Long-term Platinum Retention After Platinum-based Chemotherapy in Testicular Cancer Survivors: A 20-Year Follow-up Study

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Abstract. *Aim: Evaluation of long-term platinum (Pt) retention in testicular cancer survivors (TCSs) treated with platinum-based chemotherapy to elucidate possible mechanisms of developing late effects. Patients and Methods: 458 TCSs treated 1980-1994 participated in a national follow-up study (2007-2008). Four treatment groups were evaluated for long-term serum Pt levels: surgery (n=135), cumulative cisplatin ≤850 mg (n=252), cisplatin >850 mg (n=57) and carboplatin (n=14). Results: The median observation time was 20 (range=13-28) years. The median Pt level according to treatment group was: surgery, 50 ng/l; cisplatin ≤850 mg, 85 ng/l; cisplatin>850 mg, 106 ng/l; carboplatin, 40 ng/l. The risk for having a Pt level in the highest quartile was positively associated with cisplatin dose (Ordinal regression (OR)=1.29, per 100 mg increase in cisplatin dose, 95% Confidence interval (CI)=1.20-1.38), and negatively associated with follow-up time (OR=0.50 per 5-year increase in follow-up time, 95% CI=0.37-0.68). Conclusion: Pt levels are significantly elevated in serum at a median of 20 years after cisplatin-based chemotherapy for testicular cancer.*

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Germ cell testicular cancer (TC) is the most common malignancy among young men, and its incidence is rising (1). The 5-year cancer-specific survival rate has increased considerably during the past decades, and currently exceeds 95% in Norway (1, 2). This improved prognosis is primarily due to cisplatin [*cis*-diaminedichloridoplatinum (II)], the cornerstone in the treatment of metastatic TC, which was introduced in the late 1970s (3, 4). Earlier detection, multimodal treatment and more correct staging with computed tomographic scans and tumor markers have also contributed to improved survival.

Cisplatin is a first-generation platinum cytotoxic agent. Its major mechanism of action is assumed to be platination of DNA followed by formation of several intra- and interstrand crosslinks (5, 6). The renal elimination of cisplatin is incomplete and platinum levels are still elevated several years after treatment discontinuation (7, 8). Moreover, *ex vivo* experiments have shown that up to 10% of circulating platinum remains reactive (9).

During the past two decades, concerns have emerged about possible long-term adverse effects of cisplatin-based chemotherapy, with cardiovascular disease and second malignancies being the most feared adverse health effects (10-12).

Measurements of serum platinum reveal up to 1,000-fold higher concentrations in patients treated with cisplatin compared to controls 8-75 months after chemotherapy (9). Thus, long-term persistence of platinum in serum may be associated with long-term toxicity, as indicated in a previous study of a subgroup of our patients (n=169) at a median of 12 years of follow-up (8). Other studies evaluating long-term retention of platinum

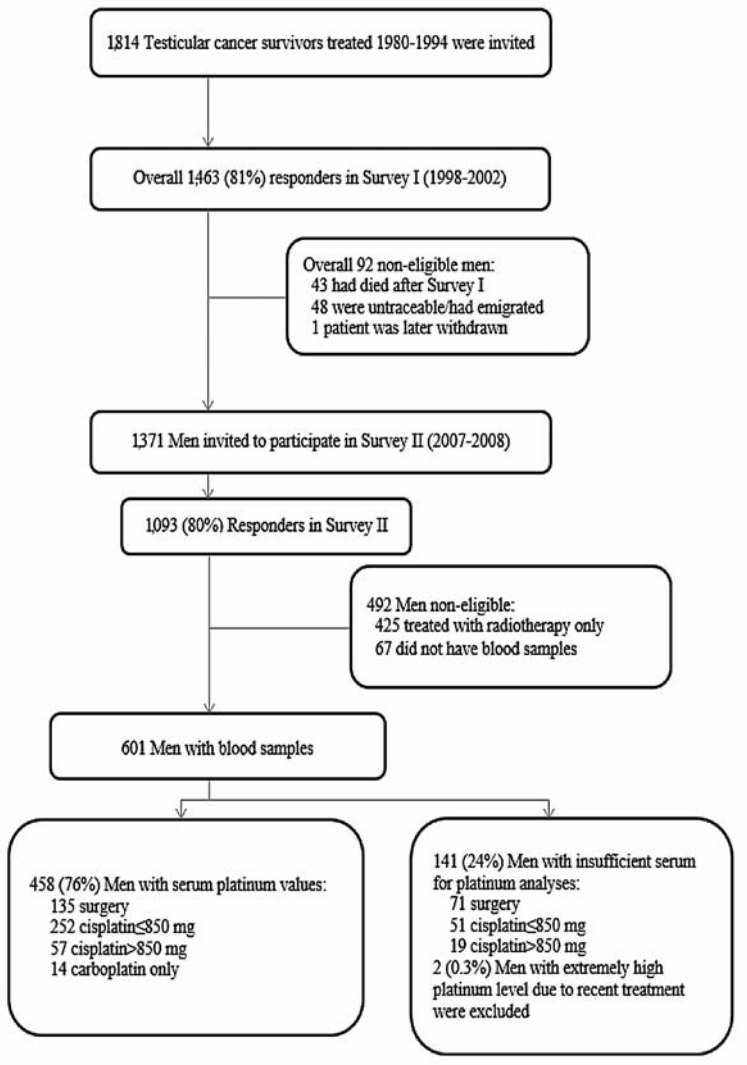

Figure 1. *Flowchart for men available for long-term Platinum analysis.*

after treatment for TC have rarely included patients with more than a median of 10 years of follow-up (7-9). Furthermore, knowledge about long-term platinum retention in patients treated with carboplatin is limited.

The major aim of our ongoing project is to evaluate the associations between serum platinum levels and long-term adverse effects in survivors of TC. As a first step, we present observations on serum platinum levels at a median of 20 years after treatment for TC. The aim of the present study was to evaluate the serum platinum level according to treatment group, cumulative cisplatin dose and time since therapy.

Patients and Methods

Study population and design. All Norwegian long-term survivors (n=1,814) of unilateral germ-cell TC aged 18-75 years and treated 1980-1994 were identified through the Cancer Registry of Norway, and invited to participate in two subsequent follow-up studies at all five university hospitals. The surveys consisted of mailed questionnaires and outpatient clinical examinations that included laboratory tests (13, 14). In survey I (1998-2002), 1463 (81%) had an outpatient visit at the responsible hospital. At one hospital, deep-frozen serum was stored for supplementary analyses, including of platinum level (8). Overall 1371 of the men from the first survey were invited to participate in a second survey during 2007-2008 (survey II, Figure 1), in which 1093 (80%) participated. In survey II, blood samples were drawn at the general practitioner's office and transported to the Oslo University Hospital for storage at −70˚C.

Primary data regarding treatment, relapse, staging and histology were retrieved from the patients' medical records. The study was approved by the Committee for Medical Research Ethics, the Southern Health Region of Norway (approval number S-98094 S05368). All participants provided informed written consent.

Among the 1093 participants in survey II, 635 were excluded from the current study: 425 men were treated with radiotherapy, 67 did not have blood samples and 141 had insufficient serum for platinum

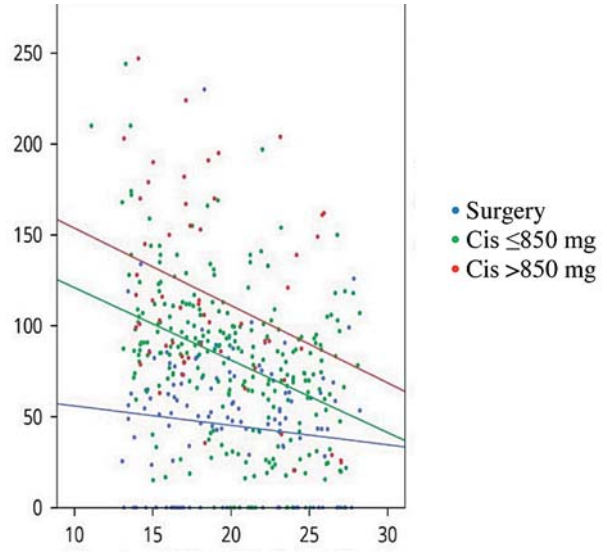

Figure 2. *Scatterplot showing individual platinum levels according to follow-up time. Cis: Cisplatin*

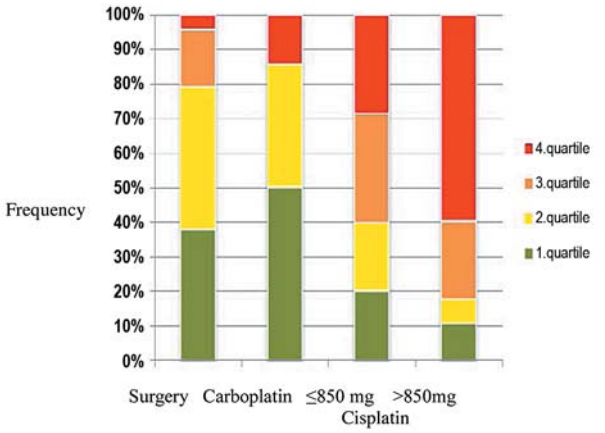

Figure 3. *The distribution of platinum quartiles according to treatment group.*

analysis. Two men had ongoing platinum-based chemotherapy as the blood samples in survey II were drawn (treatment for colorectal cancer, relapse from TC). Thus, the remaining 458 men treated with surgery and chemotherapy with sufficient serum for platinum analyses constitute the present study population (Figure 1).

Standards of treatment 1980 to 1994 and treatment groups. After orchiectomy, patients were staged according to the Royal Marsden Hospital System (15). Most patients were either treated within the Swedish-Norwegian Testicular Cancer Group (SWENOTECA) (16, 17), according to the European Organization for Research and Treatment for Cancer, or the Medical Research Council protocols (18). Details regarding treatment according to histological type and stage have been reported (14).

Patients with advanced disease received chemotherapy; typically cisplatin and bleomycin combined with either vinblastine (CVB) or etoposide (BEP). Some patients received treatment with dose-intensive BEP (19). In addition, 19 men were treated with carboplatin-based chemotherapy within research protocols (20-22), of whom five received both carboplatin-based and cisplatin-based chemotherapy during their treatment period.

With respect to the therapeutic dose of the two drugs, an equivalent amount of carboplatin was in this study considered 4-fold less potent than cisplatin (23). In five men who had received both platinum agents, we calculated the corresponding cisplatin doses by dividing the carboplatin dose by four before adding to their cumulative cisplatin dose. Based on the specific treatment, including relapse treatment, men were categorized into four treatment groups: surgery only (n=135, reference group), carboplatin only (n=14), cumulative cisplatin dose ≤850 mg (n=252) and >850 mg (n=57).

Quantification of serum platinum levels. In total, 458 serum samples were analyzed during March 2011 and March 2012 (386 and 72 samples in each time period, respectively). The samples were kept on dry-ice when shipped from Oslo University Hospital to St. Olav University Hospital in Trondheim, Norway for analyses of platinum. After arrival they were kept at −20˚C before being thawed for three hours at room temperature, and analyzed for total platinum.

Calibrators at three concentrations (25, 250 and 1.000 ng/l) were produced by spiking platinum-free plasma with certified platinum solution (Spectrapure, Oslo, Norway) and used for external calibration. Quality controls at two levels (100 and 1.000 ng/l) were prepared by spiking platinum-free plasma with an oxaliplatin solution (St. Olavs Univeristy Hospital Pharmacy, Trondheim, Norway). Aliquots of 100 µl of serum sample were pipetted into vials (2 ml precleaned polypropylene; Sarsted, Nűmbrecht, Germany) and diluted with 400 µl purified water (produced from a MilliQ Element unit, Millipore, Molsheim, France) and 500 µl dilution reagent containing 1% (v/v) HNO_3, 1% (v/v) HCl and 2.000 ng Ir/l in water (HNO_3 and HCl from Chem Scan AS, Elverum, Norway). Each vial was inverted five times to ensure proper mixing. For evaluation of accuracy, a serum sample from a quality management program (QMEQAS, Quebec, Canada) with an assigned platinum content was analyzed in every sequence.

Platinum determinations in serum samples were carried out with a high-resolution inductively coupled plasma-sector field mass spectrometry (ICP-SFMS) (Element 2; Thermo Scientific, Bremen, Germany). The monitoring of platinum was performed at low resolution (300m/Δm at valley definition). Platinum has six naturally occurring isotopes and the isotope ^{195}Pt was chosen because of its highest abundance and lack of potential spectral interferences. Internal standardization (^{193}Ir) was used to compensate for analytical issues such as drift and matrix effects.

The lower limit of quantification (LOQ) for this method was 15 ng/l. This limit was based on 10 times the standard deviation of a series of blanks. Linearity was proved for the concentration range from 15 to 10.000 ng/l. Intra-sequence precision at low level (100 ng/l) and high level(1.000 ng/l) based on controls were estimated to be 3.6% and 1.2%, respectively. Inter-sequence precision at low level and high level were estimated to 9.9% and 5.1%, respectively. Analysis of a serum sample from a quality management program resulted in a mean value (n=8) of 651±30 ng/l; this is in good agreement with a reported consensus value of 600±103 ng/l.

Table I. *Characteristics for 458 testicular cancer survivors according to treatment group.*

Characteristic	Surgery only	Carboplatin	Cisplatin	
			≤850 mg	>850 mg
	N=135	N=14	N=252	N=57
Median age (range), years				
At diagnosis	29 (17-64)	31 (19-55)	29 (15-64)	29 (15-62)
At follow-up	50 (32-84)	47 (35-71)	50 (31-82)	47 (31-83)
Median observation time (range), years	20 (13-28)	16 (14-20)	20 (11-28)	18 (13-27)
Initial disease stage*, no. (%)				
I	131 (97)	3 (21)	86 (34)	6 (11)
IMk+/ II	4 (3)	9 (64)	124 (49)	22 (39)
III		0	9 (4)	5 (9)
IV		2 (14)	33 (13)	24 (42)
Histology				
Seminoma	4 (3)	7 (50)	43 (17)	7 (12)
Non-seminoma	131 (97)	7 (50)	209 (83)	50 (88)
Surgery, no. (%)				
RPLND	86 (64)	4 (29)	168 (67)	48 (84)
Other	3 (2)		4 (2)	4 (7)
Treatment period, no. (%)				
1980-1984	40 (30)		70 (28)	13 (23)
1985-1989	43 (32)	2 (14)	92 (37)	15 (26)
1990-1994	52 (39)	12 (86)	90 (36)	29 (51)
Additional RT, no. (%)	0	5 (36)	19 (7.5)	6 (11)
Relapse, no. (%)				
After surgery only	1		28 (11)	2 (3.5)
After previous chemotherapy			3 (1.2)	7 (12)

RPLND: Retroperitoneal lymph node dissection; no.: number; RT: radiotherapy. *Disease staging by Royal Marsden Hospital Staging system.

Statistical analysis. Categorical variables are presented as counts and proportions, and continuous variables are presented as the median and range. Positive serum platinum concentrations below the LOQ were set to a value of zero. Crude associations between platinum level and observation time according to treatment group were assessed using scatter plots, Pearson correlation coefficients and simple linear regression.

Serum levels of serum platinum were categorized into quartiles. Logistic regression was used to evaluate the risk of having serum platinum in the highest quartile vs. the other quartiles according to treatment group, with the surgery group as reference, and presented as odds ratios (OR) with 95% confidence intervals (CIs). Age was included as a covariate in all regression models. All p-values are two-sided and statistical significance was set at $p<0.05$. Statistical analyses were performed using SPSS 21.0 (SPSS, Chicago, IL, USA).

Results

Patients and treatment characteristics. Details concerning patients' characteristics according to treatment group are presented in Table I. Overall, the median age at TC diagnosis was 29 (range=15-64) years and at follow-up (survey II) 50 (range=31-84) years. The median observation time was between 16 and 20 years across the different groups (range=11-28 years). The majority of participants in the current study had non-seminomatous cancer (87%), and 51% had metastatic disease at diagnosis. A total of 41 men experienced relapse during the follow-up period. One patient with relapse in the surgery-only group went through a new surgical intervention. There were 31 relapses in the group treated with ≤850 mg cisplatin and nine in those treated with >850 mg cisplatin.

Details regarding chemotherapy are presented in Table II. The majority of patients treated with ≤850 mg cisplatin received standard BEP (52%) or CVB (38%) as primary treatment, and 99% of them received four or fewer platinum-based cycles. The majority of men in the group treated with >850 mg cisplatin received BEP (33%) or other chemotherapy combinations (54%). Among the patients receiving BEP, 7 (2.8%) and 17 (30%) men received dose-intensive BEP in the group treated with ≤850 mg cisplatin and that treated with >850 mg, respectively. In total, 304 men received cisplatin as the only platinum agent, 14 received carboplatin-only, while five received both carboplatin and cisplatin.

Table II. *Chemotherapy regimens for 323 men.*

Characteristic	Carboplatin	Cisplatin	
		≤850 mg	>850 mg
	N=14	N=252	N=57
Median platinum level (range), ng/l	40 (0-1140)	85 (0-725)	106 (21-247)
Median cisplatin dose (range), mg	0	730 (190-850)	1120 (600-3095)
Median carboplatin dose (range), mg	2573 (710-3710)		
Chemotherapy regimen, no. (%)			
BEP alone, any combination		131 (52)	19 (33)
CVB alone		95 (38)	4 (7)
Both BEP and CVB		7 (3)	3 (5)
Other combinations		19 (8)	31 (54)
Carboplatin monotherapy, no. (%)	7 (50)		
CEB, no. (%)	7 (50)		
Dose-intensive regimens, no. (%)		7 (2.8)	17 (30)
Platinum-based cycles, no. (%)			
1-2	6 (36)	20 (7.9)	
3-4	8 (57)	231 (92)	19 (33)
>4	0	1 (0.4)	38 (66)

BEP, Bleomycin, etoposide, cisplatin; CVB, cisplatin, vinblastine, bleomycin; CEB, carboplatin, etoposide, bleomycin; no., number; dose-intensive regimens refers to chemotherapy regimens where cisplatin was scheduled over a shorter time span or with higher cumulative cisplatin dose compared to regular BEP.

Platinum levels. The median platinum level for all men treated with platinum-containing chemotherapy was 87.3 (range=0-725) ng/l. The median platinum level for the surgery-treated group was 50 (range=0-230) ng/l, that for the carboplatin-treated group was 40 (0-1140) ng/l, the ≤850 mg cisplatin-treated group was 85 (range=0-725) ng/l and that for the >850 mg cisplatin-treated group was 106 (range=21-247 ng/l). Overall, the platinum level was negatively associated with follow-up time ($p<0.001$), and positively associated with cisplatin dose ($p<0.001$) and cisplatin treatment groups ($p<0.001$). Correlations between platinum level and follow-up time were significant in both cisplatin-treated groups (≤850 mg, $p<0.001$; >850 mg, $p=0.014$), but non-significant in the carboplatin-treated group ($p=0.18$). Figure 2 shows a scatterplot with individual platinum levels according to follow-up time for all treatment groups except the carboplatin-treated group. The scatter plot for the latter group is not shown due to a limited number of cases and one extreme outlier.

In the surgery-treated group, 4.4% had a platinum level in the highest quartile, while corresponding numbers were 14% and 29% for the carboplatin-treated and ≤850 mg cisplatin-treated groups, respectively (Figure 3). In the group treated with >850 mg cisplatin, as many as 60% had platinum levels in the highest quartile. The OR for having a platinum level in the highest quartile was positively associated with cisplatin dose (OR=1.29 per 100 mg increase in cisplatin dose, 95% CI=1.20-1.38). The cisplatin-treated groups were also associated with an increased risk of having a platinum level in the highest quartile, with an OR of 9.4 (95% CI=3.9-22.5) for the ≤850 mg group and of 31.2 (95% CI=11.6-84.1) for the >850 mg group, in comparison to the surgery-treated group. The platinum level was negatively associated with follow-up time, with an OR of 0.50 (95% CI=0.37-0.68) per every five additional years.

Discussion

We have demonstrated that circulating platinum is still detectable in plasma up to 28 years after cisplatin-based chemotherapy. We found that platinum levels were significantly associated with previous cisplatin therapy, with respect to both dose and time from treatment. Furthermore, the risk for detecting a platinum level in the highest quartile increased with increasing cumulative cisplatin dose.

The major strength of this study is the large cohort of survivors of TC with extensive follow-up time and detailed information regarding cancer therapy and patient characteristics. Furthermore, the fact that TC treatment beyond orchiectomy was restricted to the five Norwegian university hospitals, and the high participation rates of ~80% for both surveys, minimizes the risk of selection bias. Prior studies focusing on long-term platinum retention have included smaller patient cohorts (maximum 169) and were restricted to a median of 14 years of follow-up (7-9). Hence, our cohort is the largest and with the longest follow-up within this research field.

Nevertheless, some of our treatment groups were relatively small, which limited statistical power. Moreover, calculations of the platinum elimination rates were not possible since we only had one measurement for the majority of patients. Elimination will be studied separately for the subgroup of patients with two platinum measurements. Furthermore, kidney function prior to and after treatment was not investigated thoroughly enough to elucidate elimination pharmacokinetics.

Most prior studies on platinum retention included TC patients treated with regimens similar to that of our study (7-9), and with cumulative cisplatin doses ranging between 350-400 mg/m^2, comparable with those applied in our cohort. In line with results from earlier studies (7-9), we observed a significant relationship between the administered cisplatin

dose and the platinum level measured later, and an inverse relationship between time until platinum assessment and platinum level.

The higher levels documented in two earlier studies (8, 9) when compared to ours is clearly due to shorter follow-up times. The study by Gietema *et al*. (7) had a six-year shorter follow-up time when compared to the present study, and a slightly longer follow-up when compared to the previous Norwegian study by Sprauten *et al*. (8). Both the study by Sprauten *et al*. (personal communication, M. Sprauten) and the data presented herein demonstrate higher platinum levels in controls when compared to the studies by Gietema *et al*. and Brouwers *et al*. (9). The reason for this is unclear, but may be due to different analytical methods or environmental factors.

The discussed studies differ with respect to analysis methods as the Gietema group used high-pressure decomposition of plasma samples followed by adsorptive voltammetric measurement, while the others all used ICP-MS. Analyses of platinum were also performed on different specimens (blood, serum and plasma) which may complicate comparisons. ICP-MS is a well-known, sensitive technique for quantification of platinum in different sample types. The sensitivity of the method is not only given by the instrument performance, but also by the complete sample preparation, including the quality of chemicals and equipment such as sample tubes. Reduction of the sample is important to avoid interference and maintain good analytical quality. We decided to utilize a method based on dilution to reduce the concentration of the organic matrix, hence minimizing the contamination risk and maintaining a high detection power.

Our reference group had a median platinum level of 50 ng/l. In a report from 1992 using voltammetric methods, the natural level of platinum in human blood and plasma was estimated to range between ≤0.8 and 6.9 ng/l (24). The high levels of platinum in our Norwegian male reference group may be a result of a platinum group element (PGE) bioaccumulation. The worldwide production of PGEs has increased significantly since 1970 , and is largest in Europe, attributable to a more prevalent use of catalysts for vehicle exhaust (25). High levels of PGE in soil bordering areas with heavy traffic are well documented (26, 27), and both diet and inhalation of PGE-containing particulate matter are important exposure routes for PGE in humans (28). Although Norway is a country consisting of large rural districts, approximately 80% of the population lives in urban communities where soluble platinum of roadside dust enters the food chain through water, sediments and soil. An explanation for the lower platinum levels reported in earlier studies (7, 9) may be that their population has been exposed to PGEs for a shorter period of time, and the fact that earlier studies were conducted in a period with less PGE pollution and accumulation.

A possible contributing explanation for the discrepancy in platinum levels between the Dutch and the Norwegian cohorts may be their genetic differences. It has been demonstrated that genetic polymorphisms in glutathione-S-transferase P1 and M1 gene, (*GSTPI* and *GSTMI*) are associated with chemotherapy-induced toxicities (29), and genetic differences may lead to diverse pharmacokinetics and cytotoxic drug tolerance among patients.

Apart from treatment burden, mechanisms explaining the increased risk for late effects after cisplatin-based chemotherapy have not been clarified (10). It is assumed that persistent exposure of endothelial cells to circulating platinum may cause endothelial dysfunction, which has been demonstrated to occur up to seven years after chemotherapy administration (30, 31). The study by Sprauten *et al*. is the first and so far the only one to demonstrate a correlation between chemotherapy-related long-term serum platinum levels and severity of neurotoxicity (2).

We did not find any association between platinum levels and follow-up time for carboplatin-treated patients. The number of patients was, however, limited and no conclusions could be drawn regarding retention of carboplatin.

In conclusion, we are the first to document that survivors previously treated with cisplatin for TC have increased platinum levels at a median of 20 years after cisplatin-based chemotherapy when compared to controls treated with surgery only. Further studies should explore the impact of residual platinum with respect to late effects in order to clarify underlying mechanisms. The role of different genotypes for platinum detoxification should also be examined to elucidate individual differences with respect to late toxicity.

Acknowledgements

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