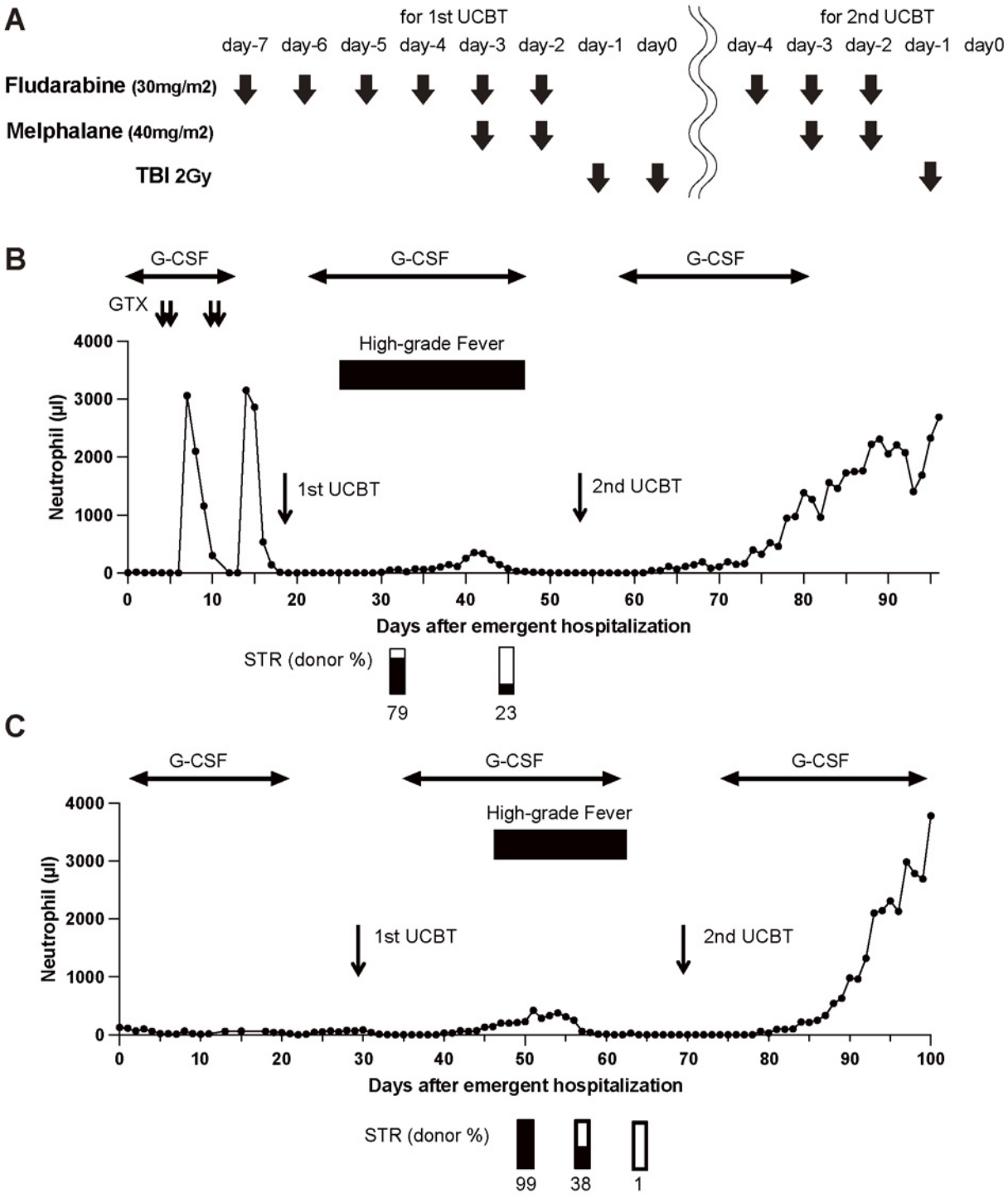

Figure 1. Conditioning regimens and clinical courses in the two presented cases
(A) The conditioning regimens used for umbilical cord blood transplantation (UCBT) in these two cases. The conditioning regimen of upfront UCBT for fulminant aplastic anemia is shown on the left. Short-term melphalan-containing reduced-intensity conditioning for 2nd UCBT after primary graft failure is shown on the right. (B and C) Clinical course of case 1 (B) and case 2 (C). Donor chimerism (%) was analyzed using multiplex amplification of short tandem repeat markers.
G-CSF, granulocyte colony-stimulating factor; GTX, granulocyte transfusion; UCBT, umbilical cord blood transplantation; STR, short tandem replication; TBI, total body irradiation.

second allo-HSCT can reduce the risk of serious infections and improve patient outcomes.

Some studies have proposed shortened conditioning regimens prior to the second UCBT for hematologic malignancies[9,10], which typically include Flu (30mg/m^2/day for 1-3 days), CY (2g/m^2 for 1 day), and TBI (2-4 Gy), collectively referred to as the one-day regimen[9,10]. In this study, we replaced CY with Mel (40mg/m^2/day

for 2 days) as part of a short-term reduced-intensity conditioning regimen. Specifically, we consistently used conditioning regimens comprising Mel (80 mg/m^2) plus Flu/low-dose TBI in both the first and second UCBTs. The original Flu/Mel (80 mg/m^2)/low-dose TBI regimen used in the first UCBT has been demonstrated as a reduced-intensity conditioning protocol for UCBT in hematologic malignancies[11]. Yamamoto et al. applied this regimen in the first UCBT for 12 SAA patients, achieving primary neutrophil engraftment in 11 of 12 cases[12]. Among these, two patients diagnosed with FAA underwent upfront UCBT without prior immunosuppressive therapy (IST), one of whom developed primary graft failure. These findings suggest that the Flu/Mel (80 mg/m^2)/low-dose TBI regimen may not be sufficient to suppress graft rejection in upfront UCBT for IST-naive SAA patients. Further research, including retrospective analyses using large registry data, is necessary to determine the optimal conditioning regimen for upfront allo-HSCT in SAA patients.

The Flu/CY-based regimen has been widely used as a reduced-intensity conditioning regimen in alternative allo-HSCT for AA patients[5]. A recent study of BMT for childhood AA using Japanese registry data reported that engraftment rates at day 60 were similar between the Flu/CY-based and Flu/Mel-based regimens. However, the Flu/CY-based regimen resulted in a higher incidence of late graft failure than the Flu/Mel-based regimen (11% vs 3%; p=0.035)[13]. Thus, Flu/Mel-based regimens may offer better long-term outcomes in UCBT, potentially reducing the incidence of late graft failure.

In conclusion, we successfully rescued two cases of primary graft failure after the first UCBT for FAA by performing a second UCBT with a short-term, melphalan-based reduced-intensity conditioning regimen. UCBT remains an important therapeutic option for SAA, particularly in cases of IST failure or graft failure after the first allo-HSCT. Further modifications to transplantation procedures should be pursued to overcome graft failure.

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Author Contributions

MMF, SY, TY, and NH were involved in the cases as the attending physician. MMF, SY and TY wrote the manuscript. TI supervised the cases and helped in writing the manuscript. All authors revised and approved the manuscript.

Conflicts of Interest

The authors declare no conflict of interest. Disclosure forms provided by the authors are available on the website.

Statement of Ethics

The cases were approved by the clinical ethics committee of the Kobe Medical Center General Hospital (Approval No. zn171211), and each patient signed a written informed consent to publish the case report.

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