Title page

Title:

Ovarian function after allogeneic hematopoietic stem cell transplantation in children and young adults given 8-Gy total body irradiation-based reduced-toxicity myeloablative conditioning

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Running head: Ovarian function after 8-Gy TBI-based HSCT

Abbreviations: ALL, acute lymphoblastic leukemia; BU, busulfan; CY, cyclophosphamide; FLAG-IDA, fludarabine, cytosine arabinoside, idarubicin and granulocyte colony-stimulating factor; FLU, fludarabine; FSH, follicle-stimulating hormone; GvHD, graft versus host disease; HRT, hormone replacement therapy; HSCT, hematopoietic stem cell transplantation; LH, luteinizing hormone; MAC, myeloablative conditioning; POI, primary ovarian insufficiency; RIC, reduced-intensity conditioning; RRT, regimen-related-toxicity; RTMAC, reduced-toxicity myeloablative conditioning; TBI, total body irradiation.

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AUTHOR CONTRIBUTIONS

Y.N. and K.H. designed the study. S.S., D.M., T.K., M.T., R.Y., K.S., K.K., K.K., K.H., and Y.N. collected the data. K.K., and K.H. analyzed the data. K.K., K.H., and Y.N. wrote the paper. K.K. and K.H. are co-first authors of this paper. All authors critically reviewed the paper and approved the final version.

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Abstract

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Background: The spectrum of late sequelae after hematopoietic stem cell transplantation (HSCT) includes infertility, which is the most frequent complication. Some reports suggested that ovarian function may be better preserved in females undergoing HSCT with reduced-intensity conditioning (RIC) than with conventional myeloablative conditioning (MAC). However, the impact of HSCT after 8-Gy based reduced-toxicity MAC (RTMAC), whose efficacy is between those of conventional MAC and RIC, on ovarian function remains unclear.

Procedure: A single-center retrospective analysis of data derived from patient

information for all the children who underwent transplantation at the Shinshu University Hospital was carried out. Patients who underwent 8-Gy total body irradiation (TBI-)-based RTMAC before HSCT were analyzed.

Results: A total of 36% (5 of 14) of the patients developed primary ovarian insufficiency (POI) during the observation period, but serum follicle-stimulating hormone (FSH) levels reduced to normal range with spontaneous menstruation in two, implying the reversal of POI. Furthermore, only one (10%) of the 10 pre-pubertal patients (71%; 10/14) at the time of HSCT suffered from POI at the last observation, but all three post-pubertal patients developed POI (100%), and two (67%) continued to suffer from POI at the last observation.

Conclusions: Taken together, 8-Gy TBI-based RTMAC before HSCT may decrease the possibility of POI compared with conventional MAC, especially in pre-pubertal patients. A longer follow-up will be required to ascertain whether a normal pregnancy and delivery can occur in such patients.

Keywords: POI, HSCT, hematological malignancies, 8-Gy TBI, children

1 | INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is an important curative treatment strategy for children with high-risk hematological malignancies. However, many patients who have undergone HSCT during childhood suffer from poor quality of life in the long-term, and the reported increasing incidence of late effects is associated with the improvement in survival rates.[1,2] The spectrum of late sequelae after HSCT affects a variety of organs, and infertility is the most frequent complication.[3,4] In long-term female survivors, recovery of ovarian function is observed in only 1% of the patients who have received high-dose busulfan (BU), whereas this number is 10%–15% in those who receive total body irradiation (TBI).[5-7]

Primary ovarian insufficiency (POI), irrespective of whether it is caused by HSCT, is also referred to as premature ovarian insufficiency, premature ovarian failure, or premature menopause, and is associated with not only infertility but also other sequelae, such as osteoporosis, cardiovascular disorders, impaired psycho-social well-being, and compromised sexual health, which are secondary to estrogen

deficiency.[8,9]

Reduced-intensity conditioning (RIC) refers to non-myeloablative conditioning that involves lower doses of chemotherapy and/or radiotherapy to reduce short-term toxicities and permit transplantation in patients who cannot undergo conventional myeloablative conditioning (MAC). Published studies on RIC before HSCT in children predominantly dealt with nonmalignant diseases;[10] however, some reports suggest that ovarian function may be better preserved in females undergoing HSCT with RIC compared to those undergoing conventional MAC,[11-14] implying that RIC is an important option for mitigating POI after HSCT. However, as data on the usefulness of RIC in children with hematological malignancies is scarce, HSCT after conventional MAC continues to be the standard therapy.

The need to reduce ovarian toxicity in children with hematological malignancies is self-evident, and RIC regimens can help reduce ovarian toxicity. Therefore, we have previously developed (in 2003) a treatment protocol, which aimed at reducing regimen-related-toxicity (RRT) while maintaining anti-tumor effect(s). Specifically,

this protocol uses a reduced-toxicity myeloablative conditioning (RTMAC) regimen consisting of 8-Gy TBI (2 Gy/day, days −7 to −4), fludarabine (FLU) (30 mg/m^2/day, days −8 to −4) and cyclophosphamide (CY) (60 mg/kg/day, days −3 and −2) for pediatric hematological malignancies, which showed low toxicity during the early post-transplant period (until day 100) and a high probability of survival in the medium-term after the transplant.[15,16] Even though previous reports state that exposure to high-dose CY, BU, or TBI is associated with gonadal failure,[17-19] these studies have used conventional MAC (12-Gy of TBI or 16mg/kg of BU), and the impact of HSCT after 8-Gy based RTMAC on ovarian function is not clear. As the median follow-up period for our initial study has exceeded 12 years, we here report the impact of HSCT after 8-Gy TBI-based RTMAC on ovarian function.

2 | METHODS

2.1 | Study design and settings

This study was approved by the ethical committee of the Shinshu University School of Medicine (title: Single-center retrospective analysis of ovarian function after allogeneic HSCT in children and young adults, approval number: 3650), and written informed consent was obtained from all patients and/or their guardians. We performed a single-center retrospective analysis of data derived from patient information until November 30, 2018, for all children who underwent transplantation. Of them, patients who underwent 8-Gy TBI-based RTMAC before HSCT from March 2003 to March 2015 were analyzed. Data collection was updated as of November 30, 2018. In principle, we applied our RTMAC conditioning to all patients with hematological malignancies, except for patients with non-complete remission acute lymphoblastic leukemia (ALL). Some patients who were eligible per our criteria did not undergo our RTMAC conditioning owing to the patient's guardians' request or enrollment into another study. Detailed clarification is included in our previous report.[15] All patients underwent HSCT at

Shinshu University Hospital, and all clinical data were collected from the clinical charts of Shinshu University Hospital.

2.2 | Patients

The study subjects comprised a series of 14 female patients with hematological malignancies who had undergone a RTMAC regimen consisting of 8-Gy TBI/FLU/CY followed by HSCT at the Shinshu University Hospital. The exclusion criteria were as follows: (1) history of radiation therapy before HSCT; (2) undergone repeat HSCT using TBI or alkylating agents after the first HSCT; (3) patients aged <13 years as of November 30, 2018 [20]; (4) and died before the last follow-up.

2.3 | Conditioning regimen and additional cranio-spinal irradiation

The preparative conditioning regimen consisted of 8-Gy TBI (2-Gy/day, days −7 to −4), FLU (30 mg/m^2/day, days −8 to −4) and CY (60 mg/kg/day, days −3 and −2).

None of the patients received antithymocyte/antilymphocyte globulin in order not to decrease the graft-versus-leukemia effect. After HSCT, patients with central nervous system disease underwent additional cranio-spinal irradiation (brain, 10-Gy; spinal, 7-Gy). Details regarding graft versus host disease (GvHD) prophylaxis and supportive care before/after HSCT have been reported elsewhere.[15,16]

2.4 | Data collection and gonadotropin assay

Relevant medical history including diagnosis, age at diagnosis, anamnesis, age at HSCT, treatment received, donor information, grades of acute/chronic GvHD, RRT using Common Terminology Criteria for Adverse Events version 4.0, anthropometric data, pubertal status using Tanner stages, menstrual history, use of hormone replacement therapy (HRT), late effects, and laboratory outcomes including serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were obtained. The FSH and LH levels were measured at random regardless of the stage of menstruation and were quantified using electro-

chemiluminescence immunoassay (Cobas e411 immunoanalyzer, Roche Diagnostics, Mannheim, Germany).

2.5 | Classification of pubertal stage

We used a combination of the Tanner stages and menarche to classify pubertal stage, as follows: (i) pre-pubertal, defined as Tanner breast stage B I; (ii) pubertal, defined as Tanner breast stage B II, B III, or B IV; or (iii) post-pubertal, defined as Tanner breast stage B V and/or menarche.

2.6 | Definition of POI and suspected POI

POI was defined as amenorrhea with elevated serum FSH levels (>30 mIU/mL).[21-24] Amenorrhea was defined as the absence of menstrual cycles for ≥4 months or primary amenorrhea by age 16 years.[9] POI in patients who had not achieved menarche before HSCT and were less than 16 years old at last observation was defined as delayed puberty with elevated FSH levels (>30 mIU/mL). Delayed

puberty was defined as the absence of initiation of puberty (Tanner stage B II development) in girls aged ≥13 years and the absence of pubertal progress for >12 months.[9] Suspected POI was defined as having at least one of two POI criteria, viz., amenorrhea/delayed puberty or elevated serum FSH levels (>30 mIU/mL).[9] Restoration of ovarian function was defined as unassisted conception and/or resumption of menstrual cycles (at least two consecutive episodes) without HRT along with normal FSH levels (<12 IU/l) after the diagnosis of POI or suspected POI.[25] The POI decision was put on hold for patients who had regular menstruation cycle with slightly elevated FSH (12 mIU/mL to 30 mIU/mL).

3 | RESULTS

3.1 | Characteristics of the patients

In total, 19 patients who received HSCT after RTMAC, 14 met our eligibility criteria (Table1) and the diagnoses in these patients were ALL (n = 4), acute myeloid leukemia (n = 6), myelodysplastic syndrome (n = 1), juvenile myelomonocytic

leukemia (n = 1), or acute leukemia of ambiguous lineage (n = 2). The median age of patients at HSCT was 9.6 years (range, 0.8–22.8 years), 8 patients received bone marrow transplantation and 6 received unrelated cord blood transplantation. None of the patients had any comorbidities/syndromes that could potentially influence the ovarian functions before HSCT. Detailed information on HSCT is summarized in Table 2. One patient with a central nervous system disease received additional cranio-spinal irradiation after HSCT (Pt. #11), and one patient with a relapse after first HSCT received second HSCT (Pt. #7). Acute GvHD, grades I, II and III was observed in five, four, and two patients, respectively, while limited and extensive chronic GvHD were observed in two and four patients, respectively.

3.2 | Gonadal status

The median follow-up period of after HSCT was 12.2 years (range, 3.7–15.7 years); 5 patients developed overt POI during the follow-up, although the ovarian

insufficiency spontaneously recovered in 2 of them; therefore, 3/14 patients had persistent clinical/biochemical signs consistent with POI at last clinical follow-up (Table 3). At the time of HSCT, 10 patients were pre-pubertal, 1 was pubertal, and 3 were post-pubertal. Figure 1 shows the clinical course of patients with POI and suspected POI, wherein 2 of the 5 POI patients (#1 and #6) showed restoration of ovarian function as their serum FSH levels had normalized, and they had started having spontaneous menstruation during the follow-up period. However, the other 3 patients (Pt. #4, #13, and #14) continued HRT. Pt. #1 received Kaufmann therapy from age 17.3 to 20.8 years, and consequently, started menstruating; importantly, the cessation of Kaufmann therapy did not affect the menstruation cycle in this patient. Pt. #7 underwent a second HSCT 1.4 years after the first HSCT; the second HSCT used the FLAG-IDA regimen (FLU, cytosine arabinoside, idarubicin and granulocyte colony-stimulating factor) without either TBI or BU, and the patient reached menarche 2.4 years after the second HSCT. Three patients were post-pubertal at HSCT (Pt. #6, #13, and #14) and all of them developed POI, but only

one (Pt. #6) recovered ovarian function. At last follow-up, three patients (Pt. #4, #13, and #14) continued to suffer from POI. Figure 2 shows the clinical course of LH/FSH ratios. Starting ages of LH/FSH ratio elevation in patients with either POI or suspected POI were higher than in those without POI.

4 | DISCUSSION

This study describes the natural history of gonadal function in 14 consecutive pubertal or young adult female patients after they had undergone 8-Gy TBI-based RTMAC and HSCT for hematological malignancies. The novel aspects and strengths of this study are the uniformity and moderate intensity of the conditioning process.

Previous studies have reported that recovery of ovarian function was observed in only 1% of the patients who received high-dose BU, whereas 10%–15% of those who received TBI regained ovarian function.[5-7] These results are predominantly based on HSCTs after conventional MAC, i.e., 12-Gy TBI and high-dose CY or

16mg/kg of BU. Recently, few studies have reported better preservation of ovarian function in females undergoing HSCT after RIC (non-myeloablative conditioning) compared to those undergoing conventional MAC.[11-14] However, the course of ovarian function in patients who undergo HSCT after RTMAC, which is in-between conventional MAC and RIC, has been scarcely described, and our study assumes importance in this scenario as it reports on the impact of HSCT after TBI-based RTMAC on ovarian function in female children, adolescents, and young adults with hematological malignancies.

Sanders *et al.* have analyzed the percentage of ovarian function recovery as a function of TBI exposure dose in adult females who underwent HSCT, and they report that the use of 12-Gy TBI-based conditioning for HSCT led to the recovery of ovarian function in 9.6% (26/270) of the patients.[5] Couto-Silva *et al.* also reported that 11% (2/18) of the patients recovered ovarian function after HSCT after being subjected to a 12-Gy TBI regimen during childhood.[26] In our study, 36% (5/14) of the patients developed POI during the observation period, but serum FSH levels

decreased to normal with spontaneous menstruation in two patients, implying the reversal of POI. Further, at last follow-up, 79% (11/14) patients had normal ovarian function. Our results suggest that, compared to 12-Gy TBI-based conventional MAC, 8-Gy TBI-based conditioning leads to lower ovarian toxicity and better preservation of ovarian function in females. The favorable results reported here may also be related to pubertal status at HSCT, as it is known that the risk of ovarian dysfunction increases with age and pubertal status at the time of radiation exposure.[20,27,28] Doses as low as 5-Gy can affect ovarian function in post-pubertal girls,[29] and doses >10-Gy confer higher risk. In pre-pubertal girls, higher radiation dose (i.e., >10-Gy) is associated with impaired ovarian function, and doses >15-Gy confer higher risk.[20] Based on these reports, it is expected that 8-Gy TBI regimen can mainly affect ovarian function in post-pubertal females but not in pre-pubertal ones. In our study, only one of the 10 patients (10%) who were pre-pubertal (71%; 10/14) at the time of HSCT suffered from POI at the last follow-up. In contrast, all the three post-pubertal patients developed POI (100%), and two of them (67%)

continued to suffer from POI. Taken together, our results are consistent with those of the previous studies.

LH/FSH ratio has been reported to be a useful examination to assess ovulation disorder, such as polycystic ovary syndrome.[30] As shown in Figure 2, starting ages of LH/FSH ratio elevation in patients with either POI or suspected POI were higher than in those without POI. However, currently, we do not have any specific conclusions about these results because of the small sample size and the short observation period. Therefore, a long-term observation and comparison with other examinations for ovulation are needed to draw conclusion about the usefulness of the LH/FSH ratio.

Chronic GvHD is also a reported risk factor for gonadal dysfunction and female infertility.[31,32] Recently, Shimoji *et al.* have demonstrated that GvHD targets the ovary and impairs ovarian function and fertility in mice by mediating donor T-cell infiltration and apoptosis of the ovarian follicle cells, leading to ovarian insufficiency and infertility.[33] Similarly, we found that 5 of the 7 patients who had POI or

suspected POI, 5 (71%) had chronic GvHD, while only one (14%) patient had chronic GvHD in the group of 7 patients with normal ovarian function. Conversely, in 6 patients with chronic GvHD, five (83%) were diagnosed with POI or suspected POI, while only 2 of the patients without chronic GvHD were diagnosed as having POI or suspected POI. Further, in these two patients, one (pt. #7) received HSCT twice, and the other (Pt. #14) was post-pubertal at the time of HSCT. At the final follow-up, there were no patients with POI or suspected POI who did not have any risk factor impairing ovarian function. Thus, these results may suggest that patients who are pre-pubertal status at HSCT and do not develop chronic GvHD have low risk of POI after 8-Gy TBI-based conditioning for HSCT. Importantly, as our results also show that 8-Gy TBI-based conditioning for HSCT does not adequately preserve ovarian function in patients who are post-pubertal at the time of HSCT; therefore, other methods should probably be considered to preserve gonadal function and fertility in these patients. In any case, further data collection is needed

to clarify the effect of chronic GvHD because the number of patients included in this study is small.

Overall, 3/5 patients (Pt. #1, #6, and #14) who received alkylating agents before HSCT developed POI, whereas only 2/9 patients (Pt. #4 and #13) who did not receive alkylating agents before HSCT conditioning developed POI. In addition, the total dose of alkylating agents, including HSCT-conditioning regimen, in patients with POI was significantly larger than in those without POI (7,362 mg/m^2 vs. 3,932 mg/m^2, p = 0.04, Mann–Whitney U- test). Chemaitilly *et al.* reported that independent risk factors for POI in childhood cancer survivors included ovarian radiotherapy and total cyclophosphamide equivalent dose ≥8,000 mg/m^2.[34] Therefore, alkylating agents may be considered as one of the gonadal toxicity factor.

Some research groups have formulated fertility preservation guidelines to improve fertility after HSCT, which recommend oocyte cryopreservation as the primary strategy to preserve fertility in patients who are post-pubertal at HSCT.[35,36] If oocyte

cryopreservation cannot be performed, then ovarian tissue cryopreservation may be attempted. However, ovarian tissue cryopreservation followed by ovarian re-transplantation in patients with hematological malignancies continues to be classified as an experimental therapy because it has been reported that xenografts of thawed human ovarian cortical tissue in mice led to the formation of leukemic tumors.[36,37] Ovarian shielding during TBI is also a viable alternative as the cumulative incidence of ovarian recovery was 68.8% at 2 years after transplantation in patients who were post-pubertal at HSCT and were provided ovarian shielding; the actual dose applied to the ovaries was 2.4–3.1-Gy.[38,39] While ovarian shielding appears to be an attractive method to preserve ovarian function; however, longer follow-up will be required to ascertain whether normal pregnancies and deliveries are possible in these patients without increasing the risk of leukemia relapse.

We could show relatively lower ovarian toxicity after 8-Gy TBI-based RTMAC in patients who were pre-pubertal. However, it is important that the patients are made

aware that their ovarian reserve may be reduced by conditioning or pre-HSCT chemotherapy and that premature menopause remains probable. Although a clear safe threshold of radiotherapy dose could not be defined,[9,20,40-42] mathematic modeling based on rates of oocyte decline predict the mean age for ovarian failure to be 26.5 and 19.7 years in patients who receive 6-Gy and 9-Gy radiation at the age of 10, respectively.[43] Moreover, because treatment with a combination of alkylating agents and radiotherapy increases the risk of POI,[28] ovarian function should be regularly monitored even in patients who do not have POI after HSCT, and oocyte cryopreservation can be considered to counter the imminent POI in patients who do not have frozen oocytes.

Recently, the anti-Müllerian hormone (AMH), which is secreted by the granulosa cells of the pre-antral and the early antral follicles, has been reported to be a marker of ovarian function and reserve after HSCT.[14,44,45] Therefore, a serum AMH assay may provide a more accurate evaluation of ovarian function in patients who have undergone HSCT.

Nevertheless, there are some limitations in our study. The first is the age at last observation, because the mean age at last observation was 19.4 years (range; 13.4–26.7 years) and none of the patients was married during the study period. A longer follow-up will be required to ascertain whether these patients can have normal pregnancies and deliveries, and if new POI occurs in the subsequent years. Second, as the number of patients who were post-pubertal at HSCT was small (n = 3) the effects of 8-Gy TBI RTMAC on the ovaries in these patients need to be verified in future studies with larger patient numbers.

In summary, 8-Gy TBI-based RTMAC before HSCT may decrease the possibility of POI compared to those undergoing conventional MAC, especially in pre-pubertal patients. A longer follow-up will be required to ascertain whether a normal pregnancy and delivery can occur in these patients and future studies are needed to clarify the impact of after 8-Gy TBI-based RTMAC in patients who are post-pubertal at HSCT.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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Legends

FIGURE 1 Clinical course of patients with POI and suspected POI after HSCT

Patient (Pt.) #1 underwent Kaufmann therapy from 17.3 to 20.8 years of age. Although Kaufmann therapy was discontinued at the age of 20.8, her menstrual cycle remained unaffected. Pt. #4 continues to receive HRT and maintains a regular menstrual cycle. Pt. #6 experienced resumption of menstruation at the age of 15.3 years, 1.8 years after HSCT. Pt. #7 underwent a second HSCT 1.4 years after first HSCT using FLAG-IDA regimen, and reached menarche at the age of 13.9 years, 2.4 years after the second HSCT. Pt. #11 experienced spontaneous menarche at the age of 14.5 years, 3.6 years after HSCT. Pt. #13 has continued to receive HRT (initiated at the age of 18.9 years), which maintains a regular menstrual cycle. Pt. #14 has continued to receive HRT from the age of 24.0 years, which maintains a regular menstrual cycle.

FIGURE 2 Clinical course of luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio after HSCT

Starting ages of LH/FSH ratio elevation in patients with either POI or suspected POI were higher than in those without POI.

TABLE 1 Patient characteristics

Pt.	Disease	Cytogenetic abnormalities	Central nervous system disease	Age at diagnosis (years)	Anamnesis	Exposure to alkylating agents before HSCT	CED (mg/m^2)	Disease status at HSCT
1	MNKL	normal karyotype	No	10.0	None	Yes	5,643	CR1
2	JMML	normal karyotype	No	0.5	Invagination	No	2,385	UPD
3	B-ALL	normal karyotype	No	3.8	Asthma	Yes	7,319	CR2
4	AML M2	t(8;21)	No	8.2	None	No	3,030	Refractory
5	ABL	MLL rearrangement	No	0.9	None	No	2,712	CR2
6	T-ALL	others	No	5.0	None	Yes	14,279	CR2
7	AML M2	t(8;21)	No	9.5	None	No	3,002	CR2
8	AML M7	monosomy 7	No	0.9	None	No	2,558	CR1
9	AML M6	trisomy 8	No	4.4	Behçet's Disease	No	2,624	CR1
10	AML M7	trisomy 8	No	3.0	None	No	2,957	CR1
11	B-ALL	normal karyotype	Yes	8.3	None	Yes	8,185	CR2
12	MDS RCMD	trisomy 8	No	10.6	Hemangioma simplex	No	3,646	UPD
13	AML M2	normal karyotype	No	15.4	Depression	No	3,959	Refractory
14	B-ALL	others	No	18.3	None	Yes	9,899	CR2

HSCT, hematopoietic stem cell transplantation; MNKL, myeloid/NK cell precursor acute leukemia; JMML, juvenile myelomonocytic leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ABL, acute biphenotypic leukemia; MDS, myelodysplastic syndrome; RCMD, refractory cytopenia with multilineage dysplasia; CR, complete response; UPD, untreated primary disease; CED, cyclophosphamide equivalent dose.

TABLE 2 Clinical information of HSCT and post-HSCT

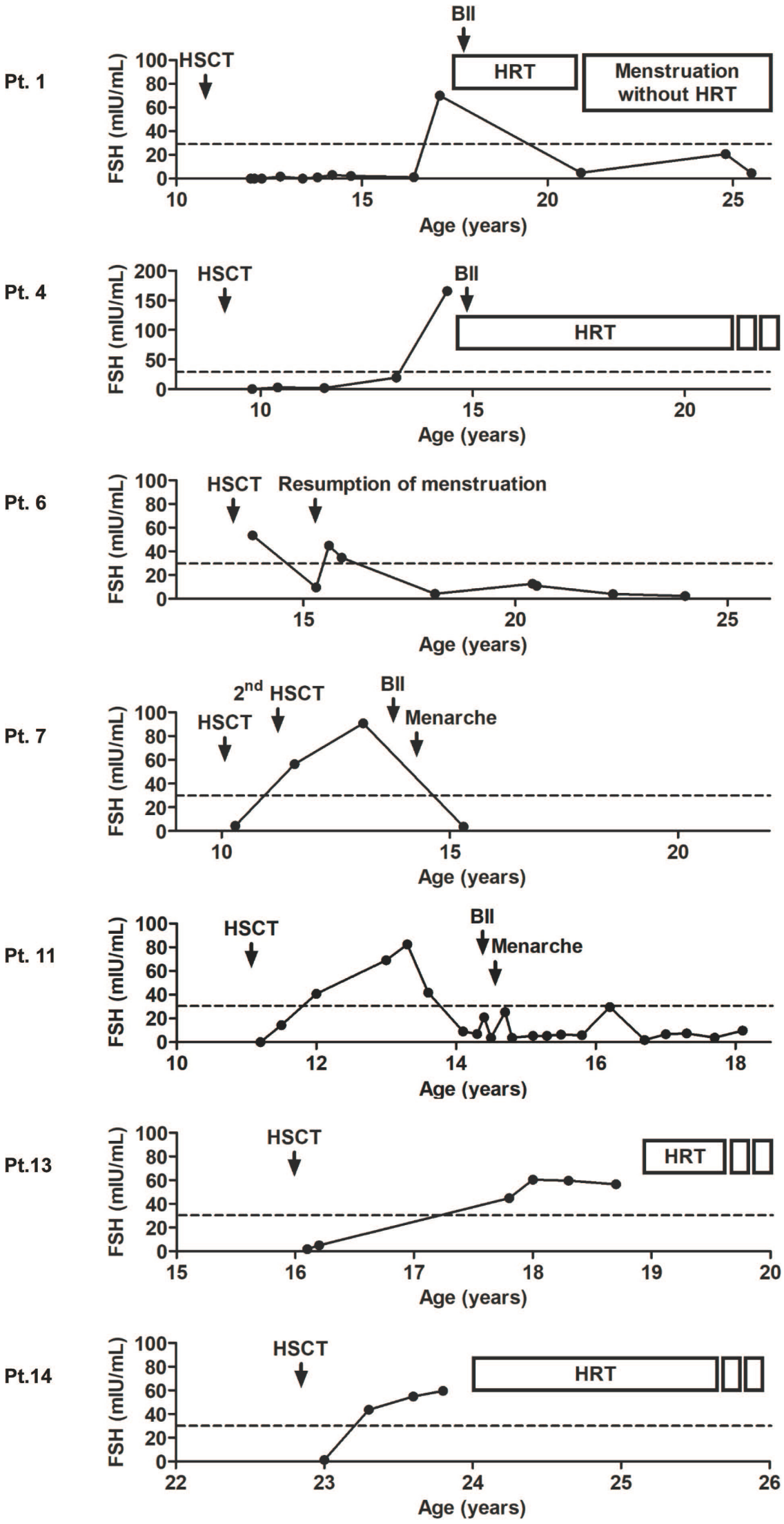
Pt.	Age at HSCT (years)	Donor	Stem cell source	HLA-matching (A, B, C, DRB1)	Infused cell dose, TNC ($\times 10^8$/kg)	Infused cell dose, CD34 ($\times 10^6$/kg)	Acute GvHD (Grade)	Chronic GvHD	RRT (CTCAE v4.0, Grade)	Additional irradiation/ chemotherapy after HSCT	Late effects other than POI
1	10.8	Related	BM	4/8	3.0	N/A	I	Extensive	0	No	Compression fracture
2	0.8	Unrelated	CB	7/8	0.234	0.220	No	No	0	No	GHD, Papillary thyroid carcinoma
3	5.6	Related	BM	7/8	5.9	4.100	II	No	0	No	None
4	9.1	Related	BM	4/8	5.36	2.680	II	Extensive	0	No	Type 2 DM, Restrictive pulmonary dysfunction, Fatty liver, Chronic hemorrhagic cystitis
5	2.4	Related	BM	7/8	15.6	13.260	III	Extensive	Gas, 1	No	GHD, Hypothyroidism, Hyperuricemia, Dysmenorrhea
6	13.5	Unrelated	BM	7/8	3.3	3.500	No	Limited	Gas, 1	No	Scoliosis
7	10.1	Related	BM	8/8	8.25	4.620	No	No	0	Yes (2nd HSCT at the age of 11.5)	Scoliosis
8	1.3	Unrelated	BM	8/8	3.6	2.120	II	No	0	No	Hyperuricemia
9	4.9	Related	BM	8/8	3.3	14.00	I	No	Gas, 1	No	None
10	3.8	Unrelated	CB	7/8	0.396	0.095	I	No	Gas, 2	No	None
11	10.9	Unrelated	CB	7/8	0.338	0.126	III	Limited	Gas, 1	CSI	GHD, Adenomatous goiter
12	10.8	Unrelated	CB	6/8	0.396	0.110	I	No	0	No	None
13	16.0	Unrelated	CB	7/8	0.204	0.102	II	Extensive	Hemorrhagic cystitis, 2	No	None
14	22.8	Unrelated	CB	7/8	0.248	0.091	I	No	Encephalomyelitis infection, 3	No	None

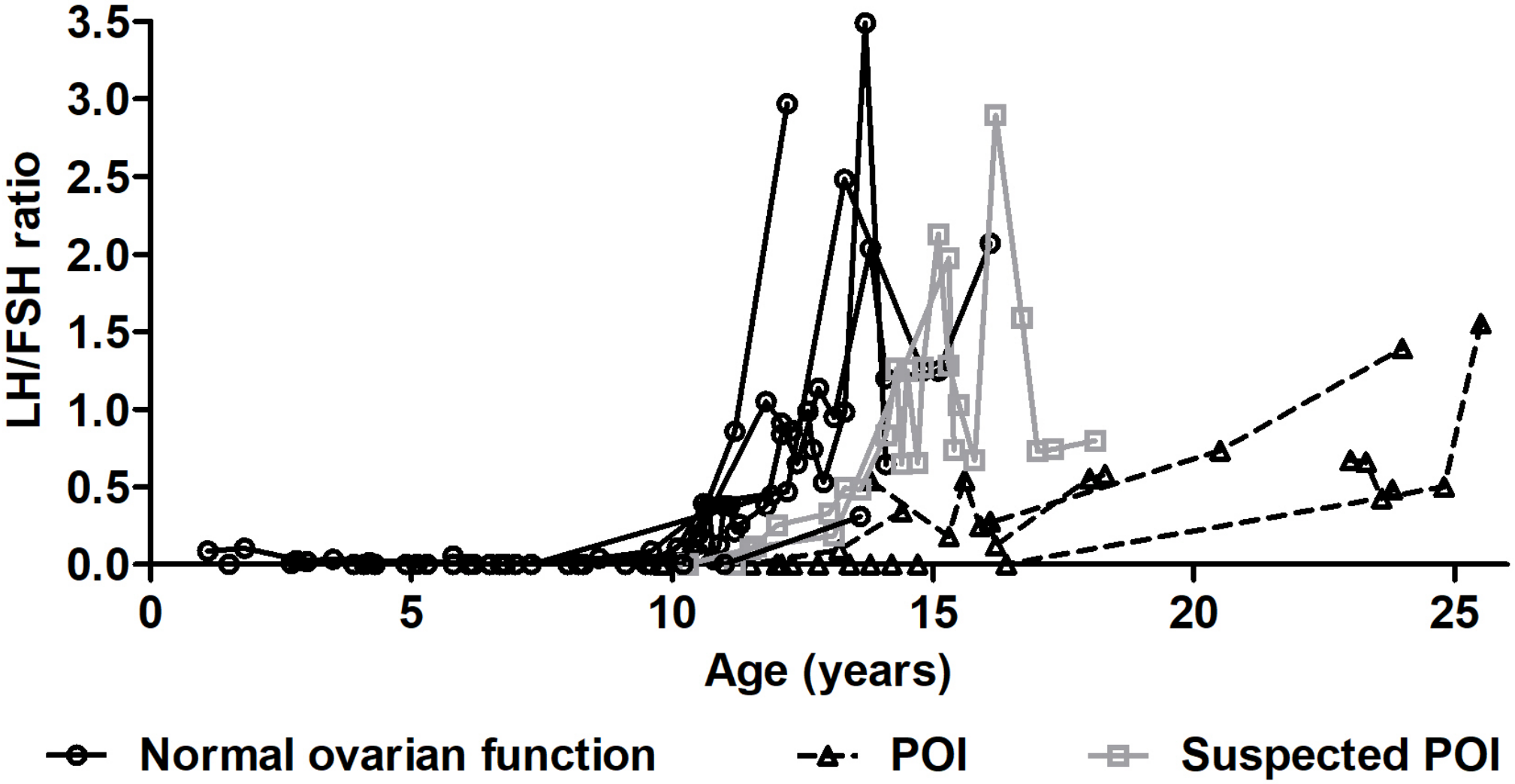
HSCT, hematopoietic stem cell transplantation; HLA, human leukocyte antigen; BM, bone marrow; CB, cord blood; GvHD, graft versus host disease; RRT, regimen-related toxicity; CTCAE, Common Terminology Criteria for Adverse Events; POI, primary ovarian insufficiency; CSI, cranio-spinal irradiation; Gas, gastrointestinal disorders; GHD, growth hormone deficiency; DM, diabetes mellitus; N/A, not applicable.

TABLE 3 Gonadal status before and after HSCT

Pt.	Pubertal status at HSCT	Age at HSCT (years)	Age at Tanner B II (years)	Age at menarche (years)	Adoption of HRT (age)	POI/ Suspected POI	Follow-up period (years)	Age at last observation (years)	Menstruation status after HSCT
1	pre-pubertal	10.8	17.6	17.4	Kaufmann (17.3-20.8)	POI >> recovered	15.7	26.5	Spontaneous menstruation have been happening after discontinuance of Kaufmann therapy.
2	pre-pubertal	0.8	10.6	11.4	No	No	14.7	15.5	Regular
3	pre-pubertal	5.6	10.7	13.7	No	No	14.3	19.9	Regular
4	pre-pubertal	9.1	14.8	17.0	Kaufmann (17.3-), Conjugated estrogen (14.5-)	POI	13.6	22.7	Regular menstruation by HRT.
5	pre-pubertal	2.4	10.7	12.3	No	No	13.7	16.1	Regular
6	post-pubertal	13.5	11.1	11.6	No	POI >> recovered	13.2	26.7	Resumption of menstruation at the age of 15.3.
7	pre-pubertal	10.1	13.3	13.9	No	Suspected POI >> recovered	12.2	22.3	Regular
8	pre-pubertal	1.3	10.9	11.8	No	No	12.1	13.4	Regular
9	pre-pubertal	4.9	10.5	12.0	No	No	11.5	16.4	Regular
10	pre-pubertal	3.8	NA	11.9	No	No	11.4	15.2	Regular
11	pre-pubertal	10.9	14.4	14.5	No	Suspected POI >> recovered	8.0	18.9	Regular
12	pubertal	10.8	10.1	11.8	No	No	5.1	15.9	Regular
13	post-pubertal	16	9.6	11.3	Estradiol transdermal (18.9-)	POI	4.2	20.2	No resumption of menstruation.
14	post-pubertal	22.8	11.7	12.7	LEP (24.0-)	POI	3.7	26.5	No resumption of menstruation.

HSCT, hematopoietic ste-m cell transplantation; HRT, hormone replacement therapy; LEP, Low dose Estrogen Progestin; POI, primary ovarian insufficiency.


Pt. 1
BII
HSCT
HRT
Menstruation without HRT
FSH (mIU/mL)
Age (years)
Pt. 4
HSCT
BII
HRT
FSH (mIU/mL)
Age (years)
Pt. 6
HSCT
Resumption of menstruation
FSH (mIU/mL)
Age (years)
Pt. 7
2nd HSCT
HSCT
BII
Menarche
FSH (mIU/mL)
Age (years)
Pt. 11
HSCT
BII
Menarche
FSH (mIU/mL)
Age (years)
Pt.13
HSCT
HRT
FSH (mIU/mL)
Age (years)
Pt.14
HSCT
HRT
FSH (mIU/mL)
Age (years)


LH/FSH ratio
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
Age (years)
Normal ovarian function
POI
Suspected POI