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Change in telomere length and cardiovascular risk factors in testicular cancer survivors

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Abstract

Background: Testicular cancer (TC) survivors cured with chemotherapy (CT) are prone to develop cardiovascular diseases, as part of an accelerated aging phenotype. A mechanism contributing to these events can be telomere shortening.

Patients and Methods: In a prospective cohort of patients with disseminated TC who received cisplatin-based CT, mean absolute leukocyte telomere length (TL) was measured before and 1 year after start of treatment. Cardiovascular risk factors, including development of the metabolic syndrome and hypogonadism, were assessed before and up to 5 years after CT.

Results: For the whole group ($n = 55$), TL did not change 1 year after CT (5.7 (2.2−13.4) vs. 5.8 kb (1.6−19.2), $P = 0.335$). At baseline, patients with a BMI >30 kg/m^2 ($n = 12$) had shorter TL (4.9 (2.2−13.4) vs. 6.3 kb (3.1−12.9), $P = 0.045$), while no age-dependent differences were measured. Patients with TL shortening after 1 year ($n = 7$) showed a significant increase in diastolic blood pressure ($P = 0.007$) and triglycerides ($P = 0.003$), compared to those with unchanged TL. There was no association between telomere shortening after 1 year or short TL at baseline ($n = 7+11$) and development of metabolic syndrome (25% vs. 21%; $P = 0.777$), or hypogonadism (38% vs. 17%; $P = 0.120$) after 5 years.

Conclusions: A small subset of TC patients treated with cisplatin-based CT showed telomere shortening 1 year after treatment. This shortening was associated to a rise in diastolic blood pressure and triglycerides, but not to newly developed metabolic syndrome and hypogonadism after 5 years.

Keywords: Cisplatin; Hypogonadism; Metabolic syndrome; Obesity; Survivorship; Telomere; Testicular neoplasms

1. Introduction

The number of patients with testicular cancer (TC) cured with cisplatin-based chemotherapy (CT) is increasing. A major clinical burden remains that TC survivors are at risk for developing early aging phenotypes with second primary malignancies, cardiovascular disease (CVD) and development of cardiovascular risk factors. A potential underlying mechanism for this early aging phenotype after cytotoxic treatment is replicative senescence, partly due to telomere shortening [1,2].

Telomeres are repeated sequences of nucleotides at the ends of DNA strings that protect the DNA from damage and prevents genomic instability. During replication,

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telomeric sequences are progressively lost due to physical impediments affecting the efficacy of DNA polymerase, resulting in telomere shortening. During treatment with CT, DNA damage is inflicted to rapidly proliferating cancer and normal cells. DNA damage can lead to cell death, and compensatory proliferation from unaffected normal cells is needed to maintain tissue homeostasis. This process accelerates telomere shortening and promotes premature senescence [3]. Senescent cells produce cytokines, chemokines, growth factors, and proteases—also called the senescence-associated secretory phenotype (SASP). The SASP maintains low-grade inflammation, which is associated with age-related morbidities [4−6].

Previous studies have suggested a potential correlation between telomere length (TL) and CVD. A meta-analysis of 32 prospective and retrospective studies in 144,610 cardiac patients or controls found that the shortest TL tertile was associated with a 39% higher risk of myocardial infarction and a 28% higher risk of cardiovascular death [7]. Another meta-analysis of 15 cross-sectional, longitudinal and retrospective studies in 7,256 CVD patients or controls, with or without diabetes, found an inversed correlation between TL and arterial pulse wave velocity (aPWV), and TL and carotid intima-media thickness (cIMT) [8].

In vitro and *in vivo* studies have shown that treatment with cisplatin-based CT resulted in shortening of TL and the inhibition of telomerase activity in germ cells, which normally has the ability to synthesize telomeric sequences [9,10]. In breast cancer survivors, measurement of telomere shortening showed no differences between standard dose CT and standard combined with high-dose carboplatin-based CT and peripheral blood stem cell transplantation [11]. A study in childhood cancer survivors treated with various CT regimens showed a relationship between telomere shortening and second primary malignancies, and also between TL and hs-CRP, a biomarker of inflammation [12,13]. Because data on correlations between telomere shortening and accelerated aging phenotypes in patients treated with cisplatin-based CT are missing, we performed a study in TC patients before and after treatment to correlate TL to cardiovascular risk factors.

2. Patients and methods

2.1. Patient selection

Patients had been included in a prospective study and had received cisplatin-based CT for metastatic testicular cancer at the University Medical Center Groningen (UMCG) between 2008 and 2012. A detailed description of this study rationale, design, and method is given elsewhere [14]. This study was approved by the ethics committee of the UMCG.

In short, patients aged 18 to 50 years were eligible if they had to start with first line (B)EP (bleomycin, etoposide, cisplatin) CT and had no history of cardiovascular events. Study visits were performed before, during and 1 year after CT to assess (sub)clinical vascular damage and CVD risk factors. 67 of 73 participants who gave informed consent for this study also consented for blood withdrawal for DNA isolation.

2.2. TL measurement

Before and 1 year after cisplatin-based CT fasting, blood was drawn in the morning, mixed with heparin and stored at −80˚C. DNA was isolated automatically using the Promega ReliaPrep large volume HT gDNA isolation system by the human genomics facility (HuGE-F) of the genetic laboratory of the Erasmus MC, Rotterdam, The Netherlands (www.glimdna.org). Mean leukocyte TL was determined using the absolute human telomere length quantification qPCR assay kit (AHTLQ Catalog #8918, ScienCell Research Laboratories, Carlsbad, California, USA). Mean absolute leukocyte TL was calculated using a reference human genomic DNA sample from this kit with known TL.

Paired samples of one individual were measured in the same assay plate. All samples were measured in duplicate to correct for intra-assay variability. In case of a difference in duplicate of >1Ct, paired samples of this individual were remeasured. Internal controls were used in each run to control for interassay variability. CV in these samples was <10%.

Telomere shortening was defined as a decrease in TL of more than 33% after 1 year compared to baseline. Short TL at the start of CT was defined as <5 kb pair (=p25).

2.3. Phenotypic data collection

Phenotypic data of the patients was collected before, and up to 5 years after CT. Data contained disease and treatment characteristics, cardiovascular events, risk factors of CVD, hypogonadism, endothelial, and inflammatory biomarkers in blood (high sensitive C-reactive protein [hs-CRP]), plasminogen activator inhibitor (PAI), soluble intercellular adhesion molecule (sICAM), growth differentiation factor 15 (GDF-15), von Willebrand Factor (vWF) and factor VIII (FVIII)), and vascular measurements baroreflex sensitivity (BRS [15]), intima media thickness (IMT [15]), and advanced glycation end products (AGE [14]) in the skin.

After completion of CT, patients visited the hospital regularly for routine follow-up. At completion of CT, 1 year later, and every 2 years thereafter, follow-up visits were extended with cardiovascular risk assessments in addition to the identification of other potential long-term effects of CT, such as hypogonadism, neuropathy, Raynaud's phenomenon or psychosocial problems. Data from the fifth year of follow-up (48−60 months) was extracted for this research. In case a visit in that time frame was missing, data from the nearest visit was used.

2.4. Outcomes

Primary outcome was a change in median TL of the whole group of TC patients between the start of cisplatin-based CT and 1 year after.

Secondary outcomes were: associations between short TL or telomere shortening and 1) risk factors of CVD, endothelial and inflammatory biomarkers, and vascular measurements, 2) metabolic syndrome after 5 years (defined according to the NCEP ATP III definition update in 2005 [16,17]), and 3) hypogonadism after 5 years (defined as testosterone <10 nmol/L or luteinizing hormone [LH] >10 U/L or use of testosterone replacement therapy [TRT]).

2.5. Statistics

TL was not normally distributed. Data are presented as medians with ranges. For correlations between continuous variables, Spearman's correlation coefficient was used. For paired measurements, Wilcoxon signed-rank test was used. For comparisons between groups, Mann-Whitney U or Chi-square test (or Fisher's exact test in case of <5 counts) was applied. Two-sided *P*-values <0.05 were considered significant. Statistical analyses were performed in SPSS Statistics 23.0 (IBM SPSS Inc, New York, USA). For posthoc power analyses GPower 3.1 (Heinrich-Heine-Universität Düsseldorf, Germany) was used.

3. Results

55 out of 67 patients (82%) were included in the study because of availability of paired measurements of TL at the start of CT and 1 year after, and no evidence of recurrent disease during follow-up (Table 1).

3.1. Telomere length before CT

Age at diagnosis was not significantly associated with TL in the whole group (n = 55) (Supplementary Fig. A1). Patients with a BMI >30 kg/m^2 (n = 12) had shorter TL than those with BMI ≤30 kg/m^2 (4.9 [2.2−13.4] vs. 6.3 kb [3.1−12.9], P = 0.045) before start of CT (Fig. 1); there was no difference in age between these groups (Supplementary Fig. B1).

3.2. Telomere shortening and cardiovascular risk factors

For the whole group, median TL did not significantly change between the start of CT and 1 year later (5.7 kb [2.2−13.4] vs. 5.8 kb [1.6−19.2], P = 0.335) (Fig. 2). However, 7 out of 55 participants showed telomere shortening. These 7 did not differ in baseline characteristics and treatment (Supplementary Table A1).

Interestingly, these 7 patients showed a significant increase in diastolic blood pressure (5 [−5−25] vs. −5 [−25−20], P = 0.007) and triglycerides (1.20 [0.14−1.82] vs. 0.04 [−1.70−1.75], P = 0.003), compared to those with unchanged TL (Fig. 3, Supplementary Table B1). They also had a larger increase in BMI, but this was not significant (Fig. 3, Supplementary Table B1).

3.3. Metabolic syndrome and hypogonadism 5 years after CT

8 out of 36 (22%) patients without metabolic syndrome at start of treatment had developed metabolic syndrome after 5 years. Telomere shortening after 1 year or short TL at the start of CT (combined group n = 18, 33%) were not significantly associated with this newly developed metabolic syndrome (25% vs. 21%; P = 0.777) (Table 2).

10 out of 43 (23%) patients without hypogonadism at start of treatment had developed hypogonadism after 5 years. Telomere shortening after 1 year or short TL at the start of CT were not significantly associated with this newly developed hypogonadism (38% vs. 17%; P = 0.120) (Table 2). 1 patient was excluded in this analysis because of hypogonadism due to bilateral orchiectomy.

When also including patients who had hypogonadism at start of CT (n = 51), we found a higher prevalence of hypogonadism in patients with short TL or telomere shortening compared to those without (44% vs. 15%, P = 0.050) 5 years after treatment. There was a significantly larger increase in waist circumference in this group (3.3 [−2.5 −13.0 vs. −1.0 [−13.5−21.0] cm, P = 0.046).

Changes in endothelial and inflammatory biomarkers, and vascular measurements were not different between patients with short TL and/or telomere shortening and those without, as were for changes in LH or testosterone (Table 2). Patients using TRT were excluded in analyses with testosterone or LH levels.

4. Discussion

This study investigated whether patients with disseminated TC treated with cisplatin-based CT had shortening of TL 1 year later. Shortening of TL may be one of the causes and predictors for the development of early aging phenotypes. In this study we focused on mean leukocyte TL. On average, there was no significant difference in TL before and 1 year after treatment. However, in patients who did show telomere shortening, there was a larger increase in diastolic blood pressure and triglycerides 1 year after CT. Short TL or telomere shortening were not predictive for newly developing the metabolic syndrome or hypogonadism, but an association with the presence of hypogonadism when including all patients was observed.

Several other studies have reported associations between telomere shortening and cardiovascular risk factors. In 8,074 participants from the community-based prevention of renal and vascular end-stage disease (PREVEND) study, telomere shortening was associated with increased BMI,

Table 1
Patient characteristics before CT and 1 and 5 years later.

	Before CT Median (range)/N (%)	+1 yr Median (range)/N (%)	*P* Before CT vs. +1 yr	+ 5 yrs Median (range)/N (%)	*P* Before CT vs. +5 yrs
Telomere length (kb)	5.7 (2.2–13.4)	5.8 (1.6–19.3)	0.335		
Age at diagnose (yrs)	30 (18–46)				
Histology					
Seminoma	11 (20)				
Nonseminoma	44 (80)				
Royal Marsden Stage					
II	44 (80)				
III	5 (9)				
IV	6 (11)				
IGCCCG prognosis group					
Good	48 (87)				
Intermediate	7 (13)				
Regimen					
3x BEP	41 (75)				
4x BEP	11 (20)				
4x EP	3 (5)				
Cardiovascular risk factors					
BMI (kg/m^2)	25.1 (18.2–42.4)	25.4 (20.3–46.4)	**0.036**	24.5 (20.6–51.3)	0.217
Waist circumference (cm)	91 (68–123)	91 (61–144)	0.642	92 (80–142)	0.065
Fasting glucose (mmol/L)	5.1 (3.5–7.1)	5.3 (3.3–8.6)	0.089	5.4 (3.3–7.8)	0.102
Systolic BP (mmHg)	130 (118–160)	120 (100–150)	**0.000**	125 (95–155)	**0.011**
Diastolic BP (mmHg)	80 (67–99)	78 (60–100)	0.129	80 (50–100)	**0.031**
Total cholesterol (mmol/L)	4.4 (3.0–7.5)	4.9 (3.3–7.9)	**0.000**	4.7 (2.4–7.5)	**0.016**
LDL-cholesterol (mmol/L)	2.9 (1.5–5.2)	3.1 (1.8–5.9)	**0.013**	3.0 (1.4–5.9)	**0.038**
HDL-cholesterol (mmol/L)	1.2 (0.6–1.9)	1.3 (0.7–1.9)	**0.008**	1.4 (0.6–2.1)	**0.000**
Triglycerides (mmol/L)	1.12 (0.57–3.87)	1.29 (0.52–4.78)	0.249	1.29 (0.40–6.55)	0.404
Endothelial/inflammatory biomarkers					
PAI (ug/L)	30 (4–125)	21 (8–55)	**0.004**	30 (7–134)	0.340
hs-CRP (mg/L)	1.2 (0.2–50.4)	1.4 (0.2–15.1)	0.170		
GDF-15 (pg/ml)	392 (198–1111)	384 (247–981)	**0.010**		
sICAM (ng/ml)	225 (116–475)	238 (109–419)	0.144		
vWF (%)	87 (44–297)	106 (44–218)	**0.005**	104 (40–369)	**0.026**
Factor VIII (%)	100 (49–153)	119 (34–197)	**0.000**	144 (48–216)	**0.000**
Metabolic syndrome[a]	10 (22)	8 (15)	0.227	15 (28)	1.000
Hypogonadism[b]	6 (12)	17 (33)	**0.000**	16 (31)	**0.039**
LH (U/L)	2.6 (.01–14.5)	6.4 (2.5–19.4)	**0.000**	5.8 (1.6–15.7)	**0.000**
Testosterone (nmol/L)	20.0 (7.9–43.0)	16.0 (6.7–33.0)	**0.000**	17.0 (3.4–38.0)	**0.001**
TRT	1 (2)	2 (4)	1.000	4 (7)	0.250

BEP = bleomycin, etoposide, and cisplatin containing chemotherapy; BMI = body mass index; CT = chemotherapy; EP = etoposide and cisplatin containing chemotherapy; hsCRP = high-sensitivity C-reactive protein; FVIII = coagulation factor VIII; GDF-15 = growth differentiation factor 15; IGCCCG = international germ cell cancer collaborative group; LH = luteinizing hormone; PAI-1 = plasminogen activator inhibitor 1; sICAM = soluble intercellular adhesion molecule 1; TL = telomere length; vWF = von Willebrand Factor; yr = year.

Bold text denotes statistical significance ($p < 0.050$).

[a] Metabolic syndrome is defined according to the NCEP ATP III definition update in 2005 in which three or more of the following factors have to be present: waist circumference >102 cm, triglycerides ≥1.7 mmol/L, HDL cholesterol <1.03 mmol/L or use of lipid lowering medication, blood pressure ≥130/85 mm Hg or use of antihypertensive drugs, fasting glucose ≥5.6 mmol/L or use of blood glucose lowering medication. Patients with a BMI >30 kg/m^2 with an unknown waist circumference were considered to have abdominal obesity and therefore, scored as one point for increased waist circumference.

[b] Hypogonadism is defined as testosterone <10 nmol/L or LH >10 U/L or using testosterone replacement therapy.

waist-hip ratio, blood pressure, glucose and triglycerides, and decreased HDL-cholesterol [18]. Results of 2 other studies were consistent with the findings in the PREVEND study [19,20]. Patients of the Netherlands study of depression and anxiety (NESDA) cohort showed a correlation between telomere shortening and greater 6-year increase in waist circumference [19]. A second study from the same authors reported an association between a 10-year increase in waist circumference and telomere shortening in patients who were part of the coronary artery risk development in young adults (CARDIA) study [20]. In addition, they showed that short TL was associated with metabolic syndrome, and that short TL at baseline was associated with unfavourable metabolic syndrome components after 2 and 6 years [21]. In our study, we did not find an association between short TL or telomere shortening and development of the metabolic syndrome after 5 years. However, the observed increase in waist circumference due to

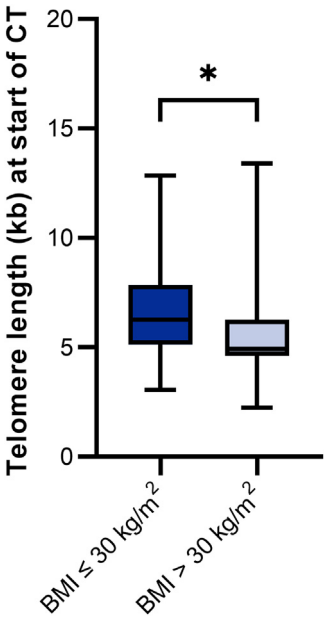

Fig. 1. Telomere length before start of chemotherapy in patients with BMI ≤30kg/m^2 vs. BMI >30 kg/m^2. BMI = body mass index; CT = chemotherapy.

accumulation of adipose tissue may precede development of the metabolic syndrome later.

Apart from the effect on diastolic blood pressure, an association between systolic blood pressure and telomere shortening was not found. This could be partially explained by the rather high systolic blood pressure of many participants at baseline, likely due to stress related to the start of intensive CT treatment, and had, therefore, lowered 1 year later.

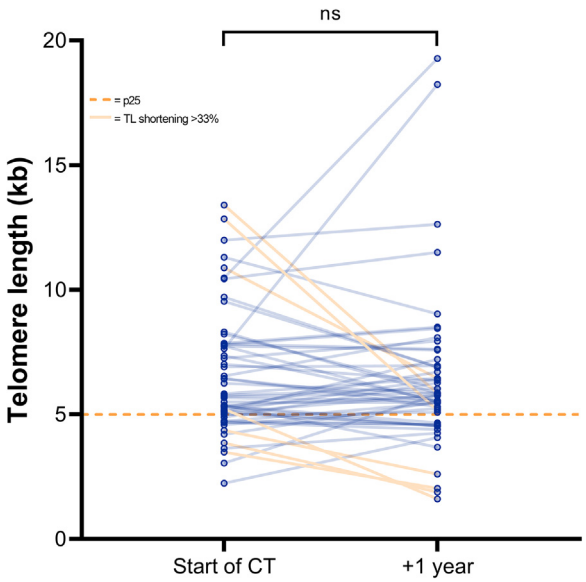

Fig. 2. Telomere length measured before start of chemotherapy and 1 year after. The orange lines represent patients that had a telomere shortening of >33%. CT = chemotherapy.

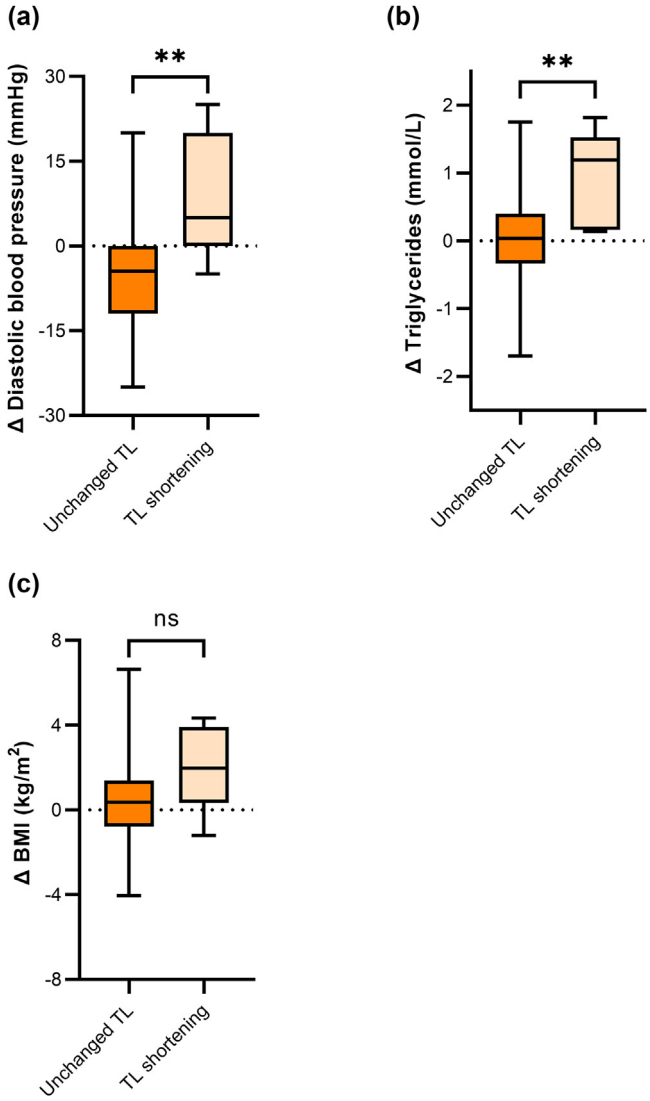

Fig. 3. Changes in diastolic blood pressure, triglycerides and BMI 1 year after start of chemotherapy in patients with >33% shortening of TL vs. unchanged TL. BMI = body mass index; TL = telomere length.

Furthermore, we observed that, before the start of CT, TC patients with a BMI >30 kg/m^2 had shorter TL. This is consistent with findings from other studies and may be explained in 2 different ways. First, patients may already be born with short telomeres, after which they are more likely to develop CVD risk factors, such as a higher BMI and waist circumference, because they are molecularly older [22]. Another explanation may be that having several years of stable obesity causes telomere shortening by low-grade inflammation and oxidative stress [23].

We also explored the development and presence of hypogonadism after CT treatment and investigated its relationship to TL, since low testosterone levels are associated with metabolic syndrome in TC survivors [24]. 5 years after CT, patients with short TL or telomere shortening had not newly developed hypogonadism more often than others. However, the presence of hypogonadism 5 years after

Table 2
Patients with short TL before CT and/or telomere shortening vs. the rest.

	n	Rest *n* = 37 N (%)/Median (min-max)	N	Short TL Before CT and/or Telomere Shortening After 1 yr *n* = 18 N (%)/Median (min - max)	*P*
Metabolic syndrome[a] at +5 yrs	37	10 (28)	18	5 (28)	1.000
Newly developed after start of CT	24	5 (21)	12	3 (25)	0.777
Hypogonadism[b] at +5 yrs	34	5 (15)	17	8 (44)	0.050
Newly developed after start of CT	30	5 (17)	13	5 (39)	0.120
Cardiovascular risk factors					
Δ BMI (kg/m^2)	37	0.3 (−4.1−6.6)	18	0.6 (−1.3−4.6)	0.216
Δ Waist circumference (cm)	30	−1.0 (−13.5−21.0)	10	3.3 (−2.5−13.0)	**0.046**
Δ Fasting glucose (mmol/L)	30	0.3 (−2.5−1.4)	15	0.2 (−1.1−1.0)	0.791
Δ Systolic blood pressure (mmHg)	36	−8 (−31−10)	15	−12 (−25−10)	0.453
Δ Diastolic blood pressure (mmHg)	36	−5 (−25−20)	15	0 (−15−25)	0.062
Δ Total cholesterol (mmol/L)	34	0.3 (−0.6−1.2)	17	0.4 (−1.2−3.3)	0.915
Δ LDL-cholesterol (mmol/L)	34	0.3 (−0.7−1.1)	15	0.1 (−0.6−2.20)	0.894
Δ HDL-cholesterol (mmol/L)	34	0.0 (−0.4−0.4)	15	0.1 (−0.2−0.8)	0.579
Δ Triglycerides (mmol/L)	34	0.05 (−1.26−1.75)	17	0.14 (−1.70−1.82)	0.572
Endothelial/inflammatory biomarkers					
Δ hsCRP (mg/L)	37	0.0 (−17.5−7.9)	18	−2.4 (−48.8−3.0)	0.083
Δ PAI-1 (ug/L)	35	−6 (−79−27)	17	−7 (−96−24)	0.711
Δ GDF-15 (pg/mL)	37	42 (−627−372)	17	13 (−356−290)	0.301
Δ sICAM (ng/mL)	35	4 (−139−57)	18	5 (−83−150)	0.764
Δ vWF (%)	35	13 (−170−83)	16	−2 (−21−48)	0.340
Δ FVIII (%)	33	24 (−46−83)	17	13 (−12−54)	0.306
Vascular measurements					
Δ AGE (AU)	21	−0.01 (−0.30−0.20)	10	−0.11 (−0.69−0.43)	0.819
Δ mean IMT (mm)	35	0.03 (−0.25−0.13)	11	−0.01 (−0.25−0.27)	0.387
Δ max IMT (mm)	35	0.02 (−0.31−0.12)	11	−0.04 (−0.30−0.32)	0.387
Δ mean BRS (ms/mmHg)	28	0.09 (−2.17−2.45)	15	0.24 (−1.03−0.70)	0.919
Hypogonadism					
Δ LH (U/L)	34	3.5 (−1.5−18.9)	14	5.2 (2.7−17.9)	0.884
Δ Testosterone (nmol/L)	34	−2.7 (−20.0−8.0)	14	−6.0 (−21.0−2.0)	0.179

AGE = advanced glycation endproduct; BMI = body mass index; BRS = baroreceptor sensitivity; CT = chemotherapy; hsCRP = high-sensitivity C-reactive protein; FVIII = coagulation factor VIII; GDF-15 = growth differentiation factor 15; IMT = intima media thickness; LH = luteinizing hormone; PAI-1 = plasminogen activator inhibitor 1; sICAM = soluble intercellular adhesion molecule 1; TL = telomere length; vWF = von Willebrand factor; yr = year.
Bold text denote sstatistical significance ($p < 0.050$).

[a] Metabolic syndrome is defined according to the NCEP ATP III definition update in 2005 in which three or more of the following factors have to be present: waist circumference >102 cm, triglycerides ≥1.7 mmol/L, HDL cholesterol <1.03 mmol/L or use of lipid lowering medication, blood pressure ≥130/85 mm Hg or use of antihypertensive drugs, fasting glucose ≥5.6 mmol/L or use of blood glucose lowering medication. Patients with a BMI >30 kg/m^2 with an unknown waist circumference were considered to have abdominal obesity and therefore scored as one point for increased waist circumference.

[b] Hypogonadism is defined as testosterone <10 nmol/L or LH >10 U/L or using testosterone replacement therapy.

treatment was higher in this group. An association between testosterone and TL has been previously described by Fang et al. [25] in a group of patients with idiopathic pulmonary fibrosis. The mechanism behind this is still unclear but may be mediated by the amount of abdominal fat, as in our study patients with short TL or telomere shortening showed a larger increase in waist circumference. These results support the hypothesis on the constant interaction between adipose tissue and testosterone [26].

The exact underlying mechanism between cardiovascular risk factors and changes in TL in this group is not known. Interpreting the results so far and by comparing them with other studies, an association with a biomarker of low-grade inflammation like hs-CRP was expected, but not found [13,27,28]. Due to small numbers of TC patients with a decrease in TL after CT (13%), only larger differences in endothelial or inflammatory biomarkers would reach significance. Also, before start of treatment, some patients have higher tumor burden than others, causing hs-CRP to be increased as a result of an inflammatory state. Therefore, it would be of interest to investigate changes in such biomarkers in a steadier state post-CT.

A strength of our study is the longitudinal study design and the pairing of genetic and phenotypic data at two time points. Also, study subjects were homogenous because of the relatively small age group, same treatment entities, and routine cardiovascular risk assessments during follow-up.

There are several limitations to this study. First, posthoc power analysis showed that the number of patients was too low to analyse subtle changes in TL after 1 year. Therefore, we chose to investigate patients with a decrease in TL of >33% vs. the rest. Furthermore, the hypothesis of

shortening of TL in all patients may have been overestimated, since not all TC patients develop long-term effects. In addition, the molecular signature of aging is the effect of a combination of several mechanisms underlying the process of aging. Therefore, not only mean leukocyte TL, but also other aging biomarkers, such as the amount of critically short telomeres, DNA methylation, or the SASP should be taken into account when investigating molecular aging [29–32]. Until additional molecular markers of aging are available, one should be cautious with firm conclusions.

5. Conclusion

In conclusion, only a small subset of TC patients treated with cisplatin-based CT showed telomere shortening 1 year after treatment, which was associated with a rise in diastolic blood pressure and triglycerides. Also, short TL at start of CT or telomere shortening after 1 year were associated with the presence of hypogonadism 5 years after treatment and a larger increase in waist circumference. Whether these are the direct effects of the treatment with cisplatin-based CT cannot be concluded.

Future research on the early aging phenotype in TC survivors and survivors that are at risk for developing CVD is needed. Research should focus on a combination of underlying mechanisms of aging, such as telomere shortening, biomarkers of senescence, the SASP or DNA methylation, to improve the identification of patients after treatment for TC that are at risk for CVD and other age-related diseases.

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Declaration of Competing Interest

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CRediT authorship contribution statement

Ellen L.D. Volders: Methodology, Formal analysis, Writing – original draft, Visualization, Project administration. **Coby Meijer:** Conceptualization, Methodology, Writing – review & editing. **Lotte S. Steeneken:** Methodology, Validation, Investigation. **Sjoukje Lubberts:** Conceptualization, Investigation. **Nynke Zwart:** Resources, Data curation. **Arie M. van Roon:** Writing – review & editing. **Joop D. Lefrandt:** Writing – review & editing. **Igle J. de Jong:** Writing – review & editing. **M. Demaria:** Writing – review & editing. **Janine Nuver:** Writing – review & editing. **Jourik A. Gietema:** Writing – review & editing, Supervision, Funding acquisition.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2023.10.010.

References

[1] Ness KK, Kirkland JL, Gramatges MM, Wang Z, Kundu M, McCastlain K, et al. Premature physiologic aging as a paradigm for understanding increased risk of adverse health across the lifespan of survivors of childhood cancer. J Clin Oncol 2018;36:2206–15. https://doi.org/10.1200/JCO.2017.76.7467.

[2] Lubberts S, Meijer C, Demaria M, Gietema JA. Early ageing after cytotoxic treatment for testicular cancer and cellular senescence: Time to act. Crit Rev Oncol Hematol 2020;151:102963. https://doi.org/10.1016/j.critrevonc.2020.102963.

[3] Gallicchio L, Gadalla SM, Murphy JD, Simonds NI. The effect of cancer treatments on telomere length: a systematic review of the literature. J Natl Cancer Inst 2018;110:1048–58. https://doi.org/10.1093/jnci/djy189.

[4] Liu Y, Zhang Z, Li T, Xu H, Zhang H. Senescence in osteoarthritis: from mechanism to potential treatment. Arthritis Res Ther 2022;24:174. https://doi.org/10.1186/s13075-022-02859-x.

[5] Hou Y, Dan X, Babbar M, Wei Y, Hasselbalch SG, Croteau DL, et al. Ageing as a risk factor for neurodegenerative disease. Nat Rev Neurol 2019;15:565–81. https://doi.org/10.1038/s41582-019-0244-7.

[6] Sun Y, Wang X, Liu T, Zhu X, Pan X. The multifaceted role of the SASP in atherosclerosis: from mechanisms to therapeutic opportunities. Cell Biosci 2022;12:74. https://doi.org/10.1186/s13578-022-00815-5.

[7] Emami M, Agbaedeng TA, Thomas G, Middeldorp ME, Thiyagarajah A, Wong CX, et al. Accelerated biological aging secondary to cardiometabolic risk factors is a predictor of cardiovascular mortality: a systematic review and meta-analysis. Can J Cardiol 2022;38:365–75. https://doi.org/10.1016/j.cjca.2021.10.012.

[8] Kosmopoulos M, Chiriacò M, Stamatelopoulos K, Tsioufis C, Masci PG, Kontogiannis C, et al. The relationship between telomere length and putative markers of vascular ageing: a systematic review and meta-analysis. Mech Ageing Dev 2022;201:111604. https://doi.org/10.1016/j.mad.2021.111604.

[9] Liu M, Hales BF, Robaire B. Effects of four chemotherapeutic agents, bleomycin, etoposide, cisplatin, and cyclophosphamide, on DNA damage and telomeres in a mouse spermatogonial cell line. Biol Reprod 2014;90:72. https://doi.org/10.1095/biolreprod.114.117754.

[10] Liu M, Maselli J, Hales BF, Robaire B. The effects of chemotherapy with bleomycin, etoposide, and cis-platinum on telomeres in rat male germ cells. Andrology 2015;3:1104–12. https://doi.org/10.1111/andr.12102.

[11] Schröder CP, Wisman GB, de Jong S, van der Graaf WT, Ruiters MH, Mulder NH, et al. Telomere length in breast cancer patients before and after chemotherapy with or without stem cell transplantation. Br J Cancer 2001;84:1348–53. https://doi.org/10.1054/bjoc.2001.1803.

[12] Gramatges MM, Liu Q, Yasui Y, Okcu MF, Neglia JP, Strong LC, et al. Telomere content and risk of second malignant neoplasm in survivors of childhood cancer: a report from the childhood cancer survivor study. Clin Cancer Res 2014;20:904–11. https://doi.org/10.1158/1078-0432.CCR-13-2076.

[13] Ariffin H, Azanan MS, Abd Ghafar SS, Oh L, Lau KH, Thirunavakarasu T, et al. Young adult survivors of childhood acute lymphoblastic leukemia show evidence of chronic inflammation and cellular aging. Cancer 2017;123:4207–14. https://doi.org/10.1002/cncr.30857.

[14] Lubberts S, Boer H, Altena R, Meijer C, van Roon AM, Zwart N, et al. Vascular fingerprint and vascular damage markers associated with vascular events in testicular cancer patients during and after chemotherapy. Eur J Cancer 2016;63:180–8. https://doi.org/10.1016/j.ejca.2016.05.022.

[15] Nuver J, Smit AJ, van der Meer J, van den Berg MP, van der Graaf WT, Meinardi MT, et al. Acute chemotherapy-induced cardiovascular changes in patients with testicular cancer. J Clin Oncol 2005;23:9130–7. https://doi.org/10.1200/JCO.2005.01.4092.

[16] Grundy SM, Becker D, Clark LT, Cooper RS, Denke MA, Howard WJ, et al. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). JAMA 2001;285:2486–97. https://doi.org/10.1001/jama.285.19.2486.

[17] Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation 2005;112:2735–52. https://doi.org/10.1161/CIRCULATIONAHA.105.169404.

[18] Huzen J, Wong LSM, van Veldhuisen DJ, Samani NJ, Zwinderman AH, Codd V, et al. Telomere length loss due to smoking and metabolic traits. J Intern Med 2014;275:155–63. https://doi.org/10.1111/joim.12149.

[19] Révész D, Milaneschi Y, Verhoeven JE, Lin J, Penninx BWJH. Longitudinal associations between metabolic syndrome components and telomere shortening. J Clin Endocrinol Metab 2015;100:3050–9. https://doi.org/10.1210/JC.2015-1995.

[20] Révész D, Verhoeven JE, Picard M, Lin J, Sidney S, Epel ES, et al. Associations between cellular aging markers and metabolic syndrome: findings from the CARDIA study. J Clin Endocrinol Metab 2018;103:148–57. https://doi.org/10.1210/jc.2017-01625.

[21] Révész D, Milaneschi Y, Verhoeven JE, Penninx BW. Telomere length as a marker of cellular aging is associated with prevalence and progression of metabolic syndrome. J Clin Endocrinol Metab 2014;99:4607–15. https://doi.org/10.1210/jc.2014-1851.

[22] Niu Z, Wen X, Buka SL, Wang M, Tian L, Loucks EB, et al. Associations of telomere length at birth with predicted atherosclerotic lesions and cardiovascular disease risk factors in midlife: a 40-year longitudinal study. Atherosclerosis 2021;333:67–74. https://doi.org/10.1016/j.atherosclerosis.2021.08.013.

[23] Zhang Y, Xu Z, Yang Y, Cao S, Lyu S, Duan W. Association between weight change and leukocyte telomere length in U.S. adults. Front Endocrinol (Lausanne) 2021;12:650988. https://doi.org/10.3389/fendo.2021.650988.

[24] Nuver J, Smit AJ, Wolffenbuttel BH, Sluiter WJ, Hoekstra HJ, Sleijfer DT, et al. The metabolic syndrome and disturbances in hormone levels in long-term survivors of disseminated testicular cancer. J Clin Oncol 2005;23:3718–25. https://doi.org/10.1200/JCO.2005.02.176.

[25] Fang C, Huang H, Zhang Q, Wang N, Jing X, Guo J, et al. Relation between sex hormones and leucocyte telomere length in men with idiopathic pulmonary fibrosis. Respirology 2020;25:1265–73. https://doi.org/10.1111/resp.13871.

[26] Fernandez CJ, Chacko EC, Pappachan JM. Male obesity-related secondary hypogonadism—pathophysiology, clinical implications and management. Eur Endocrinol 2019;15:83–90. https://doi.org/10.17925/EE.2019.15.2.83.

[27] Tucker LA. Insulin resistance and biological aging: the role of body mass, waist circumference, and inflammation. Biomed Res Int 2022;2022:2146596. https://doi.org/10.1155/2022/2146596.

[28] Gao X, Kong Y, Li S, Dong S, Huang X, Qi D, et al. Intermediate effects of body mass index and C-reactive protein on the serum cotinine—leukocyte telomere length association. Front Aging Neurosci 2022;13:827465. https://doi.org/10.3389/fnagi.2021.827465.

[29] Quintela-Fandino M, Soberon N, Lluch A, Manso L, Calvo I, Cortes J, et al. Critically short telomeres and toxicity of chemotherapy in early breast cancer. Oncotarget 2017;8:21472–82. https://doi.org/10.18632/oncotarget.15592.

[30] Telomeres LPM. Telomerase and cancer. Arch Med Res 2022;53:741–6. https://doi.org/10.1016/j.arcmed.2022.10.004.

[31] Goncalves RSDSA, Maciel ÁCC, Rolland Y, Vellas B, de Souto, Barreto P. Frailty biomarkers under the perspective of geroscience: a narrative review. Ageing Res Rev 2022;81:101737. https://doi.org/10.1016/j.arr.2022.101737.

[32] Verma AK, Singh P, Al-Saeed FA, Ahmed AE, Kumar S, Kumar A, et al. Unravelling the role of telomere shortening with ageing and their potential association with diabetes, cancer, and related lifestyle factors. Tissue Cell 2022;79:101925. https://doi.org/10.1016/j.tice.2022.101925.