Fertility recovery and pregnancy after allogeneic hematopoietic stem cell transplantation in Fanconi anemia patients

Samir K. Nabhan,[1] Marco A. Bitencourt,[2] Michel Duval,[3] Manuel Abecasis,[4] Carlo Dufour,[5] Karim Boudjedir,[1] Vanderson Rocha,[1] Gérard Socié,[6] Jakob Passweg,[7] Kumiko Goi,[8] Jean Sanders,[9] John Snowden,[10] Hiromasa Yabe,[11] Ricardo Pasquini,[2] and Eliane Gluckman[1] on behalf of Aplastic Anaemia Working Party, EBMT

[1]Eurocord Office, Hôpital Saint Louis, Paris, France, [2]Hospital de Clínicas, Curitiba, Brazil, [3]Hôpital Sainte-Justine, Montreal, QC H1T 1C9, Canada, [4]Instituto Português de Oncologia, Lisbon, Portugal, [5]Istituto Giannina Gaslini and SAAWP, Genova, Italy, [6]Hôpital Saint Louis, Paris, France and SAAWP, [7]Hôpitaux Universitaires de Genève, Geneva, Switzerland and SAAWP, [8]University of Yamanashi, Yamanashi, Japan, [9]Fred Hutchinson Cancer Research Center, Seattle, WA, USA, [10]Royal Hallamshire Hospital, Sheffield, UK, and [11]Tokai University Hospital, Kanagawa, Japan

ABSTRACT

Reduced fertility is one clinical manifestation among other well known Fanconi anemia features. Most recipients of allogeneic hematopoietic stem cell transplantation suffer from secondary infertility owing to gonadal damage from myeloablative conditioning. In order to evaluate the rate of pregnancy in Fanconi anemia transplanted patients, we performed a retrospective analysis of female patients transplanted in 15 centers from 1976 to 2008. Among 578 transplanted Fanconi anemia patients, we identified 285 transplanted females of whom 101 patients were aged 16 years or over. Ten became pregnant (4 twice). Before hematopoietic stem cell transplantation all had confirmed Fanconi anemia diagnosis. Median age at transplantation was 12 years (range 5-17 years). Conditioning regimen consisted of cyclophosphamide with or without irradiation. During follow up, 5 of 10 patients presented signs of ovarian failure. Among those, 2 patients spontaneously recovered regular menses, and 3 received hormonal replacement therapy. Pregnancy occurred from four to 17 years after hematopoietic stem cell transplantation. Three patients had preterm deliveries, one patient had a hysterectomy for bleeding. All 14 newborns had normal growth and development without congenital diseases. In conclusion, recovery of normal ovarian function and a viable pregnancy is a realistic but relatively rare possibility even in Fanconi anemia patients following hematopoietic stem cell transplantation. Mechanisms of fertility recovery are discussed.

Key words: pregnancy, Fanconi anemia, bone marrow transplantation.

Introduction

Fanconi anemia (FA) is a rare autosomal recessive disorder that belongs to the group of chromosomal instability syndromes. FA cells of all organ tissues are hypersensitive to DNA cross-linking agents and to oxygen, and have cell cycle abnormalities. Its clinical features include progressive bone marrow failure, skeletal and urogenital malformations, skin hyperpigmentation, increased susceptibility to malignancy and reduced fertility.[1,2] As base-line, in non-transplanted FA patients who reach adult age, the rate of successful pregnancy has been estimated at 15%.[3] Hematopoietic stem cell transplantation (HSCT), using an adapted attenuated conditioning regimen, represents the only curative therapy capable of restoring normal hematopoiesis in patients with FA. In FA, the conditioning has been reduced because of the toxic effect of alkylating agents.[4] For this reason, most centers use low-dose cyclophosphamide with or without irradiation assuming that this is the equivalent of a myeloablative conditioning considering the DNA repair defect related to the genetic mutation.[5] In order to describe fertility in female patients after transplant, we designed a multicenter retrospective analysis of posttransplant pregnancy in FA patients.

Design and Methods

Fifteen transplant centers from 10 different countries participated in this survey. Centers that reported pregnancies after transplant were asked to fill in a specific disease form and the MED-B EBMT form including detailed information on diagnosis, transplant procedure, gynecological and obstetrics follow up.

From 1976 to 2008, these 15 centers had transplanted 578 FA patients. Among them, there were 285 female patients but only 101 reached at least 16 years and were potentially at risk of pregnancy. The total number of reported pregnancies was 14 and we present the detailed case reports of 10 patients from 8 different centers, since 4 of them became pregnant twice after HSCT. Four patients have been previously reported (patients 4, 5, 6 and 9).[6-8]

Results and Discussion

Patient characteristics are summarized in Table 1. Their median age at diagnosis was eight years (range 4-17). They presented with different phenotypes of the disease: some had only classical hallmarks such as *café au lait* spots and thumb abnormalities but others presented more severe abnormalities such as deafness, congenital hip dysplasia and trachea-esophageal fistula. All diagnoses were confirmed by increased chromosomal breakage following exposure to DNA cross-linking agents such as mitomycin C or diepoxybutane (DEB). Mutation analysis was not performed at that time.

One of our 10 patients could be considered to have an extensive malformation syndrome, defined by the presence of abnormalities in at least 3 different sites, involving the head, limbs, gastro-intestinal, urogenital and cardiovascular abnormalities. Skin abnormalities were not included in this classification.

All patients presented with pancytopenia and non-severe or severe marrow aplasia. Five had received previous treatment with androgens and corticosteroids, with temporary or no response before transplant and were heavily transfused.

At transplantation, the median age of patients was 12 years (range 5-19). Donors were HLA matched siblings in 8 cases and unrelated matched bone marrow in 2 (patients 4 and 9). Conditioning is described in Table 1, most patients received low-dose cyclophosphamide and 4-6 Gy irradiation involving the ovaries, 3 patients received high-dose cyclophosphamide without irradiation. Graft *versus* host disease (GVHD) prophylaxis consisted of cyclosporine and methotrexate or cyclosporine alone in 2 cases. Median follow up (FU) was 14 years (range 9-20). All patients engrafted with full long-term hematologic reconstitution. Only one patient developed grade II acute skin GVHD responsive to prednisone. Finally, no patient with pregnancy had severe chronic GVHD.

Regarding their gynecological follow up, 5 patients had spontaneous puberty development after transplant. Among those 5 patients, 4 had received 4-6 Gy TBI or TAI. The other 5 patients were diagnosed with ovarian failure characterized by amenorrhea, high levels of follicle-stimulating hormone and luteinizing hormone and low levels of oestradiol. One patient also had uterine and ovarian atrophy and another presented with symptomatic menopause. However, only 3 received hormonal replacement therapy (HRT) with estrogens and progestagens for 2-4 years before developing regular menses and becoming pregnant. Two patients recovered fertility without any treatment.

Their median age at pregnancy was 21 years (range 18-23) and the median time from transplant to pregnancy was eight years (range 4-16). During pregnancy, the patients were followed regularly with blood counts, hepatic, cardiac and renal function tests; all were normal except for one patient who developed preeclampsia with hypertension and transient renal failure which resolved after delivery. No spontaneous abortion was observed among these 10 patients. Term delivery was observed in 11 cases. Three patients had preterm delivery, and 2 had a cesarean section. One patient had preeclampsia at 27 weeks. Only one post partum complication was observed with an uncontrolled uterine hemorrhage resulting in hysterectomy.

There were 14 live births (9 males and 5 females). The 27-week premature baby weighed 870 g at birth and was kept in an intensive care unit during her first 45 days of life. Ultimately her development was normal. Currently, they are all healthy children with normal growth and development without congenital abnormalities and normal blood cytogenetics.

The patients did not receive any additional treatment after delivery and their hematologic status remained stable during and after pregnancy. Unfortunately, one patient presented with tongue and esophagus squamous cell carcinoma and died as a result of this malignant complication 20 years after transplant and 15 years after her first pregnancy. The other 9 mothers are alive and well.

Pregnancy following HSCT after myeloablative conditioning is considered to be a rare event.[9-14] In the largest multicenter European retrospective study on pregnancy outcomes after transplant, 232 out of 37,362 autologous and allogeneic HSCT patients (0.6%) conceived after HSCT.[9] In Seattle, among 1,522 disease free survivors following marrow transplant between August 1971 and January 1992, 41 female patients and partners of 35 male patients had 146 pregnancies after transplant.[10] The dose of irradiation required to destroy 50% of immature oocytes has been estimated at 2 Gy[15]. Reduced ovarian volume and low inhibin B and anti-Mullerian hormone concentrations in survivors with regular menses may be markers of incipient ovarian failure.[15] In addition, uterine damage manifested by impaired growth and blood flow is a likely consequence of pelvic irradiation.[11] However, the true incidence of pregnancy and pregnancy-related complications after transplantation are unknown.[12-14]

Fanconi anemia patients posttransplant are supposed to have an even lower probability of fertility recovery compared to other transplant groups and three distinct factors can be incriminated: hypergonadotropic hypogonadism related to FA status, radiotherapy and greater toxicity from chemotherapy in cells presenting increased sensitivity to DNA damage. However, several isolated case reports of FA patients pregnancy post-HSCT have been published.[6-8] Moreover, experimental studies suggested that FA complementation groups are required for mitotic proliferation of primordial germ cells and also that hormonal problems and sterility might be associated to specific mutations.[16,17] Unfortunately, data regarding the complementation group or additional mutations were not available for most of the described patients in our study.

The magnitude of ovarian damage in females who received HSCT is drug-specific and dose-related. Furthermore, the age at exposure has a fundamental role as younger women need higher doses of irradiation and/or alkylating agents to produce irreversible ovarian failure. It is also known that few patients may recover gonad function spontaneously even receiving conditioning with CY and/or TBI after varying intervals of time.[18,19]

The increased sensitivity to DNA-damaging agents in FA requires the use of an attenuated conditioning regimen before HSCT to avoid lethal toxicity. Nevertheless, patients with FA who undergo HSCT still present toxicity comparable to that of patients transplanted for other diseases given conventional conditioning regimens. Our data do not authorize any recommendation for conditioning Fanconi anemia patients; most regimens now use fludarabine which decreases toxicity and improves engraftment. In our own series, use of fludarabine and low-dose cyclophosphamide gives good results in term of engraftment and long-term survival after HLA identical sibling

bone marrow transplant (Nabhian *et al.*, unpublished results, 2010). For unrelated bone marrow transplantation, most centers use the association of fludarabine, low-dose cyclophosphamide and TBI 2 Gy as described by the Minnesota group.[19-24]

Guardiola *et al.* suggested a significant correlation between survival, toxicity after transplant and extent of malformations of FA[25]. In this context, only one of our 10 patients could be considered to have an extensive malformation syndrome and none of them had developed chronic graft-*versus*-host-disease.

In most mammalian species, the production of ovarian oocytes is thought to cease after birth. However, this belief has been challenged by research indicating that female gonad have regenerative activity in juvenile and adult mice *in vivo*. Johnson *et al.* published a hypothesis postulating that fertility recovery after transplant might be the result of germ line stem cells supplied by the donor bone marrow.[26, 27] To test this hypothesis, we decided to analyze the genetic origin of the child of one of our FA patients who consented to a genetic analysis among mother, daughter and donor. These results showed clearly the genetic relationship between the transplanted patient and her daughter, excluding the possibility of germ cell transmission from the donor. This suggested that fertility recovery after BMT could only result from incomplete depletion of the ovarian follicle reserve.[28] Other studies have shown that bone marrow cells or other normally circulating cells are not involved in the formation of mature ovulated oocytes and that instead of germ line stem cells, putative thecal stem cells could be isolated from new born mouse ovaries. Therefore, the presence of female germ cells in mammals is still very controversial. More recently, Zou *et al.* identified and confirmed the presence of female germline stem cells in postnatal mammalian ovaries.[29]

Our findings also suggest that, just as for other diagnosis,[30] if pregnancy does occur in FA patients after transplant, outcome is likely to be favorable.

Obviously, numerous questions remain unanswered: is the pregnancy frequency of FA patients comparable to other transplant groups? Should our low intensity conditioning regimen be considered non-ablative for the ovaries? Is there a mechanism of fertility repair involved? And if this is so, what would be the donor bone marrow cells contribution to that? If the hypothesis of recovery of germ cells provided by donor cells is highly improbable, we cannot exclude the possibility that donor cells con-

Table 1. Cases of transplanted Fanconi's anemia and pregnancy.

	Fanconi Anemia diagnosis		HSCT				Pregnancy			
Case	Age	Physical examination	Age	Conditioning Regimen	Ovarian Failure	HRT	Age	Delivery and Outcome	Baby outcome	Mother's status
1	8 y	Café au lait spots, typical face and microphtalmia	14 y	CY 20 mg/Kg TBI 6 Gy + ATG	Yes	Yes	21 y	Normal	Normal	Alive
2	5 y	Low birth weight, growth retardation, café au lait spots, typical face and thumb abnormalities	6 y	CY 200 mg/Kg	No	No	18 y	Normal	Normal	Alive
3	5 y	Low birth weight, typical face, thumb abnormalities and pelvic kidney	5 y	CY 20 mg/Kg TAI 5 Gy	Yes	Yes	21 y	Normal - Atonic uterus and hysterectomy	Normal	Alive
4	7 y	Café au lait spots, typical face, microcephalia, congenital hip dysplasia	12 y	CY 20 mg/Kg TAI 5Gy + ATG	Yes	No	21 y	Cesarean 27 w - preeclampsia	870 g	Alive
5	17 y	Normal	17 y	CY 20mg/Kg TAI 5Gy + ATG	Yes	Yes	21 y	Normal	Normal	Alive
6	9 y	Low birth weight, hypopigmentation, typical face, hyperpigmentation and congenital tracheal-esophageal fistula	14 y	CY 200 mg/Kg	Yes	No 24 y Normal	20 y	Normal Normal	Normal Squamous Cell Carcinoma	Died, 35 y
7	8 y	Café au lait spots	6 y	CY 200 mg/Kg	No	No	20 y	Normal 24 y	Normal Normal	Alive Normal
8	5 y	Typical face	9 y	CY 200mg/Kg TBI 5Gy + ATG	No	No	22 y 24 y	Cesarean 34 w - placental abnormality Normal 33 w	2010 g 1930 g	Alive
9	4 y	Café au lait spots, hypopigmentation and thumb abnormalities	12 y	CY 40 mg/Kg + FLU 180 mg/m² TAI 4,5 Gy + ATG	No	No	19 y	Normal	Normal	Alive
10	10 y	Polydactyly	19 y	CY 20 mg/Kg + AraC 24 g/m² TBI 6 Gy + ATG	No	No	23 y	Normal	Normal	Alive

HRT: hormonal replacement therapy; CY: cyclophosphamide. TBI: total body irradiation; TAI: thoraco-abdominal irradiation; ATG: anti-thymocyte globulin; FLU: fludarabine; Ara-C: cytarabine.

tribute to the repopulation of granulosa secreting factors which might repair damaged oocytes. In FA, somatic mosaicism is frequent; it acts as a natural gene therapy showing that genetic correction confers a selective advantage to FA stem cell, a process which might restore potential germ line stem cells.[31,32] We can speculate that oocyte mosaicism has occurred in the patients in whom spontaneous recovery of ovarian function developed after HSCT.

For now, our conclusions are that recovery of normal ovarian function and a viable pregnancy is a realistic possibility even in FA patients following allogeneic HSCT. How best to estimate ovarian reserve clinically is highly controversial. Passive assessments of ovarian reserve include measurements of serum follicle stimulating hormone (FSH), oestradiol (E(2)), anti-Mullerian hormone (AMH) and inhibin B. Ultrasound determination of antral follicle count (AFC), ovarian vascularity and ovarian volume can also have a role.[11,15]

On this basis, we recommend that long-term follow up should always include: regular hormonal assessment, replacement therapy to prevent early and late unwanted effects after SCT and finally, patient information about puberty and fertility. Counseling before transplant and during follow up is very important; the patients should be followed in a gynecology unit specialized in sterility in order to propose other methods of procreation including cryopreservation of ovarian tissues before transplant or new techniques of *in vitro* fertilization.

Further analyses must include a fertility rate comparison of transplanted and non-transplanted FA females and future prospective studies might determine the influence of systemic factors related to transplantation on female fertility recovery.

References

1. D'Andrea AD, Grompe M. Molecular biology of Fanconi anemia: implications for diagnosis and therapy. Blood. 1997;90(5):1725-36.
2. Young NS, Alter BP. Clinical features of Fanconi's anemia. In: Young NS, Alter BP, eds. Aplastic Anemia Acquired and Inherited. Philadelphia, PA: Saunders 1994.p.275-309.
3. Alter BP, Frissora CL, Halperin DS, Freedman MH, Chitkara U, Alvarez E, et al. Fanconi's anaemia and pregnancy. Br J Haematol. 1991;77(3):410-8.
4. Berger R, Bernheim A, Gluckman E. In vitro effect of cyclophosphamide metabolites on chromosomes of Fanconi's anaemia patients. Br J Haematol. 1980;45(4):565-8.
5. Gluckman E, Auerbach AD, Horowitz MM, Sobocinski KA, Ash RC, Bortin MM, et al. Bone marrow transplantation for Fanconi anemia. Blood. 1995;86(7):2856-62.
6. Dalle JH, Huot C, Duval M, Rousseau P, Francoeur D, Champagne J, et al. Successful pregnancies after bone marrow transplantation for Fanconi anemia. Bone Marrow Transplant. 2004;34(12):1099-100.
7. Goi K, Sugita K, Nakamura M, Inukai T, Honna H, Hirose K, et al. Natural pregnan-

cy and delivery after allogeneic bone marrow transplantation in a Fanconi anaemia patient. Br J Haematol. 2006;135(3):410-1.
8. Morris ES, Darbyshire P, Fairlie F, Dalley CD, Snowden JA. Two natural pregnancies following allogeneic transplantation for Fanconi anaemia. Br J Haematol. 2008;140(1):113.
9. Salooja N, Szydlo RM, Socié G, Rio B, Chatterjee R, Ljungman P, et al. Pregnancy outcomes after peripheral blood or bone marrow transplantation: a retrospective survey. Lancet. 2001;358(9278):271-6.
10. 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(7):3045-52.
11. Liu J, Malhotra R, Voltarelli J, Stracieri AB, Oliveira L, Simoes BP, et al. Ovarian recovery after stem cell transplantation. Bone marrow Transplantation. 2008;41(3):275-8.
12. Critchley HO, Wallace WH. Impact of cancer treatment on uterine function. J Natl Cancer Inst Monogr. 2005;(34):64-8.
13. Green DM, Kawashina T, Stovall M, Leisenring W, Sklar CA, Mertens AC, et al. Fertility of female survivors of childhood cancer: a report form the childhood cancer survivor group. J Clin Oncol. 2009; 27(16):

2677-85.
14. Nakagawa K, Kanda Y, Yamashita H Nakagawa S, Sasano N, Ohtomo K, et al. Ovarian shielding allows ovarian recovery and normal birth in female hematopoietic SCT recipients undergoing TBI. Bone Marrow Transplant. 2008;42(10):697-9.
15. Sills ES, Alper MM, Walsh AP. Ovarian reserve screening in infertility: practical applications and theoretical directions for research. Eur J Obstet Gynecol Reprod Biol. 2009;146(1):30-6.
16. Nadler JJ, Braun RE. Fanconi anemia complementation group C is required for proliferation of murine primordial germ cells. Genesis. 2000;27(3):117-23.
17. Wong JC, Alon N, Mckerlie C, Huang JR, Meyn MS, Buchwald M, et al. Targeted disruption of exons 1 to 6 of the Fanconi Anemia group A gene leads to growth retardation, strain-specific microphthalmia, meiotic defects and primordial germ cell hypoplasia. Hum Mol Genet. 2003;12(16):2063-76.
18. Cohen A, Békássy AN, Gaiero A, Faraci M, Zecca S, Tichelli A, et al. Endocrinological late complications after hematopoietic SCT in children. Bone Marrow Transplant. 2008; 41 (Suppl 2):S43-8.
19. Thibaud E, Rodriguez-Macias K, Trivin C, Esperou H, Michon J, Brauner R. Ovarian

function after bone marrow transplantation during childhood. Bone Marrow Transplant. 1998;21(3):287-90.

20. Tan PL, Wagner JE, Auerbach AD, Defor TE, Slungaard A, Macmillan ML. Successful engraftment without radiation after fludarabine-based regimen in Fanconi anemia patients undergoing genotypically identical donor hematopoietic cell transplantation. Pediatr Blood Cancer. 2006;46(5):630-6

21. Wagner JE, Eapen M, MacMillan ML, Harris RE, Pasquini R, Boulad F, et al. Unrelated donor bone marrow transplantation for the treatment of Fanconi anemia. Blood. 2007;109(5):2256-62.

22. Chaudhury S, Auerbach AD, Kernan NA, Small TN, Prockop SE, Scaradavou A, et al. Fludarabine-based cytoreductive regimen and T-cell-depleted grafts from alternative donors for the treatment of high-risk patients with Fanconi anaemia. Br J Haematol. 2008;140(6):644-55.

23. Motwani J, Lawson SE, Darbyshire PJ. Successful HSCT using non radiotherapy-based conditioning regimens and alternative donors in patients with Fanconi anaemia--experience in a single UK centre. Bone Marrow Transplant. 2005;36(5):405-10.

24. MacMillan ML, Wagner JE. Haematopoietic cell transplantation for Fanconi anemia. When and how? Brit J of Haematol. 2010; 149(1):14-21.

25. Guardiola Ph, Pasquini R, Dokal I, Ortega JJ, van Weel-Sipman M, Marsh JC, et al. Outcome of 69 allogeneic stem cell transplantations for Fanconi anemia using HLA-matched unrelated donors: a study on behalf of the European Group for Blood and Marrow Transplantation. Blood. 2000; 95(2):422-9.

26. Johnson J, Bagley J, Skaznik-Wikiel M, Lee HJ, Adams GB, Niikura Y, et al. Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. Cell. 2005;122(2):303-15.

27. Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature. 2004;428(6979):145-50.

28. Veitia RA, Gluckman E, Fellous M, Soulier J. Recovery of female fertility after chemotherapy, irradiation, and bone marrow allograft: further evidence against massive oocyte regeneration by bone marrow-derived germline stem cells. Stem Cells. 2007;25(5):1334-5.

29. Zou K, Yuan Z, Yang Z, Luo H, Sun K, Zhou L, et al. Production of offspring from a germline stem cell line derived from neonatal ovaries. Nat Cell Biol. 2009; 11(5):631-6.

30. Carter A, Robison LL, Francisco L Smith D, Grant M, Baker KS, et al. Prevalence of conception and pregnancy outcomes after hematopoietic cell transplantation: report from the Bone Marrow Transplant Survivor Study. Bone Marrow Transplant. 2006; 37(11):1023-9.

31. Pinto FO, Leblanc T, Chamousset D, Le Roux G, Brethon B, Cassinat B, et al. Diagnosis of Fanconi anemia in patients with bone marrow failure. Haematologica. 2009;94(4):487-5.

32. Raya A, Rodriguez-Piza I, Guenechea H, Vassena R, Navarro S, Barrero MJ, et al. Disease corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. Nature. 2009; 460(7251):53-9.

- 238 -

LETTER TO THE EDITOR

Alternative donor marrow transplantation in children with aplastic anemia using low-dose irradiation and fludarabine-based conditioning

Allogeneic BMT is a curative treatment for patients with severe aplastic anemia (SAA). In particular, BMT from a HLA-identical sibling is an established treatment for children with acquired SAA, but the results of alternative donor (AD) transplantation have been less favorable because of the high rates of graft failure and severe acute GVHD.[1] However, recent data have shown improvements in the survival of patients receiving unrelated donor (UD) transplantation possibly due to better donor matching attributable to the use of high-resolution HLA typing data.[2] Two major studies have been performed in the setting of UD transplantation for SAA. The first is a Japanese study that reported 154 SAA patients undergoing UD transplantation using CY/TBI/anti-thymocyte globulin (ATG) and also CY/limited field irradiation/ATG: 11% showed rejection whereas 56% survived.[3] The second involves a group in the USA, which reported a study that tested the deescalating doses of TBI (from 6 to 2 Gy) in combination with CY and ATG in UD transplantation for SAA, and showed the best outcomes in patients receiving 2 Gy, with 8 of 13 patients surviving.[4] The current series presents the results of AD transplantation in 13 SAA patients undergoing BMT with the addition of low-dose irradiation to fludarabine (Flu)-based conditioning.

The characteristics of the 13 SAA patients who underwent AD BMT at Tokai University Hospital between September 2001 and May 2008 are shown in Table 1. Of these 13 patients, 12 received prior immunosuppressive therapy, and all were transfusion dependent at the time of AD transplantation. Specifically, the donors were a UD for 11 patients and an HLA mismatched family member for 2 patients. The HLA-incompatible loci are described in Table 1 and detailed data of HLA typing were received from the Japan Marrow Donor Program. Follow-up data were collected on 30 June 2010. Patients were conditioned with 3 Gy thoracoabdominal irradiation (TAI) on day −6, Flu 25 mg/m^2 once daily (i.v.) on days −5, −4, −3 and −2 (total dose 100 mg/m^2), CY 750 mg/m^2 once daily (i.v.) on days −5, −4, −3 and −2 (total dose 3000 mg/m^2) and ATG (thymoglobulin; Genzyme, Cambridge, MA, USA) 1.25 mg/kg once daily (i.v.) on days −5, −4, −3 and −2 (total dose 5 mg/kg) Unmanipulated BM was infused on day 0. Ovary and testis shielding during TAI was performed for all patients, and thyroid gland shielding was provided for five successive patients from patient 9 onwards to prevent secondary thyroid cancer. GVHD

prophylaxis was carried out with short-term MTX (15 mg/m^2 on day 1; 10 mg/m^2 on days 3, 6 and 11) and continuous i.v. infusion of tacrolimus 0.02–0.03 mg/kg. Mycophenolate mofetil 15 mg/kg per day (days 14–42) was added for patients who received antigen mismatched donor marrow. All patients were administered G-CSF from day 5 until their neutrophil counts exceeded 1.5 × 10^9/L.

The clinical outcomes of BMT are summarized in Table 2. All patients achieved neutrophil engraftment (>0.5 × 10^9/L) at a median of day +20 (range, 12–25 days), but one patient showed rejection 31 days after transplantation caused by hemophagocytic syndrome following sepsis. This patient received a second successful BMT from his haploidentical mother with Flu, melphalan and TAI conditioning. The day 30 chimerism by STR analysis of BM mononuclear cells was 100% in all but one patient (96%), and that of PBMCs was 100% in all patients more than 12 months after BMT. None of the patients showed grade III/IV regimen-related toxicity using the Bearman's criteria at any evaluation point. All patients are alive and well; their hemograms are normal with complete donor cell engraftment, and with a Lansky/Karnofsky score of 100% at a median of 64 months following BMT. There were no malignancies observed during the follow-up period.

Both graft rejection and severe acute GVHD were the major causes of failure in the AD BMT for SAA, particularly in mismatched UD.[5] The risk of rejection can be reduced with the addition of 8–10 Gy irradiation,[3] but this is associated with delayed effects on growth, pulmonary toxicity and secondary malignancies.[6] One approach to improve conditioning by the European Group for Blood and Marrow Transplantation SAA working party has been the substitution of TBI with Flu to reduce the risk of secondary tumors.[7] The results are also less favorable in older patients aged more than 15 years, with 61% survival and 32% rejection. Although radiation increases the risk of secondary tumors, very low dose irradiation (2–3 Gy) may reduce such risk, and this procedure is used in many regimens for UD transplantations such as in our study to achieve a stable engraftment.[8] We previously reported that a Flu-based conditioning regimen (low-dose TAI/Flu/CY/ATG) that was used in 27 Fanconi anemia patients for AD transplantations led to successful engraftment in 25 of 26 evaluable patients without severe toxicity.[9] The advantage of Flu may be its strong cytotoxic activity against lymphocytes, which consistently prolongs immunosuppression, thus facilitating the engraftment of hematopoietic stem cells. The combination of Flu and low-dose TAI might establish a definitive engraftment in SAA patients transplanted from an AD.

Table 1   Patient and donor characteristics

Patient no.	Age (years)	Sex	Time from diagnosis to BMT (months)	Prior treatment	Number of pretransplant transfusions		Donor type	Donor matching	
					RBC	Platelet		Identity of HLA allele	Mismatched HLA allele
1	13	F	116	ATG, HDMP, CSA, G-CSF, androgen	10	7	UD	6/10	A, C, DRB1, DQB1
2	16	M	47	ATG, HDMP, CSA, androgen	20	5	UD	8/10	C, C
3	10	M	79	ATG, HDMP, CSA, androgen	17	27	UD	7/10	A, C, DQB1
4	15	F	18	ATG, HDMP, CSA	22	10	UD	7/10	A, DRB1, DQB1
5	11	M	21	ATG × 2, HDMP, CSA	22	29	UD	6/10	C, DRB1, DQB1, DQB1
6	4	F	14	ATG × 2, HDMP, CSA	29	66	Mother	4/8	A, B, C, DRB1
7	5	M	32	ATG × 2, HDMP, CSA	10	2	UD	7/10	C, DRB1, DQB1
8	16	M	64	ATG × 2, HDMP, CSA	29	67	UD	7/10	A, C, DQB1
9	15	M	101	ATG × 2, HDMP, CSA	75	46	UD	6/6	No
10	17	M	115	CSA, androgen	9	2	UD	8/10	A, C
11	5	F	17	ATG, HDMP, CSA, G-CSF	20	73	UD	6/6	No
12	14	F	93	None[a]	4	3	Brother	5/6	A
13-1	15	M	35	ATG, HDMP, CSA, androgen	48	7	UD	6/8	B, C
13-2	—	—	—	—	—	—	Mother	4/8	A, B, C, DRB1

Abbreviations: ATG = anti-thymocyte globulin; F = female; HDMP = high-dose methylprednisolone; M = male; UD = unrelated donor.
[a]The status at diagnosis of patient 12 was mild aplastic anemia and she developed very severe aplastic anemia 91 months after diagnosis with recurrent infection.

Table 2   Outcomes of BMT

Patient no.	Status at BMT	Infused TNC (× 10⁸/kg)	ANC after BMT >0.5 × 10⁹/L	GVHD		Chimerism status (% donor cells)		Complication	Survival (months)
				Acute	Chronic	BM At the day 30 chimerism test	PB At the last chimerism test (months after BMT)		
1	SAA	2.07	20	0	No	100	100 (58)	No	>94
2	SAA/+8	3.05	14	0	No	100	100 (36)	No	>75
3	SAA	5.05	20	0	Yes	100	100 (35)	No	>73
4	SAA	3.92	21	0	No	100	100 (53)	No	>69
5	SAA	3.93	15	0	No	100	100 (29)	No	>69
6	SAA	3.95	18	I	Yes	100	100 (27)	No	>68
7	SAA	3.58	21	0	No	100	100 (24)	No	>64
8	SAA	1.12	25	II	No	100	100 (18)	Sepsis	>59
9	SAA	2.67	15	0	No	96	100 (20)	No	>48
10	SAA	1.60	21	I	No	100	100 (27)	EB duodenitis	>47
11	SAA	4.29	21	0	No	100	100 (33)	Sepsis	>43
12	VSAA	3.02	12	0	No	100	100 (16)	No	>34
13-1	VSAA	2.94	16	NA	0	0	0 (0.7)	Sepsis, HPS	>27
13-2	—	5.13	17	0	Yes	100	100 (14)	No	—

Abbreviations: EB = Epstein–Barr virus; HPS = hemophagocytic syndrome; NA = not applicable; SAA = severe aplastic anemia; TNC = total nucleated cells; VSAA = very severe aplastic anemia; +8 = trisomy 8 chromosome positive.

In addition, the total dose of CY ($3000 \, mg/m^2$) was reduced by about half from the standard dose at 200 mg/kg for SAA patients to reduce cardiac complications,[10] as many SAA patients have cardiac dysfunction due to persistent anemia and/or iron accumulation.

The second goal of this series was to prevent severe acute GVHD after AD transplantation including mismatched transplants. None of the patients in our study developed grade III/IV acute GVHD. The low-dose ATG administered prior to transplantation might exert a preventive effect on GVHD in conjunction with tacrolimus and short-term MTX.[9]

Taken together, this series demonstrated that a conditioning regimen containing low-dose irradiation and a minimum dose of Flu, CY and ATG could enable the successful engraftment of AD marrow in children with SAA. Long-term follow-up and larger studies are warranted to confirm the high engraftment rates and absence of severe GVHD and secondary malignancies.

Conflict of interest

The authors declare no conflict of interest.

M Yabe[1], T Shimizu[2], T Morimoto[2], T Koike[2],
H Takakura[2], E Suganuma[2], N Sugiyama[2], S Kato[1] and
H Yabe[1]

[1]*Department of Cell Transplantation, Tokai University
Hospital, Isehara, Kanagawa, Japan and*
[2]*Department of Pediatrics, Tokai University Hospital,
Isehara, Kanagawa, Japan*
E-mail: yabeh@is.icc.u-tokai.ac.jp

References

1 Hows J, Szydlo R, Anasetti C, Camitta B, Gajewski J, Gluckman E. Unrelated donor marrow transplants for severe acquired aplastic anemia. *Bone Marrow Transplant* 1992; **10** (Suppl): 102–106.

2 Maury S, Balère-Appert ML, Chir Z, Boiron JM, Galambrun C, Yakouben K *et al.* Unrelated stem cell transplantation for severe acquired aplastic anemia: improved outcome in the era of high-resolution HLA matching between donor and recipient. *Haematologica* 2007; **92**: 589–596.

3 Kojima S, Matsuyama T, Kato S, Kigasawa H, Kobayashi R, Kikuta A *et al.* Outcome of 154 patients with severe aplastic anemia who received transplants from unrelated donors: the Japan Marrow Donor Program. *Blood* 2002; **100**: 799–803.

4 Deeg HJ, Amylon ID, Harris RE, Collins R, Beatty PG, Feig S *et al.* Marrow transplants from unrelated donors for patients with aplastic anemia: minimum effective dose of total body irradiation. *Biol Blood Marrow Transplant* 2001; **7**: 208–215.

5 Perez-Albuerne ED, Eapen M, Klein J, Gross TJ, Lipton JM, Baker KS *et al.* Outcome of unrelated donor stem cell transplantation for children with severe aplastic anemia. *Br J Haematol* 2008; **141**: 216–223.

6 Rizzo JD, Curtis RE, Socié G, Sobocinski KA, Gilbert E, Landgren O *et al.* Solid cancers after allogeneic hematopoietic cell transplantation. *Blood* 2009; **113**: 1175–1183.

7 Bacigalupo A, Locatelli F, Lanino E, Marsh J, Socié G, Maury S *et al.* Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party. *Bone Marrow Transplant* 2005; **36**: 947–950.

8 Vassiliou GS, Webb DKH, Pamphilon D, Knapper S, Veys PA. Improved outcome of alternative donor marrow transplantation in children with severe aplastic anaemia using a conditioning regimen containing low-dose total body irradiation, cyclophosphamide and Campath. *Br J Haematol* 2001; **104**: 701–705.

9 Yabe H, Inoue H, Matsumoto M, Hamanoue S, Koike T, Ishiguro H *et al.* Allogeneic haematopoietic cell transplantation from alternative donors with a conditioning regimen of low-dose irradiation, fludarabine and cyclophosphamide in Fanconi anaemia. *Br J Haematol* 2006; **134**: 208–212.

10 Goldberg MA, Antin JH, Guinan EC, Rappeort JM. Cyclophosphamide cardiotoxicity: an analysis of dosing as a risk factor. *Blood* 1986; **68**: 1114–1118.

ORIGINAL ARTICLE

High incidence of fatty liver and insulin resistance in long-term adult survivors of childhood SCT

Y Tomita[1,5], H Ishiguro[1,5], Y Yasuda[1], H Hyodo[1], T Koike[1], T Shimizu[1], T Morimoto[1], K Hattori[1], M Matsumoto[1], H Inoue[1], H Yabe[1,2], M Yabe[1], O Shinohara[1], S Kojima[3], T Minemura[4] and S Kato[1,2]

[1]Department of Pediatrics, Tokai University School of Medicine, Isehara, Kanagawa, Japan; [2]Department of Cell Transplantation & Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan; [3]Division of Gastroenterology, Department of Internal Medicine, Tokai University School of Medicine, Isehara, Japan and [4]Department of Clinical Laboratory, Tokai University School of Medicine, Isehara, Japan

Overweight/obesity among adult survivors of childhood SCT has been considered to be predictive of eventual development of metabolic abnormalities. Fatty liver is increasingly recognized as a major cause of liver-related morbidity and mortality in the general population. However, the real incidence of fatty liver in adult survivors of SCT has not been fully elucidated. We determined whether adult survivors are at risk for overweight/obesity, metabolic abnormalities and fatty liver and whether these risks are associated with cranial radiotherapy (CRT) before SCT. Among the 51 patients (30 males), only two male patients were overweight/obese at the last evaluation. On the other hand, 9 male (30%) and 15 female (71%) patients were underweight. Fatty liver was diagnosed in 11 male (37%) and 10 female (48%) patients during the follow-up period, although patients who had fatty liver did not tend to be overweight/obese. Significantly more patients who received CRT before SCT developed fatty liver with insulin resistance than those who did not ($P < 0.05$). Even patients who are not overweight/obese may develop fatty liver and metabolic abnormalities. We recommend that healthcare professionals recognize these risks and give life-long attention to detecting, preventing and treating late complications after SCT.
Bone Marrow Transplantation advance online publication, 21 June 2010; doi:10.1038/bmt.2010.144
Keywords: fatty liver; insulin resistance; childhood cancer survivors; irradiation

Introduction

The number of long-term surviving SCT recipients has increased steadily and attention is now extended to the late endocrine complications of this procedure.[1] Overweight/obesity has been identified as a potential late effect of therapy in survivors of acute lymphoblastic leukemia (ALL) treated with conventional therapy,[2] and cranial radiotherapy (CRT) during ALL treatment has been implicated as a potential cause of excess weight gain among these survivors. Although the mechanism by which CRT leads to overweight/obesity is unknown, hypothalamic damage leading to GH deficiency and/or leptin insensitivity has been suggested.[3,4] Overweight/obesity in childhood, adolescence and young adulthood after SCT treatment is an important predictor of eventual development of hyperinsulinism and its attendant metabolic syndrome.[5] Specifically, excessive accumulation of visceral fat within the abdomen is strongly and independently associated with metabolic syndrome,[6,7] and the storage of fat in non-adipose tissue such as the liver is known to cause insulin resistance in mouse models.[8]

Fatty liver is increasingly recognized as a major cause of liver-related morbidity and mortality[9] because of its potential to progress to cirrhosis and liver failure.[10] This disease is often associated with metabolic abnormalities characterized by obesity,[11] type II diabetes mellitus,[12] dyslipidemia,[13] and hypertension,[14] and, finally, each of these abnormalities also carries a cardiovascular disease risk. Whether the risk for these metabolic abnormalities is increased in adult survivors of childhood SCT recipients has, however, not been fully elucidated. There is a possibility that identification of the risk factors for development of fatty liver in survivors is, therefore, critical for the development of strategies for prevention of and intervention in cardiovascular disease.

Although overweight/obesity in adult survivors of childhood ALL has been well evaluated, no longitudinal study that investigated metabolic abnormalities in survivors of childhood SCT has been reported,[15,16] and the mechanism of these conditions has not been completely understood. A longitudinal retrospective study of a cohort of adult

2

survivors of childhood SCT was performed to determine whether adult survivors are at risk for overweight/obesity, metabolic abnormalities and fatty liver and whether this risk is associated with CRT or other factors used in the treatment of SCT.

Materials and methods

Patients

We reviewed the clinical records of 215 patients who received allogeneic SCT at Tokai University Hospital between 1982 and 1997. Inclusion criteria of this study were survival at least 10 years after SCT, age 18 years or greater at the time of the last evaluation and no history of liver dysfunction, endocrinological and metabolic abnormalities before SCT and of treatments that affect fatty liver after SCT. Fifty-one surviving patients (30 male and 21 female patients) fulfilled these criteria. The median age of the 51 patients at SCT was 10.5 years (range, 0.9–15.9 years), the median age at the last evaluation was 26.6 years (range, 19.4–34.3 years) and the median follow-up duration after SCT was 17.4 years (range, 10.9–25.8 years).

A written informed consent was obtained from the patients and/or their parents. Patient characteristics are summarized in Table 1.

Transplantation procedure

In addition to conventional chemotherapy, nine patients who had ALL and one patient who had non-Hodgkin's lymphoma received prophylactic CRT (1, 12 Gy; 1, 15 Gy; 7, 18 Gy and 1, 24 Gy) before SCT. Conditioning regimens for 46 patients consisted of irradiation combined with/without CY and/or other drugs; 6–12 Gy of TBI for the

malignant disease group was given in 3–6 fractions, and 3–10 Gy of thoraco-abdominal irradiation (TAI) for the non-malignant disease group in 1–5 fractions. The remaining five patients received conditioning without irradiation. Prophylaxis against GVHD varied during the time period; methotrexate, CYA or a combination of both drugs were used. Because no differences were observed in the main outcome between those who received TAI and chemotherapy only, the study population was categorized into three groups according to the conditioning protocol they had received: CRT + TBI, TBI and TAI + Chemo groups.

Anthropometric measures of body composition

All patients had achieved their final height at the last evaluation. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Patients were classified as overweight/obese if their BMI was $25 kg/m^2$ or greater and as underweight if their BMI was $18.5 kg/m^2$ or less according to the World Health Organization definition of obesity.[17] Waist circumference (WC) was measured at the level of the superior iliac crest. Abdominal adiposity was defined as a waist-to-height ratio greater than 0.5.[18–20] Bioelectronical impedance analysis was performed for measurement of body fat by InnerScan (TANITA, Tokyo, Japan).

Evaluation of metabolic syndrome

Metabolic syndrome was defined according to criteria of a committee for the establishment of the definition and diagnostic criteria of metabolic syndrome in Japanese:[21] Central obesity (WC $\geqslant 85$ cm in male or $\geqslant 90$ cm in female patients) and the presence of at least two of the following factors: (1) triglyceride (TG) levels 150 mg/dL and/or high-density lipoprotein cholesterol (HDL-C) <40 mg/dL;

Table 1 Patient characteristics

	All (n = 51)	Male (n = 30)	Female (n = 21)
Median age at SCT (years)	10.5 (0.9–15.9)	11.2 (0.9–15.8)	8.8 (2.6–15.9)
Median age at last evaluation (years)	26.6 (19.4–34.3)	27.7 (19.8–34.2)	25.8 (19.4–34.3)
Median follow-up duration after SCT (years)	15.0 (6.7–24.7)	15.1 (6.7–24.7)	15.0 (9.3–19.0)
Primary disease			
Malignant disease			
ALL	14	6	8
AML	9	6	3
CML	5	3	2
NHL	5	4	1
Non-malignant disease			
AA	10	8	2
Others	8	3	5
Cranial radiation before SCT	10	5	5
Conditioning regimen for SCT			
TBI + VP-16 + other drugs	29	13	16
TBI + CY	5	4	1
TAI + CY + other drugs	12	8	4
Bu + CY + other drugs	4	4	0
Other drugs	1	1	0

Abbreviations: AA = aplastic anemia; ALL = acute lymphocytic leukemia; AML = acute myelogenous leukemia; Bu = busulfan; CML = chronic myelogeous leukemia; CY = cyclophosphamide; NHL = non-Hodgkin lymphoma; SCT = stem cell transplantation; TAI = thoraco-abdominal irradiation; TBI = total body irradiation; VP-16 = etoposide.
Data are presented as median (range).

- 243 -

(2) systolic blood pressure 130 mm Hg and/or diastolic blood pressure 85 mm Hg; and (3) fasting plasma glucose 110 mg/dL.

Evaluation of fatty liver and steatohepatitis

Fatty liver was evaluated by 460 longitudinal ultrasounds among the 51 patients during the follow-up period. The Hitachi EUB340 (Hitachi, Tokyo, Japan), Yokogawa RT2800 (GE Yokogawa Medical System, Tokyo, Japan) and Yokogawa RT3000 were used as ultrasound equipment until 1994 for evaluation of fatty liver. The Aloka SSD 650CL (Aloka, Tokyo, Japan) was used after 1995. Of the four criteria used for the diagnosis of fatty liver (hepatorenal echo contrast (HR), liver brightness (LB), deep attenuation and vascular blurring), the first two were used as definitive criteria and the last two were taken into account as needed.[22] Degree of fatty liver was classified as follows: Severe, both HR and LB were positive; Moderate, either HR or LB was positive and/or either deep attenuation or vascular blurring was positive; and Mild, neither HR, LB, deep attenuation or vascular blurring was positive. In all cases, two specialists in gastroenterology separately confirmed the diagnosis.

Abdominal computed tomography was performed in 38 patients (23 male and 15 female patients) for coordination with fatty liver diagnosed by ultrasound. Fatty liver was determined using the ratio of liver to spleen, as described in detail elsewhere.[23] The criterion for fatty liver was below 0.9 of the hepatosplenic Hounsfield Units ratio.

Liver biopsy was performed in three allografted patients who received CRT + TBI (2 male; 1 female) and in one autografted female patient who received TBI, for coordination with fatty liver diagnosed by ultrasound and histological evaluation. Markers of hepatic fibrosis was evaluated by serum Procollage III peptide (normal range, 0.3–0.8 U/mL), serum type IV collagen (<150 ng/mL) and serum hyaluronic acid concentrations (<50 ng/mL).

Evaluation of glucose and lipid metabolism profiles

An overnight fasting blood sample was obtained in all patients for the measurement of plasma glucose, plasma insulin, plasma glycosylated hemoglobin, serum TG, serum total cholesterol, serum HDL-C, serum low-density lipoprotein cholesterol, serum-free fatty acid, plasma leptin and serum adiponectin. Hypertriglyceridemia was defined as a serum TG level above 150 mg/dL and low HDL-cholesterolemia was defined as a serum HDL-C level below 40 mg/dL. The normal ranges for plasma leptin were 1.0–11.5 ng/mL in male and 2.0–20.6 ng/mL in female patients, and those for serum adiponectin were 3.8–16.6 μg/mL in male and 4.1–18.9 μg/mL in female patients.

Oral glucose tolerance test (OGTT) was performed in 48 patients (28 male and 20 female patients) for evaluation of glucose metabolism. Patients were given glucose at 1.75 g/kg (maximum 75 g) after a 12-h overnight fast, and samples for measurement of plasma glucose and plasma insulin were drawn at baseline every 30 min until 120 min. Hyperinsulinemia was defined as a fasting plasma insulin value of 20 mU/L or greater or a peak plasma insulin during OGTT of 150 mU/L or greater. Definitions of

diabetes mellitus and impaired glucose tolerance were according to the Japan Diabetes Society criteria. The presence of either type I or type II diabetes was diagnosed if fasting plasma glucose was 126 mg/dL or greater and/or if plasma glucose 2 h after glucose load was 200 mg/dL or greater. A random plasma glucose value higher than 200 mg/dL was also regarded as indicating diabetes. Results were considered normal if fasting plasma glucose was below 110 mg/dL and the 2-h plasma glucose was below 140 mg/dL. Impaired glucose tolerance was diagnosed in those who had values that were neither normal or that indicated diabetes mellitus.

The capacity of insulin secretion was evaluated by the insulinogenic index (II) using OGTT: (plasma insulin at 30 min—plasma insulin at baseline)/(plasma glucose at 30 min—plasma glucose at baseline) with a result of 0.4 or less indicated dysfunction of insulin secretion. Insulin resistance was further estimated by the homeostasis model assessment-insulin resistance: fasting plasma insulin (mU/L) × plasma glucose (mg/dL)/405 with a result of 2.5 or greater indicated IR.

Evaluation of endocrine function

Growth hormone (GH) secretion was repeatedly assessed by the insulin tolerance test and IGF-I before and annually after SCT as described in detail elsewhere.[24] GH deficiency was defined as a GH level of <10 ng/mL in response to stimulation with regular insulin.

Thyroid function was evaluated before and annually after SCT by serial measurement of basal serum thyroid-stimulating hormone (TSH) levels, serum-free triiodothyronine (FT3) levels and free thyroxine (FT4) levels. Normal values in our institute were: TSH 0.30–4.00 U/mL, FT3 2.50–4.50 pg/mL and FT4 0.75–1.75 ng/dL. Subclinical compensated hypothyroidism was defined as elevated TSH levels (4–10 U/mL) with normal FT4 levels with no clinical symptoms.

In male patients, onset of puberty was defined by a testicular volume of 4 mL.[25] Testicular volume was determined using an orchidometer, as described by Prader.[26] Testicular Leydig cell function and germinal epithelium damage were evaluated with basal serum luteinizing hormone (LH) levels, basal serum follicle-stimulating hormone (FSH) levels and serum testosterone levels. Normal basal serum LH and FSH levels at our institute were <5 mIU/mL and <9 mIU/mL, respectively. Partial Leydig cell dysfunction and partial germinal epithelium damage were defined by increased basal LH levels or basal FSH levels with normal testosterone levels. In female patients, the time of menarche and the recurrence of menstruation after SCT were recorded. Ovarian function was evaluated with basal serum LH levels, basal serum FSH levels and serum estradiol (E2) levels after SCT. Primary ovarian failure was defined by increased basal FSH levels. Endocrine tests were undertaken in a morning fasting state to avoid diurnal variation of hormones. Our definition of partial testicular or ovarian insufficiency is as follows; Testes; basal FSH 15 IU/mL, basal LH 20 IU/mL and normal testosterone. Ovary; we defined the partial ovarian failure when E2 was within normal range and when FSH was >10 IU/mL in adulthood. And we defined the

4

primary ovarian failure when FSH was >40 IU/mL or the menarche did not appear spontaneously.

Statistical analysis

As the data had a skewed distribution, median and range are presented throughout the text, tables and figures. Fisher's exact probability and the χ^2 test were used to assess the association either between or among groups. Differences in anthropometric and laboratory variables among groups were analyzed by the Kruskal–Wallis test with Dunn's multiple comparison test. All statistical analyses were performed with the statistical package GraphPad Prism for Windows (Ver. 4.03 Prism). A P-value of less than 0.05 was considered statistically significant.

Results

Anthropometrics

Patients in the CRT + TBI groups were significantly older than those in either the TBI groups or TAI + Chemo groups ($P<0.05$, Table 2). Among the 51 patients, two male patients had a greater than 25 kg/m^2 BMI (25.6 and 26.2, respectively) at the last evaluation, whereas none of the female patients were overweight/obese (Figure 1). On the other hand, 9 male (30%) and 15 female (71%) patients had a BMI less than 18.5 kg/m^2 (Figure 1). No patient satisfied the criteria for metabolic syndrome, although three male patients had a WC greater than 85 cm (91.2 cm in CRT + TBI group and 87.2 and 92.2 cm in TAI + Chemo group, Figure 1). CRT was significantly associated with an increased BMI, waist-to-height ratio and body fat in both male and female CRT + TBI groups compared with either the male TBI group or female TAI + Chemo group ($P<0.05$, respectively), although the average BMI in the three groups did not indicate overweight/obesity (Table 2).

Fatty liver

Among the 51 patients, their liver function test was within normal range during follow-up period, although HCV–RNA test was positive in two patients (one in TBI group and another in TAI + Chemo group). Information on daily alcohol consumption was obtained from all patients by the self-report. Overall, most patients were non-drinkers or drank only minimally. Abdominal computed tomography was performed in 38 patients to confirm the diagnosis of fatty liver made through ultrasound. As a strong correlation was identified between ultrasound and abdominal computed tomography ($P<0.001$), ultrasound findings were used to evaluate the presence of fatty liver in this study. Fatty liver was diagnosed in 11 male (37%) and 10 female (48%) patients by ultrasound during the follow-up period. Moreover, fatty liver was histologically confirmed by biopsy in four patients. However, patients who had fatty liver did not tend to be overweight/obese (Figure 2). The mean BMIs in male and female patients who had fatty liver were 22.6 and 19.2 kg/m^2, respectively, at the last evaluation. No relationships between the onset of fatty liver and gender of patients, age at SCT, primary disease and GVHD were observed. In all, 205 ultrasound examinations were

performed to evaluate the presence of fatty liver during the follow-up period. Concerning the mode of irradiation, a significantly greater number of patients who received CRT + TBI developed fatty liver compared with either TBI group or TAI + Chem group ($P<0.005$, Figure 2). Fatty liver in four patients (two males, two females) in the TBI group and two males in the TAI + Chemo group improved with exercise and dietary regimens by physicians and dieticians during the follow-up period. However, fatty liver did not improve in any of those with this condition in the CRT + TBI group with exercise and dietary treatments (Figure 2).

Evaluation of lipid and glucose metabolism

Studies of lipid and glucose metabolism were performed to investigate the mechanism of development of fatty liver in patients who received SCT. CRT was significantly associated with increased plasma leptin levels in the CRT + TBI group compared with either the male TBI group or with the female TAI + Chemo group ($P<0.01$ and $P<0.05$, respectively, Table 2). Moreover, serum adiponectin levels in the CRT + TBI group decreased compared with either the TBI or TAI + Chemo group, although the difference among groups was not statistically significant.

Homeostasis model assessment-insulin resistance in the male and female CRT + TBI groups increased compared with either the TBI or TAI + Chemo groups, although no statistically significant difference was recognized among groups. (Table 2). CRT was, however, significantly associated with increased insulin resistance in the CRT + TBI groups compared either with TBI or TAI + Chemo groups ($P<0.05$ in male and female patients, Table 2). None of the patients had a decreased insulinogenic index at evaluation. Plasma glucose levels in the 75 g OGTT did not differ among groups and were within normal range at each time point.

Evaluation of endocrine function

Ten patients experienced poor GH secretion (5, CRT + TBI; 4, TBI; 1, TAI + Chemo) at least twice, although a permanent GH deficiency was not observed. CRT was significantly associated with transiently poor GH secretion in the CRT + TBI group compared with either the TBI or TAI + Chemo group ($P<0.05$, respectively). Serum IGF-I levels remained in the lower half of the normal range for age throughout the follow-up period, although in male patients decreased serum IGF-I levels in the CRT + TBI group were significantly more frequent than in the TAI + Chemo group ($P<0.05$, Table 2).

Thyroid function in all but one subject was within normal range at the last evaluation, although 12 patients experienced transient subclinical compensated hypothyroidism (4, CRT + TBI; 5, TBI; 3, TAI + Chemo) during the follow-up period.

All patients had developed adult genitalia (Tanner stage V) at the last evaluation. In male patients, puberty started spontaneously in all patients according to increases in testicular volume increase (4 mL). Serum testosterone levels in all patients reached the adult level at some time point from adolescence to adulthood after SCT. CRT was,

- 245 -

Table 2 Anthropometrics, fatty liver, liver, lipid and glucose metabolism and endocrine function in adult survivors of childhood stem cell transplant

	Male				Female			
	CRT+TBI (n=5)	TBI (n=13)	TAI+Chemo (n=12)	P-value	CRT+TBI (n=5)	TBI (n=12)	TAI+Chemo (n=4)	P-value
Age at SCT (years)	11.7 (7.4–15.4)	11.5 (0.9–15.8)	10.5 (4.0–15.5)	0.59	9.1 (4.3–15.9)	10.2 (3.4–15.3)	4.5 (2.6–15.0)	0.34
Age at last evaluation (years)	28.6 (20.8–30.1)	24.6 (18.9–33.0)	21.9 (18.0–28.7)	0.04	24.3(18.9–31.3)	23.7(19.3–31.9)	21.4 (18.0–24.3)	0.31
Follow-up duration after SCT (years)	14.7 (11.3–21.2)	16.8 (7.6–24.7)	11.3 (6.7–17.4)	0.18	15.3 (12.4–16.8)	14.2 (9.3–19.0)	15.7 (9.3–18.0)	0.93
Anthropometric measures of body composition								
Height (cm)	163.5 (152.7–168.0)	158.6 (150.6–171.6)	165.0 (144.7–173.4)	0.85	154.2 (146.7–163.9)	152.2 (143.5–157.3)	151.2 (130.5–161.8)	0.93
Weight (kg)	53.6 (44.9–69.6)	43.8 (30.7–61.9)	52.3 (32.5–72.8)	0.12	46.7 (36.7–60.5)	39.3 (32.2–44.2)	37.6 (32.0–42.2)	0.06
Waist circumference (cm)	77.3 (70.7–91.2)	63.0 (55.5–76.0)	65.0 (53.9–92.2)	*0.04	74.5 (67.5–81.5)	60.7 (56.5–71.4)	59.6 (58.3–65.7)	**0.02
BMI (kg/m²)	23.0 (18.6–25.6)	17.7 (13.5–21.3)	19.4 (14.6–26.2)	*0.04	21.2 (17.1–24.2)	17.1 (14.6–18.2)	16.4 (16.1–16.4)	**0.01
Waist circumference to height ratio	0.51 (0.45–0.54)	0.39 (0.35–0.45)	0.38 (0.36–0.55)	*0.03	0.50 (0.46–0.52)	0.40 (0.38–0.47)	0.41 (0.39–0.43)	*0.02
Body fat (%)	20.6 (18.0–28.8)	10.0 (5.0–22.6)	12.2 (5.2–24.1)	*0.02	30.7 (21.3–33.7)	22.8 (13.7–30.2)	18.9 (16.4–21.4)	0.05
Liver dysfunction								
AST (U/L)	34 (21–75)	25 (17–68)	25 (18–60)	0.57	30 (23–76)	26 (21–161)	24 (15–28)	0.24
ALT (U/L)	33 (15–118)	21 (12–99)	30 (10–159)	0.54	36 (19–125)	31 (12–158)	18 (12–19)	0.12
γGTP (U/L)	61 (15–254)	31 (15–175)	37 (19–107)	0.79	55 (17–118)	23 (7–273)	18 (14–23)	0.25
Fatty liver								
P-III-P (U/mL)	1.0 (0.7–1.7)	0.9 (0.6–2.3)	1.2 (0.7–3.1)	0.15	0.9 (0.8–1.3)	1.0 (0.8–1.6)	1.3 (0.8–2.6)	0.48
Type IV collagen (ng/mL)	127 (78–161)	139 (88–238)	141 (91–355)	0.69	117 (110–152)	120 (76–236)	174 (97–204)	0.66
Hyaluronic Acid (ng/mL)	21 (0–21)	16 (0–36)	20 (0–41)	0.71	0 (0–14)	15 (0–24)	18 (10–28)	0.07
Lipid and glucose metabolism								
Leptin (ng/ml)	16.2 (8.7–20.8)	4.3 (1.9–8.6)	4.9 (2.4–11.3)	*0.01	23.2 (16.3–25.6)	13.8 (5.6–21.5)	6.4 (3.3–8.3)	**0.01
Adiponectin (mg/mL)	5.6 (4.1–8.9)	6.4 (2.7–16.4)	8.0 (2.6–29.5)	0.55	4.8 (2.7–7.4)	5.6 (2.1–16.5)	11.8 (10.1–19.7)	0.12
Triglyceride (mg/dL)	212 (41–751)	78 (47–199)	86 (49–409)	0.17	90 (70–127)	86 (52–138)	57 (44–95)	0.16
Total cholesterol (mg/dL)	216 (165–273)	173 (82–250)	174 (148–294)	0.20	213 (166–290)	218 (166–241)	205 (137–235)	0.89
HDL-cholesterol (mg/dL)	58 (29–108)	48 (33–89)	60 (39–81)	0.74	63 (45–80)	62 (50–109)	82 (64–103)	0.23
LDL-cholesterol (mg/dL)	140 (55–163)	109 (81–163)	114 (71–892)	0.88	129 (76–208)	128 (96–312)	112 (64–126)	0.25
Free-fatty acid (mEq/L)	0.8 (0.2–1.0)	0.3 (0.2–0.8)	0.7 (0.3–1.3)	0.08	0.6 (0.4–0.9)	0.4 (0.3–0.7)	0.3 (0.3–0.3)	0.06
Glucose (mg/dL)	94 (90–110)	93 (85–134)	91 (64–111)	0.73	96 (85–111)	93 (84–143)	98 (79–107)	0.89
IRI (mU/L)	13.8 (3.0–52.8)	5.9 (3.9–10.4)	8.3 (2.2–17.4)	0.20	10.6 (5.2–30.5)	5.2 (2.6–29.7)	6.2 (3.9–10.3)	0.09
HOMA-IR	3.2 (0.7–12.3)	1.3 (0.8–2.5)	2.3 (0.5–3.8)	0.09	2.7 (1.1–8.4)	1.2 (0.6–8.4)	1.1 (1.0–2.5)	0.11
Hb A1c (%)	4.9 (4.5–6.0)	5.0 (4.4–5.4)	4.7 (4.2–6.9)	0.70	5.2 (4.8–6.6)	5.0 (4.6–5.4)	5.2 (4.5–5.4)	0.37
Insulinogenic index	1.1 (1.0–2.7)	1.4 (0.2–4.2)	0.4 (0.0–2.0)	0.07	1.0 (0.1–2.9)	1.1 (0.5–2.1)	0.7 (0.3–1.2)	0.92
Oral glucose tolerance test								
Glu 0 min (mg/dL)	88 (86–92)	86 (54–98)	91 (85–114)	0.54	89 (78–99)	85 (79–92)	82 (82–87)	0.40
Glu 30 min (mg/dL)	122 (121–128)	100 (85–164)	123 (90–189)	0.15	144 (124–179)	137 (71–166)	155 (117–161)	0.48
Glu 60 min (mg/dL)	140 (124–191)	134 (113–157)	126 (94–206)	0.35	151 (81–214)	132 (91–185)	146 (135–148)	0.79
Glu 90 min (mg/dL)	128 (106–213)	158 (113–182)	145 (101–187)	0.57	148 (92–185)	119 (74–134)	113 (77–117)	0.28

Table 2 Continued

	Male				Female			
	CRT+TBI (n=5)	TBI (n=13)	TAI+Chemo (n=12)	P-value	CRT+TBI (n=5)	TBI (n=12)	TAI+Chemo (n=4)	P-value
Glu 120 min (mg/dL)	159 (104–174)	125 (92–173)	146 (114–195)	0.42	140 (76–149)	108 (101–153)	105 (98–113)	0.35
IRI 0 min (mU/L)	12.3 (5.4–69.2)	7.5 (2.3–11.3)	10.2 (4.5–20.6)	0.19	19.4 (15.7–26.4)	5.8 (4.9–33.6)	6.1 (4.5–6.8)	0.03
IRI 30 min (mU/L)	78.6 (38.0–166.7)	12.9 (7.1–91.0)	52.2 (12.5–158.2)	0.04	137.6 (32.1–241.0)	39.3 (2.2–199.1)	47.5 (41.2–70.5)	0.17
IRI 60 min (mU/L)	85.8 (47.9–313.8)	44.0 (35.3–92.2)	47.1 (17.1–146.5)	0.08	114.0 (66.7–162.3)	81.1 (21.0–267.6)	57.9 (47.5–58.4)	0.12
IRI 90 min (mU/L)	88.8 (25.3–366.8)	100.2 (41.7–166.9)	91.2 (52.0–139.6)	0.97	149.2 (91.9–257.3)	77.6 (15.5–175.3)	30.6 (23.1–45.2)	0.07
IRI 120 min (mU/L)	278.8 (62.6–452.3)	58.0 (25.5–112.4)	94.8 (31.2–174.6)	*0.03	225.4 (39.3–316.7)	80.6 (44.7–270.3)	31.2 (27.3–35.6)	**0.02
Endocrine function								
IGF-I (ng/mL)	189 (168–205)	188 (119–324)	271 (198–473)	**0.02	145 (141–149)	203 (137–220)	273 (254–292)	0.08
TSH (mU/mL)	3.6 (2.1–6.2)	2.2 (0.1–5.4)	2.2 (0.7–6.2)	0.22	1.7 (1.6–2.3)	2.1 (1.3–3.6)	0.5 (0.0–2.0)	***0.04
FT3 (pg/mL)	3.6 (3.4–4.0)	3.6 (0.8–4.5)	3.8 (3.2–4.7)	0.92	3.3 (3.0–3.6)	3.2 (2.8–3.7)	3.3 (2.8–3.8)	0.84
FT4 (ng/dL)	1.1 (0.9–1.2)	1.0 (0.8–1.6)	1.1 (0.7–4.8)	0.19	1.0 (0.9–1.2)	1.0 (0.7–1.3)	1.1 (0.9–1.3)	0.76
Testosterone (mg/dL)	278 (16–397)	429 (171–833)	404 (261–712)	0.12				
LH (mIU/L)	14.7 (7.9–19.4)	9.3 (3.6–18.5)	4.5 (1.1–13.6)	**0.01				
FSH (mIU/L)	38.2 (11.4–51.0)	31.2 (7.8–58.2)	13.0 (3.5–24.0)	*0.05				

Abbreviation: SCT = stem cell transplantation.
Data are presented as median (range).
*CRT + TBI vs TBI.
**CRT + TBI vs TAI + Chemo.
***TBI vs TAI + Chemo.

however, associated with decreased serum testosterone levels in the CRT + TBI group compared with either the TBI or TAI + Chemo group, although statistically significant differences were not recognized among groups (Table 2). All patients experienced raised basal LH and FSH levels with normal serum testosterone levels while approaching adolescence, indicating the presence of partial Leydig cell dysfunction and partial damage of the testicular germinal epithelium at some time during the follow-up period.

In female patients who received SCT before the age of 10 years, 9 of 11 patients who had not manifested menarche before SCT entered puberty spontaneously after SCT and subsequently had menarche at a median age of 13.3 years (range, 12.3–15.3 years), which was an appropriate age for healthy Japanese girls. These patients who received SCT before the age of 10 had normal E2 levels during the pubertal period without hormone replacement therapy (data not shown), although partial ovarian failure was observed in these patients. On the other hand, 3 of 10 patients who received SCT after the age of 10 had manifested menarche after SCT spontaneously. The remaining seven patients were diagnosed as having primary gonadal dysfunction after SCT, and six patients were administered hormone replacement therapy.

Discussion

This is the first report on adult survivors of childhood SCT to indicate longitudinal changes in fatty liver after SCT. The novel findings are that significantly more patients who received CRT before SCT developed fatty liver than those who did not, independent of sex, age at SCT, primary disease and GVHD. In addition, the difficulty of improving fatty liver in patients who received CRT even with appropriate exercise and dietetic treatment was revealed. Furthermore, the incidence of fatty liver was not associated with overweight/obesity in these patients who received SCT. These results underscore the increased health risk among adult survivors treated with CRT.

Reports on long-term survivors of childhood ALL have claimed that overweight/obesity, using the definition of WHO of a $BMI > 25\,kg/m^2$, is common. Sklar et al.[2] reported that leukemia survivors treated with CRT were more likely to be overweight/obese at attainment of final height than those treated with chemotherapy only. Although the mechanism by which ALL survivors become overweight/obese remains unclear, several explanations have been proposed including GH insufficiency, use of corticosteroids and reduced energy expenditure.[27–29] One proposed mechanism to explain the association between CRT and overweight/obesity in survivors of childhood ALL is leptin insensitivity. Leptin is an adipocyte-derived hormone that binds to the biologically active long form of its receptor in the hypothalamus.[30] It has been speculated that radiation-induced damage to the pituitary–hypothalamus axis may result in a disruption of leptin signal, which eventually results in obesity.[4]

Ross et al.[31] also reported that leptin insensitivity may influence obesity in survivors of childhood ALL, particularly
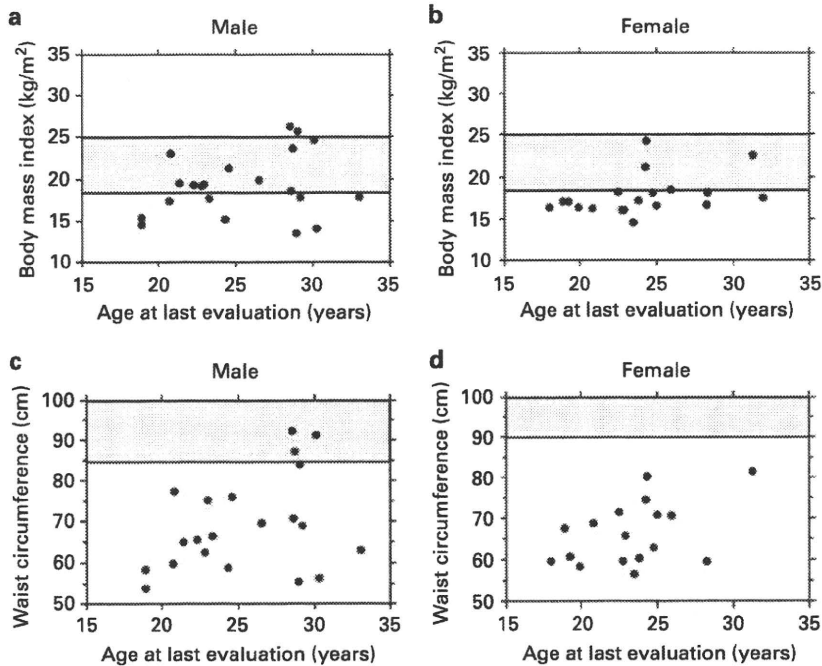
Figure 1 Body mass index (**a**, **b**) and waist circumference (**c**, **d**) in male and female adult survivors of childhood SCT recipients. The gray zones on Figure 1 were normal range of BMI and abdominal circumstance. Only two of the patients were BMI above 25 kg/m², and the abdominal circumstance was above normal range only in three patients.
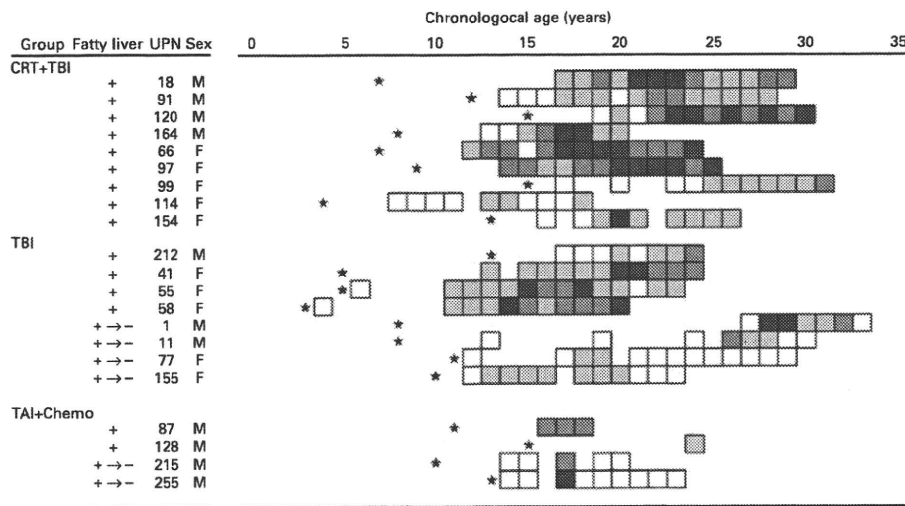

Figure 2 Changes in fatty liver in adult survivors of childhood SCT. UPN, unique patient number; Degree of fatty liver classified as follows: □, absence; ▨, mild; ▦, moderate; ■, severe; +, existence; + → −, improved; ★, SCT.

those exposed to cranial radiation and leptin polymorphism. In this study, CRT was significantly associated with an increased plasma leptin level in the CRT + TBI groups compared with either the male TBI group or female TAI + Chemo group. Our data provide support that CRT affects the pituitary–hypothalamus, causing leptin receptor insensitivity. However, it is surprising that the average BMI in all three groups did not show a shift toward overweight/ obesity. The National Health and Nutrition Survey of

Japan in 2004 reported that the frequency of BMI less than 18.5 kg/m² in the general Japanese male and female population in their 20s and 30s were 6.1 and 18.5%, respectively.[32] In this study, 9 males (30%) and 15 females (71%) had a BMI less than 18.5 kg/m², and they did not manifest chronic GVHD and a nutritional disorder during the follow-up period. Therefore, these patients were almost 4 to 5 times as likely to be underweight as the general Japanese population even though they had received CRT

8

before SCT and their plasma leptin levels increased during the follow-up period. It is difficult to explain a change in body composition after SCT only by a leptin increase and to adopt the concept of the metabolic syndrome for these patients after SCT.

An in vivo mouse experiment performed by Ablamunits et al.[33] clarified the relationship between SCT including TBI and body composition. Leptin-deficient ob/ob mice were exposed to TBI followed by SCT to rescue hematopoiesis and their body composition was monitored. TBI/SCT completely arrested body weight gain as early as 2 months after irradiation. Body composition measurements showed that body weight arrest was because of both retardation of lean mass accumulation and the inability to accumulate fat mass. Bingham et al.[34] also created ventromedial hypothalamic nucleus leptin receptor knockout (Lepr KO^{VMH}) mice to evaluate the relationship between leptin level and body composition. Lepr KO^{VMH} mice on normal mouse chow showed a twofold increase in plasma leptin and insulin levels relative to wild-type littermates. However, Lepr KO^{VMH} mice did not show a significant weight increase over their wild-type littermates. Nuclear magnetic resonance analysis demonstrated that the increased weight in Lepr KO^{VMH} mice entirely reflects increased adiposity and histological evidence also supported the nuclear magnetic resonance body composition data. Examination of white adipose tissue from Lepr KO^{VMH} mice revealed a marked cellular hypertrophy, whereas examination of brown adipose tissue showed a significant increase in the size of cellular vacuoles. Thus, despite comparable body weights, Lepr KO^{VMH} mice showed increased lipid accumulation in adipocytes relative to wild-type littermates.

According to the results of these experiments and our current study, changes in body weight after SCT may be influenced not only by increased leptin levels caused by radiation-induced damage to the pituitary–hypothalamus axis but also by direct exposure of adipocytes to radiation. Although visceral fat was not quantified by abdominal CT in this study, the WC as an indication of abdominal adiposity in these patients did not indicate a significant increase in visceral fat. Therefore, direct exposure of abdominal adipocytes to radiation may participate in the mechanism of the arrested body weight in survivors of SCT.

Fatty liver is increasingly recognized as a major cause of liver-related morbidity and mortality, because of its potential to progress to cirrhosis and liver failure. We have investigated the prevalence of fatty liver in Japan over the past 12 years.[22] The subjects of the previous study were 39 151 individuals who visited the Tokai University Hospital Health Checkup Center for the first time during the 12-year period from 1989 to 2000. In all cases, the diagnosis of fatty liver was based on abdominal ultrasonography with the same ultrasound equipment and diagnostic criteria as used in this study. Prevalence of fatty liver in subjects in their 20s and 30s was 18–27% and 4–7% in male and female patients, respectively. In contrast, the current study reports an incidence of fatty liver, that is, in 37% of male and in 48% of female adult survivors who were not overweight/obese during the follow-up period, respectively. These results indicated that SCT may affect the prevalence

of fatty liver in adult survivors and that they were more likely to develop fatty liver compared with the general Japanese population. It was impossible or very difficult for us to collect sufficient information on all drugs, which were used before SCT, because most patients were referred from many hospitals with a summary of treatment, which did not contain the exact total doses of all drugs. We tried to collect data on steroids, which were used pre-SCT and post-SCT periods, and analyzed if there was relation between steroid and development of fatty liver. We could not find any significant relation between steroid use or dosage and fatty liver.

We performed liver biopsy in four patients in whom diagnosis of severe fatty liver was made by echography. Atypical non-alcoholic steatohepatitis was suspected in one of these patients, and fatty liver was diagnosed histological in all patients. As the computed tomography also supported the echographical diagnosis of fatty liver in all patients including these four patients, and taking the risk of liver biopsy into consideration, we decided to evaluate and to follow up these patients mainly by echography.

Insulin normally inhibits the production of glucose and very low-density lipoprotein in the liver.[35] Fat accumulation in the liver is known to cause insulin resistance in mouse models that have neither s.c. nor visceral fat[8] and is observed in insulin-resistant mice[33] and human subjects with lipoatrophy.[36] Therefore, insulin resistance is a key player in the pathogenesis of fatty liver. Kotronen et al.[37] showed that liver fat accumulation was 4-fold higher in subjects with than without metabolic syndrome and the best correlate of liver fat was the fasting serum insulin level. This strong association may be explained by the decrease in hepatic insulin clearance in subjects with increased hepatic fat content.[38] Indeed, a 51% decrease in liver fat achieved by rosiglitazone therapy has been shown to increase insulin clearance by 20%.[39] Bingham et al.[34] demonstrated that Lepr KO^{VMH} mice showed significantly increased insulin resistance, hyperleptinemia, adipose mass and fatty liver on a low-fat diet. By 20 weeks of age, the livers of Lepr KO^{VMH} mice fed normal mouse chow weighted approximately 50% more than wild-type littermates and contained approximately 50% more triacylglycerol. In this study, CRT was significantly associated with increased insulin resistance. Therefore, insulin resistance may be one possible explanation of the relationship between adult survivors without overweight/obesity and high prevalence of fatty liver. However, clinical investigations and further studies in animal models should elucidate the underlying mechanism.

In conclusion, we found that significantly more patients who received CRT before SCT developed fatty liver and insulin resistance than those who did not and that improvement in fatty liver in patients with increased leptin levels was difficult with appropriate treatments. Even patients who do not tend to be overweight/obese may develop metabolic abnormalities and fatty liver after SCT so in the future we must think about treatment of these conditions, including application of medical therapy. We, therefore, recommend that healthcare professionals recognize these risks and pay life-long attention to detecting, preventing and treating the late complications after SCT.

References

1 Nandagopal R, Laverdière C, Mulrooney D, Hudson MM, Meacham L. Endocrine late effects of childhood cancer therapy: a report from the Children's Oncology Group. *Horm Res* 2008; **69**: 65–74.

2 Sklar CA, Mertens AC, Walter A, Mitchell D, Nesbit ME, O'Leary M et al. Changes in body mass index and prevalence of overweight in survivors of childhood acute lymphoblastic leukemia: role of cranial irradiation. *Med Pediatr Onco* 2000; **35**: 91–95.

3 Link K, Moëll C, Garwicz S, Cavallin-Ståhl E, Björk J, Thilén U et al. Growth hormone deficiency predicts cardiovascular risk in young adults treated for acute lymphoblastic leukemia in childhood. *J Clin Endocrinol Metab* 2004; **89**: 5003–5012.

4 Brennan BM, Rahim A, Blum WF, Adams JA, Eden OB, Shalet SM. Hyperleptinaemia in young adults following cranial irradiation in childhood: growth hormone deficiency or leptin insensitivity? *Clin Endocrinol* 1999; **50**: 163–169.

5 Facchini FS, Hua N, Abbasi F, Reaven GM. Insulin resistance as a predictor of age-related diseases. *J Clin Endocrinol Metab* 2001; **86**: 3574–3578.

6 Fujimoto WY, Bergstrom RW, Boyko EJ, Chen KW, Leonetti DL, Newell-Morris L et al. Visceral adiposity and incident coronary heart disease in Japanese-American men. The 10-year follow-up results of the Seattle Japanese-American Community Diabetes Study. *Diabetes Care* 1999; **22**: 1808–1812.

7 Kuk JL, Katzmarzyk PT, Nichaman MZ, Church TS, Blair SN, Ross R. Visceral fat is an independent predictor of all-cause mortality in men. *Obesity* 2006; **14**: 336–341.

8 Moitra J, Mason MM, Olive M, Krylov D, Gavrilova O, Marcus-Samuels B et al. Life without white fat: a transgenic mouse. *Genes Dev* 1998; **12**: 3168–3181.

9 Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221–1231.

10 Teli MR, James OF, Burt AD, Bennett MK, Day CP. The natural history of nonalcoholic fatty liver: a follow-up study. *Hepatology* 1995; **22**: 1714–1719.

11 Bellentani S, Saccoccio G, Masutti F, Crocè LS, Brandi G, Sasso F et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000; **132**: 112–117.

12 Gupte P, Amarapurkar D, Agal S, Baijal R, Kulshrestha P, Pramanik S et al. Non-alcoholic steatohepatitis in type 2 diabetes mellitus. *J Gastroenterol Hepatol* 2004; **19**: 854–858.

13 Assy N, Kaita K, Mymin D, Levy C, Rosser B, Minuk G. Fatty infiltration of liver in hyperlipidemic patients. *Dig Dis Sci* 2000; **45**: 1929–1934.

14 Donati G, Stagni B, Piscaglia F, Venturoli N, Morselli-Labate AM, Rasciti L et al. Increased prevalence of fatty liver in arterial hypertensive patients with normal liver enzymes: role of insulin resistance. *Gut* 2004; **53**: 1020–1023.

15 Cohen A, Duell T, Socié G, van Lint MT, Weiss M, Tichelli A et al. Nutritional status and growth after bone marrow transplantation (BMT) during childhood: EBMT Late-Effects Working Party retrospective data. European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* 1999; **23**: 1043–1047.

16 Nysom K, Holm K, Michaelsen KF, Hertz H, Jacobsen N, Müller J et al. Degree of fatness after allogeneic BMT for childhood leukaemia or lymphoma. *Bone Marrow Transplant* 2001; **27**: 817–820.

17 WHO. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation. *World Health Organ Tech Rep Ser* 2000; **894**: i–xii, 1–253.

18 Ashwell M, Cole TJ, Dixon AK. Ratio of waist circumference to height is strong predictor of intra-abdominal fat. *BMJ* 1996; **313**: 559–560.

19 Savva SC, Tornaritis M, Savva ME, Kourides Y, Panagi A, Silikiotou N et al. Waist circumference and waist-to-height ratio are better predictors of cardiovascular disease risk factors in children than body mass index. *Int J Obes Relat Metab Disord* 2000; **24**: 1453–1458.

20 Hsieh SD, Yoshinaga H, Muto T. Waist-to-height ratio, a simple and practical index for assessing central fat distribution and metabolic risk in Japanese men and women. *Int J Obes Relat Metab Disord* 2003; **27**: 610–616.

21 Matsuzawa Y. Metabolic Syndrome-definition and diagnostic criteria in Japan. *J Atheroscler Thromb* 2005; **12**: 301.

22 Kojima S, Watanabe N, Numata M, Ogawa T, Matsuzaki S. Increase in the prevalence of fatty liver in Japan over the past 12 years: analysis of clinical background. *J Gastroenterol* 2003; **38**: 954–961.

23 Davidson LE, Kuk JL, Church TS, Ross R. Protocol for measurement of liver fat by computed tomography. *J Appl Physiol* 2006; **100**: 864–868.

24 Shinagawa T, Tomita Y, Ishiguro H, Matsumoto M, Shimizu T, Yasuda Y et al. Final height and growth hormone secretion after bone marrow transplantation in children. *Endocr J* 2001; **48**: 133–138.

25 Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 1976; **51**: 170–179.

26 Prader A. Testicular size: assessment and clinical importance. *Triangle* 1966; **7**: 240–243.

27 Oeffinger KC, Mertens AC, Sklar CA, Yasui Y, Fears T, Stovall M et al. Obesity in adult survivors of childhood acute lymphoblastic leukemia: a report from the Childhood Cancer Survivor Study. *J Clin Oncol* 2003; **21**: 1359–1365.

28 Van Dongen-Melman JE, Hokken-Koelega AC, Hählen K, De Groot A, Tromp CG, Egeler RM. Obesity after successful treatment of acute lymphoblastic leukemia in childhood. *Pediatr Res* 1995; **38**: 86–90.

29 Warner JT, Bell W, Webb DK, Gregory JW. Daily energy expenditure and physical activity in survivors of childhood malignancy. *Pediatr Res* 1998; **43**: 607–613.

30 Strosberg AD, Issad T. The involvement of leptin in humans revealed by mutations in leptin and leptin receptor genes. *Trends Pharmacol Sci* 1999; **20**: 227–230.

31 Ross JA, Oeffinger KC, Davies SM, Mertens AC, Langer EK, Kiffmeyer WR et al. Genetic variation in the leptin receptor gene and obesity in survivors of childhood acute lymphoblastic leukemia: a report from the Childhood Cancer Survivor Study. *J Clin Oncol* 2004; **22**: 3558–3562.

32 The Ministry of Health, Labor and Welfare of Japan. The National Health and Nutrition Survey of Japan in 2004.

10

33 Ablamunits V, Weisberg SP, Lemieux JE, Combs TP, Klebanov S. Reduced adiposity in ob/ob mice following total body irradiation and bone marrow transplantation. *Obesity* 2007; **15**: 1419–1429.

34 Bingham NC, Anderson KK, Reuter AL, Stallings NR, Parker KL. Selective loss of leptin receptors in the ventromedial hypothalamic nucleus results in increased adiposity and a metabolic syndrome. *Endocrinology* 2008; **149**: 2138–2148.

35 Yki-Järvinen H. Fat in the liver and insulin resistance. *Ann Med* 2005; **37**: 347–356.

36 Agarwal AK, Garg A. Congenital generalized lipodystrophy: significance of triglyceride biosynthetic pathways. *Trends Endocrinol Metab* 2003; **14**: 214–221.

37 Kotronen A, Westerbacka J, Bergholm R, Pietiläinen KH, Yki-Järvinen H. Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab* 2007; **92**: 3490–3497.

38 Goto T, Onuma T, Takebe K, Kral JG. The influence of fatty liver on insulin clearance and insulin resistance in non-diabetic Japanese subjects. *Int J Obes Relat Metab Disord* 1995; **19**: 839–840.

39 Tiikkainen M, Häkkinen AM, Korsheninnikova E, Nyman T, Mäkimattila S, Yki-Järvinen H. Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance, and gene expression in adipose tissue in patients with type 2 diabetes. *Diabetes* 2004; **53**: 2169–2176.

- 251 -