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

Table 1 Characteristics of patients and cord blood units

Characteristics	
Patients, *n*	33
Male/female, *n*	22/11
Median age, years (range)	42 (19–52)
Median wt, kg (range)	55 (41–75)
Median no. of cryopreserved nucleated cells ($\times 10^7$ cells per kg (range))	2.51 (1.71–4.60)
Median no. of cryopreserved CD34-positive cells ($\times 10^5$cells per kg (range))	0.91 (0.21–2.14)
Median time from diagnosis to transplantation, months (range)	9 (2–223)
Recipient CMV status, positive/negative, *n*	27/6
Diagnosis	
RAEB, *n*	7
MDS-related secondary AML, *n*	26
Cytogenetic subgroups	
Standard, *n*	23
Adverse, *n*	10
Conditioning regimen	
TBI + Ara-C/G-CSF + CY, *n*	33
GVHD prophylaxis	
CyA + MTX, *n*	33
No. of HLA-A, -B and -DRB1 mismatches	
1, *n*	5
2, *n*	15
3, *n*	11
4, *n*	2
Year of transplant	
1998–2003, *n*	15
2004–2009, *n*	18

Abbreviations: RAEB = refractory anemia with excess blasts; MDS = myelodysplastic syndrome; G-CSF = recombinant human G-CSF.

Table 2 Multivariate analysis of factors associated with hematopoietic recovery and acute GVHD

Outcome/variables	*Hazard ratio (95% CI)*	P-*values*
Neutrophil recovery		
Diagnosis		
sAML	2.48 (1.04–5.92)	0.04
RAEB	1	
Plt recovery		
Recipient CMV status		
Positive	0.21(0.09–0.48)	0.00021
Negative	1	
Acute GVHD (grade II–IV)		
No. of HLA mismatches		
3 or 4	3.04 (1.19–7.78)	0.021
1 or 2	1	

Abbreviations: CI = confidence interval; sAML = myelodysplastic syndrome-related secondary acute myelogenous leukemia; RAEB = refractory anemia with excess blasts.

(CI), 79.7–100%). In multivariate analysis, neutrophil recovery was significantly faster for sAML patients ($P = 0.04$) (Table 2). A self-sustained plt count of more than 50×10^9 cells per l was achieved in 29 patients at a median time of 51 days (range, 30–179 days). The cumulative incidence of plt recovery at day 200 was 88% (95% CI, 75.1–100%). In multivariate analysis, plt recovery was significantly faster for CMV seronegative patients ($P < 0.001$) (Table 2).

aGVHD and cGVHD

Acute GVHD occurred in 28 of 30 evaluable patients. The grading of aGVHD was grade I in 12 patients, grade II in 10, grade III in 4 and grade IV in 2. The cumulative incidence of grade II–IV and grade III and IV aGVHD at day 100 after CBT was 67% (95% CI, 34.0–90.8%) and 41% (95% CI, 5.5–75.5%), respectively. In multivariate analysis, degree of HLA mismatch had a significant impact on the incidence of grade II–IV aGVHD ($P = 0.021$) (Table 2). cGVHD occurred in 25 of 28 evaluable patients. Among 25 cGVHD patients, 11 patients were of the extensive type. The cumulative incidence of overall and extensive-type cGVHD was 76% (95% CI, 60.4–91.2%) and 34% (95% CI, 17.4–51.2%), respectively. No factor was associated with the incidence of overall and extensive-type cGVHD.

Relapse

The cumulative incidence of relapse at 5 years was 16% (95% CI, 2.7–29.1%). No factor was associated with the incidence of relapse.

TRM and causes of death

The cumulative incidence of TRM at day 100 and at 5 years was 6% (95% CI, 0–14.4%) and 14% (95% CI, 0.8–27.0%), respectively. No factor was associated with TRM. Eight patients died. Of the eight patients, four died of relapse. In the remaining four patients, the causes of death were treatment related (organ failure ($n = 1$), infection ($n = 1$), cGVHD with or without infection ($n = 2$)).

EFS

Of 33 patients, 24 are alive and event-free after CBT. Median follow-up of event-free survivors was 59 months (range, 5–137 months). The probability of EFS at 5 years was 70% (95% CI, 55.3–88.9%) (Figure 1). No factor was associated with EFS.

Discussion

Several studies have suggested the promising results of unrelated CBT after myeloablative conditioning for adult patients.[7–15] Recently, reports of disease-specific outcomes for adult patients with acute leukemia after CBT have been published;[21–24] however, there have been no reports detailing the long-term follow-up results of disease-specific outcomes of adult advanced MDS patients treated with CBT after myeloablative conditioning. Although we have previously reported the results of 19 adult patients with advanced MDS who received CBT after myeloablative conditioning,[16,17] the follow-up duration after CBT was

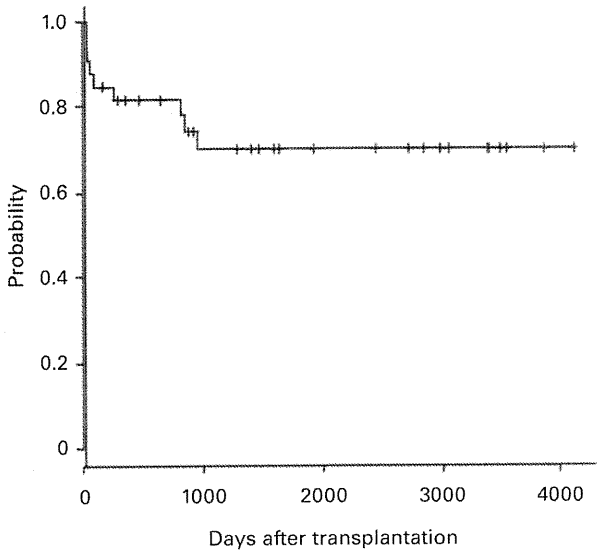


Figure 1 Probability of EFS after cord blood transplantation.

short and risk factors associated with transplant outcomes were not analyzed. In addition, our recent report of the results of 77 adult patients with AML who received CBT included 20 patients with sAML;[21] however, the details of sAML-specific outcomes were not fully analyzed. Therefore, we updated the results of unrelated CBT after myeloablative conditioning for 33 adult patients with advanced MDS and identified pretransplant factors related to the transplant outcomes on long-term follow-up.

In this study, the cumulative incidence of neutrophil recovery at day 50 was 91% and the median time to neutrophil recovery was 22 days. Of 33 patients, only 3 patients experienced primary engraftment failure. The diagnosis of sAML was identified as a significant factor affecting faster neutrophil recovery. One reason may be that two of the three patients who experienced primary engraftment failure had refractory anemia with excess blasts (2 of 7 refractory anemia with excess blasts patients experienced primary engraftment failure). The cumulative incidence of plt recovery at day 200 was 88%, and plt recovery was significantly faster for CMV seronegative patients. This finding is consistent with our previous report.[25] The cumulative incidence of grade II–IV aGVHD, grade III and IV aGVHD, overall and extensive-type cGVHD was 67, 41, 76 and 34%, respectively. As described before,[13] because immunosuppressants for CBT recipients tended to be discontinued faster in our institution, the incidence of aGVHD and cGVHD was relatively higher than other reports of adult CBT. However, the cumulative incidence of TRM at day 100 and at 5 years was very low (6 and 14%, respectively), and aGVHD and cGVHD were not related to TRM. In multivariate analysis, degree of HLA mismatch had a significant impact on the incidence of grade II–IV aGVHD. Recently, Arcese *et al.*[14] reported the updated results of a large series of adult patients with different hematopoietic malignancies transplanted in 63 centers of the Eurocord group. In total, 171 adult patients received CBT after myeloablative conditioning. They analyzed outcomes and risk factors after CBT. The cumulative incidence of grade II–IV aGVHD was 32%, and no factor was found to significantly influence the development or severity of aGVHD in multivariate analysis. Our previous studies[21,22] and those of others,[7,24] as well as that of Arcese *et al.*[14] reported that HLA matching had no effect on aGVHD after CBT in adults; however, the degree of HLA mismatch had a significant impact on aGVHD in this study. The reasons for this finding remain unclear because of the limited number of patients. The cumulative incidence of relapse at 5 years was 16% and the probability of EFS at 5 years was 70%. No factor was associated with relapse and EFS. Recently, a transplantation-specific cytogenetics grouping scheme for patients with MDS has been reported.[18] Under this scheme, abnormalities of chromosome 7 and complex karyotype are considered to be adverse risks, whereas all others are considered standard risk. Although, we performed univariate and multivariate analyses according to this transplantation-specific grouping, cytogenetics had no impact on the incidence of relapse and EFS. In this analysis, unrelated CBT after myeloablative conditioning can cure ~70% of the adult patients with advanced MDS. As previously described,[13,15,21] a lower TRM rate and the use of G-CSF-combined preparative regimen,[21] which was capable of reducing the post transplant relapse rate in refractory myeloid malignancies, may be associated with encouraging outcomes in this study. In addition, Japanese patients might have some advantages in the setting of HLA-mismatched transplantation because of HLA or non-HLA immune genetics.

In summary, we updated the results of unrelated CBT for adult advanced MDS patients. These results suggest that unrelated CBT after myeloablative conditioning could be safely and effectively used for adult patients with advanced MDS without suitable related or unrelated BM donors.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank the physicians and nurses who cared for patients in this study.

References

1 de Witte T, Suciu S, Peetermans M, Fenaux P, Strijckmans P, Hayat M *et al.* Intensive chemotherapy for poor prognosis myelodysplasia (MDS) and secondary acute myelogenous leukemia following MDS of more than 6 months duration. A pilot study by the leukemia cooperative group of the european organisation for research and treatment in cancer (EORTC-LCG). *Leukemia* 1995; **9**: 1805–1810.

2 Anderson JE, Anasetti C, Appelbaum FR, Schoch G, Gooley TA, Hansen JA *et al.* Unrelated donor transplantation for myelodysplasia (MDS) and MDS-related acute myeloid leukemia. *Br J Haematol* 1996; **93**: 59–67.

3 Arnold R, de Witte T, van Biezen A, Hermans J, Jacobsen N, Runde V *et al.* Unrelated bone marrow transplantation in patients with myelodysplastic syndromes and secondary acute myeloid leukemia: an EBMT survey. *Bone Marrow Transplant* 1998; **21**: 1213–1216.
4 de Witte T, Hermans J, Vossen J, Bacigalupo A, Meloni G, Jacobsen N *et al.* Haematopoietic stem cell transplantation for patients with myelodysplastic syndromes and secondary acute myeloid leukaemias: a report on behalf of the chronic leukaemia working party of the european group for blood and marrow transplantation (EBMT). *Br J Haematol* 2000; **110**: 620–630.
5 de Witte T, Pikkemaat F, Hermans J, van Biezen A, Mackinnan S, Cornelissen J *et al.* Genotypically nonidentical related donors for transplantation of patients with myelodysplastic syndromes: comparison with unrelated donor transplantation and autologous stem cell transplantation. *Leukemia* 2001; **15**: 1878–1884.
6 Deeg HJ, Storer B, Slattery JT, Anasetti C, Doney KC, Hansen JA *et al.* Conditioning with targeted busulfan and cyclophosphamide for hemopoietic stem cell transplantation from related and unrelated donors in patients with myelodysplastic syndrome. *Blood* 2002; **100**: 1201–1207.
7 Laughlin MJ, Barker J, Bambach B, Omeb NK, Rizzieri DA, Wagner JE *et al.* Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N Engl J Med* 2001; **344**: 1815–1822.
8 Gluckman E. Current status of umbilical cord blood hematopoietic stem cell transplantation. *Exp Hematol* 2000; **28**: 1197–1205.
9 Sanz GF, Saavedra S, Planelles D, Senent L, Cervera J, Barragan E *et al.* Standardized, unrelated donor cord blood transplantation in adults with hematologic malignancies. *Blood* 2001; **98**: 2332–2338.
10 Long GD, Laughlin M, Madan B, Kurtzberg J, Gasparetto C, Morris A *et al.* Unrelated umbilical cord blood transplantation in adult patients. *Biol Blood Marrow Transplant* 2003; **9**: 772–780.
11 Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE *et al.* Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med* 2004; **351**: 2265–2275.
12 Rocha V, Labopin M, Sanz G, Arcese W, Schwerdtfeger R, Bosi A *et al.* Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med* 2004; **351**: 2276–2285.
13 Takahashi S, Iseki T, Ooi J, Tomonari A, Takasugi K, Shimohakamada Y *et al.* Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematological malignancies. *Blood* 2004; **104**: 3813–3820.
14 Arcese W, Rocha V, Labopin M, Sanz G, Iori AP, de Lima M *et al.* Unrelated cord blood transplants in adults with hematologic malignancies. *Haematologica* 2006; **91**: 223–230.
15 Takahashi S, Ooi J, Tomonari A, Konuma T, Tsukada N, Oiwa-Monna M *et al.* Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem cell transplantation from related donors in adult patients with hematological malignancies after myeloablative conditioning regimen. *Blood* 2007; **109**: 1322–1330.
16 Ooi J, Iseki T, Takahashi S, Tomonari A, Ishii K, Takasugi K *et al.* Unrelated cord blood transplantation for adult patients with advanced myelodysplastic syndrome. *Blood* 2003; **101**: 4711–4713.
17 Ooi J. The efficacy of unrelated cord blood transplantation for adult myelodysplastic syndrome. *Leuk Lymphoma* 2006; **47**: 599–602.
18 Armand P, Deeg HJ, Kim HT, Lee H, Armistead P, de Lima M *et al.* Multicenter validation study of a transplantation-specific cytogenetics grouping scheme for patients with myelodysplastic syndromes. *Bone Marrow Transplant* (e-pub ahead of print 28 September 2009; doi:10.1038/bmt.2009.253).
19 Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA *et al.* Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation* 1974; **18**: 295–304.
20 Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE *et al.* Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med* 1980; **69**: 204–217.
21 Ooi J, Takahashi S, Tomonari A, Tsukada N, Konuma T, Kato S *et al.* Unrelated cord blood transplantation after myeloablative conditioning in adults with acute myelogenous leukemia. *Biol Blood Marrow Transplant* 2008; **14**: 1341–1347.
22 Ooi J, Takahashi S, Tomonari A, Tsukada N, Konuma T, Kato S *et al.* Unrelated cord blood transplantation after myeloablative conditioning in adults with ALL. *Bone Marrow Transplant* 2009; **43**: 455–459.
23 Atsuta Y, Suzuki R, Nagamura-Inoue T, Taniguchi S, Takahashi S, Kai S *et al.* Disease-specific analyses of unrelated cord blood transplantation compared with unrelated bone marrow transplantation in adult patients with acute leukemia. *Blood* 2009; **113**: 1631–1638.
24 Sanz J, Sanz MA, Saavedra S, Lorenzo I, Montesinos P, Senent L *et al.* Cord blood transplantation from unrelated donors in adults with high risk acute myeloid leukemia. *Biol Blood Marrow Transplant* 2010; **16**: 86–94.
25 Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Kato S *et al.* Impact of cytomegalovirus serostatus on outcome of unrelated cord blood transplantation for adults: a single-institute experience in Japan. *Eur J Haematol* 2008; **80**: 251–257.

Japanese Journal of Transfusion and Cell Therapy, Vol. 57. No. 3 57(3) : 139—145, 2011

—[Original]— 原 著—

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

Miki Yuzawa[1], *Tokiko Nagamura-Inoue*[1], *Ikuo Ishige*[1], *Kazuo Ogami*[1], *Tomoki Tamura*[1], *Atsuko Takahashi*[2], *Hideki Kodo*[2], *Satoru Yamaguchi*[3] *and Arinobu Tojo*[1]

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-

1) Department of Cell Processing and Transfusion, The Institute of Medical Science, The University of Tokyo
2) Tokyo Cord Blood Bank
3) Yamaguchi Hospital
〔Received: 2010/08/27, Accepted: 2010/10/15〕

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.

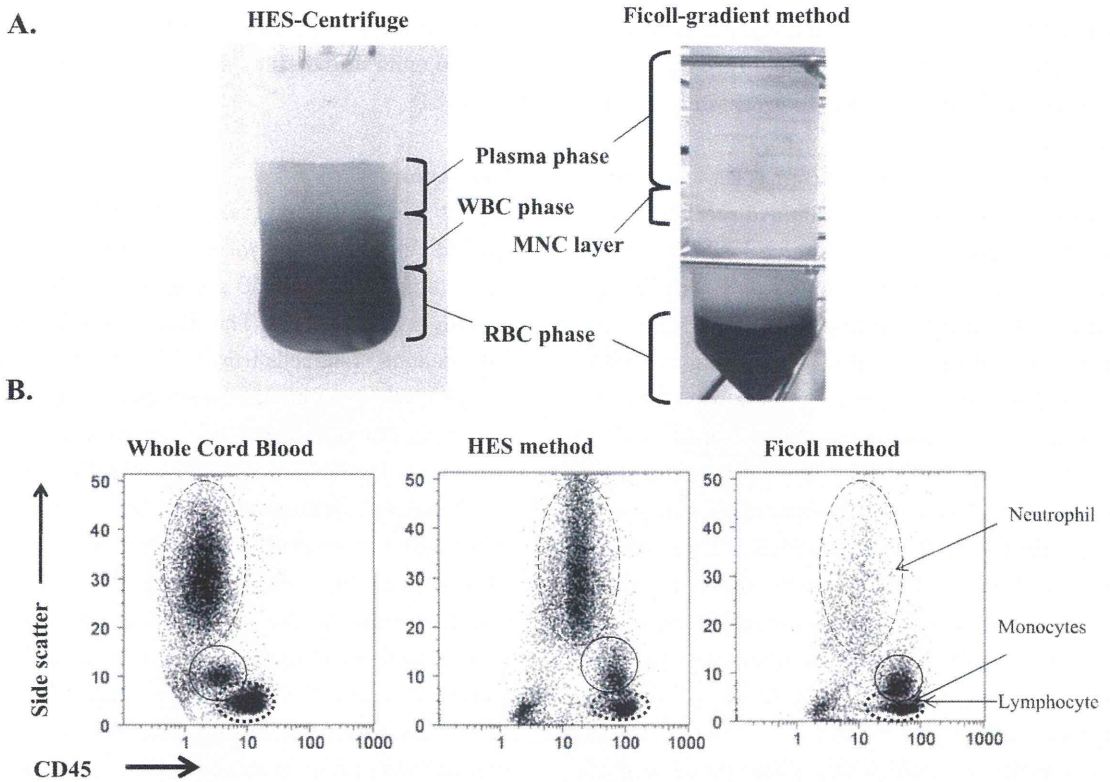


Fig. 1　Comparison of the processing in cord blood

A: Differential display of CB cells and MNCs isolated using a buffy coat layer HES centrifugation method and using a Ficoll-Paque (F/P) density-gradient method. The HES centrifugation method shows a cloudy broad band and the F/P density-gradient method shows a relatively sharp white band. B: Flow cytometric analysis after CB processing using HES centrifugation and density-gradient methods. The X-axis shows CD45 expression, and the Y-axis shows side-scatter. Neutrophils in the CB-MNC layer were reduced in samples processed using the density-gradient method.

NC count and differential white blood cell count

Differential white blood cells (WBC) were counted using an automated hemocytometer XE-2100 (Sysmex Corporation, Kobe, Japan). Total NCs include total WBC and NRBC. MNCs are defined as the sum of lymphocytes and monocytes and the proportion of MNCs is calculated as the sum of them in the TNCs.

$CD34^+$ cell count and viability analysis

We measured $CD34^+$ cells following the ISHAGE single platform method. We stained and analyzed the pre- and post- processed samples using CD45 FITC- and CD34 PE-conjugated antibodies (BD, Franklin Lakes, NJ) and Via-Probe 7-Amino-Actinomycin D (7-AAD) (BD) in TruCount tube (BD). After lysis of RBC by a PharmLyse reagent (BD), we analyzed cells using a flow cytometry FACS Calibur (BD). For cell viability analyses, viability was defined as the proportion of $CD45^+$ $7\text{-}AAD^-$ viable WBCs within the total population of $CD45^+$ cells.

Recovery rate of TNCs, MNCs and $CD34^+$ cells was defined as the percentage that the post-processing cell number was divided by the pre-processing cell number.

Statistical analysis

Data are presented as the mean±SD or the median with range. Comparisons between two groups were performed using the Student T test, and Pearson correlation coefficients were calculated to evaluate correlations between groups using by JMP software (version 6.0.2, Cary, NC) and R software, respectively, and p values less than 0.05 were considered to be significant.

Results

The HES centrifugation method isolates a cloudy broad band and the density-gradient method isolates a MNC layer which is relatively sharp and white band (Fig. 1.A). According to the flow cytometric analysis of CD45/side scatter after CB processing, neutrophils in

the CB-MNC layer were reduced in samples processed by the density-gradient method (Fig. 1.B).

Effect of time from collection to processing on sample quality

To evaluate the effect of time from collection to processing on sample quality, we examined recovery rates of NCs, MNCs, and $CD34^+$ cells using a density-gradient protocol. Median collection volume was 81.0 m*l* (range 60.0 m*l* to 133.7 m*l*), including 28 m*l* of CPD, and median concentration of NCs before processing was 0.96×10^7/m*l* (range 0.60×10^7 to 1.7×10^7/m*l*). Median time from CB collection to processing was 14.0 h (range; 6-38 h). Recovery rate of MNCs before and after processing was 71.3±10.3%. This rate decreased significantly with time ($p = 0.0006$; Fig. 2.A). Interestingly, the percentage of neutrophils in the MNC layer increased significantly in proportion to the time from collection to processing ($p<0.0001$; Fig. 2.B). No significant difference was found in the recovery rates of NCs (Fig. 2.C) or $CD34^+$ cells over time (Fig. 2.D).

The viability of $CD45^+$ WBCs before processing decreased significantly with increasing time from collection to processing ($p = 0.0085$; Fig. 2.E), although the viability was relatively kept high (mean±SD 96.3±2.6%) in this study.

Effect of storage temperature on sample quality

To evaluate the effect of processing time and storage temperature on neutrophil contamination, we divided CB units into 4 samples of equal volume. One sample was immediately processed, and the remaining three were stored at 4℃, 15℃, or RT (25℃) for further processing. As expected, neutrophil contamination increased with increasing storage temperature (20±11% at 4℃, 27±12% at 15℃, and 46±15% at RT; Table 1). Samples processed immediately (within 12 h) contained less neutrophil contamination (10±4%) than those stored for 30 h at any temperature. Samples processed immediately and samples stored at 4℃ had significantly lower neutrophil contamination than those stored at RT. There was no significant difference in the recovery rate of NCs in samples stored at the various temperatures.

Because CB units in obstetrics were stored for various time and at uncontrolled temperature, we introduced the refrigerator to store the collected CB units at 10℃ in the storage room of the obstetrics. Contamination of neutrophils in the MNC layer (Fig. 2.F) stored at 10℃ (n=85) was significantly decreased compared with that stored at RT ($p < 0.0001$) in the obstetrics. The CB units stored at RT are the same units as those in Fig. 2.B.

Discussion

Most CB banks have now adopted to the HES method[9)10)] within 48 h of collection in obstetrics according to the guidelines of CB processing for CB banking in many other countries. The HES method is sufficient for obtaining stem cells from a limited volume of CB, but generally most hematopoietic progenitor cells are present in the MNC fraction[11)12)]. Furthermore, tissue-specific stem cells can be found in the MNC population of CBs isolated using a density-gradient method in the regenerative experiments[1)~4)], except the special population such as very small ES like cells[13)].

In the present study, we found that prolonged time from CB collection to processing at RT resulted in a significant increase of neutrophil contamination in the MNC layer using a density-gradient protocol. In addition, prolonged time from collection to processing CB resulted in a decrease in recovery rate of MNC (lymphocytes and monocytes), although the final TNC numbers were not affected. These results suggest that prolonged time from CB collection to processing decreases neutrophil density, whereas the density of lymphocytes and monocytes increases after CB processing. Therefore, the final product seems same, as far as the TNC are manually or automatically counted without WBC differentials. Although obtaining mesenchymal stem cells from CB seemed more difficult than we expected[14)15)], our results show that prolonged time from collection to processing and preserved temperature might affect the composition of CB samples. To our knowledge, this is the first report that clearly describes the relationship between the time from collection to processing and the composition of CB samples isolated using a density-gradient method. Our results also suggest that these phenomena might occur in other blood- and bone marrow-derived MNCs isolated using a density-gradient method. Although a change in cellular density or the alteration of cell metabolites may explain neutrophil contamination in the MNC layer, the mechanism of this contamination is still unknown.

We also found that CB storage at lower temperatures prevented an increase in neutrophil contamination in the MNC layer when time from collection to processing was prolonged. Some researchers have re-

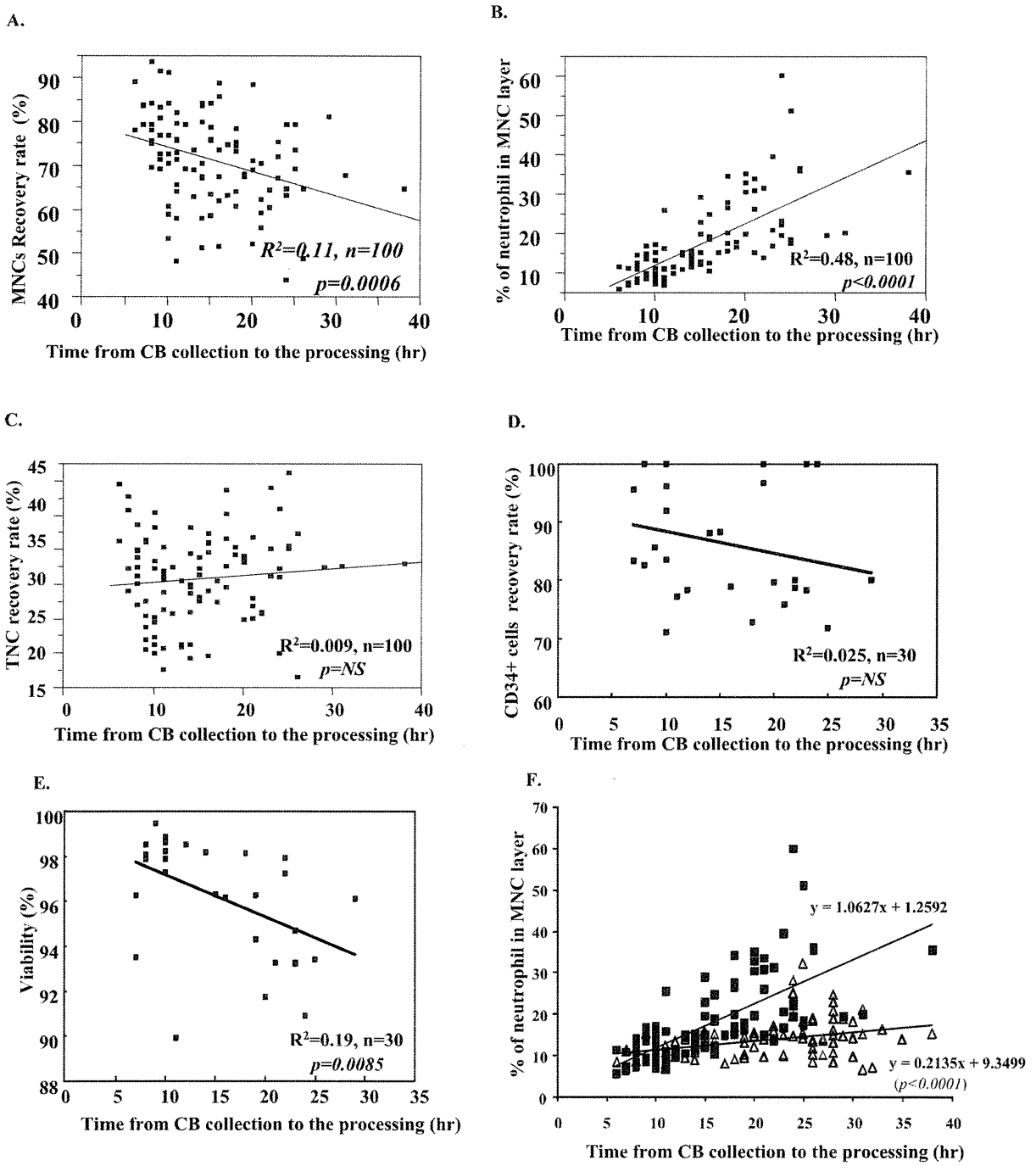


Fig. 2　Influences of time from collection to processing and temperature on the composition of MNC layer

A: MNC recovery rate. The recovery rate of CB-MNCs after F/P density-gradient processing decreased significantly with time from CB collection to processing (in hours; $p=0.0006$). B: The percentage of neutrophils in the MNC layer. The percentage of neutrophils in the CB-MNC layer (neutrophil contamination) increased significantly in proportion to time from CB collection to processing ($p<0.0001$). C: Recovery rate of NCs. The recovery rate of NCs in the CB-MNC layer showed no significant change according to the indicated time from CB collection to processing ($p=NS$). D: Recovery rate of absolute numbers of $CD34^+$ cells. $CD34^+$ cells in the CB-MNC layer were counted using a flow cytometry-based ISHAGE method. The recovery rate of $CD34^+$ cells in the CB-MNC layer showed no significant decline according to the indicated time from collection to processing ($p=NS$). E: Viability of white blood cells after processing using an F/P density-gradient method. Viability is the percentage of 7-AAD^- viable cells within the total $CD45^+$ white blood cell population ($p=0.0085$). F: The percentage of neutrophils in the MNC layer. ■ indicates CB units stored at RT in obstetrics (n=100) and △ indicates CB units stored under temperature control at 10℃ in obstetrics (n=85) ($p<0.0001$).

Table 1 Effects of time and temperature on CB-MNC processing.

Time after collection	Immediately processed*	At 30 h Stored temperature		
		4℃	15℃	RT (25℃)
In MNC layer (%)				
Monocytes	18±7	15±8	15±6	10±3
Lymphocytes	72±11	66±18	57±8	45±8
Neutrophils	10±4[†]	20±11[†]	27±12	46±15[†]

*CB units, which were transported faster to the processing facility (IMSUT-CRC) and were immediately processed into MNCs within 12 hours after collection. Data are shown as mean±SD. n=4, [†]$p<0.05$.

ported that storage of platelets at 4℃ inhibits cytokine accumulation and bacterial growth[16]. Maintaining platelet function is not important for processing CB. In our hand, we did not find any de novo aggregation in CB samples stored at 4℃ overnight and returned to RT just before processing (data not shown). Generally, RT is thought to have wide range such as 4 to 25℃, but our results suggest the need to store CB at lower temperature and process it immediately. Furthermore, when we set up a refrigerator in the storage room of obstetrics to keep the collected CB units at 10℃, contamination of neutrophils seemed to be prevented in the CB-MNC layer, as tested by the Ficoll density method, when compared with samples stored at RT (Fig. 2F). This may suggest that a lower fixed temperature (not broad range, RT) to store CB units in obstetrics is better for the fresh storage of CB. Time from collection to processing in clinical CBB requires re-evaluation.

In conclusion, storage temperature and time from collection to processing influences the quality of CB-MNCs products processed using a Ficoll-Paque density-gradient method.

Acknowledgement

We thank all staff in Tokyo Cord Blood Bank for the official work. We also thank the all staff in Obstetrics for their continuous corporation with the cord blood bank project. This project was supported by the project of realization of regenerative medicine, organized by the Ministry of Education, Culture, Sports, Science, and Technology.

Disclosure of Interest

We have no disclosure of interests.

References

1) Barachini S, Trombi L, Danti S, et al: Morpho-functional characterization of human mesenchymal stem cells from umbilical cord blood for potential uses in regenerative medicine. Stem Cells Dev, 18: 293—305. 2009.
2) Bieback K, Kern S, Klüter H, et al: Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. Stem Cells, 22: 625—634, 2004.
3) Bieback K, Klüter H: Mesenchymal stromal cells from umbilical cord blood. Curr Stem Cell Res Ther, 2: 310—323, 2007.
4) Lee OK, Kuo TK, Chen WM, et al: Isolation of multipotent mesenchymal stem cells from umbilical cord blood. Blood, 103: 1669—1675, 2004.
5) Tablin F, Oliver AE, Walker NJ, et al: Membrane phase transition of intact human platelets: correlation with cold-induced activation. J Cell Physiol, 168: 305—313, 1996.
6) Tablin F, Walker NJ, Klein SD, et al: Animal models for studies on cold-induced platelet activation in human beings. J Lab Clin Med, 135: 339—346, 2000.
7) Winokur R, Hartwig JH: Mechanism of shape change in chilled human platelets. Blood, 85: 1796—1804, 1995.
8) Reid TJ, LaRussa VF, Esteban G, et al: Cooling and freezing damage platelet membrane integrity. Cryobiology, 38: 209—224, 1999.
9) Rubinstein P, Dobrila L, Rosenfield RE, et al: Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc. Natl. Acad. Sci. USA, 92: 10119—10122, 1995.
10) Nagamura-Inoue T, Shioya M, Sugo M, et al: Wash-out of DMSO does not improve the speed of engraftment of cord blood transplantation: follow-up of 46 adult patients with units shipped from a single cord blood bank. Transfusion, 43: 1285—1295, 2003.
11) Almici C, Carlo-Stella C, Mangoni L, et al: Density separation of umbilical cord blood and recovery of hemopoietic progenitor cells: implications for cord blood banking. Stem Cells, 13: 533—540, 1995.
12) Almici C, Carlo-Stella C, Wagner JE, et al: Density separation and cryopreservation of umbilical cord blood cells: evaluation of recovery in short-and long-term cultures. Acta Haematol, 95: 171—175, 1996.
13) Halasa M, Baskiewicz-Masiuk M, Dabkowska E, et al: An efficient two-step method to purify very small embryonic-like (VSEL) stem cells from umbilical cord blood (UCB). Folia Histochem Cytobiol, 46: 239—243, 2008.

14) Secco M, Zucconi E, Vieira NM, et al: Multipotent stem cells from umbilical cord: cord is richer than blood! Stem Cells, 26: 146—150, 2008.
15) Ishige I, Nagamura-Inoue T, Honda MJ, et al: Comparison of mesenchymal stem cells derived from arterial, venous, and Wharton's jelly explants of human umbilical cord. Int J Hematol, 90: 261—269, 2009.
16) Currie LM, Harper JR, Allan H, et al: Inhibition of cytokine accumulation and bacterial growth during storage of platelet concentrates at 4 degrees C with retention of in vitro functional activity. Transfusion, 37: 18—24, 1997.

臍帯血単核球分離における採取後経過時間と保管温度条件の影響の検討

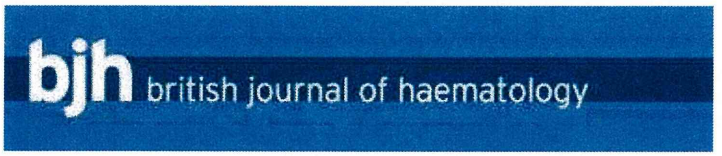
湯沢　美紀[1)]　長村(井上)登紀子[1)]　石下　郁夫[1)]　尾上　和夫[1)]　田村　友樹[1)]
高橋　敦子[2)]　幸道　秀樹[2)]　山口　暁[3)]　東條　有信[1)]
[1)]東京大学医科学研究所附属病院セルプロセッシング・輸血部
[2)]財団法人献血供給事業団東京臍帯血バンク
[3)]医療法人成和会山口病院

要旨：
近年，臍帯血は造血幹細胞のみならず組織幹細胞ソースとしても注目されている．臨床使用の臍帯血処理方法であるHES遠心分離法による有核細胞の分離について，海外のガイドラインにおいては採取から細胞処理までの時間は48時間以内，室温での保管および搬送がよいとされている．一方，組織幹細胞用の細胞処理として多くの論文で採用されているフィコール法による単核球の分離についての検討報告はない．本研究では，臍帯血採取後の経過時間や温度条件が，フィコール法による単核球分離に及ぼす影響について検討した．その結果，採取から細胞処理開始までの経過時間（採取後経過時間）によって有核細胞，CD34陽性細胞の回収率に有意差は認めないものの，採取後経過時間が長いほど有意に単核球回収率および細胞生存率の低下を認めた．特に，フィコール処理後の単核球層の好中球混入率は，採取後の時間経過に伴い有意に増加した．さらに，臍帯血の保管温度の検討において，採取後30時間でのフィコール処理後単核球層の好中球混入率は，比較的低温で保管した場合のほうが室温保管に比べ有意に低かった．今回の検討において，経過時間や温度等の保管条件が，臍帯血単核球処理後の細胞組成に影響を及ぼすことが分かり，採取後の臍帯血管理状態の重要性が再確認された．

キーワード：
臍帯血，単核球，分離方法，フィコール法，保管温度，細胞生存率，回収率

Journal Web Site: http://www.jstmct.or.jp/jstmct/

British Journal of Haematology



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

Journal:	*British Journal of Haematology*
Manuscript ID:	BJH-2011-00322.R1
Manuscript Type:	Ordinary Papers
Date Submitted by the Author:	n/a
Complete List of Authors:	Morio, Tomohiro; Tokyo Medical and Dental University Graduate School of Medical and Dental Sciences, Department of Pediatrics and Developmental Biology Atsuta, Yoshiko; Nagoya University School of Medicine, Department of Hematopoietic Stem Cell Transplantation Data Management/Biostatistics Tomizawa, Daisuke; Tokyo Medical and Dental University Graduate School of Medical and Dental Sciences, Department of Pediatrics and Developmental Biology Nagamura-Inoue, Tokiko; Research Hospital, Institute of Medical Science, University of Tokyo and Tokyo Cord Blood Bank, Department of Cell Processing & Transfusion Kato, Koji; Japanese Red Cross Nagoya First Hospital, Department of Pediatrics Ariga, Tadashi; Hokkaido University Graduate School of Medicine, Department of Pediatrics Kawa, Keisei; Osaka Medical Center and Research Institute for, Department of Hematology/Oncology Koike, Kazutoshi; Ibaraki Children's Hospital, Department of Pediatric Hematology Tauchi, Hisanobu; Ehime University Graduate School of Medicine, Department of Pediatrics Kajiwara, Michiko; Service Medical Hospital Tokyo Medical and Dental University, Department of Blood Transfusion Hara, Toshiro; Kyushu University Graduate School of Medical Sciences, Department of Pediatrics Kato, Shunichi; Tokai University, School of Medicine, Department of Cell Transplantation & Regenerative Medicine
Key Words:	CORD BLOOD TRANSPLANTATION, PRIMARY AND SECONDARY IMMUNE DEFICIENCIES, SCID, WISCOTT-ALDRICH, CONDITIONING

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

Tomohiro Morio[1], Yoshiko Atsuta[2], Daisuke Tomizawa[1], Tokiko Nagamura-Inoue[3], Koji Kato[4], Tadashi Ariga[5], Keisei Kawa[6], Kazutoshi Koike[7], Hisamichi Tauchi[8], Michiko Kajiwara[9], Toshiro Hara[10], Shunichi Kato[11] and for the Japanese Cord Blood Bank Network

1 Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University Graduate School of Medical and Dental Sciences, Tokyo, Japan

2 Department of Hematopoietic Stem Cell Transplantation Data Management/Biostatistics, Nagoya University School of Medicine, Nagoya, Japan

3 Department of Cell Processing & Transfusion, Research Hospital, Institute of Medical Science, University of Tokyo and Tokyo Cord Blood Bank, Tokyo, Japan

4 Department of Pediatrics, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan

5 Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo, Japan

6 Department of Hematology/Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan

7 Department of Pediatric Hematology, Ibaraki Children's Hospital, Ibaraki, Japan

8 Department of Pediatrics, Ehime University School of Medicine, Ehime, Japan

9 Department of Blood Transfusion, Tokyo Medical and Dental University Medical Hospital, Tokyo, Japan

10 Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

11 Department of Cell Transplantation and Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan

Running title:

Cord blood transplantation for primary immunodeficiency

Correspondence to:

Tomohiro Morio, MD, PhD

Department of Pediatrics and Developmental Biology

Tokyo Medical and Dental University Graduate School of Medical and Dental Sciences,

1-5-45 Yushima Bunkyo-ku, Tokyo 113-8519, Japan

Tel. & Fax: 81-3-5803-5245

Email: tmorio.ped@tmd.ac.jp

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; 40 patients with severe combined immunodeficiency (SCID), 23 with Wiskott–Aldrich syndrome (WAS), 7 with chronic granulomatous disease, 5 with severe congenital neutropaenia (SCN) and 13 with other immunodeficiencies.

Five-year overall survival (5Y-OS) for all patients was 69% (95% confidence interval [CI], 57%–78%). 5Y-OS for SCID and WAS was 71% and 82%, 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 leukocyte 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 HLA-matched sibling. Controlling pre-transplant infection and selecting HLA-matched donors will lead to a better outcome.

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

Introduction

Allogeneic haematopoietic stem cell transplantation (HSCT) has been successfully used as a curative therapy for most severe forms of primary immunodeficiency (PID) (Antoine, *et al* 2003, Cuvelier, *et al* 2009, Dvorak and Cowan 2008, Griffith, *et al* 2008, Kobayashi, *et al* 2006, Mazzolari, *et al* 2007, Rao, *et al* 2005, Sakata, *et al* 2004, Zeidler, *et al* 2000). Stem cell transplantation from a human leukocyte 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 (Cuvelier, *et al* 2009, Dvorak and Cowan 2008, Griffith, *et al* 2008).

Umbilical cord blood grafts from unrelated donors have been successfully used, primarily in children and subsequently in adults (Gluckman, *et al* 1997, Kurtzberg, *et al* 1996, Laughlin, *et al* 2004, Rocha, *et al* 2004, Rocha, *et al* 2000, Rubinstein, *et al* 1998, Wagner, *et al* 1996). Theoretically, unrelated cord blood transplantation (UCBT) has the following distinct advantages in PID patients: (1) the cord blood product is rapidly accessible in most cases; (2) the incidence and severity of graft-versus-host disease (GVHD) is not excessive, even in mismatched transplantation and (3) 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) (Bhattacharya, *et al* 2003, Bhattacharya, *et al* 2005, Fagioli, *et al* 2003, Knutsen, *et al* 2003, Knutsen and Wall 2000, 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 the UCBT 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 a 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 4 (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, 5 were 3-antigen mismatched and 3 were 4-antigen mismatched.

Immunosuppressive prophylaxis against GVHD after UCBT consisted of cyclosporin 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 0.5×10^9/L for 3 consecutive days. Platelet recovery was defined by a count of at least 20×10^9/L; recovery was unsupported by transfusion for 7 days. Reticulocyte recovery was defined by a count of at least 20‰.

Patients without conditioning or with only anti-thymocyte globulin (ATG) were categorised as the those receiving no conditioning. Patients administered BU/CY ± TBI or TLI, BU/CY + ATG ± TLI, BU/CY + fludarabine (Flu) or CY/etoposide/high-dose AraC were categorised as those receiving myeloablative therapies (MATs). CY dose ranged from 120 mg/kg to 240 mg/kg (median, 200 mg/kg) in patients receiving MAT.

TBI <4 Gy was classified as 'low-dose TBI'. Patients administered Flu/melphalan (L-PAM) ± low-dose TBI or TLI, Flu/BU ± TLI or Flu/CY (50–60 mg/kg) ± low-dose TBI/TLI, Flu +