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Updated Clinical Outcomes of Hematopoietic Stem Cell Transplantation Using Myeloablative Total Body Irradiation with Ovarian Shielding to Preserve Fertility

Masahiro Ashizawa[1], Yu Akahoshi[2], Hirofumi Nakano[1], Shunto Kawamura[2], Junko Takeshita[2], Nozomu Yoshino[2], Yukiko Misaki[2], Kazuki Yoshimura[2], Ayumi Gomyo[2], Masaharu Tamaki[2], Machiko Kusuda[2], Kazuaki Kameda[2], Hidenori Wada[2], Koji Kawamura[2], Miki Sato[2], Kiriko Terasako-Saito[2], Aki Tanihara[2], Shun-ichi Kimura[2], Hideki Nakasone[2], Shinichi Kako[2], Keiko Akahane[3], Masaru Wakatsuki[4], Katsuyuki Shirai[3], Yoshinobu Kanda[1,2,*]

[1] *Division of Hematology, Department of Medicine, Jichi Medical University, Tochigi, Japan*
[2] *Division of Hematology, Jichi Medical University Saitama Medical Center, Saitama, Japan*
[3] *Division of Radiology, Jichi Medical University Saitama Medical Center, Saitama, Japan*
[4] *Department of Radiology, Jichi Medical University, Tochigi, Japan*

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A B S T R A C T

Myeloablative conditioning regimens are associated with severe gonadal toxicity. To preserve ovarian function, we have been investigating ovarian shielding during total body irradiation (TBI) with a myeloablative dose. In this report, we update the clinical outcomes. Female patients with standard-risk hematologic diseases, aged 40 years or younger, who desired to have children, were included (n = 19). The conditioning regimen consisted of TBI at 12 Gy with ovarian shielding and cyclophosphamide (120 mg/kg) or cytarabine (24 g/m^2). Ovarian shielding reduced the actual irradiation dose applied to the ovaries from 12 Gy to 2 to 3 Gy. The median age at hematopoietic stem cell transplantation (HSCT) was 24 years (range, 19 to 33 years). With a median follow-up period of 1449 days (range, 64 to 3694) after HSCT, 5-year overall survival and 1- and 5-year relapse rates were 67%, 17%, and 31%, respectively. Only 2 of 14 patients with acute myeloid or lymphoid leukemia in remission have relapsed thus far. The 6-month and 1-year cumulative rates of menstrual recovery were 42% and 78%, respectively. In all patients with menstrual recovery, menstruation recovered within 1 year. The serum anti-Müllerian hormone (AMH) level tended to gradually increase after menstrual recovery. Three patients with extensive chronic graft-versus-host disease experienced delayed recovery of menstruation and serum AMH. Five pregnancies in 3 patients resulted in normal delivery in 1, selective cesarean operation in 1, current pregnancy in 1, and natural abortion in 2. These results suggest that a myeloablative TBI regimen with ovarian shielding could preserve fertility after HSCT without an apparent increase in relapse in standard-risk patients. Because serum AMH recovered gradually over time, the AMH level during the early phase after HSCT may have little value as a marker of ovarian reserve.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) has been established as a curative therapeutic procedure for refractory hematologic disorders. However, permanent infertility frequently occurs in patients who receive myeloablative conditioning regimens, including total body irradiation (TBI) or high-dose busulfan [1]. Cryopreservation of embryo, unfertilized eggs, or ovarian tissues has been investigated to preserve reproductive potential in female patients who undergo chemotherapy with strong gonadal toxicity [2,3]. However, these invasive procedures are difficult to perform in patients who have hematologic disorders due to bleeding, infection, or a need to start urgent chemotherapy. In addition, subsequent reimplantation of ovarian tissue may carry a risk of introducing leukemic cells into the patient [4,5]. On the other hand, it is difficult to collect enough good-quality eggs in patients who have received multiple cycles of chemotherapy [6]. Consequently, most patients with hematologic disorders often undergo HSCT without any fertility preservation strategies before HSCT.

To solve this problem, we investigated a conditioning regimen consisting of high-dose cyclophosphamide (CY) and TBI

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* Correspondence and reprint requests: Yoshinobu Kanda, MD, Division of Hematology, Department of Medicine, Jichi Medical University, 3311-1, Yakushiji, Shimotsuke-city, Tochigi 329-0498, Japan.
E-mail address: ycanda-tky@umin.ac.jp (Y. Kanda).

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with ovarian shielding [7,8]. The rationale of this regimen is that young patients who undergo HSCT for aplastic anemia with high-dose CY alone often show ovarian recovery after HSCT [1]. Metallic ovarian shielding reduced the actual irradiation dose applied to the ovaries from 12 Gy to 2 to 3 Gy [9], which, by itself, is unlikely to result in permanent infertility [10]. Therefore, a conditioning regimen that consists of high-dose CY and TBI with ovarian shielding might theoretically maintain fertility in young female patients who undergo HSCT. We previously reported the preliminary fertility outcomes in patients who underwent HSCT using ovarian shielding [8]. In this study, we analyzed not only the fertility data but also the outcome of HSCT with a longer follow-up period in a greater number of patients.

PATIENTS AND METHODS

Study Population

Nineteen female patients with standard-risk hematologic diseases, aged 40 years or younger, who desired to have children, underwent HSCT with ovarian shielding between April 2007 and June 2018 at Jichi Medical University Saitama Medical Center. Standard risk was defined as acute leukemia in first or second complete remission (CR), myelodysplastic syndrome (MDS) without leukemic transformation, or non-neoplastic disorder. The patients' data were collected from medical records and patient questionnaires. This study was approved by the institutional review board of Jichi Medical University Saitama Medical Center.

Transplantation Procedure

The conditioning regimen consisted of CY (60 mg/m^2/d for 2 days) and TBI at 12 Gy (2 Gy twice daily for 3 days) with ovarian shielding. In 1 patient, CY was changed to cytarabine (3 g/m^2 twice daily for 4 days) because of cardiac dysfunction. Prophylaxis for graft-versus-host disease (GVHD) consisted of a combination of a calcineurin inhibitor and short-term methotrexate. Cyclosporine or tacrolimus was administered as a 24-hour continuous infusion. The doses of cyclosporine and tacrolimus were adjusted to maintain target blood concentrations of 300 to 500 ng/mL and 12 to 15 ng/mL, respectively [11,12]. In vivo T cell depletion with antithymocyte globulin or alemtuzumab was used in patients who underwent HSCT from an HLA-mismatched donor [13]. Prophylaxis against bacterial, fungal, and *Pneumocystis jiroveci* infection consisted of levofloxacin, fluconazole or itraconazole, and sulfamethoxazole/trimethoprim or inhalation of pentamidine, respectively. Acyclovir was administered as prophylaxis against herpes simplex virus (HSV) infection and varicella-zoster virus (VZV) reactivation [14,15].

Ovarian Shielding

All patients received TBI by the long source-to-axis distance method. The details of the irradiation procedure are given elsewhere [9]. Briefly, patients were treated in a lateral position alternately facing and turning their back to the beam, which resulted in beam delivery in the anterior-posterior and posterior-anterior directions. The source-to-axis distance was 400 cm. The position of the ovaries was checked by magnetic resonance imaging and/or computed tomography and marked on the patient's skin. A pair of columnar metal blocks attached to an acrylic board was placed in front of the patient's body. With this method, the actual irradiation dose applied to the ovaries was reduced from 12 Gy to 2.4 Gy [9].

Assessment of Ovarian Function

The anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), and estradiol 2 concentrations were measured with frozen plasma samples stored at −80°C or fresh serum samples. Information on menstrual recovery, pregnancy, and delivery was obtained from medical records and questionnaires regularly distributed to patients. The measurement of these hormones and the questionnaire survey were performed before the start of the conditioning regimen; at 3 months, 3 to 9 months, 9 to 15 months, 15 to 21 months, and 21 to 30 months after HSCT; and every year thereafter up to 10 years after HSCT. In addition, available cryopreserved serum samples were used if remained for patients who underwent HSCT before 2013.

Statistical Analysis

Overall survival and disease-free survival were estimated according to the Kaplan-Meier method. The probabilities of nonrelapse mortality and relapse were calculated by treating relapse and death without relapse, respectively, as competing events. The recovery rate of menstruation was calculated by treating relapse of the underlying disease and death without ovarian recovery as competing events. All statistical analyses were performed with EZR version 1.40 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (version 3.5.2; R Foundation for Statistical Computing, Vienna, Austria) [16].

RESULTS

Patient Characteristics

The clinical characteristics of patients with ovarian shielding are summarized in Table 1. Nineteen patients with a median age of 23 years (range, 19 to 33 years) at HSCT were included in this study. The patients' underlying disease included acute myeloid leukemia (AML) (n = 9), acute lymphoblastic leukemia (ALL) (n = 5), myelodysplastic syndrome (MDS) (n = 2), acute unclassified leukemia (AUL) (n = 1), blastic plasmacytoid dendritic cell neoplasm (BPDCN) (n = 1), and aplastic anemia (AA) (n = 1). Cytogenetic abnormalities included t(8;21) in 1 AML (patient 2), t(15;17) in 1 AML (patient 5), inv(16) in 2 AMLs (patients 17 and 18), complex karyotype in 1 AML (patient 14), hyperdiploid in 1 ALL (patient 10), and t(3;21) in 1 MDS (patient 15). All patients except for 1 with MDS and AA received multiple courses of chemotherapy before HSCT. One patient with AA had exacerbated pancytopenia after immunosuppressive therapy and received HSCT from the syngeneic donor thereafter. Three of 4 evaluable patients achieved molecular remission and the other patient with t(8;21) achieved cytogenetic remission. Seventeen patients received allogeneic graft, whereas 1 each received autologous and syngeneic graft. The conditioning regimen was a combination of TBI and CY in all patients except for 1 who received TBI combined with cytarabine. Antithymocyte globulin or alemtuzumab was combined with the conditioning regimen in 2 patients each who were transplanted from an HLA-mismatched donor. No patients received maintenance therapies after HSCT.

Transplantation Outcome

Neutrophil engraftment was achieved in all patients at a median of 22.5 days after HSCT (range, 11 to 38 days). Twelve of the 17 allogeneic transplant recipients developed acute GVHD: grade I in 8, grade II in 3, and grade IV in 1. Five patients developed chronic GVHD, including 3 with extensive form. With a median follow-up period of 1449 days after HSCT (range, 64 to 3694 days), 5-year overall survival and 5-year progression-free survival were 67% (95% confidence interval [CI], 37% to 85%) and 64% (95% CI, 36% to 82%). The 1- and 5-year relapse rates were 17% (95% CI,[illegible]% to 37%) and 31% (95% CI, 10% to 54%) (Figure 1). Relapse was confirmed in 5 patients, with background diseases including AML in first CR (CR1), ALL in CR1, AUL in CR1, BPDCN in CR1, and MDS with excess of blasts 1. Two patients died of nonrelapse mortality including bronchiolitis obliterans and post-transplant lymphoproliferative disorder.

Assessment of Fertility

Hormone replacement therapy was not performed after HSCT in all but 1 patient (patient 3). The 6-month and 1-year cumulative rates of menstrual recovery were 42% (95% CI, 20% to 63%) and 78% (95% CI, 47% to 92%), respectively, and menstrual recovery was observed exclusively within 1 year after HSCT (Figure 2). Menstruation was not recovered in 2 of the 13 patients who survived over 1 year. One patient (patient 3) without menstrual recovery started to receive hormone replacement therapy at 5 years after HSCT.

The serum AMH level fell below measurable limits (0.1 ng/mL) after HSCT in all 14 evaluable patients (Figure 3). The serum AMH level tended to increase gradually following menstrual recovery and normalization of serum Luteinizing hormone (LH), FSH, and estradiol 2. In some patients, it has

Table 1
Patient Characteristics

Patient No.	Age at HSCT	Disease	Disease Status	Stem Cell Source		Conditioning Regimen	Engraftment, d	Acute GVHD	Chronic GVHD	Outcome, mo
1	20	ALL	CR1	Unrelated	BM	CY (120 mg/kg) + TBI 12 Gy	17	Grade IV	Extensive	Death from TRM (80)
2	21	AML	CR2	Unrelated	BM	CA (24 g/m^2) + TBI 12 Gy	26	Grade I	No	Alive in CR (123)
3	23	AML	CR1	Unrelated	BM	CY (120 mg/kg) + TBI 12 Gy	17	No	No	Alive in CR (90)
4	19	AML	CR1	Related	PB	CY (120 mg/kg) + TBI 12 Gy	25	Grade II	Not evaluable	Relapse (3)
5	23	AML	CR2	Autologous	PB	CY (120 mg/kg) + TBI 12 Gy	11	—	—	Alive in CR (96)
6	31	AML	CR1	Related	PB	CY (120 mg/kg) + TBI 12 Gy	25	Grade I	Limited	Alive in CR (98)
7	20	AUL	CR1	Unrelated	BM	CY (120 mg/kg) + TBI 12 Gy	29	Grade I	No	Relapse (4.5)
8	19	AML	CR1	Related	PB	CY (120 mg/kg) + TBI 12 Gy	19	No	No	Alive in CR (90)
9	26	BPDCN	CR1	Related	PB	CY (120 mg/kg) + TBI 12 Gy	30	No	Limited	Relapse (36)
10	20	ALL	CR1	Related	PB	CY (120 mg/kg) + TBI 12 Gy	12	Grade I	Extensive	Alive in CR (60)
11	26	ALL	CR1	Unrelated	BM	CY (120 mg/kg) + TBI 12 Gy	20	Grade I	No	Relapse (13)
12	20	MDS	EB2	Related	PB	CY (120 mg/kg) + TBI 12 Gy + alemtuzumab (0.5 mg/kg)	15	Grade II	Not evaluable	Death from TRM (4)
13	30	ALL	CR1	Unrelated	BM	CY (120 mg/kg) + TBI 12 Gy + ATG (2.5 mg/kg)	21	Grade I	No	Alive in CR (25)
14	23	AML	CR2	Related	PB	CY (120 mg/kg) + TBI 12 Gy	20	No	Extensive	Alive in CR (27)
15	32	MDS	EB1	Unrelated	BM	CY (120 mg/kg) + TBI 12 Gy + ATG (2.5 mg/kg)	25	No	No	Relapse (4.3)
16	28	ALL	CR1	Related	PB	CY (120 mg/kg) + TBI 12 Gy	12	Grade I	No	Alive in CR (15)
17	27	AML	CR2	Unrelated	CB	CY (120 mg/kg) + TBI 12 Gy	29	Grade II	Not evaluable	Alive in CR (2.1)
18	24	AML	CR2	Related	PB	CY (120 mg/kg) + TBI 12 Gy + alemtuzumab (0.5 mg/kg)	38	Grade I	No	Alive in CR (7)
19	33	Primary AA	Moderate	Syngeneic	PB	CY (120 mg/kg) + TBI 12 Gy	36	—	—	Alive (56)

BM indicates bone marrow; TRM, treatment-related mortality; CA, cytarabine; PB, peripheral blood; EB2, excess blasts 2; EB1, excess blasts 1; ATG, antithymocyte globulin.

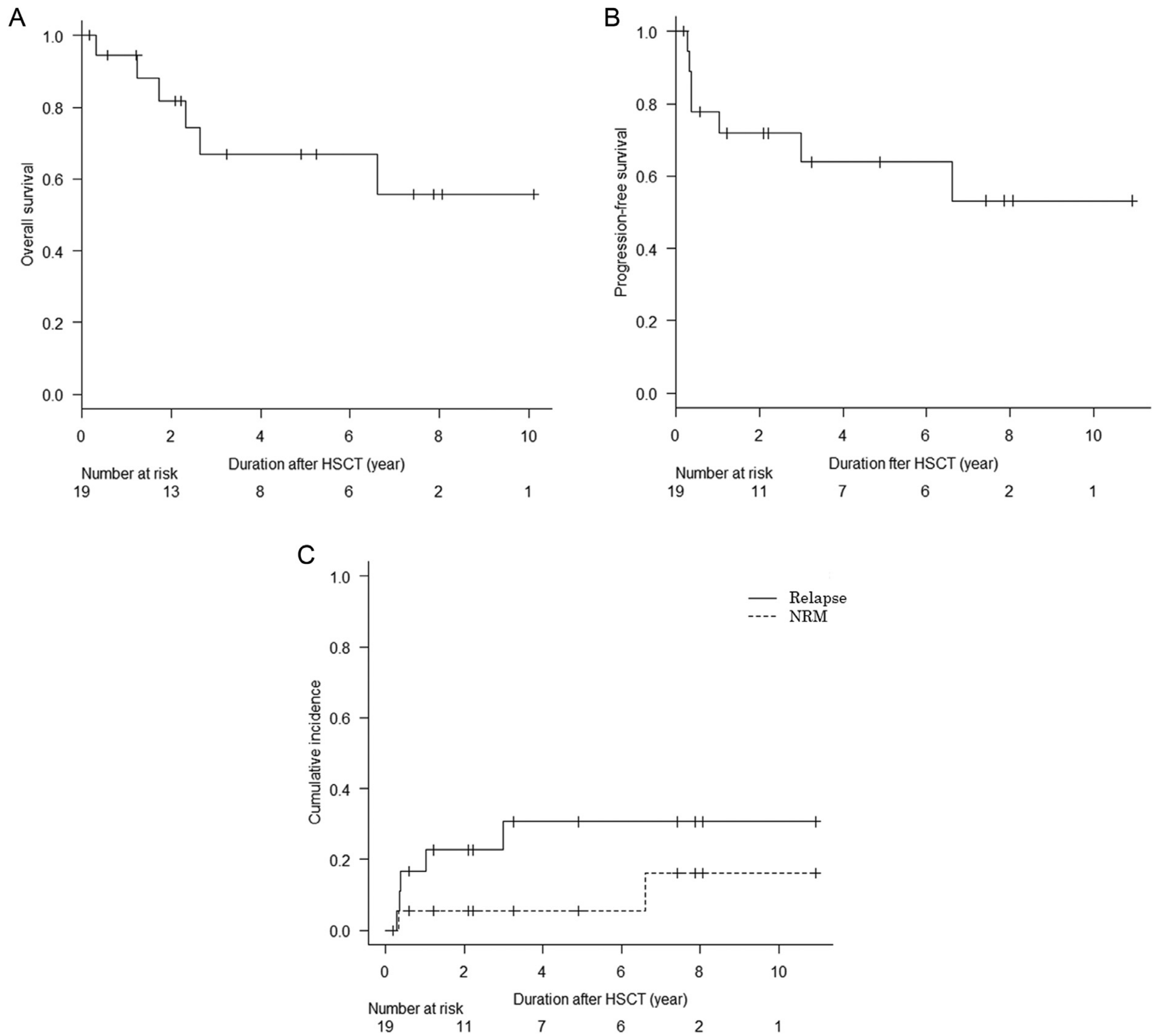

Figure 1. Probabilities of overall survival (A) and progression-free survival (B) and cumulative incidences of relapse and nonrelapse mortality (NRM) (C).

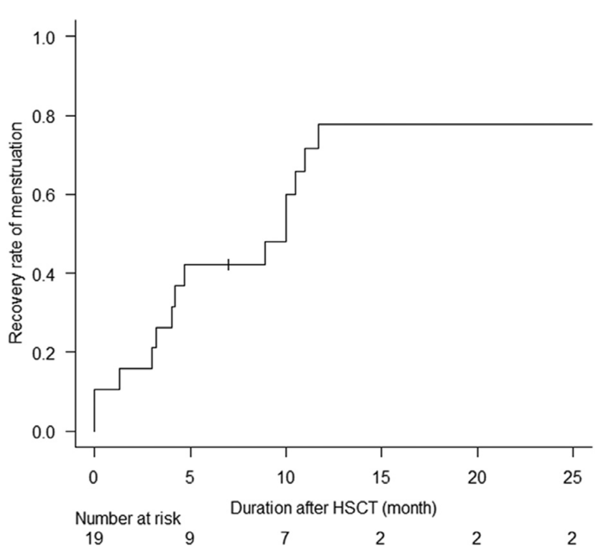

Figure 2. Cumulative incidence of menstrual recovery after HSCT.

continued to increase for several years after HSCT. Two and 3 patients developed limited and extensive chronic GVHD, respectively. All patients with extensive chronic GVHD exhibited delayed recovery of menstruation and serum AMH, although patients with limited chronic GVHD did not. In particular, patient 14 has not yet achieved menstrual recovery or elevation of the serum AMH level after HSCT. Although patient 1 recovered menstruation once, she has since become amenorrheic again with the re-elevation of serum LH and FSH, following the development of extensive chronic GVHD. Although patient 10 exhibited menstrual recovery immediately, elevation of the serum AMH level was confirmed more than 3 years after HSCT.

Five spontaneous pregnancies in 3 patients resulted in 2 deliveries (Table 2). Patient 2 gave birth to a healthy child by elective cesarean section, despite no major complication before or during pregnancy. Patient 6 delivered a healthy child without complications by vaginal birth at 68 months after HSCT and since then has had 2 spontaneous abortions. Patient 13 is currently pregnant with no complications. Two patients (patients 2 and 13) conceived spontaneously even with low AMH levels, which is generally considered associated with a low likelihood of pregnancy.

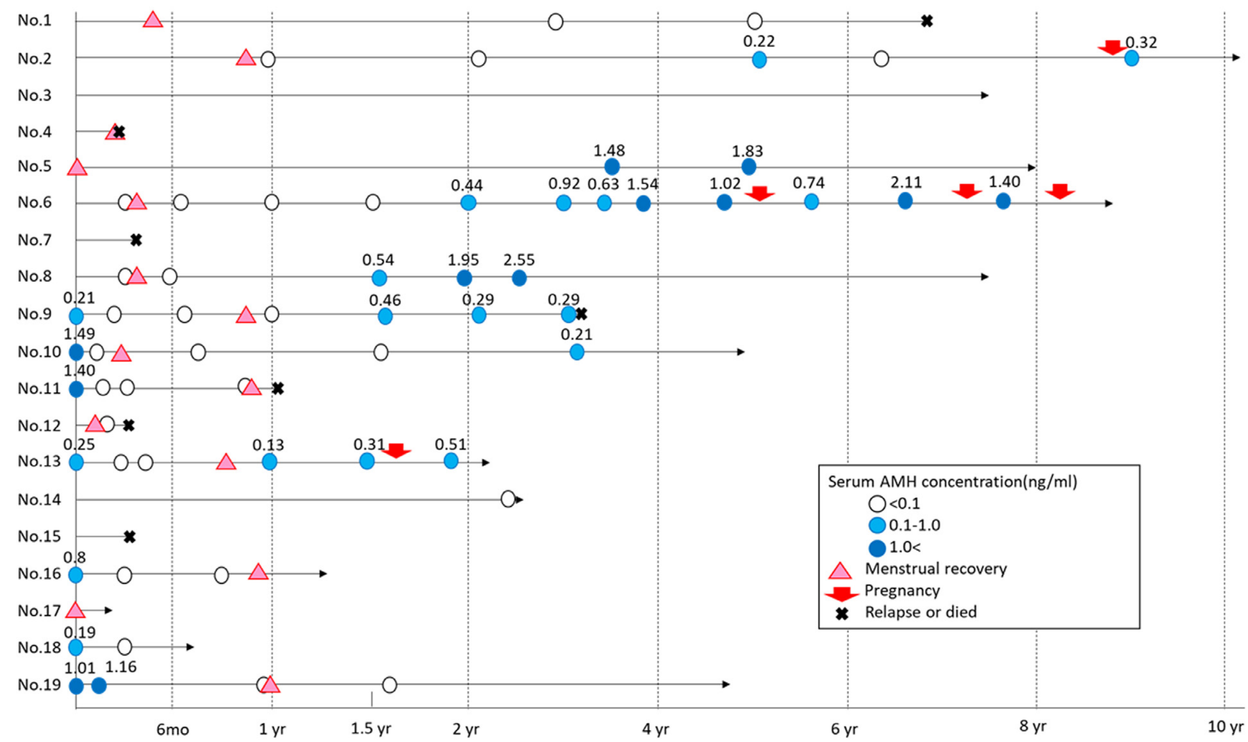

Figure 3. Relationship between menstrual recovery, serum AMH level, and pregnancy.

DISCUSSION

In the current study, ovarian shielding in TBI-containing conditioning regimens was associated with preserved fertility with a relapse rate comparable to that of standard-risk patients who received conventional conditioning regimens. In the report by the Seattle group, the relapse rate was not increased by a nonmyeloablative conditioning regimen that included only 2 Gy of TBI among patients with MDS or AML in remission transformed from MDS [17]. On the basis of this report, we considered that the relapse rate would not increase in standard-risk patients, as the ovaries are irradiated with at least 2 Gy in our regimen [9]. However, we took special care to minimize shielding of the pelvic bone. Five of 19 patients developed relapse, including 2 with AML or ALL in CR1, 1 with

Table 2
Fertility Assessment after HSCT.

Patient No.	Menstrual Recovery after HSCT, mo	Recovery of AMH after HSCT*	Marriage	Pregnancy after HSCT, mo	Childbirth
1	Yes (4.7)	No	No	No	
2	Yes (10.5)	Yes	Yes	Yes (108)	Elective cesarean section
3	No	Not evaluable	No	No	
4	Yes (3.2)	Not evaluable	No	No	
5	Not stopped	Yes	No	No	
6	Yes (4)	Yes	Yes	Yes (68, 86, 99)	Normal delivery, 2 spontaneous abortions
7	No	Not evaluable	No	No	
8	Yes (4.2)	Yes	Yes	No	
9	Yes (10)	Yes	Yes	No	
10	Yes (3)	Yes	Yes	No	
11	Yes (10)	No	No	No	
12	Yes (1.3)	No	No	No	
13	Yes (8.9)	Yes	Yes	Yes (21)	Currently pregnant
14	No	No	No	No	
15	No	Not evaluable	No	No	
16	Yes (11)	No	No	No	
17	Not stopped	Not evaluable	No	No	
18	No	No	No	No	
19	Yes (11.7)	No	Yes	No	

* The AMH concentration was considered to have recovered when it rose above 0.1 ng/mL.

AUL in CR1, 1 with MDS in excess of blasts 1, and 1 with BPDCN in CR1. When the patients were grouped according to the background diseases, relapse was observed in only 2 of 14 patients with AML or ALL, whereas the 2 patients with AUL and BPDCN both relapsed. Considering the response to chemotherapy before HSCT, 2 of the 3 patients who relapsed in CR1 with acute leukemia (patients 4 and 7) did not achieve CR by initial induction chemotherapy and required salvage therapy. The patient with MDS in excess of blasts 1 (patient 15) did not receive chemotherapy before HSCT. Although it is difficult to draw a definitive conclusion from this small cohort, the application of ovarian shielding should be considered carefully in patients with acute leukemia after primary induction failure or in patients with MDS in excess of blasts, because these patients experienced early relapse after HSCT with ovarian shielding.

In the previous literature, the incidence of gonadal recovery was 10% to 13.5% in female patients who received a TBI-containing conditioning regimen [18,19]. Moreover, in a report from the European Society for Blood and Marrow Transplantation, the overall incidence of pregnancy was very low (<2%), except for patients transplanted for severe AA [1]. In contrast, ovarian shielding resulted in a high menstrual recovery rate of about 80% at 1 year after HSCT. To date, there have been 3 pregnant patients among 7 married long-term survivors. A longer follow-up period could increase the number of pregnancies, based on a favorable transplantation outcome.

AMH is secreted from granulosa cells of the preantral and small antral follicles in women. AMH levels decrease steadily over time in healthy women [20]. The antral follicles are susceptible to anticancer drugs and radiation. In a previous report, all patients who received HSCT with a TBI-containing conditioning regimen developed premature ovarian failure, and their serum AMH concentrations were undetectable for several years after HSCT [21]. In contrast, the serum AMH level tended to increase gradually, starting at more than 1 year after HSCT, in our current and previous studies [8]. Although antral follicles had disappeared due to the conditioning regimen, remaining primordial follicles protected by ovarian shielding may have matured into antral follicles several years after HSCT, leading to the re-elevation of serum AMH. Patient 2 became pregnant while her AMH level was recovering from below the measurable limit to 0.32 ng/mL. Patient 13 became pregnant while her AMH level was recovering from 0.31 to 0.51 ng/mL. In patient 6, who became pregnant 3 times, the first pregnancy was confirmed while her AMH level was fluctuating from 1.02 to 0.74 ng/mL. In a previous report, a serum AMH level of 1.06 ng/mL or greater was associated with an improved live-birth rate [22]. In the early period after HSCT, the AMH level may not accurately reflect the “true” number of remaining follicles. In our previous report, we had not yet observed pregnancies in patients with acute leukemia. However, in the current study, we confirmed pregnancies and live births in patients with acute leukemia, as observed with ovarian shielding using other methods [7,8].

Previously, we reported that menstruation that had recovered after HSCT stopped again following the development of severe chronic GVHD in a patient [8]. In this study, 2 other patients with extensive chronic GVHD were associated with delayed recovery of menstruation and serum AMH. Tauchmanovà et al. [23] reported that ovarian and uterine volumes in women with GVHD were significantly lower than those in women without GVHD. Shimoji et al. [24] demonstrated donor T cell infiltration in close proximity to apoptotic granulosa cells in the ovarian follicles, resulting in impaired AMH production and maturation of ovarian follicles after HSCT in a mouse model. These reports support the notion that chronic GVHD impairs fertility. Therefore, the control of chronic GVHD may be important, as is reducing gonadal toxicity by the conditioning regimen, to preserve fertility.

One patient (patient 3) has not yet achieved menstrual recovery despite having neither relapse nor GVHD. Although it is unclear why menstruation has not recovered in this patient, she had received a high cumulative dose of anthracyclines as part of chemotherapy before HSCT. Anthracyclines not only reduce oocytes through direct injury but also damage the blood vessel wall and reduce blood flow to the ovaries and their volume [25].

In conclusion, ovarian shielding may preserve fertility without increasing the likelihood of relapse in selected female patients of reproductive age. Based on the current data and the results of a decision analysis [26], ovarian shielding could be a viable option for female patients of reproductive age with acute leukemia in remission under the condition that the patients understand the limitations. The AMH level during the early phase after HSCT may have little value as a marker of ovarian reserve.

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