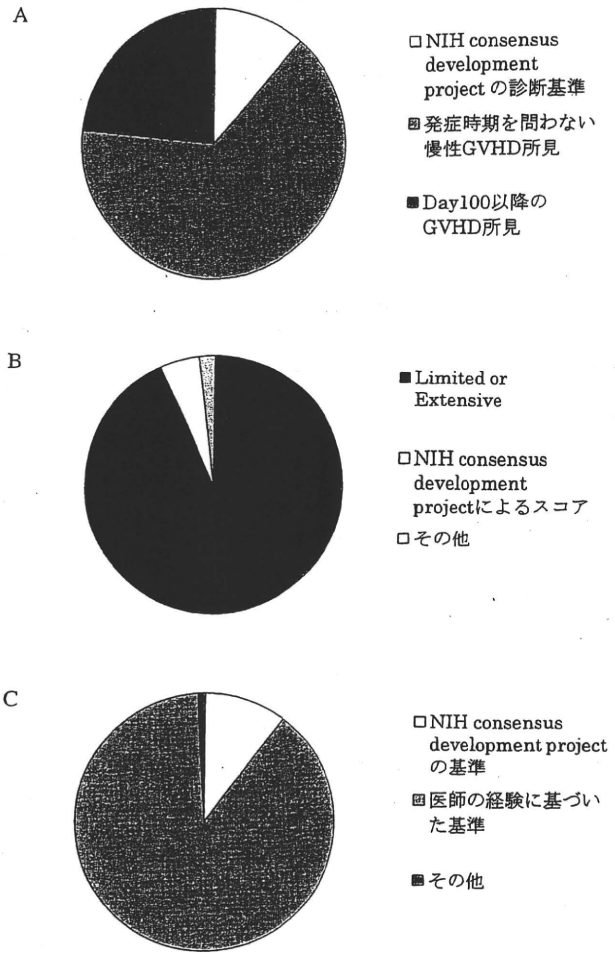

図1 A: 慢性 GVHD の診断について，各施設で実際に使用している診断基準
B: 慢性 GVHD の重症度について，実際に使用している基準
C: 慢性 GVHD の治療効果判定について，実際に使用している基準

あった。連携がある施設において，連携の方法は，退院サマリー・申し送り書などの文書 44.8%，カンファレンス・連絡会 17.2%，病棟看護師が外来へ行く 13.8%，外来看護師が病棟を訪問 17.2%，その他 17.3%などであった。

外来で施行している具体的なケアの内容は，ドライアイについての点眼指導，口腔乾燥対策，感染予防，日焼け予防，ホルモン補充療法の指導，IVH 管理，セクシャリティに関する相談，生活指導，メンタルケア，家族支援，福祉制度の紹介などが挙げられた。

II．自由記述

移植後長期フォローにおける現状の問題点と今後の在り方について自由記述の回答を集計した。集計は自由記述文からキーワードを抜き出し，キーワードの頻出順にまとめた。医師の意見（図 3A, C）と看護師の意見（図 3B, D）はそれぞれ別個に集計した。

〈構造的問題〉

医師からの回答で頻度の高い意見としては，医師が不足している。専従の看護師がいない。移植に関わるコメディカルが少ない。一般診療の中で移植患者も診ている。多忙である。などの問題点が挙げられた。

その他の意見としては，移植医の育成が必要。他科との連携が不足している。施設内に余裕がない。保険適用外の検査・治療が多い。地域毎に決まった施設に移植患者を集中させるべき。専門診療に報酬をつけるべき。などが挙げられた。

看護師からの回答で頻度の高い意見は，外来に移植患者用の場所がほしい，専門知識を持った専従の看護師が必要，看護師の人材不足，病棟と外来での看護の継続性が必要，配置転換が多く経験が積みにくいなどであった。

〈診療プロセス〉

医師からの回答では，長期フォローに関する日本のガイドラインを充実させる必要がある，関連する他科との連携が必要，多職種によるフォロー体制が必要，NIH 基準は実施が困難である，移植に関わる人材の教育・育成が必要，その他の意見としては，専門外来が必要，病院間の連携が必要，専門看護師の必要性などがあった。

看護師からの回答では，多職種との連携が少ない，看護・ケアのガイドラインや慢性 GVHD などに対するケアのマニュアルがほしいなどであった。

〈移植に関する専門外来設置についての意見〉

医師の回答では，

設置したいが人員確保が難しい，外来枠がない，スタッフの教育が必要，採算に疑問，移植患者が少ない。

看護師の回答では，

ケアの継続ができる体制が必要，専従の看護師が必要，必要性はあるが今の業務体制では困難。などの意見が寄せられた。

その他，専門外来の必要性・利点に関する医師からの意見としては，

- 複数の医師・診療科が同時に診察できるような専門外来が必要。
- 診断基準・マニュアルに従い診療・評価も可能になる。
- 客観的・正確な評価が可能になるため患者の QOL・生存の向上につながると思う。
- 患者一人にかける時間を増やせる。
- 診療が円滑になる。

看護師からの意見では，

- 移植患者に時間をかけて関われる。

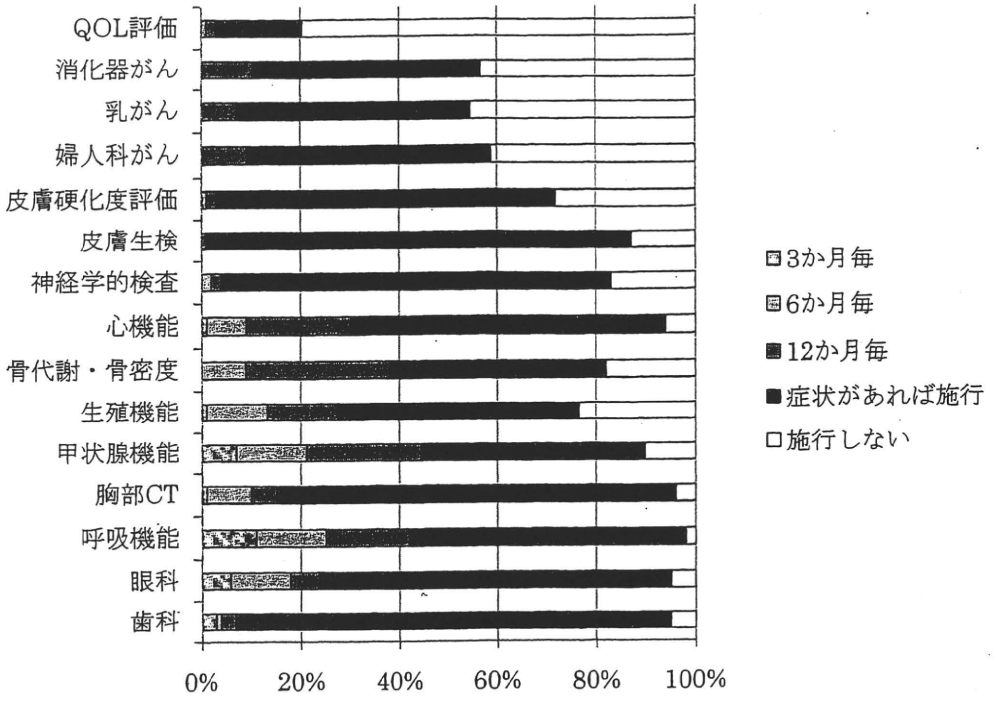

図2　晩期合併症のスクリーニング頻度：造血幹細胞移植後の晩期合併症に関する検査あるいは診察の頻度を集計した。

・専門職が関わることで患者も安心できる。

などが挙げられた。

専門外来設置に関する障害については，

医師の回答では，

・人材確保が困難であり，実現は難しい。
・移植以外の患者が多く，設置は困難。
・移植医の育成と教育が必要。
・専門職としての看護師が必要。
・人材が確保できない。施設に余裕がない。

看護師の回答では，

・看護師の知識や技術を向上させる必要がある。
・専門知識をもったスタッフの確保が難しい。
・現状の設備では無理。

などの意見が挙げられた。

考　案

近年，臍帯血移植，骨髄非破壊的造血幹細胞移植，新規免疫抑制剤などの新しい移植技術の進歩により移植適応患者は増加している。特に従来は移植適応外とされていた60歳代の患者に対する移植も積極的に施行されるようになり，移植による合併症管理の重要性と必要性は今まで以上に高まっている。特に，移植後長期生存者における慢性GVHDをはじめとする晩期合併症の管理は，移植後の長期予後を左右する要素である。しかし，移植後長期フォローは患者毎の個別の対応が必要な場合が多く，その診療には十分な時間と人手を要する。

今回の実態調査により明確になったことは，移植医療の構造的な問題として①一施設当たりの同種移植患者数は血縁・非血縁，臍帯血移植を含めて10例程度である。②施設内で移植に携わる医師数が少ない。③一般診療が多忙であり移植患者の診療に費やせる時間が外来で中央値12.5分しかない。④医師以外の多職種によるフォロー体制が不十分であること。⑤他科や他の医療機関との連携が不十分等。診療プロセスの問題として①NIH基準を使用している施設は少なく，実際に診療に用いることは困難。②晩期合併症のスクリーニングを決まったスケジュールで実施している施設は少ない。③長期フォロー患者の診療は，個々の医師の判断に基づいて施行されることが多くマニュアルを定めている施設は少ない。などであった。

多くの施設において造血幹細胞移植後の長期フォローは多忙な血液内科一般診療の合間に行われることが多く，十分な時間をかけて個々の患者に対して必要十分な診療を提供できているとは言えない状況である。長期

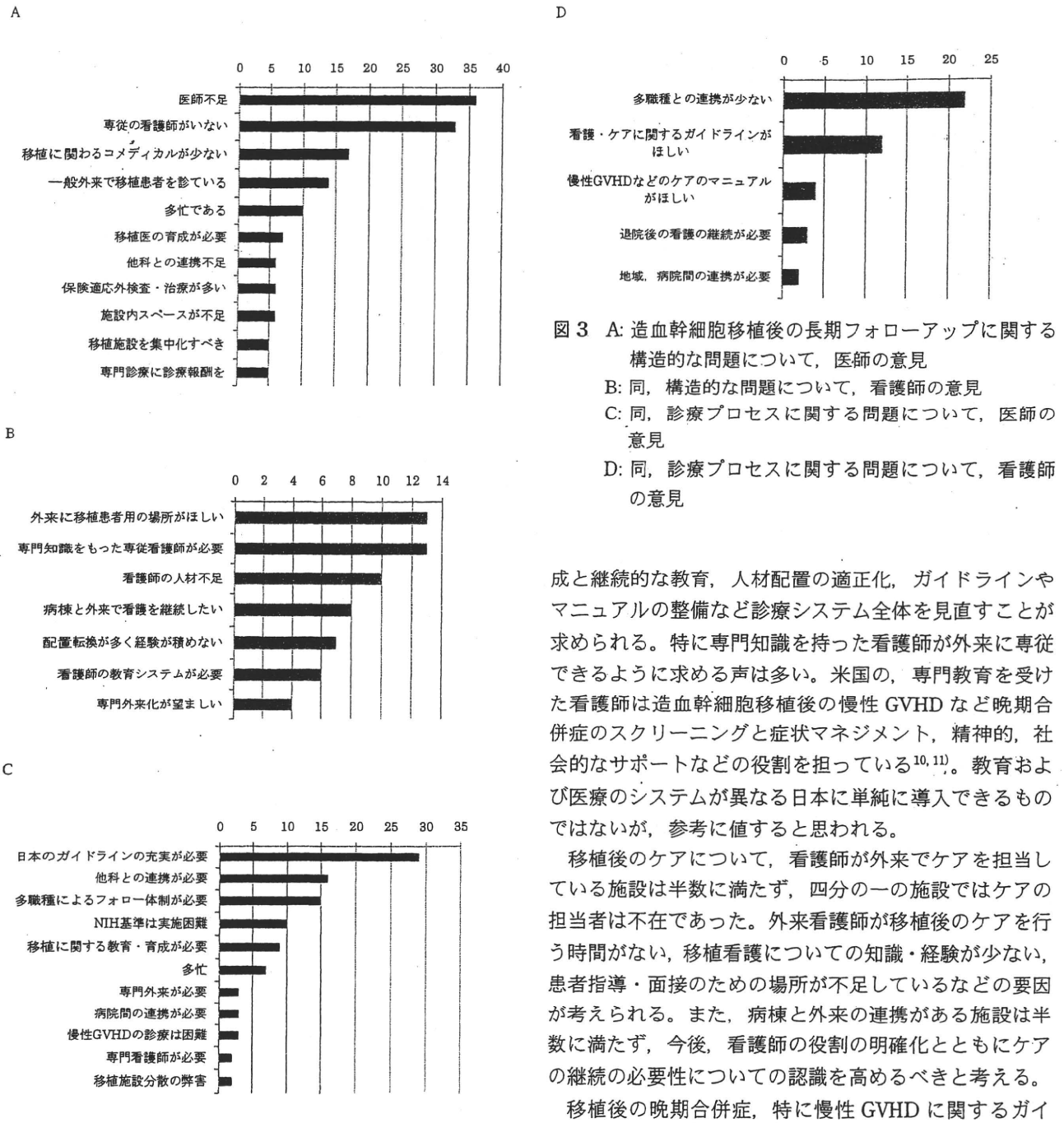

図3 A: 造血幹細胞移植後の長期フォローアップに関する構造的な問題について，医師の意見
B: 同，構造的な問題について，看護師の意見
C: 同，診療プロセスに関する問題について，医師の意見
D: 同，診療プロセスに関する問題について，看護師の意見

フォローにおける構造的問題として医師不足が上位に挙げられた。しかし，今回の調査においては，長期フォロー患者一人当たりの診療時間は，施設規模，移植実施数，移植に携わる医師総数などと有意に相関せず，診療に中央値の倍である25分以上を当てている11施設でも医師総数は同じであった。また，これら11施設の多くは医師以外の職種が長期フォローの診療に関与していることから，適切なチーム医療・人的資産の活用が医師不足を補える可能性が示唆される。他部門・多職種の協力体制や看護師をはじめとする移植に関わるスタッフの養成と継続的な教育，人材配置の適正化，ガイドラインやマニュアルの整備など診療システム全体を見直すことが求められる。特に専門知識を持った看護師が外来に専従できるように求める声は多い。米国の，専門教育を受けた看護師は造血幹細胞移植後の慢性GVHDなど晩期合併症のスクリーニングと症状マネジメント，精神的，社会的なサポートなどの役割を担っている[10,11]。教育および医療のシステムが異なる日本に単純に導入できるものではないが，参考に値すると思われる。

移植後のケアについて，看護師が外来でケアを担当している施設は半数に満たず，四分の一の施設ではケアの担当者は不在であった。外来看護師が移植後のケアを行う時間がない，移植看護についての知識・経験が少ない，患者指導・面接のための場所が不足しているなどの要因が考えられる。また，病棟と外来の連携がある施設は半数に満たず，今後，看護師の役割の明確化とともにケアの継続の必要性についての認識を高めるべきと考える。

移植後の晩期合併症，特に慢性GVHDに関するガイドラインは，NIH基準が発表されているが，日本では，ほとんど診療に用いられていないことが示された。本来，NIH基準は臨床試験での使用を目的に開発されたものであり，診療現場での日常的な適用は困難と思われた。実際の診療で使いやすく，医師間・施設間での差異が生じにくいような客観的なガイドラインや診断基準を充実させてゆくことが必要と思われる。また，EBMT/CIBMTR/ASBMTの合同勧告では，神経・内分泌代謝・二次がん等の晩期合併症に関するスクリーニングを定期的に行うことを推奨している[9]が，このようなスクリーニングを計画的に施行している施設は多くなかった。推

測される理由としては，時間的な余裕がない，他科・他部門との連携がしにくい等があげられる。十分な診療の質を担保するには，全科横断的な協力体制の構築と，晩期合併症に関する知識の普及が必要と思われる。また，看護師からの要望として移植看護・ケアに関するガイドラインやマニュアルの充実が挙がっており，今後の課題である。

移植患者のために特化した外来枠や移植後長期フォローアップのための外来枠を設けている施設は少数であるが，施設内で移植患者を集約することによって同一の診断基準を適用し，同質のケアを効率よく提供することが可能になるため診療の質向上に寄与するなど利点が多いと思われる。

欧米での移植医療は少数のセンター施設によって集約的に行われており，移植の各工程により担当する部門を特化させることにより専門性の高い効率のよいシステムが構築されている。NIH 基準および EBMT/CIBMTR/ASBMT 合同勧告などは，長期フォローシステムが確立された施設では効果的な運用が可能と思われる。しかし日本における造血幹細胞移植医療は多数の中小規模施設が主体をなしており集約化は進んでいない。本調査でも明かになったように各施設における移植例数は多くない反面，移植患者の診療に携わる人材の不足や時間的制約などの問題が生じている。少数の造血幹細胞移植施設への集約化は，このような問題の解決法と思われるが，大都市に患者が集中することで患者の医療へのアクセス性が阻害される恐れも生じる。実際，平成 21 年 5 月末までの都道府県別累計非血縁者間同種移植患者数を人口で除した指数を求めると，東京，愛知，福岡など大都市圏で高く，人口の少ない地域と比較すると 5 倍から 10 倍の格差が既に存在している[1, 12]。

造血幹細胞移植後の患者 QOL を向上させるためには，日本の良い面を生かしつつ長期フォローのための基盤を整備し，人材育成と資源配分を見直す必要があると考える。

本調査は，厚生労働省科学研究費補助金がん臨床研究事業「治療関連合併症を減少させて同種造血幹細胞移植後の生存率の向上を目指す標準的治療法の開発研究」班における分担研究として行われた。

文　献

1) 骨髄移植推進財団. 骨髄バンクデータ集 (http://www.jmdp.or.jp/data/). Accessed 2009 Nov 11.
2) Flowers MED, Deeg HJ. Delayed complications after hematopoietic cell transplantation. In: Blume KG, Forman SJ, Applebaum FR, eds. Thomas' Hematopoietic Cell Transplantation. Third editon. Malden, MA: Wiley-Blackwell; 2004: 944-961.
3) Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005; **11**: 945-56.
4) Shulman HM, Kleiner D, Lee SJ, et al. Histopathologic diagnosis of chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on Criteria for Cinical Trials in Chronic Graft-versus-Host Disease: II. Pathology Working Group Report. Biol Blood Marrow Transplant. 2006; **12**: 31-47.
5) Martin PJ, Weisdorf D, Przepiorka D, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: VI. Design of Clinical Trials Working Group report. Biol Blood Marrow Transplant. 2006; **12**: 491-505.
6) Couriel D, Carpenter PA, Cutler C, et al. Ancillary therapy and supportive care of chronic graft-versus-host disease: national institutes of health consensus development project on criteria for clinical trials in chronic Graft-versus-host disease: V. Ancillary Therapy and Supportive Care Working Group Report. Biol Blood Marrow Transplant. 2006; **12**: 375-396.
7) Pavletic SZ, Martin P, Lee SJ, et al. Measuring therapeutic response in chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: IV. Response Criteria Working Group report. Biol Blood Marrow Transplant. 2006; **12**: 252-266.
8) Schultz KR, Miklos DB, Fowler D, et al. Toward biomarkers for chronic graft-versus-host disease: National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: III. Biomarker Working Group Report. Biol Blood Marrow Transplant. 2006; **12**: 126-137.
9) Rizzo JD, Wingard JR, Tichelli A, et al. Recommended screening and preventive practices for long-term survivors after hematopoietic cell transplantation: Joint recommendations of the European Group for Blood and Marrow Transplantation, Center for International Blood and Marrow Transplant Research, and the American Society for Blood and Marrow Transplantation (EBMT/CIBMTR/ASBMT). Bone Marrow Transplant. 2006; **37**: 249-261.
10) Buchsel PC. Survivorship issues in hematopoietic stem cell transplantation. Semin Oncol Nurs. 2009; **25**: 159-169.
11) Rice RD, Bailey G. Management issues in hematopoietic stem cell transplantation. Semin Oncol Nurs. 2009; **25**: 151-158.
12) 総務省統計局. 統計でみる都道府県のすがた 2009 (http://www.stat.go.jp/data/ssds/5a.htm). Accessed 2009 Nov 11.

Long-term follow-up of patients undergoing allogeneic hematopoietic stem cell transplantation in Japan

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Key words : Long-term follow-up, Chronic GVHD, Stem cell transplantation

The number of hematopoietic stem cell transplantations and recipients with late complications from transplantation are both increasing; therefore, we investigated the status of the long-term follow-up system for hematopoietic stem cell transplantation survivors in Japan using a mail questionnaire; 100 of 194 institutions replied. The median examination time for each patient was 12.5 min. Five percent of institutions had an outpatient transplantation clinic, 1% had a manual for long-term follow-up after stem cell transplantation, and 11% used NIH criteria for the diagnosis of chronic GVHD. The lack of human resources, such as doctors, nurses, and other co-medical staff for transplant patients, was a structural problem. In addition, the development of guidelines for Japanese patients and staff education are also required in the clinical process. Thus, a long-term follow-up system, training of human resources, and appropriate reallocation of funds for medical services are required.

Brief report

Successful sustained engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with severe aplastic anemia

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We retrospectively analyzed 12 consecutive adult severe aplastic anemia patients who received unrelated umbilical cord blood transplantation after a reduced-intensity conditioning regimen (RI-UCBT). The conditioning regimen consisted of 125 mg/m^2 fludarabine, 80 mg/m^2 melphalan, and 4 Gy of total body irradiation. The median infused total nucleated cell number and CD34$^+$ cell number were 2.50 × 10^7/kg and 0.76 × 10^5/kg, respectively. Eleven of the 12 patients achieved primary neutrophil and platelet engraftment. All patients who achieved engraftment had complete hematologic recovery with complete donor chimerism, except for one patient who developed late graft failure 3 years after RI-UCBT. Two of the 12 patients died of idiopathic pneumonia syndrome, and the remaining 10 patients are alive, having survived for a median of 36 months. Our encouraging results indicate that RI-UCBT may become a viable therapeutic option for adult severe aplastic anemia patients who lack suitable human leukocyte antigen-matched donors and fail immunosuppressive therapy. (*Blood.* 2011;117(11):3240-3242)

Introduction

Bone marrow transplantation from a human leukocyte antigen (HLA)-matched sibling is recommended as first-line therapy for younger patients with severe aplastic anemia (SAA).[1,2] However, many patients lack HLA-matched sibling donors. Bone marrow transplantation from an HLA-matched unrelated donor has been an alternative therapeutic option for patients who fail one or more courses of immunosuppressive therapy, but high rates of graft failure (GF), graft-versus-host disease (GVHD), and infection still remain to be solved.[3] The number of unrelated umbilical cord blood transplantations (UCBTs) has been increasing.[4] However, little information has been available on whether UCBT is feasible for SAA patients. We reported successful urgent UCBT using reduced-intensity (RI) conditioning for a 70-year-old SAA patient in 2003.[5] Here we present successful sustained engraftment of 11 consecutive patients with SAA who received RI-UCBT with the same RI conditioning regimen after the first report.

Methods

This study included 12 consecutive adult patients with acquired SAA who underwent RI-UCBT at our institute from September 2002 through January 2009. The patients' characteristics and umbilical cord blood (UCB) units are summarized in Table 1. Their median age was 49 years (range, 20-70 years). Four cases of severe, 6 of very severe, and 2 of fulminant type were included according to criteria as previously reported.[2,6] Fulminant type was defined as no neutrophils in the peripheral blood at diagnosis despite administration of granulocyte-colony stimulating factor. Ten patients, except for the 2 patients with fulminant type, had failed at least one course of immunosuppressive therapy. All patients gave their written informed consent in accordance with the Declaration of Helsinki, and the study was approved by the Toramon Hospital Institutional Review Board. UCB units were obtained from the Japanese Cord Blood Bank Network, and single UCB unit was infused in all the studied patients. All UCB units were serologically typed for HLA-A, -B, and -DR antigen before selection and were tested by high-resolution DNA typing before transplantation. The degree of mismatch is expressed using antigen level at HLA-A and -B, and allele level at DRB1. ABO incompatibility was not incorporated as one of the factors used in CB unit selection. The median total nucleated cell number and CD34$^+$ cell number at cryopreservation were 2.50 × 10^7/kg (range, 1.83-4.39 × 10^7/kg) and 0.76 × 10^5/kg (range, 0.27-1.52 × 10^5/kg), respectively. Anti-HLA antibodies were screened before transplantation in 6 patients using a FlowPRA method (One Lambda), and LAB Screen PRA or Single Antigen (One Lambda) was used to identify HLA antibody specificities.[7,8] All patients were conditioned with 25 mg/m^2 fludarabine daily for 5 days, 40 mg/m^2 melphalan daily for 2 days, and 4 Gy of total body irradiation in 2 fractions in 1 day. GVHD prophylaxis consisted of cyclosporine in 2, tacrolimus in 2, and tacrolimus plus mycophenolate mofetil in 8. Assessment of engraftment, GF, chimerism, GVHD, and supportive care during transplantation were performed as previously reported.[9,10] Karnofsky performance status score was assessed as surrogate for quality of life of the survivors. Overall survival was estimated using the Kaplan-Meier method.

Results and discussion

Patients' outcomes are summarized in Table 2. Eleven of the 12 patients achieved primary neutrophil and platelet engraftment. The median times to achieve neutrophil engraftment and platelet count more than 20 × 10^9/L were 18 days (range, 12-28 days) and

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Table 1. Characteristics of patient, grafts, and GVHD prophylaxis

Case no.	Age, y	Previous treatment	Interval from diagnosis to UCBT, mo	Previous transfusion times (RBCs/platelet)	Disease status at UCBT	HLA match	HLA Ab (reactive to CB)	ABO group (R/D)	TNC $\times 10^7$/kg	CD34$^+$, $\times 10^5$/kg	GVHD prophylaxis
1	70	CSA	3	11/14	SAA	4/6	NT	A/A	4.00	1.23	CSA
2	20	ATG + CSA	78	> 20/> 20	VSAA	4/6	NT	B/O	2.65	1.07	CSA
3	22	ATG + CSA, PSL	157	> 20/> 20	SAA	4/6	NT	A/O	2.26	0.27	Tac
4	26	ATG + CSA	3	> 20/> 20	VSAA	5/6	NT	A/A	2.65	0.70	Tac
5	59	ATG + CSA	8	> 20/> 20	SAA	5/6	Positive (no)	O/O	2.15	1.52	Tac + MMF
6	49	ATG + CSA, PSL	12	> 20/> 20	VSAA	3/6	NT	A/A	2.04	0.62	Tac + MMF
7	70	None	1	5/8	Fulminant	4/6	Positive (yes)	A/O	4.39	1.29	Tac + MMF
8	52	None	1	4/6	Fulminant	4/6	NT	AB/A	3.20	0.49	Tac + MMF
9	46	ATG + CSA	45	> 20/> 20	VSAA	4/6	Positive (no)	AB/O	1.83	0.42	Tac + MMF
10	49	ATG + CSA, PSL	327	> 20/> 20	VSAA	6/6	Positive (no)	B/O	2.34	0.82	Tac + MMF
11	65	CSA	6	16/> 20	VSAA	6/6	Positive (no)	A/A	3.31	0.56	Tac + MMF
12	31	ATG + CSA, PSL	215	> 20/> 20	SAA	4/6	Positive (no)	B/O	2.09	1.26	Tac + MMF

RBC indicates red blood cell; CB, cord blood; R, recipient; D, donor; TNC, total nucleated cells; CSA, cyclosporine-A; ATG, antithymocyte globulin; PSL, prednisone; VSAA, very severe aplastic anemia; NT, not tested; Tac, tacrolimus; and MMF, mycophenolate mofetil.

42 days (range, 26-64 days), respectively. All patients who achieved engraftment had complete hematologic recovery and were free from transfusion, and they showed complete donor chimerism at the time of the first chimerism analysis (median, 14 days; range, 11-73 days). One patient developed primary GF and was later found to have antibody against mismatched HLA on donor cells. Another patient developed secondary GF 3 years after UCBT. Both patients underwent a second RI-UCBT and obtained rapid donor engraftment. The negative impact of multiple transfusions before transplantation was not detected (Tables 1-2). Among 11 evaluable patients, 2 developed grade I and 5 developed grade II acute GVHD. Of the 9 patients who survived longer than 100 days after transplantation, 3 developed limited type of chronic GVHD. No patients developed grade III-IV acute GVHD and extensive type of chronic GVHD. Two of the 12 patients died of idiopathic pneumonia syndrome, and the remaining 10 patients are alive, having survived for a median of 36 months (range, 14-91 months). The probability of overall survival at 3 years was 83.3% (Figure 1). The surviving patients had high Karnofsky performance status score with a median of 90% (range, 60%-100%).

The present study demonstrated that our RI conditioning regimen allows a sufficient sustained engraftment of UCB in adult SAA patients. The RI conditioning regimen was originally developed in our institute for UCBT for various hematologic malignancies.[9] Eleven of the 12 patients achieved primary engraftment, which compares favorably with previously reported engraftment rates of UCBT for SAA.[11-16] Our RI conditioning regimen would be more potent than the others to overcome immunologic barriers for engraftment. Cell dose has been known to significantly influence the rate of engraftment after UCBT.[14] In the present study, although the cell dose was not very large, sufficient engraftment was seen. Any significant relationship between cell dose (total nucleated cell, ≥ 2.5 vs $< 2.5 \times 10^7$/kg; CD34$^+$, ≥ 0.8 vs $< 0.8 \times 10^5$/kg) and engraftment kinetics were observed (data not shown). Thus, not just cell dose but other factors, such as the intensity of the conditioning regimen and posttransplantation immunosuppression, may be important to achieve better engraftment after UCBT for SAA patients. Interestingly, all 6 patients who were screened for HLA antibodies before transplantation had HLA antibodies, and the one case who had positive HLA antibodies against an HLA on a transplanted UCB unit was the only one who failed primary engraftment. Recently, Takanashi et al reported that, in large number of UCBT for various hematologic malignancies, the

Table 2. Outcomes of 12 patients after reduced-intensity unrelated cord blood transplantation

Case no.	Days to ANC > 0.5 × 10^9/L	Days to PC > 20 × 10^9/L	% Donor chimerism (days tested, methods)	aGVHD	cGVHD	Discontinuation of IS (mo)	Complications	Survival (mo)
1	12	52	100 (14, FISH)	Grade II (skin)	No	Yes (3)	Possible IPA	Alive (91)
2	20	64	> 90 (49, PCR-STR)	Grade II (skin)	Limited	Yes (2)	No	Alive (90)
3	26	42	100 (26, FISH)	No	No	Yes (26)	Yes	Alive (69)
4	18	53	100 (18, FISH)	No	No	Yes (5)	*Pneumocystis jirovecii*, late GF, rescued by second RI-UCBT	Alive (69)
5	16	26	96.6 (14, FISH)	Grade I (skin)	Limited	Yes (14)	Norwalk virus colitis, EBV-PTLD	Alive (39)
6	28	64	99.6 (11, FISH)	No	NE	No	IPS	Dead; IPS (3)
7	No	No	48.8 (10, FISH), 4.3 (15, FISH)	NE	NE	NE	Primary GF, rescued by second RI-UCBT	Alive (32)
8	18	28	99.2 (13, FISH)	Grade II (skin, gut)	No	Yes (7)	CMV colitis, EBV-PTLD	Alive (28)
9	28	43	> 90 (14, PCR-STR)	Grade I (skin)	NE	No	HSV pneumonia, IPS	Dead; IPS (3)
10	15	27	99 (73, FISH)	No	Limited	No	No	Alive (22)
11	15	27	100 (20, FISH)	Grade II (skin, gut)	No	No	No	Alive (22)
12	13	28	100 (14, FISH)	Grade II (gut)	No	No	No	Alive (14)

ANC indicates absolute neutrophil count; PC, platelet count; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; IS, immunosuppressant; FISH, fluorescence in situ hybridization; PCR-STR, PCR of short tandem repeat; NE, not evaluable; IPA, invasive pulmonary aspergillosis; EBV-PTLD, Epstein-Barr virus–associated posttransplantation lymphoproliferative disorder; and IPS, idiopathic pneumonia syndrome.

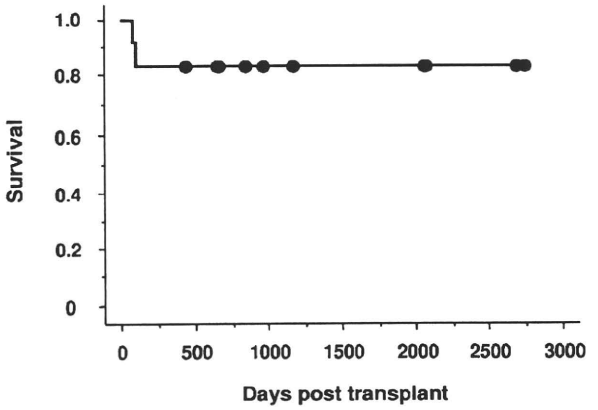

Figure 1. Survival of 12 patients with SAA undergoing unrelated cord blood transplantation.

patients with anti-HLA antibodies, when the specificity corresponding to mismatched antigen in UCB graft, showed significantly lower neutrophil or platelet recovery than those with antibody-negative or -positive but not corresponding to UCB graft.[17] Although the observations may differ from that of diverse populations and warrants further investigation, if possible, the use of a UCB unit with corresponding HLA antibodies in the recipient should be avoided.

Three-year survival in the studied patients was 83.3%. In addition to high rate of engraftment, the low risk of severe GVHD might contribute to high survival rate with good quality of life, and seems to be one of the important advantages of using a UCB unit for SAA patients. The other advantage of the use of UCB units is rapid availability. In the present study, 2 patients with fulminant type could be rescued by urgent hematopoietic stem cell transplantation using UCB units. More than 90% of recipients can find a suitable UCB unit in Japan; thus, UCB expands the chance to receive transplantation for those who need it urgently.

In conclusion, this retrospective study strongly suggests the feasibility and effectiveness of RI-UCBT for adult SAA patients. RI-UCBT may become a viable therapeutic option for those who lack suitable HLA-matched donors and who fail or relapse after immunosuppressive therapy. Although our results should be interpreted with caution because of the small number of patients and still short follow-up duration, we think that RI-UCBT with the conditioning regimen presented here deserves further evaluation in a prospective trial, hopefully in a multicenter setting.

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Authorship

Contribution: H.Y. and D.K. performed transplantation, analyzed extracted data, and contributed to writing the paper; A.Y. reviewed histopathologic sections; H.Y. and N.M. performed statistical analysis; N.U., K. Izutsu, and S. Taniguchi reviewed study design and methods; and K. Ishiwata, H.A., S. Takagi, M.T., N.N., Y.A.-M., K.M., A.W., and S.M. performed transplantation and contributed to writing the paper.

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References

1. Marsh J. Making therapeutic decisions in adults with aplastic anemia. *Hematology Am Soc Hematol Educ Program.* 2006:78-85.
2. Marsh JC, Ball SE, Cavenagh J, et al. Guidelines for the diagnosis and management of aplastic anaemia. *Br J Haematol.* 2009;147(1):43-70.
3. Passweg JR, Perez WS, Eapen M, et al. Bone marrow transplants from mismatched related and unrelated donors for severe aplastic anemia. *Bone Marrow Transplant.* 2006;37(7):641-649.
4. Brunstein CG, Setubal DC, Wagner JE. Expanding the role of umbilical cord blood transplantation. *Br J Haematol.* 2007;137(1):20-35.
5. Kusumi E, Miyakoshi S, Murashige N, et al. Successful reduced-intensity stem cell transplantation (RIST) with mismatched cord blood in a 70-year-old patient with severe aplastic anemia (SAA). *Bone Marrow Transplant.* 2003;32(11):1111-1112.
6. Camitta BM, Thomas ED, Nathan DG, et al. Severe aplastic anemia: a prospective study of the effect of early marrow transplantation on acute mortality. *Blood.* 1976;48(1):63-70.
7. Pei R, Wang G, Tarsitani C, et al. Simultaneous HLA Class I and Class II antibodies screening with flow cytometry. *Hum Immunol.* 1998;59(5):313-322.
8. Takanashi M, Fujiwara K, Tanaka H, Satake M, Nakajima K. The impact of HLA antibodies on engraftment of unrelated cord blood transplants. *Transfusion.* 2008;48(4):791-793.
9. Miyakoshi S, Yuji K, Kami M, et al. Successful engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with advanced hematological diseases. *Clin Cancer Res.* 2004;10(11):3586-3592.
10. Uchida N, Wake A, Takagi S, et al. Umbilical cord blood transplantation after reduced-intensity conditioning for elderly patients with hematologic diseases. *Biol Blood Marrow Transplant.* 2008;14(5):583-590.
11. Yoshimi A, Kojima S, Taniguchi S, et al. Unrelated cord blood transplantation for severe aplastic anemia. *Biol Blood Marrow Transplant.* 2008;14(9):1057-1063.
12. Mao P, Zhu Z, Wang H, et al. Sustained and stable hematopoietic donor-recipient mixed chimerism after unrelated cord blood transplantation for adult patients with severe aplastic anemia. *Eur J Haematol.* 2005;75(5):430-435.
13. Chan KW, McDonald L, Lim D, Grimley MS, Grayson G, Wall DA. Unrelated cord blood transplantation in children with idiopathic severe aplastic anemia. *Bone Marrow Transplant.* 2008;42(9):589-595.
14. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med.* 1998;339(22):1565-1577.
15. Lau FY, Wong R, Chui CH, Cheng G. Successful engraftment in two adult patients with severe aplastic anemia using nonmyeloablative conditioning followed by unrelated HLA-mismatched cord blood transplantation. *J Hematother Stem Cell Res.* 2001;10(2):309-311.
16. Schwinger W, Urban C, Lackner H, et al. Transplantation of related and unrelated umbilical cord blood stem cells in Austria: Austrian Working Party for Stem Cell Transplantation. Austrian Society of Hematology and Oncology. *Wien Klin Wochenschr.* 1999;111(9):348-353.
17. Takanashi M, Atsuta Y, Fujiwara K, et al. The impact of anti-HLA antibodies on unrelated cord blood transplantations. *Blood.* 2010;116(15):2839-2846.

Brief report

Successful engraftment after reduced-intensity umbilical cord blood transplantation for myelofibrosis

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Although allogeneic hematopoietic stem cell transplantation has recently been applied to patients with myelofibrosis with reproducible engraftment and resolution of marrow fibrosis, no data describe the outcomes of umbilical cord blood transplantation. We describe 14 patients with primary (n = 1) and secondary myelofibrosis (n = 13) who underwent reduced-intensity umbilical cord blood transplantation. Conditioning regimens included fludarabine and graft-versus-host disease prophylaxis composed cyclosporine/tacrolimus alone (n = 6) or a combination of tacrolimus and mycophenolate mofetil (n = 8). Thirteen patients achieved neutrophil engraftment at a median of 23 days. The cumulative incidence of neutrophil and platelet engraftment was 92.9% at day 60 and 42.9% at day 100, respectively. Posttransplantation chimerism analysis showed full donor type in all patients at a median of 14 days. The use of umbilical cord blood could be feasible even for patients with severe marrow fibrosis, from the viewpoint of donor cell engraftment. (*Blood*. 2010;116(4):649-652)

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered the only curative therapy for primary myelofibrosis (MF) and MF secondary to hematologic malignancies.[1] Myeloablative conditioning regimens are associated with high rates of transplantation-related mortality (TRM), especially among elderly patients.[2-4] Recent reports indicate that reduced-intensity conditioning (RIC) regimens can improve outcomes in such patients.[5-8] These reports also confirm the safety and effectiveness of bone marrow (BM) and mobilized peripheral blood stem cells (PBSCs) from matched related or unrelated donors as stem cell sources. In contrast, the feasibility of umbilical cord blood transplantation (CBT) for MF is unknown.

CBT is a valuable alternative to allo-HSCT for treating patients with hematologic diseases who do not have matched related or unrelated donors and who need urgent transplantation.[9-12] On the other hand, engraftment delay or failure is one of the most critical issues that can arise after CBT. The limited doses of total nucleated cells and $CD34^+$ cells in umbilical cord blood and a human leukocyte antigen (HLA) disparity influence the kinetics of hematopoietic recovery.[13-15] Considering these disadvantages of CBT, delayed engraftment or engraftment failure is a great concern for MF patients who undergo CBT.[16] The goal of this study is to evaluate the feasibility of reduced-intensity CBT (RI-CBT) for MF.

Methods

The records of all patients who underwent RI-CBT at Toranomon Hospital from August 2003 and December 2008 were reviewed to identify patients who had histologically confirmed MF before starting the conditioning regimen. Marrow fibrosis was assessed on silver-stained BM trephine biopsies and classified into 4 grades according to the World Health Organization classification.[17] All the patients were incurable using conventional approaches and lacked an HLA-identical sibling or a suitable unrelated donor from the Japan Marrow Donor Program. Cord blood units serologically matching more than or equal to 4 of 6 HLA antigens and containing at least 1.8×10^7 nucleated cells/kg of recipient body weight before freezing were obtained from the Japan Cord Blood Bank Network. Conditioning regimens were determined at the discretion of each physician according to the patients' disease, disease status, and history of prior therapy. Information about baseline demographics, clinical characteristics, transplantation, and its outcome were collected from medical records. Assessment of engraftment, chimerism (one or more times a week), pre-engraftment immune reactions, graft-versus-host disease (GVHD), and supportive care during transplantation were performed as previously reported.[18-20] Cumulative incidences were estimated for neutrophil and platelet engraftment. Overall survival was estimated using the Kaplan-Meier method, taking the interval from date of transplantation to death or last contact.[21] The Institutional Review Board of Toranomon Hospital approved the study, and written informed consent was provided by all patients to use their records in accordance with the Declaration of Helsinki.

Results and discussion

Fourteen MF patients (median age, 57.5 years; range, 46-72 years) were extracted. Table 1 shows the clinical characteristics of the patients. They had primary MF (n = 1), leukemic transformation from MF secondary to polycythemia vera or essential thrombocytosis (n = 2), or MF secondary to acute myeloid leukemia (AML; n = 11; AML with multilineage dysplasia in all patients except for one with de novo AML). All but one patient had the highest-grade

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Table 1. Patient characteristics

Patient no.	Age, y/sex	Diagnosis	Disease status	Time from diagnosis to transplantation, d	Pretransplantation MF grade	Splenomegaly	Cytogenetics
1	55/M	AML/MF/ET	PIF	1732	3	Yes	Normal
2	53/M	PMF	Untreated	307	3	Yes	NA
3	61/M	AML/MDS	PIF	116	3	Yes	Complex*
4	51/F	AML/MDS	PIF	740	3	Yes	Normal
5	61/F	AML/MDS	PIF	227	3	Yes	NA
6	55/M	AML/MDS	Untreated	299	3	Yes	Complex
7	46/M	AML/MDS	Untreated	600	3	Yes	NA
8	58/M	AML/MDS	Untreated	544	3	Yes	Complex
9	67/F	AML/MF/PV	Untreated	150	3	Yes	t(3;3)(q21;q26), −7
10	53/M	De novo AML	PIF	111	3	No	Complex
11	57/F	AML/MDS	Untreated	352	3	Yes	Complex with t(9;22)(q34;q11)
12	62/M	AML/MDS	Untreated	147	3	Yes	add(1)(p32), −7
13	72/F	AML/MDS	PIF	329	2	No	Complex with t(9;22)(q34;q11)
14	66/M	AML/MDS	Untreated	92	3	No	Normal

AML indicate acute myeloid leukemia; MF, myelofibrosis; ET, essential thrombocythemia; PIF, primary induction failure; PMF, primary myelofibrosis; AML/MDS, acute myeloid leukemia with multilineage dysplasia; NA, not available; and PV, polycythemia vera.

*Complex karyotype was defined as 3 or more abnormalities at pretransplantation evaluation.

MF. The median time from diagnosis to transplantation was 303 days (range, 92-1732 days). Table 2 shows the transplantation characteristics. All received purine analog–based conditioning regimens composing fludarabine phosphate (125-180 mg/m^2), melphalan (80-140 mg/m^2), or intravenous busulfan (12.8 mg/kg) and 0 to 8 Gy of total body irradiation. GVHD prophylaxis included tacrolimus and mycophenolate mofetil for 8 patients, tacrolimus, or cyclosporine A alone in 6. Neutrophil and platelet engraftment was achieved in 13 and 6 patients, respectively, of the 14 patients. The median time to engraftment was 23 days (range, 14-43 days) and 53 days (range, 44-102 days) for neutrophils and platelets, respectively. The cumulative incidence of neutrophil engraftment at day 60 and platelet engraftment at day 100 was 92.9% and 42.9%, respectively (Figure 1A-B). Chimerism analysis of the peripheral blood of 8 patients and the BM of 6 showed that donor chimerism was complete (donor > 90%) in all of them. The median length of time required to achieve complete donor chimerism was 14 days (range, 7-33 days; Figure 1A). Of the 14 patients, 9 (64%) developed pre-engraftment immune reactions. Five (36%) developed acute GVHD grades 2 to 4. No extensive chronic GVHD was observed in 6 evaluable patients (Table 3). Five patients remained alive at last contact, representing an estimated probability of overall survival of 28.6% at 4 years (Figure 1C). All the patients who could not achieve platelet engraftment died, whereas 4 of 7 patients (57%) who achieved platelet engraftment survived. In 9 patients who died after RI-CBT, 5 patients died of relapsed leukemia. Non–relapse-related causes of death composed infection (n = 2), GVHD (n = 1), and multiple organ failure (n = 1). Marrow fibrosis disappeared in 2 evaluated patients who survived beyond 100 days.

This study demonstrated that umbilical cord blood results in successful engraftment, even for patients with severe marrow fibrosis in the setting of the RIC regimen, which was similar to that of other stem cell sources, such as BM and PBSCs.[2-8,22] Although marrow fibrosis has historically been considered as a relative contraindication to transplantation because of concerns over an insufficient and/or dysfunctional niche in which allogeneic hematopoietic stem cell engraftment may proceed, recent outcomes of allo-HSCT for MF support the concept that marrow fibrosis is not an absolute barrier to allogeneic hematopoietic stem cell engraftment.[1] However, data from these reports are limited to transplantations with BM and PBSCs, and no information is available about umbilical cord blood. Delayed hematopoietic recovery and low engraftment rate, perhaps because of limited infused cell doses and

Table 2. Transplantation characteristics

Patient no.	TNC, ×10^7/kg	CD34$^+$, ×10^5/kg	Sex match	HLA match	Blood type match	Conditioning regimen	GVHD prophylaxis
1	2.52	0.823	MM	4/6	MM	F125/M80/TBI4	CsA
2	2.62	0.678	MM	4/6	MM	F125/M80/TBI4 + SRT	TAC
3	3.17	1.60	Match	4/6	Match	F125/M80/TBI4	TAC
4	2.43	NA	MM	4/6	Match	F125/M80/TBI4	TAC
5	3.94	2.26	MM	5/6	Match	F180/M140	TAC/MMF
6	2.31	0.887	MM	4/6	MM	F125/M80/TBI4	TAC
7	2.72	1.03	Match	4/6	MM	F125/Mel140/TBI4	TAC/MMF
8	2.46	0.773	MM	4/6	Match	F180/M140	TAC
9	1.99	1.24	MM	4/6	MM	F125/M80/TBI4 + SRT	TAC/MMF
10	3.25	0.547	MM	4/6	Match	F125/M140/TBI4	TAC/MMF
11	3.31	1.31	MM	4/6	Match	F125/M80/TBI8	TAC/MMF
12	2.37	0.873	MM	4/6	MM	F125/M80/TBI8	TAC/MMF
13	2.51	0.993	MM	4/6	Match	Flu180/B12.8/TBI2	TAC/MMF
14	2.50	0.554	MM	5/6	Match	F125/M120	TAC/MMF

TNC indicates total nucleated cell count; MM, mismatch; F, fludarabine (mg/m^2); M, melphalan (mg/m^2); TBI, total body irradiation; CsA, cyclosporine; SRT, splenic radiation; TAC, tacrolimus; MMF, mycophenolate mofetil; and B, intravenous busulfan (mg/kg).

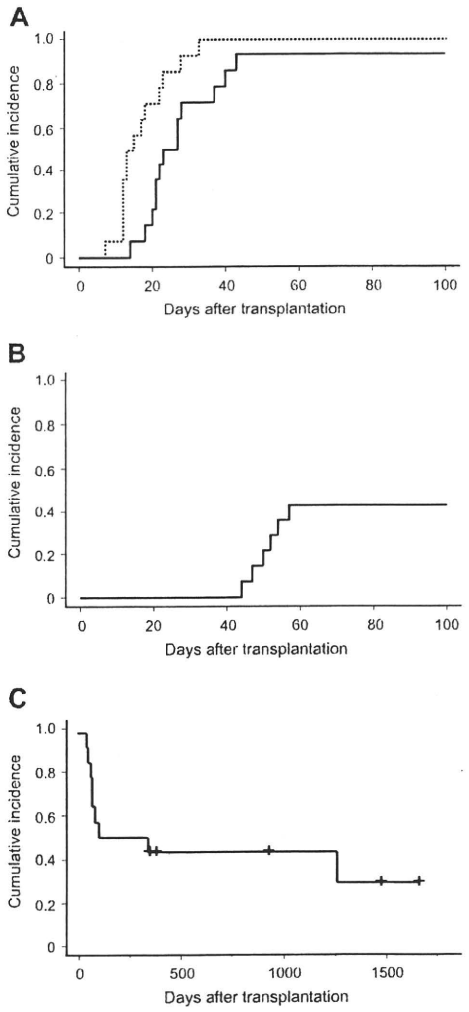

Figure 1. Cumulative incidence of engraftment. (A) Solid and broken lines indicate cumulative incidence of neutrophil engraftment and complete donor chimerism, respectively. (B) Cumulative incidence of platelet engraftment. (C) Overall survival.

HLA disparities, might limit the use of umbilical cord blood in these cases.[13-15,19] However, the present study demonstrated an equivalent or superior engraftment rate after CBT compared with allo-HSCT using other stem cell sources.[1-8] We also confirmed an early chimerism switching in the present study. All 14 patients achieved complete donor chimerism at a median of 14 days, which was much earlier than that with neutrophil engraftment. Moreover, we histologically confirmed that RI-CBT had the potential to cure marrow fibrosis in 2 evaluated patients. These data suggest that RI-CBT is an encouraging strategy for treating MF.

Despite successful engraftment, overall survival was poor in the present study compared with previous reports. However, this result does not eliminate the feasibility of RI-CBT for MF patients. Our patient series included only one primary MF. In 13 of 14 patients, MF coexisted with AML simultaneously. High prevalence of concurrent AML with MF in the present study probably made overall survival poorer. However, MF with AML is also challenging issues in real clinical settings. Physicians occasionally face rapidly growing AML cases with concurrent marrow fibrosis, especially in the elderly, for whom urgent allo-HSCT is the only curative therapy. For those patients, CBT is attractive because of its accessibility. In this viewpoint, we think that the feasibility of RI-CBT suggested in the present study is encouraging.

In conclusion, our data suggest that RI-CBT is feasible, even for patients with severe marrow fibrosis, from the viewpoint of donor cell engraftment. Especially for MF with AML, further improvements are required in the next place to overcome poor survival resulting from relapse.

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Table 3. Outcome of RI-CBT

Patient no.	Neutrophil engraftment, d	Platelet engraftment, d	Pre-engraftment immune reactions*	aGVHD 2-4	aGVHD 3-4	cGVHD	Survival	Survival from transplantation, d	Cause of death
1	27	52	No	Yes	No	No	Dead	1264	Relapse
2	22	54	Yes	No	No	NE	Alive	1672	NA
3	23	Not engrafted	Yes	Yes	Yes	NE	Dead	68	Infection
4	40	102	Yes	Yes	No	Limited	Alive	1481	NA
5	18	44	Yes	No	No	No	Dead	344	Relapse
6	14	Not engrafted	Yes	No	No	NE	Dead	78	Relapse
7	21	57	Yes	Yes	Yes	Limited	Alive	937	NA
8	Not engrafted	Not engrafted	No	No	No	NE	Dead	42	Infection
9	37	Not engrafted	Yes	No	No	NE	Dead	45	MOF
10	28	Not engrafted	Yes	Yes	Yes	NE	Dead	64	GVHD
11	27	Not engrafted	Yes	No	No	NE	Dead	61	Relapse
12	43	NA	No	No	No	Limited	Alive	392	NA
13	21	47	No	No	No	Limited	Alive	355	NA
14	20	50	No	No	No	NE	Dead	100	Relapse

aGVHD indicates acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; NE, not evaluable; NA, not applicable; and MOF, multiple organ failure.

*Pre-engraftment immune reactions were diagnosed when febrile patients developed skin eruption, diarrhea, jaundice, or body weight gain of more than 10% of baseline, with no direct evidence of infection or adverse effects of medication, developing more than 6 days before engraftment.[18]

Authorship

Contribution: S. Takagi performed transplantation, analyzed extracted data, and contributed to writing the paper; Y.O. analyzed histologic sections; N.U., K.T., K.I., M.T., H.Y., Y.A.-M., K.M., A.W., and S.M. performed transplantation and contributed to writing the paper; N.M. performed transplantation and supported statistical analysis; K.O. reviewed histologic sections and contributed to writing the paper; and S. Taniguchi reviewed the study method and organized this study.

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References

1. Zang DY, Deeg HJ. Allogeneic hematopoietic cell transplantation for patients with myelofibrosis. *Curr Opin Hematol.* 2009;16(2):140-146.
2. Guardiola P, Anderson JE, Bandini G, et al. Allogeneic stem cell transplantation for agnogenic myeloid metaplasia: a European Group for Blood and Marrow Transplantation, Societe Francaise de Greffe de Moelle, Gruppo Italiano per il Trapianto del Midollo Osseo, and Fred Hutchinson Cancer Research Center Collaborative Study. *Blood.* 1999;93(9):2831-2838.
3. Deeg HJ, Gooley TA, Flowers ME, et al. Allogeneic hematopoietic stem cell transplantation for myelofibrosis. *Blood.* 2003;102(12):3912-3918.
4. Daly A, Song K, Nevill T, et al. Stem cell transplantation for myelofibrosis: a report from two Canadian centers. *Bone Marrow Transplant.* 2003; 32(1):35-40.
5. Rondelli D, Barosi G, Bacigalupo A, et al. Allogeneic hematopoietic stem-cell transplantation with reduced-intensity conditioning in intermediate- or high-risk patients with myelofibrosis with myeloid metaplasia. *Blood.* 2005;105(10):4115-4119.
6. Snyder DS, Palmer J, Stein AS, et al. Allogeneic hematopoietic cell transplantation following reduced intensity conditioning for treatment of myelofibrosis. *Biol Blood Marrow Transplant.* 2006; 12(11):1161-1168.
7. Kroger N, Zabelina T, Schieder H, et al. Pilot study of reduced-intensity conditioning followed by allogeneic stem cell transplantation from related and unrelated donors in patients with myelofibrosis. *Br J Haematol.* 2005;128(5):690-697.
8. Merup M, Lazarevic V, Nahi H, et al. Different outcome of allogeneic transplantation in myelofibrosis using conventional or reduced-intensity conditioning regimens. *Br J Haematol.* 2006;135(3): 367-373.
9. Barker JN, Davies SM, DeFor T, Ramsay NK, Weisdorf DJ, Wagner JE. Survival after transplantation of unrelated donor umbilical cord blood is comparable to that of human leukocyte antigen-matched unrelated donor bone marrow: results of a matched-pair analysis. *Blood.* 2001;97(10): 2957-2961.
10. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med.* 2004;351(22):2265-2275.
11. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med.* 2004;351(22):2276-2285.
12. Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood.* 2004;104(12):3813-3820.
13. Wagner JE, Barker JN, DeFor TE, et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood.* 2002;100(5):1611-1618.
14. Gluckman E, Rocha V, Arcese W, et al. Factors associated with outcomes of unrelated cord blood transplant: guidelines for donor choice. *Exp Hematol.* 2004;32(4):397-407.
15. Terakura S, Azuma E, Murata M, et al. Hematopoietic engraftment in recipients of unrelated donor umbilical cord blood is affected by the CD34+ and CD8+ cell doses. *Biol Blood Marrow Transplant.* 2007;13(7):822-830.
16. Rajantie J, Sale GE, Deeg HJ, et al. Adverse effect of severe marrow fibrosis on hematologic recovery after chemoradiotherapy and allogeneic bone marrow transplantation. *Blood.* 1986;67(6): 1693-1697.
17. Thiele J, Kvasnicka HM, Tefferi A, Barosi G, Orazi A, Vardiman JW. Primary myelofibrosis. In: *World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues*, 4th edition. Lyon, France: IARC Press; 2008:44-47.
18. Kishi Y, Kami M, Miyakoshi S, et al. Early immune reaction after reduced-intensity cord-blood transplantation for adult patients. *Transplantation.* 2005;80(1):34-40.
19. Matsuno N, Wake A, Uchida N, et al. Impact of HLA disparity in the graft-versus-host direction on engraftment in adult patients receiving reduced-intensity cord blood transplantation. *Blood.* 2009; 114(8):1689-1695.
20. Takagi S, Masuoka K, Uchida N, et al. High incidence of haemophagocytic syndrome following umbilical cord blood transplantation for adults. *Br J Haematol.* 2009;147(4):543-553.
21. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc.* 1958;53:457-481.
22. Rondelli D. Allogeneic hematopoietic stem cell transplantation for myelofibrosis. *Haematologica.* 2008;93(10):1449-1450.

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LETTER TO THE EDITOR

Delayed neutrophil engraftment in cord blood transplantation with intensive administration of mycophenolate mofetil for GVHD prophylaxis

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In allo-SCT, mycophenolate mofetil (MMF) is increasingly being used for prevention of GVHD because of less mucositis and faster neutrophil engraftment compared with MTX.[1] Three-times daily administration of MMF after allo-SCT has been demonstrated to maintain higher plasma levels of the active metabolite, mycophenolic acid (MPA), and possibly result in better clinical outcomes.[2–4] We retrospectively analyzed the effect of MMF dosing on neutrophil engraftment in single-unit cord blood transplantation (s-CBT) and unrelated BMT.

A total of 45 patients who received allo-SCT using MMF between November 2004 and November 2009 were studied. The patient characteristics are shown in Table 1. All patients received allo-SCT as a primary setting. MMF was administered orally for GVHD prophylaxis in combination with tacrolimus from 4 to 6 h after allo-SCT from day 0 to day 30, and then the dose tapered depending on individual risk factors for GVHD as described previously.[5] Regardless of the stem cell source, MMF was administered twice a day (b.i.d.) at a dose of 17.5 mg/kg (range 12.2–26.0 mg/kg, maximum 3000 mg/day) or thrice a day (t.i.d.) at a fixed dose of 1000 mg (median 17.5 mg/kg, range 10.0–22.7 mg/kg, total 3000 mg/day). Neutrophil engraftment was defined as the first of two consecutive days with an ANC of $>0.5 \times 10^9$/l. Acute GVHD was graded according to the consensus grading scale. The cumulative incidence was calculated for neutrophil engraftment and acute GVHD by treating death as a competing event. All descriptive data were analyzed using the statistical package Prism 5.0 (GraphPad Software, San Diego, CA, USA).

Almost all patients, except two from the t.i.d. group receiving s-CBT, had successful neutrophil engraftment.

Table 1 Patient characteristics and engraftment

	s-CBT		*BMT*	
	b.i.d.	*t.i.d.*	*b.i.d.*	*t.i.d.*
No. of patients	13	15	9	8
Median age, years (range)	49 (21–66)	52 (20–66)	42 (32–58)	48.5 (35–59)
Sex, male/female	5/8	9/6	6/3	6/2
Diagnosis at allo-SCT				
AML	1	2	2	0
ALL	5	5	2	2
MDS	6	5	4	3
NHL	1	1	0	3
Others	0	2	1	0
Conditioning regimen				
Myeloablative with or without TBI	8/1	6/0	8/0	5/0
Nonmyeloablative with or without TBI	4/0	8/1	0/1	1/2
Donor				
Median $CD34^+$ cells, $\times 10^6$/kg (range)	0.10 (0.04–0.21)	0.09 (0.03–0.19)	1.30 (0.74–2.60)	1.05 (0.23–3.70)
P	0.67		0.53	
HLA allele typing				
6/6	1	0	7	5
5/6	3	1	2	3
4/6	6	10	—	—
3/6	3	4	—	—
Neutrophil engraftment ($>0.5 \times 10^9$/l)				
Median period, days (range)	17 (14–48)	22 (14–41)	11 (9–17)	11 (8–14)
P	0.016		0.696	

Abbreviations: MDS = myelodysplastic syndrome; NHL = non-Hodgkin's lymphoma; s-CBT = single unit umbilical cord blood transplantation.
'b.i.d.' patients received MMF twice a day until day 30. 't.i.d.' patients received MMF thrice a day.
P refers to the comparison between b.i.d. and t.i.d. patients in each stem cell source using the Mann–Whitney test for $CD34^+$ cell doses and the Gehan–Wilcoxon test for a period of neutrophil engraftment.

One died of severe bacterial pneumonia before neutrophil engraftment, and the other encountered graft failure but restored autologous hematopoiesis. As shown in Table 1, there was no significant difference in the median periods of neutrophil engraftment between the b.i.d. and t.i.d. groups of BMT. However, the intensive t.i.d. administration of MMF significantly delayed neutrophil engraftment in s-CBT, although there was no significant difference in the infused $CD34^+$ cell doses between the two groups (Table 1). MMF-induced myelosuppression has been seen during the treatment of acute GVHD.[6] Neutropenia associated with increased plasma MPA levels has been reported in systemic sclerosis.[7] MMF has been reported to inhibit the proliferation of vascular smooth muscle cells and fibroblasts.[8,9]

The cumulative incidences of grade II–IV acute GVHD in the b.i.d. and t.i.d. groups of s-CBT were 25 and 8% (log-rank $P=0.258$), respectively. Although intensive MMF administration resulting in strong immunosuppression may prevent graft rejection and severe acute GVHD, it might also inhibit the growth of donor hematopoietic cells. Such an undesirable effect might be more apparent in s-CBT, in which the $CD34^+$ cell numbers are small.

These preliminary observations require confirmation, and randomized studies are necessary to determine the best dosing scheme for MMF.

Conflict of interest

The authors declare no conflict of interest.

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References

1 Bolwell B, Sobecks R, Pohlman B, Andresen S, Rybicki L, Kuczkowski E *et al.* A prospective randomized trial comparing cyclosporine and short course methotrexate with cyclosporine and mycophenolate mofetil for GVHD prophylaxis in myeloablative allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2004; **34**: 621–625.

2 Giaccone L, McCune JS, Maris MB, Gooley TA, Sandmaier BM, Slattery JT *et al.* Pharmacodynamics of mycophenolate mofetil after nonmyeloablative conditioning and unrelated donor hematopoietic cell transplantation. *Blood* 2005; **106**: 4381–4388.

3 Okamura A, Yamamori M, Shimoyama M, Kawano Y, Kawano H, Kawamori Y *et al.* Pharmacokinetics-based optimal dose-exploration of mycophenolate mofetil in allogeneic hematopoietic stem cell transplantation. *Int J Hematol* 2008; **88**: 104–110.

4 Royer B, Larosa F, Legrand F, Gerritsen-van Schieveen P, Berard M, Kantelip JP *et al.* Pharmacokinetics of mycophenolic acid administered 3 times daily after hematopoietic stem cell transplantation with reduced-intensity regimen. *Biol Blood Marrow Transplant* 2009; **15**: 1134–1139.

5 Nishikawa S, Okamura A, Yamamori M, Minagawa K, Kawamori Y, Kawano H *et al.* Extended mycophenolate mofetil administration beyond day 30 in allogeneic hematopoietic stem cell transplantation as preemptive therapy for severe graft-versus-host disease. *Transplant Proc* 2009; **41**: 3873–3876.

6 Pidala J, Kim J, Perkins J, Field T, Fernandez H, Perez L *et al.* Mycophenolate mofetil for the management of steroid-refractory acute graft vs host disease. *Bone Marrow Transplant* 2010; **45**: 919–924.

7 Cuomo G, Abignano G, Valentini G. Early onset neutropenia after mycophenolate mofetil in systemic sclerosis. *Rheumatol Int* 2009; **29**: 1529–1530.

8 Miljkovic D, Cvetkovic I, Stosic-Grujicic S, Trajkovic V. Mycophenolic acid inhibits activation of inducible nitric oxide synthase in rodent fibroblasts. *Clin Exp Immunol* 2003; **132**: 239–246.

9 Shimizu H, Takahashi M, Takeda S, Inoue S, Fujishiro J, Hakamata Y *et al.* Mycophenolate mofetil prevents transplant arteriosclerosis by direct inhibition of vascular smooth muscle cell proliferation. *Transplantation* 2004; **77**: 1661–1667.

CLINICAL RESEARCH

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

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

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INTRODUCTION

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

In this study, we retrospectively investigated the impact of neutropenia on the development of early bloodstream and pulmonary infections in HSCT recipients. As indexes of the severity of neutropenia, we used the D-index and c-D-index, which were recently proposed by Portugal et al. [12]. The D-index was based on a graph that showed the absolute neutrophil count during neutropenia and was calculated as the area over the neutrophil curve (Figure 1).

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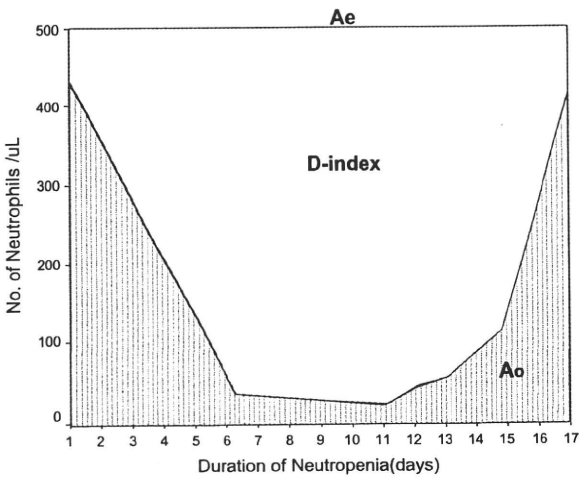

Figure 1. Area over the neutrophil curve (D-index) of a hypothetical neutropenic patient. If the duration of neutropenia is 16 days, the expected neutrophil area (shaded area, A_e) is $16 \times 500 = 8000$. If the area under the neutrophil curve calculated by the trapezoidal method (striped area, A_o) is 2062, the D-index = $8000 - 2062 = 5938$.

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

PATIENTS AND METHODS

Patients

The Transplantation Unit of Saitama Medical Center, Jichi Medical University has 3 individual rooms and 2 quad rooms (11 beds in total) that are equipped with a laminar air-flow (LAF) system with high-efficiency particulate air (HEPA) filters. In principle, allogeneic and autologous HSCTs are performed in these individual and quad rooms, respectively. We retrospectively reviewed the charts of consecutive patients who underwent autologous or allogeneic HSCT, between April 2005 and March 2009. Patients who had already developed documented infections before HSCT were excluded. Twenty-three autologous and 35 allogeneic HSCT recipients were finally included in this study.

Transplantation Procedure

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

Graft-versus-host disease (GVHD) prophylaxis in allogeneic HSCT consisted of the continuous infusion of cyclosporine A with a starting dose of 3 mg/kg/day and short-term methotrexate (10-15 mg/m^2 on day 1, 7-10 mg/m^2 on days 3 and 6, and optionally on day 11 in HSCT from a donor other than an HLA-matched sibling) [21] with the exception of 1 patient who received a continuous infusion of tacrolimus with a starting dose of 0.03 mg/kg/day and short-term methotrexate. Acute GVHD (aGVHD) was graded as previously described [22].

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

D-Index and c-D-Index Calculation

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

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

Definition of Early Bloodstream and Pulmonary Infections

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

Statistical Considerations

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

Dichotomous variables were compared using Fisher's exact test, and continuous variables were compared using the Mann-Whitney *U* test. A *P*-value of <.05 was considered to be significant. To assess the ability of the D-index, c-D-index, and duration of neutropenia to predict infections we performed a receiver operating characteristic (ROC) curve analysis and calculated the positive and negative predictive values in this patient population.

RESULTS

Patients

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

Epidemiologic and Clinical Factors

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

Table 1. Clinical and Epidemiological Characteristics of the Study Patients

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

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

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

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

Among the indexes of the severity of neutropenia over the whole duration of neutropenia, days of profound neutropenia (<100/μL) significantly affected the development of bloodstream infections (median 15 versus 7 days, $P = .031$). The D-index and days of neutropenia (<500/μL) tended to be higher or longer in patients with bloodstream infections, with borderline significance (median 7102.5 versus 3963.5 and 17.5 versus 10.5 days, $P = .055$ and .072, respectively). As indexes of the cumulative severity of neutropenia from the start of neutropenia, neither the c-D-index, cumulative duration of neutropenia, nor cumulative duration of profound neutropenia significantly affected bloodstream infections (median 3375 versus 3963.5, 8.5 versus 10.5 days, and 7 versus 7 days, $P = .443$, .397, and .900, respectively). On the other hand, both the indexes of the severity of the whole duration of neutropenia, including the D-index, days of neutropenia and days of profound neutropenia (median 9816.5 versus 3999.5, 24 versus 11 days, and 18 versus 7 days, $P = .007$, .003, and .008, respectively), and the indexes for the cumulative severity of neutropenia, including the c-D-index, cumulative duration of neutropenia, and cumulative duration of profound neutropenia, significantly affected pulmonary infections (median 7589 versus 3999.5, 20 versus 11 days, and 15 versus 7 days, $P = .028$, .020, and .024, respectively). When we focused on the 6 cases with probable, possible, or suspected pulmonary mold infections, the indexes of the severity of neutropenia over the whole duration of neutropenia significantly affected the development of invasive mold infections (median 8702 versus 4059, 23 versus 11 days, 17 versus 7 days, $P = .027$, .020, and .027, respectively), whereas the indexes for the cumulative severity of neutropenia affected it with borderline significance (median 6678 versus 4059, 18 versus11 days, and 15 versus 7 days, $P = .081$, .090, and .077, respectively).

ROC analysis revealed that the D-index, c-D-index, and duration of neutropenia were equally useful for predicting early pulmonary infections. The area under the ROC curves were 0.810, 0.801, and 0.832 for the D-index, days of neutropenia and days of profound neutropenia, respectively (Figure 2A). These

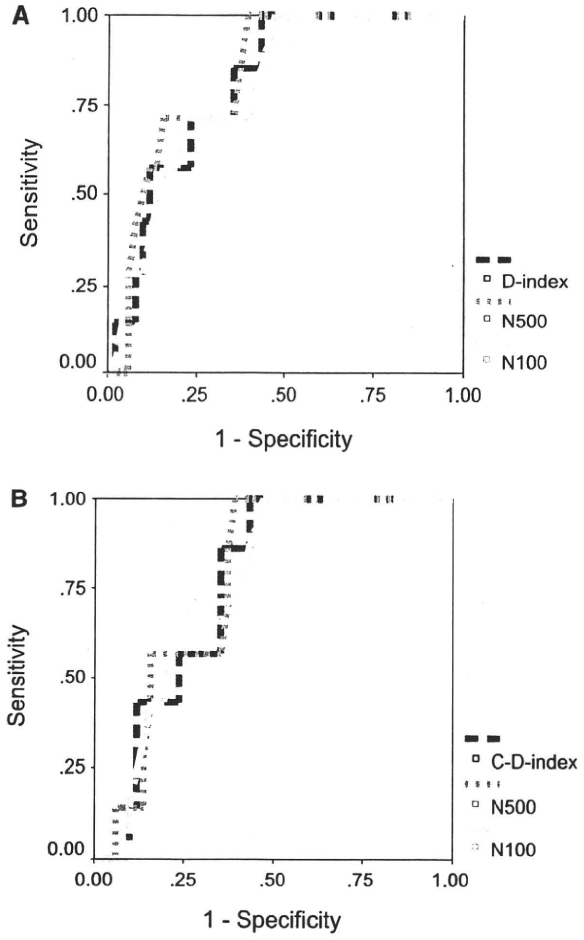

Figure 2. Receiver operating characteristic curves comparing the D-index with the days of neutropenia (<500/μL, N500) and profound neutropenia (<100 /μL, N100) (A), and comparing the cumulative D-index (c-D-index) with the cumulative durations of neutropenia (<500/μL, N500) and profound neutropenia (<100/μL, N100) (B) as predictors of pulmonary infection.

values were .756, .769, and .762 for the c-D-index, cumulative duration of neutropenia, and cumulative duration of profound neutropenia, respectively (Figure 2B). The ROC curve was closest to the left corner of the plot when the thresholds for the D-index, days of neutropenia, and days of profound neutropenia were 7600, 20, and 15, respectively. With the use of these cutoff values, the sensitivity and specificity for predicting pulmonary infections were 71.4% and 84.3%, 71.4% and 78.4%, and 71.4% and 76.5%, respectively. The positive and negative predictive values were 29.4% and 95.1%, 38.7% and 95.6%, and 31.3% and 95.2%, respectively. Similarly, the ROC curve was closest to the left corner of the plot when the thresholds for the c-D-index, cumulative duration of neutropenia, and cumulative duration of profound neutropenia were 5500, 13, and 14, respectively. With the use of these cutoff values, the sensitivity and specificity for predicting pulmonary infections were 85.7% and 74.7%, 100% and 60.8%, and 71.4% and 72.5%, respectively. The positive and negative predictive values were 31.6% and 97.4%, 25.9% and 100.0%, and 35.7% and 94.9%, respectively (Table 2).

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

DISCUSSION

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

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

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

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

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

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

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

The negative predictive value of the c-D-index for early pulmonary infections after HSCT was 97.4% using a cutoff value of 5500. This means that patients with a c-D-index less than 5500 have little probability of developing pulmonary infections and are less likely to require the empiric or preemptive administration of intensive antimold treatment, if they have received treatment in a clean room equipped with a LAF system. However, it can be difficult to calculate the c-D-index compared to the simple duration of neutropenia. Although Portugal et al. [12] reported that the D-index and c-D-index were superior to the duration of neutropenia for predicting invasive mold infections in acute myelogenous leukemia patients with chemotherapy, there seemed to be no great difference according to their ROC curves. The current study showed that the D-index and c-D-index were as effective as the duration of neutropenia for predicting early pulmonary infection in HSCT recipients, probably because neutropenia was more severe and uniform in HSCT recipients than in patients with standard chemotherapy. In fact, the D-index and c-D-index were strongly correlated with the total days of neutropenia and the cumulative duration of neutropenia, respectively, in this study (correlation coefficients 0.974 and 0.968, $P < .001$ and $< .001$, respectively).

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

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

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

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