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Gonadal status in reproductive age women after haematopoietic stem cell transplantation for haematological malignancies

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BACKGROUND: Ovarian failure is a frequent complication occurring after haematopoietic stem cell transplantion (SCT), which is generally ascribed to radiation treatment and antiblastic alkylating agents. METHODS: Ovarian morphology and function were studied in reproductive age women 12–24 months after allogeneic SCT (*n* = 23) received from an HLA identical sibling, or autologous SCT (*n* = 22). Thirteen allo-transplanted women were suffering from chronic graft-versus-host disease (cGVHD). RESULTS: Menstrual cycles recovered in two and four women in the allo- and auto-SCT groups respectively, being associated with younger age and longer period elapsed from transplant. There was no difference in previous use of alkylating agents between allo- and auto-transplantation, while corticosteroid treatment was longer and more recent in the allo-SCT group. Significantly higher gonadotrophin levels and lower estradiol were seen in the combined group of patients than in controls. In allo-transplanted women, androgens were also significantly lower than in controls. Ovarian and uterine volumes were lower in patients than in controls, and in the allo- than in the auto-transplanted women. Within the allo-SCT group, endocrine function and ovarian and uterine volumes were significantly lower in the patients suffering from cGVHD. CONCLUSIONS: Ovarian failure in SCT recipients is likely to be caused principally by myelo-ablative treatments, but the condition of gonadal and androgen insufficiency can be worsened by an altered immunomodulation in allogeneic setting.

Key words: busulphan/cyclophosphamide/graft-versus-host disease/ovarian failure/stem cell transplantation

Introduction

In recent years, autologous (auto-) and allogeneic (allo-) stem cell transplantation (SCT) have consistently improved disease-free and overall survival of haematological and non-haematological malignancies, and an increasing number of young women can expect to become long-term survivors (Kolb *et al*., 1999). However, improvement in disease control is associated with significant early and delayed side-effects, one of the most frequent being gonadal damage.

Ovarian failure associated with SCT has been generally ascribed to total body irradiation (TBI) and antiblastic alkylating agents, both widely used for the treatment of haematological malignancies and in conditioning regimens to transplant (Schubert *et al*., 1990; Shalet *et al*., 1995; Couto-Silva *et al*., 2001). Ovarian damage results in immediate menopause in most women; recovery is rare, being less frequent at older age (Schubert *et al*., 1990; Shalet *et al*., 1995). The conditioning regimen aims at eradicating the underlying malignant disease and, in the allogeneic setting, at suppressing the host immune system. Conditioning regimens avoiding TBI, such as the combination of busulphan and cyclophosphamide, have been employed more frequently in recent years: such a combination, however, still confers a consistent risk for developing ovarian failure, by altering germ cell viability and gonadal hormone production (Sanders *et al*., 1988; Afify *et al*., 2000; Sklar *et al*., 2001). Additionally, a 'cytokine storm' is released in the peri-transplantation period (Jadus *et al*., 1992), and a prolonged immune deficiency usually occurs due to delayed and incomplete recovery of the immune system in both allogeneic and autologous SCT recipients.

Allogeneic graft compromises the host immune system more severely than the autologous one due to a prolonged treatment by immunosuppressive drugs needed to avoid the 'reverse' rejection. Indeed, acute or chronic graft-versus-host disease

Table I. Clinical features in 22 women after allogeneic stem cell transplantation (SCT), 23 women after autologous SCT and in 45 healthy women as controls

	Allo-SCT (n = 22)	Auto-SCT (n = 23)	Controls (n = 45)
Age at evaluation (years)	27.6 ± 2.3	28.2 ± 2.6	28.5 ± 2.4
Time from transplant (months)	18.9 ± 1.2	17.8 ± 1.7	–
BMI (kg/m^2)	23.4 ± 0.9	25 ± 1.0	24.2 ± 0.8
Diagnosis:			
AML	9	10	–
ALL	4	–	–
CML	7	–	–
HD	2	6	–
NHL	–	4	–
CLL	–	3	–
Previous treatment history:			
AML	Idarubicin/mitoxantrone (MIT)/cytarabine (CYT)/etoposide		
ALL	Vincristine/daunorubicin/cyclophosphamide/methotrexate/MIT/CYT/asparaginase		
CML	Hydroxyurea/interferon-α		
HD	Vincristine/adriblastine/bleomycin/etoposide		
NHL	Methotrexate/adriblastine/cyclophosphamide/vincristine/prednisone		
CLL	Chlorambucil/fludarabine		
Previous use of alkylating agents (no. of patients)	6/22 (27%)	13/23 (56.5%)	
Prednisone cumulative dose (g/m^2)	7.7 ± 1.8	8.2 ± 0.9	–
Prednisone treatment duration (months)	8.4 ± 1.8*	5.9 ± 0.7	–

Values are expressed as mean ± SEM. Corticosteroid cumulative dose is expressed as prednisone equivalents.
*$P < 0.001$ versus autologous setting.
BMI = body mass index; AML = acute myeloid leukaemia; ALL = acute lymphocytic leukaemia; CML = chronic myeloid leukemia; HD = Hodgkin disease; NHL = non-Hodgkin lymphoma; CLL = chronic lymphocytic leukaemia.

(aGVHD, cGVHD)—a complication affecting >50% of patients after allografting—induces additional relevant alteration in the immune system (Sullivan *et al*., 1991).

We have recently demonstrated that cGVHD profoundly impairs endocrine functions in allo-STC recipients, even in the absence of TBI (Tauchmanovà *et al*., 2002). Moreover, cGVHD was associated with lower sperm count in male long-term survivors after busulphan/cyclophosphamide conditioning (Grigg *et al*., 2000). It should be emphasized that most previous studies focused on the etiopathogenetic role of chemotherapy and TBI in inducing ovarian failure and infertility and considered allo- and auto-SCT patients as one group (Sanders *et al*., 1988; Chatterjee and Goldstone, 1996; Schimmer *et al*., 1998; Afify *et al*., 2000; Grigg *et al*., 2000; Couto-Silva *et al*., 2001; Tauchmanovà *et al*., 2002). Conversely, we observed more frequent recovery of menstrual cycles in women after auto-SCT than in those all-transplanted.

To investigate whether differences in ovarian residual function exist in the allogeneic and autologous setting treated with similar conditioning regimen without TBI, we designed this observational, analytical, prospective, controlled study.

Materials and methods

Subjects

Fifty consecutive reproductive age women (24 allo- and 26 auto-transplanted, aged 21–45 years, median 29) who had received SCT for malignant haematopoietic disorders in our institution were enrolled in this pilot study between 12 and 24 (median, 17) months after SCT. Inclusion criteria were: (i) disease-free at the time of evaluation with complete haematological reconstitution; (ii) ⩾12-month period elapsed since SCT. Exclusion criteria were: (i) history of radiation therapy; (ii) previous history of menstrual cycle disorders or endocrine diseases; (iii) previous HRT.

Five women were excluded because of hyperthyroidism (n = 1), delayed puberty (n = 1), history of oligomenorrhoea prior to the disease (n = 2) and radiation treatment (n = 1). In the remaining 45 patients, primary diseases were acute or chronic myelogenous leukaemia (AML, n = 19 and CML, n = 7 respectively), acute lymphoblastic leukaemia (ALL, n = 4), chronic lymphocytic leukaemia (CLL, n = 3), Hodgkin disease (HD, n = 8) and non-Hodgkin lymphoma (NHL, n = 4). Allo- and auto-transplanted patients with AML received SCT in their first complete remission, patients with ALL, HD, NHL and CLL were transplanted in second complete remission. Profile at study entry of the patients is summarized in Table I.

Forty-five healthy pre-menopausal women age- and body mass index (BMI)-matched with the patients agreed to participate in this study and were included as controls. All of them had regular menstrual cycles. Exclusion criterion for control women was the use of estrogen/progesterone use as contraceptive or replacement treatment.

Informed consent was given by all patients and the study was designed in accordance with the Helsinki II Declaration.

Treatments

Both allo- and auto-transplanted patients for AML, CML and ALL received the same conditioning regimen BUCY2, including busulphan (16 mg/kg in 4 days) and cyclophosphamide (120 mg/kg in 2 days). Patients with HD, NHL and CLL were conditioned with the BEAM protocol, consisting of carmustine (300 mg/m^2 in 1 day), etoposide (200 mg/m^2 in 4 days), cytarabine (400 mg/m^2 in 4 days) and melphalan (140 mg/m^2 in 1 day). Allo-SCT recipients received bone marrow-derived stem cells from HLA-identical siblings. After allografting, aGVHD prophylaxis was performed with cyclosporin A (1 mg/kg i.v. from day –1 to +21, then 10 mg/kg p.o. for 6 months) and short course methotrexate (10 mg/kg for four doses). Acute GvHD was treated by high-dose methylprednisolone (2–10 mg/kg for 10 days),

Table II. Univariate predictors of cycle recovery

	Cycle recovery	No cycle recovery	*P*
Age at SCT (years)	21.7 ± 2.3	28.5 ± 2.5	0.013
BMI at SCT (kg/m^2)	23.6 ± 1.6	23.9 ± 0.9	NS
BMI at evaluation (kg/m^2)	22.8 ± 1.8	24.7 ± 1.4	NS
Corticosteroid dose (mg/m^2)	8.9 ± 2.7	8.1 ± 1.1	NS
Corticosteroid duration (months)	9.6 ± 2.2	7.3 ± 1.9	NS
Time from SCT (months)	20.2 ± 1.7	17.1 ± 1.8	0.047
Patients previously treated with alkylating agents (*n*/total)[a]	3/6 (50%)	16/39 (41%)	NS

Corticosteroid dose was calculated as prednisone equivalents.
[a]χ^2 test was used for comparison of the frequency.
Student's *t*-test was used to compare mean values.
BMI = body mass index; SCT = stem cell transplantation; NS = not significant.

followed by slow dose tapering in the following 6 months, monitoring patients' clinical conditions. Chronic GVHD was treated by prednisolone at doses of 1–2 mg/kg, associated with CsA at doses ranging from 1 to 8 mg/kg/day. Previous treatment history included alkylating agents for patients with ALL, CLL, HD and NHL (Table I) and consisted in a mean 6 month duration of chemotherapy for all diagnoses. Acute GVHD was considered according to the Glucksberg *et al*. (1974) grading system, and cGVHD was graded as either limited or extensive, based upon clinical severity and target organ involvement (Shulman *et al*., 1980). Nine women had been affected by aGVHD of global grade 1–3 and 13 women were affected by cGVHD (six limited and seven extensive form). Three women with the limited form had liver involvement and the other three skin manifestation. Liver, skin, intestine and eyes were the most common cGVHD sites in the extensive form with variable combination [liver and gastrointestinal ($n = 2$), liver and eyes ($n = 2$), skin, gut and eyes ($n = 2$), skin, gut and kidney ($n = 1$)]. Liver manifestation included an increase of liver enzymes in all women (ALT: 50–1100 U/l; AST: 82–761 U/l; γ-glutamyltransferase: 90–350 U/l) and reversible cholestasis with alkaline phosphatase (487–785 U/l) and bilirubin increase (5–9 mg/dl) associated with icterus in two. Skin lesions included lichen planus and focal epidermal atrophy. Ocular dryness (Sjogren-like syndrome) was the major ophthalmological manifestation, while oral mucosa dryness and lichenoid lesions were the upper gastrointestinal tract symptoms. Diarrhoea and malabsorption were present in two women. One woman developed reversible membranous glomerulonephritis with nephrotic syndrome. All these patients were treated as stated above for a period ranging 6–24 months (Table I). After evaluation, hormone substitution treatment with estradiol and medroxyprogesterone was initiated in all women with amenorrhoea unless contraindicated or refused.

Design

Previous medical records were reviewed to obtain complete information on patients' outcome, including data on menstrual history pre-transplantation. Post-transplantation data were recorded prospectively. BMI (kg/m^2) was determined in all subjects. In the control group the endocrine and ultrasonography was performed in the early follicular phase of the menstrual cycle (2–3 day).

Endocrine evaluation

In all subjects, blood samples were obtained between 08:00 and 10:00 a.m. Circulating FSH, LH, prolactin, 17β-estradiol, testosterone and Δ_4-androstenedione levels were assayed to assess the hypothalamic–pituitary–ovarian function. Dehydroepiandrosterone sulphate (DHEAS) was also measured as the major androgen of adrenal origin. All measurements were performed by commercially available kits: 17β-estradiol by radioimmunoassay (RIA) (Nichols Institute Diagnostics, USA), total testosterone and DHEAS using Immulite, solid phase chemiluminescent enzyme immunoassay (DPC, USA); FSH and LH with RIA Biodata S,p.A. kits (Rimini, Italy); androstenedione using RIA Diagnostic Systems Laboratories kit (Webster, USA); at least two different samples were taken in all subjects and the average value was calculated for each hormone. Detection limits were 3 pg/ml for 17β-estradiol, 5 ng/dl for testosterone, 10 ng/dl for Δ_4-androstenedione and 2 μg/dl for DHEAS. Intra-assay and inter-assay coefficients of variation were <7 and <15% respectively, for all endocrine determinations.

Table III. Predictors of cycle recovery by regression analysis

	Odds ratio (95% CI)	*P*
Age at SCT <21 years	6.8 (0.191–0.829)	< 0.05
Allogeneic setting	0.47 (–0.337–0.116)	NS
Previous alkylating treatment	0.72 (–0.303–0.143)	NS
Time from SCT >18 months	2.1 (–0.116–0.336)	NS

SCT = stem cell transplantation; NS = not significant.

Ultrasonographic evaluation

Pelvic ultrasonography was performed in all patients and controls using a 7.5 MHz transvaginal transducer. The study was performed by a single well-trained operator (S.P.), blind in respect to patient or control examination. Ovarian and uterine volumes were estimated using the ellipsoid formula (longitudinal×antero-posterior×transversal diameter×0.523) (Pavlik *et al*., 2001). The mean ovarian volume for each woman was calculated. Number of follicles and endometrium thickness were also recorded.

Statistical analysis

Student's *t*-test for paired and unpaired data was used to compare the group of patients and controls, and different subgroups of patients respectively. The non-parametric Mann–Whitney *U*-test was used to compare hormone levels in patients and controls as well as in allo- versus auto-SCT patient group, when Wilk–Shapiro's test was not consistent with the Gaussian distribution of the data. To assess the dependence between endocrine and ultrasonographic features of the patients, the Pearson's correlation coefficient was calculated. As possible predictor variables of menstrual cycle recovery, age at transplant, BMI, time from SCT, previous use of alkylating agents and type of transplant were considered. Univariate and regression analysis was performed using the cycle recovery as a binary outcome variable. Two-sided $P < 0.05$ was taken as statistically significant. Data are expressed as mean ± SEM.

Table IV. Endocrine and ultrasound findings in women after allogeneic or autologous stem cell transplantation (SCT) and in controls

	Allo-SCT (n = 22)	P versus controls	Auto-SCT (n = 23)	P versus controls	Controls (n = 45)	Reference intervals
FSH	127 ± 16[b]	< 0.001	63.6 ± 7.9	< 0.001	6.8 ± 3.2	2–13 IU/l
LH	75 ± 10[b]	< 0.001	28.2 ± 3.7	< 0.001	10.6 ± 2.6	2–15 IU/l
17β-Estradiol	48.8 ± 6.7[a]	< 0.001	73.05 ± 8.8	< 0.001	183.6 ± 19.2	140–734 pmol/l
Testosterone	0.55 ± 0.07[a]	< 0.001	1.32 ± 0.14	0.551	1.35 ± 0.14	0.68–2.8 nmol/l
Δ_4-Androstenedione	1.74 ± 0.14[a]	< 0.001	3.84 ± 0.17	0.947	4.1 ± 1.22	3.4–10 nmol/l
Dehydroepiandrosterone sulphate	3.3 ± 0.29	< 0.05	4.3 ± 0.7	NS	4.7 ± 0.3	1.4–7 μmol/l
Prolactin	8.8 ± 0.89	NS	9 ± 1.2	NS	9.5 ± 1.3	2–15 μg/l
Ovarian volume (cm^3)	1.35 ± 0.14[c]	< 0.001	3.3 ± 0.14	< 0.001	8.2 ± 0.17	5.7–10 cm^3*
Uterine volume (cm^3)	27.2 ± 0.76[c]	< 0.001	47.3 ± 1.56	< 0.001	53.0 ± 1.9	–
No. of follicles per ovary	0.4 ± 0.7[c]	< 0.001	3 ± 2	< 0.001	8 ± 2	–
Endometrial thickness (mm)	3.5 ± 0.15	< 0.05	3.9 ± 0.14	< 0.05	6.4 ± 0.16	5–8 mm*

Values are expressed as mean ± SEM. Ovarian and uterine volumes were calculated according to the ellipsoid formula (multiplication of 3 diameters×1/3π).
*Data relative to the early follicular phase of menstrual cycle.
[a]$P < 0.05$, [b]$P < 0.01$ and [c]$P < 0.001$ versus auto-SCT.
NS = not significant.

Results

Menstrual history data indicated that the disappearance of menstrual cycles was caused by the myelo-ablative conditioning therapy in 30 women, while the other 15 had amenorrhoea after conventional chemotherapy shortly before SCT. Recovery of menstrual cycles appeared slightly, but not statistically, more frequent in the group of auto-transplanted patients (4/23 versus 2/22 in the allo-SCT group). Women who experienced recovery of the ovarian function were younger than those who did not, and were transplanted from a longer period ($P < 0.05$). BMI, corticosteroid cumulative dose and duration of treatment did not differ in women with or without cycle recovery (Table II). By the logistic regression, however, only young age at SCT was a positive predictor of cycle recovery ($P < 0.05$), while previous use of alkylating agents, type of transplant, BMI and time elapsed from transplant did not reach statistical significance (Table III).

At study entry, the groups of allo- and auto-transplanted women had similar age and follow-up period after transplantation, while they differed in the underlying haematological disease. Patients with AML and CML were not treated by alkylating agents, all patients with CML received allogeneic SCT, while patients with AML were treated by allo- or auto-SCT in a similar proportion (9 versus 10). Consequently, before transplant alkylating agents were more frequently used in the auto-SCT group. The dose of corticosteroids was similar in both patient groups, although most allo-transplanted patients were treated for longer periods, and treatments were stopped more recently, as in the auto-SCT group steroid treatment was included in chemotherapeutic regimens previous to the transplant (Table I).

Endocrine evaluation

Endocrine parameters of the two patients' groups and controls are shown in Table IV. Serum gonadotrophin levels were significantly higher ($P < 0.001$) while 17β-estradiol, Δ_4-androstenedione, testosterone ($P < 0.001$) and DHEAS ($P < 0.05$) were lower in allo-SCT patients than in controls. The auto-SCT women had significantly higher ($P < 0.001$) gonadotrophin values and lower 17β-estradiol ($P < 0.001$) than controls while Δ_4-androstenedione and testosterone levels were similar. Serum gonadotrophin levels were significantly higher ($P < 0.001$) and 17β-estradiol, Δ_4-androstenedione and testosterone significantly lower ($P < 0.05$) in the allo-SCT compared with the auto-SCT group. Prolactin levels were within the normal range in all subjects, without any difference between patients and controls. Similar results with the same statistical significance were obtained when only women affected by AML were compared (10 auto- and 9 allo-transplanted, data not shown). When allo-SCT patients were divided according to presence of cGVHD, ovarian failure was found to be slightly more severe in women with cGVHD (Table V).

Table V. Clinical features, endocrine and ultrasound evaluation in women after allogeneic SCT according to chronic GVHD occurrence

	Chronic GVHD (n = 13)	No chronic GVHD (n = 9)	P
Age (years)	26.6 ± 3.1	25.2 ± 3.8	NS
Time since transplant (months)	18.5 ± 1.1	19 ± 1.3	NS
Body mass index (kg/m^2)	21.2 ± 2.2	24.1 ± 1.9	NS
FSH (IU/l)	140 ± 22.3	106.3 ± 6.6	< 0.05
LH (IU/l)	78.6 ± 12.5	66.3 ± 2.4	NS
17β-estradiol (pmol/l)	37.4 ± 4.26	58 ± 5.1	< 0.05
Prolactin (μg/l)	7.65 ± 1.4	7.9 ± 1.5	NS
Testosterone (nmol/l)	0.4 ± 0.1	0.55 ± 0.11	NS
Δ_4-Androstenedione (nmol/l)	1.57 ± 0.17	1.85 ± 0.23	NS
DHEAS (μmol/l)	2.7 ± 0.32	3.5 ± 0.23	< 0.05
Ovarian volume (cm^3)	1.05 ± 0.14	1.4 ± 0.15	< 0.001
Uterine volume (cm^3)	23.1 ± 0.9	34 ± 1.9	< 0.001

Values are expressed as mean ± SEM.
Ovarian and uterine volumes were calculated according to ellipsoid formula (multiplication of 3 diameters×1/3π).
GVHD = graft-versus-host disease; DHEAS = dehydroepiandrosterone sulphate.

Ultrasonographic evaluation

Ultrasonographic results in the patients' groups and in controls are shown in Table IV. Ovarian size in our control group was within the normal range of pre-menopausal women with similar age (Wehba *et al*., 1996; Flaws *et al*., 2000; Pavlik *et al*., 2001). Volumes of ovaries and uterus were lower in the

patients, both allo- and auto-transplanted, when compared with the controls ($P < 0.001$). Furthermore, both uterine and ovarian volumes were significantly ($P < 0.001$) smaller in the allo- than in the auto-SCT group.

In the patient group, the mean number of follicles was significantly ($P < 0.001$) lower than in the controls; and allo-transplanted women had fewer follicles than those auto-transplanted ($P < 0.001$). In particular, four to 12 small and larger follicles (diameter, <12 mm) per ovary were observed in the control group, several small ovarian follicles (diameter <6 mm) were found in 12 women after auto-SCT and in only four after allo-SCT. In the allo- and auto-SCT groups, without difference between groups, the mean endometrial thickness was significantly ($P < 0.05$) lower in comparison with controls (Table IV). In fact, endometrial thickness was very low or undetectable in most SCT patients.

With regard to the presence of cGVHD, ovarian and uterine volumes were significantly lower in women with than in those without GVHD ($P < 0.001$; Table V).

As expected, in the patient group considered as a whole, 17β-estradiol ($r = 0.51$; $P < 0.05$) and $Ä_4$-androstenedione ($r = 0.55$; $P < 0.05$) values correlated with ovarian volume; 17β-estradiol was also inversely correlated with FSH levels ($r = –0.67$; $P < 0.05$). Uterine volume correlated with ovarian volumes ($r = 0.77$; $P < 0.01$). On the other hand, no correlation was found between age, BMI, time elapsed from transplantation and endocrine or ultrasonographic parameters.

Discussion

The results of the present study show that reproductive age women undergoing allogeneic or autologous SCT have a premature ovarian failure (secondary hypergonadotrophic amenorrhoea), that persists up to 24 months after transplant in most cases (87%). Only younger age (<21 years) at SCT was an important predictor of ovarian function recovery. Although cycles recovered more frequently in auto-SCT women, the difference when compared with the allo-SCT group was not significant. The longer period elapsed between SCT and evaluation in women who experienced recovery of ovarian function, suggests that recovery is not an early event and may occur long after SCT. By regression analysis, the type of transplant, BMI, previous use of alkylating agents and steroids were not associated with a different probability of spontaneous recovery of ovarian function. However, some of these factors can become significant in larger populations of these particular patients. In fact, age, weight gain, disease stage and use of systemic chemotherapy were also important predictors of menopause onset in other female neoplastic populations (Goodwin *et al*., 1999).

Ovarian failure is reported to be a rather frequent event after myelo-ablative therapy followed by SCT in postpubertal patients (Sanders *et al*., 1988; Schubert *et al*., 1990; Shalet *et al*., 1995; Chatterjee and Goldstone, 1996; Grigg *et al*., 2000). However, whether it is due to high-dose alkylating agents and/or TBI used in the conditioning regimens, to conventional chemotherapy before myelo-ablative treatment, to the combination of all three treatment approaches (Keilholtz *et al*., 1997; Couto-Silva *et al*., 2001) or to other unknown factors is still to be fully elucidated. Most previous studies (Sanders *et al*., 1988; 1996; Chatterjee and Goldstone, 1996; Schimmer *et al*., 1998; Afify *et al*., 2000; Grigg *et al*., 2000; Couto-Silva *et al*., 2001; Tauchmanovà *et al*., 2002), having as their endpoints spontaneous recovery of ovarian function, infertility or pregnancy rates, have focused on the role of different treatments, such as TBI and chemotherapy, in inducing ovarian failure. Additionally, in previous studies no distinction between allo- and auto-SCT subjects has been performed, and different conditioning regimens, also including TBI, were considered altogether. In an attempt to overcome these limitations, we included patients receiving a similar conditioning treatment, excluding TBI, and analysed auto- and allo-SCT patients separately.

One of the relevant finding of the current study, is that allo-transplanted recipients had lower 17β-estradiol and androgen circulating levels and lower ovarian volume than in the auto-transplanted ones. Importantly, among allo-transplanted women those who developed cGVHD had lower 17β-estradiol, $Δ_4$-androstenedione, testosterone and DHEAS and higher FSH and LH levels than those who did not develop GVHD. The potential role of a lower BMI (indicating less fat mass after cGVHD) in inducing androgen aromatization to estrogens seems to be negligible, considering the lower circulating androgen levels and the absence of correlation between BMI and endocrine parameters. It should also be noted that auto-transplanted women had an ovarian volume similar to that observed in women after a corresponding interval from physiological menopause; in contrast the ovarian volume of allo-SCT was lower (Wehba *et al*., 1996; Flaws *et al*., 2000; Pavlik *et al*., 2001).

The differences in endocrine and ultrasound parameters between auto- and allo-transplanted patients were similar in the subgroup including only women with AML, indicating that results did not depend on the underlying disease, but likely on different transplant procedures. Chemotherapy is known to induce ovarian failure by apoptotic changes in pre-granulosa cells causing follicle loss (Warne *et al*., 1973; Chabner *et al*., 1996). In particular, alkylating drugs have been shown to alter base pairs, leading to DNA cross-links and single-strand breaks (Epstein, 1990). The combination of high-dose cyclophosphamide and busulphan is one of the most potent conditioning regimens to induce ovarian failure (Brennan and Shalet, 2002). Primordial follicles were absent in some patients at ovarian biopsy, likely due to damage of oocytes and proliferating as well as resting follicles supporting granulosa cells (Warne *et al*., 1973).

An additional finding in our cohort of patients was that, besides having lower 17β-estradiol levels, allo-SCT women had lower testosterone levels than both auto-SCT and control women. The ovarian secretion of estrogens is reported to decline faster than that of androgens after physiological menopause, likely due to progressive follicular atresia with persistent androgen production from stromal ovarian tissue (Lindgren *et al*., 2000). Consequently, the ovaries were hypothesized to become primarily androgen-producing glands, capable of maintaining gonadotrophin responsiveness for many

years (Lindgren *et al*., 2000). As a matter of fact, different studies had shown that ovarian testosterone production remains relatively constant, thereby increasing the relative ovarian contribution to the global testosterone production and androgen:estrogen ratio (Judd *et al*., 1974; Adashi, 1994). Conversely, absent gonadotrophin receptors and insignificant 17β-estradiol and androgen production have been recently shown in post-menopausal women after cessation of menstrual cycles for ⩾5 years (Couzinet *et al*., 2001). The different period of time elapsing from menopause onset to evaluation could explain the difference between these apparently contrasting findings. Serum 17β-estradiol, Δ_4-androstenedione and testosterone levels in our cohort of allo-SCT patients were significantly decreased, indicating both follicles and stromal endocrine cell damage, with signs of more severe damage in women affected by cGVHD. Conversely, in auto-SCT patients only serum 17β-estradiol levels were decreased, while Δ_4-androstenedione and testosterone levels were normal 12–24 months after transplantation. The ovarian contribution to lower serum androgens is confirmed by the correlation between ovarian volume and 17β-estradiol and androgen levels. However, since lower DHEA-S values were found in allo-SCT patients, particularly in those with cGVHD, effects of longer and more recent corticosteroid use on adrenal androgen secretion cannot be ruled out. The degree of immune system involvement might be hypothesized as a major difference between the two groups.

While the toxic effects of antiblastic agents have been widely described as detrimental, the impact of immune system damage on endocrine function in transplanted women is not clear. Allogeneic SCT is an exceptional condition in that a massive number of donor immunological cells are infused into a host, which can recognize the host as 'non-self'. The best-known target organs for such a 'graft versus host reaction' are skin, liver, gut and lung, but any other organ can be targeted including gonads, and the neoplastic tissue itself. The prophylaxis and treatment of GVHD require immunosuppressive drugs, which further compromise immune system function and whose effects cannot be separate from those of the immune system dysregulation itself. Azoospermia related to cGVHD (Grigg *et al*., 2000) was also reported in allo-SCT recipients, which is in line with our hypothesis on a possible involvement of the immune system derangement in the gonadal damage.

In the current study, we did not investigate circulating autoantibodies against ovary since the existence of a correlation between anti-ovarian antibody titre, cellular immune dysfunction and histological evidence of inflammation in women with autoimmune ovarian failure is still undefined. Circulating anti-ovary autoantibodies are not correlated with clinical activity of autoimmune oophoritis and their pathogenic role remains questionable (Kinch *et al*., 1965; Hoek *et al*., 1997).

In conclusion, ovaries after auto-SCT show features similar to those found after physiological menopause, whereas allo-SCT seems to be associated with major injury, including lower estradiol and androgen production and smaller ovarian size. Chronic GVHD following allografting further worsens this condition. Ovarian failure in SCT recipients seems to be related principally to the myelo-ablative conditioning regiments; however, ovarian steroid levels and size can be further influenced in the allogeneic setting, likely by a major deregulation of the immune system and its treatments. The impact of a lower residual estrogen and androgen steroid secretion after allo-transplantation on women's health, especially in terms of osteoporosis, cardiovascular risks and quality of life remains to be established as well as any future potential therapeutic implication.

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