CLINICAL STUDY

Risk factors for post-treatment hypogonadism in testicular cancer patients

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Abstract

Objectives: Testicular germ-cell cancer (TGCC) patients are at risk of developing hypogonadism but no risk factors have yet been defined.

Methods: Blood was collected from 143 TGCC patients (after orchidectomy, prior to further therapy (T0) and 6, 12, 24, 36 and 60 months (T6, T12, T24, T36 and T60) after therapy). Biological hypogonadism (BH) was defined as: serum testosterone below 10 nmol/l and/or LH > 10 IU/l; odds ratios (ORs) for BH with BH at T0, age, stage of disease, testicular characteristics, and androgen receptor polymorphism as predictors were calculated as well as the OR for developing BH post-treatment (one to two cycles of adjuvant chemotherapy (ACT) versus three to four cycles of higher dose chemotherapy (HCT) versus adjuvant radiotherapy (RT)).

Results: HCT increased the OR for BH at T6 (OR 22, 95% confidence interval (CI) 4.4–118) and T12 (OR 5.8, 95% CI 1.5–22). RT increased the OR at T6 (OR 10, 95% CI 2.1–47) and at T12 (OR 3.9, 95% CI 1.1–14). Microlithiasis predicted BH at T0 (OR 11, 95% CI 1.2–112), T12 (OR 3.9, 95% CI 1.1–13), T24 (OR 3.0, 95% CI 1.0–8.8), T36 (OR 5.4, 95% CI 1.7–17) and T60 (OR 4.4, 95% CI 1.2–16). BH at T0 was a risk for BH at T6 (OR 53, 95% CI 19–145), T12 (OR 125, 95% CI 37–430), T24 (OR 88, 95% CI 26–300) and T36 (OR 121, 95% CI 32–460).

Conclusions: It is clinically relevant that BH at T0 and testicular microlithiasis were predictive factors for post-treatment BH. HCT and RT gave temporary BH.

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Introduction

Testicular germ-cell cancer (TGCC) patients are at risk of developing Leydig cell insufficiency (1, 2). Orchidectomized TGCC patients were reported to have subnormal testosterone values in contrast to orchidectomized non-cancer patients (2), indicating that the disease *per se* is affecting the Leydig cell function. Different treatment modalities, such as orchidectomy, chemotherapy and radiotherapy may also affect the Leydig cell function, although the data are rather conflicting. Furthermore, although serum testosterone levels may decrease following orchidectomy (3), a compensatory, time-related improvement in Leydig cell function has been postulated (4). It may, therefore, imply that men being hypogonadal shortly after testis removal may recover and should, therefore, not be offered androgen replacement therapy immediately.

A Leydig cell impairment, with subnormal/normal s-testosterone values and/or increased luteinizing hormone (LH), was found in patients who had undergone chemotherapy (5–8). However, in other studies, no difference in testosterone or LH after chemotherapy was found (9, 10).

These discrepancies might be due to differences in the chemotherapy used, doses and also the length of follow-up time.

Many studies have focused on testosterone levels in men treated for TGCC, only few dealt with risk factors for development of hypogonadism. In a 10-year follow-up study, Nord *et al.* found that men treated for TGCC had a three- to fourfold risk to develop hypogonadism and that the risk increased with age and treatment intensity (11, 12). Another study reported increased risk of gonadal dysfunction in long-term survivors of testicular cancer and there was a dose–response effect following chemotherapy (12).

Our prospective longitudinal study was carried out to identify potential patient, disease and treatment-related factors associated with testicular dysfunction. Pre-treatment hormone levels, age, stage of disease, treatment and contralateral testicular status in terms of volume, consistency and ultrasound pattern were investigated. Furthermore, polymorphic variations in the androgen receptor (AR) gene, which have an impact on androgen sensitivity (13, 14) and therefore might influence the risk of developing hypogonadism, were also studied.

We assessed the predictive value of above-mentioned characteristics in relation to risk for post-treatment biochemical signs of androgen deficiency.

Patients and methods

Patients

All patients with TGCC, who had been diagnosed after March 1996 at the Department of Oncology, Lund University Hospital and were under the age of 50, were eligible for the study (15). Six time points were defined for hormonal analysis: after orchidectomy, but before further treatment (T0) and 6, 12, 24, 36 and 60 months (T6, T12, T24, T36 and T60) after completed therapy. Testosterone and LH were measured at each time point.

The patients were included from March 2001 until the end of the year 2005 and during this period, 200 men were eligible. Out of these, 28 denied to participate and 23 were excluded due to comorbidity. Of the remaining 149 men, 6 patients did not deliver any blood samples. From all other patients ($n=143$), one or more blood samples were collected (Figs 1 and 2).

All participated with written informed consent according to protocols approved by the ethical review boards of Lund University.

Cancer treatment

Treatment was given according to the Swedish Norwegian Testicular Cancer Group (Swenoteca) protocols (16, 17).

The surveillance group (SO) consisted of 13 men, 5 with seminomas and 8 with non-seminomas. These men did not receive any further therapy after orchidectomy. Patients with clinical stage I (CSI) non-seminomas (NSGCT) were offered either adjuvant chemotherapy (ACT) or surveillance. Patients with CSI seminoma (SGCT) received adjuvant radiotherapy (RT) or chemotherapy (ACT) or entered a surveillance programme. Patients with more advanced disease were treated with chemotherapy. Standard chemotherapy of NSGCT was the BEP regimen (Bleomycin 30 000 IU day 1.5 and 15; Etoposide 100 mg/m² day 1–5; Cisplatin 20 mg/m² day 1–5, given every third week). In six NSGCT patients given ACT, Etoposide was replaced with Vinblastin 0.15 mg/kg, max 11 mg/day, day 1–2 (CVB regimen). ACT for SGCT consisted of one to two cycles of Carboplatin, AUC 7.

ACT (BEP/CVB/Carboplatin) was given to 43 patients. Among these, 37 patients got one cycle and 6 got two cycles of chemotherapy. Forty-two patients with metastatic disease received three or four cycles of chemotherapy, defined as high dose CT (HCT). Among patients with NSGCT, 15 received three cycles and 19 received four cycles of BEP. Four cycles of EP (BEP minus Bleomycin) were given to eight patients with advanced SGCT. Nine patients (six NSGCT and three SGCT) received more intensive treatment (HDT).

Thirty-six patients with seminomas received adjuvant radiotherapy (RT). It was administered to para-aortic and ipsilateral iliac lymph nodes. A target dose of 25.2 Gy was given in 14 fractions. The total dose to the remaining testicle was estimated retrospectively to be 0.04–0.43 Gy in seven randomly selected men.

An overview of patient characteristics is presented in Table 1.

Figure 1 Flow chart describing the patient material: from six patients, we did not collect any samples; one did not come for follow-up, one moved to another region immediately after inclusion, three have not yet delivered their first sample after inclusion and one died of progressive disease.

Figure 2 Flow chart describing the number of serum testosterone samples analysed. The arrows connect samples from the same patients. Post-orchidectomy samples (T0); T6, T12, T24, T36 and T60 indicate 6, 12, 24, 36 and 60 months post-treatment. The figures differ slightly from those in Table 2 since nine patients with testosterone values have missing LH values and therefore cannot be grouped as hypogonadal.

Hormone analysis

Blood sampling was performed between 0800 and 1500 h. During the study period, methodological changes of the hormonal analyses were made. Two methods of assessing serum values of testosterone were applied. Since May 2001, a luminometric technique has been applied (Access; Beckman Coulter, Chaska, MN, USA) and previously a RIA, an ‘in-house’ method developed at the department of clinical chemistry in Malmö, was used. RIA values were converted to Access (Access value = 1.19 × RIA value = −1.07).

After 5 April 2000, LH in serum was determined with the Elecsys LH immunoassay (Roche Diagnostics). Before that date, LH was determined with an immunofluorometric assay (DELFLIA LH, Wallac Oy, Turku, Finland). A conversion between the methods was hence necessary to carry out (Elecsys LH immunoassay value = 0.953 ×

DELFLIA value = +0.602). Furthermore, five LH samples were analysed in another laboratory using an immunofluorometric assay (Immuno1, Bayer AG). The conversion factor between the two laboratories was calculated based on parallel measurements done on serum from 30 healthy fishermen under the age of 50 (Elecsys LH immunoassay value = 1.79 × Immuno1 value = +0.05).

In a pooled dataset, after conversion of the values, no difference in hormone levels was found when samples analysed by different methods were compared with each other.

AR gene polymorphism analysis

Blood samples for DNA analysis were available from 83 men. These subjects did not differ from the remaining 66 regarding age, disease, stage, histological type, treatment

Table 1 Characteristics of 143 patients with testicular germ-cell cancer, who have delivered at least one blood sample for hormonal analysis.

	No therapy after orchidectomy	ACT	HCT	HDT	RT	Total
No. of patients	13	43	42	9	36	143
Age (median) ^a	29	29	27	29	35.5	30
Seminoma	5	2	8	3	36	54
Non-seminoma	8	41	34	6	0	89
Stage I	13	43	9 ^b	2 ^c	36	103
Stage II	0	0	21	2	0	23
Stage III	0	0	4	2	0	6
Stage IV	0	0	8	3	0	11

Royal Marsden Hospital (RMH) staging system has been used (30). ACT, one to two cycles of adjuvant chemotherapy; HCT, three to four cycles of chemotherapy; HDT, more intense treatment, more than four cycles of chemotherapy; RT, adjuvant radiotherapy.

^aAt the time of diagnosis.

^bRecurrent disease including four patients with marker elevation.

^cRecurrent disease.

or sperm concentration at T0 (data not shown). Genomic DNA was prepared from peripheral leukocytes, and the CAG (83 subjects) and GGN repeats of the AR gene (81 subjects) were amplified by PCR and subsequently analysed externally on a Beckman Coulter CEQ 2000XL (Beckman Coulter) sequencing gear, as previously described (18).

Ultrasound examination

Of the 143 patients, 107 subjects underwent an ultrasound of the remaining testicle between 6 and 12 months after inclusion, performed by the same radiologist. The outcome was presence or absence of microlithiasis, defined as at least five uniform, non-shadowing echogenic foci of 1–3 mm, scattered throughout the testicular parenchyma (19, 20) (Fig. 7). For determination of testicular volume, we used the formula for the volume of ellipsoid: $1/3 \times \pi \times \text{length} \times \text{wide} \times \text{thickness} \times 1/2$ (21). The consistency was investigated by palpation and assessed as being normal or soft.

Statistical analysis

Statistical analyses were performed using SPSS 11.0 software (Chicago, IL, USA).

Regarding hypogonadism at T0, patients being hCG positive at this time point were excluded from the statistical analysis. Seven patients receiving testosterone replacement were considered hypogonadal from the time the replacement was given. Two of them were in the ACT group, two in the HCT group and two in the RT group. The last one was in the group receiving more intensive treatment (more than four cycles of chemotherapy).

Biochemical hypogonadism (BH) was defined as s-testosterone < 10 nmol/l and/or s-LH $\geq$ 10 IU/l (22).

Since the blood samples were taken between 0900 and 1500 h and the levels of testosterone but not LH decrease during the day, for all statistically significant findings the possible associations were re-tested with LH > 10 IU/l as the only indicator of BH.

Primarily, using binary logistic regression analysis, the odds ratio (OR) for BH was calculated for TGCC patients using the ACT group as a reference group. A separate comparison was done for each treatment modality (HCT/RT) and post-treatment time point. Furthermore, the risk for the whole group of TGCC men for developing BH was tested at different post-treatment time points, with respect to the following potential predictive factors: BH at T0 (yes/no), AR CAG (<21, 21–22, >22) and GGN (<23, 23, >23) repeat number, stage of disease (I–IV), testicular consistency (normal/soft), ultrasound (+/– microlithiasis), volume of the contralateral testis (<15 vs $\geq$ 15 ml) and age (<30 vs $\geq$ 30 years). Patients presenting with microlithiasis were complementary analysed after excluding those who were hypogonadal at T0. The method of hormone analysis was used as potential confounder.

All analyses were performed separately for each time point.

$P < 0.05$ was considered as statistically significant.

Results

Absolute hormone levels

The absolute testosterone and LH values with medians and ranges are measured for all different therapy arms and at all the different follow-up time points (Tables 3 and 4).

BH in relation to treatment

For the HCT group, a significantly increased OR for hypogonadism, when compared with the ACT group, was found after 6 (OR: 22, 95% confidence interval (CI) 4.4–118) and 12 (OR 5.8, 95% CI 1.5–22) months post-therapy. Also in the RT group, a significantly increased OR was observed when compared with the ACT group after 6 (OR 10, 95% CI 2.1–47) and after 12 (OR 3.9, 95% CI 1.1–14) months post-therapy. The number of patients from both surveillance and the HDT group were too few to analyse (Figs 3 and 4).

Post-treatment BH in relation to BH at T0

BH at T0 strongly predicted BH at T6 (OR 53, 95% CI 19–145), T12 (OR 125, 95% CI 37–430), T24 (OR 88, 95% CI 26–300) and T36 (OR 121, 95% CI 32–460). At T0, 22 men were hypogonadal. After 1 year, 12 out of 20 and after 2 years 6 out of 11 had normal hormone levels of those 22 initially being hypogonadal (Table 2 and Fig. 5).

BH in relation to stage, age and AR polymorphisms

No statistical significance was observed for stage of disease or age. AR polymorphisms of CAG and GGN repeat did not have any effect on the risk of developing BH.

BH in relation to testicular characteristics

Pathological ultrasound with presence of microlithiasis was predictive for BH at these time points: T0 (OR 11, 95% CI 1.2–112), T12 (OR 3.9, 95% CI 1.1–13), T24 (OR 3.0, 95% CI 1.0–8.8), T36 (OR 5.4, 95% CI 1.7–17) and T60 (OR 4.4, 95% CI 1.2–16; Fig. 6). When excluding patients being hypogonadal at T0 a similar trend was observed, reaching statistical significance at T36 (OR 5.2, 95% CI 1.5–19) and T60 (OR 4.7, 95% CI 1.0–21). Testicular volume or consistency of the contralateral testicle was not associated with any increased risk for BH.

For all above-mentioned statistically significant associations, the same trends were found when LH > 10 IU/l was used as the only indicator of BH (Fig. 7).

Figure 3 Therapy-dependent odds ratios of developing biological hypogonadism with the group given adjuvant chemotherapy as reference in patients treated with three to four cycles of chemotherapy. Post-orchidectomy samples (T0); T6, T12, T24, T36 and T60 indicate 6, 12, 24, 36 and 60 months post-treatment.

Discussion

We found microlithiasis in the remaining testis as significant risk factors for BH, defined as low total testosterone and/or high LH levels in serum. In addition, BH prior to chemo- or radiotherapy and treatment modality seems to play a role in this context, higher doses of chemotherapy as well as adjuvant radiation being the most harmful to Leydig cells.

The issue of testosterone levels in men treated for TGCC was previously addressed by others (23–25). However, these studies did not clearly discriminate between treatment modalities and/or the post-treatment investigations were not performed at specific time points, allowing for more precise mapping of the process of Leydig cell recovery (Tables 3 and 4).

A significant increase in the OR for BH was observed in patients receiving three to four cycles of chemotherapy 6 and 12 months post-treatment. The same observation was made in the RT group. The choice of reference group was made on the assumption that one to two cycles of BEP only

has limited impact on Leydig cell function. Another option had been to use the T0 group or surveillance patients. However, with respect to T0, possible post-orchidectomy compensatory improvements of Leydig cell function and also possible temporary dysfunction after orchidectomy could not be taken into consideration. The number of surveillance patients was not sufficient to be used as reference and if men without TGCC were used as controls, the impact of therapy could not be discriminated from the negative effect of TGCC *per se* on Leydig cell function.

We have shown that Leydig cell function may occur over time, since no difference between the therapy groups was observed at T24 or later. This phenomenon is similar to that recently reported for sperm production, with a significant decline in sperm concentration after 6–12 months and after subsequent recovery to the pre-treatment level (15). The time-related post-treatment recovery of Leydig cell function is in discordance with the data published by Nord *et al.* who reported an increased risk of hypogonadism 10 years after completion of TGCC treatment (11). However, the longer observation time and

Figure 4 Therapy-dependent odds ratios of developing biological hypogonadism with the group given adjuvant chemotherapy as reference in patients treated with radiotherapy. Post-orchidectomy samples (T0); T6, T12, T24, T36 and T60 indicate 6, 12, 24, 36 and 60 months post-treatment.

Table 2 Characteristics of the 143 testicular germ-cell cancer (TGCC) patients included in the study, with and without biochemical signs of hypogonadism.

	No. hypogonadal/all (% hypogonadal)					
	T0	T6	T12	T24	T36	T60
Age, years						
< 30	9/32 (28%)	12/27 (44%)	13/32 (41%)	14/39 (36%)	14/33 (42%)	10/38 (26%)
≥ 30	21/44 (48%)	22/44 (50%)	22/42 (52%)	15/32 (47%)	13/31 (42%)	6/24 (25%)
Stage						
I	24/56 (43%)	21/54 (39%)	26/58 (45%)	19/54 (35%)	23/47 (49%)	12/39 (31%)
II	4/11 (36%)	6/8 (75%)	5/9 (56%)	6/10 (60%)	3/12 (25%)	3/11 (27%)
III	1/2 (50%)	2/3 (67%)	1/2 (50%)	0/1 (0%)	0/1 (0%)	0/3 (0%)
IV	1/7 (14%)	5/6 (83%)	3/5 (60%)	4/6 (67%)	1/4 (25%)	1/3 (33%)
Hypogonadal at T0						
Yes	22/22 (100%)	9/21 (43%)	12/20 (60%)	6/11 (55%)	3/7 (43%)	0/0
No	0/36 (0%)	8/29 (28%)	6/26 (23%)	10/26 (38%)	9/15 (60%)	2/3 (67%)
No. of CAG repeats						
< 21	4/11 (36%)	3/7 (43%)	7/12 (58%)	7/16 (44%)	11/20 (55%)	5/19 (26%)
21–22	3/12 (25%)	4/11 (36%)	4/12 (33%)	11/18 (61%)	9/18 (50%)	5/10 (50%)
> 22	6/15 (40%)	5/13 (38%)	6/15 (40%)	4/19 (21%)	4/16 (25%)	4/14 (29%)
No. of GGN repeats						
< 23	1/4 (25%)	1/2 (50%)	0/3 (0%)	0/5 (0%)	2/6 (33%)	1/6 (17%)
= 23	10/25 (40%)	10/23 (43%)	15/26 (58%)	15/31 (48%)	15/30 (50%)	7/16 (44%)
≥ 23	2/9 (22%)	1/6 (17%)	2/10 (20%)	7/17 (41%)	7/18 (39%)	6/19 (32%)
Microlithiasis						
Yes	14/32 (44%)	12/29 (41%)	17/28 (61%)	19/32 (59%)	19/29 (66%)	11/23 (48%)
No	3/20 (15%)	9/18 (50%)	8/24 (33%)	9/26 (35%)	7/27 (26%)	5/29 (17%)
Testicular consistence						
Soft	3/4 (75%)	2/3 (67%)	3/5 (60%)	2/5 (40%)	1/4 (25%)	1/3 (33%)
Normal	26/71 (37%)	31/67 (46%)	31/68 (46%)	27/66 (41%)	26/59 (44%)	15/52 (29%)
Volume						
< 15 ml	12/31 (39%)	14/28 (50%)	16/32 (50%)	18/33 (55%)	13/33 (39%)	9/25 (36%)
≥ 15 ml	3/18 (17%)	5/16 (31%)	7/18 (39%)	8/24 (33%)	13/22 (59%)	7/25 (28%)
Treatment						
Surveillance	1/3 (33%)	5/7 (71%)	3/5 (60%)	1/4 (25%)	2/4 (50%)	3/4 (75%)
ACT	9/27 (33%)	3/24 (12%)	6/25 (24%)	8/27 (30%)	9/23 (39%)	3/14 (21%)
HCT	7/21 (33%)	13/18 (72%)	12/18 (67%)	9/18 (50%)	7/19 (37%)	4/20 (20%)
RT	11/20 (55%)	10/18 (56%)	12/22 (55%)	7/17 (41%)	8/14 (57%)	5/17 (29%)
HDT	2/5 (40%)	3/4 (75%)	2/4 (50%)	4/5 (80%)	1/4 (25%)	1/1 (100%)

ACT, one to two cycles of chemotherapy; HCT, three to four cycles of chemotherapy; RT, radiotherapy; HDT, more than four cycles of chemotherapy; RMH, Royal Marsden Hospital staging system has been used (30).

therefore an age-dependent deterioration cannot be excluded in the latter study.

Although stage of disease and treatment are closely related, we did not find any association between

disease stage and the risk of BH. However, stage I patients can either be treated by ACT or by RT that are modalities apparently differing in their impact on Leydig cell function.

Figure 5 Odds ratios for biological hypogonadism for TGCC patients in relation to biological hypogonadism at T0. Post-orchidectomy samples (T0); T6, T12, T24 and T36 indicate 6, 12, 24 and 36 months post-treatment. After 60 months, statistical analysis was not done due to insufficient number of subjects.

Figure 6 Odds ratios for biological hypogonadism in relation to microlithiasis of the remaining testicle. Post-orchidectomy samples (T0); T6, T12, T24, T36 and T60 indicate 6, 12, 24, 36 and 60 months post-treatment.

Scrotal ultrasound is routinely used in the diagnosis of TGCC. Ultrasonically detected microlithiasis in the contralateral testis was associated with post-treatment risk of BH with an OR of 3–11. Microlithiasis has earlier been reported in patients with testicular dysgenesis syndrome (TDS) (26) and is believed to be associated with confirmed testicular cancer (27). Since Leydig cell dysfunction is considered as a part of the TDS, our results could support this observation. An increased risk of post-treatment hypogonadism in men with microlithiasis could be due to significant risk of androgen deficiency prior to chemo- or radiotherapy, which is also found to be a predictor of BH at T6 and at subsequent time points. However, microlithiasis remained a predictor of BH after

exclusion of the subgroup of men who were hypogonadal at T0. To our knowledge, there are no studies indicating that cancer therapy can induce microlithiasis and therefore we assume that the pathological ultrasonic pattern was also present prior to treatment.

Sensitivity to androgens is genetically determined by the polymorphisms in the AR gene. Lower androgen sensitivity, with higher LH levels, was found in men with long CAG tracts (28). It could be anticipated that the risk of hypogonadism is modified by the length of this repeat. However, we found no association between any of the AR polymorphisms and biochemically defined hypogonadism. Similarly, no significant associations were found for testicular volume or consistency.

Figure 7 Ultrasonographic picture of microlithiasis in a testicle. The white spots represent microcalcifications.

Table 3 Total testosterone levels in relation to treatment given and time at follow-up.

	Median – testosterone – nmol/l (n) range					
	T0	T6	T12	T24	T36	T60
Surveillance	17 (3) 11–22	9.8 (7) 6.4–15	10 (5) 6.8–14	11 (4) 9.6–14	9.8 (4) 8.4–16	9.5 (4) 6.7–22
ACT	14 (26) 6.7–23	14 (24) 10–22	14 (25) 7.7–21	14 (27) 9.1–24	13 (24) 8.2–22	12 (14) 6.3–20
HCT	15 (20) 4.0–29	13 (18) 1.5–22	10 (17) 5.4–21	13 (18) 4.4–23	14 (19) 4.8–33	14 (20) 7.9–21
RT	12 (18) 7.5–20	12 (19) 8.1–21	12 (23) 6.9–24	12 (17) 7.1–18	11 (14) 1.3–23	13 (17) 3.0–18
HDT	13 (4) 12–18	15 (4) 9.1–33	11 (5) 8.2–43	10 (5) 6.8–12	10 (4) 10–13	7.8 (1) 7.8
All patients	14 (71) 4.0–29	12 (72) 1.5–33	12 (75) 5.4–43	12 (71) 4.4–24	12 (65) 1.3–33	13 (56) 3–22

ACT, one to two cycles of adjuvant chemotherapy; HCT, three to four cycles of chemotherapy; HDT, more intense treatment. More than four cycles of chemotherapy; RT, adjuvant radiotherapy; n, amount of patients.

The signs of hypogonadism are non-characteristic and good biochemical markers of androgen deficiency are lacking. The clinical diagnosis is usually based on the combination of symptoms and serum levels of testosterone and LH. In this study, we have only used biochemical parameters in defining hypogonadism. The levels of 10 nmol/l for total testosterone and 10 IU/l for LH are generally accepted as useful markers of hypogonadism in younger males (22). A shortcoming in current study is the change in the methods for testosterone and LH measurements. However, conversion of the results from the method used in the first part of the study to the one applied during the second part was based on more than 30 subjects tested with both methods. Furthermore, no statistically significant difference between measurements performed with the different methods was found at any time point. Finally, the type of method applied for hormone measurement was included as confounding factor in the statistical analysis. We therefore believe that our results are reliable despite the methodological drawback.

Blood samples for hormone analysis were collected between 0900 and 1500 h. Due to the diurnal variation

in testosterone concentration, sampling should ideally be performed before 1000 h. However, the time of blood sampling was randomly distributed among the patients and not related to any of the possible predictors of hypogonadism tested. Furthermore, for all statistically significant associations, same trends were found when LH > 10 IU/l was used as the only indicator of hypogonadism. Unlike testosterone, LH does not decrease during the daytime.

Although all subjects with increased post-orchidectomy hCG were excluded from the T0 analysis, we did not have access to the information regarding increased pre-orchidectomy hCG normalizing following removal of the tumour-bearing testis. Due to the length of the time period between surgery and initiation of adjuvant treatment (at least 6 weeks), we do not expect that testosterone and LH levels were influenced in these men by previous hCG elevation. However, any reminiscent hCG effect would tend to change to classification of a subject at T0 from hypo- to normogonadal and, therefore, rather reduce the power of hypogonadism at T0 as predictor of post-treatment androgen deficiency.

Table 4 Total luteinizing hormone (LH) levels in relation to treatment given and time at follow-up.

	Median – LH values – IU/l (n) range					
	T0	T6	T12	T24	T36	T60
Surveillance	5.9 (3) 5.5–11	7.2 (7) 4.0–18	5.5 (5) 4.0–14	5.2 (4) 2.8–7.8	5.3 (4) 4.0–6.0	5.0 (4) 3.0–9.0
ACT	5.6 (25) 1.4–15	6.7 (25) 2.2–19	6.6 (24) 2.4–25	5.8 (27) 2.0–15	6.6 (23) 3.0–17	5.8 (14) 3.0–11
HCT	3.8 (20) 0.1–67	12 (18) 5.4–33	11 (19) 2.5–24	7.8 (18) 0.3–23	6.3 (19) 0–22	5.0 (20) 2.0–12
RT	5.2 (18) 1.6–21	8.4 (18) 0.2–30	6.6 (22) 2.5–30	5.3 (17) 3.4–31	5.4 (14) 0–12	5.2 (17) 0–16
HDT	6.0 (4) 0.2–12	13 (4) 7.0–23	12 (4) 7.2–27	10 (5) 1.9–21	7.6 (4) 6.0–14	13 (1) 13
All patients	5.4 (70) 0.1–67	8.2 (72) 0.2–33	7.1 (74) 2.4–30	6.4 (71) 0.3–31	6.0 (64) 0–22	5.2 (56) 0–16

ACT, one to two cycles of adjuvant chemotherapy; HCT, three to four cycles of chemotherapy; HDT, more intense treatment. More than four cycles of chemotherapy; RT, adjuvant radiotherapy; n, amount of patients.

Finally, it should be kept in mind that for some of the analyses the number of subjects included was relatively low and lack of statistical significance due to a type 2 error cannot be excluded.

In summary, men presenting with TGCC have an increased risk of androgen deficiency (29) but the risk of developing hypogonadism has been more or less overseen in this category of patients. Measuring of testosterone and LH concentration is not a general clinical routine during the follow-up of TGCC men. The identification of risk factors for development of BH is, therefore, important for proper management of these patients. Current study shows that BH prior to oncological treatment and in those presenting with microlithiasis by ultrasound are strong predictors of subsequent hypogonadism. Three to four cycles of chemotherapy and adjuvant radiotherapy also implies an increased risk of being hypogonadal, at least during the first post-treatment year. This should be taken into consideration in the pre-treatment investigation and follow-up of men treated for TGCC.

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