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ORIGINAL ARTICLE

Unrelated cord blood transplantation after myeloablative conditioning in adults with advanced myelodysplastic syndromes

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We analyzed the disease-specific outcomes of adult patients with advanced myelodysplastic syndrome (MDS) treated with cord blood transplantation (CBT) after myeloablative conditioning. Between August 1998 and June 2009, 33 adult patients with advanced MDS were treated with unrelated CBT. The diagnoses at transplantation included refractory anemia with excess blasts ($n=7$) and MDS-related secondary AML (sAML) ($n=26$). All patients received four fractionated 12 Gy TBI and chemotherapy as myeloablative conditioning. The median age was 42 years, the median weight was 55 kg and the median number of cryopreserved nucleated cells was 2.51×10^7 cells per kg. The cumulative incidence of neutrophil recovery at day 50 was 91%. Neutrophil recovery was significantly faster in sAML patients ($P=0.04$). The cumulative incidence of plt recovery at day 200 was 88%. Plt recovery was significantly faster in CMV seronegative patients ($P<0.001$). The cumulative incidence of grade II–IV acute GVHD (aGVHD) and extensive-type chronic GVHD was 67 and 34%, respectively. Degree of HLA mismatch had a significant impact on the incidence of grade II–IV aGVHD ($P=0.021$). TRM and relapse at 5-years was 14 and 16%, respectively. The probability of EFS at 5 years was 70%. No factor was associated with TRM, relapse and EFS. These results suggest that adult advanced MDS patients without suitable related or unrelated BM donors should be considered as candidates for CBT.

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Keywords: cord blood transplantation; adult; myelodysplastic syndrome; myeloablative conditioning

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Introduction

The prognosis of advanced myelodysplastic syndrome (MDS) is poor. Although some patients with advanced MDS achieve remission with standard intensive chemotherapy, the duration is usually limited.[1] Therefore, Allo-SCT is considered as the only curative therapy for MDS patients. Alternative donor sources other than HLA-identical siblings have been used as allogeneic stem cell sources.[2–6] Recently, umbilical cord blood from unrelated donors has been used as an alternative stem cell source for adult patients.[7–15] However, reports of disease-specific outcomes for adult patients with advanced MDS after cord blood transplantation (CBT) are still limited. We have previously reported the results of a group of 19 adult patients with advanced MDS who received unrelated CBT.[16,17] Here, we have updated the results of unrelated CBT after myeloablative conditioning for 33 adult patients with advanced MDS. The main purpose of this retrospective single-center study was to confirm the safety and efficacy of unrelated CBT for adult advanced MDS patients after a myeloablative conditioning regimen, as well as to identify pretransplant factors related to the transplant outcomes on long-term follow-up.

Patients and methods

Patients

This was a retrospective single-center analysis. Between August 1998 and June 2009, 33 adult patients with advanced MDS were treated with unrelated CBT at The Institute of Medical Science, University of Tokyo. The diagnosis of MDS was made for all patients according to the World Health Organization classification. The diagnosis at transplantation included refractory anemia with excess blasts (1/2) ($n=7$) and MDS-related secondary AML (sAML) ($n=26$). MDS-related sAML was defined as AML that developed during the follow-up period of MDS. The cytogenetic subgroups according to a transplantation-specific cytogenetics grouping for MDS[18] were adverse risk (abnormalities of chromosome 7 and complex karyotype) in 10 patients and standard risk (all others) in 23 patients. Written informed consent for treatment was obtained from all patients.

Cord blood unit selection
The HLA-A and HLA-B Ags were identified by serological typing. HLA-DRB1 alleles were determined by high-resolution molecular typing using PCR sequence-specific primers. Patients without a suitable, closely HLA-matched family donor were eligible for CBT if a matched unrelated BM donor was unavailable as a first treatment option. If there was insufficient time for an unrelated BM donor search because of disease status or because preliminary search indicated a low likelihood of obtaining a matched unrelated BM donor, we attempted to locate cord blood grafts. Preferred cord blood units matched three or more of six HLA loci and contained a minimal cell count of 1.5×10^7 nucleated cells per kg before freezing. All but one cord blood units were obtained from cord blood banks in the Japan Cord Blood Bank Network.

Conditioning
All patients received four fractionated 12 Gy TBIs on days −9, −8 or on days −8 and −7, recombinant human G-CSF combined Ara-C and CY. Ara-C was administered i.v. over 2 h at a dose of 3 g/m^2 every 12 h on days −6 and −5 or on days −5 and −4 (total dose, 12 g/m^2). G-CSF was administered by continuous infusion at a dose of 5 μg/kg per day. Infusion of G-CSF was started 12 h before the first dose of Ara-C and stopped at the completion of the last dose. CY was administered i.v. over 2 h at a dose of 60 mg/kg once daily on days −4 and −3 or on days −3 and −2 (total dose, 120 mg/kg). At 2 or 3 days after the completion of conditioning, patients received a CBT.

GVHD prophylaxis
All patients received standard CYA and MTX as GVHD prophylaxis. CYA was given i.v. every day starting on day −1 at a dose of 3 mg/kg per day. MTX (15 mg/m^2 i.v.) was given on day 1, and 10 mg/m^2 of MTX was administered on days 3 and 6. Once oral intake could be tolerated, patients were administered oral CYA at a dose of 1:2, in two divided doses per day, on the basis of the last i.v. dose. In the absence of GVHD, CYA was tapered beginning between weeks 6 and 9 until it could be discontinued in the absence of chronic GVHD (cGVHD) between 6 and 12 months after transplantation. CYA was reduced when serum creatinine levels rose 1.5 times above baseline or when other serious agent-associated toxicities occurred. Physicians could freely modify the CYA dose for patients experiencing severe acute GVHD (aGVHD) or risk of disease relapse. Corticosteroid-based treatment was considered when grade II or higher severe aGVHD occurred (0.5–2 mg/kg).

Supportive care
All patients received G-CSF by i.v. infusion starting on day 1 until durable granulocyte recovery was achieved. The supportive care regimen, including prophylaxis for infection, was the same as previously reported.[13]

End points and definitions
Myeloid engraftment was defined as the first of the 3 consecutive days during which the ANC was at least 0.5×10^9 cells per l. Plt recovery time was achieved on the first of 3 days when the plt count was higher than 5×10^9 cells per l without transfusion support. The chimerism status after CBT was determined either by FISH with a Y chromosome probe for sex-mismatched CBT or by quantitative PCR analysis for microsatellite DNA markers. Primary engraftment failure was defined as the absence of donor-derived myeloid cells on the day of death, the day of relapse and on day 60 in patients surviving after CBT. A second allogeneic transplantation or autologous hematopoietic reconstitution before donor-derived myeloid recovery was considered as primary engraftment failure. Both aGVHD and cGVHD were graded according to the previously published criteria.[19,20] TRM was defined as death from any cause except relapse. Relapse was defined by morphological evidence of disease in peripheral blood, BM or extramedullary sites. EFS was defined as the time from CBT to graft failure, relapse, death or the last observation.

Statistical analysis
Cumulative incidences were estimated for hematopoietic recovery, GVHD, TRM and relapse to take competing risks into account. The probability of EFS was estimated from the time of CBT according to the Kaplan–Meier method. Variables considered in univariate analysis were body weight, age, recipient sex, degree of HLA matching, recipient CMV serology, diagnosis (MDS-related sAML or others), cytogenetic subgroups, year of transplant, total nucleated cell dose and CD34-positive cell dose. Variables with a value of $P<0.1$ for each end point were tested in multivariate analysis. End points were calculated at the last contact, the date of the last follow-up being 1 December 2009.

Results

Characteristics of patients and cord blood units
The characteristics of 33 patients and cord blood units are shown in Table 1. Among the patients, the median age was 42 years (range, 19–52 years), the median weight was 55 kg (range, 41–75 kg), the median number of cryopreserved nucleated cells was 2.51×10^7 cells per kg (range, 1.71–4.60×10^7 cells per kg), and the median number of cryopreserved CD34-positive cells was 0.91×10^5 cells per kg (range, 0.27–2.14×10^5 cells per kg). All patients received a single and HLA-mismatched cord blood unit.

Hematopoietic recovery
A total of 30 patients had myeloid reconstitution, and the median time to achieve more than 0.5×10^9 cells per l ANC was 22 days (range, 18–35 days). Three patients experienced primary engraftment failure. Of the three patients, one relapsed at day 53 and two died at days 24 and 28 without myeloid engraftment. The cumulative incidence of neutrophil recovery at day 50 was 91% (95% confidence interval

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Time from cord blood collection to processing and temperature influence the quality of mononuclear cell products isolated using a density-gradient protocol

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Background: For clinical cord blood (CB) transplantation, CB is processed using a standard hydroxyethyl starch protocol generally within 48 h of collection at room temperature. However, for tissue stem cell research, mononuclear cells (MNCs) were isolated from CB using a Ficoll-Paque density-gradient method. Here we report the effect of storage temperature and time from CB collection to processing on the cord blood mononuclear cells (CB-MNCs) isolated using a density-gradient method.

Methods: We processed CB using a Ficoll-Paque density-gradient method to collect the cells in the MNC layer. Cells were analyzed using an automatic blood cell counter, and $CD34^+$ cells were counted according to the ISHAGE method.

Results: The recovery rate of viable MNCs in the CB-MNC layer was inversely related to the time from collection to processing of CB samples. However, recoveries of total nucleated cell and $CD34^+$ were not affected by the time from collection to processing. The percentage of neutrophil contamination in the MNC layer increased significantly with increasing time from CB collection to processing (n=100, $p<0.0001$). Furthermore, CB stored at low temperatures had significantly less neutrophil contamination in the MNC layer than those stored at room temperature for 30 h after CB collection.

Discussion: Storage temperature and time from collection to processing influence the composition of CB-MNCs products processed using a Ficoll-Paque density-gradient method.

Keywords: cord blood (CB), mononuclear cells (MNCs), cord blood (CB) processing, density-gradient method, storage, viability, recovery

第58回日本輸血・細胞治療学会総会座長推薦論文

Introduction

Cord blood (CB) cells are of interest not only as a source of hematopoietic stem cells but also tissue stem cells, which have potential therapeutic applications in regenerative medicine. The National Research Cord Blood Stem Cell Resource Bank (http://scb.ims.u-tokyo.ac.jp/), the first stem cell bank established in the world, began in 2003 as part of a project to realize the potential of regenerative medicine. It is organized by the Ministry of Education, Culture, Sports, Science and Technology in Japan. The research CB banks isolated cord blood nucleated cells (CB-NCs), $CD34^+$ cells, and mononuclear cells (CB-MNCs) from nonconforming CBs for clinical use, and supplies CB in research-use for domestic researchers via Riken Bioresource Center. In this project, we first processed CB-NCs using a hydroxyethyl starch (HES) centrifugation method, as used clinically for CBB, and provided the frozen CB-NCs units to researchers. However, when frozen CB-NCs units are thawed by the ordinary thawing method, such as by mixing with a large volume of medium, aggregation often occurs due to a large quantity of residual neutrophils and red blood cells. In this situation, general researchers find it difficult to process and con-

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tinue the culture of frozen CB-NCs. In addition, many researchers in the field of regenerative medicine have reported that CB-MNCs contain a potential source for regeneration[1)~4)]. Therefore, since 2008, we have started to release CB-MNCs products for researchers. CB-MNCs are processed by the well-known density-gradient method based on differences in cell size and density. To assure the quality of CB-MNC products for research use, we set the following quality criteria: 1) informed consent from the mother, 2) negative for infection and genetic background, 3) initiation of CB processing within 36 h of CB collection in obstetrics, 4) neutrophil contamination in the CB-MNCs products of less than 20%, and 5) more than 1×10^7 of MNC per tube. In this setting, we found that neutrophil contamination often exceeded 20%. Here, to resolve the problem of neutrophil contamination, we investigated potential factors, including time and temperature, which may be critical to the excess of neutrophil contamination.

CB stored at room temperature (RT) is preferred to CB stored at a low temperature because platelets in CB units may aggregate at low temperatures[5)~8)]. Guidelines for the store period of CB samples differ. According to the guidelines (http://www.factwebsite.org/Standards/) of many countries except Japan, CB samples should be processed within 48 h of collection in obstetrics, whereas guidelines released by the Japan Cord Blood Bank Network suggest that CB samples be processed within 24 h (https://www.j-cord.gr.jp/ja/bank/technical.html). However, the effects of storage time and temperature on the isolation of CB-MNCs using a density-gradient method are not fully understood. Here we show that time from collection to processing and storage temperature influence the quality of CB-MNC products processed using a Ficoll-Paque density-gradient method.

Materials and Methods

CB collection, store and transportation to IMSUT-CRC

The study for Research Cord Blood Stem Cell Resource Bank Project was approved by the Ethical Committee of the Institute of Medical Science, The University of Tokyo (IMSUT), Japan, and by the Tokyo Cord Blood Bank (Tokyo CBB). Informed consent was obtained from the mothers involved in this project. CB was collected in collection bag (Kawasumi, KBS-200CA 8, Japan) and stored at RT (around 20℃) in a plastic store box setting in a delivery room of obstetrics until the CB units were picked up at around 8 : 30 am every day. Therefore, some CB units collected in the prior day were stored overnight (up to 24 hours), and others collected in the early morning (until CB units were picked up) were stored shortly in the obstetrics. The collected CB units in the obstetrics were transported to Tokyo CBB at RT by the courier every morning. It almost constantly took less than one hours to transport CB from obstetrics to Tokyo CBB. CB units were determined to be conforming or not for transplantation in Tokyo CBB. The nonconforming CB units were anonymized in the Tokyo CBB and subsequently transported to the research cord blood stem cell bank, namely, IMSUT-Cell Resource Center (IMSUT-CRC). The transportation was carried by the courier or immediately transported by the staff of IMSUT-CRC or Tokyo CBB. During transportation, CB bags were placed at RT in foam polystyrene boxes with cushions. After reception of the bags, the bags were preserved at 15℃ until processing.

CB processing

One hundred CB units were processed into MNCs using the bioclean cabinet installed in the IMSUT-CRC. The time from CB collection to processing was defined as the total time elapsed since CB collection at obstetrics and evaluation of conformity at Tokyo CBB until the initiation of CB processing into MNCs at IMSUT-CRC.

After 0.5-m*l* pre-processed CB sample was drawn for the testing, the CB was diluted to the appropriate volume (up to the multiples volume of 30 m*l*) with saline. Then 30 m*l* of CB was poured onto filters inside 50 m*l*-LeucoSep tubes (Greiner bio-one GmbH, Frickenhausen, Germany) that had been pre-filled with 15 m*l* of Ficoll-Paque PLUS (GE Healthcare UK Ltd., Buckinghamshire, UK). Then, the tubes were centrifuged at 1,000 x g at 20℃ for 20 min. After centrifugation, the MNC layer was collected and washed twice with 30 m*l* of PBS. The cell suspension was adjusted to 10 m*l* with PBS, and 0.5 m*l* of the post-processing sample was used to calculate cell numbers and differential.

To estimate the effect of storage temperature, we divided CB units into four equal parts, respectively. One group was initiated to be processed at 12 h after CB collection, whereas the remaining three were stored for 30 h elapsed since CB collection to processing at 4℃, 15℃, and RT, respectively.

bjh research paper

Outcome of unrelated umbilical cord blood transplantation in 88 patients with primary immunodeficiency in Japan

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Summary

We report the results of umbilical cord blood transplantation (UCBT) performed in 88 patients with primary immunodeficiency (PID) between 1998 and 2008 in Japan; severe combined immunodeficiency (SCID, $n = 40$), Wiskott–Aldrich syndrome (WAS, $n = 23$), chronic granulomatous disease ($n = 7$), severe congenital neutropaenia (SCN, $n = 5$) and other immunodeficiencies ($n = 13$). Five-year overall survival (5-year OS) for all patients was 69% [95% confidence interval (CI), 57–78%], and was 71% and 82% for SCID and WAS, respectively. The main cause of death before day 100 was infection (17/19), while that after day 100 was graft-versus-host disease (GVHD) (5/7). Using multivariate analyses, pre-transplant infection, no conditioning, ≥2 human leucocyte antigen (HLA) mismatches or diagnosis other than SCID, SCN or WAS were all associated with poor prognosis. Reduced-intensity conditioning was associated with decreased overall mortality compared with myeloablative therapy. The cumulative incidence of grade 2–4 acute GVHD at day 100 was 28% (95% CI, 19–38%), and that of chronic GVHD at day 180 was 13% (95% CI, 7–23%). We conclude that UCBT should be considered for PID patients without an HLA-matched sibling. The control of pre-transplant infection and selection of HLA-matched donors will lead to a better outcome.

Keywords: primary immunodeficiency, severe combined immunodeficiency, Wiskott-Aldrich syndrome, cord blood transplantation, reduced-intensity conditioning.

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Allogeneic haematopoietic stem cell transplantation (HSCT) has been successfully used as a curative therapy for most severe forms of primary immunodeficiency (PID) (Zeidler *et al*, 2000; Antoine *et al*, 2003; Sakata *et al*, 2004; Rao *et al*, 2005; Kobayashi *et al*, 2006; Mazzolari *et al*, 2007; Dvorak & Cowan, 2008; Griffith *et al*, 2008; Cuvelier *et al*, 2009). Stem cell transplantation from a human leucocyte antigen (HLA)-identical family donor provides better prognosis than bone marrow transplantation from an unrelated donor (Antoine *et al*, 2003). Survival with this type of transplantation from a matched unrelated donor has improved significantly over the years in patients with severe combined immunodeficiency (SCID), whereas no improvement in survival has been observed with this transplantation in non-SCID patients (Antoine *et al*, 2003). The optimal stem cell source for PID patients with no HLA-identical sibling remains to be determined (Dvorak & Cowan, 2008; Griffith *et al*, 2008; Cuvelier *et al*, 2009).

Umbilical cord blood grafts from unrelated donors have been successfully used, primarily in children and subsequently in adults (Kurtzberg *et al*, 1996; Wagner *et al*, 1996; Gluckman *et al*, 1997; Rubinstein *et al*, 1998; Rocha *et al*, 2000, 2004; Laughlin *et al*, 2004). Theoretically, unrelated cord blood transplantation (UCBT) has the following distinct advantages in PID patients: (i) the cord blood product is rapidly accessible in most cases; (ii) the incidence and severity of graft-versus-host disease (GVHD) is not excessive, even in mismatched transplantation and (iii) the risk of latent viral transmission is low. The disadvantages of UCBT include slower haematopoietic/immunological reconstitution and graft failure, which have been observed with UCBT for malignant disorders, and naivety of lymphocytes to pathogens (Brown *et al*, 2008; Griffith *et al*, 2008; Szabolcs *et al*, 2008). Rapid immune reconstitution is particularly important in PID patients with ongoing infection who undergo UCBT.

The limited data available show that UCBT can be a curative measure in patients with SCID, Wiskott–Aldrich syndrome (WAS), chronic granulomatous disease (CGD) and severe congenital neutropaenia (SCN) (Knutsen & Wall, 2000; Bhattacharya *et al*, 2003, 2005; Fagioli *et al*, 2003; Knutsen *et al*, 2003; Kobayashi *et al*, 2006). Most of the available data have come from a single centre, and thus, detailed information on the outcome and problems associated with UCBT in PID patients is still lacking. In this study, we report the results of UCBT performed in 88 PID patients between 1998 and 2008 in Japan.

Methods

Collection of data

All UCBTs carried out for PIDs through the Japan Cord Blood Bank Network (JCBBN) between August 1998 and January 2008 was enrolled in this study. Eighty-eight patients with PID underwent UCBT during this period. All data were provided by JCBBN, which collects recipients' clinical information at day 100 after transplantation. Recipients' data on survival, disease status and long-term complications are renewed annually by administering follow-up questionnaires. Latest data acquisition was performed in November 2009. The present study was approved by the institutional ethical and data management committees of JCBBN.

Patients

A summary of patients enrolled in this study is shown in Table I. Forty patients had SCID (45%) and 48 (55%) had non-SCID. Patients with familial haemophagocytic syndrome were not included in this study. The median age at the time of transplantation was 10 months (range, 0–248 months).

Procedures

Cryopreserved, unrelated cord blood cells were used as the source of haematopoietic stem cells. The type of conditioning used and median cell dose infused are shown in Table I.

In most cases, HLA matching was performed by both serological and DNA typing for HLA-A, HLA-B and HLA-DRB1. In this study, HLA mismatch was defined according to serological or low-resolution molecular typing for HLA-A and HLA-B and high-resolution molecular typing for HLA-DRB1. Of the UCB donors, 29 (33%) were HLA fully compatible. Of the mismatched donors, 40 (45%) were 1-antigen mismatched, 15 (17%) were 2-antigen mismatched and four (5%) were 3-antigen mismatched (Table I). In 48 patients in whom high-resolution genotypical typing was performed for HLA-A, HLA-B and HLA-DRB1, 11 were fully matched, 13 were 1-antigen mismatched, 16 were 2-antigen mismatched, five were 3-antigen mismatched and three were 4-antigen mismatched.

Immunosuppressive prophylaxis against GVHD after UCBT consisted of ciclosporin A (CyA)- and tacrolimus-based regimens in 48 and 35 patients, respectively. Five patients were not administered any immunosuppressive drug after UCBT.

Various techniques including karyotyping, HLA typing and fluorescence *in situ* hybridization for the XY chromosome and variable number of tandem repeats were used to confirm the engraftment of donor cells.

Definitions

Neutrophil recovery was defined by an absolute neutrophil count of at least $0{\cdot}5 \times 10^9$/l for three consecutive days. Platelet recovery was defined by a count of at least 20×10^9/l, unsupported by transfusion for 7 d. Reticulocyte recovery was defined by a count of at least 20‰.

Patients without conditioning or with only anti-thymocyte globulin (ATG) were categorized as receiving no conditioning. Patients administered busulfan (BU)/cyclophosphamide (CY) ± total body irradiation (TBI) or total lymphoid irradiation

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Cord Blood Transplantation from Unrelated Donors for Children with Acute Lymphoblastic Leukemia in Japan: The Impact of Methotrexate on Clinical Outcomes

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Cord blood transplantation (CBT) from an unrelated donor is recognized as one of the major treatment modalities in allogeneic stem cell transplantation (SCT) for children with hematologic malignancies. We analyzed the clinical outcomes of CBT for children with acute lymphoblastic leukemia (ALL) in Japan and identified the risk factors for the transplant outcomes. From 1997 to 2006, 332 children with ALL underwent CBT from unrelated donors, 270 of which had no prior transplant. Their disease statuses at transplant were first complete remission (CR) (n = 120), second CR (n = 71), and more advanced stages (n = 75). As preconditioning for SCT, total body irradiation (TBI) was given to 194 patients and, for the prophylaxis of graft-versus-host disease (GVHD), methotrexate (MTX) was given to 159 patients. The cumulative incidents of neutrophil and platelet recovery (>20 K) were 88.5% and 78.4%, respectively. The incidents of grade II-IV, III-IV acute GVHD (aGVHD), and chronic GVHD (cGVHD) were 45.6%, 20.4%, and 19.2%, respectively, and treatment-related mortality was 22.6%. The 5-year event-free survival (EFS) and overall survival (OS) at CR1, CR2, and advanced status were 47.4%, 45.5%, 15.0%, and 63.7%, 59.7%, and 20.7%, respectively. Multivariate analysis revealed that MTX with calcineurin inhibitor (CNI) was associated with decreased incidence of grade II-IV GVHD (CNI alone: hazard ratio [HR] = 1.74, 95% confidence interval [CI] = 1.06-2.83, $P = .027$; CNI + prednisolone (PSL), HR = 1.61, 95% CI = 1.03-2.50, $P = .036$), III-IV aGVHD (CNI alone: HR = 3.02, 95% CI = 1.55-5.91, $P = 0.001$; CNI + PSL, HR = 1.89, 95% CI = 0.93-3.83, $P = .078$), or cGVHD (CNI alone: HR = 1.78, 95% CI = 0.83-3.82, $P = .143$; CNI + PSL, HR = 2.44, 95% CI = 1.24-4.82, $P = .01$), compared with CNI alone or CNI + PSL. At an advanced stage of disease, GVHD prophylaxis with MTX + CNI is associated with improved OS compared with CNI alone (CNI alone: HR = 3.20, 95% CI = 1.43-7.15, $P = .005$; CNI + PSL, HR = 1.47, CI = 0.67-3.20, $P = .332$). Our retrospective study showed that CBT for children with ALL is feasible and GVHD prophylaxis with MTX + CNI is associated with significant favorable outcomes in prevention of aGVHD and cGVHD as well as survival advantage in advanced cases.

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KEY WORDS: Cord blood transplantation, Acute lymphoblastic leukemia, HLA, Methotrexate

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INTRODUCTION

Multiagent chemotherapy for children with acute lymphoblastic leukemia (ALL) has achieved excellent clinical outcomes in recent years [1,2]. However, those patients who relapsed during or after chemotherapy or those with very high-risk features, such as Philadelphia chromosome-positive ALL (Ph+ALL) or infant ALL with mixed lineage leukemia (MLL) gene rearrangement, are proposed as candidates for allogeneic stem cell transplantation (SCT) [3-6] at their first remission. Patients and donors are required to be compatible in terms of human leukocyte antigen (HLA) for better transplant outcome and, if they lack an HLA-identical related donor, they have options of alternative donors, such as bone marrow transplantation (BMT), peripheral blood stem cell transplantation (PBSCT), cord blood transplantation (CBT) from an unrelated donor, or transplantation from an HLA-haploidentical family donor [7-9]. Out of these four treatment modalities, CBT has advantages such as immediate availability of a CB unit for an urgent transplant, lower risks of severe acute and chronic graft-versus-host disease (aGVHD, cGVHD), and a less stringent requirement of HLA compatibility than unrelated or haploidentical BMT. In Japan, the Japan Cord Blood Bank Network (JCBBN) was established in 1999, and 11 local cord blood banks are affiliated to JCBBN where more than 7000 CBT were performed by the end of 2010. Here, we report the clinical outcomes and risk factors of children with ALL who underwent CBT in Japan.

PATIENTS AND METHODS

Patient and Donor Characteristics

From 1997 to 2006, 332 unrelated CBT were performed for children with ALL and 270 transplantations were undertaken as the first SCT in Japan. Because the overall survival (OS) of patients who underwent transplantation as the first SCT was significantly better than that of those with prior SCT (50.3% vs 12.7%, $P < .001$), we restricted this analysis to only patients with no prior SCT in order to interpret the exact risk factors of CBT. The patient and donor characteristics are shown in Table 1. Patients transplanted at first complete remission (CR1) (n = 120) include 41 infant ALL and 17 patients with Ph +ALL.

The HLA typing of cord blood units was performed in each CB bank by low-resolution molecular typing of HLA-A and B, combined with high-resolution molecular typing of DRB1. The high-resolution molecular typing for 3 loci of HLA-A, B, and DRB1 was performed in 187 patients.

In JCBBN, CB units of 0 to 2 HLA antigen mismatches with the patient were allowed for transplantation, and the minimum number of nucleated cells recommended for transplantation was 2×10^7/kg of patient body weight at cryopreservation.

Transplantation

All CB units were provided from the 11 local CB banks affiliated to JCBBN, and all transplant institutions were required to meet the minimum requirements of JCBBN in terms of experience of allogeneic SCT. The numbers of transplanted cells, preconditioning, as well as GVHD prophylaxis are shown in Table 1. Supportive care after transplantation, such as gut decontamination, empirical administration of antibiotics, prophylaxis or treatment of cytomegalovirus (CMV) infection, was performed according to each institutional protocol. Grading of GVHD was performed according to the standard criteria [10].

Definition and Statistics

The median duration of follow-up was 438 days (range: 10-3293 days). In this study, rates of neutrophil and platelet engraftment, incidents of aGVHD and cGVHD, leukemic relapse, nonrelapse mortality (NRM), and probabilities of event-free survival (EFS) and OS were analyzed. The variables evaluated included recipient age, sex, sex mismatch, disease status at transplants (CR1/CR2 vs advanced disease), ABO compatibility, HLA matching by low- and high-resolution typing, number of nucleated cells, colony-forming unit-granulocyte-macrophage (CFU-GM) and CD34-positive cells of the cord blood units at cryopreservation conditioning regimens with total body irradiation (TBI), administration of granulocyte-colony stimulating factor (G-CSF), GVHD prophylaxis (calcineurin inhibitor [CNI] alone, CNI + methotrexate [MTX] versus CNI + prednisolone [PSL]), mixed lineage leukemia (MLL) gene rearrangement, t(4;11), and transplantation year. Because the information of high-resolution DNA typing was only available for a limited number of patients, it was not included in the multivariate analysis. The day of neutrophil engraftment was defined as the first day of 3 consecutive days with absolute neutrophil count (ANC) $\geq 500/mm^3$, and that of platelet engraftment was the first day of platelet count over $20,000/mm^3$ without transfusion. The treatment-related mortality was defined as all causes of nonleukemic deaths after transplantation. The EFS was defined as patients who are alive in CR with engraftment. The probabilities of OS and EFS were calculated by the method of Kaplan and Meier. The log-rank test was used for group comparisons. Time-to-event outcomes for neutrophil and platelet engraftment, treatment-related mortality, relapse,

ASBMT
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Comparison of Unrelated Cord Blood Transplantation and HLA-Mismatched Unrelated Bone Marrow Transplantation for Adults with Leukemia

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Recent advances in unrelated cord blood transplantation (UCBT) and high-resolution typing of human leukocyte antigen (HLA) from an unrelated donor have increased choices in alternative donor/stem cell source selection. We assessed HLA-mismatched locus-specific comparison of the outcomes of 351 single-unit UCB and 1,028 unrelated bone marrow (UBM) adult recipients 16 years old or older at the time of transplantation who received first stem cell transplantation with myeloablative conditioning for acute leukemia or myelodysplastic syndromes. With adjusted analyses, HLA 0 to 2 mismatched UCBT showed similar overall mortality (relative risk [RR] = 0.85, 95% confidence interval [CI], 0.68-1.06; $P = .149$) compared with that of single-HLA-DRB1-mismatched UBMT. UCBT showed inferior neutrophil recovery (RR = 0.50, 95% CI, 0.42-0.60; $P < .001$), lower risk of acute graft-versus-host disease (RR = 0.55, 95% CI, 0.42-0.72; $P < .001$), and lower risk of transplantation-related mortality (RR = 0.68, 95% CI, 0.50-0.92; $P = .011$) compared with single-HLA-DRB1-mismatched UBMT. No significant difference was observed for risk of relapse (RR = 1.28, 95% CI, 0.93-1.76; $P = .125$). HLA 0 to 2 antigen-mismatched UCBT is a reasonable second alternative donor/stem cell source with a survival outcome similar to that of single-HLA-DRB1-mismatched or other 7 of 8 UBMT.

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KEY WORDS: Unrelated cord blood transplantation, HLA-mismatched unrelated bone marrow transplantation

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a widely used, curative treatment for hematologic malignancies. When available, a human leukocyte antigen (HLA)-identical sibling is the donor of choice. However, only about 30% of candidates eligible for allogeneic HSCT will have such a donor. In addition, older patients with older siblings have more difficulty finding such a donor capable of stem cell donation. High-resolution donor-recipient HLA matching has contributed to the success of unrelated donor marrow transplantation, and the current first recommended alternative donor after an HLA-matched sibling for HSCT is an HLA-A, -B, -C, and -DRB1 8 of 8-allele-matched unrelated donor [1-4]. However, there are still a significant number of patients for which finding an HLA 8 of 8-matched unrelated donor is difficult and for whom a second alternative donor/stem cell source should be found.

The effect of HLA mismatches after bone marrow transplantation from unrelated donors (UBMT) has been well studied, and single mismatched UBM donors are usually selected as a second alternative donor/stem cell source [1-4]. Lee al. [3] showed that a single mismatch, antigen-level, or high-resolution, at HLA-A, -B, -C, or -DRB1 loci was associated with higher mortality and decreased survival. However, the reduction in survival may be acceptable in comparison with the survival rates for currently available alternative treatments. Analyses from the Japan Marrow Donor Program (JMDP) showed better survival in HLA class II mismatched recipients; thus, single-DRB1-mismatched UBM donor is currently a second alternative in Japan [1,2,5].

Recent advances in unrelated cord blood transplantation (UCBT) have provided patients with increased choices for a second alternative donor/stem cell source [6]. Clinical comparison studies of cord blood transplantation and HLA-A, -B, and -DRB1 6 of 6 allele-matched bone marrow transplantation for leukemia from unrelated donors in adult recipients showed comparable results [7-9]. More recently, promising outcomes of UCBT were shown compared with HLA-A, -B, -C, and -DRB1 8 of 8 allele-matched UBMT, the current first alternative donor/stem cell source [10-12].

The aim of this study was to determine the utility of UCBT as a second-alternative donor source in adult patients with acute leukemia or myelodysplastic syndromes. It is common today to perform high-resolution typing of HLA for donor selection of unrelated donors; thus, we performed mismatched-allele-specific analyses for comparison of HLA-mismatched UBMT and UCBT in terms of overall survival (OS) and other HSCT outcomes, setting single-DRB1-mismatched UBMT, the current second alternative, as the reference.

PATIENTS AND METHODS

Collection of Data and Data Source

The recipients' clinical data were provided by the Japan Cord Blood Bank Network (JCBBN) and the JMDP [13]. Peripheral blood stem cell donation from unrelated donors was not permitted in Japan during the study period. All 11 cord blood banks in Japan are affiliated with JCBBN. Both JCBBN and JMDP collect recipients' clinical information at 100 days posttransplantation. Patients' information on survival, disease status, and long-term complications including chronic graft-versus-host (cGVHD) disease and second malignancies is renewed annually using follow-up forms. This study was approved by the institutional review board of Nagoya University Graduate School of Medicine.

Patients

The subjects were adult patients of at least 16 years of age with acute myeloid leukemia, acute lymphoblastic leukemia, and myelodysplastic syndromes, who were recipients of first UBMT or UCBT with myeloablative conditioning. All patients in the UCBT cohort received a single-unit CB. Transplantation years were between 1996 and 2005 for UBMT and between 2000 and 2005 for UCBT to avoid the first 3 years of a pioneering period (1993-1995 for UBMT and 1997-1999 for UCBT). There were no statistically significant differences between UBMT in 1996-1999 and UBMT in 2000-2005 in probabilities of OS (41% versus 44%, at 3 years posttransplantation; $P = .86$) and in relapse-free survival (RFS) (40% versus 40%, at 3 years posttransplantation; $P = .93$).

Among 2,253 UBMT recipients with complete HLA high-resolution data, the following recipients with HLA -A, -B, -C, and -DRB1 8 of 8 allele match (n = 1,079) and more than three mismatches (5 of 8 allele match [n = 117], 4 of 8 allele match [n = 24], 3 of 8 allele match [n = 4], 2 of 8 allele match [n = 1]) were excluded. There were no statistically significant differences in risk of mortality or treatment failure (RFS) associated with single high-resolution (allele) versus single low-resolution (antigen) mismatches (data not shown), so in the analyses, allele and antigen mismatches were considered equivalent. HLA matching of cord blood was performed using low-resolution molecular typing methods for HLA-A and -B, and high-resolution molecular typing for HLA-DRB1. Of 557 recipients of CB with complete HLA data, 105 recipients with three mismatches and nine recipients with four mismatches were excluded. A total of 1,028 UBMT recipients (248 HLA class II locus mismatched, 424 HLA class I locus mismatched, and 356 HLA 2 loci mismatched) and 351 UCBT recipients (20 HLA-A, -B, low-resolution and -DRB1 matched, 87

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—【報　告】— Report —

院内における血液細胞処理のための指針

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日本輸血・細胞治療学会細胞治療委員会 Cell Processing 基準小委員会

既に治療法として確立している造血幹細胞移植に用いる細胞処理のガイドラインを，日本輸血・細胞治療学会と日本造血細胞移植学会との共同指針として今回初めて策定した．欧米の FACT-JACIE 基準を参考にする一方で，移植施設が小規模で分散し移植に係るコメディカルの少ないわが国の状況を踏まえて，多くの施設が受容し得る内容とした．ただし，必ずしも現在の大半の施設が満たす基準ではなく「目指すべき基準（理想的な基準）」の内容も含めた．構成は，1 目的，2 対象，3 細胞の採取，4 責任者と作業者，5 設備・機器，6 細胞処理（プロセシング），7 払い出し，8 保存と解凍，9 検体保存，10 投与，11 廃棄，12 雑則からなり，教育的観点から「付」として代表的な細胞処理法に関して，解説，標準作業手順書(SOP)サンプル，記録シートサンプル，結果シートサンプルなどを設けた．今後この指針が多くの場面で運用されるように努めるとともに，現場に即した指針となるように改訂を重ねていく必要がある．また，将来的に欧米の最新の指針と同等の基準となり，また輸血・細胞処理部門認定や有害事象の監視体制を構築するのに活用されることが期待される．

キーワード：造血幹細胞移植，細胞処理，指針，SOP

はじめに

医療施設内で処理・製造される洗浄血小板や造血幹細胞等の院内血液細胞製剤は輸血医療や細胞治療にいまや不可欠である．しかしながら，これらの院内製剤は，Good Manufacturing Practice（GMP）の下で日本赤十字社等から供給される血液製剤[1)2)]と異なり，その安全性や品質の保証は担保されていない．従って，院内血液細胞製剤の扱いは，血液法のもと行政に残された喫緊の課題である．そこで，院内血液細胞製剤を扱う国内のあらゆる施設が遵守すべき最小限の基準をここに作成し，血液細胞製剤(生物製剤，生物由来製品，臨床研究用細胞：組織製剤等）における院内血液細胞製剤の規制上の位置づけを明確にするとともに，血液法に則り，院内血液製剤の安全性の向上，適正使用の推進，そして安定供給の確保への行政ならびに医療機関の取り組みを促すことを目標とする[3)4)]．

本基準は，日本輸血・細胞治療学会が主体となり日本造血細胞移植学会の協力を得て作成された．また，FACT-JACIE2006 年第 3 版(Part C および D)[5)]を参考とした．ただし，輸血・細胞処理部門のわが国の現状

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を考慮し[6]，必ずしも多くの施設が満たす基準ではなく「目指すべき基準」の内容も含めた．これに関しては，文末に「望ましい」という表現で示した．指針は本文部分に加え，「付」として，代表的な細胞処理についての解説，標準作業手順書（standard operating procedures；SOP）サンプル，記録用紙サンプル，結果用紙サンプルなどを提示した（http://www.jstmct.or.jp/jstmct/Guideline/List.aspx）．

今後，特に欧米の指針と同等の基準となるべく，改定を加えることができるようにする．また，将来的に輸血・細胞処理部門認定や有害事象の監視体制を構築することも可能と考えられる．

指針本文概要

1　目的

本基準は，細胞の性状を変えることなく医療施設内で処理・製造される院内血液細胞（以下，「血液細胞製剤」と称し，主に造血幹細胞等を意味する）の製造工程において，安全で高い品質を確保し，また製剤に問題があった場合に原因等の遡及調査を可能にすることを目的とする．

2　対象

主に造血幹細胞移植に関連して院内で実施される細胞採取・処理・凍結保管を本指針の対象とする．すなわち，①同種および自家末梢血幹細胞の凍結保存と解凍，②同種および自家骨髄の赤血球除去，血漿除去および単核球の分離，凍結保存と解凍，③ドナーリンパ球輸注（donor lymphocyte infusion；DLI）のためのリンパ球の採取・凍結保存と解凍，④臍帯血移植における細胞保存と解凍．ただし，臨床研究として行われる細胞療法や再生治療に関わる細胞処理は対象としない．なお，上記の処理を院内で実施する全ての病院を本指針の対象とする．

3　細胞の採取

採取施設は以下の規定に従うこと．すなわち，非血縁者骨髄採取は骨髄バンクの「ドナー適格性判定基準」と「骨髄採取マニュアル」を遵守する．末梢血幹細胞採取では「末梢血幹細胞動員・採取に関するガイドライン」（日本造血細胞移植学会，日本輸血・細胞治療学会）を遵守する．非血縁ドナーDLIでは「ドナーリンパ球輸注（DLI）コーディネートマニュアル」（骨髄バンク）を遵守する．血縁ドナーもこれらに準ずることが望ましい．血縁ドナーでは必ず採取前に「血縁造血幹細胞ドナー（骨髄/末梢血）団体傷害保険」の説明を行うこと．

4　責任者と作業者

責任体制を明確にするため，総括責任者，細胞採取責任者，細胞処理責任者，品質管理責任者をおくこと．各責任者はそれぞれ別が望ましいが兼任可能とする．総括責任者は製造された血液細胞製剤を用いて治療を行う診療科長・部長等の医師で，これらの基準が適切に運用されるよう努めること．細胞採取責任者は細胞採取に習熟した医師で，細胞が適切に採取されるよう努め，適宜作業者の教育を行うこと．細胞処理責任者は細胞処理に習熟した医師で，細胞が適切に処理・管理されるよう努め，適宜作業者の教育を行うこと．品質管理責任者はこれらの基準が適切に運用されるよう体制を整え維持し，適宜作業者の教育を行うこと．作業者は予め細胞プロセッシングに係る十分な教育訓練を受け，全ての工程に習熟していること．

5　設備・機器

閉鎖系で細胞処理を行う場合は専用の機器を用いること．開放系で行う場合はクリーンベンチなどを完備する．設備と機器は定期的に点検を行い，その記録を保管すること．

6　細胞処理（プロセシング）

細胞処理を行う場所は，照明，換気，給排水が整備され，十分広く清潔で専用とし，部外者の立ち入りが制限され，必要な機器や物品が機能的に配置されていることが望ましい．複数の検体を同じ場所で同時に扱わず，出庫前の製剤を保管する場所を設置することが望ましい．

安全管理のため，作業者等の危険性を最小限にするよう配慮すること．作業中は手袋，ヘアーキャップ，マスク，専用衣を着用し，伝染性微生物，有害な化学薬品，放射性危険物に作業者が暴露した場合の対応方法を安全マニュアルに整備すること．医療廃棄物は適切に処理すること．

細胞処理では各作業のSOPを整備すること．SOPには目的，機器と消耗品，作業工程，指示書，工程記録等を含むことが望ましい．担当者はSOPをいつでも参照できること．新規・改定では責任者が事前に内容を確認すること．特定生物由来製品を使用した場合，薬事法で定める事項を記録し20年間保存すること．

担当医からの申込書等があること．凍結した場合は解凍後の生細胞率を評価することが望ましい．工程手順が新規・改定された場合は，事前にテストランを行うことが望ましい．処理は無菌的に行い，開放系での処理はクリーンベンチ内等で実施すること．作業工程記録書を作成し記録することが望ましい．重要な試薬，消耗品のロット番号，使用期限，重要な機器の種類等を記載すること．検査として，総有核細胞数と生細胞率（凍結した場合），末梢血幹細胞のCD34陽性細胞数，細菌・真菌検査を含むことが望ましい．菌検査が陽性の場合は，責任者等に速やかに連絡し，事前に定めた対処法に準じて対応すること．出庫の基準を各施設で

定めること．検査方法や機器の保守・点検の検査も含むことが望ましい．

ラベルは取り違いのないように運用し，細胞等の受入・出庫には2人以上で照合すること．処理途中のバッグや検体にも識別番号，製剤名，採取日時等のラベルを貼付等すること．

7 払い出し

出庫までに細胞処理責任者は工程記録を審査すること．出庫時には2人以上で外観，ラベルや名前等を確認し，工程記録に記録することが望ましい．

8 保存と解凍

製剤を保存する場合は施錠等し，部外者の立ち入りは制限されていること．

製剤ごとに保管期間・温度等を定めること．保存庫は警報システム等により24時間対応できる体制であること．温度を継続的に記録できることが望ましい．完全に液体窒素内に浸された製剤では継続的な温度モニターは不要だが，液体窒素量を継続的に監視するシステムがあること．

血液細胞の解凍は37℃急速解凍を原則とする．解凍のためのSOP，工程記録を定めること．必要に応じて解凍サンプルの検査を行い，患者担当医に報告すること．

9 検体保存

処理後の細胞の一部を保存することが望ましい．検体には専用のラベルを貼付し専用の台帳で管理すること．

10 投与

輸血・細胞処理部門から搬送された製剤は，原則として担当医が速やかに患者に投与すること．患者への投与前に，担当医および看護師は，ベッドサイド等で，輸血製剤に準じた方法で指示書と患者氏名，ドナー氏名，ID，製剤名，採取日，容量等の照合をすること．

11 廃棄

細胞廃棄の基準を定め，予め廃棄承諾書をドナー（および患者）から得ること．

12 雑則

この指針は，細胞療法の進歩や医学的，社会的情勢の変化等を勘案して，必要に応じ，又は施行後5年を目途として見直しを行うものとする．なお，この指針は平成22年5月27日より施行する．

考　案

既に確立している造血幹細胞移植に用いる細胞処理について，日本造血細胞移植学会と日本輸血・細胞治療学会の共同指針を初めて策定した．従来，移植に用いられる細胞に関しては法的規制も学会指針もなかった．たとえば自家末梢血幹細胞移植は難治性悪性リンパ腫などの治療法として小規模病院でも実地臨床として行われるが[6]，細胞処理には専門の技術・管理が必要で，重大な事故が起こり得る．この工程を管理することは，最終産物の質を担保するだけでなく，これに係る医療従事者の責任も保証することでもあり，その必要性は自明である．

欧米では既に国レベルで細胞処理を規制・管理している[5]．わが国では移植施設が小規模で分散している点が欧米と異なり，施設によっては少数の血液内科医が不十分な設備で移植を何とかこなして地域医療に貢献していることもある[6]．指針策定の目的は，各施設でSOPを整備して再現性・計画性のある細胞処理・管理が行われること，処理工程や結果が適切に記録され必要時に遡及調査が可能なこと，責任体制を明確化することである．一方，グローバル化した現代においては欧米のFACT-JACIE基準[5]とも整合性を保つ必要がある．最終的に，これらさまざまな立場の関係者が受容し得る指針を作成した．このためには，複数の施設の移植に携わる医師および臨床検査技師からなる小委員会で原案を作成し，関連学会・シンポジウムなどで検討を重ね，さらに日本輸血・細胞治療学会および日本造血細胞移植学会のホームページでパブリックコメントを求めて最終版を作成した．今後これが多くの場面で運用されるように努めるとともに，現場に即した指針となるように改訂を重ねていく必要がある．

謝辞：本指針策定に際しては，日本輸血・細胞治療学会細胞治療委員会および日本造血細胞治療学会ガイドライン委員会の委員をはじめとして多くの先生方の御意見を参考にさせていただきましたことを深謝いたします．なお，本研究は厚生労働省科学研究費補助金（H20-医薬-一般-006）の補助を受けた．

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GUIDELINE FOR PROCESSING CELLULAR THERAPY PRODUCTS ROUTINELY USED FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION IN JAPAN

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Abstract:

In Japan, about 4,000 hematopoietic stem cell transplantations (HSCT) are currently performed for various hematologic and non-hematologic disorders in about 200 hospitals per year. However, there have been no regulations or professional standards or guidelines for the processing of cellular therapy products routinely used for HSCT. Therefore, the Japan Society of Transfusion Medicine and Cell Therapy (JSTMCT), in collaboration with the Japan Society for Hematopoietic Cell Transplantation (JSHCT), have established guideline, titled 'the Japanese Standards for Processing Cellular Therapy Products Routinely Used for Hematopoietic Stem Cell Transplantation', for all hospitals and related personnel performing HSCT. According to a nation-wide survey performed by JSTMCT, it is likely that the number of medical staff and equipment is insufficient in many hospitals. Although these guidelines are based on the world-wide standard, the FACT-JACIE 3rd edition, and are intended to be minimum standards, some modifications were made to reflect the present situation of most hospitals. The guidelines include; 1 Objective, 2 Application, 3 Product Collection, 4 Personnel, 5 Equipment and Facility, 6 Policies and Procedures, 7 Distribution, 8 Storage and Thawing, 9 Sample Storage, 10 Infusion, 11 Disposal, and 12 Provision. Appendices include outlines of each procedure related to transplantation and examples of standard operation procedures (SOPs) and record forms. The established standards are to be uploaded to the JSTMCT website so that individuals can access and download the SOPs and record forms, which can be revised for use at each hospital. An accreditation system is also planned to be established in the near future.

Keywords:

hematopoietic stem cell transplantation, cell processing, guideline, SOP

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