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Microalbuminuria, decreased fibrinolysis, and inflammation as early signs of atherosclerosis in long-term survivors of disseminated testicular cancer

J. Nuver[a], A.J. Smit[b], D.Th. Sleijfer[a], A.I. van Gessel[b], A.M. van Roon[b], J. van der Meer[c], M.P. van den Berg[e], J.G.M. Burgerhof[f], H.J. Hoekstra[g], W.J. Sluiter[d], J.A. Gietema[a,*]

[a]*Department of Medical Oncology, University Medical Center Groningen, Hanzeplein 1, PO Box 30.001, 9700 RB Groningen, The Netherlands*
[b]*Department of Vascular Medicine, University Medical Center Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands*
[c]*Department of Haematology, University Medical Center Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands*
[d]*Department of Endocrinology, University Medical Center Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands*
[e]*Department of Cardiology, University Medical Center Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands*
[f]*Department of Epidemiology and Statistics, University Medical Center Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands*
[g]*Department of Surgical Oncology, University Medical Center Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands*

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Abstract

Testicular cancer patients have an increased risk for coronary artery disease more than ten years after cisplatin-based chemotherapy. We investigated whether vascular changes, including endothelial dysfunction, are present earlier. Ninety chemotherapy-treated testicular cancer patients (median follow-up of seven years) were compared with 44 patients after orchidectomy only and 47 healthy men. Microalbuminuria was present in 10 (12%) chemotherapy patients, one stage I patient and none of the controls. Chemotherapy patients had higher levels of fibrinogen, C-reactive protein (hs-CRP), von Willebrand factor (vWF), plasminogen activator inhibitor (PAI-1), and tissue-type plasminogen activator (t-PA). Chemotherapy patients with elevated PAI-1 (25/90) showed clustering of cardiovascular risk factors resembling the metabolic syndrome. In conclusion, cured testicular cancer patients showed a high prevalence of microalbuminuria and increased plasma levels of endothelial and inflammatory marker proteins, which might progress to more severe endothelial dysfunction and overt atherosclerosis.

Keywords: Testicular neoplasms; Arteriosclerosis; Endothelium; Albuminuria; Inflammation; Fibrinolysis; Plasminogen activator inhibitors; von Willebrand Factor

1. Introduction

Testicular cancer is the most common malignancy in men between 20 and 40 years of age. Over recent decades, the number of survivors of disseminated testicular cancer has increased, due to an increase in incidence and a decrease in mortality as a result of the introduction of cisplatin- and bleomycin-containing chemotherapy. Testicular cancer patients are assumed to have a normal life expectancy once they reach a durable complete remission. However, several studies have demonstrated treatment-related factors that may affect morbidity and mortality in the long term.

Cardiovascular complications of standard chemotherapy have been reported in testicular cancer patients several years after treatment. Cardiovascular risk factors, like hypertension, hypercholesterolaemia, overweight [1–3], and insulin resistance [4] occur frequently. Furthermore, recent studies have demonstrated an increased risk for cardiovascular events ten or more years after cisplatin- and bleomycin-containing chemotherapy [4,5].

Endothelial damage is thought to play an important role in the development of late vascular toxicity following

* Corresponding author. Tel.: +31-50-3612821; fax: +31-50-3614862.
E-mail address: j.a.gietema@int.azg.nl (J.A. Gietema).

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chemotherapy for testicular cancer. *In vitro* studies have provided some clues for the existence of both bleomycin- and cisplatin-induced endothelial damage [6,7]. Furthermore, Raynaud's phenomenon, occurring in up to 37% following bleomycin- and cisplatin-containing chemotherapy [2], has been associated with endothelial dysfunction.

While an increased risk of cardiovascular events has been established in testicular cancer patients more than ten years after chemotherapy, it is unknown whether atherosclerotic changes are present earlier. Early detection of vascular changes is important for early therapeutic intervention with the aim to reduce cardiovascular risk. We performed a cross-sectional study to investigate whether atherosclerotic changes, endothelial dysfunction in particular, are present in cured testicular cancer patients after cisplatin-based chemotherapy. Chemotherapy-treated patients were compared with stage I testicular cancer patients who had not received chemotherapy, but had been treated with orchidectomy only and with healthy men of comparable age.

2. Patients and methods

2.1. Patients

All patients with disseminated non-seminomatous testicular cancer who had been successfully treated with cisplatin-containing chemotherapy at the University Hospital Groningen, The Netherlands, between July 1988 and April 1999 were approached to participate. Radiotherapy, a history of cardiovascular events before diagnosis, and age above 55 years at the start of chemotherapy were exclusion criteria. Patients with stage I non-seminomatous testicular cancer, free of disease after orchidectomy only, and apparently healthy men, who had been recruited from the same geographical area through advertisements, were also asked to participate. The study was approved by the local ethics committee. Written informed consent was obtained from each participant.

2.2. Measurements

Information was obtained regarding medication, smoking status, and family history of cardiovascular disease (angina pectoris, myocardial infarction, or stroke in at least one first-degree relative before the age of 65 years). A physical examination was performed with measurements of weight and height.

Urinary albumin excretion was determined in 24-h urine samples using nephelometry (Dade Behring Diagnostic, Marburg, Germany) with a threshold of 2.3 mg/l and intra- and inter-assay coefficients of variation <4.3 and 4.4%, respectively [8]. Microalbuminuria was defined as a urinary albumin excretion of 30–300 mg/24 h. Albumin excretion data from 3348 men (age range 28–65 years) from a Dutch population study (all inhabitants of the city of Groningen) [9], were used to estimate the prevalence of microalbuminuria in the general male population, using an adjusted weighting approach in which non-response was taken into account [10]. Variance estimation was performed using the jack-knife procedure [10].

Blood samples were drawn after an overnight fast and analysed for lipid, glucose and insulin levels. The insulin-to-glucose ratio was calculated by dividing fasting serum insulin (pmol) by fasting plasma glucose (mmol). Fibrinogen was measured using the Clauss functional assay (reference values 1.7–3.5 g/l). High-sensitivity C-reactive protein (hs-CRP; lower limit of detection 0.16 mg/l) was measured using the BNII Nephelometer (Dade Behring, Brussel, Belgium). Von Willebrand factor (vWF, reference values 50–150%), plasminogen activator inhibitor type 1 antigen (PAI-1, reference values 4–43 ng/ml), and tissue-type plasminogen activator antigen (t-PA, reference values 1–10 ng/ml) were measured using an enzyme-linked immunosorbent assay (ELISA).

An ambulatory blood pressure device (Spacelab 90207; SpaceLabs Inc., Redmond Washington, USA) was used to document blood pressure every 30 min during a 24-h period. Hypertension was defined as mean 24-h blood pressure $>135/85$ mmHg.

A wall tracking system consisting of an ultrasound scanning device with a 7.5 MHz linear array transducer (Wall Tracking System 2.0, Pie Medical Scanner 200, Maastricht, The Netherlands) was used to assess flow-mediated vasodilation (FMD) and nitroglycerin (NTG)-mediated vasodilation [8]. FMD was calculated as the percent maximal post-ischaemic increase in arterial diameter compared with the average of two baseline diameters. NTG-mediated vasodilation was expressed as the percent maximal post-NTG increase in brachial artery diameter compared with the post-ischaemic baseline diameter. All measurements were performed by a single skilled technician in a quiet and temperature-controlled room in the morning and were recorded and analysed off-line.

Intima-media thickness (IMT) of the common carotid artery (CCA) was measured using the Wall Tracking System. The posterior wall of the left CCA was assessed approximately one centimeter proximal to the carotid bifurcation. Recorded data were processed using Wall Tracking System 2.0 software (Pie Medical, Maastricht, The Netherlands).

CCA distensibility ($\Delta D/D_d \cdot 100$), compliance ($\Delta D/\Delta P$ in μm/mm Hg), and stiffness ($\beta = (\ln(P_s/P_d) \cdot 100)/((D_s - D_d)/D_d)$) were calculated as measures of CCA function [11]. Changes in vessel diameter were derived from Wall Tracking System recordings; pulse pressure (ΔP) and values of systolic and diastolic blood pressure

(P_s and P_d) from simultaneous Finapres (FINger Arterial PRESsure; Ohmeda 2300 E; Ohmeda, Liberty Corner, NJ, USA) non-invasive blood pressure recordings and ambulatory blood pressure measurements, respectively.

2.3. Statistical analysis

Differences between groups were evaluated by analysis of ranks (Kruskal–Wallis), using Duncan's method for correction of multiple testing. Double-sided *P*-values <0.05 were considered to indicate significance. For $\alpha=0.05$, this study had a power of 85% to detect a difference in IMT, FMD, and arterial stiffness between chemotherapy patients and controls of 0.059 mm, 3.5%, and 1.11, respectively.

3. Results

Ninety chemotherapy patients (89% of 101 eligible patients), 44 stage I patients with a similar follow-up, and 47 healthy male controls participated (Table 1).

3.1. Cardiovascular risk factors and events

Cardiovascular risk factors are shown in Table 1. Microalbuminuria was present in 10 (12%) chemotherapy patients. This was significantly different from the controls (0%), but also from a larger group of men from the Dutch population study [9], in whom microalbuminuria prevalence was 4.6% (95% Confidence Interval (CI) for difference in proportions 0.3%–13.7%).

Eighteen chemotherapy patients (22%) had hypertension; nine already used antihypertensive medication at the time of investigation and in nine raised blood pressure was established. Three chemotherapy-treated patients had suffered a cardiovascular event: one myocardial infarction, two ischaemic strokes. None of the stage I patients or controls had suffered an event.

3.2. Endothelial and inflammatory marker proteins

Chemotherapy patients had higher levels of vWF, t-PA, PAI-1, fibrinogen and hs-CRP than the controls

Table 1
General characteristics and cardiovascular risk factors

		Chemotherapy	Stage I	Controls
Number of subjects		90	44	47
Age (years)		37 (20–65)	36 (24–63)	37 (22–55)
Median (range)				
Follow-up duration (years)[a]				
Median (range)		7 (3–13)	7 (3–13)	na
Chemotherapeutic regimen[b] (*N*,%)				
BEP		73 (81)	na	na
EP		8 (9)		
BEP/VIP		4 (5)		
BOP/VIP		3 (3)		
BEP/PVB		2 (2)		
	Normal value	Number (%) of persons with abnormal result		
Blood pressure[c]	<135/85 mmHg	18 (22)	9 (23)	5 (11)
LDL-cholesterol[d]	<3.4 mmol/l	64 (71)*	26 (59)	21 (45)
Body mass index	<27.8 kg/m^2	18 (22)	12 (27)	5 (11)
Urinary albumin excretion	<30 mg/24 h	10 (12)*	1 (2)	0 (0)
Smoking status				
Current smoker		35 (39)	17 (39)	13 (28)
Former smoker		6 (7)	8 (18)	4 (8)
Non-smoker		46 (51)	19 (43)	30 (64)
Unknown		3 (3)		
Positive family history		18 (20)	9 (20)	17 (36)

*Chemotherapy versus controls $P<0.05$. LDL, low-density lipoprotein.

[a] Years since chemotherapy or orchidectomy for chemotherapy and stage I groups, respectively.

[b] (B)EP: (bleomycin), etoposide, cisplatin; BOP: bleomycin, vincristine, cisplatin; PVB: cisplatin, vinblastine, bleomycin; VIP: etoposide, ifosfamide, cisplatin; na: not applicable.

[c] Hypertension: high mean 24-h systolic and/or diastolic blood pressure or use of antihypertensive medication.

[d] Hypercholesterolaemia: abnormal LDL-cholesterol or use of cholesterol-lowering medication.

(Table 2). Stage I patients had higher levels of vWF, t-PA, hs-CRP and fibrinogen than controls.

3.3. Vascular structure and function

Mean common carotid IMT, FMD and NTG-mediated vasodilation did not differ between the patients and controls (Table 3). CCA stiffness was higher in the chemotherapy-treated than the stage I patients.

3.4. Cardiovascular structure and function in relation to the marker proteins

Chemotherapy patients with PAI-1 >43 ng/ml ($N=25$, 28%) had higher concentrations of triglycerides and t-PA, a higher body mass index, a higher insulin-to-glucose ratio, higher systolic and diastolic blood pressure, decreased CCA distensibility, and increased CCA stiffness compared with controls (Table 4). Differences in age between the subgroups confounded the results for PAI-1 in stage I patients and for t-PA and hs-CRP in both chemotherapy and stage I patients. No significant differences were found between the patients with normal and high plasma levels of vWF and fibrinogen.

4. Discussion

Patients with disseminated testicular cancer have an increased risk for cardiovascular disease more than ten years after chemotherapy [4,5]. We investigated whether signs of atherosclerosis are present earlier. We found a high prevalence of microalbuminuria and high levels of markers of inflammation and endothelial activation in testicular cancer patients after a median follow-up of only seven years.

Microalbuminuria, an indicator of generalised endothelial dysfunction [12], was present in 12% of testicular cancer patients a median of seven years post-chemotherapy and has been reported in up to 22% a median of 14 years after treatment [4]. Microalbuminuria was significantly more prevalent in chemotherapy patients than in a male population of comparable age. A high prevalence of microalbuminuria is an important finding

Table 2
Endothelial and inflammatory marker proteins

		Chemotherapy		Stage I		Controls	
	Normal value	Median (range)	Number (%) of persons with abnormal result	Median (range)	Number (%) of persons with abnormal result	Median (range)	Number (%) of persons with abnormal result
Fibrinogen	⩽3.5 g/l	3.0 (1.9–4.1)[a,b]	13 (14)	2.8 (1.7–3.8)[c]	2 (5)	2.5 (1.9–3.4)	0 (0)
hs-CRP	<10 mg/l	1.40 (0.16–11.10)[a]	1 (1)	0.90 (0.16–4.70)[c]	0 (0)	0.59 (0.16-2.60)	0 (0)
vWF	⩽150%	108 (28–296)[a]	21 (24)	113 (52–220)[c]	6 (14)	91 (43–304)	4 (9)
PAI-1	⩽43 ng/ml	26.5 (3.0–183.0)[a]	25 (28)	18.8 (4.7–118.0)	10 (23)	15.5 (2.5–67.0)	7 (15)
t-PA	⩽10 ng/ml	7.6 (1.5–21.0)[a]	27 (30)	6.9 (3.2–23.0)[c]	8 (19)	5.6 (2.3–15.0)	3 (7)
PAI-1/t-PA ratio	na	3.45 (0.5–31.6)	na	2.76 (1.2–9.4)	na	2.62 (0.7–12.1)	na

hs-CRP, high-sensitivity C-reactive protein; PAI-1, plasminogen activator inhibitor type 1; t-PA, tissue-type plasminogen activator; vWF, von Willebrand factor; na, not applicable.
[a] Chemotherapy versus controls $P<0.05$.
[b] Chemotherapy versus stage I $P<0.05$.
[c] Stage I versus controls $P<0.05$.

Table 3
Vascular structure and function

	Chemotherapy	Stage I	Controls
CCA IMT (mm)	0.614±0.106	0.628±0.119	0.601±0.110
Baseline BA diameter (μm)	5066.4±650.2	4950.7±557.1	4921.7±509.8
Absolute post-ischaemic BA dilation (μm)	315.8±272.4	304.2±290.1	311.5±324.4
FMD (%)	6.6±5.9	6.3±6.4	6.5±7.0
Absolute post-NTG BA dilation (μm)	427.4±241.7	347.0±226.9	430.2±314.2
NTG-mediated vasodilation (%)	8.3±5.0	6.8±4.6	9.0±6.9
Carotid compliance (μm/mmHg)	11.1±5.0	11.5±5.4	11.9±4.6
Carotid distensibility	7.4±2.5	8.2±2.8	8.2±2.9
Carotid stiffness	7.1±2.0[a]	6.6±2.4	6.5±2.0

BA, brachial artery; CCA, common carotid artery; FMD, flow-mediated vasodilation; IMT, intima-media thickness; NTG, nitroglycerin. Values are means±standard deviations (S.D.).
[a] Chemotherapy versus stage I $P<0.05$.

Table 4
Cardiovascular risk factors and damage in chemotherapy patients with elevated (>43 ng/ml) and normal PAI-1 and controls

	Chemotherapy Elevated PAI-1 $N=25$	Chemotherapy Normal PAI-1 $N=65$	Controls $N=47$
Age (years)	38.4±9.3	38.1±10.3	37.4±8.9
LDL-cholesterol (mmol/l)	3.87±1.04	3.75±0.91	3.50±0.99
Triglycerides (mmol/l)	2.32±1.14[a,b]	1.39±0.70	1.20±0.74
Insulin-to-glucose ratio	19.5±9.5[a,b]	13.2±6.4[c]	11.2±5.6
Body mass index (kg/m^2)	28.0±4.4[a,b]	24.3±2.7	24.0±2.9
Systolic blood pressure (mmHg)	132.0±9.7[a,b]	121.3±8.9	123.0±8.3
Diastolic blood pressure (mmHg)	82.9±7.6[a,b]	73.4±7.4	74.9±5.9
Carotid distensibility	6.6±2.0[a]	7.6±2.6	8.2±2.9
Carotid stiffness	7.7±2.3[a]	6.9±1.9	6.5±2.0

LDL, low-density lipoprotein; PAI-1, plasminogen activator inhibitor type 1. Values are means±standard deviation (S.D.).
[a] High PAI-1 versus controls $P<0.05$.
[b] High PAI-1 versus normal PAI-1 group $P<0.05$.
[c] Normal PAI-1 group versus controls $P<0.05$.

in this young patient population, since microalbuminuria is a predictor of cardiovascular events [9].

Increased concentrations of vWF and of PAI-1 and t-PA antigens have been associated with a higher risk of coronary artery disease [13,14]. Levels of these proteins were higher in chemotherapy patients than controls, suggesting endothelial activation and/or injury. Since PAI-1 and t-PA antigens bind 1:1 in plasma to form a non-functional complex, excess of PAI-1 over t-PA implies decreased fibrinolytic potency. The difference in PAI-1 between chemotherapy patients and controls was much larger than the difference in t-PA, suggesting decreased fibrinolysis, endothelial dysfunction, and an increased risk of atherothrombosis.

PAI-1 has been associated with several components of the metabolic syndrome [13]. In the present study, chemotherapy patients with elevated PAI-1 showed a clustering of cardiovascular risk factors suggesting a metabolic syndrome-like state, which has earlier been reported in testicular cancer patients more than ten years after chemotherapy [4]. Compared with controls, chemotherapy patients with elevated PAI-1 also had decreased CCA distensibility, and increased CCA stiffness, suggesting more extensive atherosclerotic changes as well. In this respect, the high number of patients with elevated PAI-1 is worrisome.

Atherosclerosis is considered as an inflammatory disease. Elevated levels of inflammatory proteins are associated with the risk of coronary heart disease and severity of atherosclerosis [15]. Both fibrinogen and hs-CRP were increased in chemotherapy patients compared with controls, suggesting an inflammatory state. During chemotherapy, testicular cancer patients suffer leucopenia, which might lead to a higher frequency of chronic infections with microorganisms that are associated with a higher risk of coronary artery disease, e.g. *Chlamydia pneumoniae* [16]. Otherwise, endothelial activation, as shown by increased levels of vWF and t-PA, may elicit a cytokine response with subsequent release of inflammatory proteins.

Interestingly, patients treated with orchidectomy only also had higher levels of vWF and fibrinogen than controls. This may be explained by hormonal or metabolic changes due to orchidectomy or by some unknown testicular cancer-related factor, rather than by cisplatin or bleomycin toxicity alone. Lower levels of testosterone have been associated with raised insulin concentrations, higher body mass index, and decreased HDL-cholesterol in men [17]. Furthermore, testosterone has been described to have anti-ischaemic effects [18]. Therefore, testosterone deficiency induced by orchidectomy and chemotherapy [19] may contribute to endothelial dysfunction and atherosclerotic changes in testicular cancer survivors.

Despite high levels of endothelial and inflammatory marker proteins, vascular tone and vessel wall structure did not seem affected, except for CCA distensibility and stiffness in chemotherapy patients with elevated PAI-1. FMD measures large vessel nitric oxide release. At the early stages of vasculopathy, only microvascular endothelial function might be disturbed, while macrovascular endothelial function may be preserved, as illustrated by normal FMD [8]. We chose to study the common carotid IMT, since this segment is best visualised and shows the least variability in results [20]. However, atherosclerosis has been reported to occur later in the common carotid segment than in the internal carotid or carotid bulb segment [21]. Therefore, early atherosclerotic lesions may have been missed. Previous studies have shown that higher baseline levels of vWF are associated with progression of CCA IMT in patients with peripheral arterial disease [22] and that elevated CRP is a risk factor for ischaemic stroke, independent of atherosclerosis severity as measured by IMT [23]. Therefore, elevated plasma levels of endothelial and inflammatory proteins may precede measurable alterations

in FMD and IMT. Longer follow-up will be needed to find out whether increased levels of plasma marker proteins predict changes in endothelial function and morphology in our group of patients.

This is the first study in cured testicular cancer patients demonstrating a high prevalence of microalbuminuria and increased levels of endothelial and inflammatory marker proteins a median of seven years post-chemotherapy. These early changes may point to an accelerated atherosclerosis and an increased risk for future cardiovascular disease, considering that cardiovascular events seem to occur increasingly after a longer follow-up [4]. Early cardiovascular changes may prove to be important targets for early therapeutic intervention.

5. Conflict of interest statement

No conflicts of interest.

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