

ORIGINAL ARTICLE

Preservation of ovarian function by ovarian shielding when undergoing total body irradiation for hematopoietic stem cell transplantation: a report of two successful cases

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The purpose of this study was to evaluate the possibility of preserving ovarian function by ovarian shielding to reduce the irradiation dose in total body irradiation (TBI). The subjects in the study were females aged less than 40 years, who were undergoing allogeneic hematopoietic stem cell transplantation using a TBI-based regimen and who desired to have children after transplantation. For ovarian shielding, abdominal computed tomography (CT) and skin marking were performed in both the supine and prone positions, prior to the TBI. A pair of columnar blocks was placed just above the patient's body. Thus far three patients have been treated. The serum estradiol level decreased to an undetectable level (<8.5 pg/ml) after transplantation and the follicle-stimulating hormone (FSH) level increased above 90 mIU/ml in all patients and they became amenorrheic. However, regular menstruation recovered in patients no. 1 and 2 about 800 and 370 days after transplantation, respectively, with a decrease in the serum FSH level. Menstruation did not recover in patient no. 3, and serum estradiol was transiently detected above 20 pg/ml. The preservation of ovarian function was made possible by ovarian shielding. However, a longer follow-up is needed to know if normal pregnancy and delivery can occur.

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Introduction

The conditioning regimen before allogeneic hematopoietic stem cell transplantation is intended to eradicate tumor cells and to promote immunosuppression to prevent graft rejection. A combination of cyclophosphamide and total body irradiation (TBI) is the most widely used regimen in transplantation for leukemia. However, this regimen causes severe germ cell injury and infertility.^{1–12} On the other hand, patients who have received cyclophosphamide alone for aplastic anemia frequently recover ovarian function after transplantation. Considering that the dose of cyclophosphamide in transplantation for aplastic anemia is usually higher than that in transplantation for leukemia (200 vs 120 mg/kg), we explored the possibility of preserving ovarian function by reducing the irradiation dose by ovarian shielding.

Patients and methods

Patients

Three female patients aged less than 40 years, who were undergoing allogeneic hematopoietic stem cell transplantation using a TBI-based regimen and who desired to have children after transplantation, were the subjects of this study. The study was approved by the Ethics committee of the University of Tokyo Hospital and all patients gave informed consent to participate in this study.

Transplantation procedure

The preparative regimen was a combination of cyclophosphamide at 60 mg/kg/day for 2 days and TBI at 2 Gy twice daily for 3 days. In patient no. 3, the dose of cyclophosphamide was reduced to 40 mg/kg/day for 1 day and etoposide at 20 mg/kg/day for 2 days was added instead, because of impaired cardiac function before transplantation. Cyclosporin A was administered as a continuous infusion at a dose of 3 mg/kg/day combined with short-term methotrexate (10–15 mg/m² on day 1 and 7–10 mg/m² on days 3 and 6, and optionally on day 11) to prevent GVHD. Patient no. 3, who underwent transplantation from a

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two-locus-mismatched sibling donor, received alemtuzumab at 0.2 mg/kg/day from day -8 to day -3.¹³ Methylprednisolone at 1–2 mg/kg/day was added for patients who developed grade II–IV GVHD. Prophylaxis against bacterial, fungal and *Pneumocystis carinii* infection consisted of fluconazole, ciprofloxacin, and sulfamethoxazole/trimethoprim. For prophylaxis against herpes simplex virus infection, acyclovir was given 750 mg/day intravenously or 1000 mg/day orally from days -7 to 35, followed by long-term low-dose (400 mg/day) oral administration until the end of immunosuppressive therapy. A cytomegalovirus antigenemia assay using C10/C11 antibody was performed at least once a week after engraftment. Ganciclovir was started when more than two positive cells were detected on two slides.

TBI and ovarian shielding

Patients were treated in a mobile box made of 10 mm thick polymethyl methacrylate 600 mm wide by 2000 mm long by 400 mm high. The box is capable of moving up to 250 cm forward and backward on the rails with a constant speed. Beam intensity and moving velocity defined dose rate in TBI.¹⁴ Normally, beam opening of the linac is $400 \times 10 \text{ cm}^2$. Leukemia patients were usually treated in the supine position for three fractions in the morning and in the prone position for three fractions in the evening.

The center of the mobile box was selected to be a reference point to attain the prescribed dose. Beam intensity and moving velocity were determined based on the measurement of the doses in Mix-DP slab phantoms with an ionization chamber, but no corrections for patient body size were required due to the use of the mobile box.

In TBI for the leukemia patients, most commonly, a pair of customized metal blocks was placed on the mobile box for lung shielding. The blocks were fabricated according to the lung shape, which was obtained by use of the X-ray film taken in the box. Lung shielding was performed in a fraction of TBI out of six fractions for three consecutive days in most cases.

For ovarian shielding, abdominal magnetic resonance imaging (MRI) and computed tomography (CT) were performed prior to the TBI. Position of the ovaries was checked with T2-weighted image of MRI and was projected and marked onto the patient's skin. Trans-abdominal ultrasound on the day of treatment was performed for the accurate positioning of the shields. As the ovarian shielding was performed in all six fractions, CT scan and skin marking were performed both in supine and prone positions. A pair of columnar blocks (8 cm in height and 5 cm in diameter) was placed just above the patient's body, as demonstrated in Figures 1 and 2. For ovarian shielding, beam opening was $40 \times 2 \text{ cm}^2$ to decrease penumbra. Figure 3 shows a portal image taken during an actual TBI with ovarian shielding.

Actual measurement for humanoid phantom

Actual doses to the ovary were measured with glass dosimeters within a humanoid phantom. Doses of 2 Gy in the supine position and 2 Gy in the prone position were given for total body with the tracking technique. Twelve glass dosimeters were placed at the ovarian position of the humanoid phantom under shielding (Figure 4).

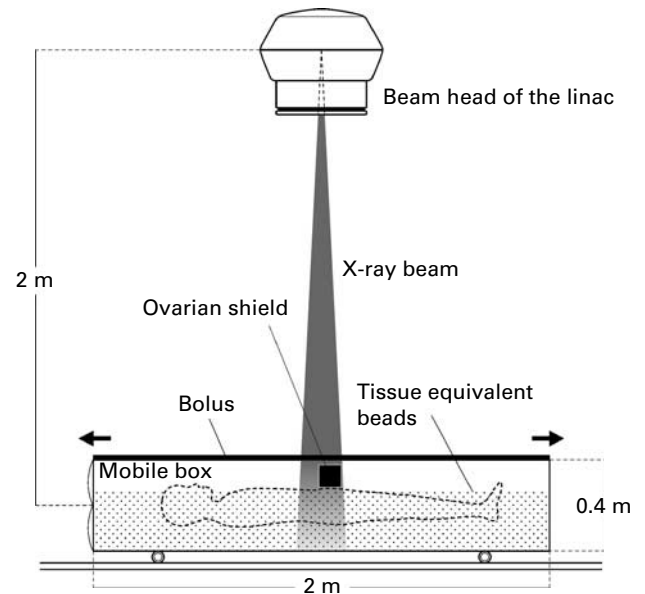


Figure 1 A schematic illustration of ovarian shielding in TBI.



Figure 2 A pair of columnar blocks with dimensions of 8 cm in height and 5 cm in diameter. It was placed just above the patient's body.



Figure 3 A portal image taken during an actual TBI with ovarian shielding.

Results

Patients

Thus far, three patients have been treated (Table 1). Two had chronic myelogenous leukemia in first chronic phase and had not received intravenous of antineoplastic agents before transplantation. The other patient had acute lymphoblastic leukemia in second remission and had received multiple courses of intensive chemotherapy. The donors were a matched unrelated donor, an HLA-identical sibling donor, and a two-locus-mismatched sibling donor in patients no. 1, 2, and 3, respectively. Patients no. 1 and 2 had regular menstruation before transplantation, but patient no. 3 already had chemotherapy-induced amenorrhea.

Transplantation outcome

All three patients had donor cell engraftment between days 15 and day 31 after transplantation. Acute GVHD was observed in only patient no. 1. She developed grade II acute GVHD limited to the skin, which was followed by extensive chronic GVHD. Patients no. 1 and 2 are alive without leukemia on days 1163 and 1055 after transplantation, respectively. However, patient no. 3 had a relapse of leukemia on day 223 and died on day 522.

Ovarian function after transplantation

The serum estradiol level decreased to an undetectable level (<8.5 pg/ml) after transplantation and the follicle-stimu-

lating hormone (FSH) level increased above 90 mIU/ml in all patients and they became amenorrheic (Figure 5). However, patients no. 1 and 2 recovered regular menstruation about 800 and 370 days after transplantation, respectively, with a decrease in serum FSH level. In patient

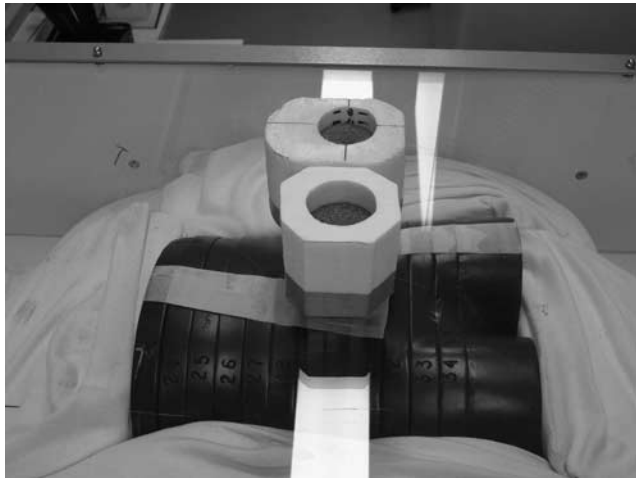


Figure 4 Humanoid phantom experiment.

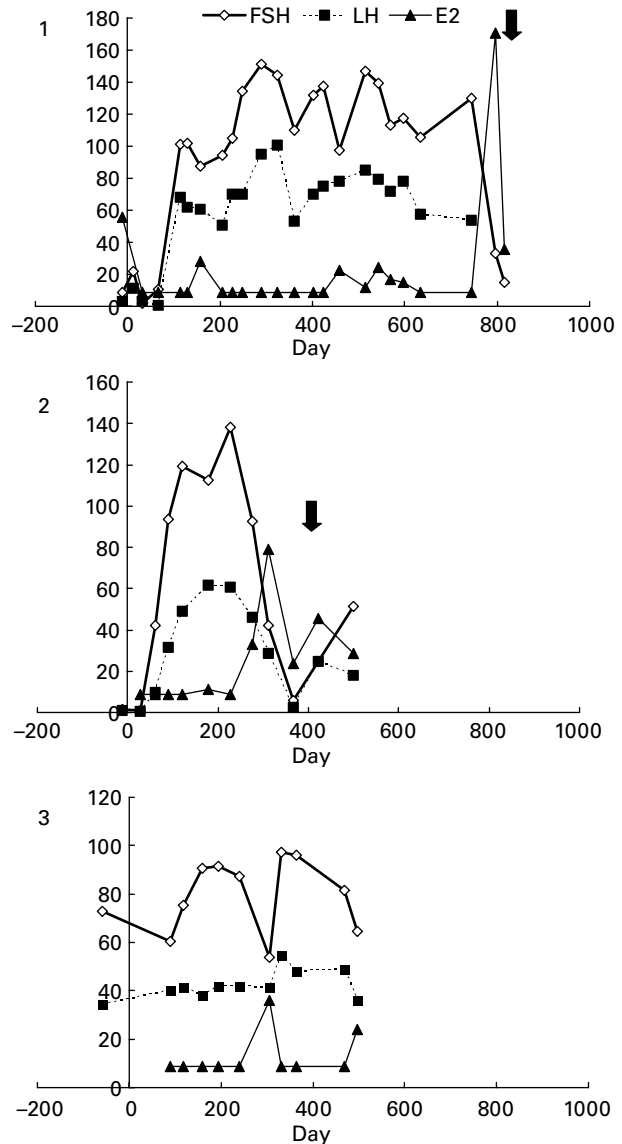


Figure 5 Ovarian function after transplantation. Arrows indicate the day of menstruation recovery.

Table 1 Patient characteristics

	Age ^a	Diagnosis	Duration ^b	Prior Tx	Regimen	TBI dose (Gy)	Donor
1	20	CML	7 years	HU, IFN	Cy/TBI	12	MUD
2	25	CML	6 months	HU	Cy/TBI	12	ISD
3	27	ALL	6 years	CCT	ETP/Cy/TBI	12	PMRD

^aAge at transplantation.

^bDuration from diagnosis to transplantation.

CML = chronic myelogenous leukemia; ALL = acute lymphoblastic leukemia; HU = hydroxyurea; IFN = interferon alpha; CCT = multiple courses of combined chemotherapy; Cy = cyclophosphamide; TBI = total body irradiation; ETP = etoposide; MUD = matched unrelated donor; MSD = HLA-identical sibling donor; PMRD = partially mismatched related donor.

no. 3, serum estradiol was transiently detected above 20 pg/ml, but she did not resume menstruating.

Assessment of basal body temperature and monitoring of follicle growth by sonohysterography would be useful to assess ovarian function of patients undergoing ovarian shielding in TBI.

Actual measurement for phantom

The mean and median actual doses measured by means of the glass dosimeters, which were inserted in the position of the ovaries in the humanoid phantom were between 1.041 and 1.042 Gy, respectively with a prescribed dose of 4 Gy. The range was 9.98–1.096 Gy. The results meant that the average total dose of the ovary was reduced from 12 to 3.123 Gy (74% less).

Discussion

The dose-limiting toxicity of TBI is interstitial pneumonia. Although the incidence of interstitial pneumonia has been significantly reduced by the use of fractionated irradiation compared to single dose irradiation,¹³ 15% of patients still develop interstitial pneumonia after fractionated TBI. Therefore, lung shielding has been investigated to decrease lung toxicity of TBI. In a small nonrandomized study, the incidence of interstitial pneumonia was lower in patients who underwent TBI with lung shielding than in those who did not have shielding.¹⁴ TBI may also affect renal function after transplantation. Therefore, Lawton *et al.*¹⁵ attempted to protect renal function by renal shielding decreasing the total dose to the kidneys from 14 to 12 Gy, and the incidence of late renal dysfunction decreased from 26 to 6%.

The ovary is an organ sensitive to irradiation and the number of antral follicles per ovary has been shown to be reduced by ovarian irradiation in long-term survivors of childhood cancer.¹⁶ Also, Shuck *et al.*¹⁷ reported that all patients who received irradiation to the ovaries at greater than 15 Gy developed hormone failure. The radiation doses that cause 5 and 50% complications to the ovaries are about 3 and 10 Gy, respectively.¹⁸ In this study, the irradiation dose to the ovaries was decreased by 75% by ovarian shielding and the total dose to the ovaries was estimated at about 3 Gy. Considering that recovery of ovarian function is frequently observed after a conditioning regimen of cyclophosphamide at 200 mg/kg only, the combination of cyclophosphamide at 120 mg/kg and TBI at 12 Gy with ovarian shielding should be reasonably protective to the ovaries.

Although patients who have received a conditioning regimen of cyclophosphamide and TBI may have spontaneous recovery of ovarian function long after transplantation, the incidence is less than 15% and it takes a median of 5 years for recovery of ovarian function after transplantation.¹⁹ In this study, regular menstruation recovered in two of the three patients within 2 years after transplantation, showing the protective effect of ovarian shielding. However, spontaneous recovery of ovarian function is rarely seen after a combination of busulfan and cyclophos-

phamide, another major conditioning regimen for leukemia.^{19–22} The risk of persistent alopecia is also more frequent after a busulfan-containing regimen.²³ Therefore, the combination of busulfan and cyclophosphamide should be avoided in young female patients, unless the patient has a condition that precludes the use of TBI, such as previous high-dose irradiation to a major organ.

It remains to be seen whether the recovery of ovarian function in these patients will allow a normal pregnancy and normal live birth. Recently, Carter *et al.*²⁴ analyzed pregnancy outcomes of female recipients and female partners of male recipients after hematopoietic stem cell transplantation. Seven females reported 13 pregnancies and 21 males reported 34 pregnancies. Most pregnancies were uncomplicated and resulted in 40 live births. Pregnancy outcomes were compared with those of their nearest-age siblings. The incidence of miscarriage or stillbirth was similar between the two groups. However, a larger study from the European Group for Blood and Marrow Transplantation²⁵ showed that the incidences of caesarean section, preterm delivery, and low birthweight singleton birth offspring were higher compared to those in the normal population. Therefore, pregnancies in transplant recipients should be treated as high risks for maternal and fetal complications. In addition, the freezing of ovarian tissues or embryos might have a role as a back-up method of fertility treatment for the patient with ovarian failure after TBI.

We have shown that ovarian function could be preserved by ovarian shielding. However, a longer follow-up is needed to know whether this will allow normal pregnancy and delivery. Also needed is a larger study to evaluate the possible risk of increased relapse of leukemia after transplantation. In addition, the freezing of ovarian tissues or embryos might have a role as a back-up method of fertility preservation for patients who undergo hematopoietic stem cell transplantation and should be evaluated in the future.

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