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Original article

Associations between long-term serum platinum and neurotoxicity and ototoxicity, endocrine gonadal function, and cardiovascular disease in testicular cancer survivors

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Abstract

Objective: To evaluate the associations between long-term serum levels of platinum (se-Pt) and neurotoxicity and ototoxicity (NTX), endocrine gonadal function (endocrine-GF), and cardiovascular disease (CVD) in testicular cancer survivors.

Material and methods: A total of 292 cisplatin-treated testicular cancer survivors (1980–1994) participated in a national follow-up study (2007–2008). Se-Pt was quantified by inductively coupled plasma mass spectrometry, and categorized in quartiles. Symptoms of NTX were assessed with scale for chemotherapy-induced neurotoxicity (SCIN), with each symptom in 4 categories and total SCIN score categorized in quartiles. Endocrine-GF was categorized according to cutoff values for the 25, 50, and 75 percentiles of luteinizing hormone (LH) and testosterone within each decadal age group established from a control group. CVD was defined as ischemic heart disease, stroke, or artery occlusion. Associations between se-Pt levels and NTX, endocrine-GF, or risk for CVD, were analyzed with ordinal logistic regression and Cox regression, respectively.

Results: Median follow-up was 19 years (range: 13–28). In ordinal regression analyses, increasing quartiles of se-Pt were significantly associated with increasing quartiles of SCIN (P for trend $=$ 0.05), increased tinnitus ($P<0.001$), and increased hearing impairment ($P = 0.04$). The association remained significant for tinnitus when adjusting for cisplatin dose. Increasing LH quartiles was associated with increasing se-Pt quartiles ($P = 0.04$). No association between se-Pt in quartiles and CVD was established.

Conclusion: Median 19 years after treatment, increasing quartiles of se-Pt are associated with increasing SCIN score, tinnitus, hearing impairment, and increasing LH levels. However, these associations remained significant only for tinnitus and LH when adjusting for administered cisplatin dose.

Keywords: Testicular cancer; Long-term follow-up; Cisplatin retention

1. Introduction

Cisplatin remains the cornerstone in the treatment of advanced testicular cancer (TC) [1], and life expectancy of TC survivors (TCS) is presumed to be near normal.

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Well-documented cisplatin-related late effects include increased risk of hypertension, obesity, metabolic syndrome [2–4], and disturbed endocrine gonadal function (endocrine-GF) with impaired testosterone production and compensatory increased luteinizing hormone (LH) levels [5], and neurotoxicity and ototoxicity (NTX) including peripheral paresthesias/neuropathy, Raynaud phenomenon, hearing impairment, and tinnitus [6]. Nevertheless, the mechanisms behind these effects partly remain unclear.

Increasing cumulative cisplatin doses are positively associated with serum platinum (se-Pt) levels median 20 years later [7–9]. Further research to clarify associations between long-term se-Pt and late effects in TCS has been recommended [10]. An association between increasing se-Pt levels median 12 years after treatment and increasing severity of NTX was described for the first time by our research group. The assumption that reactive se-Pt several years after chemotherapy exposition may contribute to vascular and organ damage was then hypothesized [11].

The aim of the present study was to evaluate the association between long-term se-Pt median 19 years after treatment and NTX, endocrine-GF, and cardiovascular disease (CVD) risk factors and events, in TCS treated with platinum-based chemotherapy. Additionally, we wanted to address the possible effect of smoking on these late effects.

2. Patients and methods

2.1. Study population and design

All Norwegian long-term survivors of unilateral TC aged 18 to 75 years, treated in the period 1980 to 1994, were invited to participate in a national multicenter follow-up survey performed at 5 university hospitals. Of 1,814 eligible men, 1,463 (81%) participated in Survey I (SI) (1998–2002) [6]. During 2007 to 2008, a second Survey (SII) was conducted with 1,093 (80% of eligible men, Fig. 1) of the same TCS participants. SII included a physical examination and blood samples at the general practitioner, as well as a questionnaire.

The present study is restricted to 292 TCS previously treated with cisplatin- or carboplatin-based chemotherapy for which se-Pt measurements were available (Fig. 1). All men receiving testosterone substitution ($N = 17$) were excluded for analyses of se-Pt and hormone levels, leaving 275 men assessable for hormone analyses. The Committee for Medical Research Ethics, the Southern Health Region of Norway, approved both surveys.

2.2. Standards of treatment, 1980 to 1994

All patients with TC initially underwent unilateral orchiectomy and staging according to the Royal Marsden Hospital System [12]. Principles for the cytotoxic treatment of TC in Norway between 1980 and 1994 have been described previously [13]. Cisplatin-based chemotherapy was combined with bleomycin and either etoposide (BEP) or vinblastine (CVB) in most patients.

All patients receiving carboplatin had corresponding cisplatin doses calculated, by dividing carboplatin doses by 4, giving equivalent clinical doses [14].

2.3. Assessments and definitions

The questionnaire in SII included a validated 6-item scale for chemotherapy-induced neurotoxicity (SCIN) addressing neuropathy, Raynaud-like phenomena in hands and feet, tinnitus, and impaired hearing. The item scores ranged from not at all (0) to very much (3), and summation of the 6 items yielded a total SCIN score ranging from 0 to 18 [6]. Additionally, the questionnaire addressed comorbidities, medication use, and smoking habits. All self-reported CVD events were validated.

Resting blood pressure was measured with an automatic device or manually. Fasting blood samples were drawn by venipuncture before 11 AM at the TCS general practitioner office to assess levels of blood lipids, glucose, LH, testosterone, and se-Pt. All routine blood samples were analyzed at Oslo University Hospital. Total testosterone and LH were determined using a commercial immunoassay [15]. Se-Pt was quantified at St. Olavs University Hospital by inductively coupled plasma mass spectrometry, with limit of quantification 15 ng/l [7]. Se-Pt concentrations measured below limit of quantification had values set to zero.

Levels of testosterone and LH from 599 controls were categorized from 1 to 4 according to cutoff values for the 25, 50, and 75 percentiles within each decadal age group to establish reference intervals [15,16]. Testosterone and LH levels in the 275 TCS were assigned to one of these 4 categories, based on the percentiles derived from the controls. Levels between 2.5 and 97.5 percentiles in healthy controls determined the reference range.

CVD was defined as ischemic heart disease (angina and myocardial infarction), stroke, or artery occlusion based on data from the questionnaire. Hypertension, obesity, and metabolic syndrome were defined by National Cholesterol Education Program Criteria [17]. Diabetes was defined as previously diagnosed diabetes based on information in the questionnaire or serum glucose ≥ 11 mmol/l. Information regarding smoking, current antihypertensive, and lipid-lowering treatment and diabetes was retrieved from the questionnaire, and missing data were categorized as being a never smoker, without such treatment, or nondiabetic, respectively. Smoking was categorized as current, former, and never smoking.

2.4. Statistical methods

Pearson correlation was used to assess univariate associations among continuous variables. Spearman correlation

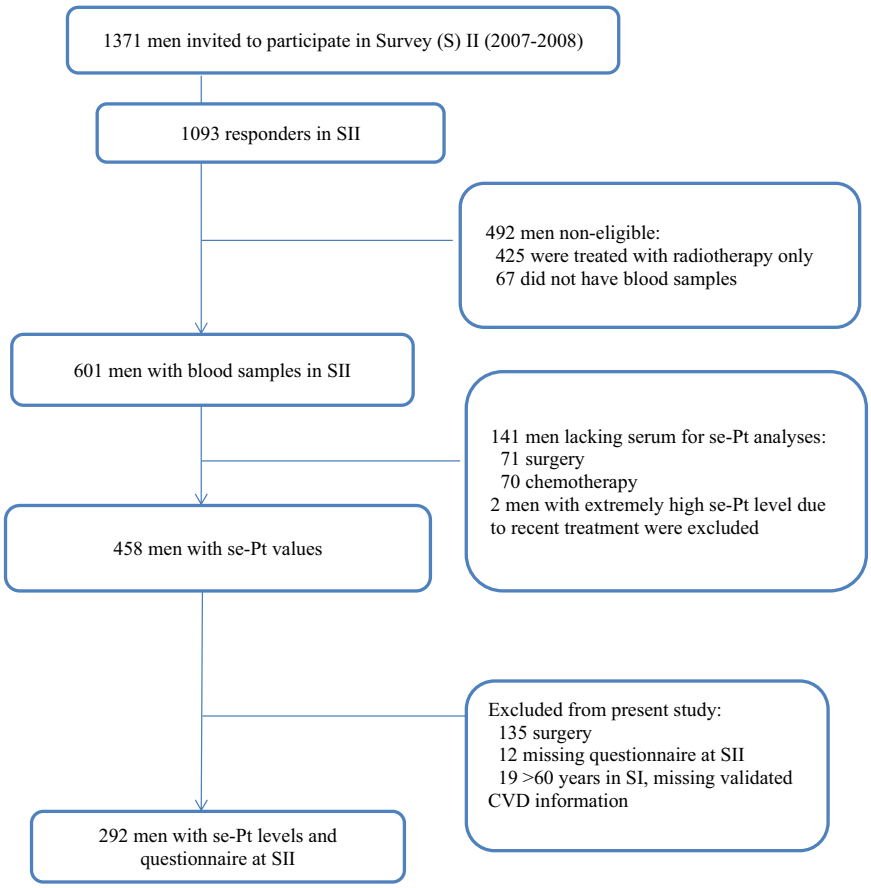

Fig. 1. Flowchart for testicular cancer survivors with long-term serum platinum (se-Pt) levels included in the study. (Color version of figure is available online.)

was used to assess univariate associations between se-Pt and both NTX and hormones and between current smoking and NTX. Mann-Whitney *U* test was used to evaluate the se-Pt distributions among different groups of TCS based on their different NTX symptoms and CVD risk factors. Se-Pt levels were categorized into quartiles and used as an explanatory variable, with the lowest quartile as reference group, and the toxicity of interest (NTX, endocrine-GF or CVD) being the dependent variable in appropriate multivariate regression models.

Associations between se-Pt levels and NTX or hormone levels were analyzed with multivariate ordinal logistic regression, both according to the separate symptom scores for all 6 NTXs and the total SCIN score or hormone levels categorized into quartiles. A test of parallel lines confirmed that the proportional assumption was met in all ordinal analyses.

The associations of se-Pt and cumulative cisplatin on the incidence of CVD were analyzed by Cox proportional hazard regression models. The observation time was calculated from the date of TC diagnosis until the date of first CVD event of interest or until the date of SII. The proportional hazard assumption was verified by visual inspection of log minus log of survival curves.

All the statistical multivariable models were adjusted for administered cumulative cisplatin and age. Additional adjustments for current smoking status (yes vs. no) were performed for analyses of NTX. All *P* values were 2-sided and statistical significance was set at $P < 0.05$. There were no multicollinearity between cisplatin and se-Pt. The data were analyzed by using SPSS 21.0 (SPSS, Chicago, IL).

3. Results

3.1. Characteristics and overall se-Pt levels

Demographics of patients and details regarding chemotherapy regimens are listed in Table 1. The 292 men studied did not differ from the original cohort invited to SII, according to age, diagnosis, histology, and follow-up time; data not shown. Median se-Pt was 86 ng/l (0–725). Median se-Pt according to number of platinum-based chemotherapy cycles are illustrated in Supplementary Figure S1.

The median se-Pt level was significantly higher for TCS reporting the highest level of tinnitus vs. the 3 lowest levels (Mann-Whitney *U* test, $P = 0.02$), but did not differ for any of the other SCIN variables.

Table 2 presents the risk factors for CVD and prevalent CVD (yes/no) with corresponding median se-Pt levels.

Table 1
Demographics and details regarding chemotherapy regimens, for 292 testicular cancer survivors

Characteristic	
Age, y, median (range)	
At TC diagnosis	29.0 (14.5–51.2)
At survey	49.3 (31.2–67.5)
Follow-up time, years, median (range)	19.4 (13.1–28.2)
Histology, *n* (%)	
Seminoma	44 (15)
Nonseminoma	248 (85)
Royal Marsden Stage, *n* (%)	
I	86 (30)
IMk and II	144 (49)
III	9 (3)
IV	53 (18)
Smoking, *n* (%)	
Never	127 (43)
Former	102 (35)
Current	62 (21)
1 missing	
Cisplatin-containing chemotherapy regimens, *n* (%)	
BEP alone	139 (48)
CVB alone	91 (31)
Both BEP and CVB	10 (3.4)
Other combinations	52 (18)
Dose-intensive regimens, *n* (%)	
BOP/VIP	6 (2.1)
BEP 40, 50, and 60	17 (5.8)
CVB 60	1 (0.3)
Carboplatin-based regimens, *n* (%)	
Carboplatin monotherapy	7 (2.4)
CEB	8 (2.7)
Other	1 (0.3)
Cisplatin dose, mg, median (range)	770 (190–3,095)
Carboplatin dose, mg, median (range)	2,950 (710–3,710)
Platinum-based cycles, *n* (%)	
1–2	23 (7.9)
3–4	238 (82)
>4	31 (11)

BEP = bleomycin, etoposide, cisplatin; BOP = bleomycin, vincristine, cisplatin; CEB = carboplatin, etoposide, bleomycin; CVB = cisplatin, vinblastine, bleomycin; *n* = number; VIP = etoposide, ifosfamide, cisplatin.
Dose-intensive, accelerated cisplatin dose or double cisplatin dose/m². Overall, 276 men received cisplatin, 16 received carboplatin, and of these only 4 men received both carboplatin and cisplatin.

3.2. Se-Pt and NTX

3.2.1. Univariate analyses

We observed a univariate association between se-Pt quartiles and SCIN quartiles (Spearman correlation, $P = 0.09$), although not statistically significant (Fig. 2). Total SCIN score was positively associated with cumulative cisplatin dose ($P < 0.01$). Significant positive correlations between current smoking and total score of SCIN ($P = 0.03$), paresthesias in hands ($P < 0.01$), and Raynaud phenomenon in hands ($P < 0.01$) and feet ($P < 0.01$) were established. No significant univariate correlations were revealed between current smoking and tinnitus or hearing impairment.

3.2.2. Multivariate analyses

In multivariate ordinal regression analyses, increasing quartiles of se-Pt was significantly associated with increasing quartiles of SCIN (P for trend $= 0.05$), increased tinnitus ($P < 0.001$), and increased hearing impairment ($P = 0.04$) (Table 3).

The association between se-Pt levels and tinnitus remained significant after adjusting for administered cisplatin dose (Table 3).

Men reporting current smoking (vs. never smoking) at the time of SII had significantly increased severity of paresthesias in hands (odds ratio [OR] = 2.85, 95% CI: 1.60–5.06), feet (OR = 1.76, 95% CI: 0.99–3.14) (near significant), and Raynauds phenomenon in hands (OR = 2.50, 95% CI: 1.43–4.39) and feet (OR = 2.42, 95% CI: 1.37–4.28) in multivariate models including age, se-Pt, and cisplatin dose. Current smoking did not have an effect on tinnitus and hearing impairment in multivariate ordinal regression analyses.

3.3. Se-Pt and endocrine-GF

At SII, the proportion of TCS with LH above the 75th percentile or testosterone below the 25th percentile was 59% and 47%, respectively. LH quartiles were univariate associated with se-Pt quartiles (Spearman correlation, $P = 0.02$, Fig. 3). LH quartiles were also positively correlated with cumulative cisplatin dose ($P = 0.01$).

The odds for being in the upper quartiles of LH increased by increase in quartiles of se-Pt, with ORs at 1.64 (95% CI: 0.86–3.13), 2.50 (95% CI: 1.28–4.87), and 1.76 (95% CI: 0.92–3.36) for second, third, and fourth se-Pt quartile, respectively (P for trend $= 0.04$). These ORs were attenuated after adjusting for cumulative cisplatin dose (3. se-Pt quartile OR = 2.13, 95% CI: 1.08–4.20 and 4. se-Pt quartile OR = 1.27, 95% CI: 0.65–2.50). Increase in administered cumulative cisplatin dose was associated with higher LH levels (OR = 1.17, 95% CI: 1.06–1.30 per 100 mg cisplatin). There was no association between testosterone quartiles and se-Pt levels, or between current smoking and hormone levels (data not shown).

3.4. Se-Pt and CVD

Overall, 24 (8.2%) TCS had experienced one or more CVD events during follow-up. There were 32 events in total, with angina ($N = 9$) and myocardial infarction ($N = 9$) as the most frequent events. The first event occurred median 16 years (range: 8–18) after TC diagnosis.

No significant associations between any of the risk factors for CVD were listed in Table 2, and se-Pt levels were

Table 2
Serum platinum (se-Pt in ng/l) levels according to neurotoxicity and ototoxicity and cardiovascular risk factors for 292 testicular cancer survivors

Characteristic	Yes Median se-Pt (range) (*n*)	No Median se-Pt (range) (*n*)
Highest quartile of total SCIN score vs. the 3 lowest	89.3 (0–247) (78)	84.75 (0–725) (214)
Smoking, current	86.5 (0–244) (62)	86.0 (0–725) (230)
Hypertension, SBT > 130 or DBT > 85 or blood pressure medication	84.2 (0–244) (193)	89.0 (0–725) (93)
Obesity, waist circumference >102 cm	85.1 (0–247) (71)	86.0 (0–725) (212)
Metabolic syndrome, 3 or more risk factors (obesity, hypertension, fasting glucose ≥110 mg/dl, triglycerides ≥150 mg/dl)	86.6 (0–244) (95)	85.9 (0–725) (189)
Cardiovascular disease	81.6 (0–195) (24)	86.0 (0–725) (268)

DBT = diastolic blood pressure; *n* = numbers; SBT = systolic blood pressure.
Hypertension, obesity, and metabolic syndrome are defined by NCEP (National Cholesterol Education Program) criteria.
There are missing values for some of the variables: hypertension, missing 6; obesity, missing 9; metabolic syndrome, missing 8; total SCIN score in quartiles, missing 1. One man did not answer the SCIN questions, and is labeled missing for all analyzes concerning SCIN; 2 men answered some of the SCIN questions, and the questions not answered were set to 0.

established. Furthermore, there was no association between se-Pt in quartiles and CVD in Cox regression analysis (4. se-Pt quartile vs. 1. se-Pt quartile, HR = 0.74, 95% CI: 0.22–2.49). Increasing cumulative cisplatin dose, per 100 mg, tended to be associated with CVD with an HR at 1.12 (95% CI: 0.99–1.26). There was no significant association between current smoking and CVD (data not shown).

4. Discussion

Median 19 years after treatment, increasing se-Pt is significantly associated with increasing SCIN score, tinnitus, hearing impairment, and increasing LH levels. However, these associations remained significant only for tinnitus and LH when adjusting for administered cisplatin dose.

A thoroughly characterized cohort of TCS with substantial long-term follow-up and detailed information regarding cancer therapy is the major strength of our study. The large control group providing the hormone reference intervals [16], which permitted age-adjustment of TCS hormonal levels, provides a further strength in this study.

An apparent limitation of our study is the reliance on self-reported symptoms at SII. The SCIN is a validated screening instrument for chemotherapy-induced neurotoxicity [18]; however, neurologic tests or audiometries were not performed. Earlier studies observed that, in comparison to self-reporting, prevalence of neurologic adverse effects could be higher when measured objectively [18]. This may imply that the SCIN underestimates the prevalence of NTX problems among our TCS.

Owing to small subgroups of SCIN symptoms and hormone percentiles, statistical power is limited. The CVD analyses are underpowered by the relatively low median age at follow-up at 49 with a low CVD incidence in the present study, as CVD predominantly occurs later in

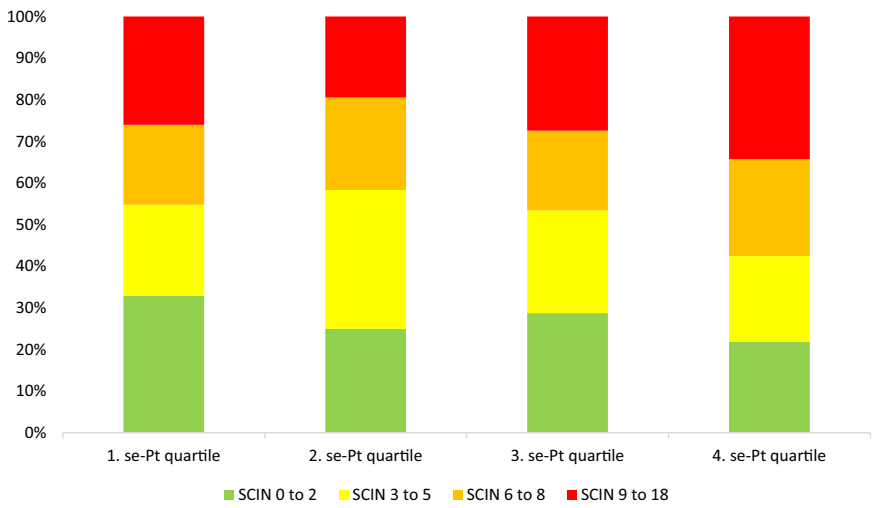

Fig. 2. Quartiles of scale for chemotherapy-induced long-term neurotoxicity (SCIN) according to serum platinum (se-Pt) quartiles. (Color version of figure is available online.)

Table 3
Odds ratios of higher levels of scale for chemotherapy-induced neurotoxicity (SCIN) symptoms at Survey II according to serum platinum (se-Pt) quartiles, administered cisplatin dose, and age*

	Model 1 Age-adjusted OR [95% CI]	*P* for trend	Model 2 Age- and cisplatin-adjusted OR [95% CI]
SCIN, quartiles			
Se-Pt 25–50	1.07 [0.60–1.93]	0.05	1.02 [0.56–1.83]
Se-Pt 50–75	1.17 [0.65–2.10]		1.07 [0.59–1.92]
Se-Pt > 75	1.81 [1.00–3.26]		1.44 [0.78–2.66]
Cisplatin, 100 mg			1.10 [1.02–1.19]
Parestesias, hands			
Se-Pt 25–50	0.99 [0.54–1.82]	0.26	0.95 [0.52–1.75]
Se-Pt 50–75	1.04 [0.57–1.90]		0.96 [0.52–1.76]
Se-Pt >75	1.41 [0.77–2.57]		1.19 [0.63–2.23]
Cisplatin, 100 mg			1.07 [0.99–1.15]
Paraesthesias, feet			
Se-Pt 25–50	1.56 [0.84–2.88]	0.09	1.47 [0.79–2.72]
Se-Pt 50–75	1.48 [0.80–2.74]		1.36 [0.73–2.52]
Se-Pt > 75	1.80 [0.97–3.33]		1.46 [0.77–2.77]
Cisplatin, 100 mg			1.10 [1.02–1.19]
Raynauds, hands			
Se-Pt 25–50	0.98 [0.55–1.76]	0.93	0.96 [0.53–1.73]
Se-Pt 50–75	0.70 [0.39–1.26]		0.67 [0.37–1.21]
Se-Pt > 75	1.16 [0.64–2.08]		1.05 [0.57–1.94]
Cisplatin, 100 mg			1.04 [0.97–1.12]
Raynauds, feet			
Se-Pt 25–50	1.35 [0.74–2.45]	0.89	1.33 [0.73–2.42]
Se-Pt 50–75	0.95 [0.52–1.72]		0.91 [0.50–1.67]
Se-Pt > 75	1.19 [0.65– 2.18]		1.09 [0.58–2.05]
Cisplatin, 100 mg			1.04 [0.97–1.12]
Tinnitus			
Se-Pt 25–50	1.20 [0.63–2.31]	<0.00	1.14 [0.59–2.18]
Se-Pt 50–75	2.46 [1.31–4.61]		2.21 [1.17–4.16]
Se-Pt > 75	2.65 [1.41–4.99]		1.91 [0.99–3.67]
Cisplatin, 100 mg			1.14 [1.05–1.24]
Hearing impairment			
Se-Pt 25–50	0.83 [0.45–1.54]	0.04	0.79 [0.42–1.46]
Se-Pt 50–75	1.11 [0.60–2.02]		1.00 [0.54–1.83]
Se-Pt > 75	1.76 [0.96–3.22]		1.28 [0.68–2.41]
Cisplatin, 100 mg			1.12 [1.04–1.21]

*From ordinal logistic regression models with first quartile of se-Pt as reference level.

life. The cross-sectional design based on assessments in SII only, hampers calculations of se-Pt elimination rates and their possible associations with long-term effects.

It has previously been established that the neurotoxic side effects of cisplatin are related to both cumulative cisplatin dose and treatment intensity [19]. Our study demonstrates side effects related to long-term sequestration of se-Pt, and it partly corroborates the results by Sprauten et al. [11], which was based on a part ($N = 169$, follow-up 12 y) of the same Norwegian cohort as our study. The Sprauten study demonstrated a stronger association between NTX and retained se-Pt than with cumulative cisplatin dose. The diminished association between se-Pt and NTX when cisplatin dose was included in the analyses in the present study could be explained by the longer observation time (median 19 y) than in the Sprauten et al. study. As far as we know, longitudinal se-Pt elimination rates have not yet been established for long-term follow-up. Other conceivable explanations may be differences in treatment and se-Pt analyzing methods.

Most organs store platinum to some extent [20], and platinum concentrations regarding neuro-ototoxicity are highest in dorsal root ganglia and lowest in the brain [21]. Dysfunctions of the outer hair cells might be important in the generation of tinnitus [22]. If the tissue platinum stored in nerves or the cisplatin-induced initial damage during treatment is responsible for outer hair cell dysfunction, is yet to be investigated. As far as we know, there are no substance that relive the neurotoxicity of platinating agents [23].

Sprauten et al. suggested that ongoing exposure to low-level se-Pt on neural tissue might limit the resolution of the acute and dose-dependent sensory neuropathy, contributing to ongoing damage. The absence of the association between se-Pt and NTX after adjusting for cisplatin in our cohort might suggest that the cisplatin-based treatment burden has a stronger effect on NTX development than retained se-Pt. Se-Pt possibly reflects the burden of treatment that has initiated these types of sequelae.

The high prevalence of hypogonadism in TCS may be explained by orchiectomy, testicular dysgenesis syndrome, postorchiectomy therapy, and aging [15]. Corresponding with our study, an increased risk of premature hormonal aging with higher LH levels was significantly associated with administered cumulative cisplatin dose [15]. To the best of our knowledge, this is the first study to describe an association between increasing long-term se-Pt levels and increasing LH levels median 19 years after cisplatin-based chemotherapy. Testicles contain more postchemotherapy-retained platinum compared to other hormone producing organs outside the brain [20]. The endocrine hypogonadism associated with higher se-Pt can result from ongoing exposure to low-level platinum in testicles, hypothetically contributing to ongoing damage.

TCS have an increased risk for CVD several years after the treatment [4]. Inflammation and endothelial dysfunction are fundamental factors of atherosclerosis development, and chemotherapy exposure inducing the atherosclerotic process, both on short term and long term have been documented [24,25]. Chemotherapy-treated TCS retain se-Pt in circulation up to 20 years after exposure [7], additionally approximately 10% of the se-Pt compounds remain reactive [9]. Thus, the possible relationship between long-term se-Pt levels and endothelium damage has been postulated as a conceivable premise resulting in CVD. Herein, we established an association between CVD and administered cisplatin dose only, but statistical power was limited because of few CVD events in our study population.

Inflammation is the most prominent hallmark of normal aging [26], along with imbalance in DNA damage and repair and a variety of intracellular and intercellular dysfunctions. Chemotherapy treatment affects almost every

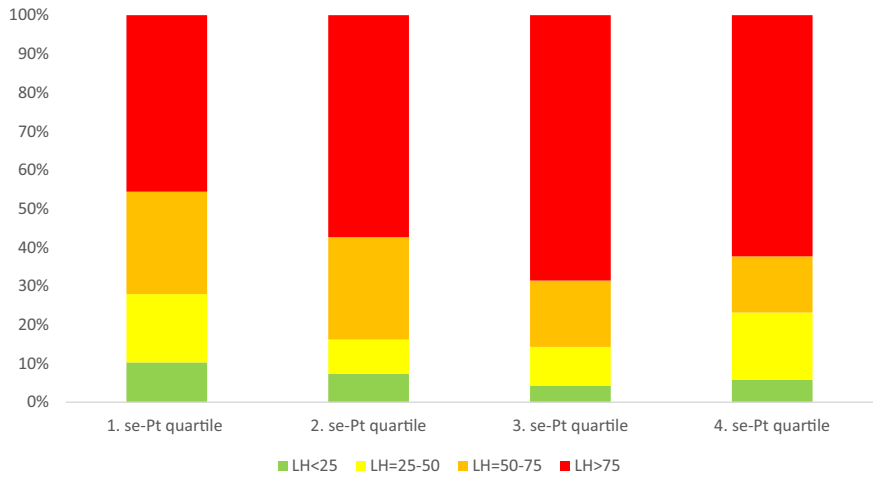

Fig. 3. Quartiles of luteinizing hormone (LH) scores according to serum platinum (se-Pt) quartiles. (Color version of figure is available online.)

organ system and may produce a "premature aging syndrome" [27], with long-term and late effects mimicking those of aging. Cisplatin-based chemotherapy causes DNA damage [28], possibly leading to a state of accelerated aging. The association between cisplatin dose and late effects explored in this study supports the hypothesis that the amount and type of chemotherapy leads to the development of late effects by inducing endothelial and tissue damage from the very beginning of treatment.

As after chemotherapy, the destructive effect of smoking is seen within most organs [29,30]. Our study finds disturbingly strong associations between smoking and neurotoxicity, in line with earlier findings within our group [6]. Thus, we recommend that TCS should constantly be motivated to quit smoking.

In summary, we have shown that increasing se-Pt is associated with increasing SCIN score, severity of tinnitus and hearing impairment, and increasing LH levels. Future studies should evaluate se-Pt elimination rates and their impact on long-term and late effects in TCS.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.urolonc.2016.06.012.

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Supplementary figure, paper II

Illustrations of median se-Pt according to number of administered platinum-based chemotherapy-cycles.