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The pituitary-Leydig cell axis before and after orchiectomy in patients with stage I testicular cancer

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ABSTRACT

Introduction: This study investigates the pituitary-Leydig cell axis in patients with stage I testicular germ cell cancer (TGCC) followed with surveillance only, in order to evaluate the risk of Leydig cell dysfunction one year after orchiectomy.

Patients and methods: A retrospective evaluation of reproductive hormones in patients with unilateral stage I TGCC ($N = 72$) without relapse diagnosed between 1990 and 2008. A group of healthy males ($N = 706$) served as controls.

Results: Before orchiectomy there were no significant differences in luteinizing hormone (LH) and testosterone (T) levels between human chorionic gonadotropin (hCG)-negative patients and controls, although 33% of the patients were outside the 97.5 percentile when using bivariate LH/T evaluation. At 1-year follow-up there was a significant increase in LH ($\Delta LH = 2.04$ IU/L, $p < 0.001$), and 57% of the patients had an LH/T relation outside the 97.5 percentile.

Conclusion: Patients with stage I TGCC are at increased risk of having an LH/T relation outside the normal range one year after orchiectomy, suggesting insufficient Leydig-cell function. Whether a proportion of these patients will develop manifest hypogonadism and benefit from androgen therapy is yet to be clarified.

1. Introduction

Around 50% of TGCC patients will be cured by orchiectomy alone without further treatment, and the other half will need either chemotherapy or radiotherapy due to disseminated disease.[1] TGCC is highly sensitive to both treatment modalities and most patients will be long-term survivors. This fact has led to an increased focus on long-term complications of TGCC treatment. Several studies have investigated long-term complications of different regimens of chemotherapy and radiotherapy and shown that these treatments cause an increased risk of secondary malignancy,[2] cardiovascular disease[3–5] and changes in fertility and reproductive hormones.[6–11] However, side-effects related to the removal of one testicle without further therapy, has not been critically examined. Removal of one testicle due to TGCC leads to a considerable decrease in the number of the T producing Leydig cells and whether or not the remaining Leydig cells are able to maintain serum T within its normal range on the long term has not been thoroughly investigated. If the Leydig cells fail to compensate for the reduced amount of cells, despite an increased LH drive, manifest primary hypogonadism may evolve and associated conditions like obesity,[12] decreased bone mineral density,[13,14] decreased muscle mass,[15] dyslipidemia[16] and psychological symptoms can develop.[17,18] A recent study has shown that TGCC patients treated with orchiectomy and radiotherapy to the contralateral testicle due to carcinoma in situ were at particularly high risk of developing primary hypogonadism.[19]

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Thus, approximately 60% of patients receiving 20 Gray to the testicle with carcinoma in situ needed androgen substitution in the years following treatment. The majority of patients developed hypogonadism within the first years following treatment, but in some patients, hypogonadism became evident even 5–10 years after therapy.

The aim of the present study was to investigate changes of the pituitary-Leydig cell axis in patients with unilateral TGCC treated with orchiectomy alone in order to detect whether these patients have an increased risk of primary hypogonadism. It has previously been suggested that joint evaluation of LH and T by the use of bivariate charts is a more sensitive expression of the pituitary-Leydig cell axis than evaluation of either of the hormones alone[6,20] and, therefore, this method was used in the present study. Elevated levels of hCG can be observed in both stage I seminomas and stage I non-seminomas before orchiectomy.[21] This can have a significant influence on hormonal levels and for this reason we divided the patients into hCG-positive and hCG-negative groups.

2. Patients and methods

2.1. Patients

Data were extracted from The Testicular Cancer Database at Rigshospitalet, where all subjects with unilateral TGCC stage I treated with orchiectomy and surveillance and diagnosed between 1990 and 2008 were eligible ($N = 436$). In order to compare hormone analyses between patients, we chose to evaluate all patients who had at least one hormone measurement before orchiectomy and one measurement between 6 and 18 months after orchiectomy. If a subject had more than one hormone measurement taken in this time interval, the sample closest to 12 months after orchiectomy was chosen for statistical analysis.

Patients were excluded if any of the following was present: no available hormone measurements before orchiectomy ($N = 245$), no hormone measurements between 6 and 18 months after orchiectomy ($N = 108$), unknown histology, unknown level of hCG at baseline or loss of follow-up ($N = 11$). Thus, 72 patients were included in the study.

2.2. Controls

A cohort of 706 healthy males served as controls in this study and the reference-curves for LH, T and estradiol (E2) as well as the bivariate reference curve for T in correspondence with LH were constructed on the base of this cohort (Figs. 1–3 and 5). In order to obtain age-match between controls and patients, 547 subjects (median age 29 years, range 20–50) were chosen from the cohort for comparison of reproductive hormones at baseline (Table 1). The controls were chosen randomly without prior knowledge of fertility and body mass index (BMI) as described by Aksglaede et al.[20]

2.3. Hormone analyses

Blood samples for hormone analyses were drawn between 8 AM and 12 PM. LH and follicle stimulating hormone (FSH) were measured by time-resolved immunoflourometric assay (DELFIA; Wallac, Inc., Turku, Finland) with detection limits of 0.05 and 0.06 IU/L, respectively. Intra- and inter-assay coefficients of variation (CV) were both below 5% in the LH and FSH assays. Sex hormone binding globulin (SHBG) was measured using time-resolved flouroimmunoassay (DELFIA, Wallac) with detection limits of 0.23 nmol/L, intra- and inter-assay CVs were less than 5.1% for SHBG. Inhibin B was determined using a specific two-sided enzyme immunometric assay from Oxford Bio-Innovation Ltd. (Oxford, UK). The sensitivity of the inhibin B assay was 20 pg/ml, and the intra and interassay CVs were less than 12% and less than 17%, respectively. E2 was measured by RIA (Pantex, Santa Monica, CA (before 1998 distributed by Immuno Diagnostic Systems, Boldon, UK)). The detection limit was 18 pmol/litre, the intra- and inter-assay CVs were less than 8% and 13%, respectively. HCG-β was determined by immunoflourometric assay calibrated against the international standard (WHO75/551) (B.R.A.H.M.S. Kryptor).

2.4. Statistical analyses

Comparison of medians between controls and patients at baseline was performed using Man–Whitney *U*-Test, while paired *t*-test was used for comparing means between baseline and follow-up, except for LH where Wilcoxon Log Rank test was used. *p*-values <0.05 were considered significant.

The reference curves for T, LH and E2 versus age were constructed based on the 706 controls and made by local linear regression smoothing, and similarly the 2.5 and 97.5 percentiles were obtained from smooth variance estimates as described by Aksglaede et al.[20] Bivariate reference charts of T in conjunction with the corresponding LH were constructed based on the 706 controls as described by Aksglaede et al.[20] One can read the 2.5 and 97.5 percentiles of LH and T as the horizontal and vertical lines, respectively. The PC based package of SPSS 17.0 was used for the statistical analyses.

3. Results

Median follow-up time after orchiectomy was 11.2 months, range (6.1–17.5 months). Baseline characteristics of the patients are presented in Table 1.

3.1. LH

At baseline LH was significantly decreased in hCG-positive patients as compared with healthy controls, while there were no significant differences between hCG-negative patients and controls (Table 1). At baseline nine of the hCG-positive patients (82%) had LH levels below the 2.5 percentile, whereas one patient (9%) had LH >97.5 percentile. At follow-up LH had normalised in all patients but four (three with LH levels above the 97.5 percentile). At baseline most hCG-negative patients had normal LH levels (three had LH levels >97.5 percentile (5%) and two (3%) <2.5 percentile). At follow-up, 16 had LH above the 97.5 percentile (28%) and none of the patients were below the 2.5 percentile (Fig. 1). As shown in Fig. 4 there was a highly significant increase in LH between baseline and follow-up in both groups of patients.

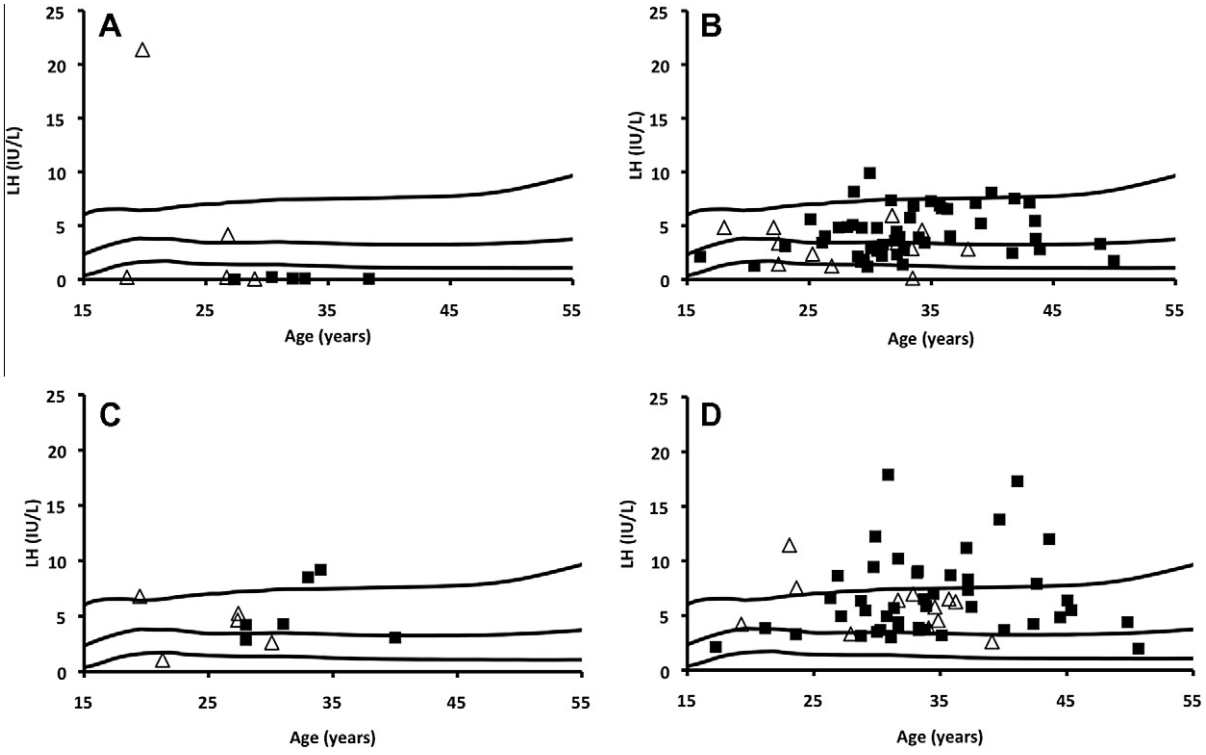

Fig. 1 – Serum concentration of LH before (A and B) and after (C and D) orchiectomy in unilateral stage I TGCC patients according to age and type of cancer (■: seminomas, Δ: non-seminomas) with (A and C) or without (B and D) hCG raised at baseline. Lines represent 2.5, 50 and 97.5 percentiles.

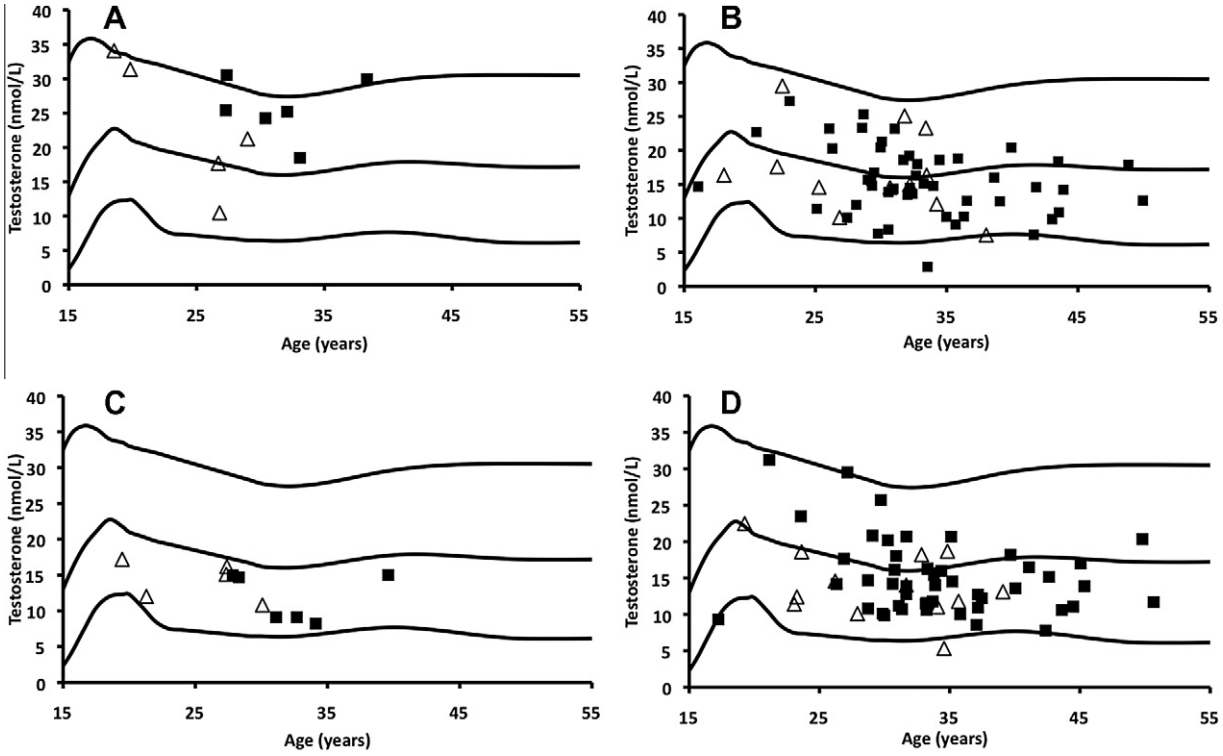

Fig. 2 – Serum concentration of T before (A and B) and after (C and D) orchiectomy in unilateral stage I TGCC patients according to age and type of cancer (■: seminomas, Δ: non-seminomas) with (A and C) or without (B and D) hCG raised at baseline. Lines represent 2.5, 50 and 97.5 percentiles.

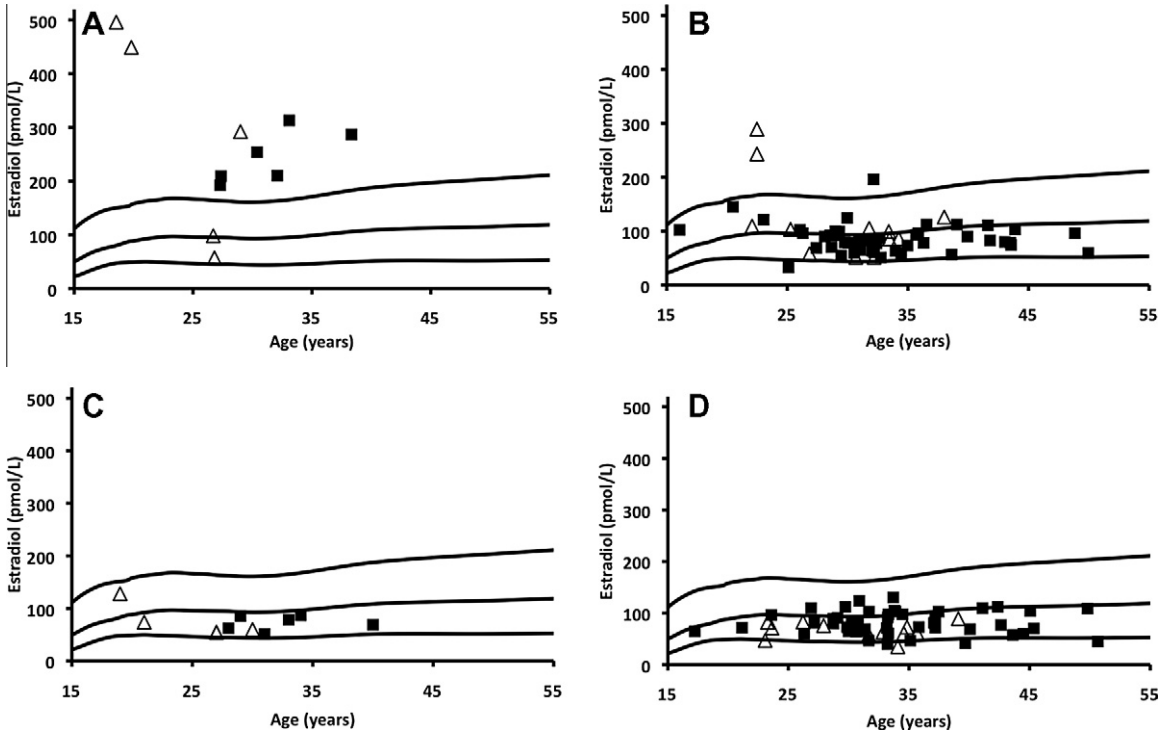

Fig. 3 – Serum concentration of (E2) before (A and B) and after (C and D) orchiectomy in unilateral stage I TGCC patients according to age and type of cancer (■: seminomas, Δ: non-seminomas) with (A and C) or without (B and D) hCG raised at baseline. Lines represent 2.5, 50 and 97.5 percentiles.

3.2. *Testosterone*

At baseline T was significantly raised in hCG-positive patients, while there was no significant difference between hCG-negative patients and controls (Table 1). At baseline, three of the hCG-positive patients (25%) had T levels >97.5 percentile and none of the patients were below the 2.5 percentile. All hCG-negative patients had T levels within the normal range at baseline as well as at follow-up, except one patient with a subnormal T (Fig. 2). As shown in Fig. 4 there was a highly significant decline in T between baseline and follow-up for hCG-positive patients, while there were no significant changes in T for hCG-negative patients.

3.3. *LH/T*

The bivariate LH/T reference charts demonstrated that 19 of the hCG-negative patients (33%) were outside the 97.5 percentile at baseline, while 33 of the patients (57%) were outside the 97.5 percentile at follow-up (Fig. 5).

3.4. *Estradiol*

At baseline the serum concentration of E2 was significantly elevated in hCG-positive patients, while it was significantly lowered in hCG-negative patients compared to controls (Table 1). Ten of the hCG-positive patients (83%) had E2 levels >97.5 percentile at baseline, while at follow-up none of these patients were outside the limits of normality. Most hCG-negative patients had E2 levels within the reference ranges at baseline and at follow-up (Fig. 3). As shown in Fig. 4 there was a highly significant decline in serum E2 in hCG-positive patients between baseline and follow-up, while there was an apparent but non-significant decline ($p = 0.07$) in the hCG-negative patients.

3.5. *Other reproductive hormones*

At baseline FSH was significantly lower in hCG-positive patients, while it was significantly elevated in hCG-negative patients when compared to controls. Inhibin B was significantly lower in both groups of patients when compared to controls. T/E2 index was significantly lower in hCG-positive patients. Free androgen index (FAI) was significantly lower in hCG-negative patients, while it was significantly higher in hCG-positive patients when compared to controls (Table 1).

4. Discussion

In this single-centre study of 72 stage I TGCC patients we found significant changes of the pituitary-Leydig cell axis one year following unilateral orchiectomy and we, therefore, suggest that some of these patients may carry an increased risk of developing primary hypogonadism.

Few studies have investigated Leydig cell function before orchiectomy in TGCC patients.[10,22] In the present study of 61 hCG-negative TGCC patients, T and LH levels were similar when compared to 547 age-matched controls. This contrasts previous findings on a smaller group of patients from our group.[10] In that study LH was significantly lower at

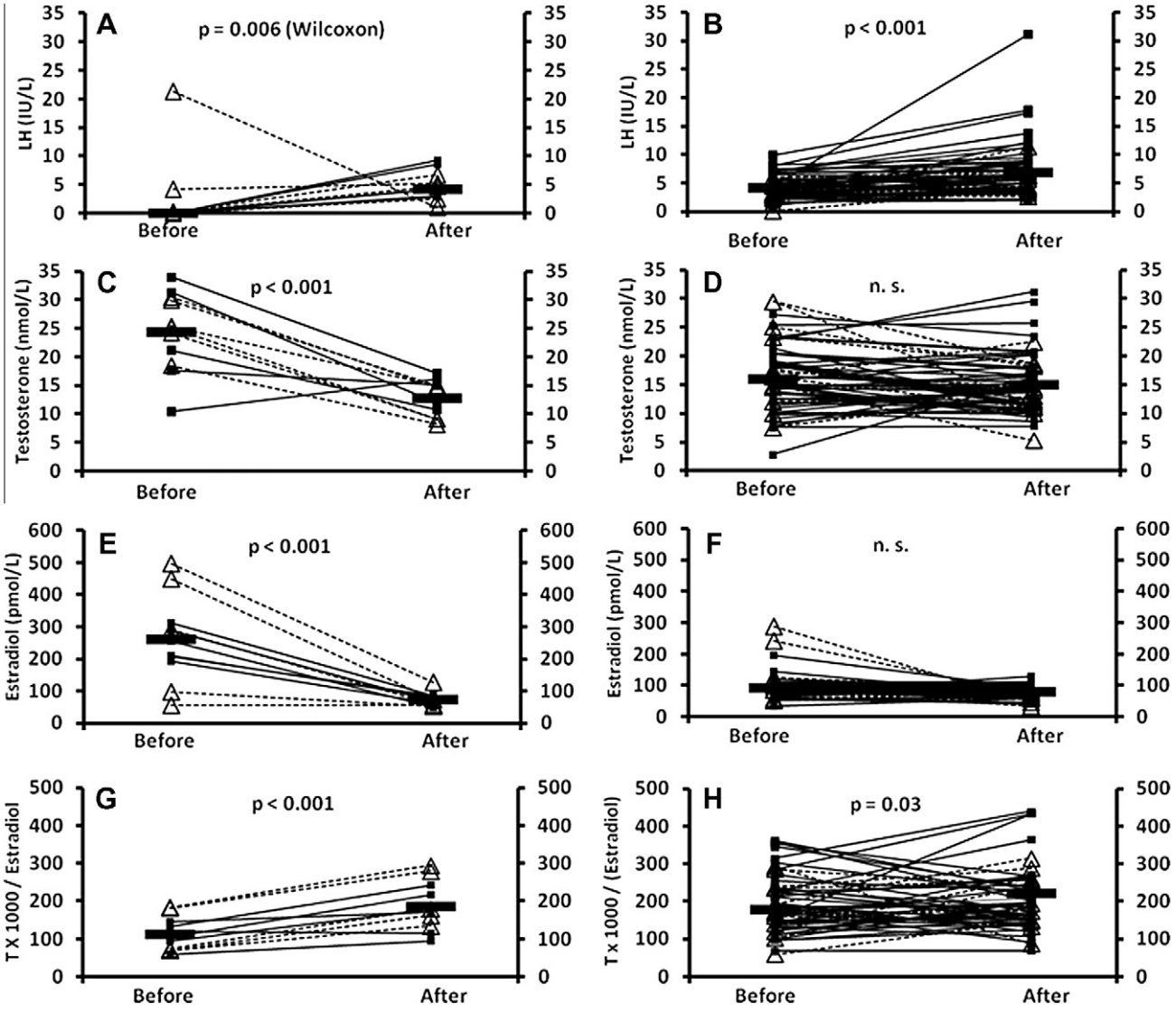

Fig. 4 – Serum concentration of LH (A and B), T (C and D), E2 (E and F) and T/E index (G and H) before and after orchiectomy in patients with (A,C,E,G) and without (B,D,F,H) hCG raised at baseline. Bars represent means, except in (A) where bars represent medians.

diagnosis in 30 TGCC patients when compared to 193 controls. The median LH in patients was 3.6 IU/L and 3.73 IU/L in the two studies, respectively, whereas median LH of the control populations differed markedly (4.7 and 3.36 IU/L, respectively). Thus, the apparently conflicting results may be explained by differences in control groups. Our present control population is larger, and median LH levels are similar to that found by others (median LH 3.3 IU/L).[8] However, when using bivariate LH/T charts it became evident that more patients than expected (33%) were outside the 97.5 percentile before orchiectomy suggesting subtle disturbances of the pituitary-Leydig cell axis already present at the time of diagnosis.

While no significant difference in serum T levels between hCG-negative patients and controls was found, E2 was significantly lower in patients as compared with controls. We have no obvious explanation for the slightly lower E2 levels despite similar T levels in these patients.

Patients with elevated hCG levels before orchiectomy presented with a markedly disturbed pituitary-Leydig cell axis at baseline with suppressed LH, elevated T and E2 as compared to controls. In one patient, however, LH was markedly elevated. This may be due to analytical cross-reaction between LH and hCG because their biochemical structures are very similar with common alpha subunits and beta subunits showing about 80% homology.[23] The elevated hCG stimulates the LH/hCG receptors on the Leydig cells resulting in elevated T.[24] Furthermore, hCG stimulates aromatase activity in the testes and periphery resulting in high E2 levels and a relatively higher E2 than T leading to a significantly lower T/E2 ratio which is in concordance with the findings of Petersen et al.[10]

The obvious differences in LH, T, E2 and T/E2 ratio between patients with hCG producing germ cell tumours and those with germ cell tumours without hCG production clearly demonstrate that it is mandatory to evaluate hCG-levels in testicular cancer patients when evaluating reproductive hormones.

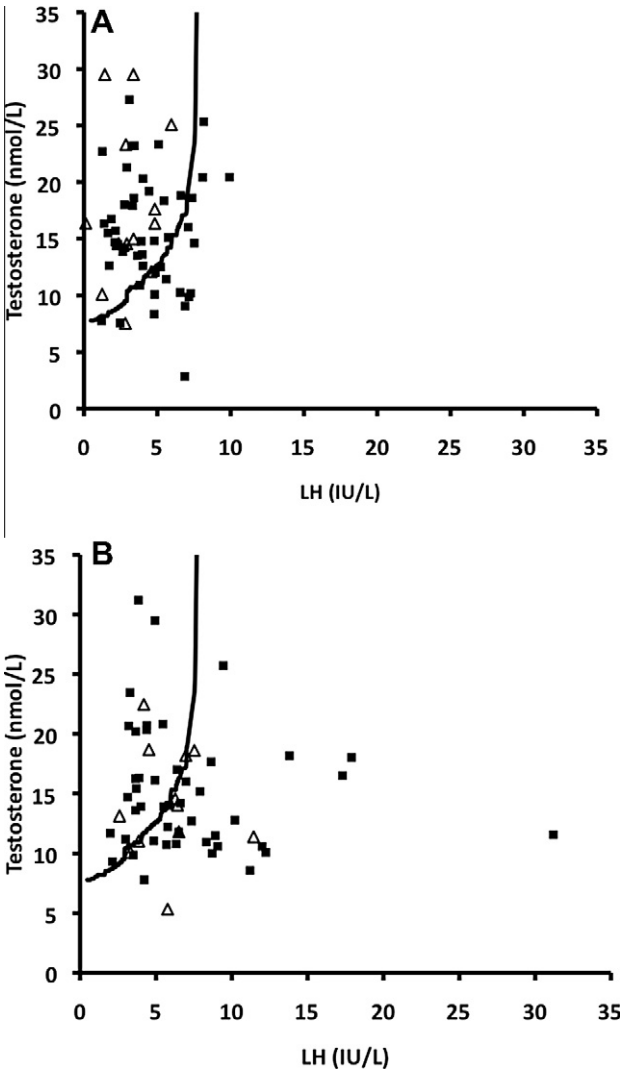

Fig. 5 – Serum T plotted against LH in two-dimensional reference charts before (A) and after (B) orchiectomy in hCG negative TGCC patients. Lines represent 97.5 percentile. (■: seminomas, Δ: non-seminomas)

In the 61 hCG-negative patients our finding of a highly significant increase in LH between baseline and 1-year follow-up as well as 28% of the patients being above the 97.5 percentile is in concordance with the findings in previous studies.[7,8,11] Some studies find a higher risk of decreased serum T in long term survivors of TGCC treated with orchiectomy only,[7,11,25] though in the present study there was no significant decline in T in hCG-negative patients, and none of the patients received androgen substitution one year after orchiectomy. However, 57% of the patients without hCG elevation were outside the 97.5 percentile at 1-year follow-up using bivariate LH/T evaluation suggesting Leydig cell insufficiency despite the fact that none of the patients had received radiotherapy or cytotoxic therapy.

It should be kept in mind that the secretion of LH from the pituitary gland is pulsatile and that T secretion shows a diurnal rhythm with values being highest in the morning.[26] Accordingly, the detection of an LH/T relation outside the 97.5 percentile should always be confirmed by a second measurement before considering further evaluation of androgen deficiency. In addition it should be emphasised that isolated measurement of the LH/T relation without investigating signs and symptoms of hypogonadism should not form the basis of androgen therapy.[20] We find it, however, of interest that a large proportion of the patients have biochemical values outside the limits of normality, and thus closer surveillance of these patients in order to detect development of manifest androgen deficiency might be advocated. We suggest assessments of T and LH 1 year postoperatively in TGCC patients, and probably annually hereafter. Prospective studies on the risk of hypogonadism and associated conditions in TGCC survivors are needed.

In conclusion most patients with unilateral hCG-negative stage I testicular cancer have LH and T levels within the normal range, although one third of the patients showed subtle disturbances of the pituitary-Leydig cell axis already at the time of diagnosis. One year after unilateral orchiectomy, a marked increase in LH was noted, and 57% of the patients were outside the 97.5 percentile when using bivariate LH/T evaluation. We suggest that TGCC patients treated with

Table 1 – Baseline characteristics.

	hCG-negative patients	hCG-positive patients	Controls
No. of patients	61	11	547
Age (years)	32 (16–50)	27 (19–38)	29 (20–50)
hCG (IU/L)	–	127 (4–1730)	–
LH (IU/L)	3.73 (0.12–9.92)	0.1 (0.01–21 38)**	3.36 (0.47–20 7)
T (nmol/L)	14.89 (2.85–29 50)	24.72 (10.49–34 07)**	16.50 (1 23–38.9)
FSH (IU/L)	6.78 (0.01–30.2)**	0 01 (0.01–4.86)**	3.56 (0.62–43 9)
Inhibin B (pmol/L)	113 (64–135)**	97 (43–147)**	161 (19–380)
Estradiol (pmol/L)	85 (33–289)*	232 (57–496)**	97 (18–275)
SHBG (nmol/L)	35 (6–76)	23 (19–40)	34(8–184)
T/E2[a]	177 (60–362)	100 (59–184)**	170 (10–736)
FAI[b]	44 (5–355)*	102 (26–179)**	50 (2–144)

[a] (T × 1000)/estradiol.
[b] Free androgen index (T/SHBG) × 100.
* $p < 0.05$ when compared to controls.
** $p < 0.005$ when compared to controls.

orchiectomy only, need close surveillance in order to detect early signs of androgen deficiency preferably before clinical signs become evident.

Conflict of interest statement

None declared.

REFERENCES

1. Krege S, Beyer J, Souchon R, et al. European consensus conference on diagnosis and treatment of germ cell cancer: a report of the second meeting of the European Germ Cell Cancer Consensus group (EGCCCG): part I. *Eur Urol* 2008;**53**:478–96.
2. Travis LB, Fossa SD, Schonfeld SJ, et al. Second cancers among 40, 576 testicular cancer patients: focus on long-term survivors. *J Natl Cancer Inst* 2005;**97**:1354–65.
3. Haugnes HS, Wethal T, Aass N, et al. Cardiovascular risk factors and morbidity in long-term survivors of testicular cancer: a 20-year follow-up study. *J Clin Oncol* 2010;**28**:4649–57.
4. Huddart RA, Norman A, Shahidi M, et al. Cardiovascular disease as a long-term complication of treatment for testicular cancer. *J Clin Oncol* 2003;**21**:1513–23.
5. Sagstuen H, Aass N, Fossa SD, et al. Blood pressure and body mass index in long-term survivors of testicular cancer. *J Clin Oncol* 2005;**23**:4980–90.
6. Brennemann W, Stoffel-Wagner B, Helmers A, et al. Gonadal function of patients treated with cisplatin based chemotherapy for germ cell cancer. *J Urol* 1997;**158**:844–50.
7. Huddart RA, Norman A, Moynihan C, et al. Fertility, gonadal and sexual function in survivors of testicular cancer. *Br J Cancer* 2005;**93**:200–7.
8. Nord C, Bjoro T, Ellingsen D, et al. Gonadal hormones in long-term survivors 10 years after treatment for unilateral testicular cancer. *Eur Urol* 2003;**44**:322–8.
9. Howell SJ, Radford JA, Ryder WD, Shalet SM. Testicular function after cytotoxic chemotherapy: evidence of Leydig cell insufficiency. *J Clin Oncol* 1999;**17**:1493–8.
10. Petersen PM, Skakkebaek NE, Rorth M, Giwercman A. Semen quality and reproductive hormones before and after orchiectomy in men with testicular cancer. *J Urol* 1999;**161**:822–6.
11. Gerl A, Muhlbayer D, Hansmann G, Mraz W, Hiddemann W. The impact of chemotherapy on Leydig cell function in long term survivors of germ cell tumors. *Cancer* 2001;**91**:1297–303.
12. Stanworth RD, Jones TH. Testosterone in obesity, metabolic syndrome and type 2 diabetes. *Front Horm Res* 2009;**37**: 74–90.
13. Finkelstein JS, Klibanski A, Neer RM, et al. Osteoporosis in men with idiopathic hypogonadotropic hypogonadism. *Ann Intern Med* 1987;**106**:354–61.
14. Greenspan SL, Neer RM, Ridgway EC, Klibanski A. Osteoporosis in men with hyperprolactinemic hypogonadism. *Ann Intern Med* 1986;**104**:777–82.
15. Katznelson L, Finkelstein JS, Schoenfeld DA, et al. Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism. *J Clin Endocrinol Metab* 1996;**81**:4358–65.
16. Isidori AM, Giannetta E, Greco EA, et al. Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. *Clin Endocrinol (Oxf)* 2005;**63**:280–93.
17. Davidson JM, Camargo CA, Smith ER. Effects of androgen on sexual behavior in hypogonadal men. *J Clin Endocrinol Metab* 1979;**48**:955–8.
18. Nieschlag E, Behre HM, Bouchard P, et al. Testosterone replacement therapy: current trends and future directions. *Hum Reprod Update* 2004;**10**:409–19.
19. Bang AK, Petersen JH, Petersen PM, et al. Testosterone production is better preserved after 16 than 20 Gray irradiation treatment against testicular carcinoma in situ cells. *Int J Radiat Oncol Biol Phys* 2009;**75**:672–6.
20. Aksglaede L, Andersson AM, Jorgensen N, et al. Primary testicular failure in Klinefelter's syndrome: the use of bivariate luteinizing hormone-testosterone reference charts. *Clin Endocrinol (Oxf)* 2007;**66**:276–81.
21. Daugaard G. The clinical use of tumour markers in germ cell cancer. *J Tumour Marker Oncology* 2001;**16**:39–48.
22. Petersen PM, Skakkebaek NE, Vistisen K, Rorth M, Giwercman A. Semen quality and reproductive hormones before orchiectomy in men with testicular cancer. *J Clin Oncol* 1999;**17**:941–7.
23. Stenman UH, Alfthan H, Hotakainen K. Human chorionic gonadotropin in cancer. *Clin Biochem* 2004;**37**:549–61.
24. Cailleux-Bounacer A, Reznik Y, Cauliez B, et al. Evaluation of endocrine testing of Leydig cell function using extractive and recombinant human chorionic gonadotropin and different doses of recombinant human LH in normal men. *Eur J Endocrinol* 2008;**159**:171–8.
25. Puhse G, Secker A, Kemper S, Hertle L, Kliesch S. Testosterone deficiency in testicular germ-cell cancer patients is not influenced by oncological treatment. *Int J Androl* 2010. doi:10.1111/j.1365-2605.2010.01123.x [Epub ahead of print].
26. Carlsen E, Olsson C, Petersen JH, Andersson AM, Skakkebaek NE. Diurnal rhythm in serum levels of inhibin B in normal men: relation to testicular steroids and gonadotropins. *J Clin Endocrinol Metab* 1999;**84**:1664–9.