A minimal dose of unmanipulated bone marrow infusion without conditioning for T-B+NK- severe combined immunodeficiency

Eun Sang Yi (✉ holypresent@naver.com)

Korea University Guro Hospital <https://orcid.org/0000-0001-8214-2106>

Hee Young Ju

Korea University Guro Hospital

Hee Won Cho

Korea University Guro Hospital

Ji Won Lee

Korea University Guro Hospital

Ki Woong Sung

Korea University Guro Hospital

Hong Hoe Koo

Korea University Guro Hospital

Eun-Suk Kang

Sungkyunkwan University School of Medicine

Kang Mo Ahn

Sungkyunkwan University School of Medicine

Yae-Jean Kim

Sungkyunkwan University School of Medicine

Keon Hee Yoo

Sungkyunkwan University <https://orcid.org/0000-0002-5980-7912>

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Abstract

Purpose Hematopoietic stem cell transplantation (HSCT) is the standard method of reconstituting immune function in severe combined immunodeficiency (SCID); however, current conditioning recommendations and optimal cell doses lack consensus. In this study, we investigated HSCT outcomes and immune reconstitution, following minimal dose (MD) HSCT in T cell-negative (T-), B cell-positive (B+), natural killer-cell negative (NK-) SCID patients.

Methods We retrospectively reviewed patients with SCID who received HSCT between 2002–2018. Standard dose (SD) and MD were classified based on a total nucleated cell count (TNC) of 1.0×10^8 /kg or more and less.

Results Seven patients with SCID received HSCT, of which four belonged to the MD group. Patients in the MD group were administered 5 mL or less of bone marrow without conditioning, with median TNC and CD34+ cell counts of 0.49×10^8 /kg and 0.62×10^6 /kg, respectively. T cells recovered within a year after HSCT, and immunoglobulin supplementation was discontinued at a median of 3.5 months after HSCT in all MD recipients. All MD recipients were alive without disease recurrence at a median of 126.9 months after HSCT, exhibiting donor chimerism in the range of 10.1%–100%. Although grade II–III graft-versus-host diseases occurred, these were manageable in all patients. One of the three patients in the SD group died of cytomegalovirus infection, while another was dependent on intravenous immunoglobulin until 41 months after HSCT.

Conclusion In patients with T-B+NK- SCID, sufficient therapeutic effects were safely obtained with minimal dose of bone marrow infusion without conditioning.

Introduction

Severe combined immunodeficiency (SCID) collectively refers to a heterogeneous group of rare genetic disorders of the immune system [1]. It is characterized by substantially decreased or functionally deficient T cells, B cells, or natural killer (NK) cells, depending on the nature of the genetic abnormalities. Patients with SCID are vulnerable to life-threatening infections; hence, treatment typically focuses on immune system reconstitution. Although gene therapies have been attempted in patients with certain types of SCID [2-5], allogeneic hematopoietic cell transplantation (HSCT) remains the definitive treatment in most cases.

Owing to the absence of functional T cells in patients with SCID, graft rejection is rare following HSCT. Immunoablative conditioning is often omitted, particularly in human leukocyte antigen-matched sibling donor (MSD) HSCT for T-B+NK- SCID [6, 7]. It is also believed that even a small amount of hematopoietic stem cells (HSCs) can induce immune reconstitution in such cases, as deficient NK cell-related immunity makes it unable to reject the donor HSCs. However, there is no established minimal effective HSC dose for successful immune reconstitution in patients with SCID.

In light of the above, our center has been using infusions of unmanipulated HSCs in small doses since 2002. According to our clinical experience, only a small number of HSCs are needed for T cell engraftment; moreover smaller doses with lower T cell counts may lower the incidence and severity of graft-versus-host disease (GVHD). In addition, mismatched cells and plasma are not likely to cause clinically significant transfusion reactions when only a small blood volume is infused; hence, the need to deplete red blood cells and plasma is obviated.

In this study, we aimed to determine the clinical and immunological outcomes of T-B+NK- SCID patients transplanted with small doses of cells without conditioning and to compare the outcome with those obtained using standard doses of HSCT. To the best of our knowledge, this paper is the first research article regarding minimal dose (MD) infusion of HSC in SCID patients, showing successful results.

Methods

Study patients and transplantations

We retrospectively reviewed a total of seven patients who received allogeneic HSCT for SCID at Samsung Medical Center, Seoul, Korea from 2002–2018. Data on patient demographics; transplantation characteristics; and clinical course information, including survival, infection, and the incidence of GVHD, were obtained. This study was approved by the Institutional Review Board of Samsung Medical Center (IRB No. 2020-03-014). The need for informed consent was waived by the board.

Evaluation of immune reconstitution following transplantation

We conducted an HSCT engraftment analysis using short tandem repeat (STR)-based chimerism testing on post-treatment days 28, 56, 100, 180, 365, and beyond, if needed. The STR-based chimerism analysis was performed using peripheral blood at 56 days and bone marrow (BM) samples at other time points. Lymphocyte subsets, including T cells (CD3⁺), B cells (CD19⁺), NK cells (CD16⁺56⁺3⁻) and immunoglobulin levels, were assessed on post-treatment days 28, 100, 180, 365, and beyond, as needed.

Definitions and statistics

Patients were placed into an MD group or a standard dose (SD) group. MD was defined as a total nucleated cell count (TNC) lower than $1.0 \times 10^8/\text{kg}$, while the SD was defined as TNC $1.0 \times 10^8/\text{kg}$ or more. Unlike other transplantations, cells are generally infused in quantities to the 10th power for cord blood transplantation (CBT); the MD of CBT was defined as TNC $< 1.0 \times 10^7/\text{kg}$.

The diagnosis and grading of GVHD were based on the standard clinical criteria [8, 9]. Complete donor chimerism was defined as $> 99\%$ of the deoxyribonucleic acid (DNA) in a sample specimen from the recipient being derived from the donor. When 1–99% of the recipient's DNA was derived from the donor, mixed chimerism was considered to have occurred [10].

T cell count recovery was defined as a CD3⁺ count $> 800 \text{ cells}/\text{mm}^3$, while CD4⁺ and CD8⁺ recovery were defined as $> 400 \text{ cells}/\text{mm}^3$ and $200 \text{ cells}/\text{mm}^3$, respectively [11]. B cell count and function recovery were defined as a CD19⁺ $> 400/\text{mm}^3$ and independence from intravenous immunoglobulin (IVIg) therapy. The time to IVIg independence described the time from HSCT to the last day of IVIg infusion.

Results

Characteristics of study patients and transplantation

The characteristics of the patients have been summarized in Table 1. A total of seven patients with T-B+NK- SCID received HSCT from 2002–2018. All patients were male, and the median age at transplantation was 4.7 months (range, 2.3–7.0). All seven patients were diagnosed with T-B+NK- SCID using immunological tests. Interleukin-2 receptor γ chain deficiency was genetically confirmed in four patients. Genetic testing was not performed in two patients (M1, S2). One patient (S3) underwent next-generation sequencing analysis; however, we could not find disease-causative genetic abnormalities.

Four patients received MD HSC, while three patients received SD HSC. Among the former, three received HSCT from an MSD, while one patient (M3) received the transplantation from a familial mismatched donor (FMMD). Transplantation was performed without conditioning and GVHD prophylaxis in the MD group. Three patients in this group received 5 mL of BM aspirate, while the remaining patient received 2 mL of BM aspirate. The median TNC, CD34⁺ cells, and CD3⁺ cell doses were $0.49 \text{ (range, } 0.32\text{--}0.84) \times 10^8/\text{kg}$, $0.62 \text{ (range, } 0.38\text{--}0.84) \times 10^6/\text{kg}$, and $0.44 \text{ (range, } 0.11\text{--}1.14) \times 10^7/\text{kg}$, respectively.

Among the three patients receiving SD HSCT, two received peripheral blood stem cell transplantation from FMMD that was manipulated using the *ex vivo* CD3⁺ T-cell receptor (TCR) $\alpha\beta^+$ depletion technique without conditioning or GVHD prophylaxis, and the other patient (S3) received mismatched, unrelated CBT with reduced-intensity conditioning. Patient S3, the younger brother of patient S2 and born as a dizygotic twin, received CBT at three months after birth without a Bacillus Calmette-Guérin (BCG) vaccination.

Excluding patient S3, who received transplantation closest to the time of birth, the other six patients all experienced severe infections prior to transplantation. Four patients (M2, M3, M4, and S2) suffered from disseminated BCG infection prior to HSCT. Patient M1 received transplantation while under ventilator care for severe pneumonia at the intensive care unit. Ventilation was discontinued six days after HSCT. Patient M3 suffered rotavirus viremia after vaccination for rotavirus. Patients M4 and S2 developed *Pneumocystis jirovecii* pneumonia. Patient M4 also experienced recurrent diarrhea. Patient S1 frequently suffered from upper respiratory tract infections and otitis media. Patient S2 had cytomegalovirus (CMV) disease of the lung and central nervous system at the time of SCID diagnosis.

Donor chimerism and survival outcome

All patients who received MD HSCT showed donor-derived cells that ranged from 7.3%–26.4% at 28 days after HSCT. This range decreased to 1.4–8.7% 100 days after HSCT before gradually increasing to 10.12%–100% at the final evaluation. Although patient M3

showed 2.7% donor chimerism 100 days after HSCT, complete donor chimerism was obtained following a stem cell boost with 1 mL of BM aspirate from the same donor 119 days after the first HSCT for uncontrolled, disseminated BCG infection. The serial STR results of the MD group have been presented in Figure 1. All patients were alive without infections related to disease recurrence at a median of 127 months (range, 21–203 months) after HSCT.

In the SD group, patient S2 experienced primary graft failure and received a second HSCT from the same FMMD 34 days after the first HSCT, albeit unsuccessfully. A third transplantation was performed using unrelated cord blood after busulfan-based conditioning, and complete donor chimerism was accomplished. However, this patient ultimately died of disseminated CMV disease 68 days after the third HSCT, which was refractory to treatment with ganciclovir (Roche Korea), foscarnet (Fresenius Kabi), and CMV immunoglobulin (Biotest pharm). Patient S1 and S3 achieved donor chimerism of 48.11% and 88.72% at 3 years and 1 year after HSCT, respectively.

Graft-versus-host disease

The patient outcomes after HSCT have been summarized in Table 2. Three of four patients in the MD group experienced grade II acute GVHD. One case was resolved without specific treatment, while the other two patients underwent GVHD treatment with corticosteroids and cyclosporine. Patient M3 developed extensive chronic GVHD, involving the lungs, skin, and mouth, after received FMMD transplantation and boost infusion. He was treated with corticosteroid and rituximab but became free from immunosuppressive agents 63 months after HSCT.

Two of the three SD HSCT recipients experienced acute GVHD of grades II and III, respectively. The grade III patient recovered following corticosteroid treatment.

Immune reconstitution after HSCT

Changes in the numbers of various immune cells and serum immunoglobulin levels of each patient after HSCT have been presented in Figure 2 and Supplementary Table S1. Patient M3, who underwent immunosuppressive treatments for extensive chronic GVHD, had a reduced T cell count 1 year after HSCT. The B cell count decreased to < 200 cells/mm³ in patients M1 and M3 during the first 3 months after HSCT; however, the patients recovered after 6 months and 1 year, respectively. IVIg supplementation was discontinued in all MD patients at a median of 4 months (range, 1–37 months) following HSCT.

In the SD group, both the T cell and B-cell counts recovered in patient S1 6 months after HSCT; however, IgG, IgA, and IgM did not reach the normal range. This patient is on IVIg supplementation at 41 months after HSCT. Patient S3, who underwent CBT after conditioning, achieved B cell and T cell recovery at 3 and 6 months, respectively.

Discussion

The optimal number of TNC and CD34⁺ cells required for HSCT in SCID patients has not yet been established, but previous studies have reported higher doses of infused cells than those used in this study. Reports by the Primary Immune Deficiency Treatment Consortium (PIDTC) and European Blood and Marrow Transplant Society (EBMT) have used doses with median TNCs of $5.5\text{--}7.98 \times 10^8/\text{kg}$ for MSD and $4.8 \times 10^8/\text{kg}$ in matched unrelated donor HSCTs for SCID [12, 13]. In our study, the MD group received infusions with lower median TNC doses than the smallest doses reported in previous studies. All MD HSCT patients survived with no clinical evidence of disease recurrence. Sufficient immune reconstitution was accomplished, and all patients discontinued IVIg supplementation.

Our strategy of infusing low doses of HSC without conditioning had several implications. First, successful transplantation in T-B+NK-SCID can be determined not by complete engraftment, but by recovery of immunity and a minimal amount of stem cell infusion without conditioning is sufficient to achieve it. Conditioning treatment is used to create BM niche space and achieve immune suppression in order to prevent graft rejection. However, the need for conditioning in patients with SCID is debated, as T cell reconstitution can be achieved without conditioning [14, 15]. In NK+ SCID patients, the BM space is full of HSCs and precursor B-lymphocytes, and the thymus is full of precursor T-lymphocytes; thus, conditioning is required to empty these niches to facilitate engraftment and consequent B- and T-lymphocyte progenitor development [6, 16]. In contrast, T-B+NK- SCID arises following impaired T- and NK cell development. The vacant BM niches of such patients are thought to be receptive and permissive of the engraftment of donor-derived precursors [6]. In this study, three patients in the MD HSC group showed stable mixed chimerism, but none experienced graft rejection. As lineage-specific donor chimerism analysis was not measured in our study, the engraftment of each cell lineage was unknown. However, our data showed that an extremely low level of unsorted donor chimerism in the early post-HSCT phase could induce durable donor engraftment,

suggesting the possibility of a graft-versus-marrow effect. In addition, the chimerism level displayed dynamic changes, indicating that chimerism alone should not be used as the sole indicator of graft failure, especially when concomitant and clinically relevant immune reconstitution is observed. Unlike HSCT in hematologic malignancies, in which mixed chimerism is a predictor of rejection, SCID is thought to be correctable with mixed chimerism; furthermore, complete donor chimerism may be unnecessary for cure.[17, 18] Instead, mixed chimerism is associated with a lesser degree of GVHD than complete donor chimerism.[18] Although most patients in the MD group showed mixed chimerism, their cellular and humoral immune reconstitutions were excellent even when extremely low levels of donor chimerism were present. Although donor chimerism was only 1.4% in patient M1 100 days after HSCT, lymphocyte counts were normal, and the B cell count was only slightly low. The PIDTC study demonstrated that the presence of higher CD4⁺ cell counts (above 500/mm³) at 6 and 12 months post-HSCT was predictive of improved long-term T cell reconstitution and better long-term overall survival.[19] In our study, CD4⁺ counts exceeded 500/mm³ at either 6 or 12 months after MD HSCT in all patients. In addition, all MD patients achieved independence from IVIg therapy post-HSCT, with three patients becoming independent in 4 months. Recovery of immunity and reduction of infection, the main goals of HSCT in SCID, were achieved using MD HSCT. One patient in this study (S2) died of infectious cause, who was a recipient of SD HSC.

Second, MD HSC infusion holds the advantage of a low incidence of transplantation-related morbidity in terms of infection, long-term GVHD, and impaired growth or fertility. Conditioning toxicity is one of the major causes of death in SCID patients [19, 20]. In countries where newborn screening testing for SCID is not used, the majority of cases without a family history of SCID are complicated by serious infection before HSCT can be administered. Four patients in our study developed disseminated BCG infection before HSCT since BCG administration is routinely recommended during the neonatal period in Korea. In addition, one patient experienced rotavirus infection after live attenuated rotavirus vaccine administration. In these cases, the use of conditioning and immunosuppressive agents for GVHD prophylaxis can actually exacerbate the infection. We found that the administration of MD HSCT without conditioning or GVHD prophylaxis was able to control pre-existing infectious complications.

By reducing the HSC infusion dose, the risk of GVHD decreases due to lower donor T cell doses. In this study, the T cell count of the infusion was almost a tenth of those used in the PIDTC and EBMT reports [12]. There were no patients with grade III or IV acute GVHD following MD HSCT, and those with grade II acute GVHD were easily managed. However, caution is still warranted, as extensive chronic GVHD developed in one patient who underwent MD HSCT from an FMMD and an HSC boost.

GVHD is one of the major causes of mortality during HSCT in patients with primary immune deficiencies [17]; moreover, and the incidence in SCID has been reported to be 28.3%– 55.3% [12, 14, 19, 21]. Unlike patients with hematologic malignancies, who may benefit from the graft-versus-leukemia effect of donor cells, SCID patients derive no clinical benefit from GVHD. On the other hand, the increased incidence of viral infections and poor immune recovery associated with GVHD prophylaxis are clinically concerning. Our MD HSCT strategy, coupled with a lack of conditioning-induced tissue damage, likely rendered routine GVHD prophylaxis unnecessary.

Third, the MD HSCT method is simple, safe, and accessible in the treatment of SCID. Only 5 mL of BM aspirate is required from donors, and the treatment can be administered to recipients at bedside, thereby avoiding delays arising during the scheduling of an operation room. Since conditioning is omitted, the need for specialized facilities with laminar flow systems to protect HSCT recipients from contagious pathogens is not mandatory. This could be securely tried even in patients suffering from serious infections and those living in resource-limited countries. Patient M1 underwent HSCT at the intensive care unit with mechanical ventilator support and had no apparent complications. Additionally, if chimerism is not adequately achieved after MD HSCT, an additional boost using a small amount of HSCs is possible. Patient M3 underwent MD HSCT from FMMD, receiving an additional 1 mL of BM infusion for poor graft performance; complete chimerism occurred thereafter.

There were some limitations to our study. This was a retrospective descriptive study using a small population and, as such, statistically significant conclusions likely cannot be drawn. Moreover, genetic mutational profiles were not available in some patients, and lineage-specific chimerism could not be tested.

Despite these limitations, the authors believe this to be the first study to report detailed HSCT information pertaining to T-B+NK- SCID patients treated with small doses of BM-derived HSCs without conditioning. Based on our data, the minimal effective dose required for HSCT without conditioning is likely to be much lower than those used in current practices. In conclusion, this study demonstrated the feasibility of MD HSCT without conditioning in T-B+NK- SCID patients.

Declarations

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Conflicts of Interest

None of the authors have any conflicts of interest to report.

Availability of data and material

The data that support the study findings are available from the corresponding author upon reasonable request.

Authors' contributions

KH Yoo and HH Koo designed the research study. HW Cho, JW Lee, and KW Sung contributed for data collection. ES Kang, GM Ahn, Y Kim supported laboratory study and resources. ES Yi and HY Ju analyzed the data. ES Yi and HY Ju wrote the original draft. All of the authors reviewed and edited the paper. KH Yoo supervised the study.

Ethics approval

This study was approved by the Institutional Review Board of Samsung Medical Center (IRB No. 2020-03-014).

Consent to participate

The need for informed consent was waived by the board.

Consent for publication

The need for informed consent was waived by the board.

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Authorship Contributions

KH Yoo and HH Koo designed the research study. HW Cho, JW Lee, and KW Sung contributed for data collection. ES Kang, GM Ahn, Y Kim supported laboratory study and resources. ES Yi and HY Ju analyzed the data. ES Yi and HY Ju wrote the original draft. All of the authors reviewed and edited the paper. KH Yoo supervised the study

Disclosure of Conflicts of Interest

None of the authors have any conflicts of interest to report.

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Tables

Table 1. Characteristics of study patients and transplantation

No.	Sex	Age at HCT (months)	Genetic type	Donor type	HLA match	Stem cell source	ABO type, Donor/Recipient	Infused volume (ml)	TNC dose (×10 ⁸ /kg)	CD34 cell dose (×10 ⁶ /kg)	CD3 cell dose (×10 ⁷ /kg)	Conditioning regimen	Ex-vivo TCR αβ depletion	GVHD prophylaxis
Minimal cell dose														
M1	M	5	NA	MSD	8/8	BM	O+/O+	5	0.84	0.67	1.14	None	No	None
M2	M	4	<i>IL2RG</i>	MSD	8/8	BM	A+/A+	5	0.32	0.38	0.37	None	No	None
M3	M	5	<i>IL2RG</i>	FMMD	5/10	BM	B+/B+	2	0.43	0.57	0.11	None	No	None
M4	M	7	<i>IL2RG</i>	MSD	10/10	BM	AB+/A+	5	0.54	0.84	0.51	None	No	None
Standard cell dose														
S1	M	4	<i>IL2RG</i>	FMMD	5/10	PB	A+/A+	55	8.57	5.10	0.02 [†]	None	Yes	None
S2	M	4	NA	FMMD	6/10	PB	A+/A+	90	12.87	6.85	0 [†]	None	Yes	None
S3	M	2	NA [‡]	UCB	9/10	CB	O+/A+	23	0.90	0.10	1.59	BuFluATG	No	Yes

[†]TCR α+β+ T cell depletion, [‡]Next-generation sequencing was performed for patient S3, but no significant relevant mutation was detected.

HSCT, hematopoietic cell transplantation; HLA, human leukocyte antigen; TNC, total nucleated cell count; GVHD, graft-versus-host disease; NA, not available; MSD, matched sibling donor; FMMD, familial mismatched donor transplantation; UCB, unrelated cord blood; BM, bone marrow; PB, peripheral blood; CB, cord blood; Bu, busulfan; Cy, cyclophosphamide; CSA, cyclosporine; MTX, methotrexate

Table 2. Outcomes after HCT

Case No.	Additional HCT or stem cell boost	Grade of Acute GVHD	Chronic GVHD	Survival	Cause of death	Free of IVIg	Months from HSCT at Last IVIg	CMV infection	CMV disease	Follow-up duration (months from HSCT)
Minimal cell dose										
M1	No	II	No	Alive		Yes	4	No	No	203
M2	No	No	No	Alive		Yes	1	No	No	127
M3	Yes [†]	II	Extensive	Alive		Yes	37	No	No	72
M4	No	II	No	Alive		Yes	3	No	No	21
Standard cell dose										
S1	No	III	No	Alive	-	No	42	No	No	42
S2 [‡]	Yes	II	N/A	Deceased	Refractory CMV disease	NA	5	Yes	Yes	5
S3	No	No	No	Alive		Yes	2	No	No	21

[†]Patient M3 received 1 ml of additional bone marrow without conditioning. [‡]Patient S2 patient received second and third HCT treatments. HCT, hematopoietic cell transplantation; CMV, cytomegalovirus; GVHD, graft-versus-host disease; IVIg, intravenous immunoglobulin, N/A, not applicable

Figures

M1	2	26.4	1.4	6.2	75.7 (17 yr)
M2		7.3		35.1	
M3	73.9	8.7	13.1	2.7	21.8
M4	36.8	8.8	11.5	8.7	10.1

Figure 1

Changes in donor chimerism of recipients receiving minimal doses of hematopoietic stem cells after transplantation.

Figure 2

Changes in the numbers of peripheral immune cells in recipients receiving minimal dose of hematopoietic stem cells transplantation. A–D indicate patients M1–M4, respectively.