Gonadal failure among female patients after hematopoietic stem cell transplantation for non-malignant diseases

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Abstract. In addition to malignant diseases, hematopoietic stem cell transplantation (HSCT) is also a vital option as a curative therapy for non-malignant diseases, such as immunodeficiency, and other hematological disorders. Not only for malignant diseases, but for non-malignant diseases, cytotoxic therapy of conditioning regimens are associated with high risks of adverse effects; however, clinical details regarding the long term outcomes of cytotoxic therapy for non-malignant diseases are not documented yet. To clarify the endocrinological consequences of pediatric HSCT for non-malignant disease patients, we conducted a retrospective analysis. From 1983 to 2014, 75 patients that underwent HSCT for non-malignant diseases were selected for this study. Of these, 23 patients (19 men, 4 women) were continuously followed up in our institute, with regular health check-ups for late effects. Based on a multiple linear regression analysis, the glucocorticoid treatment duration for chronic graft-versus-host disease (cGVHD) and the conditioning regimen were found to be independent predictors of growth retardation. All four female patients developed hypogonadism, and required hormone replacement therapy. The conditioning regimen for the four female patients with hypogonadism was based on the use of alkylating agents, and two female patients were treated with a reduced-intensity conditioning (RIC) regimen. Our study revealed that even the RIC regimen was toxic for the gonads in female patients, and that the survivors of both non-malignant and malignant diseases should be followed up carefully after pediatric HSCT.

Key words: gonadal failure, chronic graft-versus-host disease (cGVHD), reduced-intensity conditioning regimen, immunodeficiency, hematopoietic stem cell transplantation (HSCT)
**Introduction**

Hematopoietic stem cell transplantation (HSCT) is crucial for treating pediatric patients with blood- or bone marrow-related malignant diseases; due to technical advances in HSCT, the number of survivors who have undergone HSCT has been increasing rapidly (1–3). Since HSCT involves a conditioning therapy with intensive chemotherapy and/or radiation, many survivors have been revealed to show long-term consequences resulting from the therapy (3, 4). These consequences seriously affect the quality of life of the patients, and are considered one of the major medical concerns of the therapy. Thus, pediatric malignant disease patients have been called childhood cancer survivors (CCS), and regular examinations of the physical and psychological conditions of these patients are recommended (5).

HSCT is a vital option as a curative therapy for not only pediatric malignancies, but also for non-malignant and other related pediatric disorders, such as immunodeficiency syndromes, hemophagocytic lymph histiocytosis (HLH), and storage-related diseases (6). Especially, HSCT is a standard approach for treating infants with notable immunodeficiency syndromes, since poor prognosis is likely without appropriate intervention (7). Alkylating agents are key drugs used in the conditioning regimens for HSCT; however, the long-term consequences of these treatments have not yet been fully documented.

In the present study, we aimed to clarify the clinical details of the long-term endocrinological consequences of HSCT for non-malignant pediatric diseases; this would provide valuable insights into planning HSCT procedures for children with non-malignant diseases.

**Materials and Methods**

The present study was approved by the ethical committee of the Tokyo Medical and Dental University (TMDU).

From 1983 to 2014, 153 patients had undergone HSCT at our institute, and in 75 (63 male, 12 female) of those patients, the basal condition was a non-malignant disease or another related disease. We excluded 51 subjects who died during the observation; they had been transferred to other hospitals and discontinued visiting our hospital. Twenty-four subjects were regularly followed up at our institute for at least 2 yr. One patient was excluded because of his age at HSCT (> 20 yr old). Further, 23 patients (19 male, 4 female) were deemed to be eligible for the present study (Table 1).

The age of the subjects ranged from 2–43 yr, and their age at HSCT ranged from 3 mo–19 yr (median: 3 yr old). The underlying non-malignant diseases of the patients were as follows: severe combined immunodeficiency (SCID) (n = 4), severe congenital neutropenia (SCN) (n = 3), Wiskott-Aldrich Syndrome (WAS) (n = 4), X-linked hyper IgM syndrome (XHIM) (n = 4), aplastic anemia (AA) (n = 3), ectodermal dysplasia with immunodeficiency (EAD-ID) (n = 2), chronic mucocutaneous candidiasis (CMC) (n = 1), PIK3CD mutation (PIK3CD) (n = 1), and hemophagocytic syndrome (HPS) (n = 1) (Table 1).

We regularly checked the presence of chronic graft-versus-host disease (cGVHD), anthropometric data of height and weight, and blood pressure, and examined the endocrinological profiles and glucose and lipid metabolic profiles, i.e., thyroid function (TSH, fT3, fT4, and Tg), adrenal function (ACTH, cortisol, DHEA-S), gonadal function (LH, FSH, and testosterone or estradiol), IGF-1, PRL, HbA1c, fasting plasma glucose, T-chol, TG, and LDL cholesterol. The criteria for hypogonadism in our present study were as follows: a serum testosterone level of less than 3 ng/ml in men over the age of 14 or a basal serum FSH level of more than 8 IU/l in women of all ages.

In the present study, when the conditioning therapy included at least one of the following protocols: 1) TBI exceeding 5 Gy at once or 8
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Gy via divided irradiation, 2) busulfan dose exceeding 9 mg/kg in total, and 3) L-PAM dose exceeding 140 mg/m\(^2\) in total, we defined the regimen as myelo-ablative conditioning (MAC) regimen. Other conditioning regimens were considered reduced-intensity conditioning (RIC) regimens.

For GVHD, we used a standardized protocol of glucocorticoid therapy. First, 1 mg/kg/d of prednisolone was administered to initiate the therapy. The dosage was increased to up to 2 mg/kg/d if necessary. When the symptoms and/or signs of GVHD were attenuated, we discontinued the treatment after reducing the dosage by 10% every week.

For statistical analysis, we used the JMP13 software (SAS institute Inc.). For assessing the relationship between the risk factors (non-contiguous data) and contiguous data, Wilcoxon analysis was performed (Fig. 1a, c, and d). For comparing the differences between two sets of contiguous data, we performed Pearson’s correlation analysis (Fig. 1b). In the multiple linear regression analysis shown in Table 2, \( p \) values less than 0.05 were considered statistically significant.

### Results

#### Most subjects were male

X-linked congenital diseases, such as SCID, WAS, and XHIM, were major causes that

| No | Disorders | Conditioning | Sex | Age | After HSCT (yrs) | Height (SDS) | T (ng/ml) or E2 (pg/ml) | LH (mIU/ml) | FSH (mIU/ml) |
|----|-----------|--------------|-----|-----|----------------|-------------|-------------------------|-------------|--------------|
| 1  | SCID      | FLU + BU     | RIC | M   | 4 yr          | 3 mo        | 4                       | -0.66       | 0.04         | 0.4           | 2            |
| 2  | SCID      | FLU + BU     | RIC | M   | 5 yr          | 7 mo        | 5                       | -1.56       | 0.04         | <0.2          | <1.0         |
| 3  | SCID      | FLU + L-PAM  | MAC | M   | 8 yr          | 1 yr 1 mo   | 7                       | 0.25        | 0.04         | <0.2          | 1.5          |
| 4  | SCID      | FLU + BU     | RIC | M   | 3 yr          | 10 mo       | 2                       | -1.12       | 0.04         | 0.3           | 1.2          |
| 5  | SCN       | FLU + BU + ATG/FLU + CY | RIC | M   | 5 yr          | 2 yr        | 3                       | -3.1        | 0.04         | <0.2          | 2            |
| 6  | SCN       | TBI + FLU + CY | RIC | M   | 7 yr          | 2 yr        | 5                       | 1.28        | 0.04         | <0.2          | 1.6          |
| 7  | SCN       | FLU + BU     | MAC | F   | 12 yr         | 10 yr       | 2                       | 0.38        | <5           | 59.9          | 159.5        |
| 8  | WAS       | Unknown -    | M   | 35 yr | 19 yr       | 16          | NA                      | 9.59        | 2            | 3.1           |
| 9  | WAS       | BU + CY + ATG | MAC | M   | 5 yr          | 1 yr 1 mo   | 4                       | -4.9        | 0.04         | <0.2          | 2.1          |
| 10 | WAS       | BU + CY + ATG | MAC | M   | 12 yr         | 1 yr 5 mo   | 11                      | -5.13       | 0.04         | 0.2           | 2.8          |
| 11 | WAS       | BU + CY     | MAC | M   | 2 yr          | 5 mo        | 2                       | -2.69       | 0.04         | 0.3           | 1.1          |
| 12 | XHIM      | BU + CY     | MAC | M   | 9 yr          | 5 yr        | 4                       | -0.92       | 0.04         | <0.2          | <1.0         |
| 13 | XHIM      | BU + CY     | MAC | M   | 20 yr         | 3 yr        | 17                      | -0.22       | 6.38         | 5.5           | 20.2         |
| 14 | XHIM      | Unknown -   | M   | 19 yr | 3 yr        | 16          | -0.93                   | 4.15        | 8.1          | 14.1         |
| 15 | XHIM      | BU + CY     | MAC | M   | 17 yr         | 3 yr        | 14                      | -0.29       | 5.52         | 2.4           | 8.1          |
| 16 | AA        | TBI + FLU + CY + ATG | RIC | M   | 15 yr         | 12 yr       | 3                       | -1.21       | 7.25         | 2             | 1.7          |
| 17 | AA        | FLU + L-PAM + ATG | MAC | M   | 15 yr         | 9 yr        | 6                       | -0.38       | 5.57         | 3.62          | 40.9         |
| 18 | AA        | CY + TLI   | RIC | F   | 43 yr         | 14 yr       | 29                      | 0.7         | <5           | <0.2          | 1.4          |
| 19 | EDA-ID    | FLU + L-PAM + ATG | MAC | M   | 6 yr          | 1 yr 3 mo   | 5                       | 0.57        | 0.04         | <0.2          | 1.5          |
| 20 | EDA-ID    | FLU + BU + ATG | MAC | M   | 15 yr         | 12 yr       | 3                       | -2.96       | 8.11         | 2.2           | 6.3          |
| 21 | CMC       | FLU + BU + ATG | RIC | F   | 16 yr         | 12 yr       | 4                       | -1.39       | <5           | 17.5          | 69           |
| 22 | PIK3CD    | FLU + BU + ATG / FLU | RIC | M   | 11 yr         | 8 yr /9 yr  | 2                       | -0.62       | 0.04         | <0.2          | 2.4          |
| 23 | HPS       | BU + CY     | MAC | F   | 13 yr         | 1 yr        | 12                      | 0.11        | <5           | 34.4          | 148          |

HSCT: hematopoietic stem cell transplantation, SCID: severe combined immunodeficiency, SCN: severe congenital neutropenia, WAS: Wiskott-Aldrich Syndrome, XHIM: X-linked hyper IgM syndrome, AA: aplastic anemia, EDA-ID: ectodermal dysplasia with immunodeficiency, CMC: chronic mucocutaneous candidiasis, PIK3CD: PIK3CD mutation, HPS: hemophagocytic syndrome, ATG: Anti-thymocyte globulin, BU: busulfan, CY: cyclophosphamide, Flu: Fludarabine, L-PAM: melphalan, TBI: total body irradiation, TLI: total lymphoid irradiation, RIC: reduced-intensity conditioning, MAC: myelo-ablative conditioning.
necessitated HSCT; therefore, 19 out of 23 patients in our study were men. Most of the conditioning regimens (18/21; 86%) were based on the use of alkylating antineoplastic agents, since this is the most commonly used regimen in both reduced-intensity and myeloablative transplant procedures for treating immune-deficiency. The RIC regimen was used for nine patients (#1, #2, #4, #5, #6, #16, #18, #21, and #22). There were no patients whose data suggested hypothyroidism, adrenal insufficiency, diabetes mellitus, and GH deficiency. Two patients (#20 and #22) developed hypercholesterolemia, requiring treatment with antihyperlipidemic agents.
The potential risk of MAC for growth retardation

The thyroid function and the serum level of IGF-1 of the patients were within the normal range for their age and sex, suggesting that the growth retardation observed in our subjects was due to factors other than endocrinopathy. The durations of the glucocorticoid therapy for the subjects with cGVHD were significantly longer than those for subjects without cGVHD (Fig. 1a) and negatively associated with the changes in height standard deviation score (ΔHtSDS) ($R = -0.62, p$-value = 0.005) (Fig. 1b), suggesting that prolonged glucocorticoid therapy is a risk for growth retardation. Compared to the subjects treated with the RIC regimen, the ΔHtSDS was significantly reduced in those treated with the MAC regimen (Fig. 1c, $p$-value = 0.012), suggesting the MAC regimen may be a potential risk for reducing the ΔHtSDS after HSCT. In the MAC treatment group, two subjects received prolonged glucocorticoid therapy for more than two years. However, even after excluding these two subjects from the analysis, a significant difference in the ΔHtSDS was obtained (data not shown). Furthermore, we observed no significant difference between the durations of glucocorticoid therapy in the patients subjected to the two conditioning regimens (RIC and MAC) (Fig. 1d). These data suggest that in addition to prolonged glucocorticoid therapy, the MAC regimen is a factor that affects the growth of patients after HSCT.

To examine whether the duration of the glucocorticoid therapy and the conditioning regimen are independently associated with the growth retardation observed after HSCT, we performed a multiple linear regression analysis (Table 2), which revealed that these two factors are independent predictors of ΔHtSDS after HSCT.

High prevalence of primary gonadal failure in female subjects

The serum levels of testosterone were examined in seven post-pubertal male subjects (#8, #13–#17, and #20), and no abnormal findings were identified. In contrast, all post-pubertal female patients (#7, #18, #21, and #23) developed hypergonadotropic hypogonadism, requiring hormone replacement therapy (HRT) (Table 3). The conditioning regimens for all female patients were based on the use of alkylating antineoplastic agents, which are toxic to ovarian tissues; two out of four female patients (#18 and #21) were treated with the RIC regimen. Except in the case for patient #18, whose endocrinological data were obtained

### Table 3. Profiles of female subjects with gonadal failure

| Case No. | Conditioning | Radiation | Age | Sex | Tanner stage | LH (mIU/ml) | FSH (mIU/ml) | E2 (pg/ml) | AMH | Note |
|----------|--------------|-----------|-----|-----|-------------|-------------|-------------|-----------|-----|------|
| 7        | MAC          | –         | 12 yr | F   | B2-3/PH3    | 59.9        | 159.5       | < 5       | undetectable | HRT since 20 yr |
| 18       | RIC          | –         | 41 yr | F   | No data    | < 0.2*      | 1.4*        | < 5*      | undetectable |                 |
| 21       | RIC          | –         | 14 yr | F   | No data    | 17.5        | 69          | < 5       | No data       |                 |
| 23       | MAC          | –         | 11 yr | F   | B1         | 34.4        | 148         | < 5       | undetectable |                 |

* Data were obtained after the introduction of hormone replacement therapy (HRT). RIC: reduced-intensity conditioning, MAC: myelo-ablative conditioning, E2: estradiol, AMH: anti-Müllerian Hormone.
after the introduction of HRT, the profiles for all other patients suggested that the female patients developed primary ovarian failure, with markedly elevated gonadotropin levels and reduced anti-Müllerian hormone (AMH) and estradiol (E2) levels.

**Discussion**

Our study revealed two clinical points. First, the reduction of the ΔHtSDS is a possible complication of HSCT, and is caused by prolonged glucocorticoid therapy for cGVHD and the MAC regimen, independently. Second, gonadal failure is highly prevalent among female patients after HSCT. Therefore, this necessitates the need for HRT to induce pubertal development.

As observed after HSCT for malignant diseases (8–15), our study revealed that the intensity of the conditioning regimen affects the growth of patients with non-malignant diseases after HSCT. Due to the limited number of the subjects, precise analyses were not performed; however, we speculate that TBI and chemotherapy, including CY or the combination of BU and CY, could affect growth in a dose-dependent manner. For elucidating the details of the pathophysiology of growth retardation without endocrine disorders after HSCT, an accumulation of cases is essential.

Our study revealed that primary ovarian failure would be a major complication after pediatric HSCT for non-malignant diseases. Alkylating agents, including busulfan, have been reported to impair the ovarian follicles (16–19), and the recovery of gonadal function has been reported to be extremely rare (9, 18). Although the RIC regimen was used for patients #18 and #21 in our study, both patients developed ovarian failure, suggesting that even reduced dosages of alkylating agents would still have severe toxicity to the ovaries. The age at which the patients with ovarian failure underwent HSCT ranged from 1–14 yr old, suggesting that the toxicity of alkylating agents could affect the ovaries at any age.

Unlike the case in male patients, the high prevalence of gonadal failure in the female subjects is explained by the different mechanisms underlying the development of the ovaries and testes. In the ovaries, the follicular structures, including oocytes, are essential for the differentiation and maintenance of the granulosa and theca cells, which are the steroidogenic cells in the ovaries. Therefore, oocyte depletion due to alkylating agents subsequently causes the loss of steroidogenic cells, resulting in primary ovarian failure. In contrast, Leydig cells, the steroidogenic cells in the testes, are viable without the presence of germ cells. Therefore, androgen synthesis in male patients is maintained even after the depletion of germ cells caused by the exposure to alkylating agents.

The above findings show that the normal synthesis of androgen does not indicate the presence of germ cells in the testes, i.e., even developing secondary sexual characteristics, future fertility of the male subjects is not warranted. Indeed, in patients #13, #14, and #17, the serum levels of FSH were higher than the normal range, and we cannot exclude the possibilities that these subjects will show impaired spermatogenesis in the future. It has been reported that the administration of alkylating agents seems to involve a higher risk of infertility in males than in females (20). Although the ultimate effect of busulfan on male fertility is currently not known, careful evaluation of reproductive prognosis in male and female patients is essential.

Even after conditioning using the RIC regimen, fertility preservation would be beneficial for all pediatric patients before HSCT. However, most patients with non-malignant diseases receive HSCT before they attain the reproductive age, resulting in limited options for the preservation of fertility, such as the cryopreservation of gonadal tissues. However, at present, cryopreservation of gonadal tissues is considered an experimental technique (21,
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22), and this procedure may only be utilized for carefully selected patients as an experimental protocol.

Study Limitations

Our present study is based on a small number of the subjects, retrospective analysis, and short-term observations; additionally, careful interpretation of the data is required. However, our study revealed that HSCT for non-malignant patients, even those conditioned with RIC regimens, potentially caused late effects, especially hypogonadism in female patients. To further clarify the details of these late effects, studies involving a large number of cases are essential.

Conclusion

In conclusion, our study revealed the high prevalence of ovarian failure and growth retardation due to long-term glucocorticoid therapy after HSCT and treatment with the MAC regimen for non-malignant diseases. In addition to HSCT for malignant and related diseases, the pediatric patients receiving HSCT for non-malignant diseases should be followed up carefully, and careful consideration for preserving fertility before HSCT is essential.

Conflict of interest: The authors report no conflict of interest.

References

1. Hasle H. A critical review of which children with acute myeloid leukaemia need stem cell procedures. Br J Haematol 2014;166: 23–33. [Medline] [CrossRef]
2. Möricke A, Reiter A, Zimmermann M, Gadner H, Stanulla M, Dördelmann M, et al. German-Austrian-Swiss ALL-BFM Study Group. Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. Blood 2008;111: 4477–89. [Medline] [CrossRef]
3. Hashmi S, Carpenter P, Khera N, Tichelli A, Savani BN. Lost in transition: the essential need for long-term follow-up clinic for blood and marrow transplantation survivors. Biol Blood Marrow Transplant 2015;21: 225–32. [Medline] [CrossRef]
4. Clark CA, Savani M, Mohty M, Savani BN. What do we need to know about allogeneic hematopoietic stem cell transplant survivors? Bone Marrow Transplant 2016;51: 1025–31. [Medline] [CrossRef]
5. American Academy of Pediatrics Section on Hematology/Oncology Children’s Oncology Group. Long-term follow-up care for pediatric cancer survivors. Pediatrics 2009;123: 906–15. [Medline] [CrossRef]
6. Sullivan KM, Parkman R, Walters MC. Bone Marrow Transplantation for Non-Malignant Disease. Hematology (Am Soc Hematol Educ Program) 2000;2000: 319–38. [Medline] [CrossRef]
7. Kang E, Gennery A. Hematopoietic stem cell transplantation for primary immunodeficiencies. Hematol Oncol Clin North Am 2014;28: 1157–38. [Medline] [CrossRef]
8. Cohen A, Rovelli A, Bakker B, Uderzo C, van Lint MT, Esperou H, et al. Final height of patients who underwent bone marrow transplantation for hematological disorders during childhood: a study by the Working Party for Late Effects-EBMT. Blood 1999;93: 4109–15. [Medline] [CrossRef]
9. Cohen A, Békássy AN, Gaiero A, Faraci M, Zecca S, Tichelli A, et al. EBMT Paediatric and Late Effects Working Parties. Endocrinological late complications after hematopoietic SCT in children. Bone Marrow Transplant 2008;41(Suppl 2): S43–8. [Medline] [CrossRef]
10. Sklar C, Boulad F, Small T, Kernan N. Endocrine complications of pediatric stem cell transplantation. Front Biosci 2001;6: G17–22. [Medline] [CrossRef]
11. Myers KC, Howell JC, Wallace G, Dandoy C, El-Bietar J, Lane A, et al. Poor growth, thyroid dysfunction and vitamin D deficiency
remain prevalent despite reduced intensity chemotherapy for hematopoietic stem cell transplantation in children and young adults. Bone Marrow Transplant 2016;51: 980–4. [Medline] [CrossRef]

12. Clement-De Boers A, Oostdijk W, Van Weel-Sipman MH, Van den Broeck J, Wit JM, Vossen JM. Final height and hormonal function after bone marrow transplantation in children. J Pediatr 1996;129: 544–50. [Medline] [CrossRef]

13. Afify Z, Shaw PJ, Clavano-Harding A, Cowell CT. Growth and endocrine function in children with acute myeloid leukaemia after bone marrow transplantation using busulfan/cyclophosphamide. Bone Marrow Transplant 2000;25: 1087–92. [Medline] [CrossRef]

14. Wingard JR, Vogelsang GB, Deeg HJ. Stem cell transplantation: supportive care and long-term complications. Hematology (Am Soc Hematol Educ Program) 2002;2002: 422–44. [Medline] [CrossRef]

15. Wei C, Albanese A. Endocrine disorders in childhood cancer survivors treated with haemopoietic stem cell transplantation. Children (Basel) 2014;1: 48–62. [Medline]

16. Fallat ME, Hutter J, American Academy of Pediatrics Committee on Bioethics American Academy of Pediatrics Section on Hematology/Oncology American Academy of Pediatrics Section on Surgery. Preservation of fertility in pediatric and adolescent patients with cancer. Pediatrics 2008;121: e1461–9. [Medline] [CrossRef]

17. Teinturier C, Hartmann O, Valteau-Couanet D, Benhamou E, Bougneres PF. Ovarian function after autologous bone marrow transplantation in childhood: high-dose busulfan is a major cause of ovarian failure. Bone Marrow Transplant 1998;22: 989–94. [Medline] [CrossRef]

18. Sanders JE, Hawley J, Levy W, Gooley T, Buckner CD, Deeg HJ, et al. Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. Blood 1996;87: 3045–52. [Medline]

19. Grigg AP, McLachlan R, Zaja J, Szer J. Reproductive status in long-term bone marrow transplant survivors receiving busulfan-cyclophosphamide (120 mg/kg). Bone Marrow Transplant 2000;26: 1089–95. [Medline] [CrossRef]

20. Byrne J, Mulvihill JJ, Myers MH, Connelly RR, Naughton MD, Krauss MR, et al. Effects of treatment on fertility in long-term survivors of childhood or adolescent cancer. N Engl J Med 1987;317: 1315–21. [Medline] [CrossRef]

21. Practice Committee of American Society for Reproductive Medicine. Ovarian tissue cryopreservation: a committee opinion. Fertil Steril 2014;101: 1237–43. [Medline] [CrossRef]

22. Onofre J, Baert Y, Faes K, Goossens E. Cryopreservation of testicular tissue or testicular cell suspensions: a pivotal step in fertility preservation. Hum Reprod Update 2016;22: 744–61. [Medline] [CrossRef]