

Time-related arterial changes after allogeneic hematopoietic stem cell transplantation in children

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BACKGROUND: Metabolic syndrome is a frequent late effect in young adults after hematopoietic stem cell transplantation (HSCT) performed in childhood.

METHODS: To further study the signs of cardiovascular changes in HSCT patients, we performed noninvasive vascular ultrasonic measurements of arterial stiffness and endothelial function in 25 children (median age: 11.2 y) and in 22 healthy matched controls.

RESULTS: The distensibility of the common carotid artery (CCA) was significantly lower in the patients than in the controls (mean = 0.48, SD = 0.19 vs. mean = 0.64 mm Hg⁻¹ × 10⁻², SD = 0.28; *P* = 0.024). The distensibility decreased with time passed after HSCT (*P* = 0.009). The compliance of the CCA was decreased (mean = 0.10, SD = 0.04 vs. mean = 0.13 mm² × mm Hg⁻¹, SD = 0.05; *P* = 0.041), and the incremental elastic modulus (*E* inc) was higher in the patients than in the controls (mean = 2.05, SD = 0.7 vs. mean = 1.6 mm × 10³, SD = 0.6; *P* = 0.019). *E* inc was associated with time passed after HSCT (*P* = 0.036). The size of the brachial artery and flow-mediated dilation did not differ between the groups.

CONCLUSION: Early mechanical changes of the arterial wall were found at young age after HSCT. Ultrasonography may offer a noninvasive method to find early alterations of the vascular bed and to optimize prevention of atherosclerosis in HSCT patients.

The number of long-term survivors after hematopoietic stem cell transplantation (HSCT) has increased, and, consequently, secondary morbidity and quality-of-life issues have become more and more important, especially in subjects who undergo transplantation in childhood (1,2). Endocrinopathies, including growth hormone deficiency, hypothyroidism, and hypogonadism, are the most common late effects after HSCT (1,3). However, metabolic (4) and cardiovascular morbidity (5), as newer entities in the field, may deserve more emphasis with elapsing time after transplantation. We observed insulin resistance, impaired glucose tolerance, and dyslipidemia in a time-correlated manner in one-third of the young adults studied 3–18 y after HSCT in childhood (4). Metabolic abnormalities have also been reported in adult female recipients of

stem cell transplantation (6). Metabolic disturbances can be seen as a prologue to atherosclerosis, and even more cardiovascular morbidity may be anticipated in the middle-aged survivors of childhood malignancies.

Ultrasonography, as a noninvasive method, has been a valuable tool in revealing early changes in the endothelial function even in young children beyond 6 y with known underlying cardiovascular risk factors. In familial hypercholesterolemia, endothelial function is disturbed, and the carotid wall is stiffer from early childhood as compared with healthy controls (7–10). In children with low birth weight, endothelial dysfunction and altered stiffness of the common carotid artery (CCA) are found as young as the age of 9 y (9,10). In addition, chronic hyperglycemia impairs endothelial function (11), and the intima–media layer is thicker in diabetic children than in healthy age-matched controls (12). Both reduced flow-mediated vasodilation and increased intima–media thickness (IMT) have been proven to correlate with late atherosclerosis and cardiovascular morbidity and mortality in adults (13,14).

The aim of this study was to evaluate whether HSCT performed in childhood, in addition to metabolic abnormalities, also implies early changes of arterial wall and endothelial function.

RESULTS

The anthropometric, hemodynamic, and biologic characteristics of the patients and controls are shown in **Table 1**. No differences in age or sex were found between the two groups, but the patients were shorter (*P* = 0.005) and weighed less (*P* = 0.04) than the age-matched controls. The patients with hormone deficiency were on appropriate substitution.

Intima–Media Thickness

The diameter or the IMT of the CCA did not differ between patients and controls (**Table 2**). However, intima–media was thicker in the male than in the female patients (mean = 0.50, SD = 0.058 vs. mean = 0.43, SD = 0.049; *P* = 0.003). In the controls, there was no significant difference between the males and the females.

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Table 1. Anthropometric, hemodynamic, and biological characteristics of the HSCT patients and the controls

	Study patients	Controls	P value
n (F/M)	25 (12/13)	22 (12/10)	
Age, y (range)	11.0 (6.4–14.9)	11.3 (6.7–15.5)	0.621
Weight, kg (range)	35.4 (18.5–70.0)*	43.1 (22.3–71.8)	0.040
Height, cm (range)	139 (116–163)*	152 (117–183)	0.005
BMI, kg/m ² (range)	17.8 (12.6–27.6)	18.3 (15.0–23.1)	0.559
SBP, mm Hg, SD	106 ± 11	107 ± 9	0.844
PP, mm Hg, SD	49 ± 8	50 ± 8	0.651
EF, %	71.9 ± 5.4	71.6 ± 6.9	0.893
Total cholesterol, mmol/l	4.5 ± 0.8	4.4 ± 0.7	0.779
HDL cholesterol, mmol/l	1.6 ± 0.4	1.7 ± 0.3	0.573
HDL/total cholesterol	0.37 ± 0.08	0.40 ± 0.07	0.422
LDL cholesterol, mmol/l	2.3 ± 0.7	2.3 ± 0.6	0.92
Triglycerides, mmol/l	1.1 ± 0.7*	0.7 ± 0.3	0.026
fb-Gluc, mmol/l	4.9 ± 0.5	4.8 ± 0.4	0.765
fb-insulin, mmol/l	14.2 ± 30.3	5.8 ± 3.8	0.224

EF, ejection fraction; F, female; fb-Gluc, blood glucose after an overnight fast; fb-insulin, blood insulin after an overnight fast; HDL, high-density lipoprotein; HSCT, hematopoietic stem cell transplantation; LDL, low-density lipoprotein; M, male; PP, pulse pressure; SBP, systolic blood pressure.

*P < 0.05.

Table 2. Dimensions, mechanical properties of the CCA, and flow-mediated brachial dilation in HSCT patients and controls (mean ± SD)

	Study patients	Controls	P value
ESD, mm	5.68 ± 0.57	5.81 ± 0.38	0.253
EDD, mm	5.09 ± 0.49	5.04 ± 0.32	0.843
ΔD, mm	0.59 ± 0.19*	0.77 ± 0.24	0.004
ΔD/EDD, %	11.7 ± 3.7*	15.4 ± 5.3	0.004
IMT, mm	0.47 ± 0.06	0.47 ± 0.05	0.845
CCA distensibility, mm Hg ⁻¹ × 10 ⁻²	0.48 ± 0.19*	0.64 ± 0.28	0.024
CCA compliance, mm ² × mm Hg ⁻¹	0.10 ± 0.04*	0.13 ± 0.05	0.041
E inc, mm Hg × 10 ³	2.05 ± 0.7*	1.6 ± 0.6	0.019
Flow-mediated dilation, %	6.5 ± 5.6	6.3 ± 4.2	0.866

CCA, common carotid artery; EDD, end-diastolic diameter; E inc, incremental elastic modulus; ESD, end-systolic diameter; HSCT, hematopoietic stem cell transplantation; IMT, intima-media thickness; ΔD = ESD – EDD.

*P < 0.05.

Mechanical Properties of the CCA

The distensibility of the CCA was significantly lower in patients than in controls (mean = 0.48, SD = 0.19 vs. mean = 0.64 mm Hg⁻¹ × 10⁻², SD = 0.28; P = 0.024) (Figure 1) and decreased with the time passed after HSCT (P = 0.009) (Figure 2). Distensibility was dependent on age only in the control group (P = 0.034). Compliance was also decreased in patients as compared with controls (mean = 0.10, SD = 0.04 vs. mean = 0.13 mm² × mm Hg⁻¹, SD = 0.05; P = 0.041). Incremental elastic modulus (E inc) was higher in HSCT patients than in controls (mean = 2.05, SD = 0.7 vs. mean = 1.6 mm × 10³, SD = 0.6; P = 0.019) (Figure 3) and was

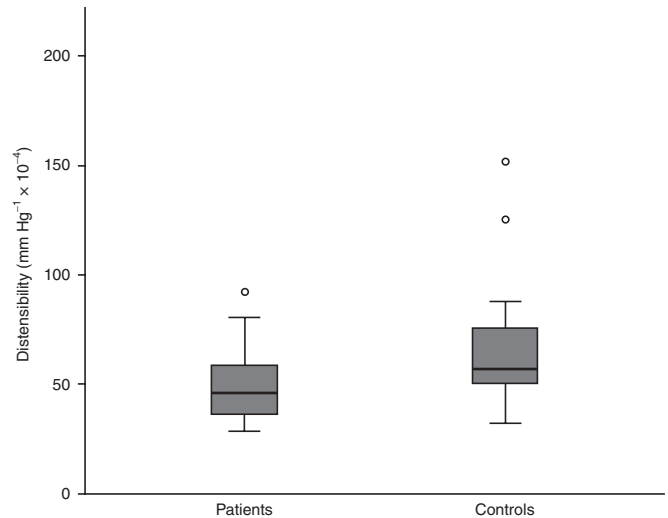


Figure 1. Distensibility in patients and controls. Circles are outliers (one in patient group and two in control group; difference to mean now 2 SDs).

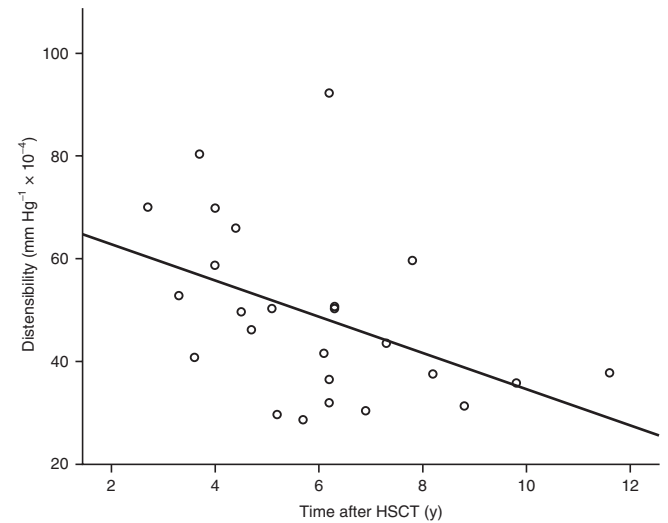


Figure 2. Distensibility in patients (circles) is associated with the time since HSCT. HSCT, hematopoietic stem cell transplantation.

dependent on the time since HSCT (P = 0.036) (Figure 4). No correlation was observed between age and E inc.

Flow-Mediated Dilation

There was no difference in the size of the brachial artery (mean = 3.04 mm, SD = 0.47 vs. mean = 3.06, SD = 0.41) or in the flow-mediated dilation (FMD) between the two groups.

Patient Characteristics and Arterial Wall Changes

Flow-mediated vasodilation tended to be slightly inferior in the 10 of 25 patients with hypothyroidism, although these patients were on appropriate hormone substitution (mean = 4.1%, SD = 3 vs. mean = 8.2%, SD = 5.9; P = 0.06). There was a negative correlation between total body irradiation (0–14 Gy) and distensibility of the CCA (P = 0.048), and also between total body irradiation and plasma high-density

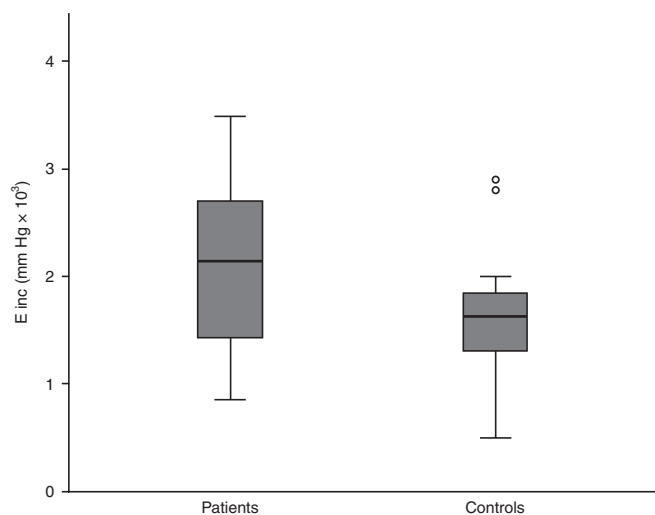


Figure 3. Incremental elastic modulus in patients and controls. In the control group, there were two outliers (circles; difference to mean more than 2 SDs).

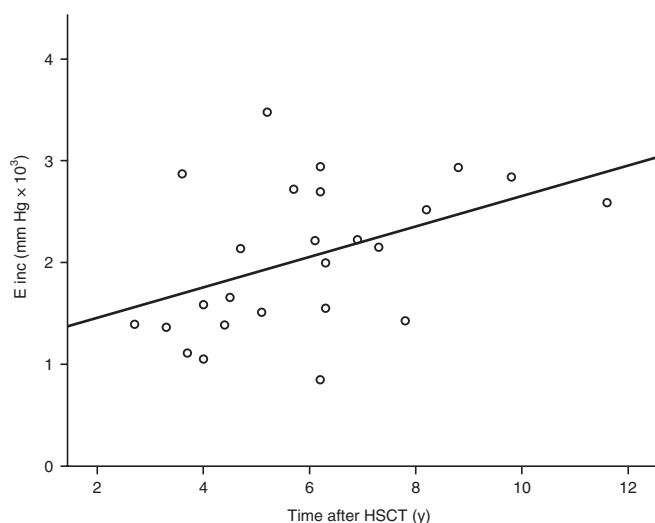


Figure 4. Incremental elastic modulus in patients (circles) is associated with the time after HSCT. HSCT, hematopoietic stem cell transplantation.

lipoprotein values ($P = 0.004$). Growth hormone deficiency, hypogonadism, microangiopathy, or clinical severity of acute or chronic graft-vs.-host disease (GVHD) did not correlate with IMT of the CCA or the elastic properties of the arterial wall.

Plasma triglyceride level, although within normal range, was higher in patients as compared with controls ($P = 0.026$) (Table 1). The mean plasma levels of total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, fasting glucose, and serum insulin did not differ between the two groups (Table 1). Plasma high-density lipoprotein cholesterol and the triglycerides correlated with distensibility of the CCA. In the patient group but not in the control group, triglyceride levels correlated with IMT. There was no correlation between insulin or glucose levels or the homeostasis model assessment index and CCA distensibility.

DISCUSSION

Our main observation is that the distensibility and the compliance of the CCA were significantly inferior in children and adolescents after HSCT as compared with healthy controls. These findings, together with higher values of E inc, indicate increased stiffness of the arterial wall. To our knowledge, this is a novel observation. Consequently, HSCT with adjuvant treatment may turn out to be an atherogenic stimulus to children and adolescents later in life.

Healthy endothelium is not only a passive lining of the blood vessels but also an active metabolic organ. Endothelium is a highly selective barrier for macromolecules as well as a surface maintaining the fluidity of blood (15). The endothelial cells control the tone of the vascular smooth muscle, control the activity of the coagulation status, and act in the immune response through an interaction with circulating leukocytes. Disturbances in arterial mechanical properties have been shown to relate to endothelial dysfunction and risk of cardiovascular events (16), and in our HSCT study population, these disturbances were observed at a very young age. Our findings also suggest that the changes in the arterial wall may increase with time passed since HSCT. Further studies are needed to evaluate whether these alterations in arterial stiffness are an end result of a transplant-related toxic insult or a cross-sectional image of an ongoing progressive process in the endothelial cells or in the arterial wall.

As a whole, HSCT affects the vascular endothelium. The endothelial disorders associated with HSCT can be divided into acute and chronic ones (15). The clinical manifestations of the acute endothelial damage are thrombotic microangiopathy, veno-occlusive disease, and capillary leak syndrome. Chronic GVHD is considered to be associated with chronic endothelial damage (15). Treatment-related toxic effects frequently trigger early vascular damage. Total body irradiation and chemotherapy, given either earlier before transplant or as part of the conditioning regimen, are associated with increased number of circulating endothelial cells as a marker of endothelial damage (17). A negative correlation between total body irradiation and arterial wall distensibility was also observed in our study; this suggests a toxic effect of total body irradiation on arterial wall. In addition, GVHD (18) and calcineurin inhibitors used in the prophylaxis of GVHD (19) may contribute to endothelial damage. In our study, neither acute-phase microangiopathy nor chronic GVHD were associated with changes in vascular endothelial function during the follow-up period. However, the number of patients in these subgroups was too small to allow statistically relevant considerations.

We did not observe any difference in FMD, an early marker of atherosclerosis, in our study cohort. By contrast, Chow *et al.* (20) found in a pilot study a significant decrease in flow-mediated vasodilation in 14 young cancer patients treated with anthracyclines (300 mg/m^2 or more). Dengel (21) showed decreased FMD response in young adults treated for acute lymphoblastic leukemia during childhood with chemotherapy with or without cranial radiation. In both of these studies, the patients were older than our patients, all of whom were younger

Table 3. Transplant-related characteristics of the patient population

	HSCT patients
Diagnosis	
ALL	10
AML, MDS, CML	8
Nonmalignant hematopoietic disease	4
Other ^a	3
Age at HSCT, y (range)	5.2 (1.1–10.3)
Conditioning	
Cyclophosphamide	11
Cytarabine	13
Other ^a	1
TBI	20
10 Gy	13
12–14 Gy ^b	7
Donor	
HLA-identical sibling	13
HLA-matched registry donor	12
TMA	3
Graft	
Bone marrow	22
Umbilical cord blood	3
GVHD	
Acute, grade 2–4 ^c	7
Chronic	11
Limited	5
Extensive	6
Late effects ^d	
Hypothyroidism	10
Growth hormone insufficiency	6
Hypogonadism	4

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; GVHD, graft-vs.-host disease; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; MDS, myelodysplastic syndrome; TBI, total body irradiation; TMA, thrombotic microangiopathy.

^aFamilial hemophagocytic histiocytosis, non-Hodgkin lymphoma, Chediak-Higashi syndrome, one of each. ^bDose of TBI. ^cGrade of acute graft-vs.-host reaction from mild (2/4) to severe (4/4). ^dThe patients with hormone deficiency were on appropriate substitution.

than 16 y. As changes in the CCA seemed to advance with time since HSCT, it may be that our patients were too young to show any changes in the brachial artery. In addition, the timing of the measurement after cuff deflation may explain our negative result. Järvisalo *et al.* (22) has found a great variation in the FMD as a function of the time after cuff deflation in healthy children. We chose to delay the measurement until 80–90 s after deflation to identify the late responders also, but the relatively large SD of the FMD may result from the variance in the response time in our patients. Continuous registration of the vessel diameter from 30 to 150 s is recommended to overcome the problem of the individual variation in FMD (22).

In 1992, Celermayer *et al.* (14) had already described a noninvasive method for testing endothelium-dependent vasodilation with high-resolution ultrasound. Nowadays, echographic methods are commonly used to assess not only the flow-mediated vasodilation of the brachial artery but also vascular changes in the wall of the carotid artery (12,23). However, the ultrasonic method for the evaluation of the endothelial function is not yet clinical routine. As a noninvasive method, ultrasound is applicable, practical, and informative in a pediatric population (beyond the age of 6 y), provided that the cooperation of the patient is good.

The core signs of metabolic syndrome—hypertriglyceridemia, hyperinsulinemia, and abdominal obesity—have been observed in one-third of the young long-term survivors after allogeneic HSCT (4,24). Metabolic syndrome characterizes, at least when left untreated, a process from single disturbances in lipid and glucose metabolism to hypertension, type 2 diabetes, and severe cardiovascular disease (25). In our study, young HSCT patients had a higher mean levels of plasma triglycerides than controls, and in a former study, we showed that the metabolic disturbances may proceed over the time after HSCT (4).

The arterial wall changes, together with metabolic abnormalities, may contribute to the premature development of cardiovascular disease. It could be useful to perform vascular ultrasound for these patients in adolescence and repeat it after 5 y, and at the same time to evaluate lipid and glucose metabolism. We also recommend that these young patients avoid lifestyle risk factors such as smoking and overweight.

Heterogeneity of the diagnoses reflects the clinical reality, but it is also a source of bias in our study. In addition, a larger number of patients with nonmalignant diseases would have given the tools to compare the impact of pretransplant and conditioning chemotherapy on the vascular wall changes. All the ultrasonographic measurements were performed by the same experienced examiner, which we consider a strength of our study.

In conclusion, early alterations of the arterial wall were found after HSCT. Time after HSCT and total body irradiation seemed to affect arterial stiffness. The evaluation of early vascular changes in childhood offers the option of preventive treatment and counseling to avoid secondary morbidity, especially premature atherosclerosis.

METHODS

Subjects

This study was performed at the Children's Hospital, Helsinki University Central Hospital and University of Helsinki, Finland, in 2003–2006. The study population consisted of 25 children who had undergone allogeneic HSCT and 22 age- and gender-matched controls. All subjects were recruited after informed written consent from the parent(s) and from the age-appropriate children. The study was conducted according the declaration of Helsinki, and the study protocol was approved by the institutional review board of Children's Hospital, Helsinki University Central Hospital.

The inclusion criteria for the patients were age of 6–15 y, a post-transplant follow-up of minimum of 2 y, and no ongoing immunosuppression. The invitation letter was sent to 50 consecutive patients visiting our follow-up clinic and fulfilling the criteria. Eleven patients

were excluded later: 1 had a secondary malignancy and 10 would have been older than 15 y during the study period. Twenty-five of the 39 eligible patients (64%) were willing to participate. The patient group comprised 13 males and 12 females, and the median age was 5.2 y (range: 1.1–10.3 y) at HSCT and 11.0 y (range: 6.4–14.9 y) at the study. Detailed patient characteristics are presented in Table 3. Those who refused to participate did not differ from the study patients with respect to transplant-related demography.

Control subjects were healthy children, 10 boys and 12 girls (median age: 11.9 y; range: 6.7–15.5 y). Two control subjects were invited among healthy siblings of children followed up for congenital heart defect from our outpatient Department of Pediatric Cardiology and 20 among age- and gender-matched children of the personnel in Children's Hospital. All control subjects were normotensive, and their echocardiography was normal.

Measurements

Ultrasound studies and blood pressure measurements. Brachial blood pressure was measured with an oscillometric method (Critikon Dinamap, GE, Waukesha, WI) in the left arm after at least 10 min of rest in the supine position. The pulse pressure was calculated as the difference between systolic and diastolic blood pressure. One experienced investigator (M.I.T.) performed the ultrasound examination of the heart and the carotid and brachial arteries.

We used a real-time B-mode ultrasound imager (Vivid 7; GE Vingmed AS, Horten, Norway) to examine the right CCA. The segment of right CCA 1–2 cm proximal to the carotid bulb was scanned with a 12L vascular probe (GE Medical Systems, Milwaukee, WI), and the resolution box function was used to magnify the arterial far wall. Off-line analysis was performed from the digitally stored data using an automated computerized analyzing system AMS II, v.1.1331 (T. Gustafsson, Gothenburg, Sweden). The IMT and end-diastolic diameter (EDD) were measured from the end-diastolic frames (corresponding to the R wave). The end-systolic diameter (ESD) was measured from the corresponding systolic maximal diameter during the same cardiac cycles. The mean value of three automatic measurements was used.

Mechanical properties of the CCA. Several parameters are available to characterize the ability of the carotid artery to expand and contract with cardiac pulsation and relaxation. Arterial compliance is defined as an absolute area change for a given pressure step at fixed vessel length. Arterial distensibility is the relative area change for a pressure increment. The E inc is a pressure step required from resting diameter to theoretical 100% stretch at fixed vessel wall length and is independent of its geometry.

We used the following formulas:

$$\text{CCA compliance} = \pi (\text{ESD}^2 - \text{EDD}^2) / 4 (\text{PP}) \text{ (mm}^2 \times \text{mm Hg}^{-1}) \text{ (26)}$$

$$\text{Pulse pressure (PP)} = P_{\text{systolic}} - P_{\text{diastolic}}$$

$$\text{CCA distensibility} = (\text{ESD}^2 - \text{EDD}^2) / (\text{EDD}^2 \times \text{PP}) \text{ (mm Hg}^{-1} \text{ 10}^{-2}) \text{ (26)}$$

$$\text{CCA E inc} = [3(1 - \text{LCSA}/\text{WCSA})] / \text{CCA distensibility} \text{ (mm Hg} \times \text{10}^3) \text{ (27)}$$

$$\text{Lumen cross-sectional area (LCSA)} = \pi (\text{CCA diameter}^2) / 4 \text{ (mm}^2)$$

$$\text{Wall cross-sectional area (WCSA)} = \pi (\text{CCA diameter} / 2 + \text{IMT})^2 - \pi (\text{CCA diameter} / 2)^2 \text{ (mm}^2) \text{ (28)}$$

Flow-mediated dilation. The endothelial-dependent FMD of the brachial artery was measured after at least 30 min of rest with a 12L ultrasound probe (Vivid 7, GE, Horten, Norway) as previously described (14,28). The AMS program was used for measuring the changes in the diameter of the brachial artery. The brachial artery was scanned 2–10 cm above the elbow in a longitudinal section. The center of the artery was identified with the cursor when the clearest images of both posterior and anterior walls were obtained. All the ultrasound scans were stored and automatically analyzed. The mean value was calculated from three cardiac cycles incident with R wave. The machine settings were chosen to optimize the lumen to vessel wall detection and kept constant during the study. Blood flow in the right arm was restricted distal to the scanned brachial artery by an adult-size blood

pressure cuff inflated to 300 mm Hg in the forearm for 4 min. After the cuff was deflated, the brachial diameter was scanned for 90 s. The ultrasound scans during 80–90 s were used for the calculation of the maximal dilation.

Laboratory and Clinical Parameters

Samples for plasma glucose (glucose oxidation reaction), serum insulin (radioimmunoassay), and plasma lipid measurements were drawn at 8–9 AM after an overnight fast. Insulin resistance was estimated by the homeostasis model assessment from the formula homeostasis model assessment index = fasting glucose (mmol/l) \times fasting insulin (mU/l)/22.5 (29,30).

Enzymatic assays were used for measuring plasma total cholesterol, cholesterol in high-density lipoprotein cholesterol, and plasma total triglycerides (TG kits; Boeringer Diagnostica, Mannheim, Germany). Low-density lipoprotein cholesterol was calculated according to the Friedewald formula (31).

Along with the anthropometric measurements, chronic GVHD was evaluated according to the modified Seattle approach (32). GVHD was defined as “limited” in case of limited skin involvement with or without signs of hepatic dysfunction. Symptoms and organ manifestations beyond that were considered to be extensive chronic GVHD.

Data Analysis and Statistical Methods

Arterial diameters were measured by one observer from digitally saved images with an automated system corresponding to at least 100 measurements. Patient characteristics are shown as mean (range). Results are expressed as mean (SD) unless stated otherwise. Comparison between groups was performed by one-way ANOVA. For nonparametric data, the Mann–Whitney *U* test was used (plasma triglycerides and serum insulin). Univariate linear regression analysis was used to examine the association between continuous variables.

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