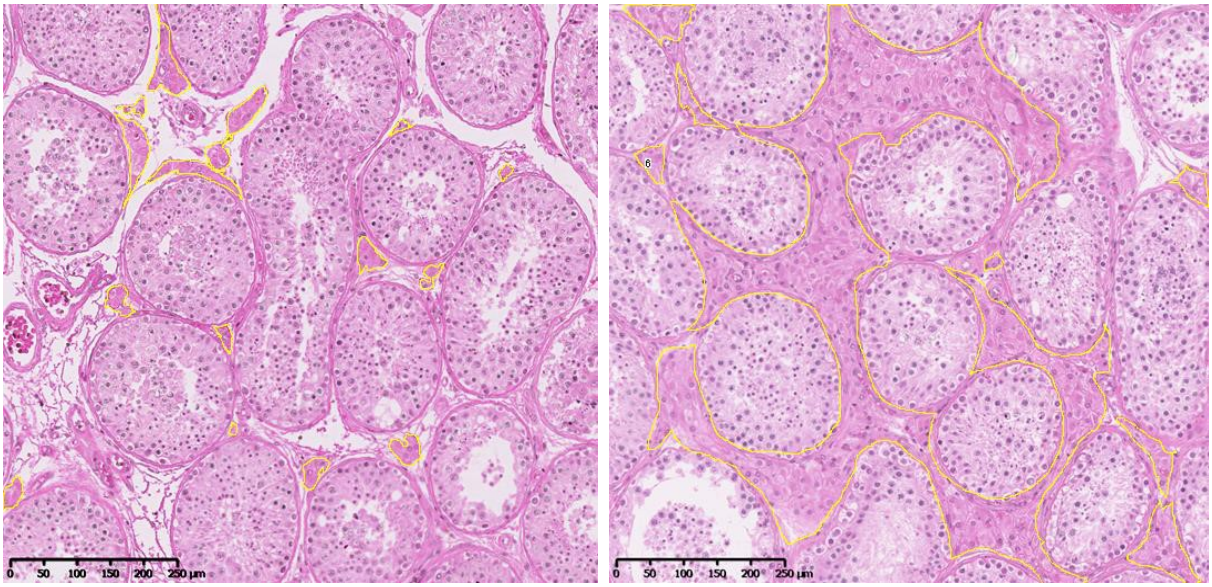
UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

Leydig cell function in testicular cancer patients: characterization, treatment-related changes and clinical implications



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The Faculty of Health and Medical Sciences at the University of Copenhagen has accepted this dissertation for public defence for the doctoral degree at Copenhagen University Hospital Rigshospitalet, Copenhagen, May 28, 2021.

ISBN 978-87-973039-0-0

Publications

List of publications included in the thesis

1) Bandak Mikkel, Jørgensen Niels, Juul Anders, Lauritsen Jakob, Kier Maria Gry Gundgaard, Mortensen Mette Saksø, Daugaard Gedske. *Preorchiectomy Leydig cell dysfunction in patients with testicular cancer.* Clinical Genitourinary Cancer 2017, Volume 15, issue 1, pages 37-43[1]

2) Bandak Mikkel, Aksglæde Lise, Juul Anders, Rørth Mikael, Daugaard Gedske: *The pituitary-Leydig cell axis before and after orchiectomy in patients with stage I testicular cancer.* European Journal of Cancer, 2011. Volume 47, issue 17, pages 2585-91[2]

3) Bandak Mikkel, Jørgensen Niels, Juul Anders, Lauritsen Jakob, Kier Maria Gry Gundgaard, Mortensen Mette Saksø, Daugaard Gedske. *Longitudinal changes in serum levels of testosterone and luteinizing hormone in testicular cancer patients after orchiectomy alone or bleomycin, etoposide, and cisplatin.* European Urology Focus, 2018, Volume 4, issue 4, pages 591-598[3]

4) Bandak Mikkel, Jørgensen Niels, Juul Anders, Vogelius Ivan Richter, Lauritsen Jakob, Kier Maria Gry Gundgaard, Mortensen Mette Saksø, Glovinski Peter, Daugaard Gedske. *Testosterone deficiency in testicular cancer survivors - a systematic review and meta-analysis.* Andrology, 2016. Volume 4, issue 3, pages 382-388[4]

5) Tarsitano Maria Grazia, Bandak Mikkel, Jørgensen Niels, Skakkebaek Niels Erik, Juul Anders, Lenzi Andrea, Daugaard Gedske, Rajpert-De Meyts Ewa. *Quantification of the Leydig cell compartment in testicular biopsies and association with biochemical Leydig cell dysfunction in testicular cancer survivors.* Andrology 2018. Volume 6, issue 5, pages 748-755[5]

6) Bandak Mikkel, Jørgensen Niels, Juul Anders, Lauritsen Jakob, Oturai Peter Sandor, Mortensen Jann, Højman Pernille, Helge Jørn Wulff, Daugaard Gedske. *Leydig cell dysfunction, systemic inflammation and metabolic syndrome in long-term testicular cancer survivors*. European Journal of Cancer 2017. Volume 84, pages 9–17[6]

7) Bandak Mikkel, Jørgensen Niels, Juul Anders, Lauritsen Jakob, Kier Maria Gry Gundgaard, Mortensen Mette Saksø, Oturai Peter Sandor, Mortensen Jann, Højman Pernille, Helge Jørn Wulff, Daugaard Gedske, *Reproductive hormones and metabolic syndrome in 24 testicular cancer survivors and their biological brothers.* Andrology 2017. Volume 5, issue 4, pages 718-724[7]

8) Bandak Mikkel, Lauritsen Jakob, Johansen Christoffer, Kreiberg Michael, Skøtt Julie Wang, Agerbæk Mads, Holm Niels Vilstrup, Daugaard Gedske. *Sexual function in a nationwide cohort of 2260 testicular cancer survivors after 17 years follow-up.* Jornal of Urology 2018. Volume 200, issue 4, pages 794-800[8]

9) Skøtt Julie Wang, Lauritsen Jakob, Kreiberg Mikael, Daugaard Gedske, Bandak Mikkel. *Quality of life in long-term testicular cancer survivors with compensated Leydig cell dysfunction*. Clinical Genitourinary Cancer 2019, Volume 17, issue 1, pages 65-71[9]

10) Bandak Mikkel, Lauritsen Jakob, Johansen Christoffer, Kreiberg Michael, Skøtt Julie Wang, Agerbaek Mads, Holm Niels Vilstrup, Daugaard Gedske. *Sexual function and quality of life in a national cohort of survivors of bilateral testicular cancer*. European Urology Focus 2020, Volume 6, issue 4, pages 711-719[10]

Acknowledgements

My engagement with oncology, and with testicular cancer more specifically began in 2009 where I decided to write my master's thesis (OSVAL II) in oncology. A hopeful email was sent to professor Mikael Rørth who referred me to professor Gedske Daugaard. Shortly after, I was handed an excel sheet with hormone data on stage I testicular cancer patients - Rows and columns with confusing values and personal identification numbers without any specific order as far as I could see.
However, step by step and with the precious help of former PhD-student Lise Aksglæde and professor Anders Juul, I managed to make some order and complete a master's thesis, which was later refined and published as a part of this thesis.
While continuing part-time research, I started full time in 2014 and had the chance to be part of wonderful research group at the Department of Oncology at Copenhagen University Hospital, Rigshospitalet with Mette Saksø Mortensen, Gry Gundgaard and Jakob Lauritsen headed by Gedske Daugaard. During the following 3.5 years most of the research presented in the present thesis was performed in close collaboration with the other members of the group.
I will like to give specific thanks to Gedske Daugaard who has been a perfect supervisor, always supportive, not performing micromanagement but being able to draw the broader outlines to keep me on track.
Secondly, I would like to thank Jakob Lauritsen who has been a valuable partner, always ready for a scientific discussion often while still wearing the bike helmet after having rushed to work.

I would also like to acknowledge the important collaboration and supervision from colleagues and friends from Copenhagen University Hospital, Rigshospitalet:
From Department of Oncology: Gry Gundgaard, Mette Saksø Mortensen and Michael Kreiberg from the testicular cancer group and Christoffer Johansen, Ivan Vogelius, and research fellows from the research group at the department. Thanks to Head of the Department, Hans Von der Maase and Ulrik Lassen.
From Department of Growth and Reproduction I would like to thank Niels Jørgensen and Anders Juul for supervision and guidance concerning the andrological aspects of the thesis. Also, I would like to acknowledge the great help from the skilled laboratory technicians, Jannie Hougård Hennings and Maiken Probst.
From Department of Clinical Physiology, Nuclear Medicine and PET, I would like to thank Peter Sandor Oturai and Jann Mortensen.

From Centre of Physical Activity Research, and Centre of Inflammation and Metabolism, Pernille Højman who tragically passed away in April 2019.
From Department of Oncology at Odense University Hospital I would like to thank Niels Holm, and from Department of Oncology at Aarhus University Hospital, Mads Agerbæk.
From Center of Healthy Ageing, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark I would like to thank Jørn Wulff Helge.

Finally, I would like to thank all testicular cancer patients and brothers to patients who have spent their time participating in the research projects constituting the backbone of the present thesis.

Contents

1.0 Introduction

Leydig cells are endocrine cells located in the testes. Their prime function is production of testosterone which is the main male hormone with a variety of functions throughout the human body. Testosterone production by the Leydig cells is regulated by luteinizing hormone (LH) secretion from the pituitary gland which binds to LH receptors on Leydig cells. In testicular cancer, the tumour bearing testicle is surgically removed. This reduces the amount of testosterone producing Leydig cells by half. In addition, around 50% of patients receive treatment with cisplatin-based chemotherapy or abdominal radiotherapy due to disseminated disease. These treatments might have a deleterious effect on Leydig cells increasing the risk of varying degrees of testosterone deficiency. While it is well known that serum levels of testosterone well below the normal values of healthy men are associated with physiological and phycological side effects, little is known about the consequences of borderline low testosterone levels accompanied by elevated LH levels.

In this thesis the influence of testicular cancer and testicular cancer treatment on Leydig cell function is characterized. Furthermore, physiological and phycological side effects of testicular cancer treatment and different degrees of testosterone deficiency after testicular cancer treatment is elucidated.

2.0 Background: Leydig cell function and testicular cancer

2.1 Leydig cells: Structure, function and dysfunction

Franz Leydig was the first to describe the cells which were later named "Leydig cells" in a comparative study of mammalian reproductive histology published in 1850 [11]. In the microscope, he found clusters of cells located in the testes between the seminiferous tubules adjacent to blood vessels. After many years of dispute, it was clarified that Leydig cells which are characterized by a dominant smooth endoplasmic reticulum and many mitochondria are endocrine cells and the prime source of testosterone which is the major circulating androgen in men [12]. There are around 500 million Leydig cells in the human testes constituting few percent of the testicular volume and producing around 95% of circulating testosterone [13]. Testosterone is synthesized in a step-wise manner with cholesterol from the plasma membrane as the prime substrate. In plasma, testosterone is predominantly bound to sexual hormone binding globulin (SHBG) and albumin while around 2% is unbound constituting the free fraction of testosterone [14,15]. It is supposed that only this fraction is biologically active as it can diffuse across the plasma membrane [14]. Testosterone exhorts its action by binding to the intracellular androgen receptor with the complex binding to the androgen response element on DNA resulting in a change in DNA transcription, while in some target organs testosterone is metabolized to the active metabolites dihydrotestosterone or estradiol.

Testosterone production is initiated by GnRH-secretion from the hypothalamus which stimulates Luteinizing Hormone (LH) secretion from the anterior pituitary. LH is a short polypeptide with a 92 amino-acids alfa-unit which is identical to the alfa-unit of human chorionic gonadotropin (hCG) and a beta-unit with 120 amino acids. LH binds to the LH receptor on the Leydig cells initiating an intracellular signal cascade resulting in testosterone production.

Under normal circumstances there is a homeostasis between LH secretion and testosterone production due to a negative feedback loop, where serum testosterone or estradiol regulates LH secretion from the pituitary resulting in both hormones being in the physiologically normal range[16]. (Figure 1a)

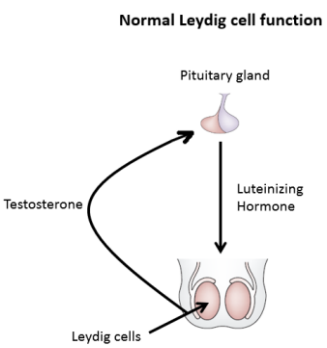


Figure 1a

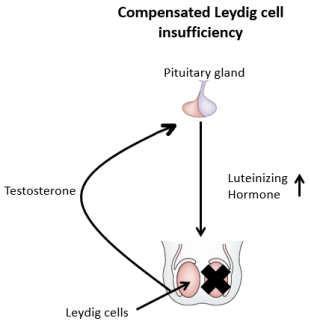


Figure 1b

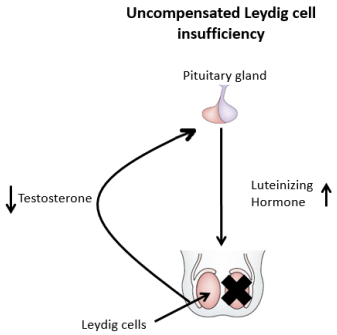


Figure 1c

In Leydig cell dysfunction due to genetic defects or environmental factors, testosterone production will drop resulting in a compensatory increase in LH secretion to maintain serum testosterone in the normal range. This condition where LH is elevated while testosterone levels are normal is known as “compensated Leydig cell insufficiency”, “mild Leydig cell failure” or “subclinical hypogonadism” (Figure 1b). In uncompensated primary Leydig cell dysfunction, increasing serum levels of LH are not sufficient to maintain normal testosterone resulting in increased LH levels and testosterone dropping below the physiologically normal levels. This condition is known as “uncompensated Leydig cell insufficiency”, “primary hypogonadism” or “primary Leydig cell failure” (Figure 1c).

Due to circadian variations as well as day-to-day variations it is generally recommended to assess Leydig cell function by drawing blood in the morning with measurements performed on two separate days [17,18] .

2.2 Leydig cell function and testicular cancer

According to the hypothesis of testicular dysgenesis, a combination of genetic defects, environmental exposure, lifestyle factors and epigenetic factors during fetal life are causing Leydig cell dysfunction and Sertoli cell dysfunction which is linked to impaired spermatogenesis, cryptorchidism, hypospadias, decreased testosterone production, impaired germ cell differentiation, and germ cell neoplasia in situ and testicular germ cell cancer later in life [19–21].

To support the hypothesis of a fetal origin of the above-mentioned disorders it would be expected that men who develop testicular cancer have Leydig cell dysfunction even before the initiation of carcinogenesis. However, only a single study including 30 TC patients have investigated Leydig cell function before testicular cancer treatment [22]. In this study, impaired spermatogenesis was reported while there was no evidence of Leydig cell dysfunction, which could be due to the small sample size. Thus, it remains to be clarified if testicular cancer patients have Leydig cell dysfunction prior to orchiectomy which would support the hypothesis of testicular dysgenesis.

2.3 Treatment of testicular cancer and the effect on Leydig cell function

In Denmark, the incidence of testicular cancer is around 10/100.000 pr. year and there are around 300 annual cases of testicular germ cell cancer [23]. At disease presentation, around 70% of patients have stage I disease where the disease is confined to the testicle [23,24]. In these patients, orchiectomy alone and follow-up in a 5-years surveillance programme has been the treatment of choice since 1984 in Denmark. In patients with seminoma histology, the 5-year relapse rate is around 20% [25] while in patients with non-seminoma histology the relapse rate is around 30% [26]. The 5-year overall survival in patients presenting with stage I disease is >99% [27].

In case of primary disseminated disease or in case of relapse after initial stage I disease, patients are divided into a poor, intermediate and good prognostic groups according to clinical risk factors [28]. Patients belonging to the good prognostic group are treated with three courses of bleomycin, etoposide and cisplatin (BEP) while patients in the intermediate and poor prognostic groups are treated with 4 courses of BEP. In case of residual disease in the retroperitoneum after BEP, post-chemotherapy surgery may be performed [29]. In case of pure seminoma histology and limited retroperitoneal spread, abdominal radiotherapy is a treatment option.

Germ cell neoplasia in situ (GCNIS) is a precursor of the majority of testicular cancers [30,31,32] and it is believed that GCNIS will develop into cancer in the majority of cases over time [33]. Therefore, a national screening program for contralateral GCNIS was gradually introduced in the 1980's in Denmark. GCNIS is present in around 5% of contralateral testicles [34,35]. Radiotherapy to the contralateral testicle with the purpose of decreasing the risk of a metachronous tumour is the preferred treatment. Bilateral synchronous testicular cancer is present in <2 % of cases [36,37] and in these cases bilateral orchiectomy is the preferred treatment option.

In unilateral testicular cancer, around half of the Leydig cells are mechanically removed at orchiectomy. Leydig cell function can be further exacerbated in patients with metastatic disease as animal studies suggest a toxic effect of chemotherapy [38] and of radiotherapy even in very low doses [39,40].

Several small studies have examined Leydig cell function in testicular cancer patients [41–46], while seven studies with more than 200 patients and a median follow-up between 2 months and 18 years since treatment have investigated the effect of testicular cancer treatment on Leydig cell function [47–53]. It has generally been reported that serum levels of LH are elevated compared to healthy men and patients treated with cisplatin-based chemotherapy and abdominal radiotherapy have elevated LH levels compared to patients treated with orchiectomy alone. The effect of TC treatment on testosterone levels appears to be

less pronounced, with some of the above-mentioned studies finding increased risk of low serum levels of testosterone and use of testosterone substitution, while others have not. This finding indicates that many TC survivors might have compensated Leydig cell insufficiency.

Little is known about longitudinal changes in Leydig cell function after TC treatment, and little is known about risk factors for Leydig cell dysfunction after TC treatment.

2.4 Signs and symptoms of Leydig cell dysfunction

Classical signs of *uncompensated Leydig cell insufficiency in* adult men are decreased muscle mass [54,55], anaemia [56] and decreased bone mineral density [55,57]. Recent studies have suggested that testosterone deficiency is associated with systemic inflammation defined by elevated plasma levels of C-reactive protein (CRP), tumour necrosis factor alpha (TNF-α), interleukin 1β and interleukin 6 as well as metabolic syndrome, which is a constellation of metabolic disturbances including abdominal obesity, hypertension, insulin resistance and dyslipidaemia. However, it is debated whether testosterone deficiency is causing systemic inflammation and metabolic syndrome or the other way around [58–63]. The classical subjective symptom of testosterone deficiency is decreased sexual interest with improvement after testosterone substitution [64–66]. The association between testosterone deficiency and depression and cognitive dysfunction is less clear and some studies have found improvement with testosterone substitution while others have not [67,68].

The pathophysiological effects of *compensated Leydig cell insufficiency* are less clear. Results from the European Aging Male Study have suggested that compensated Leydig cell insufficiency in men without testicular cancer is associated with erectile dysfunction and limited walking distance [69], while a Danish prospective population-based study found increased overall mortality, suggesting that it is a marker of underlying disease [70].

The proportion of men who progress from compensated Leydig cell insufficiency to uncompensated Leydig cell insufficiency over time is not known and it is not known if findings from normal men can be extrapolated to testicular cancer patients who have been treated with unilateral orchiectomy.

2.5 Signs and symptoms of Leydig cell dysfunction in testicular cancer patients

In population-based prospective studies elevated plasma levels of the inflammatory marker CRP has been reported as a moderate predictor of cardiovascular disease [71]. In TC patients, three cross-sectional studies have investigated systemic inflammation by assessing plasma levels of inflammatory markers including high sensitivity CRP focusing on the effect of different treatment modalities [72–74]. Results have been inconclusive, and no studies have investigated if Leydig cell dysfunction is associated with systemic inflammation.

In population-based studies, presence of metabolic syndrome has been shown to increase the risk of cardiovascular disease [75,76]. In TC survivors, cisplatin-based chemotherapy is associated with increased risk of cardiovascular disease [74,77–80] and it has been hypothesized that the increased risk is mediated through metabolic syndrome which in turn could be caused by treatment induced Leydig cell dysfunction [81]. Since 2005, eight cross-sectional studies have evaluated the prevalence of metabolic syndrome in testicular cancer survivors compared to healthy men and according to treatment modality [52,72,74,81–85]. The results have been conflicting, and it remains unclear if testicular cancer patients have increased risk of metabolic syndrome compared to healthy men and if chemotherapy increases the risk of metabolic syndrome compared to orchiectomy alone. However, all studies found associations between testosterone deficiency and metabolic syndrome, though, as they were cross-sectional, it remains unclear if Leydig cell dysfunction is causally related to metabolic syndrome in TC patients.

Sexual dysfunction in cancer survivors is a complex condition that may result from psychological and physical causes [86,87]. Since 2005, five large studies have evaluated self-reported sexual function in long-term testicular cancer survivors focusing on the effect of treatment induced changes [50,87–90]. Apart from increased risk of self-reported orgasmic dysfunction in patients treated with post-chemotherapy abdominal surgery due to residual tumour in the retroperitoneum, there has been no clear indication of sexual dysfunction according to treatment modality. Thus, it remains unknown how todays TC treatment affects long-term sexual function. Two studies have investigated the association between uncompensated Leydig cell dysfunction and sexual dysfunction [50,51] while no studies have investigated the association between compensated Leydig cell dysfunction and sexual dysfunction and impaired quality of life.

Furthermore, sexual function and other aspects of quality of life remains unexplored in TC patients treated for unilateral TC with contralateral GCNIS or bilateral orchiectomy du to bilateral TC.

2.6 Aims of the thesis

The aims of the thesis were to characterize Leydig cell function in testicular cancer patients with focus on:

1. Leydig cell function before and after orchiectomy
2. risk factors for Leydig cell dysfunction
3. influence of treatment for metastatic disease (chemotherapy (BEP) and radiotherapy) on Leydig cell function including longitudinal changes in Leydig cell function
4. whether Leydig cell dysfunction and testicular cancer treatment is associated with long-term risk of systemic inflammation and metabolic syndrome
5. impact of testicular cancer treatment on sexual function after long-term follow-up

6. impact of Leydig cell dysfunction on sexual function and aspects of quality of life after long-term follow-up
7. Sexual function and quality of life after long-term follow-up in bilateral testicular cancer patients and patients treated for germ cell neoplasia in situ (GCNIS) in the contralateral testicle

An attempt to illustrate possible causal relations investigated in the thesis are schematically summarized in Figure 2

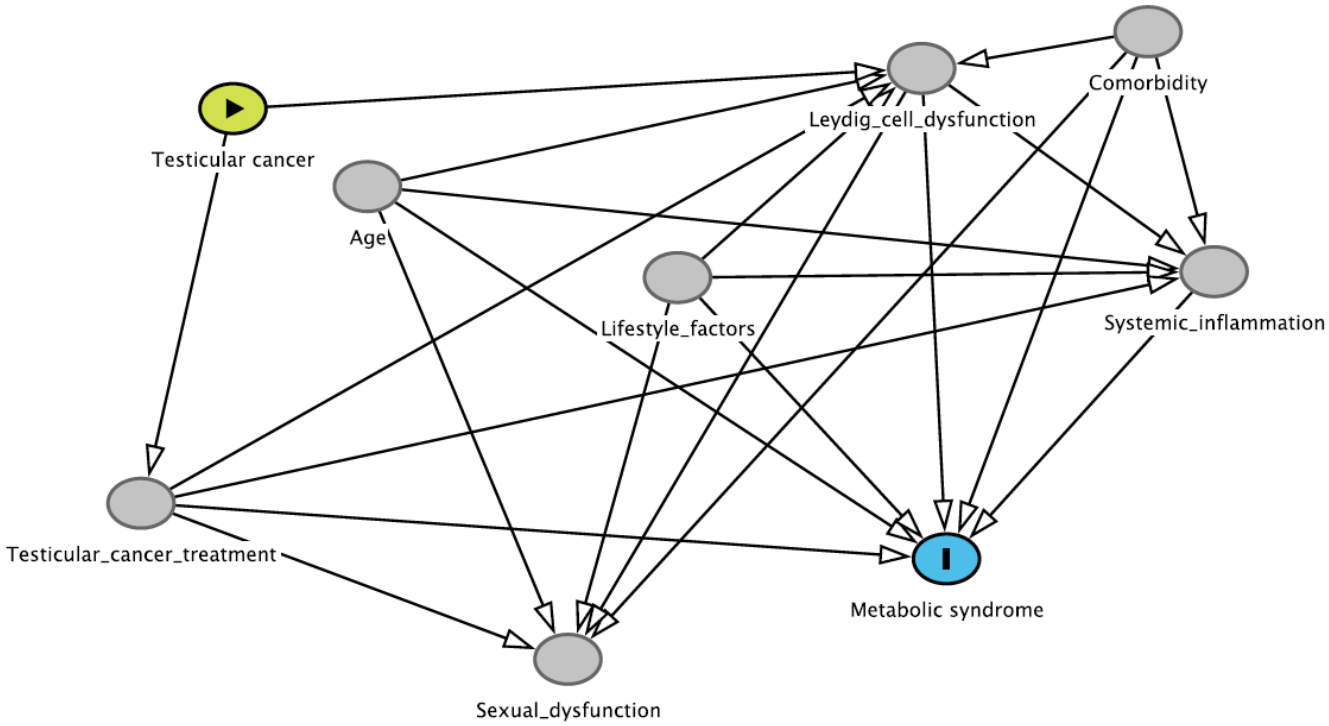


Figure 2. Schematic presentation of possible causal relations investigated in the thesis**.** Arrows represent possible causal direction.

3.0 Patients and methods

The following data sources were used to elucidate the aims of the thesis:

- *The Danish Testicular Cancer (DaTeCa) database*[91] is a nationwide database that comprises around 6000 male germ cell cancer patients diagnosed in Denmark between January 1, 1984 and December 31, 2007. Patients have been identified from the Danish National Patient Registry and hospital files and a germ cell cancer diagnosis has been confirmed by manual review of pathology reports. The coverage of DaTeCa is around 80%. The database hosts information on more than 300 variables including detailed treatment information and has been linked with several National registries such as the Danish National Patient Registry [92] The Register of causes of Death [93], and The Danish National Prescription Registry [94].

- *The Danish Testicular Cancer Late Treatment Effects Cohort (DaTeCa-LATE)*[95]

Between 2014 and 2016, a questionnaire survey including all eligible testicular cancer survivors in DaTeCa was conducted. In total, 4271 eligible testicular cancer survivors were approached by mail and 2572 agreed to participate in the survey (response rate = 60%). Comparison of responders and non-responders are presented in[95]. All study participants answered a 167-item questionnaire including six validated questionnaires. For the publications presented in the present thesis the three main questionnaires were: 1) The International Index of Erectile Function-15 (IIEF-15) [96], which covers 5 domains of sexual function: erectile function, sexual desire, orgasmic function, intercourse satisfaction, overall satisfaction, 2) The Hospital Anxiety and Depression Scale (HADS) [97] with two separate scales for depression and anxiety, 3) The Multidimensional Fatigue Inventory (MFI-20) [98], which covers five dimensions of fatigue: general fatigue, physical fatigue, mental fatigue, reduced motivation, and reduced activity.

The DaTeCa database and DaTeCa-LATE were used to elucidate if testicular cancer treatment is associated with long-term risk of sexual dysfunction and impaired quality of life (anxiey, depression, fatigue) in patients with unilateral testicular cancer, unilateral testicular cancer with contralateral GCNIS and patients with bilateral testicular cancer (aim 5 and aim 7 of the thesis). Details about statistical methods applied can be found in [8,10].

- *Symptoms and Clinical Signs of Hypogonadism in Testicular Cancer Survivors (National Clinical Trial 02240966)*

This study was conducted between 2014 and 2016 to elucidate the long-term risk of systemic inflammation and metabolic syndrome in testicular cancer patients with Leydig cell dysfunction. In total, 158 long-term testicular cancer survivors from the DaTeCa database were included based on Leydig cell function within the routine follow-up program for testicular cancer: In total, 28 patients with uncompensated Leydig cell insufficiency, 59 patients with compensated Leydig cell insufficiency and 71 patients with normal Leydig cell function within the follow-up program were included. Testosterone substitution was an exclusion criterion. In addition, 24 biological brothers to the included patients were included as a comparison group. After an overnight fast, assessment of systemic inflammation, metabolic syndrome, self-reported quality of life including sexual function was performed.

In addition, the Leydig cell compartment in the contralateral testicular biopsy performed at the time of diagnosis was microscopely assessed in a subgroup of 50 included patients and compared to 10 testicular cancer patients with markedly elevated serum levels of hCG and 10 men with obstructive azoospermia and normal Leydig cell function.

National Clinical Trial 02240966 was used to elucidate risk factors for Leydig cell dysfunction (aim 2), the association between Leydig cell dysfunction and long-term risk of systemic inflammation and metabolic syndrome (aim 4) and the association between Leydig cell dysfunction and sexual dysfunction and impaired quality of life (aim 6). Details about statistical methods applied can be found in [5–7,9].

Aim 1 was elucidated in the two studies [1,2] where Leydig cell function (serum levels of testosterone and LH) were assessed *before and after orchiectomy* in testicular cancer patients treated at Copenhagen University Hospital, Rigshospitalet. As hCG alters the pituitary-Leydig cell axis due to its structural similarity with LH[99], patients with hCG elevation were analysed separately.

Aim 3 was elucidated by performing a systematic review of the literature and a meta-analysis[4], where studies assessing the ratio of patients with low serum levels of testosterone or using testosterone substitution according to treatment intensity (orchiectomy alone, abdominal radiotherapy, cisplatin-based chemotherapy, more intensive treatment) were included. Longitudinal changes in Leydig cell function was assessed in [3] were testicular cancer patients who were treated with orchiectomy alone or cisplatin-based chemotherapy at Copenhagen University Hospital, Rigshospitalet, and had at least two evaluations of LH and testosterone performed during the follow-up program, were included.

Evaluation of Leydig cell structure and function

Before September 2014, serum levels of total testosterone were assessed with radioimmunoassay (RIA, Siemens Coat-a-count) with inter-assay variation of 12.8 % and intra-assay variation of 17% and lower detection limit of 0.23 nmol/L. After September 2014, total testosterone was assessed with immunoassay (Access 2, Beckman Coulter) with inter-assay variation of 5.18 % and intra-assay variation of 4.10% and lower detection limit of 0.35 nmol/L. Calculation of free testosterone was performed as described in[100]. Serum levels of LH were assessed with immune-assay (Delfia, Perkin Elmer) with inter-assay variation of 1.94% and intra-assay variation of 3% and lower detection limit of 0.05 IU/L. All analyses of hormone values were done at the laboratory of Department of Growth and Reproduction, Copenhagen University Hospital, Rigshospitalet.

The Leydig cell compartment in contralateral testicular biopsies was assessed with the open-source imaging program ImageJ (V.1.48, NIH) after selected slides had been scanned with the slide scanner NanoZoomer 2.0 HT (Hamamatsu Photonics, Herrsching am Ammersee, Germany) and analysed using the software NDPview version 1.2.36 (Hamamatsu Photonics), further details are described in [5].

Evaluation of systemic inflammation and metabolic syndrome

Inflammatory markers in plasma (tumour necrosis factor alpha, interleukin-1b, IL-6 and IL-8) were analysed by Meso Scale Discovery multiplex (Meso Scale Discovery, USA). Metabolic syndrome was assessed according to the definitions of The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII)[101] and the definitions of the International Diabetes Federation (IDF)[102]. The definitions are presented in Appendix 1.

Reference groups of men from the Danish background population for evaluation of Leydig cell function

As testosterone declines with age while LH increases with age [103], a reference group of 839 men from two population-based studies [104,105] were used as controls. Based on these men, reference curves for testosterone and LH (mean, lower limit, upper limit) vs chronological age were constructed. In addition, bivariate reference charts of LH in combination with testosterone were constructed. The bivariate LH/TT curve separates individuals with an abnormal LH/TT combination from individuals with a normal LH/TT combination [106].

4.0 Results and discussion

4.1 Leydig cell function in testicular patients before and after orchiectomy

Leydig cell function before orchiectomy

To assess if Leydig cell function is compromised even before testicular cancer treatment is initiated, Leydig cell function was assessed by evaluating serum levels of testosterone (total and free) and LH within 30 days *before* orchiectomy in 374 TC patients without detectable serum levels of hCG [1]. The results were compared to an age-matched reference group of healthy men. It was found that TC patients had significantly lower serum levels of total testosterone and free testosterone while there was no significant difference in LH as shown in table 1

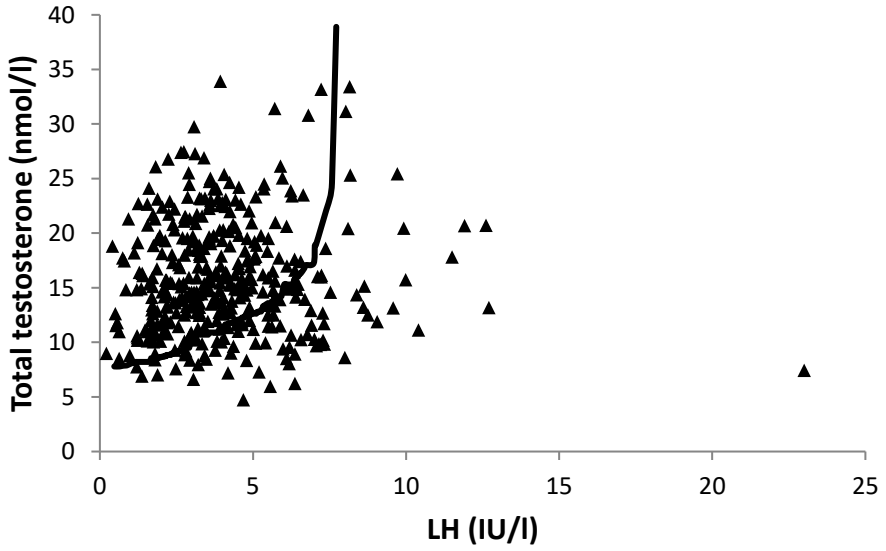
Table 1

	hCG-negative patients (n= 374)	**Controls (n= 564)**	**P-value***
LH (IU/L) (median, IQR)	3.6 (2.6 -5.1)	3.3 (2.5-4.5)	0.07
Testosterone (nmol/L) (median, IQR)	15.2 (3.6-33.9)	16.5 (13.2-20.6)	0.004
Free testosterone (pmol/L) (median, IQR)	309 (107-797)	343 (275-414)	<0.001

*Compared with controls with independent samples t-test, IQR=Interquartile range

When using combined evaluation of LH and TT, 25% of testicular cancer patients had Leydig cell dysfunction defined as combined LH/TT outside the 97.5 percentile of healthy men. This is shown in Figure 3.

Figure 3 Bivariate evaluation of LH and total testosterone before orchiectomy in hCG-negative patients. the triangles below and to the right of the curved bold line representing the 97.5 percentile of healthy men represent TC patients with Leydig cell dysfunction.



In the hCG-negative patients, increasing tumour size and presence of germ cell neoplasia in situ of the contralateral testicle was significantly associated with Leydig cell dysfunction, while there was no association between advanced clinical stage and Leydig cell dysfunction [1].

As hCG is known to disrupt the pituitary-Leydig cell axis, due to its structural similarity with LH, Leydig cell function was assessed separately in 187 testicular cancer patients who had elevated serum levels of hCG. In this sub-group, serum levels of LH were markedly depressed while total and free testosterone were highly elevated [1]. The results are shown in Table 2.

Table 2

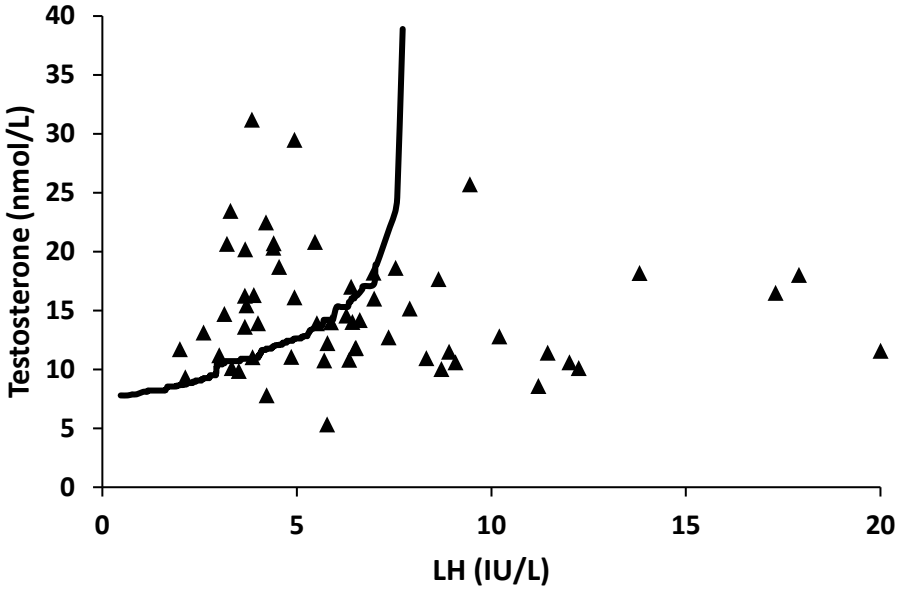
	hCG-positive patients (n= 187)	**Controls (n=564)**	**P-value***
LH (IU/L) (median, IQR)	0.49 (0.01-34.9)	3.3 (2.5-4.5)	<0.001
Testosterone (nmol/L) (median, IQR)	22.4 (16.5-30.2)	16.5 (13.2-20.6)	<0.001
Free testosterone (pmol/L) (median, IQR)	470 (118-1240)	343 (275-414)	<0.001

*Compared with controls with independent samples t-test, IQR=Interquartile range

Leydig cell function after orchiectomy

To evaluate changes in Leydig cell function after surgical removal of the tumour bearing testicle, Leydig cell function was assessed before diagnosis and 1 year after orchiectomy in 61 stage I testicular cancer patients without detectable serum levels of hCG. There was a significant increase in LH (ΔLH = 2.04 IU/L, $p<0.001$) while there were no significant changes in serum levels of testosterone[2]. When using combined evaluation of LH and TT, 57% of patients had Leydig cell dysfunction 1 year after orchiectomy. This is shown in Figure 4, where the triangles below and to the right of the curved bold line represent patients with Leydig cell dysfunction

Figure 4 Bivariate evaluation of LH and total testosterone 1 year after orchiectomy in hCG-negative patients. the triangles below and to the right of the curved bold line representing the 97.5 percentile of healthy men represent TC patients with Leydig cell dysfunction.



The present findings concerning pre-orchiectomy Leydig cell function and changes after orchiectomy, should be interpreted in the context of two studies by Petersen *et al.* from the late 1990´s [22,107]. They investigated pre-orchiectomy Leydig cell function in 30 hCG-negative TC patients compared to a group of 45 patients with malignant lymphoma and a control-group of 193 healthy men. In contrast to the present findings they did not find significant differences in testosterone levels between testicular cancer patients and healthy men and surprisingly they reported lower median LH levels in TC patients (3.6 IU/L) than in the healthy controls (4.9 IU/L). The median LH among the 30 included TC patients (3.6 IU/L) is in line with the present findings. However, median LH levels were more than 1 IU/L higher in their control group than in our control group (4.7 IU/L vs 3.3 IU/L). Thus, the conflicting findings might be due to an unrepresentative control group in the prior study.

The findings of LH increasing after orchiectomy while testosterone remains unchanged are a validation of the findings of Petersen *et al*. in their study of 48 hCG-negative TC patients [107] showing that a large proportion of patients have compensated Leydig cell insufficiency following orchiectomy.

As there was no association between advanced clinical stage and Leydig cell dysfunction, pre-orchiectomy Leydig cell dysfunction in testicular cancer patients does not seem to be due to a general cancer effect causing systemic inflammation, which have been hypothesised by others [108]. The present findings support

the hypothesis of the testicular dysgenesis syndrome (TDS) where impaired Leydig cell function as well as testicular cancer is caused by a common etiological factor [109]. The main environmental factors in TDS have been suggested to be exposures to environmental factors like endocrine disrupting substances that have an estrogenic or anti-androgenic effect on the developing testes in utero [110].

However, as an increasing tumour size was associated with Leydig cell dysfunction, it cannot be excluded, that Leydig cell dysfunction was caused by a direct tumour effect, in which the testicular tumour mechanically compromised Leydig cell function. To exclude a direct tumour effect, Leydig cell function should ideally have been evaluated before the development of a testicular tumour. As testicular cancer is a rare disease, a study investigating Leydig cell function before initiation of carcinogenesis in future TC patients would be almost impossible to conduct. Thus, the present findings demonstrate that pre-orchiectomy Leydig cell dysfunction is present in TC patients and that Leydig cell dysfunction is common in TC patients 1 year after orchiectomy. However, it would require a study investigating Leydig cell function before initiation of carcinogenesis to clarify if Leydig cell dysfunction is caused by testicular dysgenesis.

4.2 Risk factors for Leydig cell dysfunction

To characterize histological aspects of the Leydig cell compartment in testicular biopsies and to investigate if an enlarged Leydig cell compartment (Leydig cell hyperplasia) was a risk factor for long-term Leydig cell dysfunction, we investigated 50 hCG-negative patients, 10 TC patients with markedly elevated serum levels of hCG and 10 men with obstructive azoospermia and normal Leydig cell function, who served as controls [5].

We found that it is technically feasible to quantify the Leydig cell compartment in a reproducible manner by manual delineation of each Leydig cell area in a slide using an image handling software programme. As expected from earlier published studies [111] [112], patients with markedly elevated hCG had a significantly larger Leydig cell area than controls with obstructive azoospermia. In Figure 5a an example of a testicular biopsy from a control with obstructive azoospermia and normal Leydig cell area is shown with the Leydig cell area delineated in yellow, while in Figure 5b an example of a patient with highly elevated hCG and an enlarged Leydig cell area is shown.

Figure 5a A testicular biopsy from a man with obstructive azoospermia and normal Leydig cell area. The Leydig cell area is delineated in yellow.

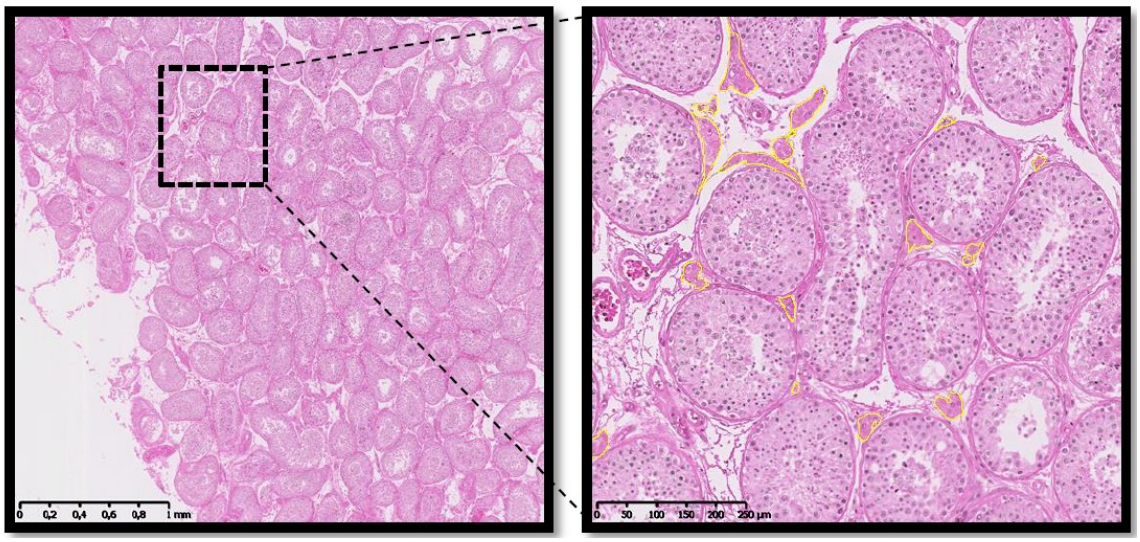
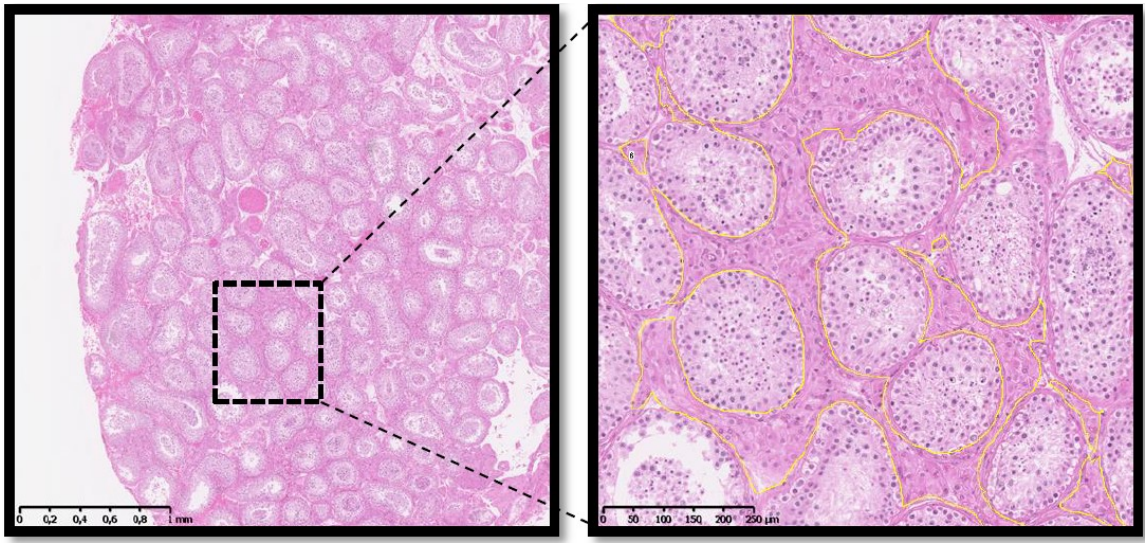


Figure 5b A testicular biopsy from a testicular cancer patient with highly elevated hCG and an enlarged Leydig cell area. The Leydig cell area is delineated in yellow.



In the 50 hCG-negative TC patients, we found an association between increasing Leydig cell area of the non-tumour bearing testicle and long-term biochemical Leydig cell dysfunction [5].

Our findings of increased Leydig cell area being associated with biochemical Leydig cell dysfunction is in line with findings in men with Klinefelter's syndrome and from infertile men [113–115], and suggests that evaluating

the Leydig cell area of the contralateral testicle in TC patients might be useful in clinical practice in order to identify patients at risk of Leydig cell dysfunction.

The Leydig cell compartment was manually delineated using an image processing software program. This was a time-consuming procedure that could possibly be automatized to make it feasible in clinical practice.

However, the clinical utility of determining whether Leydig cell hyperplasia is present remains uncertain as Leydig cell function is easily and non-invasively assessed by measuring serum levels of LH and testosterone in a blood sample.

Furthermore, small testicular size evaluated by ultrasound examination appears to be associated with Leydig cell hyperplasia [113], and small testicular size is strongly associated with Leydig cell dysfunction [6]. An ultrasonic evaluation of testicular size is easily and non-invasively performed, and thus it remains to be seen if presence of pre-orchiectomy Leydig cell hyperplasia carries independent prognostic information on the risk of future biochemical Leydig cell dysfunction.

To clarify if Leydig cell hyperplasia of the contralateral testicle is an independent risk factor for Leydig cell dysfunction and testosterone deficiency which would be of clinical utility, a prospective study including assessment of Leydig cell function and testicular size by ultrasound would be needed.

4.3 Long-term effects of testicular cancer treatment on Leydig cell function

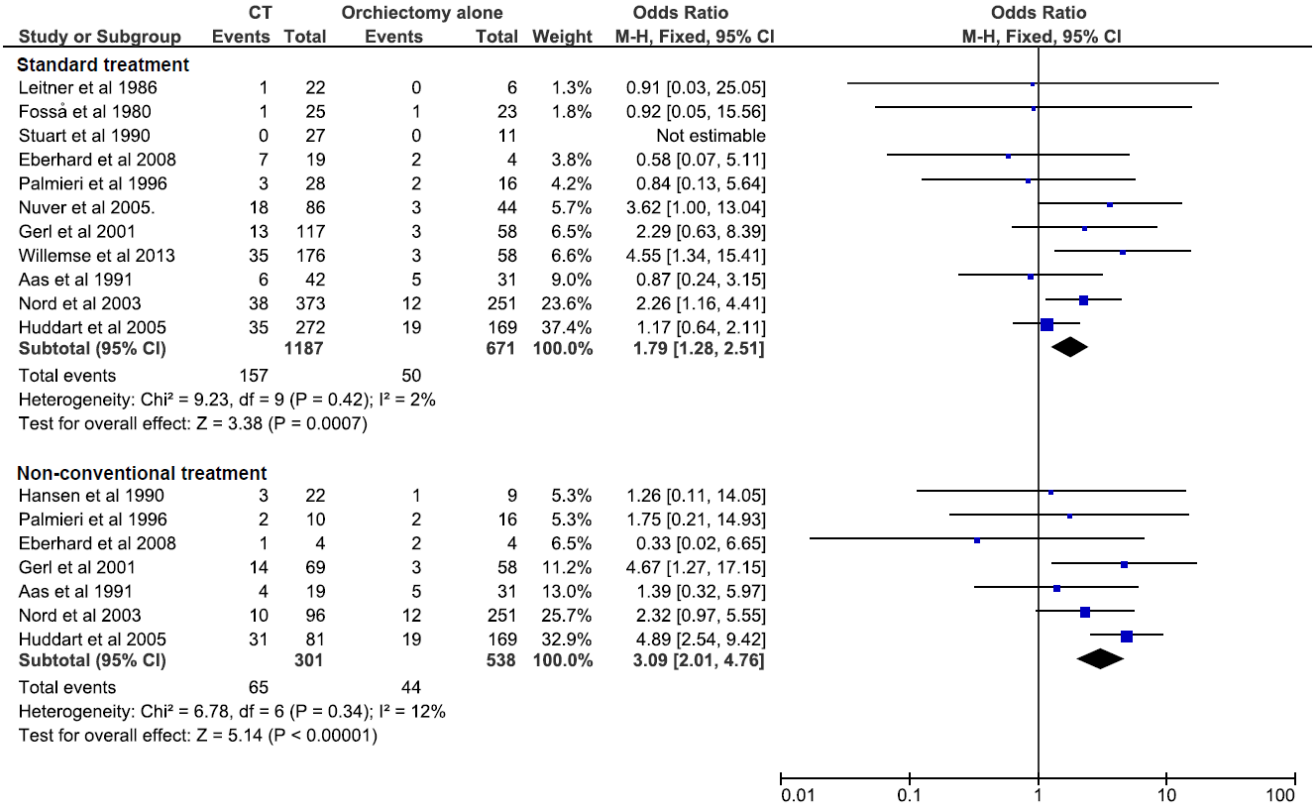
Influence of treatment for metastatic disease (chemotherapy (BEP) and radiotherapy) on Leydig cell function

To evaluate long term risk of biochemical testosterone deficiency and use of testosterone substitution in TC patients according to treatment modality, we performed a systematic review and meta-analysis [4] including 12 cross-sectional studies [41–45,48–50,52,81,116,117]..

Using stage I patients treated with orchiectomy and surveillance as reference, published data showed an increased risk of low levels of testosterone or use of testosterone substitution in patients treated with standard dose cisplatin-based chemotherapy as well as more intense chemotherapy regimens (non-conventional treatment). The results of the meta-analysis are shown in figure 6, where the combined odds ratio of testosterone deficiency or testosterone substitution is 1.79, 95% confidence interval (1.28-2.51) when comparing standard dose chemotherapy with orchiectomy alone, while the combined odds ratio,

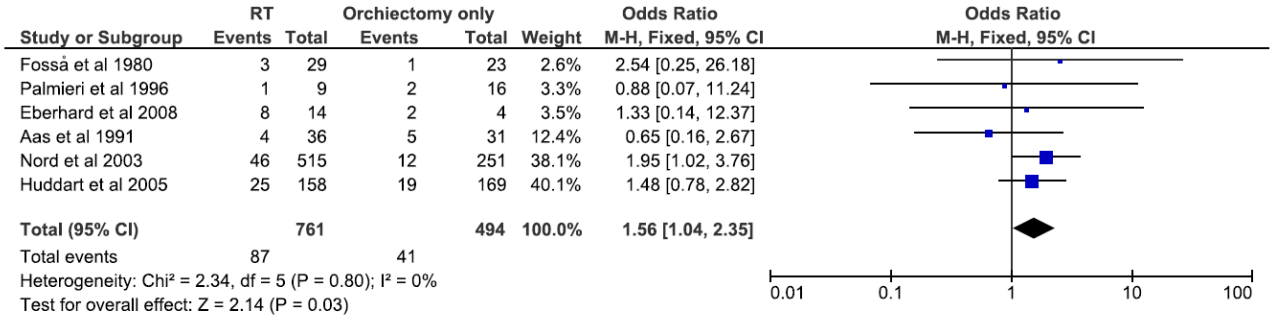
when comparing non-conventional treatment with orchiectomy alone is 3.09, 95% confidence interval (2.01-4.76)

Figure 6: Meta-analysis of studies where the risk of testosterone deficiency in patients with cisplatin-based chemotherapy or non-conventional treatment is compared with patients treated with orchiectomy alone.



In addition, an increased risk of testosterone deficiency or use of testosterone substitution was observed when comparing patients treated with abdominal radiotherapy with patients treated with orchiectomy alone (odds ratio 1.56, 95% confidence interval (1.04-2.35)). The results of the meta-analysis are shown in Figure 7.

Figure 7 Meta-analysis of studies where the risk of testosterone deficiency in patients treated with abdominal radioterapy is compared with patients treated with orchiectomy alone.



The results of the meta-analysis indicate that cisplatin-based chemotherapy and a scatter dose of radiotherapy to the remaining testicle from an abdominal radiation field pose a risk of damaging Leydig cells and thereby increases the long-term risk of testosterone deficiency.

The effect size was largest in TC patients treated with high-dose chemotherapy, chemotherapy in combination with RT and more treatment lines (non-conventional treatment). This suggests that the most heavily treated patients should be more closely followed after termination of TC treatment to detect and treat uncompensated Leydig cell insufficiency.

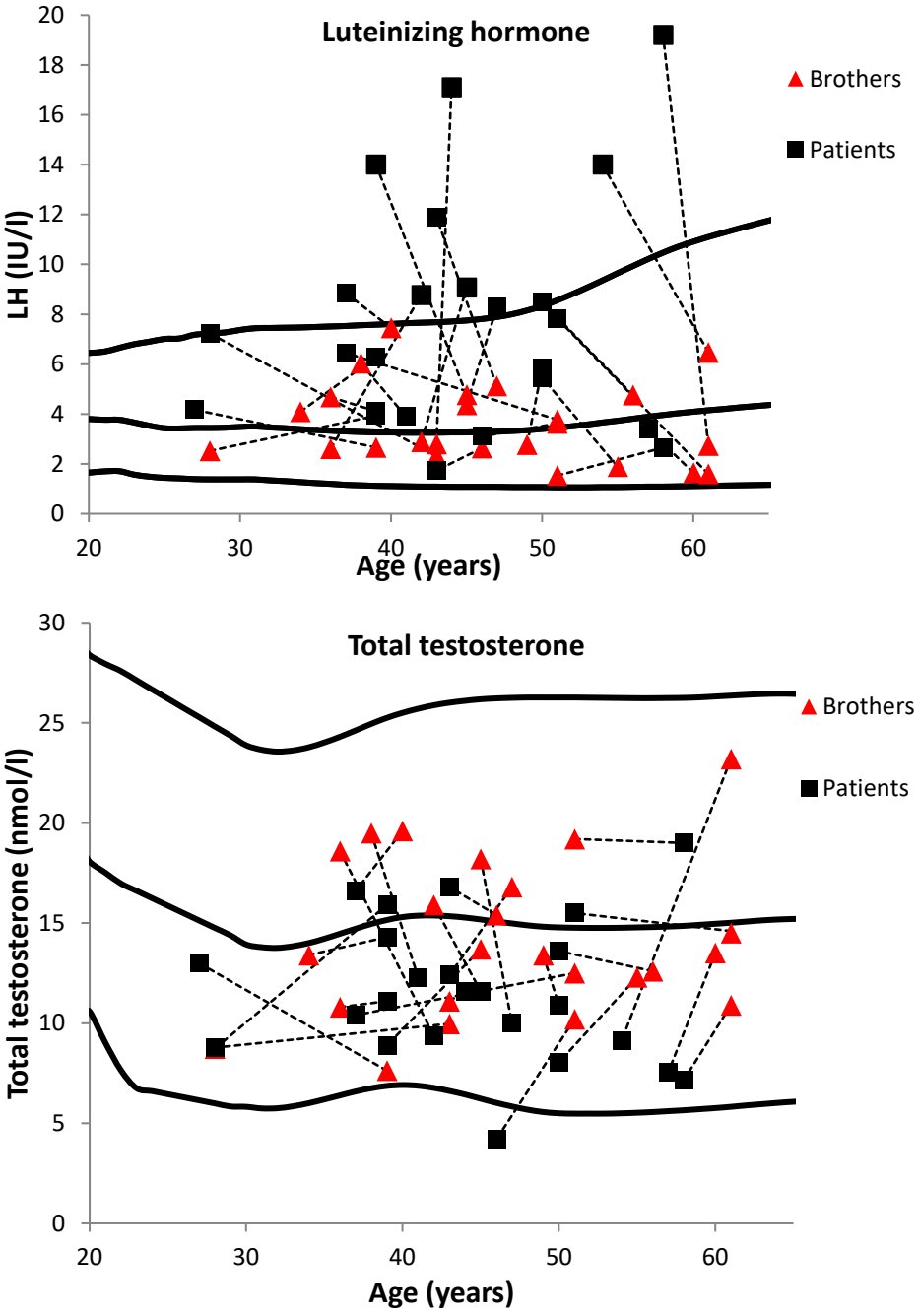
Many studies in the meta-analysis included patients treated with former radiation techniques with larger radiation doses and larger radiation fields. It has been shown that a para aortic radiation field, results in less testicular scatter dose than a dog leg field including the inguinal area [40]. It could be expected that the testicular radiation dose with modern treatment techniques including image guided therapy is less, resulting in less risk of Leydig cell dysfunction.

Comparison of Leydig cell function between TC patients and their biological brothers

In order to further investigate the testicular dysgenesis hypothesis, Leydig cell function was evaluated in 24 long-term TC survivors and compared to biological brothers and a control-group of healthy men [7]. The hypothesis was that TC survivors would have impaired Leydig cell function compared to their biological brothers, and, secondly, that brothers of TC survivors would have impaired Leydig cell function when compared to healthy men as the brothers might have shared the same environment in utero as TC patients. We found elevated LH levels and lower total testosterone levels in TC patients compared to their biological brothers [7]. The individual values of LH and total testosterone of patients (□) and brothers (Δ) are shown in

Figure 8, where each brother pair is connected with a dotted line, while the horizontal lines represent the 2.5 percentile, mean and 97.5 percentile of healthy men.

Figure 8 Serum levels of luteinizing hormone and total testosterone in 24 long-term testicular cancer survivors and their biological brothers. Lines represent the 2.5 percentile, median and the 97.5 percentile from healthy controls. (Δ represent brothers, □ represent patients, broken lines connect each brother pair).



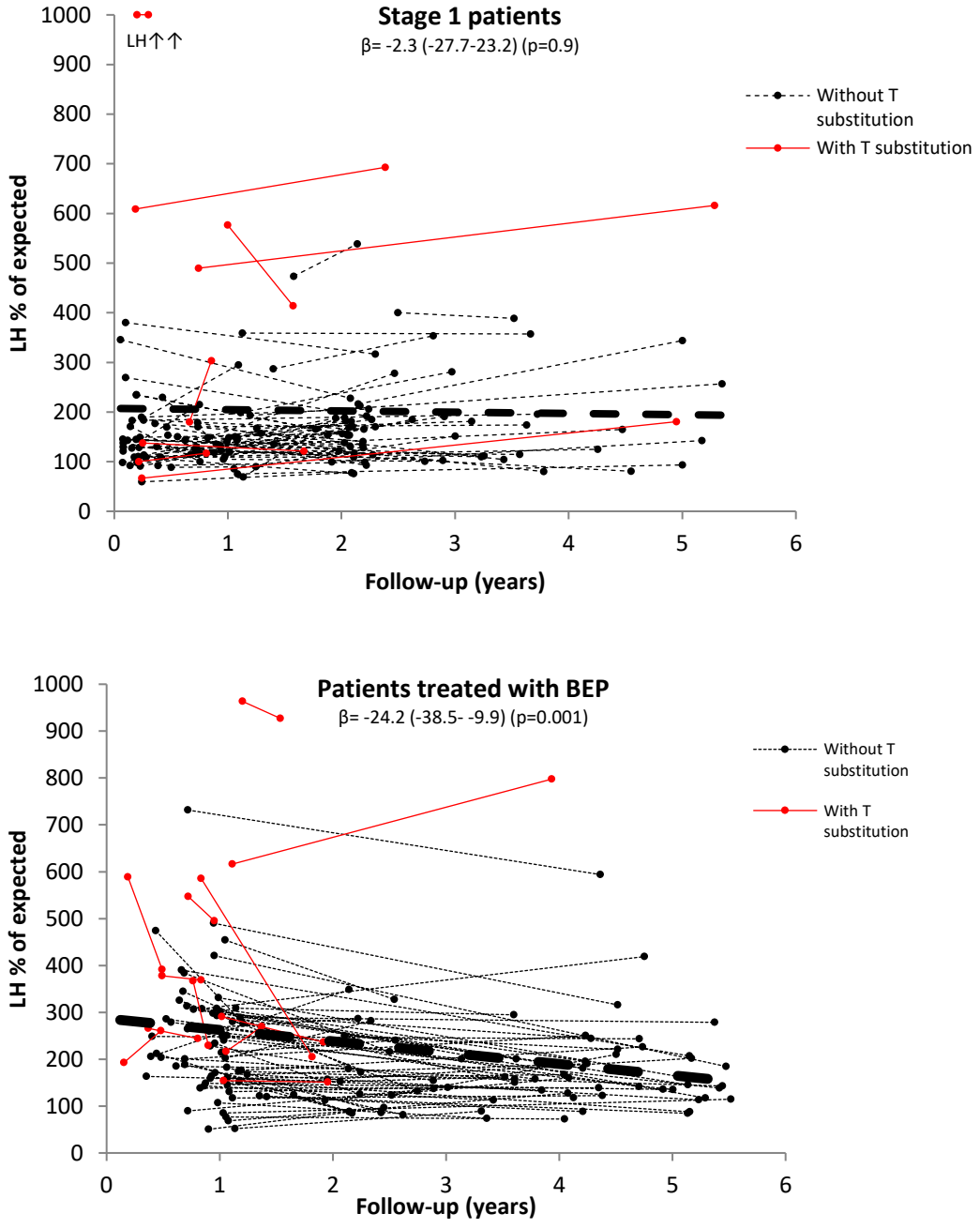
However, there was no difference in Leydig cell function between brothers of TC patients and age-matched healthy men [7].

As we did not find impaired Leydig cell function in the biological brothers to testicular cancer patients when compared to healthy men we did find support of a prenatal cause of Leydig cell dysfunction. A reason might be differences in the intrauterine environment during the pregnancies of TC patients and brothers, which could be due to temporary exposure to rapidly metabolized compounds or changes in maternal lifestyle. Ideally, to account for temporal changes in intrauterine environment and changes in lifestyle, a study with twins to TC patients should be performed. However, as there are very few such cases, an investigation would need a large international collaboration.

Longitudinal changes in Leydig cell function

In most studies on Leydig cell function in TC patients only a single assessment of testosterone and LH has been performed after TC treatment. This might be problematic, as it has been suggested that there is a degree of Leydig cell recovery in the years following TC treatment [50]. To elucidate possible changes in Leydig cell function over time, we retrospectively assessed changes in LH and TT within the 5-years follow-up program in 75 stage I TC patients and 81 TC patients treated with BEP [3]. In stage I patients, LH was around 150% of expected according to age and remained elevated throughout the follow-up period, without signs of recovery. In patients treated with BEP, LH was around 200% according to age shortly after treatment and there was a significant decline in LH to around 150% of expected according to age at the end of 5-years follow-up. The longitudinal changes in LH in the two groups of patients are shown in Figure 9, where the bold dotted line represent the mean value of all patients, while the red lines represent patients who later initiated testosterone substitution. The findings indicate an additional toxic effect of BEP on Leydig cell function with a degree of Leydig cell recovery, although LH remained considerably elevated. Testosterone remained at around 80% of expected in the two groups throughout the 5-years follow-up program [3].

Figure 9: Changes in total testosterone and luteinizing hormone (LH; % of predicted from age-matched controls) within 5-yr follow-up. Each black dotted line represents the change of an individual patient who did not initiate testosterone (T) substitution, while each red line represents the change of a patient who initiated T substitution during follow-up (total T and LH values before initiation of T substitution). The fat dotted-line represents the results of a linear regression analysis, with the beta-value representing the annual change with corresponding 95% confidence interval and p value.



In total, 11% of stage I patients and 15% of TC patients treated with BEP initiated testosterone substitution with the majority experiencing hormonal deterioration shortly after treatment.

When using bivariate evaluation of LH and TT, TC patients with a normal Leydig cell function within the first year of TC treatment had very little risk of future testosterone substitution. Thus, only 4% of TC patients with normal combined evaluation of LH and TT initiated testosterone substitution within the 5-years follow-up program [3].

Our findings of persistently elevated LH levels after orchiectomy alone and after BEP is in line with several of the above-mentioned studies [49–51,53] and suggest that an increased LH-drive is continuously needed after TC treatment to keep testosterone within its normal range. However, as there was a degree of Leydig cell recovery in BEP treated patients, referral to andrological evaluation could be deferred to after 6 months after TC treatment in these patients to allow for the pituitary Leydig cell axis to adjust. Other studies have shown a similar partial recovery in patients treated with abdominal RT [50,53]. The negative predictive value of 96% of an LH/T relation within the 97.5 percentile of healthy men and future risk of testosterone substitution, suggests that routine assessment of Leydig cell function might be omitted in TC patients with normal Leydig cell function within the first year after TC treatment. However, the data is retrospectively collected and should preferably be validated in a prospective study.

In Denmark, Leydig cell function is regularly assessed in TC patients within the 5-years follow-up programme. In case of persistently low levels of testosterone in combination with elevated LH and symptoms compatible with testosterone deficiency, patients will be offered testosterone substitution. In our questionnaire survey of around 2500 TC survivors after a median of 18 years follow-up,5% of patients received testosterone substitution [118]. This proportion is in line with Norwegian findings in 1235 long-term TC survivors where 52 (4%) received testosterone substitution after a median follow-up of 11 years. However, as Leydig cell function was not routinely assessed in the follow-up program before around the year 2000 we do not know if all TC patients with testosterone deficiency are treated. According to the present findings[3,4], we estimate that around 5-15% of unilateral TC survivors will need testosterone substitution in the years following TC treatment with increasing risk according to treatment intensity [118].

Based on the present evidence we propose that Leydig cell function is routinely assessed according to the following schedule in TC patients within the routine 5-years follow-up program after testicular cancer treatment.

	6 months	1 year	2 years	3 years	4 years	5 years
LH and testosterone	**x**	**x**				**x**

- Unless there are clear symptoms compatible with testosterone deficiency within the 1 year after treatment, reference for andrological evaluation can be omitted to >1 year after treatment as a degree of Leydig cell recovery could be expected.

- If Leydig cell function is within the 97.5 percentile of healthy men 1 year after treatment, routine assessment of Leydig cell function might be omitted

- In case of symptoms of testosterone deficiency any time within the 5-years follow-up program, Leydig cell function should be evaluated.

4.4 Is Leydig cell dysfunction and testicular cancer treatment associated with systemic inflammation and metabolic syndrome?

Leydig cell dysfunction, testicular cancer treatment and risk of metabolic syndrome

As described in the previous chapter, Leydig cell dysfunction, especially persistently elevated LH with slightly decreased testosterone, is common in TC patients. If Leydig cell dysfunction is associated with increased long-term risk of systemic inflammation, metabolic syndrome and subsequently cardiovascular disease, it could be suggested that testosterone substitution should be initiated in these patients to avoid future side effects.

To elucidate this issue we investigated 158 testicular cancer patients of whom 28 had uncompensated Leydig cell insufficiency, 59 had compensated Leydig cell insufficiency and 71 had normal Leydig cell function within the routine 5-years follow-up program [6]. None of the patients received testosterone substitution. We used two different definitions of metabolic syndrome: The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) definition [101], which is most widely used , and the newer International Diabetes Federation (IDF) definition [102]. Briefly, the main difference between the two definitions (NCEP-ATPIII vs IDF) is cut-off vales for elevated fasting blood glucose (≥6.1 mmol/L vs ≥5.6 mmol/L) and abdominal obesity (>102 cm vs >94 cm). Using the NCEP-ATPIII definition, 11% of patients with uncompensated Leydig cell insufficiency, 7% of patients with compensated Leydig cell insufficiency and 17% of patients with normal Leydig cell function *within the routine follow-up program* had metabolic

syndrome after a median of 10 years follow-up. When using the IDF criteria for definition of metabolic syndrome, the prevalence was 33%, 12%, 27% in the three groups. The difference between TC patients with compensated Leydig cell insufficiency and TC patients with normal Leydig cell function (12% vs 27%) (IDF-definition) was statistically significant (p=0.04) [6], while the difference between patients with uncompensated Leydig cell insufficiency and normal Leydig cell function (33% vs 27%) was not statistically significant (p=0.5). There was no difference between the groups when using the NCEP-ATP III definition of metabolic syndrome [6]. The individual metabolic syndrome components and the prevalence of metabolic syndrome in the three groups are presented in table 3

Table 3

Outcomes	**Uncompensated Leydig cell insufficiency (n=28)**	**Compensated Leydig cell insufficiency (n =59)**	**Normal Leydig cell function (n=71)**	**P-value***
Metabolic syndrome outcomes				
Systolic blood pressure (mmHg)	127 (121-137)	127 (120-137)	127 (120-141)	0.6/0.8
Diastolic blood pressure (mmHg)	83 (79-86)	80 (73-86)	81 (75-86)	0.6/0.7
Waist circumference (cm)	99 (91-107)	89 (86-96)	93 (87-101)	0.3/0.02
Fasting blood glucose (mmol/l)	5.4 (5.0-6.0)	5.3 (4.9-5.5)	5.3 (5.0-5.7)	0.3/0.4
HDL-cholesterol (mmol/l)	1.3 (1.1-1.6)	1.4 (1.2-1.6)	1.3 (1.1-1.6)	0.7/0.5
Triglyceride (mmol/l)	1.1 (0.9-1.9)	1.0 (0.8-1.2)	1.0 (0.8-1.6)	0.2/0.4
Metabolic syndrome (NCEP-ATPIII)	3 (11%)	4 (7%)	12 (17%)	0.5/0.08
Metabolic syndrome (IDF)	9 (33%)	**7 (12%)**	**19 (27%)**	0.5/**0.04**

*Uncompensated Leydig cell insufficiency vs normal Leydig cell function/ Compensated Leydig cell insufficiency vs normal Leydig cell function,

It is a surprising finding that TC patients with compensated Leydig cell insufficiency had *decreased* long-term risk of metabolic syndrome compared to patients with normal Leydig cell function. Obesity and insulin resistance are the core components in metabolic syndrome and it has been shown that severely obese men with BMI>40 have low LH levels [119]. However, on the contrary it is not known if a *low* BMI is associated with increased LH, which could explain the present finding. In [6] low LH within the follow-up program was not significantly associated with long-term risk of metabolic syndrome, although the odds ratio was pointing in that direction (odds ratio= 0.93, 95% confidence interval 0.85-1.009), p=0.08.

Testicular cancer treatment and metabolic syndrome

Eight cross-sectional studies and [6,7] have evaluated the prevalence of metabolic syndrome in testicular cancer survivors according to treatment modality with or without a comparison group of healthy men. The studies differ in median follow-up time since TC treatment (5 years to 19 years), in definition of metabolic syndrome and there are important differences in the characteristics of control groups. Most studies used the NCEP-ATP III criteria for definition of metabolic syndrome, while some studies used a modified definition of NCEP-ATP III as fasting blood glucose was not available. The characteristics and findings of the studies are summarized in Table 4.

Table 4

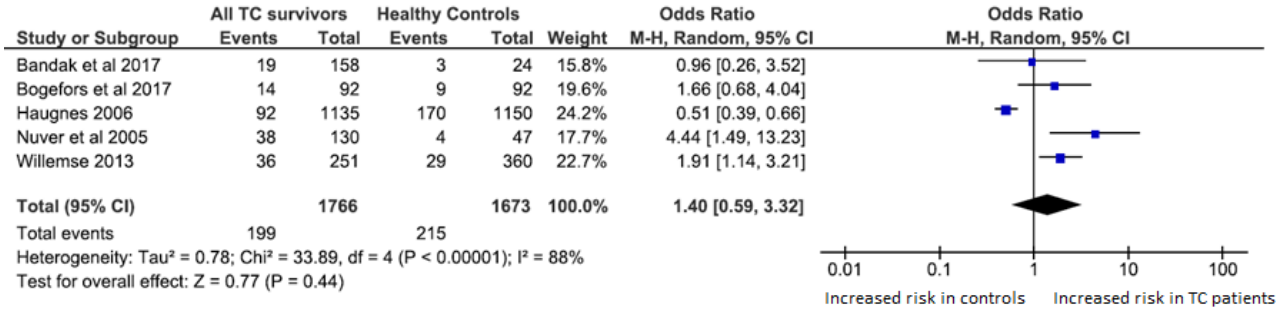
Parameters	**Nuver, 2005**[81]	**Haugnes, 2006**[82]	**Wethal, 2007**[72]	**De Haas, 2013**[83]	**Willemse,2013**[52]	**Zaid, 2018**[85]	**Bogefors, 2017**[84]	**Bandak, 2018**[6,7]	**Haugnes, 2010**[74]
Number of patients Mean age (years) Mean follow-up (years) Number of healthy subjects	130 38 (10) 7 (3-13) 47	1135 40 11 1150	589 42 11 8835	173 37 (19-59) 5 (3-20) 1085	251 40 (18-70) 8 (0.1-30) 360	486 38 (19-68) 5 (0.4-24) 486	92 40 (7) 9 92	158 43(38-50) 10 (4-17) 24	862 51 (31-69) 19 (13-28)
Definition of metabolic syndrome	NCEP-ATPIII	*Modified NCEP-ATPIII*	*Modified NCEP-ATPIII*	American Heart Association	NCEP-ATPIII	*Modified NCEP-ATPIII*	NCEP-ATPIII	NCEP-ATPIII	NCEP-ATPIII
Prevalence of metabolic syndrome (%)									
Healthy subjects	4 (9)	170 (15)	Around 15%	145 (13)	29 (8)	109 (22)	9 (10)	3 (13)	
All included TC survivors	38 (29)	92 (8)			36 (14)		14 (15)	19 (12)	281 (33)
Surveillance	16 (36)	15 (7)	9 (6)		5 (9)		1 (8)		56 (32)
Carboplatin single dose					2 (10)		2 (33)		
Chemo (standard dose)	22 (26)	30 (8)	39 (18)	44 (25)	29 (17)	102 (21)	0 (0)		105 (34)
High dose chemotherapy/ Chemo + RT		11 (13)							10 (34)
Radiotherapy		37 (8)	35 (15)						110 (32)

The first published study by Nuver *et al*. in 2005 [81] was characterized by a low prevalence of metabolic syndrome in the 47 healthy subjects constituting the control group (9%) in combination with a high prevalence among TC survivors (29%), especially in the surveillance group (36%), which was higher than in the group of patients treated with cisplatin-based chemotherapy (26%). In this study, the NCEP-ATPIII definition was applied. The next published study was by Haugnes *et al.* in 2006 [82] including 1135 TC survivors with a median age of 40 years investigated after 11 years of follow-up and compared to 1150 men from a population based study. Here, a modified NCEP-ATPIII definition was applied as fasting plasma glucose was not available. The study was characterized by a generally lower prevalence of metabolic syndrome and a relatively high prevalence of metabolic syndrome in the control group (15%), compared to the included TC survivors (8%). Haugnes *et al* performed a second analysis of the same cohort in 2010 with a median age of 51 years among included TC survivors and 19 years of follow-up[74]. In this study, fasting blood glucose was available, and a strict NCEP-ATP-III definition was applied. The prevalence of metabolic syndrome was now 33%, with no difference according to treatment modality. Even when taken older age into account (median age 51 years vs median age 40 years), the difference in prevalence of metabolic syndrome (33% vs 8%) is considerable, emphasizing the limitations of comparison studies with different definitions of metabolic syndrome.

To summarize the evidence on metabolic syndrome in TC patients according to treatment modality and in comparison with age-matched men, three separate meta-analyses were performed: 1) A meta-analysis including studies with TC patients irrespectively of treatment compared to control-groups of healthy men, 2) a meta-analysis including studies with TC patients treated with cisplatin-based chemotherapy vs healthy men, and 3) a meta-analysis including studies with TC patients treated with cisplatin-based chemotherapy versus stage I patients treated with orchiectomy alone. The studies by Wethal *et al* [72], and Haugnes *et al 2010* [74] were not included in the meta-analysis as they included the same patients as Haugnes *et al* 2006 [82].

The results of studies comparing TC patients irrespectively of treatment with healthy controls are presented in Figure 10a.

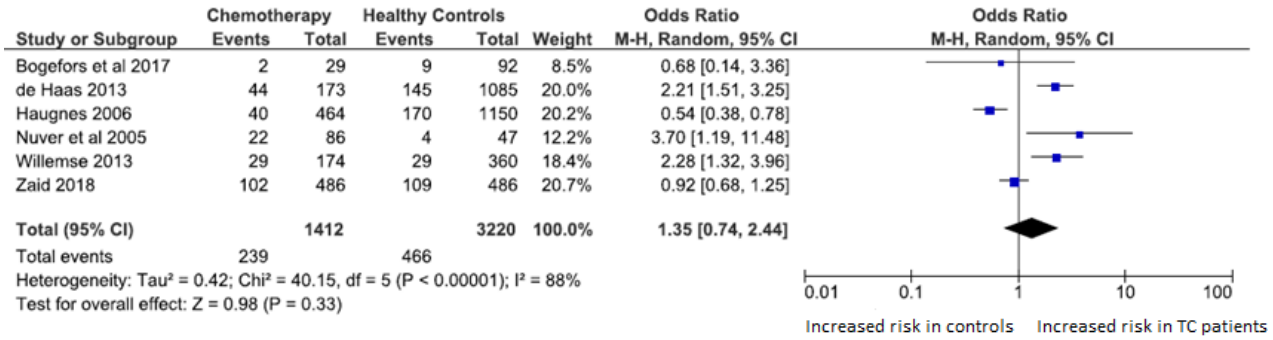
Figure 10a Meta-analysis of studies comparing the risk of metabolic syndrome in TC patients irrespectively of treatment with healthy controls



The summarized odds ratio is 1.40 95% confidence interval (0.59-3.32) suggesting that there is no clear evidence of an increased risk of metabolic syndrome in TC survivors compared to age-matched men. It should be noted that there is a high degree of heterogeneity between studies suggested by the I^2 of 88% and therefore a random effect model was applied. As it can be seen from table 1 and figure 1, the heterogeneity appears mainly to be driven by the findings by Haugnes *et al* 2006 where the prevalence of metabolic syndrome was 15% among healthy controls compared to 8% among included TC survivors (odds ratio 0.51 95% CI (0.39-0.66))

The results of studies comparing TC patients treated with cisplatin-based chemotherapy with healthy controls are presented in Figure 10b.

Figure 10b Meta-analysis of studies comparing TC patients treated with cisplatin-based chemotherapy with healthy controls

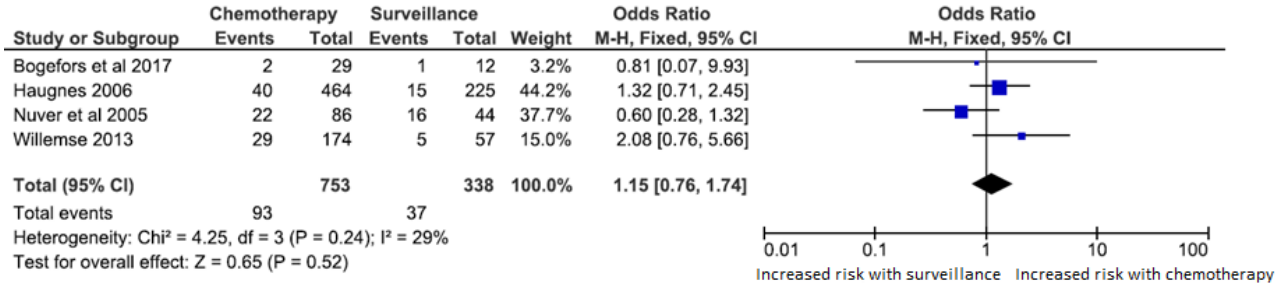


The summarized odds ratio is 1.35 95% CI (0.74-2.44) suggesting that there is no clear evidence of increased risk of metabolic syndrome in TC survivors treated with cisplatin-based chemotherapy compared

to healthy men. Again, there is a high degree of heterogeneity between studies suggested by the I^2 of 88% and therefore a random effects model was applied. In the three Dutch studies, there is a significantly increased risk of metabolic syndrome, while again, in the study by Haugnes et al 2006, the risk is significantly lower among TC-survivors.

Finally, the results of studies comparing TC patients treated with cisplatin-based chemotherapy with TC patients treated with orchiectomy alone are presented in Figure 10c.

Figure 10c Meta-analysis of studies comparing TC patients treated with cisplatin-based chemotherapy with TC patients treated with orchiectomy alone



The summarized odds ratio is 1.15 95% CI (0.76-1.74), suggesting that there is no clear evidence of an increased risk of metabolic syndrome in TC patients treated with cisplatin-based chemotherapy compared to orchiectomy and surveillance. There is less heterogeneity between studies suggested by the I^2 of 29%, and here, a fixed effect model was applied.

Thus, based on the current evidence, it cannot be generally concluded that testicular cancer patients have increased risk of metabolic syndrome when compared to age-matched non-cancerous men, neither can it be concluded that cisplatin-based chemotherapy increases the risk of metabolic syndrome compared to orchiectomy alone.

Testosterone deficiency and metabolic syndrome

In all included studies of the meta-analysis as well as in [6] there was a significant association between low levels of total testosterone and metabolic syndrome. This association has also been reported in several population-based studies [60,62,63,120]. However, there are some important caveats when interpreting the relationship between testosterone and metabolic syndrome: In TC survivors, it was reported in cross-sectional studies only, limiting the ability to draw causal conclusions, and in [6] there was no significant association between low levels of testosterone within the follow-up programme and future risk of metabolic syndrome. Furthermore, it is well established that obesity and insulin resistance which are the

core components of metabolic syndrome are associated with low levels of total testosterone and low levels of SHBG [121]. The mechanism appears to be inhibition of hepatic SHBG synthesis and inhibition of testosterone production by the Leydig cells by increasing plasma levels of insulin [122,123]. Accordingly, obesity and increased plasma insulin might be causing low levels of total testosterone rather than the other way around. This causal relationship is partly supported by the findings of a meta-analysis of individual participant data of cross-sectional and prospective studies evaluating the association between testosterone, SHGB and metabolic syndrome [59]. In this study, the association between low testosterone and low SHBG and metabolic syndrome was much weaker in prospective studies than in cross-sectional studies suggesting that low testosterone and low SHBG might be a result of metabolic syndrome rather than low testosterone and SHBG causing metabolic syndrome. On the contrary, it is well established that men with prostate cancer who are treated with medical or surgical castration are at increased risk of developing abdominal obesity and insulin resistance (refs) and several randomized clinical trials have found improvements of metabolic syndrome components in hypogonadal men treated with testosterone substitution [124–128]. However, on the other hand, randomized studies in men with metabolic syndrome have found increasing levels of testosterone after weight loss [129,130].

In conclusion, the current evidence suggests that there is a bidirectional relationship between testosterone and metabolic syndrome [131].

Systemic inflammation

We evaluated systemic inflammation by assessing the inflammatory markers high sensitivity CRP, tumour necrosis factor-α, interleukin 1β, interleukin 6, interleukin 8 and micro-albuminuria after 10 years follow-up in patients who had compensated Leydig cell insufficiency, uncompensated Leydig cell insufficiency, or normal Leydig cell function within the follow-up program [6]. The findings are summarized in table 5.

Table 5

Outcomes	**Uncompensated Leydig cell insufficiency (n=28)**	**Compensated Leydig cell insufficiency (n =59)**	**Normal Leydig cell function (n=71)**	**P-value***
Inflammatory markers				
Tumor necrosis factor alpha (pg/ml)	2.1 (1.7-2.4)	2.1 (1.8-2.5)	2.2 (2.0-2.6)	0.2/0.1
Interleukin 1β (pg/ml)	0.03 (0.01-0.04)	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.8/0.5
Interleukin 6 (pg/ml)	0.4 (0.3-0.6)	0.4 (0.3-0.5)	0.3 (0.3-0.6)	0.9/0.9
Interleukin 8 (pg/ml)	4.1 (3.3-5.6)	4.2 (3.4-5.4)	3.7 (2.9-4.8)	0.7/0.3
Micro-albuminuria (mg/l)				
<3	13 (46%)	24 (41%)	24 (34%)	
3-10	8 (29%)	25 (42%)	29 (41%)	
>10	7 (25%)	10 (17%)	18 (25%)	0.4/0.5
High-sensitivity C-Reactive Protein (mg/l)				
<1	**9 (33%)**	34 (58%)	**46 (65%)**	
≥1-3	**14 (52%)**	18 (31%)	**16 (23%)**	
>3	**4 (15%)**	7 (12%)	**9 (13%)**	**0.01**/0.6

*Uncompensated Leydig cell insufficiency vs normal Leydig cell function/ Compensated Leydig cell insufficiency vs normal Leydig cell function

In total, 64% of patients with uncompensated Leydig cell insufficiency had CRP levels ≥ 1 mg/L, while 36% of patients with normal Leydig cell function had CRP levels ≥ 1 mg/L. This difference was statistically significant, while there was no difference in the other inflammatory markers between the groups.

Patients with metabolic syndrome did not have higher levels of inflammatory markers compared to patients without metabolic syndrome, which is presented in table 6.

Table 6

Outcomes	**Metabolic syndrome absent (n=121)**	**Metabolic syndrome present (n=35)**	**P-value**
Inflammatory markers			
Tumor necrosis factor alpha (pg/ml)	2.19 (1.81-2.54)	2.17 (1.77-2.77)	0.3
Interleukin 1β (pg/ml)	0.02 (0.01-0.04)	0.02 (0.01-0.03)	0.4
Interleukin 6 (pg/ml)	0.36 (0.28-0.54)	0.37 (0.28-0.72)	0.7
Interleukin 8 (pg/ml)	4.01 (3.16-5.17)	4.18 (2.80-5.40)	0.5
Micro-albuminuria (mg/l)			
<3	52 (43%)	9 (26%)	
3-10	46 (38%)	16 (46%)	
>10	24 (20%)	10 (29%)	0.2
Hs-CRP (mg/l)			
<1	70 (58%)	19 (54%)	
≥1-3	38 (31%)	10 (29%)	
>3	13 (11%)	6 (17%)	0.6

We did not find a clear indication of Leydig cell dysfunction being associated with systemic inflammation in TC patients, and no other studies have evaluated this association in TC patients.

The association between uncompensated Leydig cell insufficiency and long-term risk of elevated hsCRP, should preferably be validated in prospective trial.

Testicular cancer treatment and systemic inflammation

Three relatively large cross-sectional studies have investigated systemic inflammation and serum markers of endothelial dysfunction in long-term testicular cancer survivors [72–74].

In a cross-sectional study of 134 Dutch TC survivors of whom 90 were treated with cisplatin-based chemotherapy and 44 with orchiectomy alone a after median 7 years follow-up and a control-group of 47 healthy men, Nuver et al. reported increased prevalence of microalbuminuria and elevated levels of high sensitivity CRP and increased levels of serum markers of endothelial damage (Fibrinogen, von Willebrand factor, PAI-1, t-PA) in patients treated with chemotherapy compared to healthy controls [73].

In a cross-sectional study of 589 Norwegian long-term TC survivors of whom 140 were treated with surveillance, 231 with radiotherapy and 218 with cisplatin-based chemotherapy, Wethal et al. found increased levels of hsCRP and vWF in patients treated with radiotherapy compared to surveillance while there was no difference in hsCRP and vWF between TC patients treated cisplatin-based chemotherapy and surveillance [72].

Similarly, in a cross-sectional study of 990 Norwegian patients after a median follow-up of 19 years, of whom 206 were treated with surveillance, 386 with radiotherapy, 364 with chemotherapy only and 34 with combined RT + chemotherapy, Haugnes et al. reported increased serum levels of hs-CRP in patients treated with radiotherapy and radiotherapy + chemotherapy, while hs-CRP was not increased in patients treated with chemotherapy alone [74].

Thus, it remains unclear if testicular cancer survivors treated with cisplatin-based chemotherapy have increased risk of systemic inflammation and endothelial dysfunction compared to patients treated with orchiectomy alone. Also, it remains unclear if metabolic syndrome, endothelial dysfunction and systemic inflammation is a causally linked to cardiovascular disease in testicular cancer patients treated with cisplatin-based chemotherapy. Large prospective trials are needed to elucidate causal relations between testicular cancer treatment, Leydig cell dysfunction, systemic inflammation and metabolic syndrome.

4.5 Impact of testicular cancer treatment on sexual function

To clarify different aspects of sexual function in TC patients according to treatment modality, we performed a cross-sectional study of 2260 long-term testicular survivors assessing self-reported sexual function using the International Index of Erectile Function-15 (IIEF-15) questionnaire at a median follow-up of 17 years

since diagnosis [118]. Around 1000 patients treated with orchiectomy and surveillance alone served as control group. We found increased risk of erectile dysfunction in patients treated with radiotherapy, BEP with or without post chemotherapy retroperitoneal surgery, and in patients who had received more than one treatment line compared to surveillance alone. The prevalence of erectile dysfunction in surveillance alone was 15% compared to 30% in patients who had received more than one treatment line. In addition, we found increased risk of orgasmic dysfunction in all treatment groups except in patients treated with BEP without post chemotherapy surgery when compared to surveillance. We did not find decreased sexual desire, decreased intercourse satisfaction and decreased overall satisfaction according to treatment modality, apart from decreased overall satisfaction in patients treated with abdominal RT [118]. The findings are summarized in table 7

Table 7

IIEF-15 outcomes	**Adjustment for age** Odds Ratio (95% confidence interval)
Erectile dysfunction	
Surveillance (reference)	1
BEP alone	1.5 (1.0-2.1) P<0.05
BEP with post chemo surgery	2.1 (1.4-3.4) P<0.005
Radiotherapy	1.7 (1.1-2.5) P<0.05
More than one line of treatment	3.2 (1.6-6.3) P<0.005
Orgasmic dysfunction	
Surveillance (reference)	1
BEP alone	1.2 (0.9-1.6)
BEP with post chemo surgery	1.5 (1.0-2.1) P<0.05
Radiotherapy	1.4 (1.0-1.9) P<0.05
More than one line of treatment	2.8 (1.6-4.7) P<0.005
Decreased overall satisfaction	
Surveillance (reference)	1
BEP alone	1.1 (0.9-1.4)
BEP with post chemo surgery	1.0 (0.8-1.4)
Radiotherapy	1.4 (1.1-1.9) P<0.05
More than one line of treatment	1.6 (1.0-2.6)
Decreased intercourse satisfaction	
Surveillance (reference)	1
BEP alone	1.2 (0.9-1.6)
BEP with post chemo surgery	1.3 (0.8-1.9)
Radiotherapy	0.9 (0.6-1.4)
More than one line of treatment	1.7 (0.9-3.4)
Decreased sexual desire	
Surveillance (reference)	1
BEP alone	1.2 (0.9-1.6)
BEP with post chemo surgery	1.1 (0.7-1.7)
Radiotherapy	1.0 (0.7-1.5)
More than one line of treatment	1.2 (0.6-2.3)

Apart from two reviews around the millennium [132133] and several studies with less than 200 participants or using unvalidated questionnaires [134–140] , four studies with >200 patients have assessed sexual function in TC survivors with validated questionnaires addressing the impact of different treatment modalities with or without comparison with a control group [50,88–90]. The results of the four studies are summarized in table 8:

Table 8

	Huddart, 2005[50]	**Dahl, 2007**[88]	**Rossen, 2012**[89]	**Kim, 2012**[90]	**Bandak, 2017**[8]	**Pallotti, 2019**[87]
Study design	Cross-sectional	Cross-sectional	Cross-sectional	Case-control	Cross-sectional	Longitudinal
Total number of patients	680	1084	401	246	2260	241
Age[1] years (range/SD)	44 (23-78)	43 (8)	47 (10)	18-50+ (mean unknown)	53 (46-61)	31 (at diagnosis)
Follow-up[1] years (range/SD)	10 (0-20)	11 (5-21)	12 (3)	14	17 (12-24, IQR)	Baseline, 6, 12, 24, 48 months and 8 years
-Surveillance	169	104	204	Not stated	1098	
- RPLND		140				
-Cisplatin-based chemotherapy (with or without RPLND)	272	343	150	Not stated	788	241
-Abdominal radiotherapy	158	497	47		300	
-More lines of treatment	81				74	
-Healthy controls	n/a	929	n/a	236	n/a	223
Method used for evaluation of sexual function	EORTC-QLQ-C30, testicular module	Brief Male Sexual Function Inventory	Six questions from EORTC-QLQ-PR25	Brief Male Sexual Function Inventory	International Index of Erectile dysfunction (IIEF-15)	International Index of Erectile dysfunction (IIEF-15)
Prevalence of erectile dysfunction	n/a	8% in young testicular cancer survivors and 19% in middle-aged testicular cancer survivors	18% in all included testicular cancer survivors	n/a	18% in all included TC survivors	n/a
Sexual function better		Overall satisfaction with sexual life in young testicular cancer survivors[5]				
Sexual function not different	Feeling of masculinity[2,3,4] Sexual activity[2,3,4] Erectile function[2,3,4] Dry ejaculation[2,3,4]	Erectile function [2,3,5] Sexual drive [2,3,5]	Erectile function[2,3] Sexual enjoyment[2,3]	Sexual drive[5] Overall sexual satisfaction[5]	Sexual desire[2,3,4] Intercourse satisfaction[2,3,4] Overall satisfaction[3,4]	Orgasmic function[5]
Sexual function worse	Enjoyment with sexual life[2] Worries about fathering a child[3] Interest in sex[4]	Ejaculatory function [3,5]	Ejaculatory function[6]	Erectile function[5] Ejaculatory function[5]	Overall satisfaction[2] Erectile function[2,3] Orgasmic function[2,3] (except chemotherapy without post-chemotherapy surgery)	Erectile function[5] Sexual drive[5] Intercourse satisfaction[5] Overall satisfaction[5]

[1]Mean/median, [2]radiotherapy compared to surveillance, [3] chemotherapy compared to surveillance, [4] more lines of treatment compared to surveillance, [5] All included TC survivors compared to controls [6] After post-chemotherapy surgery compared to surveillance

In summary, the findings are conflicting and difficult to summarize: Three studies had a control group of age-matched men, and in two of these studies [88,90] sexual function was assessed with the Brief Male Sexual Function Inventory. Both of these studies found an increased risk of orgasmic problems in testicular cancer survivors, while there was no indication of decreased sexual desire or decreased overall sexual satisfaction - on the contrary, overall sexual satisfaction was higher in young TC patients than in controls in the Norwegian study[88].

Erectile dysfunction was increased in the American study, while not in the Norwegian study. However, among older patients in the Norwegian study, the odds ratio of erectile dysfunction was 1.51, 95% confidence interval (0.99–2.31), indicating a possibly increased risk.

In the third longitudinal study, all TC patients had received 1-3 cycles of BEP and sexual function was assessed with the IIEF-15 questionnaire and compared to a control group of men who were undergoing andrological screening for idiopathic primary infertility. Here, TC patients had significantly lower sexual drive, intercourse satisfaction, overall satisfaction and lower scores on erectile function scale compared to controls throughout the follow-up period, while there was no difference in orgasmic function.

Thus, the findings of the longitudinal study conflicts with the two earlier mentioned studies, which might be due to the control group, that consisted of men who were evaluated for infertility. Intuitively, it could be expected that these men had a higher prevalence of sexual dysfunction related to infertility, but they all had close to maximum scores on all the five dimensions of the IIEF-15 indicating little prevalence of sexual dysfunction, which might explain the relatively lower scores of TC-patients[87]. Another possible explanation could be differences in the questionnaire measures used for assessment of sexual function.

Concerning the impact of treatment modality, we found a clear indication of increased risk of erectile dysfunction and orgasmic dysfunction according to treatment intensity in our large cross-sectional study, where the IIEF-15 was applied for assessment of sexual function [118]. The finding of increased risk of orgasmic dysfunction in patients treated with BEP with post-chemotherapy surgery is a validation of the Norwegian findings. However, in their study, there was no increased risk of orgasmic dysfunction in patients treated with radiotherapy, and no indication of erectile dysfunction according to treatment intensity.

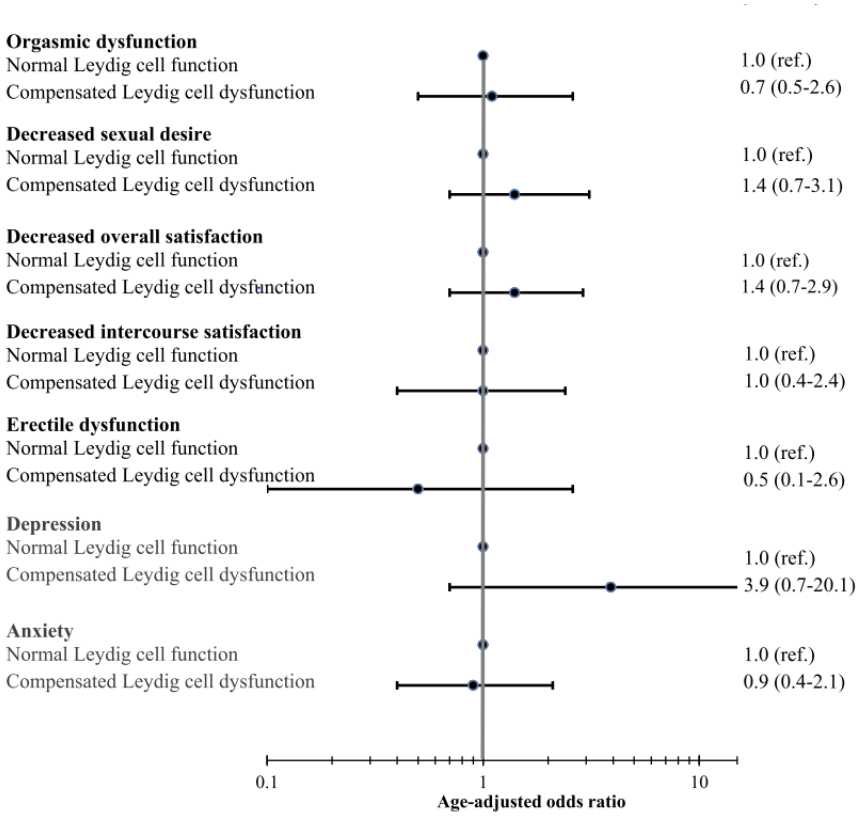
In conclusion, the findings concerning sexual dysfunction in TC survivors are not clear. However, large population-based studies seem to indicate that overall sexual satisfaction and sexual desire is not significantly different in TC survivors than in age-matched men and does not appear to be worse according to increased treatment intensity. Orgasmic function appears to be impaired compared to age-matched controls and in patients treated with post-chemotherapy retroperitoneal surgery. The findings concerning

radiotherapy and orgasmic dysfunction are mixed. Concerning erectile dysfunction, our findings from the largest study to date strongly indicate increased risk according to treatment modality.
Based on these findings we recommend screening for erectile dysfunction in TC patients who have received chemotherapy and radiotherapy in addition to orchiectomy.

4.6 impact of Leydig cell dysfunction on sexual function

To elucidate if compensated Leydig cell insufficiency is associated with sexual dysfunction and impaired quality of life, we assessed these conditions in 147 unilateral TC patients after a median follow-up of around 10 years [9]. In total, 60 of patients had Leydig cell insufficiency defined as LH > 2 standard deviations above the age-specific mean in combination with total testosterone within the age-specific normal range. We did not find impaired sexual function, increased prevalence of fatigue, anxiety or depression in in patients with compensated Leydig cell insufficiency compared to the 87 patients with normal Leydig cell dysfunction [9]. However, the study was possibly statistically underpowered to detect differences in depression, as there were only 7 cases in total. The results concerning the different aspects of sexual function and anxiety and depression are summarized in Figure 11.

Figure 11 Forest plots representing age-adjusted odds ratio of sexual dysfunction, anxiety and depression. Patients with normal Leydig cell function serve as reference.



While no studies have evaluated the risk for sexual dysfunction and impaired quality of life in TC survivors with compensated Leydig cell insufficiency, a number of studies have assessed the associations between elevated LH and sexual dysfunction/ impaired quality of life and of low levels of testosterone and sexual dysfunction/impaired quality of life [45,50,51,138,141,142].

In the above-mentioned study by Huddart et al [50], low total testosterone levels (<10 nmol/l) were associated with all aspects of sexual function (feeling less masculine, worried about fathering a child, less interest in sex, less sexually active, difficulty getting erection, sex less enjoyable, dry ejaculation) while increased LH (>12 IU/L) were associated with more worries about fathering a child and less sexual activity. Adjustment for age was not performed and only around 4 of 680 (<1%) included patients received testosterone substitution. It could be hypothesized that the associations between low testosterone and sexual dysfunction were due to pronounced untreated testosterone deficiency, which is known to be associated with sexual dysfunction in non-cancerous men [143].

In a Norwegian study of 812 long-term testicular cancer survivors, Sprauten *et al*. found an association between testosterone levels <25 percentile of age-matched men and fatigue after a median of 19 years follow-up, while there was no association between increased LH and fatigue [141]. Sexual function was not assessed in that study.

In a Polish study of 326 TC survivors investigated at a median follow-up of 5 years, low levels of testosterone (<9 nmol/L) were not associated with sexual dysfunction assessed with the Sexual Functioning Questionnaire [144] or impaired quality of life assessed with a non-validated questionnaire. Increased LH (>6.1 IU/L) was associated with sexual dysfunction assessed with Sexual Functioning Questionnaire and depression when assessed with the Beck Depression Inventory while not when assessed with the Hospital Anxiety and Depression Scale [51].

In studies by Lackner *et al* [138], Eberhard *et al* [45]. and Tal *et al* [142] there were no association between low levels of testosterone and sexual dysfunction

A British study of survivors of haematological malignancy investigated the effect of mild Leydig cell insufficiency on sexual function and fatigue at a median follow-up of 8 years since treatment [145]. A group of 36 patients with mild Leydig cell dysfunction defined as LH≥8 IU/L and total testosterone < 20 nmol/L was compared to 30 patients with normal Leydig cell function. There was no difference in prevalence of anxiety and depression assessed with HADS and no difference in fatigue assessed with MFI-20 between the two groups. Patients with mild Leydig cell dysfunction were less sexually active, while there was no difference in sexual interest and enjoyment.

In conclusion, there is no clear evidence that low levels of testosterone are associated with sexual dysfunction and impaired quality of life in testicular cancer survivors. In [9] we did not find any association between compensated Leydig cell insufficiency, impaired quality of life or sexual dysfunction, and based on the present evidence it does not appear that impaired quality of life and sexual dysfunction are important problems in TC survivors with compensated Leydig cell dysfunction. However, the possible association between compensated Leydig cell insufficiency and depression should be investigated in a large prospective trial.

4.7 Sexual function and quality of life in bilateral testicular cancer as well as patients treated for germ cell neoplasia in situ (GCNIS) in the contralateral testicle

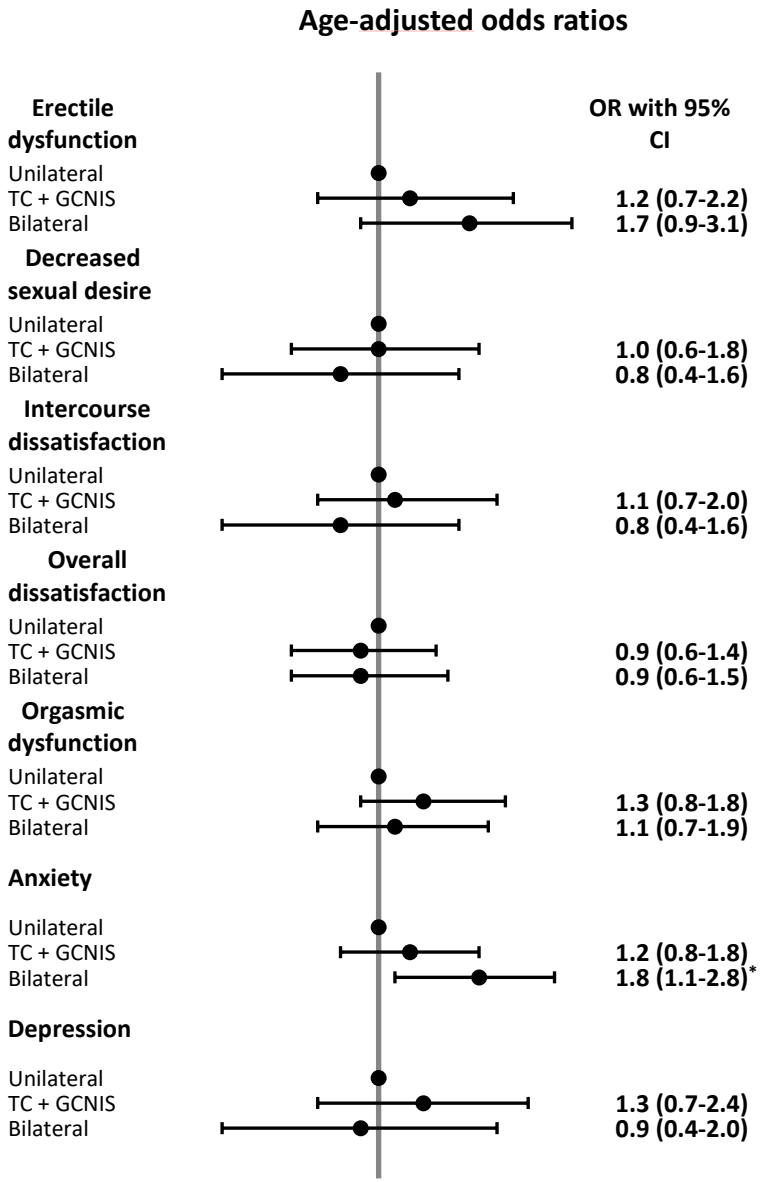
In Denmark, we have systematically screened for contralateral GCNIS since 1992 by performing a contralateral biopsy in all unilateral TC patients. The rationale has been to decrease the risk of a metachronous tumour and bilateral orchiectomy which invariably leads to testosterone deficiency and lifelong need of testosterone substitution. In few cases, systemic treatment might be avoided as well.

No large studies have assessed long term sexual function and quality of life in TC survivors where both testicles are affected, and on this background, we investigated these conditions in 126 men with unilateral TC and contralateral GCNIS treated with radiotherapy to the contralateral testicle and in 93 patients who were bilaterally orchiectomised due to bilateral TC after a median of 17 years. Our hypothesis was that patients with TC affecting both testicles would have long-term increased risk of sexual dysfunction and impaired quality of life compared to unilateral TC survivors.

In total, 95% of patients with bilateral TC received testosterone substitution while 50% of TC survivors with unilateral TC and contralateral GCNIS (TC+GCNIS) received testosterone substitution.

We found similar sexual function and increased anxiety in bilateral TC survivors compared to unilateral TC survivors. The findings are summarized in figure 12.

Figure 12 Forest plots representing the odds of sexual dysfunction, anxiety, and depression among long-term testicular cancer survivors treated for contralateral germ cell neoplasia in situ and bilateral testicular cancer. Unilateral testicular cancer survivors serve as the reference group. OR = odds ratio; CI = confidence interval; TC = testicular cancer; GCNIS = germ cell neoplasia in situ.



Among patients with TC + GCNIS, we did not find evidence of sexual dysfunction and impaired quality of life between those using testosterone substitution compared with TC survivors who did not [10].

Two small studies have investigated quality of life in bilateral TC survivors [146,147]: In a Norwegian study from 1999, quality of life was assessed with the EORTC QLQ C-30 questionnaire[148] including the testicular cancer module in 43 bilateral testicular cancer patients diagnosed from 1952 to 1994. All patients were treated with intramuscular androgen replacement. On most dimensions, quality of life was comparable when compared to a control group from the Norwegian background population. A Dutch study investigated sexual function and general wellbeing in 7 bilateral testicular cancer patients who received intramuscular androgen replacement. They found no association between testosterone levels and sexual arousal assessed by visual erotic stimulation and erectile function assessed by nocturnal penile tumescence and rigidity.

Our findings from a large national cohort of TC patients with a long follow-up do no not suggest that TC patients who have been bilateral orchiectomized are generally experiencing sexual dysfunction and impaired quality of life when compared to unilateral TC survivors, although we found increased risk of anxiety. Neither did we find a negative impact of testosterone substitution in unilateral TC survivors with contralateral GCNIS. We cannot exclude that bilateral TC survivors are experiencing other phycological problems or changes in body image which could not be detected by the questionnaires used in our study. The present findings are reassuring and are useful for evidence-based information on late effects in bilateral TC patients. The finding of increased anxiety should preferably be validated in another cohort.

5.0 Conclusions

- Testicular patients have impaired Leydig cell function even before initiation of testicular cancer treatment. This could be due to testicular dysgenesis although a direct effect of the testicular tumour compromising Leydig cell function cannot be excluded
- Testicular cancer treatment increases the risk of Leydig cell dysfunction according to treatment intensity, with patients receiving more treatment lines at highest risk of testosterone deficiency. In Denmark, the proportion of long-term unilateral TC survivors receiving testosterone substitution is around 5%, while it is 50% in patients treated with contralateral radiotherapy due to GCNIS
- In patients treated with BEP and abdominal radiotherapy, there is a degree of Leydig cell recovery within the first five years after TC treatment, although LH levels are still elevated compared to age-matched men
- Leydig cell hyperplasia of the non-tumour bearing testicle is a risk factor of long-term Leydig cell dysfunction, while TC patients with a normal Leydig cell function within the first year of the 5-years follow-up program have very little risk of future testosterone deficiency, and routine assessment of Leydig cell function might be omitted in these patients
- Compensated Leydig cell insufficiency is not associated with systemic inflammation and metabolic syndrome; on the contrary it appears that TC patients with compensated Leydig cell insufficiency have decreased risk of developing metabolic syndrome
- Low testosterone is associated with metabolic syndrome in TC survivors. However, the causal relationship is not clear and is possibly bi-directional
- Studies investigating metabolic syndrome in TC survivors are heterogenous. It does not generally appear that TC survivors have increased risk of metabolic syndrome when compared to age-matched men. Cisplatin-based chemotherapy does not appear to be associated with increased risk of metabolic syndrome compared to age-matched men or compared to orchiectomy.
- Few studies have investigated systemic inflammation in TC survivors. There is no clear indication o an increased risk of systemic inflammation in TC patients treated with cisplatin-based chemotherapy or radiotherapy.
- Leydig cell insufficiency is generally not associated with sexual dysfunction and impaired quality of life in testicular cancer survivors
- The results of studies assessing sexual function in testicular cancer patients are mixed. Cisplatin-based chemotherapy with or without post chemotherapy retroperitoneal surgery, abdominal

radiotherapy and more than one treatment line are associated with erectile dysfunction when compared to orchiectomy alone

- Cisplatin-based chemotherapy with post chemotherapy retroperitoneal surgery, abdominal radiotherapy and more than one treatment line is associated with orgasmic dysfunction when compared to orchiectomy alone
- Sexual desire and intercourse satisfaction are generally not associated with testicular cancer treatment modality
- There is no indication that survivors of bilateral TC and unilateral TC with contralateral GCNIS experience sexual dysfunction when compared to unilateral TC survivors
- Bilateral TC survivors have increased anxiety when compared to unilateral TC survivors

6.0 Perspectives

In this thesis it has been shown that treatment of testicular cancer increases the risk of Leydig cell dysfunction in long-term survivors. The association between testicular cancer treatment, Leydig cell dysfunction and metabolic syndrome remains unclear.

Large prospective trials with serial assessments of Leydig cell function, markers of inflammation and components of metabolic syndrome are needed to disentangle the causal relations between testicular cancer treatment, Leydig cell dysfunction and related diseases. Findings of such studies might also disentangle the possible mechanisms behind cisplatin-based chemotherapy and cardiovascular disease.

Cancer survivorship care plans is a relatively new concept focusing on detecting late effects in cancer survivors in a standardized manner, and the findings of this thesis are important contributions in this framework.

However, a major clinical barrier to standardizing survivorship care is the lack of specific knowledge on the management of survivors, and so far there is no evidence on the benefit of using cancer survivorship care plans to prevent late effects.

Randomized clinical trials with specific interventions to prevent specific late effects are needed to create evidence-based survivorship care. We have recently finalised a double-blind randomized clinical trial with 12-months testosterone substitution (transdermal gel (Tostran®) vs placebo in 69 testicular cancer survivors with compensated Leydig cell insufficiency defined as LH > 2 standard deviations of the age-adjusted mean in combination with free testosterone in the lower half of the age-adjusted normal range [149]. The main endpoints were systematic inflammation, components of metabolic syndrome and quality of life including sexual function. The findings of this study will possibly clarify if testosterone substitution should be considered in testicular cancer survivors with compensated Leydig cell insufficiency. In this study, a hCG-stimulation test was performed at baseline in all included patients to evaluate residual Leydig cell capacity. The hypothesis was that patients with a little residual Leydig cell capacity might benefit from testosterone substitution, and the results of this analysis might further clarify if testosterone substitution should be considered in specific subgroups of testicular cancer survivors. Research like this could hopefully pave the way for new frontiers in the prevention of some of the life-changing outcomes related to treatment of testicular cancer and add evidence to survivorship care plans.

Focused surveillance for treatment related late effects provides opportunities for early detection and implantation of health-preserving interventions. Implementation of late effect clinics for testicular cancer

survivors are currently being discussed. The findings presented in this thesis suggest that patients treated with more treatment lines are suffering most from testosterone deficiency, erectile dysfunction and orgasmic dysfunction. It could be considered to start implementation of late effect clinics focusing on this sub-group of testicular cancer survivors with possible future expansion to patients treated with BEP and abdominal radiotherapy.

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8.0 Appendix 1

Definition of metabolic syndrome according to **International Diabetes Federation (IDF)**[102]

Metabolic syndrome definition: Central obesity + 2 other components present

Components	Cutpoints
Waist circumference (central obesity)	≥ 94 cm in Europids
Raised triglycerides	>1.7 mmol/L or specific medication
Reduced HDL-cholesterol	<1.03 mmol/L or specific medication
Raised blood pressure	Systolic BP ≥ 130 mm Hg, Diastolic BP ≥ 85 mm Hg or specific medication
Raised fasting plasma glucose	≥ 5.6 mmol/L or previously diagnosed type 2 diabetes

Definition of the metabolic syndrome according to the **The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII)**[101]

Metabolic syndrome definition: 3 of 5 components present

Components	Cutpoints
Waist circumference	> 102 cm
Raised triglycerides	≥1.7 mmol/L or specific medication
Reduced HDL-cholesterol	<1.03 mmol/L or specific medication
Raised blood pressure	Systolic BP ≥ 130 mm Hg, Diastolic BP ≥ 85 mm Hg or specific medication
Raised fasting plasma glucose	≥ 6.1 mmol/L or previously diagnosed type 2 diabetes

ISBN 978-87-973039-0-0