JIMD 546 Artid 9073 Proof# 1 - 28/12/2009

t1.1 **Table 1** Blood KS evaluated by LC/MS/MS in healthy controls (SD, standard deviation)

t1.2	Age group	Number	Data	Age (years)	Gal GlcNAc (6S) (μg/ml)	Gal(6S) GlcNAc (6S) (μg/ml)	Total (μg/ml)	Gal GlcNAc (6S) (%)	Gal(6S) GlcNAc (6S) (%)
t1.3	$\chi \leq 2$	50	Average	0.5	2.2	0.4	2.6	85.7	14.2
t1.4			SD	0.5	0.7	0.2	0.9	4.5	4.6
t1.5	$2 < \chi \leq 5$	25	Average	3.3	2.1	0.5	2.6	82.5	17.5
t1.6			SD	1.0	0.7	0.2	0.8	3.7	3.7
t1.7	$5 < \chi \leq 10$	8	Average	7.5	1.8	0.4	2.2	82.6	17.3
t1.8			SD	1.7	0.6	0.2	0.8	4.7	5.0
t1.9	$10 < \chi \leq 15$	7	Average	12.4	2.4	0.5	2.9	82.2	17.6
t1.10			SD	1.5	0.7	0.1	0.8	2.1	2.2
t1.11	$15 < \chi$	37	Average	34.4	1.2	0.3	1.5	76.4	23.6
t1.12			SD	13.1	0.5	0.1	0.6	4.0	3.8
t1.13	All ages	125	Average	12.1	2.0	0.4	2.3	86.7	18.3
t1.14			SD	16.3	1.0	0.2	0.9	28.5	6.1

KS values in 5 of 19 (26.3%) MPS III patients, 3 of 6 (50%) MPS VI patients, 1 of 6 MPS VII patients, and 2 of 11 (18.2%) ML patients were above the mean + 2SD of the age-matched controls (Fig. 2).

Composition in KS The compositional ratio of Galβ1(6S)→4GlcNAc(6S) to Galβ→4GlcNAc(6S) in KS derived from the blood samples of MPS IVA patients was compared, since it was expected to be reflected by a deficiency of GALNS enzyme, which digests at the C-6 position of sulfated galactose. For the healthy control newborn infants, the ratio of Galβ1(6S)→4GlcNAc(6S) was 10.6%, and it rose between the ages of 0 and 2 years (mean 16.3%) and the concentrations stayed relatively constant until they reached 15 years. After 15 years, the compositional ratio of Galβ1(6S)→4GlcNAc(6S) increased to 23.6% and stabilized thereafter. In all ages, the ratio of Galβ1(6S)→4GlcNAc(6S) was significantly higher in patients with MPS IVA than in healthy controls (mean 22.7% vs 18%, $P<0.001$) (Tables 1 and 2, Fig. 2), suggesting that KS at the C-6 position of galactose is more sulfated in patients. When control subjects and MPS IVA patients in each age range (2–5 years, 5–10 years, 10–15 years, and over 15 years) were compared, their ratios of Galβ1(6S)→4GlcNAc(6S) to Galβ→4GlcNAc(6S) were as follows: mean 17.5% vs 19.7%; 17.3% vs 21.3%; 17.6% vs 28.5%; 23.6% vs 23.1%, respectively (Tables 1 and 2, Fig. 1).

t2.1 **Table 2** Blood KS evaluated by LC/MS/MS in MPS IVA patients (SD, standard deviation)

t2.2	Data	Number	Data	Age (years)	Gal GlcNAc (6S) (μg/ml)	Gal(6S) GlcNAc (6S) (μg/ml)	Total (μg/ml)	Gal GlcNAc (6S) (%)	Gal(6S) GlcNAc (6S) (%)
t2.3	$2 < \chi \leq 5$	12	Average	2.7	9.2	2.2	11.4	80.3	19.7
t2.4			SD	0.9	5.4	1.4	6.8	3.8	3.8
t2.5	$5 < \chi \leq 10$	13	Average	7.8	5.2	1.2	6.4	78.7	21.3
t2.6			SD	1.3	4.7	1.0	5.7	7.7	7.7
t2.7	$10 < \chi \leq 15$	9	Average	12.8	2.5	0.7	3.2	71.5	28.5
t2.8			SD	1.8	2.0	0.2	2.2	14.4	14.4
t2.9	$15 < \chi$	15	Average	30.0	1.7	0.5	2.1	76.9	23.1
t2.10			SD	16.0	1.1	0.3	1.3	7.2	7.2
t2.11	All ages	49	Average	14.3	4.6	1.1	5.7	77.3	22.7
t2.12			SD	14.1	4.7	1.1	5.8	8.8	8.8
t2.13	Severe	33	Average	12.8	5.9	1.4	7.3	79.6	20.4
t2.14			SD	13.1	5.5	1.4	6.8	6.9	6.9
t2.15	Attenuated	11	Average	19.5	1.6	0.5	2.1	71.9	28.1
t2.16			SD	19.3	1.8	0.3	2.0	13.1	13.1

t3.1 **Table 3** Blood KS evaluated by LC/MS/MS in other types of MPS and ML patients (SD, standard deviation)

t3.2	Type	Number	Data	Age (years)	Gal GlcNAc (6S) (μg/ml)	Gal(6S) GlcNAc (6S) (μg/ml)	Total (μg/ml)	Gal GlcNAc (6S) (%)	Gal(6S) GlcNAc (6S) (%)
t3.3	MPS I	31	Average	8.3	2.9	0.7	3.7	77.1	22.9
t3.4			SD	9.2	2.5	0.3	2.7	6.6	6.5
t3.5	MPS II	28	Average	13.7	5.0	1.1	6.0	81.4	18.6
t3.6			SD	9.3	3.5	0.6	4.1	3.1	2.8
t3.7	MPS III	19	Average	4.6	3.4	0.8	4.2	81.4	18.7
t3.8			SD	2.8	2.2	0.5	2.6	3.2	2.9
t3.9	MPS VI	6	Average	2.7	4.5	1.0	5.5	81.9	18.7
t3.10			SD	2.2	3.1	0.7	3.7	4.1	4.0
t3.11	MPS VII	6	Average	11.3	2.5	0.4	2.9	81.8	18.0
t3.12			SD	13.1	2.5	0.4	2.8	5.5	6.4
t3.13	ML	11	Average	1.0	3.5	0.7	4.1	84.9	15.9
t3.14			SD	2.1	3.0	0.7	3.2	4.4	3.0

Discussion

The accumulation of undegraded KS leads to damage of cartilage cells, causing systemic skeletal chondrodysplasia in patients with MPS IVA. KS, which contributes over 25% of the cartilage GAGs in adults, is one of the most important components in bone. When cartilage proteoglycans, such as KS, are not degraded properly, they are stored mainly in chondrocytes, where KS is synthesized. Pathohistological examinations of the bone and cartilage cells are useful for the diagnosis of MPS IVA; however, it is not practicable to obtain biopsy samples from MPS IVA patients. Alternatively, molecular analysis and standard enzyme assay are used as diagnostic techniques for MPS IVA. Measurements of KS concentrations in blood samples should also provide critical information about the clinical status and prognosis of MPS IVA patients, efficacy of therapies and early diagnosis as a biomarker.

The monoclonal antibody used for sandwich ELISA in previous studies (Seikagaku, Tokyo, Japan) (Tomatsu et al. 2004, 2005) is known to be specific for Galβ1(6S)→4GlcNAc(6S); both galactose and *N*-acetyl-glucosamine

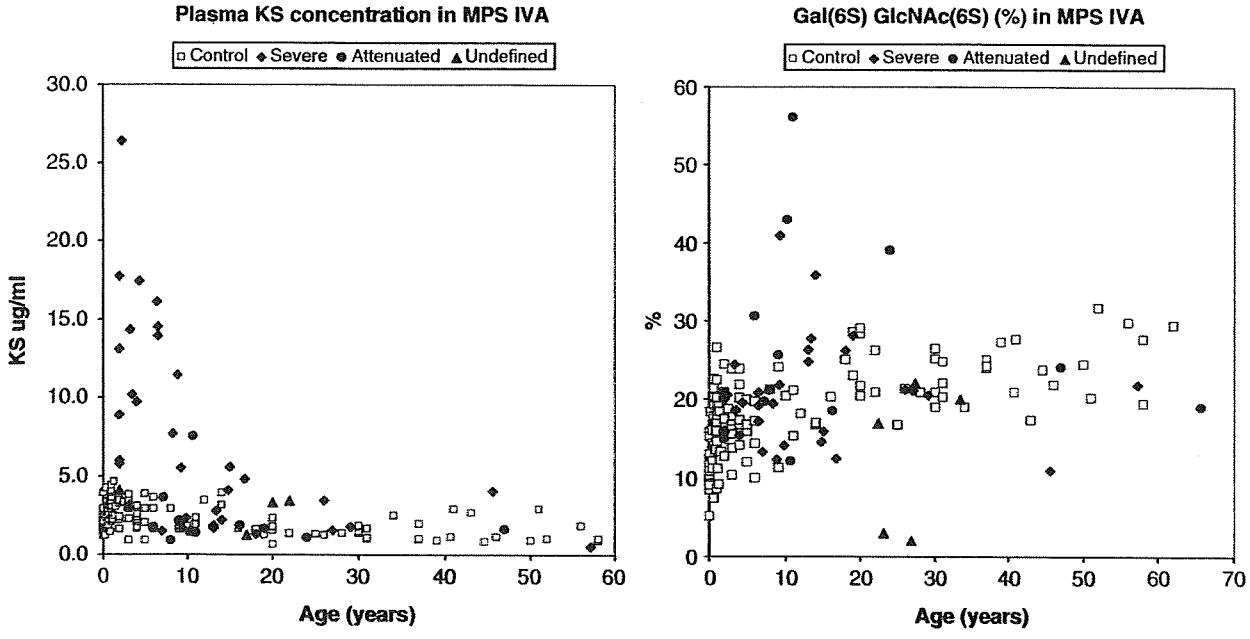

Fig. 1 Concentrations of blood KS in MPS IVA patients and healthy individuals. Results from 49 specimens from individuals with MPS IVA (severe, 33; attenuated, 11; undefined, 5) and 125 from healthy individuals are plotted with respect to age. Left panel blood, KS of patients with MPS IVA and healthy individuals. Right panel, ratio of Galβ(6S)→4GlcNAc(6S) in KS compositions (%)

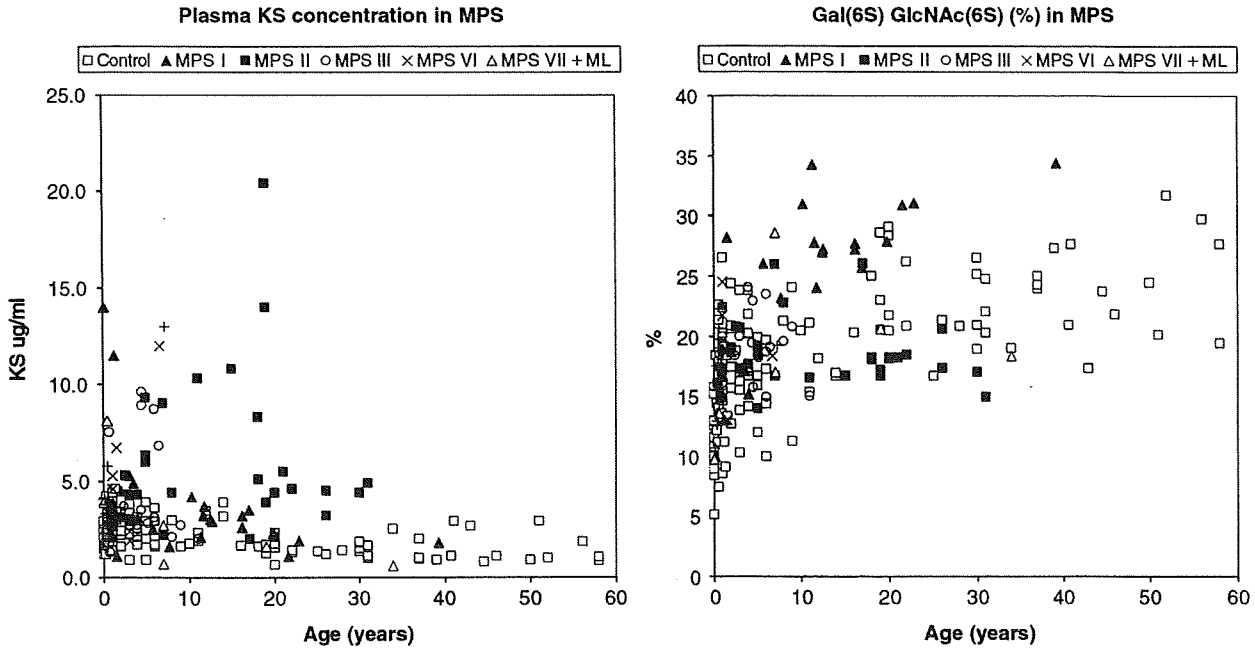

Fig. 2 Concentrations of blood KS in patients with MPS types I, II, III, VI, and VII and ML and healthy individuals. Results from 101 specimens from individuals with MPS and ML (MPS I, 31; MPS II, 28; MPS III, 19; MPS VI, 6; MPS VII, 6; ML, 11) and 125 from healthy individuals are plotted with respect to age. Left panel, blood KS of patients with MPS and ML and healthy individuals. Right panel, ratio of Galβ(6S)→4GlcNAc(6S) in blood KS compositions (%) in patients with MPS and ML and healthy individuals

have to be sulfated (Caterson et al. 1983; Mehmet et al. 1986). Therefore, this analytical method does not provide complete quantification and qualification of total KS. Concentrations of KS-derived disaccharides and their compositions in human samples are measured by the present LC/MS/MS method. Keratanase II, used for the digestion of KS, recognizes both Galβ1→4GlcNAc(6S) and Galβ1(6S)→4GlcNAc(6S), in which *N*-acetyl-glucosamine is sulfated. Thus, the current LC/MS/MS method measures the KS molecules with disaccharides of Galβ1→4GlcNAc(6S) and Galβ1(6S)→4GlcNAc(6S). Although unmeasured KS could be still present, the best choice is to use keratanase II-digested samples, since the enzyme covers the broadest range of KS saccharides, leading to 10–100 times higher KS concentration than sandwich ELISA method.

Our results of KS compositions are of great interest. The compositional ratio of Galβ1(6S)→4GlcNAc(6S) to Galβ→4GlcNAc(6S) in KS derived from blood samples of patients with MPS IVA was higher than that of the age-matched healthy controls, except for the group over 15 years old, reflected by a deficiency of the GALNS enzyme, which digests at the C-6 position of sulfated galactose. However, there was no difference in the ratio of Galβ1(6S)→4GlcNAc(6S) to Galβ→4GlcNAc(6S) in the group over 15 years old between healthy controls and MPS IVA patients. The compositional ratio of Galβ1(6S)→4GlcNAc(6S) increased even in the healthy controls. KS sulfation may increase with the patient's age, although the physiological significance of its increase remains unknown. In this study we could not clearly correlate between clinical severity and Galβ1(6S)→4GlcNAc(6S) ratio; therefore, further longitudinal studies of each individual MPS IVA patient with a different phenotype are needed.

Unaffected healthy young children would be expected to have a high cartilage turnover, resulting in higher KS level. Age-dependent changes in KS turnover (Tomatsu et al. 2005; Thonar et al. 1988) showed that blood KS level rose progressively during the first 5 years of life, remained elevated until 10–12 years of age, and then declined after teenage, until it stabilized at the age of 15 years, although longitudinal data from the individual children are required to confirm this age dependency. Elongation of the long bones during growth occurs through a process of endochondral ossification in which new cartilage is continuously laid down before it is degraded and replaced by bone. The decreased level of KS after teenage in healthy children is consistent with the fact that the growth rate begins to decline during this period.

In the initial progressive stage between ages 0 and 5 years, the mean blood KS concentration in patients with MPS IVA was the highest. After 10 years of age, the KS level in most MPS IVA patients declined to near normal or normal levels. Blood KS levels in MPS IVA could be

reflected by two factors: (1) maturation-related changes as observed in unaffected healthy children, (2) the severity of progressive chondrodysplasia. MPS IVA patients under 10 years old had markedly elevated blood KS levels, indicating that the progression of cartilage destruction in most patients is rapid in young age and that KS is released from cartilage and leached into the circulation. Patients with high levels of KS in their blood at a young age likely have the severe form of MPS IVA, where the cartilage is overloaded with undegraded KS. One may speculate that the blood concentrations in patients with MPS IVA reflect the amount of stored KS in cartilage tissues. When the growth plate has closed or torn, the synthesis of KS in cartilage will decline. In fact, the destruction of cartilage in most patients could be completed by teenage, resulting in little release of KS from the cartilage to the circulation and almost no place for the synthesis of KS.

We have reported that a newborn infant affected by MPS IVA showed lower-spine radiographs with anterior beaking of the lumbar vertebrae as well as minor anomalies on the phalanges that suggested skeletal dysplasia (Ohashi et al. 2009). Subsequent radiographs showed progression of the anterior beaking, progressive kyphosis, platyspondyly and irregularities of the vertebral bodies, which are signs characteristic of severe MPS IVA disease. Thus, one can speculate that KS accumulation in bone has already started before birth and that the KS level could already be elevated at the newborn stage. To understand this phenomenon of normalization of blood KS concentration in MPS IVA patients and to predict when elevation of KS levels will start, one needs to determine the blood KS levels sequentially at different ages for the same individuals, longitudinally.

There are well-known relationships between types of MPS and specific GAG(s) that accumulate (Neufeld and Muenzer 2001). Elevation of KS level in blood or urine was considered to be specific for MPS IV. However, we have recently demonstrated that patients with MPS and ML, other than those with MPS IV, had elevated blood KS levels in addition to the GAGs originating from the respective enzyme defect (Tomatsu et al. 2005). In this study, we also confirmed that some of the patients affected by other types of MPS and ML had elevated blood KS levels. The mechanism for the secondary elevation of KS remains unclear, since the current theory on the pathway of KS metabolism cannot explain this phenomenon.

Most patients with MPS and ML have severe bone dysplasia, as in MPS IV. Therefore, elevated levels of KS in the blood of other MPS and ML patients could be related to underlying bone disease, especially of cartilage tissues. It is noted that the mean age of patients in this study was younger in the order ML, MPS VI, MPS I, and MPS II. Generally, progressive bone disease is observed in ML, MPS VI, and MPS I at younger ages than that for MPS II. Therefore, elevation of KS in ML, MPS VI, and MPS I was observed in younger patients, while the elevation in MPS II was observed even in teenaged or older patients. It is also noteworthy that most patients with MPS and ML had a compositional ratio of Galβ1(6S)→4GlcNAc(6S) to Galβ→4GlcNAc(6S) in KS similar to that seen in the age-matched healthy controls, suggesting that the interaction between KS and GALNS in removing the sulfate in the C-6 position in galactose is not inhibited. Since KS is synthesized mainly in cartilage, the successful reduction of KS could provide more specificity for the bone pathology of MPS disease.

Assessment of urinary KS by LC/MS/MS was out of the scope of this study. However, preliminary study of urine samples from patients with MPS IVA has shown that KS levels were markedly elevated. The KS levels in both MPS IVA patients and healthy controls were age dependent. In the controls, the KS levels were highest in newborn infants, as observed from total urinary GAGs. While the degree of decline in the urinary KS concentration proportional to age was equivalent to that of blood KS concentration, urinary KS level in patients with MPS IVA remained higher than those of the controls, even after 15 years of age (unpublished data).

In summary, the determination of blood KS concentrations by LC/MS/MS should provide a useful tool to assess clinical status in patients with MPS IVA and to measure response to treatments such as enzyme replacement therapy, bone marrow transplantation, and gene therapy as observed in MPS IVA mice treated by enzyme replacement therapy (Tomatsu et al. 2008b).

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日本移植学会の倫理指針

臓器移植はドナーの存在によって初めて成立する医療であり，ドナーとレシピエントの間に介在する社会的システムがきわめて重要となってくる。移植医療に従事する医療者は高い倫理観と深い敬意をもってドナーとレシピエントの双方に接しつつ，高度の医療を提供しなければならない。

わが国の移植医療の学術団体である日本移植学会では2003年に倫理指針の大幅な改正を行い，学会員が守るべき移植医療における倫理的事項を定めた。その後，2006年に宇和島徳洲会病院における非親族間生体腎臓移植において腎臓売買や病的腎臓の移植が社会問題となったことを契機として，2007年に倫理指針の部分的な改正を行った。

ここでは，学会の倫理指針の全文(**表1**)を掲載するとともに，改正当時倫理委員会委員長としてかかわった立場からその要点について概説したい。

◎学会倫理指針の歴史的経緯

日本移植学会の最初の倫理指針は1986年に制定された。当時は「臓器移植を行うにあたって」という名称であったが，1994年に全面的な改正が行われ，正式に学会の倫理指針として位置づけられた。

当時のわが国の状況は脳死体からの移植が進まず，臓器移植に関する法律の制定を待つ直前であった。その後1997年に「臓器の移植に関する法律」(臓器移植法)が制定されたが，脳死体からの移植はきわめて限定的にしか行われていない。そのため，わが国においては生体間の臓器移植がほとんどを占めるという国際的にみても特異な状況が現在まで続いている。日本移植学会の倫理指針のほとんどは生体臓器移植に関するものである。

◎倫理指針の要点

生体臓器移植

●提供者の範囲と提供意思の第三者による確認

生体ドナーからの臓器提供においては，何よりもドナー本人の自由意思が尊重されなければならないこと，そしてその提供意思をドナーとレシピエントの双方に利害関係のない第三者が確認することを明確に規定した。

生体臓器移植が治療の選択肢になる患者においては，現実的なドナー候補者としてその直近の家族や血縁者に精神的な圧力がかかりやすいという現状がある。旧指針では提供するドナーを血縁者と家族に限定していたが，現行の指針では血縁関係を重視しつつも，提供可能な範囲を親族(6親等以内の血族，配偶者と3親等以内の姻族)までに拡大して，その範囲において自発的な提供意思を有するドナーからの移植を可能にした。

そして，ドナーの範囲を拡大する一方で，提供意思の自発性と無償性を家族以外の第三者が確認することを義務づけた。「第三者」については，「移植に関与していない者で，提供者本人の権利保護の立場にある者で，かつ倫理委員会が指名する精神科医などの複数の者」と定義した。具体的には，精神科医以外には，内科医，MSW（メディカル・ソーシャルワーカー)，移植コーディネーター，弁護士などが想定される。

●親族以外からの提供

臓器提供において最も重視すべき条件を提供者の自由意思であると規定すると，ドナーが臓器提供に伴うリスクを受容できるか否かの判断は単なる血縁関係の近さだけではなく，レシピエントとの精神的な関係の深さにもよると考えられる。法律的には血縁あるいは親族ではなくとも，「内縁関係」，「育ての親」，「長年の親友」などからの提供も例外的ながら認められることがありうるのではないかと考えられた。しかし，このような場合においては法律上の定めがなく，個々の事例ごとに慎重に判断すべきことであり，当該医療機関の倫理委員会において個別の承認を受けるとともに日本移植学会に意見を求めることとした。

●未成年者からの提供

旧指針では未成年者からの提供はまったく認められなかったが，現行の指針においては原則は同様であるが，特例として以下の条件が満たされれば16歳以上20歳未満の未成年者からの移植が認められることがあると改正された。

①成人に匹敵する判断能力を有することが精神科医などによって確認されていること

②ドナーが十分な説明を受けたうえで書面にて同意をしていること

③当該医療機関の倫理委員会が個別の事例として承認していること

④ドナーの同意とともに親権者からも書面による承諾が得られていること

●ドミノ移植における一次レシピエントがドナーとなる場合

特殊な移植形態として，ある臓器移植のレシピエント(1次)の摘出臓器を別の患者(2次レシピエント)に移植するといういわゆるドミノ移植があり，1次レシピエントは2次レシピエントのドナーとなり，ほとんどが非血縁者である。この場合は非血縁者ドナーとしてではなく，個別の移植（ドミノ移植)，個別のドナーとして当該医療機関の倫理委員会で承認を受けるものとした。

●患者の移植適応の決定とインフォームド・コンセント

移植適応決定は死体臓器移植に準じることと，患者への説明のなかにドナーにおける臓器提供に関する危険性を含めること，未成年者のレシピエントへの説明と同意において本人の署名を残すことが望ましいことなどを規定した。

●提供者の本人確認方法

2006年の宇和島徳洲会病院での臓器売買事件の際に義妹と偽ったドナーからの移植が行われていたことから，提供者が本人であることを公的証明書（免許証，パスポート，保険証など）で確認し，診療録に証明書の写しを添付することとした。

医療情報の登録と患者個人情報の保護

医学研究や医療上の個人情報の扱いに関するいくつかの重要な倫理指針が国によって作成されていることを受けて，現行の指針はこれらの倫理指針に沿って改正された。

臓器の売買と受刑者からの移植の禁止

臓器の売買，臓器に対する対価の授受，受刑者からの移植については旧指針においても厳しく禁じていたが，現行の指針ではこの禁止事項に違反した場合の罰則を明確に規定した。

おわりに

日本移植学会の倫理指針について概説をしたが，この倫理指針は移植医療を適正に推進しようという目的で作成されたものであり，移植に携わる医療者は折に触れてこの指針を読み返して理解を深められることを希望して稿を終えたい。　**(加藤俊一)**

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ORIGINAL ARTICLE

Survival after cord blood transplantation from unrelated donor as a second hematopoietic stem cell transplantation for recurrent pediatric acute myeloid leukemia

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Abstract The Japan Cord Blood Bank Network (JCBBN) reports the treatment of 22 children with acute myeloid leukemia (AML) who received umbilical cord blood transplantation from unrelated donors (CBT) as their second hematopoietic stem cell transplantation (HSCT). Provided by the JCBBN, between February 1997 and September 2006, 22 patients had CBT as a second HSCT. In the initial HSCT, eight received autologous, seven received CBT, and the remaining had allogenic BMT. At the time of CBT as a second HSCT, seven were in the second complete remission (CR2), two in the third CR (CR3), the remaining were not in remission. Reduced intensity conditioning (RIC) conducted for 10 cases and myeloablative conditioning (MAC) for 12 cases. The overall survival rate was 31.3%, 5 years after CBT. Second complete remission at second transplantation was favorable prognosis (58.3 ± 18.6%, compared with 17.1 ± 10.8% for the non-CR group. Mortality after CBT as a second HSCT accounted for 15 cases, 8 from treatment-related mortality. In conclusion, CBT combined with RIC as second HSCT may be useful against a recurrence of AML in children after the initial HSCT.

Keywords Cord blood transplantation · Hematopoietic stem cell transplantation · Second transplantation · Acute myeloid leukemia · Reduced intensity conditioning

1 Introduction

Hematopoietic stem cell transplantation (HSCT) remains the one of curative therapy for patients with high-risk leukemia. However, relapse remains a significant problem and is the major cause of post-transplantation mortality. Patients with relapsed leukemia after HSCT have a very poor prognosis and the optimal salvage therapy remains an open question. Second transplantation is often considered to be the standard of care for a patient with relapsed acute leukemia after allogeneic transplantation: it can provide a durable remission for a small number of patients who are

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eligible to receive it [1, 2]. In a report to the International Bone Marrow Transplant Registry (IBMTR), Eapen et al. [1] reported overall survivals of 41% at 1 year and 28% at 5 years after a second transplantation for treatment of relapsed acute and chronic leukemia and Meshinchi et al. [2] reported a 56% 1-year survival for 25 pediatric patients who received a second transplant for relapsed acute myelogenous leukemia (AML). Of note, younger patients and those with longer remission intervals had improved outcomes [3]. In contrast, Radich et al. [3] reported a relapse rate of 76% and a disease-free survival (DFS) rate of only 10% at 4 years for patients with relapsed AML treated with a second transplant. A second transplant is limited by the availability of donors; and co-morbidities related to the first conditioning regimen may preclude the administration of another course of high-dose cytotoxic agents.

Recently, for a source of HSCT, umbilical cord blood (CB) has become available after myeloablative therapy; and cord blood transplantation from unrelated donor (CBT) is applied for a second transplantation to treat relapsed acute and chronic leukemia [4]. Many patients have benefited from CB from unrelated donors since it was first used several years ago. Even when a suitable bone marrow (BM) donor is unavailable, CBT can be indicated for patients with specific entities because of the rapid availability of stored transplantable units, the absence of risks associated with donors, and less stringent requirements for HLA matching between the donor and recipient. However, there are only a limited number of reports on the frequencies of CBTs given as the repeat transplants for diverse types of diseases; and studies have not been conducted on AML involving children. In this regard, CBT has potential advantages for patients awaiting for a second HSCT. The current report from the Japan Cord Blood Bank Network (JCBBN) details the treatment of 22 patients with AML who received CBT from unrelated donors as a second HSCT and explores new and safer treatment approaches for patients with a scheduled second HSCT.

2 Methods

2.1 Patient characteristics

Primary data and annual follow-up reports were submitted to the data center of the JCBBN by the transplant center investigators (for centers, see Appendix). Between February 1997 and September 2006, 22 children with AML had CBT as a second HSCT. Their clinical characteristics at the first HSCT and the CBT as the second HSCT are detailed in Tables 1 and 2, respectively. One of these children was considered to have secondary AML on the basis of a history of exposure to chemotherapy. Abnormal karyotypes were classified in the favorable-risk group if t(8; 21), t(15; 17) or inv(16) was detected. In those patients lacking these favorable changes, the presence of monosomy 7, 11q23 abnormalities other than t(9;11), monosomy 5, del(5q), abnormal 3q, t(6; 9) or a complex karyotype as five or more abnormalities placed them in the poor-risk group. The remaining abnormalities made up the intermediate-risk group. In the initial HSCT, 8 of the 22 children received autologous SCT (7 had BMT and 1 had peripheral blood stem cell transplantation), 6 received CBT, and the remaining patients had allogenic BMT. At the time of CBT as a second HSCT, 6 children were in the second complete remission (CR2), 2 in the third complete remission (CR3), while 14 were not in remission. Relapse following HSCT and UCBT (as a second HSCT) ranged from 39 to 906 days with a median time of 219 days. Twenty-one out of 22 patients received various chemotherapies in different institutions before receiving the UCBT.

2.2 Donor registries and selection of grafts

Searches for unrelated CB donors were processed through the JCBBN, by which over 28,000 CB units were made available in August 2006. A preliminary search of umbilical cord blood banks was performed by using the patient's HLA phenotype, which was determined by serologic typing for class I HLA-A and HLA-B antigens and high-resolution DNA typing for class II HLA-DRB1 alleles. Preferred UCB units were those matched at 4 or more of 6 HLA loci and that contained a minimal count of 2×10^7 nucleated cells per kilogram of the recipient's body weight before freezing. Units of UCB were not depleted of T lymphocytes. The median cell number of the CBT donors was 3.7×10^7/kg of the recipient's body weight (range $2.5–12.2 \times 10^7$/kg). The $CD34^+$ cell was examined in 17 and the median $CD34^+$ cell number of the CBT donors was 1.2×10^5/kg of the recipient's body weight (range $0.36–5.9 \times 10^5$/kg). All UCB were thawed and infused without washing.

2.3 Preparative regimen and prophylaxis against GVHD

The conditioning regimen and acute GVHD prophylaxis varied according to the center policy, type of disease, prior treatment and disease status at the time of a CBT. The conditioning regimen and the GVHD prophylaxis are summarized in Table 2. The conditioning regimen was classified into two groups; 10 cases received reduced intensity conditioning (RIC) and myeloablative conditioning (MAC, including the use of radiation exceeding 10 Gy) was conducted in twenty cases [5]. After being informed of the potential risks and benefits of the procedure, the patients or their parents gave consent to the CBT. Supportive therapy

Table 1 Patient characteristics

Case #.	Gender	Age (years)	Diagnosis	FAB	Extra medullary infiltration at diagnosis	Cytogenetics	1st HSCT	Classification of preparative regimens	Site of relapse after 1st HSCT	Duration between relapse and 2nd transplantation (days)	Duration between diagnosis and relapse (days)
2055	F	7	AML	M2	Negative	t(8;21)	Auto BMT	MAC	BM	328	450
2336	M	13	AML	M4	Negative	MLLR	UCBT	MAC	BM	345	245
2341	F	2	AML	M7	Negative	Monosomy 7	UCBT	MAC	BM	NA	NA
2412	F	12	AML	M0	Negative	ND	Related BMT	MAC	BM	NA	NA
2749	F	11	AML	M0	Negative	47XX,del(21:9),t(12:13)(p12:q14)	Auto BMT	MAC	BM	358	476
2771	M	3	AML	M0	Negative	47XY, add(9)(P24), inv(9)(p11q13), del(12)(p11), del(13)(q12, q14), (del(13)(q14, q22), +21	Auto BMT	MAC	BM	191	516
2885	F	1	AML	M2	Negative	46XY, del(5)	Unrelated BMT	MAC	PB	345	106
2889	M	3	AML	M1	Negative	47XY, +8	Auto BMT	MAC	BM	220	697
2970	F	9	AML	M4	Negative	t(11;19)	Auto BMT	MAC	BM	304	609
3023	M	13	AML	M2	Negative	Normal	Auto PB	MAC	BM	167	1,530
3388	F	10	2ndary AML	M2	Negative	Normal	Unrelated BMT	MAC	BM	75	625
3505	F	14	AML	M2	Negative	46XX, t(7;11)(p15;p15)	Auto BMT	MAC	BM	190	376
3655	M	2	AML	M5	Negative	der(12),t(1;12),(q21;p13)	Unrelated BMT	MAC	BM	209	191
3667	F	10	AML	ND	Negative	Normal	Related BMT	MAC	BM	NA	NA
3754	M	10	AML	M4	Negative	Normal	Related BMT	MAC	PB	39	217
3766	F	13	AML	M4	Negative	Minor-bcr/abl = $3.6X10^3$copy/mg mRNA	Related PB	MAC	BM,CNS	906	243
3901	M	10	AML	ND	Negative	t(8;21)	UCBT	MAC	BM	NA	NA
3928	M	12	AML	M2	Negative	Normal	Unrelated BMT	MAC	BM	440	1,119
3947	F	4	AML	M1	Negative	t(1:12)	Auto PB	MAC	PB	79	570
4100	M	12	AML	M2	Negative	Normal	UCBT	MAC	BM	156	6,228
4718	M	11	AML	M5	Skin	ND	UCBT	MAC	Skin	485	356
4941	F	14	AML	ND	Kidony	Normal	UCBT	MAC	BM	74	201

UCBT unrelated cord blood transplantation, *HSCT* hematopoietic stem cell transplantation, *FAB* French-American-British classification, *NA* not available, *AML* acute myeloid leukemia, *BMT* bone marrow transplantation, *PB* peripheral blood, *CNS* central nervous system, *MLLR* mixed lineage leukemia gene rearrangement, *MAC* myeloablative conditioning

Table 2 Profiles of CBT as a Second HSCT

Case #.	NCC ($\times 10^7$/kg)	CD34 ($\times 10^5$/kg)	Body weight at UCBT	HLA disparity GVH direction	HLA disparity HVG direction	Status at UCBT	Preparative regimens	Classification of preparative regimen
2055	2.69	0.36	21	2	1	no CR	TBI(12) + CY(120) + VP(50)	MAC
2336	3.16	0.96	42	0	0	2nd CR	ATG(75) + FLU(150)	RIC
2341	15.38	5.92	10.3	1	1	2nd CR	ATG(75) + FLU(100)	RIC
2412	6.24	0.42	31	1	1	no CR	BU(16) + LPAM (6.4 mg/kg)	MAC
2749	6.36	NA	40	1	0	3rd CR	TBI(8.5) + LPAM(140) + FLU(120)	RIC
2771	5.70	NA	14.5	1	1	2nd CR	TBI(12) + CY(120)	MAC
2885	2.67	NA	12	1	1	no CR	CY(60) + CA(375) + FLU(75)	RIC
2889	31.11	NA	12	2	2	no CR	TBI(12) + CY(120) + VP(60)	MAC
2970	6.00	0.95	27	1	1	2nd CR	TBI(12) + CY(120) + VP(60)	MAC
3023	1.86	0.69	42	0	1	2nd CR	TBI(12) + CY(120)	MAC
3388	7.47	0.81	37.5	2	2	no CR	VP(50) + BU(16 mg/kg) + ACNU(400/m^2)	RIC
3505	3.98	NA	34	1	0	no CR	TBI(10) + CY(120) + VP(30) + CA(920 mg)	MAC
3655	14.35	3.88	12.1	1	1	no CR	TBI(2) + CY(50) + FLU(200) + Mylotarg 9 mg/m^2	RIC
3667	2.62	2.24	38.2	2	2	no CR	BU(21.2) + LPAM(183)	MAC
3754	4.46	0.87	26	2	2	no CR	CY(120) + BU(16) + CA(12 g)	MAC
3766	3.83	NA	48	2	2	no CR	LPAM(70) + BU(784/m^2) + TEPA(17.6) + FLU(150)	RIC
3901	7.69	1.46	25	2	2	2nd CR	TBI(2) + LPAM(90) + FLU(160)	RIC
3928	8.76	3.56	30	0	0	no CR	LPAM(140) + BU(8) + FLU(120)	MAC
3947	2.58	2.09	14.8	1	1	no CR	TBI(10) + CY(150) + VP(1,500/m^2)	MAC
4100	4.50	3.32	27	1	1	no CR	CY(120) + LPAM(70) + CA(800) + TEPA(300)	RIC
4718	4.86	0.80	31	1	1	3rd CR	BU(16) + LPAM(180)	MAC
4941	5.19	1.19	42.2	0	0	no CR	BU(8) + FLU(125)	RIC

NCC nuclear cell count, *UCBT* unrelated cord blood transplantation, *GVH* graft versus host, *MAC* myeloabrative conditioning, *CR* complete remission, *TBI* total body irradiation, *RIC* reduced intensity conditioning, *CY* cyclophosphamide, *ATG* anti-thymocyte globulin, *FLU* fludarabine, *CA* cytosine arabinocide, *BU* busulfan, *TEPA* thyotepa, *ND* not done

differed among the transplant centers. Protocols for preparative regimen and the use of CB from unrelated donors for transplantation were reviewed and approved by the institutional review boards at the transplantation centers.

2.4 Statistical methods

For this analysis, 1 July 2007 was used as the reference date (i.e., the day on which all centers locked data on patient outcomes). The outcome end points were neutrophil recovery, platelet recovery, GVHD, relapse, transplantation-related mortality (TRM), overall survival (OS) and DFS. Neutrophil recovery was defined by an absolute neutrophil count of at least 0.5×10^9/L for three consecutive days, the first of which was used as the recovery day. Platelet recovery was defined by a non-transfused platelet count of at least 20×10^9/L for seven consecutive days. Acute and chronic GVHDs were diagnosed and graded at each center according to the standard criteria [6, 7]. Relapse was defined on the basis of morphologic evidence of leukemia in the bone marrow or other extra-medullary organs. TRM was defined as any cause of non-leukemic death after transplantation. OS was the time between transplantation and death due to any cause. DFS was defined as the time interval from CBT to the first event, either relapse or death, during complete remission. These outcomes were all right-censored. For OS and DFS, the Kaplan–Meier method provided an estimate of the incidences over time, whereas the Cox models were used to evaluate the joint influence of patient-, disease- and transplant-related variables on the outcome. We fit univariate models that contain each of the variables one at a time. For comparison of group characteristics Fisher's exact test was used for univariate testing.

3 Results

3.1 Survival

Seven of the 22 patients survived and are in remission after CBT as a second HSCT (Table 3). The OS for 5 years after

CBT as a second HSCT was 31.3% ± 10.7%. In univariate analyses, the following factors were associated with a favorable prognosis of OS: (1) Status of disease at second transplantation (58.3 ± 18.6% for the CR at second transplantation, compared with 17.1 ± 10.8% for the non-CR at second transplantation; $P = 0.025$, Fig. 1a); and (2) date after January 2000 (55.1 ± 13.9% vs. 0.0 ± 0.0%, $P = 0.01$, Fig. 1b). There was a marginally significant difference between OS when the RIC was used before CBT as second HSCT (60.0 ± 15.5%) and those when the MAC was used (9.7 ± 9.1%, $P = 0.08$, Fig. 2). When conducting UCBT as a second HSCT due to AML recurrence, it was found that administration within, or following, a 219-day period leads to no statistical difference of OS. An analysis by the Fisher exact test showed that those cases treated in 1999 or earlier were characterized by a significantly larger number of those exposed to radiation therapy ($P = 0.02$) and fewer treated with RIC ($P = 0.03$), compared to those managed in 2000 or later. There was no difference in the disease status of the two groups at transplantation ($P = 0.6$). Among the subgroups, the 5-year OS was 66.7 ± 19.1% in 6 of 22 patients who received CBT as the first HSCT. By the Fisher exact test, the transplantation regimen for those treated with CBT at both the first and second HSCT (including one who received 2-Gy low radiation treatment) was that with reduced radiation intensity—RIC or one close to it (Table 2). On the other hand, there was no significant difference in OS for those treated with autologous transplantation as a first HSCT and those treated with other types of HSCT (25.0 ± 15.3% vs. 38.1 ± 14.1%, respectively, $P = 0.64$). MAC (including TBI) was conducted for eight patients who received autologous HSCT.

The DFS for 5 years after CBT as a second HSCT was 23.7 ± 10.1%. In univariate analyses, the following factors were associated with a favorable prognosis of DSF: (1) Status of disease at second transplantation (43.8 ± 18.8% for the low risk group, compared with 17.9 ± 11.0% for the high risk group; $P = 0.048$); and (2) date after January 2000 (45.9 ± 14.3% vs. 0.0 ± 0.0% $P = 0.034$). According to the univariate analysis, no significant factor related to DFS was found.

3.2 Engraftment

Four patients died within 1 month after CBT and prior to a recovery in the neutrophil count and three more patients succumbed before platelet engraftment. The cumulative incidence (CI) of neutrophil recovery at day 90 was 69.6 ± 9.6%. The median time to neutrophil recovery was 31 days (range 14–90). A univariate analysis proved that neutrophil recovery was not a significant factor. In the univariate analysis, there was marginally difference neutrophil recovery when prophylactic use of hematopoietic growth factor was present and those when the hematopoietic growth factor was not used (77.8 ± 9.8% vs. 40.0 ± 21.9%, $P = 0.07$). The association of neutrophil recovery with the nucleated cells and the $CD34^+$ cell doses was not statistically significant. The CI of platelet recovery on day 180 was 39.1 ± 10.1%. A platelet count of 20,000/mL was achieved by a median of 180 days (range 36–180). According to the univariate analysis, no significant factor related to platelet recovery was found.

3.3 Graft-versus-host disease

Acute GVHD (grade 2 or more) after CBT was conducted as a second was observed in 12 patients (6 in grade 2 and 6 in grade 3, Table 3). The 100-day cumulative incidence of acute GVHD (grade 2 or more) was 62.6 ± 12.6%. Treatment with a calcineurin inhibitor plus a steroid was possible for five patients with acute GVHD grade 3, while four with grade 1 went into remission of acute GVHD with conventional first-line steroid therapy without additional treatment. No patient suffered from acute GVHD grade 4. No patient-, disease-, or transplant-related factors that could be associated with the incidence of acute GVHD were found. Of the nine patients surviving beyond day 100 after CBT as second HSCT, three developed chronic GVHD. Three patients developed chronic GVHD (1, extensive; 2, limited).

3.4 Early transplant-related mortality

During the first 100 days after CBT, six patients succumbed to transplantation-related causes (due to infections, bleeding and other causes, respectively; Table 3). The cumulative TRM by day 100 being 24.6 ± 9.6%. From the univariate analyses, there was a marginal difference between early TRM when methotrexate (MTX) was used for GVHD prophylaxis (8.8 ± 8.0%) and those when MTX was not used (49.2 ± 17.7%, $P = .056$). Use of RIC as a conditioning regimen instead of MAC for UCBT as a second HSCT did not result in a statistical difference.

3.5 Relapse incidence

Hematologic relapses occurred in five patients after CBT as a second HSCT. The 5-year cumulative relapse incidence (RI) was 43.9 ± 14.7%. In univariable analysis, no factors that could be associated with RI were found. There was marginally difference between relapse rate when an acute GVHD (grade 2–4) was presented and those when those was not presented (22.1 ± 14.1% vs. 75.0 ± 21.0%, $P = 0.08$). The RI after CBT as second HSCT in those children presenting poor-risk cytogenetic abnormalities

Table 3 Clinical outcome after CBT as a second HSCT

Case #.	ANC recovery (≧500/μl)	Platelet (≧20,000/μl)	Acute GVHD skin	Acute GVHD GI	Acute GVHD liver	Acute GVHD overall	Therapy for acute GVHD	Chronic GVHD	Duration of survival (days)	Duration of the first remission (days)	Outcome	Cause of death
2055	NE	NE	0	0	0	0		NE	26	NA	Dead	VOD, MOF
2336	23	NE	3	0	0	2	PSL	NE	953	NA	Alive	
2341	NE	NE	2	0	0	1	PSL	NE	952	NA	Alive	
2412	31	NE	3	3	0	3	PSL + CsA	NE	341	NA	Dead	Unknown monocytosis, acute hepatic failure
2749	24	70	2	0	0	1	PSL	NE	1,412	430	Dead	Relapse
2771	24	45	0	0	0	0		NE	705	492	Dead	Relapse
2885	40	90	3	0	0	2	PSL	Limited	2,128	100	Alive	
2889	NE	NE	3	0	0	2	PSL	NE	18	637	Dead	Lung edema, alveolar bleeding
2970	14	NE	1	4	3	3	PSL + VP16	Extensive	183	NA	Dead	Bacterial infection
3023	16	36	2	0	0	2	PSL	NE	1,855	1,462	Alive	
3388	NE	NE	2	0	0	1	PSL	NE	57	576	Dead	Bleeding, bacterial infection
3505	15	90	0	0	0	0		NE	527	334	Dead	Unknown infection
3655	NE	NE	0	0	0	0		NE	38	143	Dead	Relapse
3667	21	52	3	1	0	2	PSL	NE	377		Dead	Relapse
3754	NE	NE	NE	NE	NE	NE		NE	21	5	Dead	Fungal infection
3766	22	87	3	3	0	3	PSL + FK506	NE	694	181	Alive	
3901	35	NE	0	3	0	3	FK506	NE	82	NA	Dead	IP, MOF, Bacterial infection:
3928	16	43	1	1	0	2		NE	96	385	Dead	Relapse
3947	NE	NE	NE	NE	NE	NE	CsA	NE	4	523	Dead	Unknown
4100	22	NE	0	0	0	0		NE	64		Dead	IP
4718	34	NE	3	4	2	3	PSL + CsA	Limited	140	328	Alive	
4941	15	NE	0	4	0	3	PSL + FK506	NE	101	77	Alive	

GVHD graft versus host disease, *GI* gastro-intestinal, *NE* no evidence, *VOD* veno occlusive disease, *MOF* multi organ failure, *PSL* predonisolone, *CsA* cyclosporine A, *IP* interstitial pneumonia

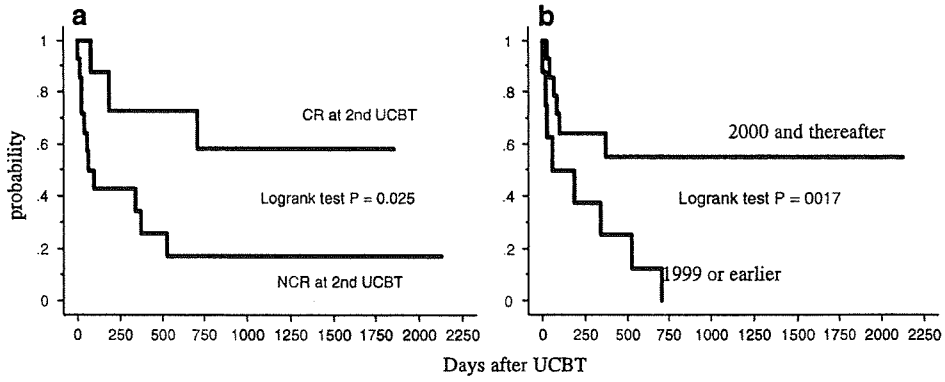

Fig. 1 Overall survival according to disease status at second transplantation (**a**) and time at transplantation (**b**)

was 50.0 ± 25%, compared with 46.1 ± 18.9% in the others ($P = 0.64$).

3.6 Causes of death for patients relapsing after CBT

Fifteen of the 22 patients succumbed after CBT that was applied as the second HSCT (Table 3), the main causes being TRM in 10, followed by a relapse of AML in 5. GVHD was not the cause of death in any of them. In 5 of the 10 patients with TRM, deaths were caused by infections (bacterial infections, 3; fungal infection, 1; and unknown, 1). One patient died from acute hepatic failure with monocytosis of unknown origin 341 days after CBT. Hemorrhagic cystitis, the most frequent complication related to the second HSCT was not observed in the 22 patients who were treated with CBT as a second HSCT.

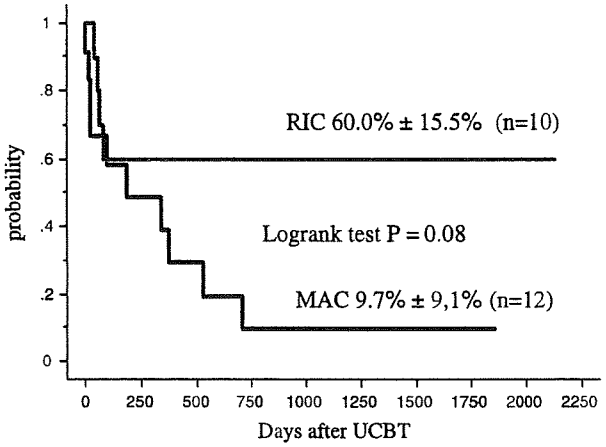

Fig. 2 There was marginally difference between overall survival (OS) when the RIC (reduced intensity conditioning) regime was used before CBT as second HSCT and those when the MAC (myeloablative conditioning) regime was used (60.0 ± 15.5% vs. 9.7 ± 9.1%, $P = 0.08$)

4 Discussion

This is the first report on the application of CB for a second transplantation attempt in children with AML. Relapse of AML after HSCT remains a significant therapeutic challenge and is the main cause of treatment failure after HSCT.

According to the results of our study, the survival rate was higher when recurrent AML was in the second remission following transplantation, if the conditioning regimen included no or only low dosage radiotherapy. For recurrences following CBT that was applied as the initial transplantation attempt, the survival rate was 66%. In cases of recurrences following an autologous or allo-BMT, the prognosis was poor if the conditioning regimen included a myeloablative procedure. A second transplantation is often recommended as the standard of care for a post-transplantation relapse of acute leukemia; but long-term (5-year) survival subsequent to the procedure was only 6–20% [6, 8]. These results highlight the need for more effective therapies for AML with a post-transplant relapse. Our observations suggest that when the CBT is the second HSCT it may be beneficial for improving post-relapse survival and may have a GVL effect for post-transplant relapse.

The results of transplantation conducted in 2000 and thereafter were generally more satisfactory than those dated 1999 or earlier. The former were characterized by less frequently conducted autografts with MAC when the transplantation was initially attempted, more frequent application of the RIC regimen at the second transplantation attempt; and fewer instances of radiotherapy, also at the second transplantation. The concept underlying HSCT with RIC in patients with malignant diseases involves a shift from the paradigm that eradicating tumors requires myeloablative chemoradiation to the theory that the donor's immune cells are utilized for tumor eradication, relying on an allogeneic graft rather than tumor effects [5]. In 2000, Champlin et al. [9] reported their experience with RIC-HSCT in adults with malignant diseases. This study demonstrated that this approach is safe and feasible even in elderly patients and those with pre-existing comorbidity

and suggested grafting to counter malignancy effects. However, there are a relatively few RIC-HSCT studies on pediatric patients with malignant diseases [10]. Although the use of the RIC regimen before the second transplants may expand the applicability of second transplants in a relapse setting, long-term follow-up is still limited [11–14]. Our report presents the first long-term result of post-transplant relapse of childhood AML, in which RIC was used as a pretreatment prior to CBT as the second HSCT. In general, the duration of remission after transplantation is an important factor in determining the prognosis following recurrences, which has been attested to in the literatures [1, 15]. The necessity for second transplants due to a recurring disease within 6 months of previous transplants resulted in higher subsequent relapse rates (80 vs. 25%). For second transplants performed within 6 months of the first, it was more difficult to save the recipients of prior allo-HSCT with second transplants than those receiving a prior autologous transplant [2]. Unfortunately, the current retrospective survey was not adequate to observe the duration of remission after the initial transplantation. As far as the selection of sources of transplants is concerned, CBT has potential advantages for patients regarding on-time availability, depending on the condition of the patient, the absence of donor risks and a low incidence of both severe GVHD and extensive chronic GVHD, despite the use of grafts with substantial donor-recipient HLA disparities.

In conclusion, the current data suggest that using CBT as a second HSCT may be an effective therapeutic option for children with AML in relapse after an initial HSCT. Those children with acute leukemias undergoing the second HSCT are at high risk for developing co-morbidities and may represent good candidates for standard myeloablative Allo SCT. CBT combined with RIC may be effective against a recurrence of AML in children after the initial HSCT.

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Appendix

Transplant centers

Transplant centers that performed CBT by the JCBBN coordination and produced follow-up reports are: Division of Pediatric Oncology, National Cancer Center Hospital, Tokyo; Division of Hematology/Oncology, Kanagawa Children's Medical Center, Yokohama; Department of Pediatrics, Ibaraki Children's Hospital, Mito; Division of pediatrics, Osaka City General Hospital, Osaka; Department of Pediatrics, Saitama Medical university, Iruma; Division of Hematology/Oncology, Saitama Children's Medical Center, Iwatsuki; Department of Hematology/Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka; Department of Pediatrics, Hamanomachi Hospital, Fukuoka; Department of Pediatrics, Hirosaki University School of Medicine, Hirosaki; Division of Pediatric Hematology/Oncology, Nagoya Red Cross First Hospital, Nagoya; Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University, Tokyo; Department of Pediatrics, Tottori University Faculty of Medicine, Yonago; Department of Pediatrics, Faculty of Medicine, University of the Ryukyus, Nishihara; Department of Pediatric Hematology and Oncology, Tohoku University School of Medicine, Sendai; Hamamatsu University School of Medicine, Hamamatsu; Department of Pediatrics, University of Occupational and Environmental Health, Kitakyushu; Department of Hematology-Oncology, Tokyo Metropolitan Kiyose Children's Hospital, Kiyose; Department of Pediatrics, University of Tokyo, Tokyo; Department of Pediatrics, Osaka City University Graduate school of Medicine.

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Incidence and Risk Factors of Early Bacterial Infections after Unrelated Cord Blood Transplantation

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Incidence and Risk Factors of Early Bacterial Infections after Unrelated Cord Blood Transplantation

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Incidence and characteristics of early bacterial infection within 100 days after unrelated cord blood transplantation (UCBT) were assessed for 664 pediatric and 1208 adult recipients in Japan. Cumulative incidence of early bacterial infection at day 100 post-UCBT was 11% (95% confidence interval [CI], 8%-13%) for children and 21% (CI, 19%-24%) for adults ($P < .0001$). Early bacterial infection in adults had a significant impact on mortality (hazard ratio [HR] = 2.1, CI, 1.7-2.6; $P < .0001$), although no significant risk factors were identified. Multivariate analysis identified older age group (6-10, and 11-15 years versus 0-5 years of age) at transplant (HR = 2.0 and 2.7, CI, 1.1-3.5 and 1.4-4.9; $P = .020$ and .002, respectively) as an independent risk factor of early bacterial infection for children. Early bacterial infection in children did not have a significant impact on mortality when adjusted. Of 315 bacteremia, 74% were caused by Gram-positive microorganisms. Pneumonia occurred in 39 patients including 13 cases of *Stenotrophomonas maltophilia* pneumonia. Early bacterial infection had a negative effect on survival for adults and the median day of development was 10 days after transplant, suggesting that the prevention of bacterial infection in the very early post-UCBT phase is important.

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KEY WORDS: Early bacterial infection, Cord blood transplantation, The Japan Cord Blood Bank Network, Risk factor for infection, Urelated donor

INTRODUCTION

Infection is 1 of the major causes of morbidity and mortality for patients undergoing bone marrow transplantation (BMT) and peripheral blood stem cell transplantation (PBSCT) [1,2]. Recently, use of cord blood transplantation (CBT) from unrelated donors has increased for patients who do not have suitable donors for BMT or PBSCT, yielding promising results [3-7]. However, neutrophil recovery has been significantly delayed in unrelated CBT patients compared to unrelated BMT patients. Bacterial infection remains 1 of the most common problems after unrelated cord blood transplantation (UCBT) [5,8-10].

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In this paper, we report the results of our analysis of early bacterial infections before day 100 following UCBT in 1872 Japanese patients. We conducted this analysis to investigate the incidence and timing of infections, causative micro organisms, potential risk factors of infections, and the influence of infection on outcome.

PATIENTS AND METHODS

Patients

Between September 1997 and September 2005, 2362 UCBT procedures were performed using a single cord blood (CB) in 175 transplantation centers with 221 transplantation units supported by 11 CB banks affiliated with the Japan Cord Blood Bank Network (JCBBN) in Japan. The subjects analyzed were 1872 patients whose initial clinical report forms (CRFs), completed 100 days after UCBT, were submitted to the JCBBN. The clinical protocols for UCBT were approved by the institutional review board of the respective institutions. Patients underwent UCBT if they had no human leukocyte antigen (HLA)-identical, 1 locus mismatched relative or an HLA-matched unrelated BM donor could not be identified within 6 to 8 weeks [11]. The patients or their parents gave their consent for UCBT after being informed of the potential risks and benefits of the procedure. All patients received conditioning chemotherapy in the sterile unit with high-efficiency particulate air filtration. The conditioning regimen, acute graft-versus-host disease (aGVHD) prophylaxis and prevention of bacterial infections varied according to the institute's policy and type of disease, although most of the institutions used oral polymyxin B or fluoroquinolone with intravenous antibiotics to prevent bacterial infections.

Selection of Grafts

Searches for unrelated CB units were processed through the JCBBN, where 25,803 CB units were available in August 2006. Suitable CB in JCBBN was selected by cell count of nucleared cell before freezing and HLA compatibility between CB and patients. Preferred unrelated CB units were those that matched at least 4 of 6 HLA antigens, based on serologic typing for class I HLA-A and HLA-B, antigens and low-resolution DNA typing for class II HLA-DR and contained a minimum cell count of 2×10^7/kg nucleated cells of the recipient's body weight before freezing.

Bacterial Infections

We analyzed bacterial infections reported in the JCBBN 100-day CRF with clinical symptoms and pathogenic micro-organisms were discovered, because it is not easy to distinguish bacteremia or pneumonia without microbiologically documented infection from preengraftment fever or capillary leak syndrome in the early post-UCBT phase.

Early bacterial infections were defined as those occurring within the first 100 days after graft infusion. If a second episode with the same organism occurred within 7 days, it was counted as a single infection episode [10].

Collection of Data

Detailed patient and clinical variables were collected by the JCBBN CRF. Its 100-day CRFs were submitted by transplantation centers or units to the 11 CB banks and checked by a data manager of each bank for missing data and inconsistent data. After the data cleaning, all CRFs were submitted from CB banks to the data center of JCBBN. Annual follow-up for each transplant case is performed to update the data on engraftment, relapse, survival, and complications. The final data set used for the analyses was fixed in March 2006.

Statistical Analysis

Because preliminary study of all patients revealed that 16 years of age and older was the sole significant variable in multivariate analysis, separate analyses were performed for children (younger than 16 years of age) and adults (16 years of age and older) to find the risk factors and to investigate the impact of infection on survival. All episodes of infection were included in the analyses to identify causative micro-organisms of infections. Various clinical factors were evaluated as potential risk factors for early bacterial infection in univariate and multivariate analyses combined with the Cox proportional-hazards regression model. Factors found to be significant ($P < .05$) or marginally significant ($P < .1$) in univariate analysis were included in the multivariate analysis using a forward stepwise method. The categorization for the analyses of risk factors was based on the rule that the smaller group of variable needed to contain at least 10% of the patients. The proportional hazards regression model with early bacterial infection as a time-dependent covariate was used to determine the effect of early bacterial infection on survival. Survival distributions were estimated with the method of Kaplan and Meier. Probabilities of early bacterial infection were calculated by means of cumulative incidence curves treating death without early bacterial infection as competing risks. Statistical analyses were performed with Stata software version 9.0 (Stata Corp., College Station, TX).

RESULTS

Characteristics of Patients

Table 1 shows the characteristics of 664 pediatric (age <16 years) and 1208 adult (age ≥16 years) patients who underwent UCBT in Japan. In the child cohort,

Table 1. Characteristics of Pediatric and Adult Patients Who Received Unrelated Cord Blood Transplantation

Variable	Child (Age <16) No. Eval (n = 664)			Adult (age ≥16) No. Eval (n = 1208)		
Sex—no. (%)						
Male	664	403	(61)*	1208	662	(55)
Female		261	(39)		546	(45)
Age group—no. (%)						
0-15	664	664		1208		
16-30					270	(22)
31-45					338	(28)
≥46					600	(50)
Disease—no. (%)						
Acute lymphoblastic leukemia	664	279	(42)	1207	211	(17)
Acute myelogenous leukemia		151	(23)		490	(41)
Adult T cell leukemia		0			65	(5)
Chronic myelogenous leukemia		8	(1)		69	(6)
Chronic lymphocytic leukemia		0			3	
Myelodysplastic syndrome		15	(2)		103	(9)
MDS/MPD		20	(3)		7	(1)
Lymphoma		28	(4)		188	(16)
Myeloma		0			32	(3)
Solid tumor		27	(4)		6	
Aplastic anemia		14	(2)		24	(2)
Immunodeficiency		47	(7)		1	
Metabolic disease		25	(4)		0	
Others		50	(8)		8	(1)
History of previous transplantation—no. (%)						
No	664	556	(84)	1208	914	(76)
Yes		108	(16)		294	(24)
Conditioning regimen—no. (%)						
Myeloablative	664	545	(82)	1208	579	(48)
Nonmyeloablative		99	(15)		621	(51)
Unknown		20	(3)		8	(1)
Total-body irradiation	664	350	(53)	1208	928	(77)
ATG/ALG	664	75	(11)	1208	38	(3)
Prophylaxis against GVHD						
Cyclosporine based	630	401	(64)	1172	846	(72)
Tacrolimus based		199	(32)		312	(27)
Others		30	(5)		14	(1)
Methotrexate used	630	362	(57)	1172	582	(50)
Prednisolone used	630	161	(26)	1172	47	(4)
Mycophenolate mofetil used	630	2		1172	78	(7)
Nucleated cell dose/kg body weight—$\times 10^{-7}$						
Median	664	5.10		1208	2.53	
Range		1.18-24.91			1.02-6.42	
HLA compatibility(GVHD direction)—no./total no. (%)						
Matched	656	162	(25)	1187	129	(11)
One-antigen mismatch		380	(58)		457	(39)
Two-antigen mismatch		106	(16)		577	(49)
Three-antigen or more mismatch		8	(1)		24	(2)

MDS/MPD indicates myelodyplasticsyndrome/myeloproliferative disease; ATG, antithymocyte globulin; ALG, antilymphocyte globulin; GVHD, graft-versus-host disease; HLA, human leukocyte antigen.
*Figures in parentheses show percentages.

108 patients (16%) had a history of previous transplantation. Myeloablative conditioning regimen was administered to 545 patients (82%). Total body irradiation (TBI) was administered to 350 of 664 patients (53%) and 311 of 545 patients (57%) who received a myeloabalative condition regimen. For GVHD prophylaxis, cyclosporine (CsA)-based prophylaxis was administered to 401 patients (64%), and tacrolimus-based prophylaxis to 199 (32%). Methotrexate (MTX) was used for GVHD prophylaxis for 362 patients (57%), and prednisolone for 161 (26%). The median dose of nucleated cells per kilogram of patient's body weight was 5.10×10^7. In the adult cohort, 600 patients (50%) were 46 years old or older, and 294 patients (24%) had a history of previous transplantation. TBI was administered to 998 of 1208 patients (77%) and 504 of 579 patients (87%) who received a myeloabalative condition regimen, and 621 patients (51%) were given a nonmyeloablative conditioning regimen [12-15]. CsA-based GVHD prophylaxis was administered to 846 patients (72%), and tacrolimus-based prophylaxis to 312 patients (27%). The median dose of nucleated cells per kilogram of patient's body weight was 2.53×10^7.

Incidence and Timing of Early Bacterial Infection

In the child cohort, 77 patients (12%) developed early bacterial infection with a cumulative incidence of 9% (95% confidence interval [CI] 7%-11%) at 50