Gonadal Function and Bone Mineral Density in Young Male Cancer Survivors

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Gonadal Function and Bone Mineral Density in Young Male Cancer Survivors

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The prognoses for testicular cancer and childhood cancer have improved significantly during recent decades, with survival rates now exceeding 95% for testicular cancer and 80% for childhood cancers. However, testicular cancer survivors (TCS) and childhood cancer survivors (CCS) have increased long-term morbidity and mortality. Among potential long-term side-effects of cancer treatment are azoospermia (the absence of spermatozoa in the ejaculate) and hypogonadism. Testosterone deficiency has previously been identified as a marker of reduced life expectancy. It has been suggested that TCS and CCS have reduced bone mineral density (BMD), but it is unclear whether this is due to hypogonadism or the effect of cancer treatment.

The first study was designed to investigate the frequency, and possible predictive factors, of azoospermia following treatment for testicular cancer. The second study was carried out to assess the frequency and possible risk factors of biochemical hypogonadism (S-testosterone <10 nmol/L and/or S-LH >10 IU/L, or ongoing testosterone replacement therapy) in TCS and CCS. Studies were then performed to investigate whether TCS or CCS had decreased BMD compared to controls, and to explore whether BMD or risk of low BMD (Z-score ≤-1) was related to hypogonadism and/or the cancer treatment received.

Possible predictive factors for azoospermia were explored in 117 TCS with confirmed sperm production after unilateral orchidectomy, but before further cancer treatment. Fasting morning blood samples were collected for the analysis of hypogonadism, and BMD was determined, in 92 TCS, 125 CCS and a corresponding number of age-matched controls from the general population. The mean follow-up time was 9 years for TCS and 24 years for CCS.

All TCS with confirmed sperm production after unilateral orchidectomy but before further cancer treatment and inhibin B levels >56 ng/L 12 months after cancer treatment had sperm production 3 years post-treatment. This finding may be important in counselling young cancer survivors regarding future fertility potential, if the findings are confirmed in future studies.

Hypogonadism was found in 26% of CCS and 36% of TCS, the risk being doubled compared to controls. Testosterone levels in cancer survivors with untreated hypogonadism were only moderately decreased; median value 9.3 nmol/L for TCS and 9.0 nmol/L for CCS. Testicular cancer survivors and CCS with untreated hypogonadism had lower hip and lumbar spine BMD than eugonadal TCS and CCS. Testicular cancer survivors with untreated hypogonadism also had increased risk of low BMD in the lumbar spine compared to eugonadal TCS. Childhood cancer survivors treated with cranial irradiation had lower hip and lumbar spine BMD than controls, but no increased risk of low BMD in the hip or lumbar spine. These findings highlight the necessity for long-term follow-up of young male cancer survivors regarding hypogonadism and BMD. The assessment of BMD in hypogonadal male cancer survivors should be considered already at moderately lowered testosterone levels, and prevention of osteoporosis should be considered an important part in future follow-up of these men.

In conclusion, two serum markers for the risk of complications resulting from cancer treatment in young male cancer survivors have been identified. Inhibin B level was found to be a good predictor of the risk of azoospermia in TCS, and low testosterone levels were found to be associated with an increased risk of low BMD in both TCS and CCS. The findings presented in this thesis can be used to improve the follow-up of young male cancer survivors.

Key words: azoospermia, testicular cancer, childhood cancer, inhibin B, hypogonadism, bone mineral density, cancer treatment

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To my family

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List of Papers

This thesis is based on the following original publications, which will be referred to in the text by their Roman numerals.

I. **Isaksson S**, Eberhard J, Ståhl O, Cavallin-Ståhl E, Cohn-Cedermark G, Arver S, Giwercman YL, Giwercman A.
Inhibin B concentration is predictive for long-term azoospermia in men treated for testicular cancer
Andrology 2014 Mar; 2(2):252-258

II. **Isaksson S**, Bogefors K, Ståhl O, Eberhard J, Giwercman YL, Leijonhufvud I, Link K, Øra I, Romerius P, Bobjer J, Giwercman A.
High risk of hypogonadism in young male cancer survivors
Clinical Endocrinology (Oxf). 2018 Mar; 88(3):432-441

III. **Isaksson S**, Bogefors K, Åkesson K, Egund L, Bobjer J, Leijonhufvud I, Giwercman A.
Risk of low bone mineral density in testicular germ cell cancer survivors: association with hypogonadism and treatment modality
Andrology 2017 Sep; 5(5):898-904

IV. **Isaksson S**, Bogefors K, Åkesson K, Øra I, Egund L, Bobjer J, Leijonhufvud I, Giwercman A.
Low bone mineral density is associated with hypogonadism in male childhood cancer survivors
Manuscript

Abbreviations

ACT	adjuvant chemotherapy
ACTH	adrenocorticotropic hormone
ALL	acute lymphoblastic leukaemia
AML	acute myeloid leukaemia
BEP	bleomycin, etoposide, cisplatin
BMD	bone mineral density
BMI	body mass index
BMT	bone marrow transplantation
CBC	cisplatin-based chemotherapy
CCS	childhood cancer survivors
CCSS	Childhood Cancer Survivor Study
CED	cyclophosphamide equivalent dose
CI	confidence interval
CNS	central nervous system
CT	computed tomography
CV	coefficient of variation
CVD	cardiovascular disease
DHT	dihydrotestosterone
DXA	dual X-ray absorptiometry
EP	etoposide, cisplatin
ETC	extensive treatment with chemotherapy
FSH	follicle-stimulating hormone
GCNIS	germ cell neoplasia *in situ*

GHD	growth hormone deficiency
GHRT	growth hormone replacement therapy
GnRH	gonadotropin-releasing hormone
HSCT	haematological stem cell transplantation
LBD	low bone mineral density
LH	luteinizing hormone
MRI	magnetic resonance imaging
NPV	negative predictive value
OR	odds ratio
PC-RPLND	post-chemotherapy retroperitoneal lymph node dissection
PPV	positive predictive value
PVB	cisplatin, vinblastine, bleomycin
RPLND	retroperitoneal lymph node dissection
SCT	standard chemotherapy
S-FSH	serum-FSH
SHBG	sex hormone-binding globulin
S-LH	serum LH
SO	surveillance only
S-testosterone	serum testosterone
SWENOTECA	Swedish and Norwegian Testicular Cancer Group
TBI	total body irradiation
TCS	testicular cancer survivors
TCS-A	testicular cancer survivors, cohort A
TCS-B	testicular cancer survivors, cohort B
TRT	testosterone replacement therapy
TSH	thyroid-stimulating hormone

Populärvetenskaplig sammanfattning (summary in Swedish)

Idag uppskattas det att var tredje person i Sverige kommer att få cancer före sin 75-årsdag. Varje år insjuknar ca 350 barn och ungdomar upp till 18 års ålder i Sverige i cancer. Av dem blir fler än 80% långtidsöverlevare. Varje år blir ca 340 män i Sverige diagnosticerade med testikelcancer, som är den vanligaste cancerformen bland män i åldrarna 15-44 år. Fler än 95% av männen med testikelcancer blir botade. Eftersom chansen till bot vid barncancer och testikelcancer är så stor, har långsiktiga biverkningar av cancerbehandlingen, t.ex. minskad chans att få biologiska barn, eller ökad risk att drabbas av andra sjukdomar senare i livet, blivit allt viktigare.

Man har länge vetat att strålbehandling och cytostatikabehandling, som båda används vid behandling av testikelcancer, kan försämra fruktsamheten. En del män blir sterila under de första månaderna efter behandling för testikelcancer, men återfår sedan förmågan att bilda spermier. Andra förblir sterila under många år, kanske livet ut. Om en grupp män får exakt samma cancerbehandling blir en del sterila och andra inte, och man har hittills inte kunnat säga något till en enskild man om just hans risk att bli steril.

För att undersöka om det gick att förutsäga vilka män som blev sterila efter testikelcancer-behandling lämnade 117 män med testikelcancer blodprover och spermaprover innan cancerbehandlingen inleddes, samt vid flera senare tillfällen upp till 3 år efter avslutad behandling. Alla hade spermieproduktion före behandlingsstart. Studien visade att ett ämne i blodet, inhibin B, kunde användas för att identifiera de män som riskerade att vara sterila 3 år efter cancerbehandling. Blodprover tagna 1 år efter cancerbehandlingen analyserades, och ett gränsvärde för inhibin B valdes. Bland dem som hade inhibin B under gränsvärdet hade en del spermieproduktion efter 3 år, medan andra var sterila. Att ha inhibin B under gränsvärdet innebar alltså inte säker sterilitet, utan bara risk för sterilitet. Däremot hade alla med inhibin B över gränsvärdet återfått spermieproduktionen 3 år efter cancerbehandling.

Män som behandlats för cancer tros också ha ökad risk för testosteronbrist. Testosteronbrist kan ge trötthet, minskad muskelmassa, försämrad potens och

nedsatt sexlust. Mycket låga testosteronvärden ger nedsatt bentäthet, men det är oklart om även lätt sänkta testosteron-nivåer påverkar bentätheten. Det har också varit oklart vilka canceröverlevare som löper högst risk för testosteronbrist, och hur mycket risken är ökad jämfört med män som inte behandlats för cancer.

En del studier har visat att unga män som behandlats för cancer har ökad risk för nedsatt bentäthet, medan andra studier inte har visat någon ökad risk. Det har också varit oklart om bentätheten i så fall har påverkats av cancerbehandlingen, eller om den har påverkats av testosteronbrist. Eftersom både testosteronbrist och nedsatt bentäthet kan behandlas, är det viktigt att ta reda på om cancerbehandlade män har ökad risk att drabbas, och hos vilka canceröverlevare risken i så fall är störst.

För att undersöka risken för testosteronbrist och risken för nedsatt bentäthet hos manliga canceröverlevare har 92 män som behandlats för testikelcancer, 125 män som behandlats för barncancer och motsvarande antal män från den allmänna befolkningen deltagit i en studie. De har fått lämna blodprover för bestämning av bl.a. testosteron, och genomgått en röntgenundersökning för bestämning av bentätheten. Resultaten visade att risken för testosteronbrist var ungefär fördubblad hos de cancerbehandlade männen. För de som behandlats för barncancer var risken störst efter strålbehandling mot hjärnan och/eller testiklarna, eller efter strålbehandling mot andra delar av kroppen kombinerat med cytostatikabehandling. För de som behandlats för testikelcancer var risken störst efter fler än 4 omgångar cytostatikabehandling, med eller utan strålbehandling mot andra delar av kroppen än testiklarna. Det visade sig också att lätt nedsatt testosteronvärde gav ökad risk för nedsatt bentäthet, medan cancerbehandlingen inte påverkade bentätheten i någon större utsträckning.

Sammanfattningsvis har två prognostiska markörer för olika långtidseffekter efter cancerbehandling identifierats. Lågt inhibin B 1 år efter behandling för testikelcancer visade på ökad risk för sterilitet 3 år efter cancerbehandling. Detta är första gången någon identifierat ett blodprov som kan visa om en enskild man riskerar att bli långvarigt steril efter cancerbehandling. Det är ännu för tidigt att använda analysen vid uppföljning av cancerpatienter. Först behövs fler studier för att se om resultatet och gränsvärdet stämmer. Man behöver också undersöka om resultatet är giltigt även efter behandling av andra cancerformer. Nedsatt testosteronvärde var ungefär dubbelt så vanligt hos cancerbehandlade män, och sänkt testosteron-nivå gav ökad risk för nedsatt bentäthet. Detta visar på hur viktigt det är att kontrollera testosteron hos män som behandlats för cancer, och att man ska överväga att undersöka bentätheten hos män med sänkta testosteron-nivåer.

Background

It is currently estimated that almost one in three people in Sweden will develop cancer before the age of 75 [1]. For every person that is diagnosed with cancer, many more lives are affected - those of parents, children, spouses, friends and colleagues. Fortunately, the prognoses for many cancers have improved during recent decades, with increasing chances of survival.

It is important that life after cancer has as high a quality as possible, and that the long-term side-effects of cancer and its treatment are either prevented, or treated if they do occur. The aim of the work presented in this thesis was to improve our ability to predict some of the possible side-effects of cancer treatment affecting gonadal function and bone mineral density (BMD) in young male cancer survivors.

Childhood cancer, background and epidemiology

Before the 1950s, the probability of surviving childhood cancer was small; cure rates in the United States being less than 10% [2]. However, survival rates following childhood cancer improved dramatically during the second half of the 20th century (Figure 1). This was mainly due to the introduction of chemotherapy to treat childhood cancers such as leukaemia during the 1950s and 1960s, and the use of combinations of several chemotherapy drugs in the 1970s [3]. In the 1970s and 1980s, treatment for childhood cancer was intensified, with the introduction of combinations of chemotherapy, irradiation and surgery. Since 2000, the overall rate of survival following childhood cancer seems to have stabilized, although the 5-year survival following neuroblastoma and tumours of the central nervous system (CNS) have continued to improve. The 5-year survival following childhood cancer in Sweden now exceeds 80% [4]. About 350 children and adolescents below the age of 18 are diagnosed with cancer in Sweden each year [5]. Leukaemias constitutes 30% of cases, CNS tumours 28% and solid tumours 42% [4].

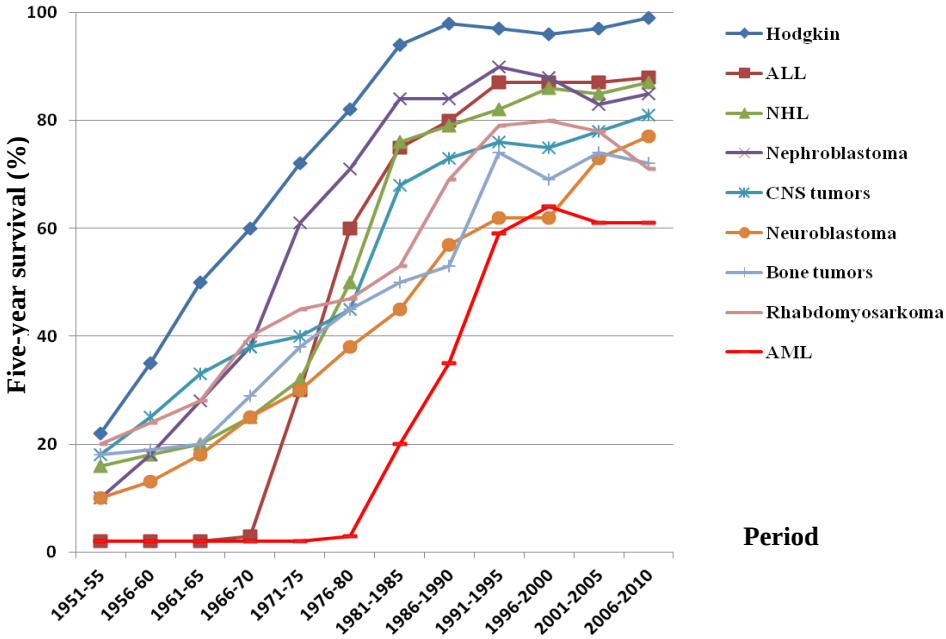

Figure 1. The estimated 5-year survival in percent over time for selected diagnostic groups of childhood cancer in Sweden. ALL= acute lymphoblastic leukaemia; NHL = Non-Hodgkin's lymphoma; CNS = central nervous system; AML = acute myeloid leukaemia. (Figure from the Swedish Childhood Cancer Soceity [4]. Printed with permission.)

Testicular cancer, background and epidemiology

The prognosis for testicular cancer has improved considerably since the 1960s, as can be seen in Figure 2. Up until the 1960s, the only forms of treatment for testicular cancer were surgery and radiation. Although patients with early stage disease could be cured, metastatic testicular cancer had a very poor prognosis, and mortality within 1 year was 90%. In the 1960s, advanced testicular cancer was treated with actinomycin-D-based chemotherapy. The combination of vinblastine and bleomycin was also introduced in the 1970s. Cisplatin was discovered in 1965, and revolutionized the treatment of testicular cancer. When tested in a phase 1 trial in the early 1970s, response, including several complete responses, was reported in 9 of 11 patients with refractory testicular cancer. The addition of cisplatin to vinblastine plus bleomycin (PVB) was first tested in 1974, and resulted in a 5-year survival rate of 64%. In the early 1980s, the combination of bleomycin, etoposide

and cisplatin (BEP) was shown to be superior to PVB, and BEP replaced PVB as the standard form of chemotherapy in 1987 [6]. Today, the prognosis for patients with testicular cancer is excellent, with mean 5-year survival of 97% in Europe [7].

Testicular cancer is a relatively rare cancer, accounting for approximately 0.7% of all male cancers globally. For reasons as yet unknown, there is a considerable geographical variation in incidence, the highest incidences being found in Northern and Western Europe, and the lowest in Asia and Africa [8]. In Sweden, testicular cancer is the most common cancer in males aged 15-44 years [9], with an age-adjusted incidence of approximately 8/100,000 men in 2015 [10].

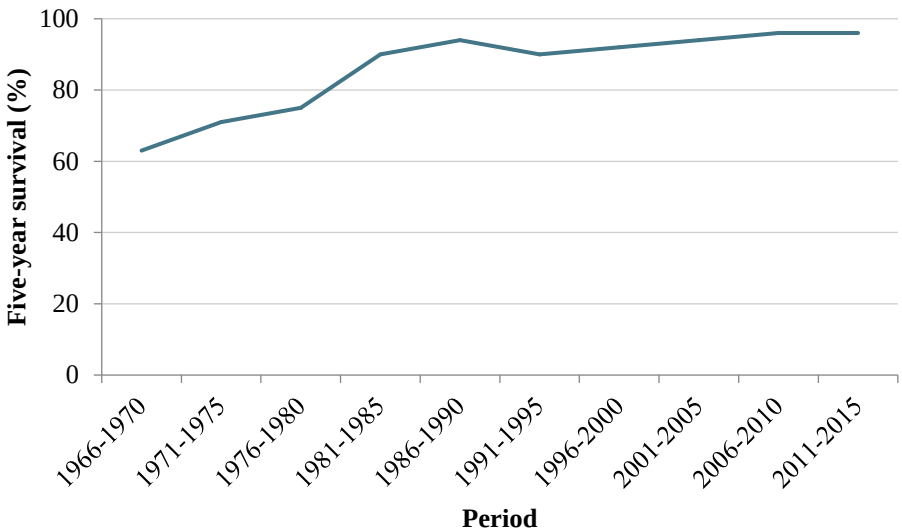

Figure 2. Five-year survival over time for patients with testicular cancer in Sweden. (Data from NORDCAN [11].)

Cancer treatment in childhood cancer and testicular cancer.

Childhood cancer treatment

Childhood cancer treatment depends on diagnosis, the stage of the disease and the child's age at diagnosis. The treatment consists mainly of chemotherapy, radiotherapy and surgery, alone or in combination.

The most common type of childhood leukaemia is acute lymphoblastic leukaemia (ALL, 80%) followed by acute myeloid leukaemia (AML). Patients with ALL are treated with intravenous, intrathecal and oral chemotherapy in addition to corticosteroids. One backbone of the treatment is high dose methotrexate, and the duration of treatment is 2.5 years. Acute myeloid leukaemia is treated with considerably more intense chemotherapy regimens for about 1 year, and some patients require high dose chemotherapy with allogeneic stem cell transplantation as primary treatment. Alkylating agents are used in cases of intermediate and high risk ALL.

Patients with Hodgkin's lymphoma require chemotherapy including alkylating agents, with or without radiotherapy. Non-Hodgkin's lymphomas are treated with intense chemotherapy including alkylating agents.

Brain tumours require neurosurgery with or without radiotherapy and/or chemotherapy, depending on the pathological diagnosis and the age of the child. In children younger than three years, radiotherapy should be avoided due to the high risk of neurological sequelae [12].

Rhabdomyosarcoma, Ewing's sarcoma and osteosarcoma are the most common sarcomas in children and adolescents. Patients with Ewing's sarcoma and osteosarcoma are treated with intense chemotherapy and surgery, with or without radiotherapy. Patients with rhabdomyosarcoma are treated with chemotherapy with or without surgery, and radiotherapy, depending on stage and response to chemotherapy.

Neuroblastoma (tumours originating from the sympathetic nervous system) and Wilms' tumour (kidney nephroblastoma) mostly affect younger children. The majority of low and intermediate risk patients with these diagnoses are treated with low dose chemotherapy and surgery. In contrast, patients with high risk pathology or high stage of disease require intense chemotherapy, including high dose chemotherapy followed by autologous stem cell transplantation for neuroblastoma. Postoperative abdominal and/or pulmonary radiotherapy is administered to high risk patients and those with metastatic disease.

Children and adolescents experiencing relapse of any kind of paediatric cancer often require toxic treatment including alkylating agents and radiotherapy. Patients with relapsed leukaemia or lymphoma, as well as specific solid tumours or brain tumours, might undergo haematological stem cell transplantation (HSCT) after conditioning regimes with high dose chemotherapy, with or without or total body irradiation (TBI). (I. Øra, personal communication, March 2018.)

Testicular cancer treatment

In Sweden and Norway, testicular cancer is treated according to the SWENOTECA (Swedish and Norwegian Testicular Cancer Group) management protocols, currently SWENOTECA VIII for non-seminomas and SWENOTECA IX for seminomas [13]. All testicular cancer patients undergo unilateral orchidectomy of the affected testicle.

In clinical stage I disease, a risk-adapted strategy is used. One cycle of adjuvant BEP is recommended for non-seminomas with a high risk of recurrence (i.e. vascular invasion of tumour cells), whereas surveillance or one cycle of adjuvant BEP are options for non-seminomas with a low risk of recurrence (i.e. no vascular invasion of tumour cells). For the treatment of clinical stage 1 seminomas with one or two risk factors (i.e. tumour >4 cm or tumour growth in the rete testis), one cycle of adjuvant carboplatin is recommended, whereas clinical stage 1 seminomas without risk factors can be managed by surveillance after orchidectomy.

Standard treatment for disseminated disease is chemotherapy, BEP, and treatment is guided by a prognostic index. One cycle of standard BEP or EP (etoposide, cisplatin) includes a cisplatin dose of 100 mg/m^2. For good prognosis disease (75 % of those with non-seminomas, >90 % of those with seminomas), the treatment is typically three cycles of BEP, whereas more advanced disease is treated with a minimum of four cycles of BEP. The treatment strategy for disseminated disease also entails an intensified multi-step treatment scheme in the case of poor treatment response, in all prognostic groups, and includes the addition of more intense chemotherapy, the most intense being tandem high dose chemotherapy with autologous stem cell support.

Radiotherapy to the para-aortic and ipsilateral iliac lymph nodes is an option for patients with seminomas with limited retroperitoneal lymph node involvement only (clinical stage IIA-IIB). Surgery is indicated for non-seminoma patients with residual disease post-chemotherapy, typically retroperitoneal lymph node dissection (RPLND).

Long-term side-effects of cancer treatment

Childhood cancer

Childhood cancer survivors (CCS) suffer from increased long-term morbidity and mortality, which increase many years after the completion of cancer treatment. In the large Childhood Cancer Survivor Study (CCSS) carried out in the United States, 18% of 5-year survivors of childhood cancer diagnosed between 1970 and 1986 had died 30 years after diagnosis. As time after diagnosis increased, death due to the recurrence or progression of the primary disease decreased, while treatment-related deaths due to secondary cancers, cardiac-related events or pulmonary events, increased [14]. When this cohort was expanded to include 5-year survivors of childhood cancer diagnosed between 1970 and 1999, a recent analysis showed a gratifying reduction in all-cause and health-related mortality 15 years after diagnosis, compared to the previous study. The reduction in health-related mortality was attributed to reductions in secondary cancers and death from cardiac- or pulmonary-related events. Reduced late mortality was associated with reduced treatment exposure for survivors of ALL and Wilms' tumour [15].

Childhood cancer survivors can be affected by a large variety of treatment-related long-term side-effects. In a publication from the CCSS, 62% of CCS had at least one chronic health condition, 28% of which had a severe or life-threatening condition, at a mean time of 17.5 years after cancer diagnosis. The adjusted relative risks compared to siblings were 3.3 for a chronic health condition and 8.2 for a severe or life-threatening condition. Thirty years after diagnosis, the cumulative incidence of chronic health conditions had increased to 73%, and to 42% for severe, disabling or life-threatening conditions [16]. A high prevalence of adverse health outcomes was also found in a study on 1713 CCS included in the St Jude Lifetime Cohort Study, after a median follow-up of 32 years. Among CCS treated with radiotherapy to the lungs, busulfan, bleomycin, carmustine/lomustine or thoracotomy, 65% had abnormal lung function. Fifty-six percent of CCS treated with anthracyclines or radiotherapy to the heart had cardiac abnormalities. Endocrine disorders were found in 62% of CCS treated with radiation of the hypothalamic-pituitary area, neck or reproductive system, or alkylating agents, while neurocognitive impairment was detected in 48% of CCS treated with cranial irradiation, neurosurgery or antimetabolite therapy [17].

Testicular cancer

Long-term toxicity after testicular cancer treatment includes both life-threatening toxicity (secondary malignancies and cardiovascular disorders) and non-life-threatening toxicity (e.g. neurotoxicity, ototoxicity, reduced fertility, hypogonadism, metabolic syndrome, pulmonary toxicity and renal toxicity) [18,19]. Testicular cancer survivors (TCS) have increased morbidity, even after more than 30 years of follow-up [20].

In studies on TCS using data from population-based cancer registries, the ratio of the number of observed to the number of expected, solid second tumours was 1.55 for 10-year survivors [21], and 1.65 for developing a secondary malignancy (including haematological malignancies) after a median follow-up of 8 years [22]. A significantly increased risk of secondary malignancies has been reported for TCS with post-orchidectomy treatment with cisplatin-based chemotherapy (CBC) only [21,23,24], infradiaphragmatic radiotherapy only [21,24,25], and combinations of chemotherapy and radiotherapy [21,24], but not for TCS treated with orchidectomy alone [23,24].

Cardiovascular disease (CVD) is a potential long-term risk in TCS. One study on 62 TCS treated with CBC reported a 7-fold increase in the risk of angina with proven myocardial ischaemia or myocardial infarction, compared to the general population [26]. Larger studies have also reported an increased risk of CVD in patients treated with CBC. In a study by van den Belt-Dusebout et al., PVB resulted in a 1.9-fold increase in the risk of myocardial infarction, and BEP in a 1.5-fold increase in the risk of CVD (95% confidence interval (CI) 1.0-2.2) a median of 18 years after treatment, compared to TCS treated with orchidectomy alone [27]. Haugnes et al. found a 5.7-fold increase in the risk of coronary artery disease compared to TCS treated with orchidectomy only, and a 3.1-fold higher risk of myocardial infarction in TCS treated with BEP at a median follow-up of 19 years compared with age-matched controls [28]. Testicular cancer survivors treated with mediastinal radiotherapy were also found to have a 3.7-fold increased risk of myocardial infarction compared to those treated with orchidectomy alone [27].

The mechanisms of cardiovascular damage in TCS are unclear, but direct vascular injury arising from chemotherapy or radiotherapy has been suggested. Raynaud's phenomenon has been detected in 37% of TCS treated with vinblastine and bleomycin ± cisplatin [29]. Increased prevalence of the metabolic syndrome has been seen in TCS treated with chemotherapy compared to the general population [30]. Recently, hypogonadal TCS have been found to have an increased odds ratio (OR) of developing metabolic syndrome, with an OR of 4.4 compared to age-matched controls, and an OR of 15 compared to eugonadal TCS, suggesting that hypogonadism may be a pathogenic link between testicular cancer, cancer therapy and the risk of CVD [31].

The male reproductive system

Testicular function

The human testicles are responsible for spermatogenesis and most androgen production. Spermatogenesis, i.e. the production of gametes, takes place within the seminiferous tubules (Figure 3a), while androgen production takes place in the Leydig cells, located in the interstitial compartments between the seminiferous tubules (Figure 3b). (For details, see the sections "Spermatogenesis" and "Steroidogenesis" below.)

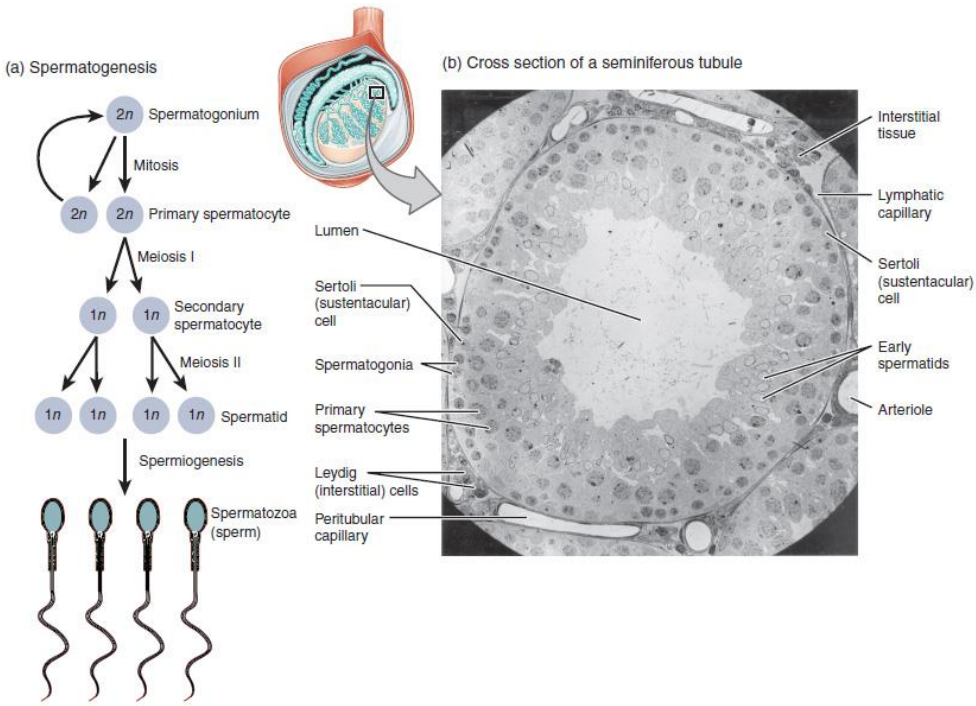

Figure 3. (a) Mitosis of a spermatogonial stem cell involves a single cell division that results in two identical, diploid daughter cells (spermatogonia to primary spermatocyte). Meiosis has two rounds of cell division: primary spermatocyte to secondary spermatocyte, and then secondary spermatocyte to spermatid. This produces four haploid daughter cells (spermatids). (b) In this electron micrograph of a cross-section of a seminiferous tubule from a rat, the lumen is the light-shaded area in the centre of the image. The location of the primary spermatocytes is near the basement membrane, and the early spermatids are approaching the lumen (tissue source: rat). EM × 900. (Micrograph provided by the Regents of University of Michigan Medical School © 2012).
Figure from Openstax CNX, Anatomy and Physiology of the Male Reproductive System [32].
Available at https://cnx.org/contents/FPtK1zmh@8.24:Nw1tEY4R@6/Anatomy-and-Physiology-of-the-

The hypothalamic-pituitary-gonadal axis

Spermatogenesis and androgen production are regulated by the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are secreted from the anterior pituitary gland (Figure 4). The biosynthesis and secretion of gonadotropins are stimulated by the pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus [33], whereas continuous GnRH release (as in pharmacologically induced castration of prostate cancer patients) inhibits gonadotropin secretion [34]. GnRH-secretion is regulated by kisspeptin. Kisspeptin also controls the onset of puberty, and participates in sex-steroid-mediated feedback in adults [35].

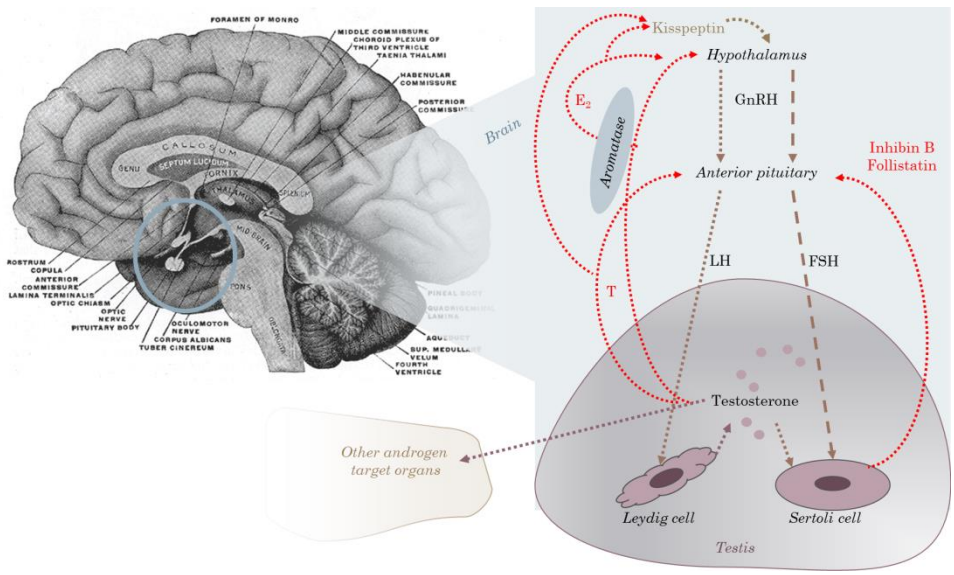

Figure 4. The regulatory pathways of the hypothalamic-pituitary-gonadal axis. Red dotted lines indicate negative feedback. GnRH = gonadotropin-releasing hormone; LH = luteinizing hormone; FSH = follicle-stimulating hormone; E_2 = oestradiol
Illustration of the brain on the left by Henry Gray (1918), Wikimedia commons.
Illustration by Magdalena Bentmar-Holgersson.

LH acts through binding to the LH receptors on the Leydig cells, stimulating steroidogenesis. Testosterone and oestradiol, a metabolite of testosterone, exert a negative feedback on both the hypothalamic and pituitary level, reducing testosterone levels [36,37]. FSH binds to the FSH receptors on the Sertoli cells, stimulating spermatogenesis. FSH secretion is controlled in a negative feedback loop by inhibin B secreted from the Sertoli cells following stimulation by FSH [38]. In the adult male, inhibin B production depends on both FSH stimulation and

spermatogenetic status, and inhibin B levels show a strong positive correlation with testicular volume and sperm count [39].

Spermatogenesis

Spermatogenesis starts in the testes at puberty. It is a complex process in which one diploid stem cell (spermatogonium) gives rise to four haploid cells, through one cycle of mitosis and two cycles of meiosis (Figure 3a) [32]. The Sertoli cells and the spermatogonia are found on the basement membrane of the seminiferous tubules. Sertoli cells are interconnected by tight junctions, forming the blood-testis barrier. The blood-testis barrier divides the seminiferous epithelium into 2 regions, with immature germ cells including the germ line stem cells in the basal region, and germ cells undergoing meiosis in the adluminal region. As the blood-testis barrier prevents substances in the circulation from reaching the adluminal region of the seminiferous tubules, spermatogenesis "above" the blood-testis barrier is dependent on the Sertoli cells for nutrition [40,41].

The Sertoli cells stop dividing at puberty [41,42]. Spermatogenesis is regulated by testosterone and FSH. As germ cells lack receptors for FSH and testosterone, these hormones exert their effect by binding to receptors on the Sertoli cells [40,43]. Testosterone is essential for spermatogenesis [44]. The Sertoli cells secrete androgen-binding protein [42], with similar steroid-binding capacities to sex hormone-binding globulin (SHBG). Androgen-binding protein binds testosterone and maintains a high testosterone concentration in the seminiferous tubules and epididymis [45]. Testicular testosterone concentrations are >80 times higher than the concentration in serum [46].

The synthesis of inhibin B depends on the interaction between the Sertoli cells and germ cells [47]. Inhibin B is a marker of spermatogenesis, and its level is related to sperm count [39,48-52], whereas levels of inhibin B below the level of detection are associated with the absence or arrest of spermatogenesis [53]. FSH is more often used than inhibin B in the assessment of males in infertile couples. FSH, but not inhibin B, is assessed in standard follow-up of testicular cancer survivors.

Azoospermia

Azoospermia is defined as the absence of spermatozoa in the ejaculate, after analysis of a centrifuged semen sample on 2 occasions [54]. Azoospermia can be caused by the failure of spermatogenesis or obstruction of the excurrent ducts of the testes. Failure of spermatogenesis can result from cryptorchidism, endocrine disorders such as hypopituitarism or hyperprolactinaemia, varicocele, or acquired causes such as chemotherapy, testicular irradiation or orchitis. Failure of spermatogenesis can also be caused by genetic abnormalities, of which Klinefelter syndrome is the most common [55]. Genetic abnormalities are found in 10-15% of azoospermic men [54].

Studies on the prevalence of azoospermia in the general population are sparse. Azoospermia has been reported in single semen samples in 1.6-2.5% of Danish men aged 20-35 years, living with a female partner who had no previous pregnancies, and neither partner had previous knowledge of fertility [48], and in 1.9% of seminal stains examined in sexual assault cases [56].

Steroidogenesis

Testosterone synthesis is initiated by the binding of LH to the LH receptors of the Leydig cells. Testosterone is synthesized from the substrate cholesterol. The testes produce more than 95% of all the circulating testosterone in the postpubertal man, the remainder being produced mainly by the adrenal glands [40].

Androgen action and metabolism

The effects of testosterone are due to testosterone itself, and its metabolites, dihydrotestosterone (DHT) and oestradiol. Following synthesis, testosterone diffuses out of the Leydig cells and into the circulation. In the bloodstream, testosterone equilibrates between free and protein-bound hormone. Only 2% of testosterone is unbound, while 44% is bound to SHBG and 54% to albumin [45]. The binding affinity of albumin is about 100 times lower than that of SHBG [45]. Since albumen binding is weak, albumin-bound testosterone, like free testosterone, is available to the tissues [57].

Testosterone is the main male sex steroid, and testosterone receptors can be found in almost every tissue. Testosterone determines the differentiation of the sexual organs in the foetus, and the development towards male phenotype during puberty. In the adult male, testosterone has a variety of functions. It is required for spermatogenesis and sexual function (e.g. libido, potency), it increases BMD and muscle mass, stimulates erythropoiesis and affects the cognitive function [58].

Approximately 5% of the circulating testosterone undergoes reduction to DHT by the enzyme 5α-reductase [59]. Testosterone and DHT both bind to the androgen receptor, but DHT binds with greater receptor affinity and also has a slower dissociation rate than testosterone [60], inducing a greater response in the target cell. 5α-reductase is found in the prostate, skin and hair follicles. DHT plays an essential role in the formation of the external genitalia during foetal development, and is the primary androgen in the prostate and hair follicles in the adult man [61].

Testosterone can also be converted to oestradiol in peripheral tissues, through a process called aromatization. Adipose tissue is one of the sites of aromatization, and obese men have increased serum oestradiol concentrations and low testosterone concentrations; these hormonal deviations being reversible with weight loss [62].

Oestradiol has several important functions in adult males, including closing the epiphyses at puberty, preserving bone mass [63], and contributing to normal libido and erectile function [64].

The testes also produce other androgens, including androstenedione, an important precursor in the production of extra-testicular oestrogens [45]. Androstenedione can also result from the peripheral conversion of testosterone, or be produced by the adrenal glands [65]. Androstenedione can be aromatized to oestrone in peripheral tissues, and oestrone can subsequently be reduced to oestradiol.

In males, approximately 20% of oestradiol is produced directly by the testes, 60% is derived from peripheral aromatization of circulating testosterone and the remainder is produced by peripheral conversion of oestrone [66]. Hence, low serum levels of testosterone lead to low serum levels of oestradiol.

Hypogonadism

Definition

The Endocrine Society defines male hypogonadism as "…a clinical syndrome that results from failure of the testis to produce physiological levels of testosterone (androgen deficiency) and a normal number of spermatozoa due to disruption of one or more levels of the hypothalamic-pituitary-testicular axis" [67]. In the definition given by the European Association of Urology, decreased sperm production is not mandatory, and male hypogonadism is defined as "…a clinical syndrome caused by androgen deficiency which may adversely affect multiple organ functions and quality of life" [68].

Hypogonadism can be further classified according to the level of hormonal disruption(s). Disturbances at the testicular level (primary hypogonadism) result in low testosterone levels, impaired spermatogenesis and elevated gonadotropin levels. Disturbances at the hypothalamic and/or pituitary level (secondary hypogonadism) result in low testosterone levels, impairment of spermatogenesis and low or inappropriately normal gonadotropin levels. Combined primary and secondary hypogonadism results in low testosterone levels, impaired spermatogenesis and normal or low gonadotropin levels, depending on whether primary or secondary hypogonadism predominates [67,68].

In recent years, an additional form of hypogonadism has been suggested. In the European Male Aging Study, 9.5% of the study subjects had elevated S-LH and normal S-testosterone levels, which led to the suggestion of compensated, or

subclinical, hypogonadism. This condition is thought to be analogous to subclinical hypothyroidism, where high thyroid-stimulating hormone (TSH) levels are seen together with normal thyroid hormone levels [69].

Symptoms

The symptoms of hypogonadism depend on the patient's age when the condition develops. If hypogonadism develops before puberty, the symptoms are those of impaired puberty with small penis, testes and prostate, scant axillary and pubic hair, disproportionately long arms and legs (due to delayed epiphyseal closure), underdeveloped muscles, gynaecomastia and lack of voice maturation [70,71]. In postpubertal males, symptoms include progressive loss of muscle mass, reduced BMD, loss of libido, erectile dysfunction, oligospermia or azoospermia, poor ability to concentrate, decreased vitality and depressed mood. Occasionally, menopause-like hot flushes can occur with acute onset of hypogonadism [70,72]. As many of these symptoms are rather unspecific, they can be misinterpreted as "normal aging", especially in elderly men. Low sexual desire is considered the symptom most commonly associated with male hypogonadism, while impotence has been considered the most common symptom causing the patient to seek medical advice [73].

Low levels of testosterone have been associated with CVD [74], the metabolic syndrome [73] and diabetes mellitus type 2 [75], while testosterone deficiency has been associated with an increased risk of all-cause mortality, both in young [76] and elderly men [77]. S-LH-levels have also been found to be positively associated with all-cause mortality [78]. Whether there is a cause-effect relationship between hypogonadism and mortality, or whether a low testosterone level is an unspecific marker of poor health, remains to be elucidated.

Diagnosis

The European Association of Urology recommends that "Hypogonadism is diagnosed on the basis of persistent signs and symptoms related to androgen deficiency and assessment of consistently low testosterone levels (on at least two occasions) with a reliable method" [68].

Testosterone concentration follows a circadian rhythm, the highest levels being found in the morning, and an almost 50% lower concentration in the evening in younger men [79-81]. Serum testosterone also decreases rapidly after glucose intake [82]. Blood samples for the evaluation of testosterone levels should therefore be collected as fasting morning blood samples. Other factors affecting

testosterone levels include smoking; higher testosterone levels being found in smokers than in non-smokers [83,84], and stress; lower testosterone levels being found after severe somatic and/or psychological stress [85].

Testosterone replacement therapy

Testosterone replacement therapy (TRT) can be started in men with symptomatic androgen deficiency, the aim being to restore testosterone levels to the normal range, restore physiological androgen-dependent functions and improve quality of life (e.g. the sense of well-being, sexual function, muscle strength and BMD). Testosterone replacement therapy is contraindicated in patients with prostate cancer, male breast cancer, active desire of fertility, haematocrit >0.54 or severe chronic cardiac failure [68].

Cardiovascular disease is not currently considered an absolute contraindication for TRT, but there is concern that TRT might have adverse cardiovascular outcomes. One randomized controlled study on TRT in hypogonadal elderly frail men was discontinued due to an excess of cardiovascular events in the treatment arm [86]. Two large observational studies have reported an increased risk of cardiovascular events with testosterone use. In an American study on male hypogonadal veterans who underwent coronary angiography, testosterone replacement therapy was found to be associated with increased risk of mortality, myocardial infarction or ischaemic stroke [87]. Another study reported an increased risk of acute non-fatal myocardial infarction after the initiation of TRT in men aged 65 years or older, as well as in younger men with pre-existing heart disease. Testosterone levels before the start of treatment or indication for TRT were not known [88]. Finally, in a meta-analysis of placebo-controlled randomized studies published in 2013, TRT was found to increase the risk of cardiovascular-related events (including a wide range of disorders) [89].

In contrast, three meta-analyses published before 2013 revealed no increased risk of cardiovascular events [90], no increased risk of cardiovascular events or death [91] and no significant difference in the rates of death, myocardial infarction, revascularization procedures or cardiac arrhythmias [92] in subjects on TRT compared to subjects given a placebo or no TRT. One meta-analysis published in 2017 found that TRT improved quality of life, libido, depression and erectile function compared to placebo, and no significantly increased risk of cardiovascular mortality, myocardial infarction, stroke, prostate cancer or heart disease was seen compared to placebo [93]. Also, two large observational studies reported a lower risk of cardiovascular death [94], and stroke and myocardial infarction [95], among hypogonadal men treated with TRT compared to untreated hypogonadal men.

In conclusion, the findings regarding TRT and CVD are conflicting. Testosterone replacement therapy should therefore be used with caution in patients with CVD, and after discussing the potential risks with the patient.

Bone mineral density

The World Health Organization (WHO) defines osteoporosis as "a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk" [96]. There is no method of measuring overall bone strength, but BMD is often used as a proxy, as it accounts for approximately 70-80% of bone strength [97,98]. Decreased BMD in adults is correlated to an increased risk of fracture [99]. Osteoporotic fractures are associated with significant morbidity and mortality. In a Swedish study, over 1.5% of all deaths after the age of 50 years were causally related to a hip fracture [100].

Bone mineral density is commonly reported as areal density in g/cm^2. To provide relative measures, T-score or Z-score are used, with T-score being used to diagnose osteoporosis. The T-score is the number of standard deviations above or below the mean value for a young healthy adult of the same sex and ethnicity as the patient, and the Z-score is the number of standard deviations above or below the mean for the patient's age, sex and ethnicity.

In the case of post-menopausal women, the WHO defines normal BMD as a T-score $\geq$-1, low bone mass (osteopenia) as a T-score between -1.0 and -2.5, and osteoporosis as a T-score $\leq$-2.5, compared to young healthy women [96]. However, it is not clear how osteopenia and osteoporosis should be defined in men and children. According to The International Society for Clinical Densitometry, the same definition of osteoporosis can be applied to men from the age of 50 years. For premenopausal females and males younger than 50 years of age, Z-scores, not T-scores, are preferred [101]. This is particularly important in children, since they have not yet reached peak bone mass.

Skeletal growth and peak bone mass

Before puberty, bone growth is largely dependent on growth hormone [102], and bone mass is acquired relatively slowly during childhood. After the onset of puberty and the adolescent growth spurt, bone mineral accretion is rapid and reaches a peak shortly after peak height gain [103]. Sex steroids are essential for the completion of epiphyseal maturation and bone mineral accrual in the teenage years. The long-term

effect of pubertal timing on peak bone mass is uncertain. In a study by Gilsanz et al., the timing of puberty was found to have a significant impact on skeletal development; early onset of puberty resulting in higher bone mass at skeletal maturity in both boys and girls. The length of puberty, however, did not significantly influence bone accretion [104]. On the other hand, Darelid et al. found a substantial catch-up in BMD, with no significant differences in BMD of the lumbar spine, femoral neck or total body in young men with early, middle or late puberty, when evaluated at 24 years of age [105].

Peak bone mass is defined as the amount of bone acquired when accrual ceases or levels off at some point after the completion of growth and development [106]. The age of peak bone mass has been debated [106]. According to a recent study based on longitudinal data, male peak bone mass is reached at about 18.5 years of age for the lumbar spine and total hip, and at about 20.5 years of age for the total body [107]. About 60-80% of the variation in peak bone mass can be attributed to heritable factors [108], hence lifestyle factors and sex hormone levels can affect 20-40% of peak bone mass. There is strong evidence that calcium intake and physical activity have positive effects on BMD, especially during late childhood and the peripubertal years. There is also good evidence of a beneficial effect of vitamin D supplementation [103,106].

Bone status in childhood has been found to be a strong predictor of bone status in young adulthood, when peak bone mass is achieved [109]. It has therefore been suggested that adult osteoporosis may be a consequence of impaired bone mass acquisition during childhood and sub-optimal peak bone mass [97,98].

Androgen impact on the adult male skeleton

Males do not show the same rapid loss of sex hormones as females after the menopause, however, the serum testosterone level declines by about 1-2% per year from the third decade onwards [110,111]. Testosterone has both direct and indirect effects on bone quality, acting directly via the androgen receptor and indirectly via peripheral conversion to oestradiol [112]. It is now believed that oestradiol is the most important sex steroid for bone homeostasis [113-115] and fracture risk [112] in men. Although testosterone has a direct effect on bone, its main influence on fracture risk may be due to its positive effects on muscle strength and physical performance [116], reducing the tendency to fall [117].

Severe hypogonadism in males leads to rapid bone loss, as seen in men treated with GnRH agonist due to prostate cancer [118]. Mild to moderate hypogonadism is a risk factor for low bone mineral density (LBD) and osteoporosis in men older than 50 years [115]. Less is known about the association between hypogonadism and BMD in younger men. Two studies carried out on men under the age of 50 have reported an

association between hypogonadism and LBD in men with sexual dysfunction or infertility [119,120].

Testosterone replacement therapy and bone mineral density

The effect of TRT on BMD has been debated. Two meta-analyses published in 2005 and 2006 showed only a moderate increase in lumbar spine BMD, but no statistically significant effect on femoral neck BMD, after TRT for up to 36 months [121,122]. However both meta-analyses included studies on subjects with normal basal testosterone levels and subjects with concurrent diseases, and the follow-up period in many of the studies was only up to 12 months. Testosterone replacement therapy has subsequently been found to increase hip BMD [123], or hip and lumbar spine BMD [124,125] in hypogonadal men after treatment for 12 [123,124] or 36 months [125]. The effect of TRT for periods longer than 36 months on BMD is not known, and no data have been reported on the relation between fracture risk and TRT.

According to The Endocrine Society's Clinical Guidelines regarding osteoporosis in men, dual X-ray absorptiometry (DXA) is recommended for the assessment of BMD in hypogonadal men aged 50-69. For hypogonadal men at high risk of fracture treated with TRT, the addition of bisphosphonate or teriparatide is suggested, due to their proven antifracture efficiency. Testosterone replacement therapy is suggested for men with borderline high risk of fracture and symptomatic testosterone deficiency (S-testosterone <6.9 nmol/L), and men with high risk of fracture, S-testosterone <6.9 nmol/L and contraindications to approved pharmacological agents for osteoporosis [126].

Cancer treatment and gonadal dysfunction

Cancer treatment can cause reduced fertility or androgen deficiency, depending on which cell type(s) are damaged. Chemotherapy and radiotherapy can cause both dose- and time-dependent impairment of testicular function. Gonadal function can also be negatively affected secondary to damage to other organs, e.g. neural damage resulting from surgery, or pituitary damage following surgery or cranial radiotherapy.

Surgery

Fertility

Sub-fertile men have an increased risk of developing testicular cancer [127,128]. All men with testicular cancer undergo orchidectomy of the affected testicle. It is unclear whether unilateral orchidectomy affects fertility. In one study, decreased sperm concentration was found in 86% of testicular cancer patients, 5 months (median) after orchidectomy, and azoospermia developed in 9% [129]. On the other hand, significant recovery of spermatogenesis was found in the majority of patients one year after unilateral orchidectomy, and the development of azoospermia only in isolated cases, in another study [130].

Retroperitoneal lymph node dissection is most often used in non-seminomas for staging in clinical stage IIA (primary RPLND), or in cases of residual abdominal mass ≥1 cm after chemotherapy, but rarely in the treatment of seminomas [13]. Retroperitoneal lymph node dissection can cause retrograde ejaculation, but ejaculation can be preserved if surgery is performed with a nerve-sparing technique. In a recent review of 176 patients who underwent primary RPLND, 97% had antegrade ejaculation [131]. Post-chemotherapy RPLND (PC-RPLND) is a more difficult operation due to fibrosis, but ejaculation can be preserved in up to 85% after modified unilateral PC-RPLND and in up to 25% after bilateral PC-RPLND [132].

Hypogonadism

Unilaterally orchidectomized testicular cancer patients have also been reported to have lower testosterone levels despite raised LH, compared to men unilaterally orchidectomized due to non-malignant diseases, indicating an underlying testicular defect in men with testicular cancer [133]. Secondary hypogonadism can result from surgery in or near the pituitary, leading to gonadotropin deficiency. Fertility can be restored with gonadotropin substitution.

Radiotherapy

Fertility

The effect of radiotherapy depends on the target area, the irradiation dose and the number of fractions administered. Radiotherapy can be given directly to the testes, as in cases of germ cell neoplasia *in situ* (GCNIS), lymphoma or leukaemia with testicular involvement, testicular relapse of leukaemia or lymphoma, or as TBI as conditioning before HSCT. During the 1970s, prophylactic testicular irradiation was used in the treatment of ALL. The testes can also receive scattered irradiation from radiotherapy aimed at targets in the abdomen or pelvis.

The germinal epithelium is very sensitive to irradiation. Recovery of spermatogenesis requires surviving stem cells, and the probability of recovery is dependent on the dose and fractionation of irradiation. Testicular irradiation with doses of 1-3 Gy cause reversible azoospermia, doses of 3-6 Gy cause azoospermia that may be reversible but unlikely, while doses of ≥ 6 Gy cause azoospermia that is likely to be permanent. After single-dose testicular irradiation, the recovery of spermatogenesis takes up to 9-18 months after doses <1 Gy, 30 months after 2-3 Gy and >5 years (if at all) after doses >4 Gy [134]. Total body irradiation with doses of 10-14 Gy is associated with a very high risk of long-term azoospermia [135]. Fractioned small doses of testicular irradiation pose a greater risk than single large doses [136], and fractioned testicular irradiation with total doses >1.2 Gy can cause long-term azoospermia (> 40 months) [137].

Sperm concentration was reported to decline 6-12 months after radiotherapy in TCS treated with 25.2 Gy in 14 fractions to the para-aortic and ipsilateral iliac lymph nodes and scattered irradiation dose to the remaining testicle estimated to be 0.04-0.43 Gy; pre-treatment levels were recovered 2-5 years after therapy [138]. Similar results were found for TCS treated with a median dose of 26 Gy in 15-20 fractions to the lumbar-aortic lymph nodes. The lowest sperm concentrations were seen 6 months after treatment, with recovery to pre-treatment values 24 months after treatment [139]. Although these studies showed recovery of sperm production after adjuvant radiotherapy, TCS treated with para-aortic and ipsilateral iliac fractioned irradiation with a median dose of 28 Gy were found to have a significantly increased risk of post-treatment infertility compared with TCS treated with chemotherapy [140].

Cranial irradiation can impair spermatogenesis by damaging the hypothalamic-pituitary-gonadal axis, resulting in lower levels of gonadotropins. (This is discussed in more detail below in the section “Cancer treatment and gonadal dysfunction, Radiotherapy”.) However, no statistically significant differences in probability of siring a pregnancy were seen for male CCS treated with 0-40 Gy or >40 Gy hypothalamic/pituitary radiation compared to CCS treated without hypothalamic/pituitary radiation [141].

Hypogonadism

The testosterone-producing Leydig cells are more resistant to radiotherapy than the germinal epithelium. The prepubertal testes are more vulnerable to radiation-induced Leydig cell damage than those in adult men [142].

The risk of hypogonadism following radiotherapy to the testicle(s) is dose-dependent. Testosterone production was found to be better preserved after 16 Gy in 8 fractions than after 20 Gy in 10 fractions to the remaining testis in unilaterally orchidectomized TCS, showing stable testosterone values after 16 Gy and an annual decrease of 2.4% after 20 Gy [143]. Testicular cancer survivors treated with a median

dose of 30 Gy infradiaphragmatic radiotherapy had 2.7 and 3.3 higher odds of developing low testosterone levels than age-matched controls, at median follow-up times of 9.3 and 17.7 years, respectively [144]. Testicular irradiation ≥12 Gy or TBI have been identified as probably leading to an increased risk of testosterone deficiency [145]. However, it has been reported that Leydig cell function was preserved, with normal testosterone levels, even after irradiation doses as high as 30 Gy in 20 fractions to the remaining testicle in adult TCS [142].

Radiation to the hypothalamic-pituitary area can cause disturbances in pituitary function, the severity and frequency of which are correlated to the total irradiation dose and length of follow-up. In children, radiation doses <30 Gy cause isolated growth hormone deficiency (GHD) in about 30% of patients. Irradiation doses of 30-50 Gy cause GHD in 50-100%, long-term LH/FSH-deficiency in >20%, long-term TSH deficiency in 3-9%, and long-term adrenocorticotropic hormone (ACTH) deficiency in 3-6%. Following cranial irradiation of >60 Gy, or 30-50 Gy for pituitary tumours, it has been reported that 30-60% develop multiple hormonal deficiencies after 10 years. Precocious puberty can occur in boys after a radiation dose of 30-50 Gy. Radiation-induced hypothalamic-pituitary dysfunction is progressive and irreversible [146].

Chemotherapy

Fertility

Most chemotherapeutic agents can damage the proliferating germinal epithelium, resulting in transient or permanent oligospermia or azoospermia. The gonadal toxicity differs between chemotherapeutic drugs; alkylating agents and cisplatin being considered the most gonadotoxic in men. The toxic effect depends on the cumulative dose, and administering combinations of chemotherapeutic drugs has an additive effect on gonadal toxicity (Table 1) [147].

Table 1. Effect of different chemotherapeutic agents on male fertility (Adapted from Puschek et al. [147])

Agent (cumulative dose)	Effect	Comment
Cyclophosphamide (19 g/m^2)	Azoospermia	
Ifosfamide (>30 g/m^2)	Azoospermia likely	Additive effect with cyclophosphamide
Procarbazine (4 g/m^2)	Azoospermia	
Chlorambucil (1.4 g/m^2)	Azoospermia	
Lomustine (500 mg/m^2)	Azoospermia likely	
Carmustine (1 g/m^2)	Azoospermia likely	
Busulfan (>600 mg/kg)	Azoospermia likely	
Cisplatin (500 mg/m^2)	Azoospermia	
Carboplatin (>2 g/m^2)	Azoospermia likely	
Doxorubicin (770 mg/m^2)	Temporary azoospermia when given alone	Azoospermia in combination with other chemotherapeutic agents
Cytarabine (1 g/m^2)	Temporary azoospermia when given alone	Azoospermia in combination with other chemotherapeutic agents
Vinblastine (50 g/m^2)	Temporary azoospermia when given alone	Azoospermia in combination with other chemotherapeutic agents
Vincristine (8 g/m^2)	Temporary azoospermia when given alone	Azoospermia in combination with other chemotherapeutic agents

Alkylating agents affect spermatogenesis in male CCS in a dose-dependent manner [148]. No lower limit for gonadotoxicity has been determined. It has been reported that cyclophosphamide at doses of <7.5 g/m^2 are associated with a decreased risk of abnormal sperm counts [149,150], while it was found in another study that normal spermatogenesis could be preserved after a cumulative cyclophosphamide dose as high as 16 g/m^2 [151]. Chlormethine (also called mechlorethamine or mustine) and procarbazine also have dose-dependent effects on spermatogenesis [152]. Conditioning before HSCT with busulfan and cyclophosphamide [153-155] or fludarabine and melphalan [155] has been associated with an increased risk of impaired

spermatogenesis, although FSH was measured as a marker of spermatogenesis in the latter study, and semen samples were not analysed.

The cyclophosphamide equivalent dose (CED) has been developed to quantify the exposure to many different alkylating agents. The algorithm used to calculate the CED is based on comparisons of the acute haematological toxicities of different alkylating agents [156]. The CED has been shown to correlate negatively with sperm concentration in male CCS. No threshold dose has been identified, but it has been found that impaired spermatogenesis is unlikely after treatment with CED < 4000 mg/m^2. Substantial overlap was found between CED associated with normospermia, oligospermia and azoospermia [157]. No studies have yet been carried out to investigate the relation between CED and hypogonadism.

Cisplatin has a dose- and time-dependent effect on spermatogenesis. It has been reported that treatment with a cumulative dose of cisplatin ≤400 mg/m^2 results in temporary impairment of spermatogenesis, with the recovery of sperm production within 4 years in the majority of patients. Longitudinal studies on TCS have shown no significant decrease in sperm concentration after 1-2 cycles of BEP or PVB [138], and that post-treatment reduction in sperm counts returned to pre-treatment values 12 months after treatment following 1-2 cycles of BEP, and 24 months after 3-4 cycles of BEP [158]. In a study by Suzuki et al., sperm recovery defined as motile spermatozoa in the ejaculate was achieved in all patients receiving 1-3 cycles of BEP, 36 months after treatment, and in all receiving 4 cycles of BEP, 48 months after treatment. Among patients receiving 5-6 cycles of BEP, recovery was not seen until 36 months after treatment, and 5 out of 6 patients had regained sperm production after 60 months; the last patient remained azoospermic 84 months after treatment [159].

A cumulative cisplatin dose of ≥600 mg/m^2 is associated with a decrease in the probability of sperm production recovery. In a study on TCS treated with a median cumulative cisplatin dose of 583 mg/m^2 (range 300-750 mg/m^2), very low median sperm concentrations and low median sperm counts were seen after chemotherapy, showing no difference between 3-8 years and 11-16 years after treatment, indicating permanent impairment of sperm production [160]. Another study on TCS reported significantly decreased sperm concentration in patients treated with cumulative doses of cisplatin ≥600 mg/m^2 compared to doses <600 mg/m^2, after median follow-up times of 59 months (range 31-103) and 79 months (range 61-103) [161]. One study on sarcoma survivors aged 16-43 years at diagnosis also identified a cumulative cisplatin dose of ≥600 mg/m^2 as a risk factor for long-term impaired spermatogenesis [162].

Carboplatin is less toxic to spermatogenesis than cisplatin. Treatment with one cycle of adjuvant carboplatin in testicular cancer patients was reported to cause no change in sperm concentration 1 or 2 years after treatment [163], and the probability of TCS

recovering spermatogenesis was found to be higher after treatment with carboplatin-based chemotherapy than with CBC [164]. However, replacing cisplatin with carboplatin in EP or BEP results in poorer treatment outcomes [165,166].

It has been debated whether pre-pubertal status offers protection against chemotherapy-induced gonadal injury or not. Age at chemotherapy has been reported to affect spermatogenesis, showing less vulnerability in pre-pubertal subjects [167]. Furthermore, male CCS aged 0-4 years at diagnosis were found to be more likely to sire a pregnancy than those aged 15-20 years at diagnosis, whereas no difference was seen between those aged 5-9, and 10-14 years at diagnosis [141]. In contrast, pre-pubertal status was found not to be protective in studies on post-treatment increased FSH levels [168] or sperm concentration [152] after chemotherapy for Hodgkin's disease. Kenney et al. found no significant association between pubertal status and spermatogenesis after treatment with alkylating agents [149], and Green et al. found no relation between age at diagnosis and risk of azoospermia or oligospermia after treatment with alkylating agents [157]. Today, prepubertal status is considered not to offer protection against chemotherapy-induced gonadal injury [136].

Hypogonadism

Leydig cells are more resistant to chemotherapy than Sertoli cells. Hence, cancer survivors can exhibit normal testosterone production despite the presence of treatment-induced azoospermia or oligospermia.

Alkylating agents have been associated with hypogonadism after treatment with high cumulative doses. Leydig cell insufficiency, resulting in elevated LH levels and normal testosterone levels, and increased LH response to GnRH stimulation, has been reported after treatment with cumulative cyclophosphamide doses with a median of 20.5 g/m^2 [149]. Ifosfamide is less gonadotoxic than cyclophosphamide. Male CCS treated with >60 g/m^2 ifosfamide have been reported to have higher LH levels than those treated with <60 g/m^2 ifosfamide, although the LH levels in both groups were within normal limits, and no differences were seen in testosterone levels [169]. In another study on male CCS treated with ifosfamide at a median dose of 54 g/m^2, 98 out of 100 had normal testosterone levels and 86 out of 100 had normal LH levels a median of 11 years after treatment [170].

Cumulative doses of cyclophosphamide in the regimens used in 2010 were 3.2-4.8 g/m^2 for Hodgkin's disease, 4.8-16.8 g/m^2 for rhabdomyosarcoma, and 8.4 g/m^2 cyclophosphamide and 63 g/m^2 ifosfamide in combination for Ewing's sarcoma [141]. This implies that hypogonadism caused by treatment with alkylating agents alone is probably rare.

Hypogonadism has also been associated with treatment for testicular cancer. No significant difference was found in testosterone levels between TCS receiving cisplatin at cumulative doses ≤400 mg/m^2, and TCS treated with orchidectomy

alone, whereas cumulative doses of cisplatin ≥400 mg/m^2 resulted in lower testosterone levels, a median of 74 and 75 months after treatment [171]. In a study by Sprauten et al., who compared TCS treated with orchidectomy alone, or combined with infradiaphragmatic radiotherapy or chemotherapy with a median cumulative cisplatin-dose of 760 mg, all groups showed an increased risk of low testosterone levels at median follow-up times of 9 and 18 years, compared to age-matched controls [144].

A**zoospermia in testicular cancer survivors**

It is not yet possible to identify those individuals who will suffer from long-term azoospermia after testicular cancer treatment. Certain cancer treatments carry a higher risk of reduced fertility, as described above, and side-effects are also modulated by individual vulnerability to cancer treatment [172].

Previous studies have provided inconclusive results regarding semen parameters as potential predictive factors of reduced fertility after testicular cancer treatment. Higher probabilities of recovering sperm production have been found in two studies on patients with normal pre-treatment sperm counts [164], and pre-treatment total sperm counts ≥39x10^6 [158]. In contrast, other studies have reported that the recovery of spermatogenesis was not related to pre-treatment sperm count [139], or pre-treatment sperm concentration [138].

Cryptorchidism is a risk factor for testicular cancer [173], and a history of cryptorchidism has also been reported to be a risk factor for self-reported post-treatment infertility in TCS [140]. However, since no semen samples were analysed in the latter study, it remains unclear whether these infertile patients had oligospermia or azoospermia. It has also been reported that highly increased levels of FSH prior to post-orchidectomy treatment were correlated with low post-treatment spermatogenesis in TCS [174].

No previous studies have been able to identify risk factors with sufficiently high sensitivity and/or specificity to predict long-term infertility in individual TCS. Bearing in mind its association to FSH and functioning spermatogenesis, inhibin B appears to be a potential predictive factor for azoospermia in TCS.

Hypogonadism in young male cancer survivors

Previous studies addressing Leydig cell function in male CCS have focused on comparing testosterone levels between patients and controls [175,176], rather than the assessment of risk factors for hypogonadism. Many studies reporting on the frequency of hypogonadism after cancer treatment also lack control groups

[17,168,171,177,178], or have controls that can be suspected of not representing the general population. Among the studies on CCS with control groups, Romerius et al. used male partners of pregnant women as controls [179], while Greenfield et al. used controls recruited by advertisement in the community and from general practitioners' surgeries [175]. Among the studies on TCS with control groups, the study by Sprauten et al. included population-based controls [144], while 200 male blue-collar workers at a smelting plant were included as controls in a study by Nord et al. [180]. Hence, further studies are required to investigate the prevalence of, and risk factors for, hypogonadism in young male CCS, compared to the general population.

Treatment for impaired fertility after cancer treatment

Cancer survivors rendered hypogonadal after cancer treatment can be treated with TRT. Testosterone replacement therapy can negatively affect spermatogenesis by suppression of the hypothalamic-pituitary-gonadal axis. Spermatogenesis can recover spontaneously after cessation of TRT, but this may take several months to several years. If spermatogenesis does not recover after discontinuation of TRT, patients can be treated with gonadotrophins in order to compensate for FSH/LH deficiency. Gonadotropins can also be added to TRT, if the patient is unwilling to discontinue TRT [181].

In cases of retrograde ejaculation, treatment with α-sympathomimetics can be tried to restore antegrade ejaculation, or spermatozoa can be retrieved from the urine for use in assisted reproductive techniques [182].

It is not known for how long after chemotherapy azoospermia can be considered as potentially reversible. Recovery of sperm production has been reported 4 and 5 years after treatment with 5-6 cycles of BEP [158], and more than 10 years after therapy in adult males treated for Hodgkin's disease [183]. However, very late recovery of sperm production may be of less clinical importance due to the limited reproductive window of a couple [184].

All post-pubertal, and even selected pubertal, male cancer patients should be offered semen cryopreservation before cancer treatment. In cases of post-treatment azoospermia due to testicular failure, without cryopreserved semen, testicular biopsy and subsequent intracytoplasmic sperm injection, if viable sperms are retrieved, is the last opportunity to achieve biological paternity.

Cancer treatment and bone mineral density

Radiotherapy

Radiotherapy can have local effects on bone. Radiation necrosis and pathologic fractures can occur after doses >50 Gy [185]. Radiotherapy can probably also have a local effect on bone after fractionated irradiation at a lower total dose. Two retrospective studies on women treated with radiotherapy have reported a high frequency of fractures near the field of irradiation. One study on women treated with curative-intent radiotherapy for cervical cancer found pelvic fractures in 9.7% of patients on post-treatment CT (computed tomography) or MRI (magnetic resonance imaging), almost half of them asymptomatic [186]. One large study on women ≥65 years reported a higher cumulative incidence of pelvic fractures, including hip fractures, in women treated with pelvic radiotherapy than in women with the same diagnosis not treated with radiotherapy. The hazard ratios for fractures were 3.16 for radiotherapy vs. no radiotherapy in women treated for anal cancer, 1.66 in women treated for cervical cancer, and 1.65 in women treated for rectal cancer, all statistically significant [187]. However, none of the studies gave any information on BMD, and it is therefore not known whether the fractures were associated with a local decrease in BMD.

A recent retrospective study reported a decrease in vertebral body BMD measured on CT in patients with abdominal cancers treated with radiotherapy, given in combination with chemotherapy in the majority of patients. No changes in vertebral body BMD were reported in patients with abdominal cancers after treatment with chemotherapy only. Irradiated patients were treated with a median of 50.4 Gy fractionated irradiation, and the reduction in BMD was found to be proportional to the radiation dose deposited to the vertebral body. Four of 42 patients had an asymptomatic vertebral compression fracture in the field of irradiation, compared to no fractures in the six patients treated with chemotherapy only, and it seems plausible that the loss in BMD may have contributed to the development of these vertebral compression fractures [188].

Radiotherapy of the hypothalamic-pituitary axis can also have an indirect effect on BMD by inducing GHD and secondary hypogonadism, as described in the section “Cancer treatment and gonadal dysfunction, Radiotherapy” above. Adults with childhood onset GHD have in some, but not all, studies been shown to have lower BMD than controls, and adults with untreated GHD with adult onset have decreased BMD [189]. Bone mineral density increases after more than 1 year of growth hormone replacement therapy (GHRT) in adults with GHD [190]. In a study on adult CCS treated with cranial radiotherapy, evaluated a mean of 27 years after cancer therapy, untreated LH/FSH-deficiency was found to be associated with low BMD (OR 2.42,

95% CI 1.10-5.30, p=0.03). Untreated GHD was not associated with low BMD in this cohort, although the association was borderline significant (OR 1.78, 95% CI 0.99-3.18, p=0.05) [191].

Chemotherapy

Long-term treatment with oral glucocorticoids is a known risk factor for osteoporosis. Glucocorticoid–induced bone loss should be suspected, and steps taken to prevent it, after treatment with prednisone equivalents of ≥5 mg/day for ≥3 months [192]. Long-term glucocorticoids are used in the treatment of childhood haematological malignancies. Increased fracture rates and higher incidences of skeletal complications during the first few years after diagnosis have been reported in children treated for ALL; most complications occurring during maintenance therapy [193]. However, it is not known whether glucocorticoid treatment in childhood causes reduced BMD or increased risk of fracture in adulthood. Cumulative methotrexate doses of >40 g/m^2, as used in the treatment of highly malignant sarcomas or high-risk ALL, have been associated with a high risk of low BMD in adulthood [194,195]. Alkylating agents have also been stated to be risk factors for osteoporosis by causing hypogonadism [196].

Bone mineral density in childhood cancer survivors

Children with ALL already have reduced BMD at diagnosis, which can decrease further during therapy [197,198]. It is unclear whether this reduction in BMD persists during adulthood. Recovery of BMD in adult survivors of ALL has been reported in a study by Gurney et al., where the Z-scores of 67% of subjects with previous BMD Z-scores ≤-2 improved by ≥1 category, a median of 8.5 years later [199].

In the large St Jude Lifetime Cohort Study, 39.3% of adult CCS (male and female together) had Z-scores <-1 on DXA (total body or lumbar spine), higher frequencies being observed among those treated with corticosteroids (45.0%), methotrexate (45.5%) and radiation to the hypothalamic-pituitary area (54.3%) [17].

Hypogonadism has been reported to be a risk factor for low BMD in CCS [136,196,200]. Studies on the association between hypogonadism and BMD in CCS often include both male and female CCS in the studied cohort [200-203] or lack controls [200,202,204], making it difficult to draw any conclusions regarding the effect of hypogonadism on BMD in adult male hypogonadal CCS. Studies on pre-pubertal or young adult CCS have revealed a correlation between hormonal levels and bone mass in a cohort of male and female CCS [201], hypogonadism and increased risk of LBD in male CCS [203], and gonadal dysfunction and LBD in a cohort of male and female CCS [200].

However, findings in pre-pubertal or young adult subjects of both sexes are not directly applicable to an adult male population.

Two studies have focused on the association between gonadal function and BMD in adult male CCS [204,205]. In both these studies, the CCS had mild hypogonadism with low or low normal testosterone levels. Holmes et al. reported a positive correlation between serum testosterone levels and BMD in the lumbar spine and femoral neck in 29 men with azoospermia treated for Hodgkin's disease [204]. Howell et al. reported lower femoral neck BMD in CCS treated for haematological malignancy exhibiting raised LH levels and low or low normal testosterone levels, compared to equally treated CCS exhibiting normal LH and testosterone levels [205].

Bone mineral density in testicular cancer survivors

Findings regarding the relation between treatment modality and hypogonadism in TCS are conflicting. Murugaesu et al. found no increased risk of osteoporosis in TSC treated with orchidectomy only or orchidectomy followed by ≥3 cycles of CBC, 5-28 years after treatment [206]. In a prospective study of newly diagnosed testicular cancer patients, Willemse et al. reported normal BMD in clinical stage I patients up to 5 years after treatment. Those with metastatic disease given ≥3 cycles of CBC exhibited a significant decrease in BMD one year after treatment, which was unrelated to gonadal status and the dose of cisplatin or corticosteroids given [207]. In a follow-up study, Willemse et al. reported TCS to have an increased prevalence of mild to moderate vertebral fractures, but no association was found between BMD, cumulative doses of cisplatin or dexamethasone, or gonadal status [208].

Lower BMD in TCS compared to controls was reported by Foresta et al., despite no biochemical signs of testosterone deficiency in the patient group [209]. In a large study on 1249 TCS, Ondrusova et al. reported that 43-51% of the TCS had osteopenia/osteoporosis. No increased risk of osteopenia/osteoporosis was seen in patients treated with unilateral orchidectomy and radiotherapy, or 2-4 cycles of chemotherapy, compared to patients treated with unilateral orchidectomy alone. Both low testosterone and high LH levels were seen more often in TCS with low BMD than in those with normal BMD, but the BMD in eugonadal and hypogonadal TCS was not compared [210].

One study has addressed the possible effect of adjuvant radiotherapy on BMD in TCS. In a study including 30 TCS treated with orchidectomy and radiotherapy (30 Gy in 15 fractions) of the para-aortic and ipsilateral iliac lymph nodes for clinical stage I seminoma, no significant difference in BMD was seen between the irradiated and non-irradiated hip a median of 28 months after radiotherapy [211].

In conclusion, there is a need for studies comparing BMD in young male cancer survivors to that in the general population, and exploring the potential association between hypogonadism and BMD in this patient category.

Current Recommendations for Long-Term Follow-up of Gonadal Function and Bone Mineral Density after Childhood or Testicular Cancer

Childhood cancer survivors

The first national guidelines for long-term follow-up after childhood cancer were published in Sweden, on-line, in April 2016 [5]. These guidelines concern all patients treated for cancer before 18 years of age, and cover side-effects after surgery, radiotherapy and chemotherapy, e.g. neurological sequelae, cardiac toxicity, pulmonary toxicity, endocrinologic disorders and female and male gonadal function.

In these guidelines, testicular irradiation at doses above 12 Gy or TBI are identified as risk factors for testosterone insufficiency, and irradiation doses above 30 Gy to the hypothalamic-pituitary area are identified as risk factors for decreased production of LH and FSH. It is recommended that male CCS with normal hormonal levels previously treated with irradiation doses exceeding 30 Gy to the hypothalamic-pituitary area or testicular irradiation should be informed about the subsequent risk of, and symptoms resulting from, testosterone deficiency, and encouraged to seek medical help if they have symptoms indicating testosterone deficiency.

Ifosfamide, vincristine, methotrexate, cisplatin, anthracyclines and purine analogues are identified as having potentially negative effects on BMD. Long-term corticosteroid treatment for graft-versus-host disease after allogenic bone marrow transplant, hypogonadism, immobilisation, nutritional defects and heredity factors predisposing to osteoporosis have also been identified as potentially leading to reduced BMD. DXA is recommended on clinical suspicion of osteoporosis, for example severe back pain, repeated fractures or fractures after minimal trauma.

Testicular cancer survivors

In Sweden and Norway, TCS are followed up according to the recommendations of SWENOTECA [212]. Patients with non-seminoma clinical stage I are followed up until 5 years after the end of treatment, with regular controls of tumour markers and radiological examinations to detect cancer recurrence, and controls of hormonal levels (testosterone, SHBG, LH and FSH), metabolic screening (lipids, fasting glucose and HbA1c) and blood pressure. The same controls are carried out up until 10 years after the end of treatment for patients with metastatic non-seminoma and all stages of seminoma. There are no recommendations to evaluate BMD.

Aims of this Work

The overall aim of the work presented in this thesis was to improve follow-up and counselling of young male cancer survivors, by investigating the prevalence, and evaluating potential risk factors, of gonadal dysfunction and decreased bone mineral density.

The specific aims were:

- to assess the frequency of long-term post-treatment azoospermia in TCS, relate the risk to the type of cancer treatment, and evaluate inhibin B and previous cryptorchidism as potential predictive markers of azoospermia (Paper I);

- to investigate the risk of biochemical hypogonadism in TCS and male CCS, compared to men from the general population, and to evaluate cancer diagnosis and type of cancer treatment as potential risk factors (Paper II);

- to assess potential differences in BMD and the risk of low bone mineral density in TCS (Paper III) and male CCS (Paper IV), compared to men from the general population, and to elucidate possible associations with biochemical hypogonadism, cancer diagnosis and type of cancer treatment.

Subjects and Methods

A summary of the subjects and methods is given in this chapter. Further details concerning the cohorts studied and the methods used can be found in the respective papers and the supplementary information to Papers I, II and IV.

Subjects

Two cohorts of TCS were studied: TCS cohort A (TCS-A, Paper I) and TCS cohort B (TCS-B, Papers II & III). The TCS-B cohort was based on re-invitation of part of the cohort from which TCS-A was derived. A cohort of CCS was also studied (Papers II & IV), and two control groups: one for TCS-B (Papers II & III) and the other for CCS (Papers II & IV). An overview of the cohorts studied is presented in Figure 5.

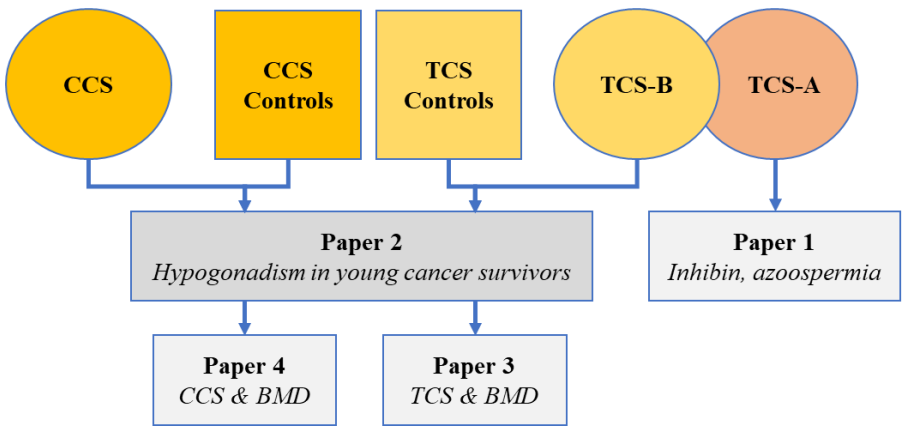

Figure 5. Overview of the cohorts studied in the work presented in this thesis. CCS = childhood cancer survivors; TCS = testicular cancer survivors; TCS-B = testicular cancer survivors cohort B; TCS-A = testicular cancer survivors cohort A; BMD = bone mineral density

All the subjects provided written informed consent at the time of inclusion in the respective studies. Ethical approval was obtained from the regional ethical review boards of Lund University and Karolinska Institute (TCS-A, Paper I), or Lund University (TCS-B, CCS and controls, Papers II-IV).

Childhood cancer survivors

The cohort of CCS was derived from 427 consecutive male CCS identified through the Swedish Cancer Registry, diagnosed with childhood cancer between 1970 and 2002, living in the region of Skåne, southern Sweden at diagnosis, and previously invited to participate in a study on reproductive function [179]. The following inclusion criteria were applied to this cohort

- all forms of extra-cranial malignant neoplasm or any CNS neoplasm before 18 years of age,
- >18 years old and alive as of 1st December 2009,
- >3 years since last cancer treatment.

Eleven men were deceased and 10 could not be located. One patient treated for testicular cancer, and included in both invited cohorts of CCS and TCS, was transferred to TCS-B. The remaining 405 men were contacted by letter, and 146 agreed to participate in the study. Six subsequently dropped out of the study, one was excluded due to management with surveillance only (diagnosed with optic glioma), six were excluded due to non-malignant disease (carcinoid of the appendix) and eight were excluded due to second malignancy or relapse within 3 years of inclusion, leaving 125 subjects (31% of those invited).

Testicular cancer survivors

TCS-A

The study described in Paper I was based on data from a cohort of TCS included in a previous study on reproductive function and hypogonadism after treatment for testicular cancer, called the Fertility Study [138,213]. The study started in 2001 in Lund, and in 2003 in Stockholm, and all men aged 18-50 years, diagnosed with testicular cancer <5 years prior to inclusion were eligible. Patients delivered semen samples after orchidectomy, but before further treatment (T_0), and 6 (T_6), 12 (T_{12}), 24 (T_{24}), 36 (T_{36}) or 60 (T_{60}) months after completion of testicular cancer treatment. Patients could enter the study at any time from T_0 to T_{60}, and were asked to provide ejaculates at the remaining times. Inclusion ceased in October 2006. A total of 464 men were eligible for the Fertility Study. Seventy-five men declined to participate and 52 men

were excluded due to mental co-morbidity, linguistic difficulties, bilateral testicular cancer or physical disability, leaving 337 participants in the Fertility Study.

Five subjects with extragonadal tumours were excluded. The following exclusion criteria were applied to the remaining 332 TCS

- azoospermia resulting from causes other than chemotherapy or radiotherapy used in testicular cancer treatment (e.g. previous vasectomy, azoospermia prior to testicular cancer or bilateral orchidectomy), or receiving radiotherapy to the remaining testicle due to GCNIS,
- relapsing disease or development of GCNIS in the remaining testicle during the study period,
- no assessable semen sample at T_{36} or T_{60} (e.g. TRT, retrograde ejaculation or no ejaculate delivered at T_{36} or T_{60}).

A total of 115 TCS were excluded, leaving 217 TCS constituting cohort TCS-A.

In order to enable the analysis of predictive factors for sperm production at T_{36} or T_{60} (T_{36-60}) in men with sperm production at T_0, two subjects with azoospermia at T_0 and 98 subjects without ejaculate at T_0 were excluded, leaving 117 TCS with longitudinal data.

TCS-B

For the TCS cohort reported on in Papers II & III, called TCS-B, the 165 TCS (including three TCS with extragonadal tumours) included in the above-mentioned Fertility Study and treated at Lund University Hospital were re-invited. Three were deceased and 1 had emigrated. The remaining 161 men were contacted by letter, and 96 agreed to participate. Two subsequently dropped out of the study, and 2 were excluded due to prostate cancer or prolactinoma diagnosed shortly after inclusion, leaving 92 subjects (57% of those invited).

Controls

An age-matched control from the general population was identified for each CCS and each subject in TCS-B. Exclusion criteria were

- previous diagnosis of tumour in the CNS or any malignant disease other than basal cell carcinoma,
- Klinefelters syndrome (47, XXY).

Controls were identified and invited to participate as follows. For each cancer survivor included in the study, a list of men matched by date of birth and living in the region of Skåne was extracted from the Swedish Population Register. Only men

living in the western part of Skåne were considered as potential controls, for practical reasons. Each of the cancer survivor was identified as the index case on the list. The man directly above the index case was invited to participate by letter. If he declined, or no reply was received within 14 days, the man directly below the index case was contacted. If he also declined, or no reply was received within 14 days, the man directly above the first invited man was invited, and so on, until a potential control agreed to participate in the study.

Of 977 invited potential controls, 240 men (25%) agreed to participate. Of these, 14 were excluded due to exclusion/drop out of the corresponding cancer survivor, 2 due to current or previous malignancy, 1 due to Klinefelters syndrome, 1 due to lack of sample material, 1 dropped out of the study and 4 were duplicates of recruited controls.

Representativeness of study participants

TCS-A

The 217 subjects in TCS-A did not differ in mean age compared to the 242 TCS excluded or declining participation (32.6 and 33.4 years, respectively). The proportion of patients with stage I disease was higher among participants than non-participants, and consequently the proportion of subjects receiving adjuvant chemotherapy was somewhat higher among the participants, whereas the opposite was true for those receiving the most extensive treatment (Figure 6).

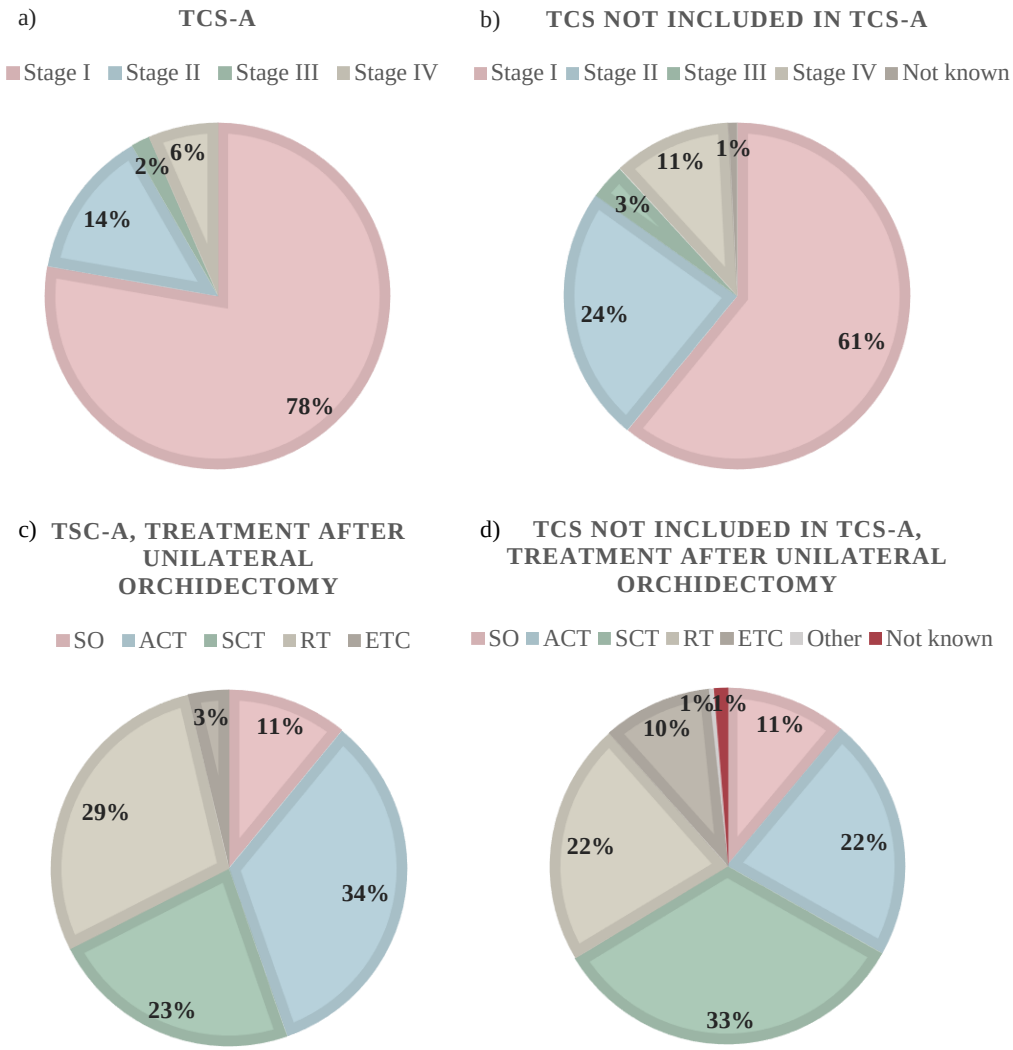

Figure 6. Distribution of testicular cancer stages: (a) in TCS-A, n=217 and (b) among eligible TCS not included in the Fertiliy Study, plus TCS included in the Fertility Study not included in TCS-A, n=242. Distribution of treatment received after unilateral orchidectomy: (c) in TCS-A, n=217 and (d) among eligible TCS not included in the Fertiliy Study, plus TCS included in the Fertility Study not included in TCS-A, n=242. SO = surveillance only; ACT = adjuvant chemotherapy, 1-2 cycles of cisplatin-based chemotherapy (CBC) or carboplatin; SCT = standard dose chemotherapy, 3-4 cycles of CBC; RT = adjuvant radiotherapy to para-aortic and ipsilateral iliac lymph nodes; ETC = extensive treatment with chemotherapy, >4 cycles of CBC or ≥4 cycles of CBC + radiotherapy to targets other than the remaining testicle.

The 117 patients for whom longitudinal data were available, were, on average, three years younger than the 100 patients without longitudinal data (31.1 and 34.4 years, respectively). There were no differences between the two groups regarding stages of disease. Treatment with 1-2 cycles of chemotherapy was more frequently seen among the patients with longitudinal data (41% vs. 26%), whereas surveillance was less frequent (2.6% vs. 20%).

TCS-B

Subjects in TCS-B were compared to TCS treated in Lund and excluded from, or declining to participate in, the Fertility Study, plus invited TCS excluded from, or declining to participate in, the current study, in total n= 141. Fewer patients had stage IV testicular cancer among those studied, than among the non-participants, whereas no major difference was seen in the distribution of treatment received after orchidectomy (Figure 7).

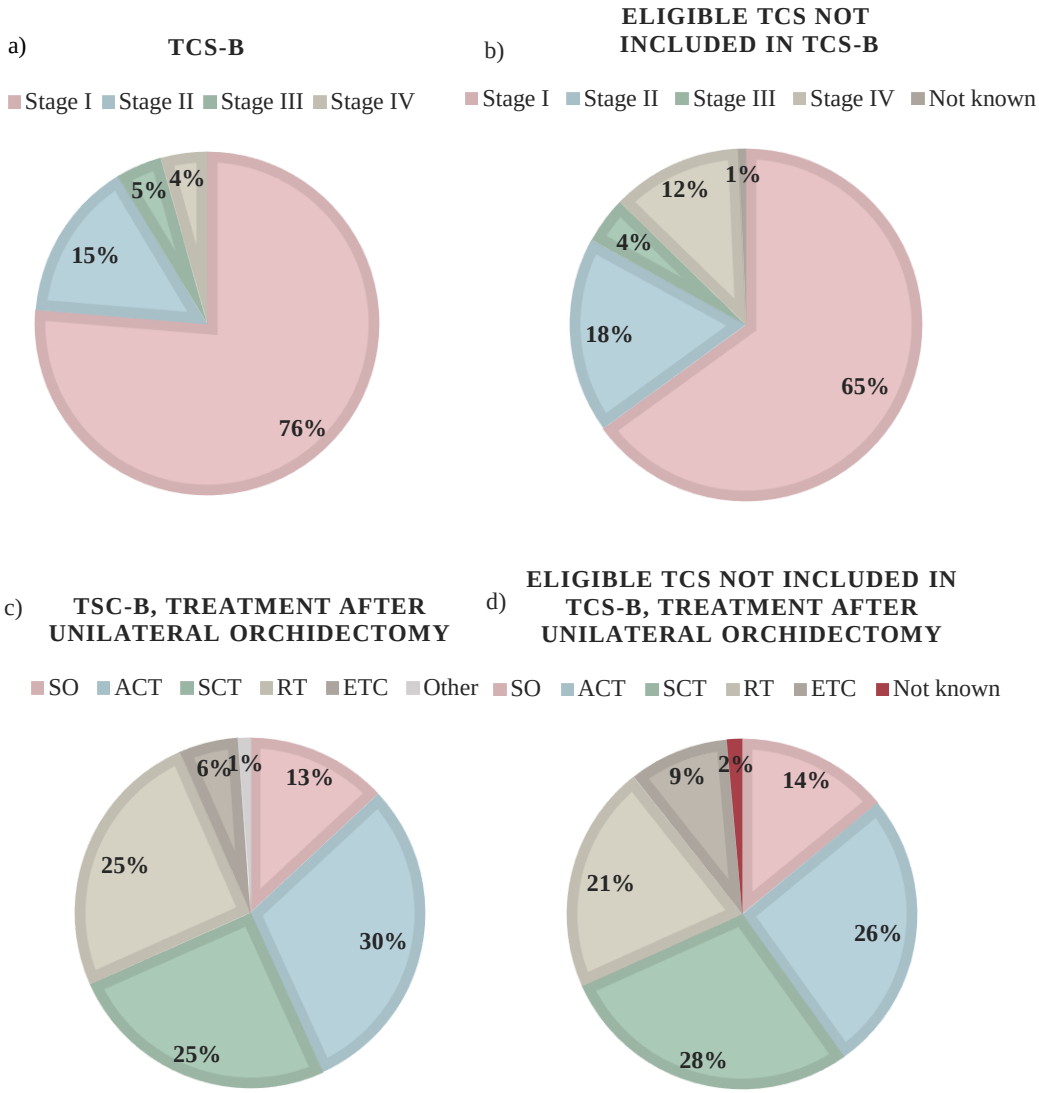

Figure 7. Distribution of testicular cancer stages: (a) in TCS-B, n=92 and (b) among TCS treated in Lund and excluded from, or declining participation in, the Fertility Study, plus TCS excluded from, or declining participation in, the current study, n=141. Distribution of treatment received after unilateral orchidectomy: (c) in TCS-B, n=92 and (d) among TCS treated in Lund and excluded from, or declining participation in, the Fertility Study, plus TCS excluded from, or declining participation in, the current study, n=141. SO = surveillance only; ACT = adjuvant chemotherapy, 1-2 cycles of cisplatin-based chemotherapy (CBC) or carboplatin; SCT = standard dose chemotherapy, 3-4 cycles of CBC; RT = adjuvant radiotherapy to para-aortic and ipsilateral iliac lymph nodes; ETC = extensive treatment with chemotherapy, >4 cycles of CBC or ≥4 cycles of CBC + radiotherapy to targets other than the remaining testicle.

Data on the number of biological children of the participants and non-participants were extracted from the Swedish Multi-Generation Register in order to evaluate possible selection bias based on fertility. The distribution of TCS having 0, 1, 2 or ≥3 children was 12%, 37%, 47% and 4% among participants, and 14%, 36%, 33% and 17% among non-participants.

Childhood cancer survivors

In order to investigate whether the CCS cohort was representative of childhood cancer patients in Sweden, the diagnosis distribution in the CCS cohort was compared to the diagnosis distribution of male cancer patients under 19 years of age at diagnosis in Sweden in 2009 (the year the study started). Some underrepresentation of patients with brain tumours and some overrepresentation of patients with other malignancies were seen (Figure 8).

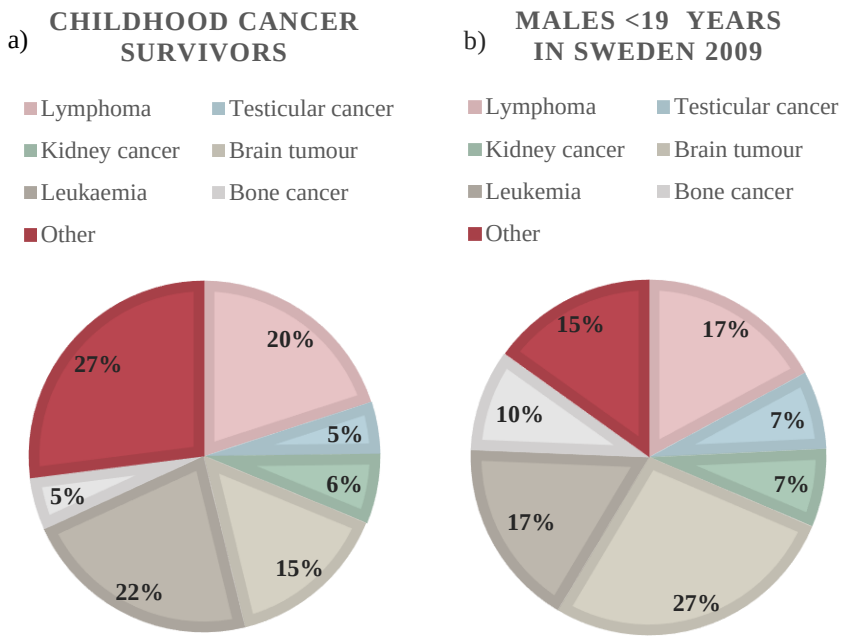

Figure 8. Distribution of diagnoses: (a) of childhood cancer survivors included in the current study, n= 125, and (b) in males <19 years of age at cancer diagnosis in Sweden 2009.
(Data from NORDCAN [1 November2013]. Available at: http://www-dep.iarc.fr/NORDCAN/SW/frame.asp)

Data on the number of biological children of participants and non-participants were extracted from the Swedish Multi-Generation Register to evaluate possible selection bias based on fertility. The distribution of CCS having 0, 1, 2 or ≥3 children was 52%, 14%, 29% and 5.3% among participants, and 65%, 14%, 17% and 5.1% among non-participants.

Controls

Data on height, weight, body mass index (BMI) and proportion of smokers among the controls for TCS-B and CCS, and corresponding data for men in similar age categories from the general population in Sweden are presented in Table 2.

Table 2. Characteristics of the 217 controls (mean age 38.4 years, range 24.1-58.8 years), and men from the general population in Sweden 2010-2011

	Controls	Men from general population 2010-2011
Height (cm)	181.6 (±0.9)	180.5 (±0.5)[#]
Weight (kg)	83.4 (±1.7)	85.2 (±1.2)[#]
Body mass index (kg/m^2)	25.2 (±0.5)	26.1 (±0.3)[#]
Smoking, current (%)	11.9 (±4.4)[†]	11.6 (±2.7)*

[#] Data on height, weight and body mass index are for men 30-39 years.
[†] Data missing for 7 controls.
* Data on smoking are for men 35-44 years.
Height, weight and body mass index are presented as means (95% confidence interval).
Smoking is presented as percent (95% confidence interval).
Data from Statistics Sweden [24 January 2018], available at:
http://www.scb.se/hitta-statistik/statistik-efter-amne/levnadsforhallanden/levnadsforhallanden/undersokningarna-av-levnadsforhallanden-ulf-silc/pong/tabell-och-diagram/halsa/halsa--fler-indikatorer/

The distribution of controls having 0, 1, 2 or ≥3 children was 12%, 42%, 35% and 11% among participants, and 21%, 46%, 23% and 10% among non-participants.

General characteristics of TCS-B, CCS and their respective controls are presented in Table 3.

Table 3. General characteristics of testicular cancer survivors cohort B (TSC-B), childhood cancer survivors (CCS), and their respective controls

	TCS-B (n=92)	Controls for TCS-B (n=92)	CCS (n=125)	Controls for CCS (n=125)
Age at inclusion (y)	40.3 (7.4)	41.2 (7.3)	33.7 (30.2-40.1)	34.4 (30.5-40.6)
Length of follow-up (y)	9.2 (2.7)	NA	24.3 (7.1)	NA
Age at diagnosis (y)	30.8 (7.2)	NA	9.6 (5.4-15.0)	NA
Height (m)	1.84 (0.06)	1.82 (0.08)	1.80 (1.75-1.86)	1.82 (1.78-1.85)
Weight (kg)	90.3 (14)	85.0 (12.6)	82.1 (72.0-91.5)	81.4 (73.3-88.6)
Body mass index (kg/m^2)	26.8 (3.9)	25.5 (3.3)	25.1 (22.8-27.6)	24.7 (22.6-26.9)
Smoking, current[#], n (%)	21 (24)	8 (9.1)	10 (8.2)	17 (14)

Age at inclusion, length of follow-up, age at diagnosis, height, weight and body mass index for TCS-B and their controls are presented as means (SD).
Age at inclusion, age at diagnosis, height, weight and body mass index for CCS and their controls are reported as medians (interquartile range) due to non-normal distribution.
NA = not applicable.
[#] Data on smoking missing for 4 TCS-B, 4 controls for TCS-B, 3 CCS and 3 controls for CCS

Cancer treatment

Testicular cancer survivors

All TCS underwent unilateral orchidectomy, including the 3 TCS with an extragonadal tumour (due to initial suspicion of testicular tumour in 2 subjects, one subject had primary extragonadal disease and subsequently developed a unilateral testicular tumour). One of the subjects in TCS-B had had a bilateral orchidectomy, and one TCS had received radiotherapy to the remaining testicle due to GCNIS. These 2 subjects were excluded from the analyses of treatment effects regarding OR for hypogonadism for therapeutic subgroups (Paper II) and BMD for therapeutic subgroups (Paper III).

Treatment was given according to the SWENOTECA protocols applied at the time of diagnosis (March 1996-March 2006). Patients with extragonadal tumours received the same treatment as for testicular cancer clinical stage IIB-IV [214]. Briefly, treatment for clinical stage I seminoma was adjuvant radiotherapy, adjuvant

chemotherapy or surveillance after orchidectomy. Adjuvant radiotherapy consisted of 25.2 Gy administered in 14 fractions to the infradiaphragmal para-aortic and ipsilateral iliacal lymph nodes. The scattered dose to the remaining testis in patients treated in Lund was estimated retrospectively in 7 randomly selected men and found to be 0.04-0.43 Gy. Adjuvant chemotherapy consisted of one cycle of carboplatin. Patients with clinical stage IIB-IV seminomas were treated with the BEP regimen (bleomycin 30,000 IU on days 1, 5 and 15 to a maximum dose of $3x10^5$ IU, etoposide 100 mg/m^2 on days 1-5 and cisplatin 20 mg/m^2 on days 1-5, every third week) or EP (BEP minus bleomycin). Patients with clinical stage I non-seminomas without vascular invasion of tumour cells were offered adjuvant chemotherapy or surveillance. Patients with clinical stage I non-seminomas with vascular invasion of tumour cells were recommended adjuvant chemotherapy. Standard chemotherapy for non-seminomas was the BEP regimen; 1-2 cycles in the adjuvant setting and 3-4 cycles for metastatic disease.

The Royal Marsden Hospital staging system was used for staging [215].

Subgroups of testicular cancer survivors according to cancer treatment, diagnosis and gonadal status

Testicular cancer survivors were grouped according to cancer treatment after unilateral orchidectomy (Papers I-III).

- Surveillance only (SO): no further treatment after unilateral orchidectomy
- Adjuvant chemotherapy (ACT): 1-2 cycles of CBC or carboplatin
- Standard dose chemotherapy (SCT): 3-4 cycles of CBC
- Adjuvant radiotherapy administered to the paraaortic and ipsilateral iliac lymph nodes
- Extensive treatment with chemotherapy (ETC): >4 cycles of CBC or ≥4 cycles of CBC + radiotherapy of targets other than the remaining testicle;
- Other (see Papers II & III)

Adjuvant radiotherapy was given with 2 parallel-opposed anterior-posterior and posterior-anterior equally weighted fields. The anatomic localisation of the irradiation fields is shown in Figure 9 [214].

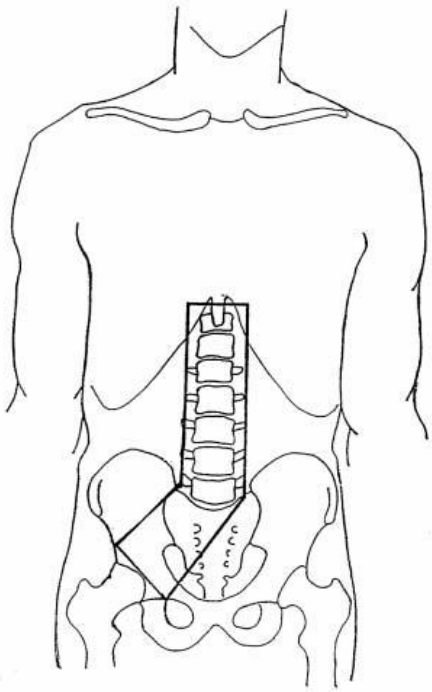

Figure 9. Target of irradiation in adjuvant radiotherapy to the infradiaphragmal para-aortic and ipsi-lateral iliacal lymph nodes. (Illustration from SWENOTECA V [214]. Printed with permission.)

Diagnostic subgroups (Paper II): Patients were categorized as seminoma or non-seminoma.

Gonadal status (Paper III): Patients were categorized as hypogonadal or eugonadal. (See "Definition of hypogonadism" below.)

Childhood cancer survivors

Childhood cancer survivors were treated with surgery, chemotherapy and radiotherapy, alone or in various combinations, based on cancer diagnosis and treatment protocols valid at the time. (For details on cancer treatment received, e.g. CED, irradiation targets etc., see supplementary information to Paper II.)

Subgroups of childhood cancer survivors according to cancer treatment, diagnosis and gonadal status

Therapeutic subgroups of CCS were identified (Paper II).

- Brain surgery, excluding radiotherapy to any target, excluding chemotherapy.
- Surgery other than brain surgery, excluding radiotherapy to any target, excluding chemotherapy.
- Chemotherapy, excluding radiotherapy to any target, in some cases combined with surgery. A subcategory of patients within this therapeutic subgroup, treated with alkylating agents to a CED of >4000 mg/m^2 was defined.
- Radiotherapy to the brain, excluding chemotherapy. All subjects also underwent brain surgery.
- Radiotherapy to the brain combined with chemotherapy, excluding cases receiving radiotherapy to the testes. In some cases combined with brain surgery, or surgery other than brain surgery.
- Radiotherapy to the testes, given as TBI or in combination with cranial irradiation. In all cases combined with chemotherapy.
- Radiotherapy to targets other than the testes and/or brain, excluding chemotherapy. In some cases combined with surgery other than brain surgery.
- Radiotherapy to targets other than the testes and/or brain, combined with chemotherapy. In some cases combined with surgery other than brain surgery.

Therapeutic subgroups were created with the intention of comparing the effect of (brain) surgery, radiotherapy to different targets and chemotherapy, alone and in combination. A CED cut-off of >4000 mg/m^2 was chosen, as treatment with alkylating agents to a CED <4000 mg/m^2 is unlikely to impair spermatogenesis [157].

In the study described in Paper IV, all CCS receiving radiotherapy to the brain ± chemotherapy and ± radiotherapy to the testes, were combined into one category called “cranial irradiation”. Similarly, all CCS receiving radiotherapy to targets other than the brain and/or testes ± chemotherapy, were combined into one category called “radiotherapy other than brain and/or testes”. This was done as the impact of chemotherapy on BMD was expected to be less pronounced than that of radiotherapy.

Diagnostic subgroups for CCS (Papers II & IV, for details see supplementary information to Paper II).

- Leukaemia (ALL and AML)
- Intracranial tumour (brain tumour, pituitary craniopharyngioma and tumour of the pineal gland)
- Lymphoma (Hodgkin's lymphoma and non-Hodgkin's lymphoma)
- Testicular cancer
- Wilms' tumour
- Bone tumour (osteosarcoma, Ewing's sarcoma and chondrosarcoma)
- Other (epipharyngeal cancer, nasopharyngeal cancer, carcinoid of the lung, malignant melanoma, neuroblastoma, ganglioneuroma, pheochromocytoma, teratoma, rhabdomyosarcoma, bladder cancer, retinoblastoma, spinal teratoma, thyroid cancer, parathyroid-adenoma, anaplastic cancer and Langerhans cell histiocytosis)

The distribution of CCS according to diagnostic and therapeutic subgroups is presented in Table 4.

Table 4. Distribution of childhood cancer survivors in subgroups according to diagnosis and treatment.

	Brain surgery[a]	Surgery other than brain surgery[a]	CT[b]	RT to brain[c]	RT to brain + CT[d]	RT to testes[e]	RT other[f]	RT other + CT[g]	Total
Leukaemia	-	-	11	-	11	5	-	-	27
Intracranial tumour	15	-	-	11	2	-	-	-	28
Lymphoma	-	1	7	-	1	-	3	9	21
Testicular cancer	-	2	3	-	-	-	-	1	6
Wilms' tumour	-	-	-	-	-	-	-	8	8
Bone tumour	-	1	2	1	-	-	-	2	6
Other	-	16	6	-	2	-	2	3	29
Total	15	20	29	12	16	5	5	23	

[a] Excluding chemotherapy and excluding radiotherapy to any target.
CT = chemotherapy; RT = radiotherapy
[b] Excluding radiotherapy to any target. In 11 cases combined with surgery other than brain surgery. 16 subjects received alkylating agents with a median CED of 4855 mg/m^2.
[c] Excluding CT, including 2 hypophysectomised cases. In all cases combined with brain surgery. Median irradiation dose 54 Gy.
[d] Excluding all cases receiving RT to testes. In 2 cases combined with brain surgery, and in 3 cases surgery other than brain surgery. Median irradiation dose 24 Gy. 14 subjects received alkylating agents with a median CED of 5000 mg/m^2.
[e] One case received TBI followed by allogeneic bone marrow transplantation (BMT), one case received RT to the testes combined with TBI followed by autologous BMT, and three cases received RT to the testes combined with RT to the cranium. Median irradiation dose to cranium 24 Gy, and to testes 20 Gy. In all cases combined with CT. 3 subjects received alkylating agents with a median CED of 4800 mg/m^2.
[f] RT to targets other than testes and/or brain, excluding cases receiving CT. In 4 cases combined with surgery other than brain surgery.
[g] RT to targets other than testes and/or brain. In 18 cases combined with surgery other than brain surgery. 12 subjects received alkylating agents with a median CED of 9593 mg/m^2. One subject received high dose chemotherapy followed by autologous BMT.

Gonadal status (Paper IV): Patients were categorized as hypogonadal or eugonadal. (See "Definition of hypogonadism" below.)

Methods

Cryptorchidism

Testicular cancer survivors in TCS-A were asked if they had a history of cryptorchidism at the time of inclusion in the Fertility Study (Paper I).

Semen analysis

Semen samples were produced by masturbation. Fresh semen samples were collected in plastic jars and analysed within one hour, according to the 1999 WHO guidelines [216].

For patients recruited in Lund, sperm analyses were performed at the Reproductive Medicine Centre, Skåne University Hospital, Malmö, apart from 24 T_0 ejaculates, which were analysed at the former Fertility Laboratory, Lund University Hospital, prior to cryopreservation. In Stockholm, all samples were analysed at the Centre for Andrology and Sexual Medicine, Karolinska University Hospital. The laboratories in Malmö and Stockholm serve as reference laboratories for the European Society of Human Reproduction and Embryology/Nordic Association for Andrology external quality control programme (Paper I).

Questionnaires and physical examination

Subjects in the TCS-B and CCS cohorts and their control groups were included in the study by researchers at the Reproductive Medicine Centre, Malmö. After signing an informed consent form, a structured interview was conducted by a research investigator regarding medical history, current medication, health status and smoking habits. A stadiometer was used for height measurements to the nearest 0.1 cm, and an electric scale for weight to the nearest 0.1 kg. The BMI was determined (kg/m^2) (Papers II-IV). No information was collected on fractures (Papers III-IV).

Bone mineral density

The subjects in the TCS-B and CCS cohorts, and their control groups, underwent DXA to assess BMD using Lunar Prodigy (GE Healthcare Lunar, Madison, Wisconsin, USA), software versions 2.15-7.70 for the majority of participants, details described below. DXA was used as it is considered the "gold standard" for BMD assessment and the diagnosis of osteoporosis [217]. Measurements were made of the femoral neck, total hip and lumbar spine, levels L1-L4, with total hip consisting of three regions measured on the proximal femur; femoral neck, trochanteric region and inter-trochanteric region (called neck, troch and inter-troch in Figure 10).

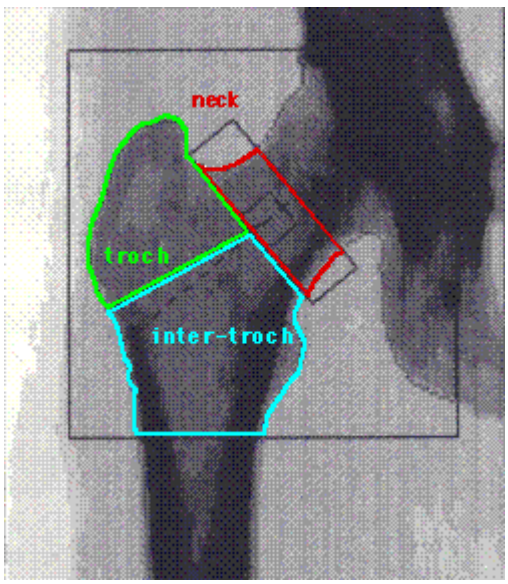

Figure 10. The three regions of the proximal femur assessed by DXA; the femoral neck ("neck"), the trochanteric region ("troch") and the inter-trochanteric region ("inter-troch"). These three regions together constitute the "total hip" on DXA. (Illustration from Dr S. M. Ott, published at https://courses.washington.edu/bonephys/. Printed with permission.)

DXA measurements were performed by the same research technicians throughout the study period. Stability and accuracy were monitored using a manufacturer-supplied phantom three times per week. The precision coefficients (CV%) for DXA have been reported previously: 0.9% for the femoral neck, 0.5% for the total hip and 0.7% for the lumbar spine L1-L4 [218].

During the study period, instrument failure obliged us to change the DXA equipment for the examination of 73 subjects. A cross-calibration was performed, and a formula obtained to adjust the values obtained from the new machine to those obtained from the original one. No inherent bias was found, and very good correspondence was found between measurements made with the two devices. (For details, see Paper III and supplementary information to Paper IV.)

Blood samples

During the inclusion of the TCS-B and CCS cohorts and their controls, the methods used for the analysis of testosterone, SHBG, LH and FSH used at the Department of Clinical Chemistry in Malmö changed. A description of the former and new methods, and the internal validation performed to compare and convert values, is presented in the supplementary information to Paper II.

Inhibin B analysis

Inhibin B was assessed in subjects from the TCS-A cohort. Blood sampling was performed between 8 a.m. and 3 p.m. Blood samples from the subjects in Lund were analysed at the Department of Clinical Chemistry, Skåne University Hospital, Malmö. Samples collected from subjects in Stockholm were analysed at the Department of Clinical Chemistry, Sahlgrenska University Hospital, Göteborg. Inhibin B was measured with the OBI inhibin B ELISA from DSL (Oxford, UK) at both laboratories [219]. The detection limit was 15 ng/L, and total CV's ranged from 15.3% at a concentration of 28 ng/L to 7.0% at a concentration of 339 ng/L. The normal range of inhibin B in postpubertal men at the two laboratories was 50-330 ng/L (Paper I).

Hormone assays

Fasting venous blood samples were drawn from subjects in the TCS-B and CCS cohorts and their controls between 8.00 a.m. and 10.00 a.m. Serum values of testosterone, LH, FSH and SHBG were measured. All analyses were performed using immuno-based methods. During inclusion in these studies (Papers II-IV), the methods of analysis of these hormones changed. Descriptions of the former and new methods, and the internal validation used for conversion, are presented in the supplementary information to Paper II.

Free testosterone was calculated as recommended by Vermeulen et al. [220].

Definition of azoospermia

Long-term azoospermia was defined as the absence of spermatozoa in the ejaculate 3 or 5 years after cancer treatment (Paper I). Azoospermia was diagnosed as follows: 2 drops (6 µg each) taken from the semen sample were put on a slide under 22 mm x 22 mm coverslips and examined at x40 magnification. If no spermatozoa were seen, the semen sample was centrifuged at 6000 rpm for 10 minutes, and the supernatant was decanted. The seminal pellet was resuspended in approximately 50 µL of the seminal plasma. Forty µL of the suspended seminal pellet was then placed on a slide under a 24 mm x 50 mm coverslip, and examined with phase-contrast optics at x200 magnification. The entire coverslip was scanned systematically. If no spermatozoa were seen, the patient was considered azoospermic.

Definition of hypogonadism

A strictly biochemical definition of hypogonadism was used (Papers II-IV). Hypogonadism was defined as S-testosterone <10 nmol/L and/or S-LH >10 IU/L, or ongoing TRT. In the study described in Paper II, hypogonadal subjects were also categorized as having primary hypogonadism (S-testosterone <10 nmol/L, S-LH and S-FSH both >10 IU/L with S-FSH > S-LH), secondary hypogonadism (S-testosterone <10 nmol/L, S-LH and S-FSH both ≤10 IU/L) [221], compensated hypogonadism (S-testosterone ≥10 nmol/L, S-LH >10 IU/L) [69] or ongoing TRT. Subjects with S-testosterone <10 nmol/L, S-LH ≤10 IU/L and S-FSH >10 IU/L were defined as having primary hypogonadism, as elevated FSH suggests testicular and not pituitary failure.

Definition of low bone mineral density

Z-scores, a comparison of an individual's BMD with that of a healthy reference population (NHANES III) of the same sex, age and weight and expressed as standard deviations, were obtained from the machine. By applying one standard deviation as the normal range, LBD was defined as a Z-score ≤-1.0. Z-score was used because of the relatively low age of the subjects.

Statistical methods

All statistical analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA), except for two-tailed Fisher's exact test and calculations of the frequency of azoospermia including 95% confidence intervals in Paper I, where an online calculator was used (www.graphpad.com). p-values <0.05 were considered statistically significant. Descriptive data are presented as mean values and standard deviations when the data are normally distributed, and medians and inter-quartile ranges when they are non-normally distributed. Categorical variables are expressed as numbers and percentages.

Frequency and predictive factors of long-term azoospermia

The results obtained from semen samples collected at T_{36} and T_{60} were combined ($T_{36\text{-}60}$), in order to obtain sufficient numbers of individuals. If semen samples from both T_{36} and T_{60} were available, the results from T_{36} were used, as more patients delivered semen samples only at T_{36} than only at T_{60}. The mean risk of long-term azoospermia in subjects in TCS-A (including 95% confidence intervals) in relation to the treatment given was calculated as the fraction of men without spermatozoa in the ejaculate at $T_{36\text{-}60}$, for all patients and for patients with longitudinal data, respectively (Paper I). Patients under surveillance only after orchidectomy were used as controls for the respective therapy groups. Two-tailed Fisher's exact test was used for comparisons between different treatment groups.

Receiver operating characteristic curve analyses were performed with previous cryptorchidism and inhibin B levels at T_0, T_6, T_{12} and T_{24} tested as potential predictors of developing azoospermia at T_{36}. Cut-off levels of inhibin B were chosen to give 100% sensitivity and maximal specificity in predicting azoospermia.

The positive predictive value (PPV) and negative predictive value (NPV) were calculated for inhibin B levels at T_0, T_6, T_{12} and T_{24} in relation to the risk of azoospermia at T_{36}. Positive predictive value was calculated as the proportion of patients with inhibin B levels below the cut-off with azoospermia at T_{36}, and NPV as the proportion of patients with inhibin B levels above the cut-off having spermatozoa in the ejaculate at T_{36}.

Hormonal levels and risk factors for hypogonadism

The mean difference (95% CI) in S-testosterone, free testosterone, S-LH, S-SHBG and S-FSH, between controls and TCS-B as well as CCS, was calculated after the exclusion of patients on TRT (Paper II). A univariate linear model was used, which

was adjusted for age and smoking, as these factors are known to affect testosterone levels [69,222].

The proportion of subjects with hypogonadism was also calculated, including those on TRT. Binary logistic regression with adjustment for age and smoking was used to assess ORs for hypogonadism in cancer survivors compared to controls. Odds ratios were calculated for hypogonadism in CCS and TCS-B, for all cancer survivors, diagnostic subgroups and therapeutic subgroups. As a lack of, or removal of, the pituitary gland inevitably leads to hypogonadism, two hypophysectomized CCS were excluded from the calculations of the ORs for hypogonadism for different therapeutic subgroups, but were included in calculations of the ORs for hypogonadism for all CCS, as well as for the diagnostic subgroups. Correspondingly, two patients in the TCS-B cohort were excluded from the calculations of the ORs for hypogonadism for therapeutic subgroups (one receiving radiotherapy to the remaining testicle due to GCNIS, and one bilaterally orchidectomized), but both were included in calculations of the ORs for all TCS as well as for the diagnostic subgroups.

Risk factors for low bone mineral density

Total hip and lumbar spine L1-4 BMD were compared for cancer survivors and controls, using linear regression models with adjustment for age, BMI and current smoking.

In the study described in Paper III, analyses of total hip and lumbar spine L-L4 BMD were performed for

- all TCS vs. controls,
- hypogonadal TCS (all hypogonadal, and those receiving and not receiving TRT, respectively) vs. eugonadal TCS,
- therapeutic subgroups of TCS (see above) vs. controls.

The two TCS receiving testicular irradiation were excluded from this analysis due to the small group size. Similar comparisons were made by calculating the ORs for LBD for total hip and lumbar spine L1-L4, using binary logistic regression. One TCS declined DXA, and his control was excluded from the BMD analyses.

In order to investigate possible local effects of scattered irradiation arising from adjuvant radiotherapy in TCS on BMD, BMD and Z-scores for the irradiated and non-irradiated hip were compared using the Wilcoxon test for paired data.

In the study described in Paper IV, analyses of total hip and lumbar spine L1-L4 BMD were performed for

- all CCS vs. controls,
- untreated hypogonadal CCS and CCS receiving TRT, respectively, vs. eugonadal CCS,
- therapeutic subgroups of CCS (see above) vs. controls,
- CCS receiving chemotherapy, excluding radiotherapy, and treated with alkylating agents, CCS receiving chemotherapy, excluding radiotherapy, and treated with methotrexate, and CCS receiving chemotherapy, excluding radiotherapy, and also treated with glucocorticoids, separately, vs. controls,
- diagnostic subgroups of CCS (see above) vs. controls.

There was considerable overlap in the subgroups of CCS receiving chemotherapy, excluding radiotherapy, and treated with alkylating agents, methotrexate or glucocorticoids, where 4 subjects were receiving 1 drug, 12 were receiving 2 drugs and 8 were receiving 3 drugs, hence, these subgroups were tested separately vs. controls.

A unilateral regression model was used, with adjustment for age, BMI and current smoking, for analyses of total hip and lumbar spine L1-L4 BMD. Similar comparisons were made by calculating ORs for LBD for total hip and lumbar spine L1-L4, using binary logistic regression.

Results

Azoospermia in testicular cancer survivors

The mean age (SD) of the TCS at the start of the study was 32.6 (7.2) years in TCS-A and 31.1 (6.1) years in the subgroup of 117 TCS with longitudinal data.

Treatment modality and risk of azoospermia

Long-term azoospermia was found in 7.8% of TCS-A and in 7.7% of the subgroup of TCS with longitudinal data (Tables 5 and 6).

When comparing TCS receiving ACT, SCT, adjuvant radiotherapy or ETC after orchidectomy, to TCS receiving SO after orchidectomy, treatment with ETC was associated with a statically significantly increased risk of azoospermia in the total patient cohort (63% vs. 4.4%, p=0.0018, Table 5). A similar frequency of azoospermia was found in TCS with longitudinal data receiving ETC (67% vs. 0%, p=0.17, Table 6). None of the other treatment categories were statistically significantly associated with azoospermia, neither in the TCS-A cohort nor in the TCS with longitudinal data.

Table 5. Azoospermia in subjects in testicular cancer survivors cohort A, 36-60 months post-treatment, according to type of treatment

Treatment	N	Azoo. (n)	Azoo. (%)	95 % CI	p*
SO	23	1	4.4	0.0-23	-
ACT	74	1	1.4	0.0-8.0	0.42
SCT	50	5	10	3.9-22	0.66
RT	62	5	8.1	3.1-18	1.0
ETC	8	5	63	30-87	0.0018
Total	217	17	7.8	4.9-12	-

N = Number of patients in each subgroup
Azoo. = Azoospermia
CI = confidence interval
* Compared to patients with no further treatment after orchidectomy
SO = Surveillance only (no further treatment after orchidectomy)
ACT = Adjuvant chemotherapy; 1-2 cycles of cisplatin-based chemotherapy (CBC) or carboplatin
SCT = Standard dose chemotherapy; 3-4 cycles of CBC
RT = Adjuvant radiotherapy administrered to the paraaortic and ipsilateral iliac lymph nodes
ETC = extensive treatment with chemotherapy; >4 cycles of CBC or ≥4 cycles of CBC + radiotherapy at targets other than the remaining testicle

Table 6. Azoospermia 36-60 months post-treatment in relation to treatment, in testicular cancer survivors with spermatozoa in the ejaculate after orchidectomy but before further treatment

Treatment	N	Azoo. (n)	Azoo. (%)	95 % CI	p*
SO	3	0	0	0.0-62	-
ACT	48	0	0	0.0-8.9	1.0
SCT	31	4	13	4.5-29	1.0
RT	29	1	3.5	0.0-19	1.0
ETC	6	4	67	30-91	0.17
Total	117	9	7.7	3.9-14	-

N = Number of patients in each subgroup
Azoo. = Azoospermia
CI = confidence interval
* Compared to patients with no further treatment after orchidectomy
SO = Surveillance only (no further treatment after orchidectomy)
ACT = Adjuvant chemotherapy; 1-2 cycles of cisplatin-based chemotherapy (CBC) or carboplatin
SCT = Standard dose chemotherapy; 3-4 cycles of CBC
RT = Adjuvant radiotherapy administrered to the paraaortic and ipsilateral iliac lymph nodes
ETC = extensive treatment with chemotherapy; >4 cycles of CBC or ≥4 cycles of CBC + radiotherapy at targets other than the remaining testicle

Inhibin B and cryptorchidism as predictors of azoospermia

Inhibin B concentrations in serum at T_0, T_6, T_{12} and T_{24} all predicted azoospermia at T_{36} with 100% sensitivity, but with different specificities, PPVs and cut-off levels (Table 7). Inhibin B at T_{12} was found to be the best predictor of long-term azoospermia, as it resulted in the highest PPV and maximal specificity for azoospermia at T_{36}. All TCS with spermatozoa in the ejaculate after orchidectomy but before further treatment, and an inhibin B level >56 ng/L 12 months after cancer treatment, had spermatozoa in the ejaculate 36 months after treatment.

Table 7. Inhibin B level as a predictor of azoospermia in testicular cancer survivors 36 months after treatment

	N	Azoo. (n)	Inhibin B cut-off, ng/L	Sens., %	Spec., %	AUC	PPV	NPV
T_0	43	3	112.00	100	40.0	0.750	0.11	1.00
T_6	51	7	49.65	100	70.5	0.883	0.35	1.00
T_{12}	62	8	55.90	100	77.8	0.911	0.40	1.00
T_{24}	77	8	97.75	100	56.5	0.899	0.21	1.00

N = Number of patients in each subgroup
Azoo. = Azoospermia 36 months after treatment
Sens. = Sensitivity
Spec. = Specificity
AUC = Area under the curve
PPV = Positive predictive value: proportion of patients with inhibin B below cut-off presenting with azoospermia at T_{36}
NPV = Negative predictive value: proportion of patients with inhibin B above cut-off having spermatozoa in the ejaculate at T_{36}

Previous cryptorchidism proved non-significant regarding the risk of azoospermia at T_{36} (data not shown).

Hypogonadism and bone mineral density in young male cancer survivors

The characteristics of TCS-B and CCS, divided into subgroups according to gonadal status, and their respective controls are given in Tables 8 and 9.

Table 8. Characteristics of testicular cancer survivors, all and divided into subgroups based on gonadal status*, and age-matched controls

	Testicular cancer survivors	Eugonadal	Hypogonadal untreated†	Testosterone replacement therapy	**Controls**
Number	92	58	23	9	92
Age at diagnosis (y)	30.8 (7.2)	30.0 (7.0)	32.7 (8.2)	31.4 (5.9)	NA
Age at inclusion (y)	40.3 (7.4)	39.7 (7.6)	41.1 (7.5)	42.0 (6.2)	41.2 (7.3)
Length of follow-up (y)	9.2 (2.7)	9.4 (2.8)	8.2 (2.4)	10.2 (2.2)	NA
Height (m)	1.84 (0.06)	1.83 (0.06)	1.84 (0.06)	1.86 (0.07)	1.82 (0.08)
Weight (kg)	90.3 (14.0)	86.6 (10.6)	96.6 (19.4)	97.2 (10.9)	85.0 (12.6)
Body mass index (kg/m^2)	26.8 (3.9)	25.8 (2.7)	28.5 (5.5)	28.4 (4.1)	25.5 (3.3)
Smoking, current	21 (23)	11 (19)	8 (35)	2 (22)	8 (9.1)
S-testosterone (nmol/L)	13.0 (10.5-15.0)	13.8 (11.9-15.7)	9.3 (8.2-11.7)	15.0 (10.6-18.8)	13.3 (10.9-17.0)
S-LH (IU/L)‡	5.1 (3.7-7.0)	4.9 (3.5-6.1)	6.8 (4.1-12.4)	NA	3.3 (2.1-4.2)

* Hormone data missing for 2 testicular cancer survivors
Hypogonadal untreated = S-testosterone < 10 nmol/L and/or S-LH>10 IU/L
† 5 cases (5.5% of testicular cancer survivors) presented with isolated high S-LH
Age at diagnosis, age at inclusion, length of follow-up, height, weight and body mass index are reported as means (SD)
Smoking is presented as number (%)
S-testosterone and S-LH are reported as medians (interquartile range) due to non-normal distribution
‡ 9 testicular cancer survivors on testosterone replacement therapy excluded
NA = Not applicable

Table 9. Characteristics of childhood cancer survivors, all and divided into subgroups based on gonadal status*, and age-matched controls

	Childhood cancer survivors	Eugonadal	Hypogonadal untreated†	Testosterone replacement therapy	**Controls**
Number	125	93	18	13	125
Age at diagnosis (y)	9.6 (5.4-15.0)	9.6 (5.3-16.0)	9.6 (5.4-14.4)	8.9 (6.5-15)	NA
Age at inclusion (y)	33.7 (30.2-40.1)	33.7 (29.8-40.0)	32.9 (29.6-31.4)	35.7 (33.1-39.1)	34.4 (30.5-40.6)
Length of follow-up (y)	24.3 (7.1)	24.4 (7.4)	24.4 (5.8)	23.2 (7.7)	NA
Height (m)	1.80 (1.75-1.86)	1.81 (1.77-1.85)	1.81 (1.76-1.84)	1.78 (1.73-1.88)	1.82 (1.78-1.85)
Weight (kg)	82.1 (72.0-91.5)	80.7 (71.1-87.1)	84.3 (75.2-102.4)	91.7 (77.4-108.4)	81.4 (73.3-88.6)
BMI (kg/m^2)	25.1 (22.8-27.6)	24.8 (22.6-26.8)	26.1 (24.5-31.0)	30.3 (26.8-31.3)	24.7 (22.6-26.9)
Smoking, current	10 (8.2)	9 (10)	-	1 (7.7)	17 (14)
S-testosterone (nmol/L)	14.1 (11.5-17.3)	14.8 (13.2-18.1)	9.0 (8.0-9.4)	14.4 (9.5-17.0)	14.7 (11.7-17.6)
S-LH (IU/L)‡	4.0 (2.5-5.6)	4.0 (2.7-5.7)	4.0 (1.9-5.3)	NA	3.1 (2.2-4.0)

* Hormone data missing for 1 childhood cancer survivor
Hypogonadal untreated = S-testosterone < 10 nmol/L and/or S-LH >10 IU/L
† 2 cases (1.6% of childhood cancer survivors) presented with isolated high S-LH
BMI = body mass index
Age at diagnosis, age at inclusion, height, weight, BMI, S-testosterone and S-LH are reported as medians (interquartile range) due to non-normal distribution
Length of follow-up is reported as means (SD)
Smoking is presented as number (%)
‡ 13 childhood cancer survivors on testosterone replacement therapy excluded
NA = Not applicable

Many CCS were taking medication that may affect BMD (immunosuppressive oral glucocorticoids, TRT, and calcium + vitamin D), or medication indicating pituitary failure (GHRT, glucocorticoid replacement and TRT), or had untreated hypogonadism. The distribution of medications and untreated hypogonadism is illustrated in Figure 11.

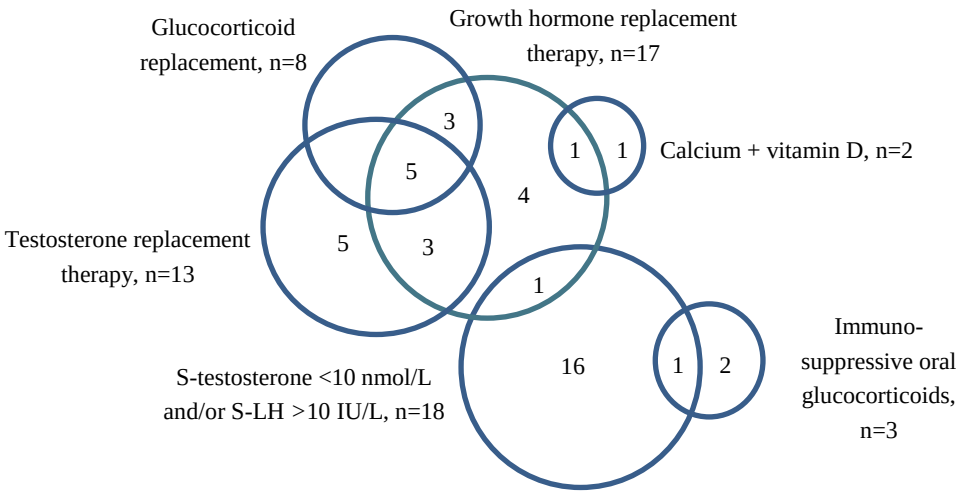

Figure 11. Distribution of medications and untreated hypogonadism among childhood cancer survivors: growth hormone replacement therapy, testosterone replacement therapy, glucocorticoid replacement, immunosuppressive oral glucocorticoids, calcium + vitamin D, or S-testosterone <10 nmol/L and/or S-LH >10 IU/L.

Hormonal levels in cancer survivors and controls

Childhood cancer survivors

Childhood cancer survivors exhibited higher S-LH levels than controls (mean difference 1.1 IU/L, 95% CI: 0.55; 1.6 IU/L, $p<0.001$). No difference was seen between the mean levels of S-testosterone and free testosterone.

Testicular cancer survivors

Testicular cancer survivors exhibited higher S-LH levels (mean ratio 1.6 IU/L, 95% CI: 1.4; 1.9 IU/L, $p<0.001$) and lower free testosterone levels (mean difference -0.023 nmol/L, 95% CI: -0.044; -0.002 nmol/L, $p=0.034$) than their controls. No difference was seen between the mean levels of S-testosterone (Paper II).

Hypogonadism in cancer survivors and controls

Childhood cancer survivors

Twenty-six percent of CCS and 14% of their controls were classified as hypogonadal. Frequencies of CCS and controls with primary, secondary or compensated hypogonadism, or TRT, are presented in Figure 12.

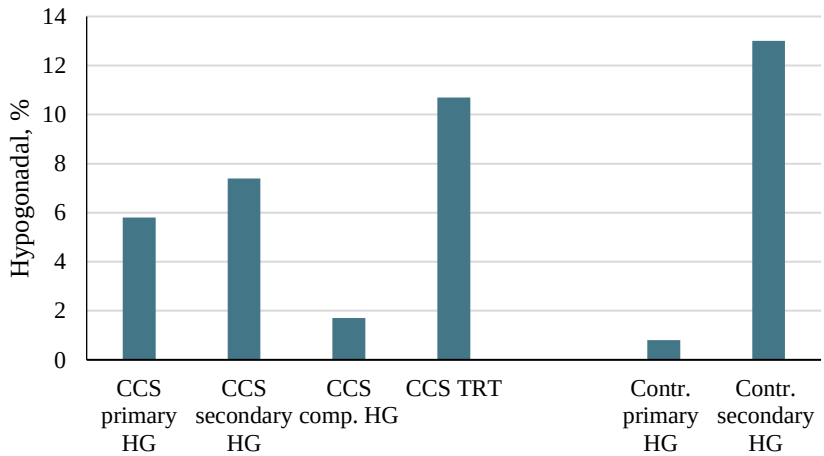

Figure 12. The frequencies of childhood cancer survivors (CCS) and controls (contr.) with primary hypgonadism (HG), secondary HG, compensated (comp.) HG or testosterone replacement therapy (TRT).

The OR for hypogonadism in CCS compared to controls was 2.1 (95% CI: 1.1; 4.1, p=0.025). The ORs for CCS, and diagnostic subgroups of CCS, are presented in Figure 13 and Paper II.

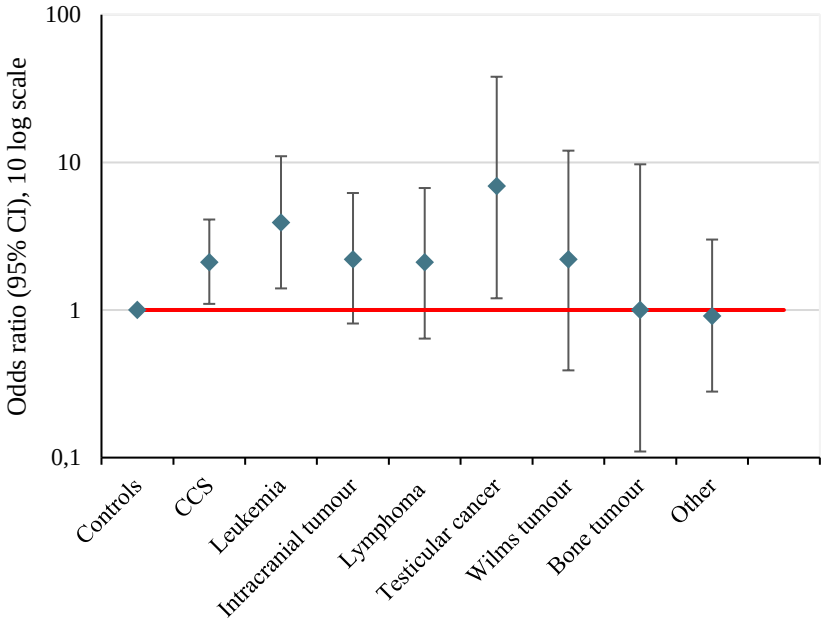

Figure 13. Odds ratios (95% CI) for hypogonadism in childhood cancer survivors (CCS), and diagnostic subgroups of CCS, compared to controls**.** Adjusted for age at inclusion and current smoking.

In CCS treated for leukaemia, intracranial tumour or lymphoma, the ORs for hypogonadism in subjects treated with or without radiotherapy were calculated (Figure 14). No hypogonadal subjects were found among CCS treated for lymphoma without radiotherapy. The OR for hypogonadism was increased in CCS with intracranial tumour treated with radiotherapy, but the OR became non-significant after the exclusion of 2 hypophysectomized cases (OR 2.7, 95% CI: 0.63; 12, p=0.18).

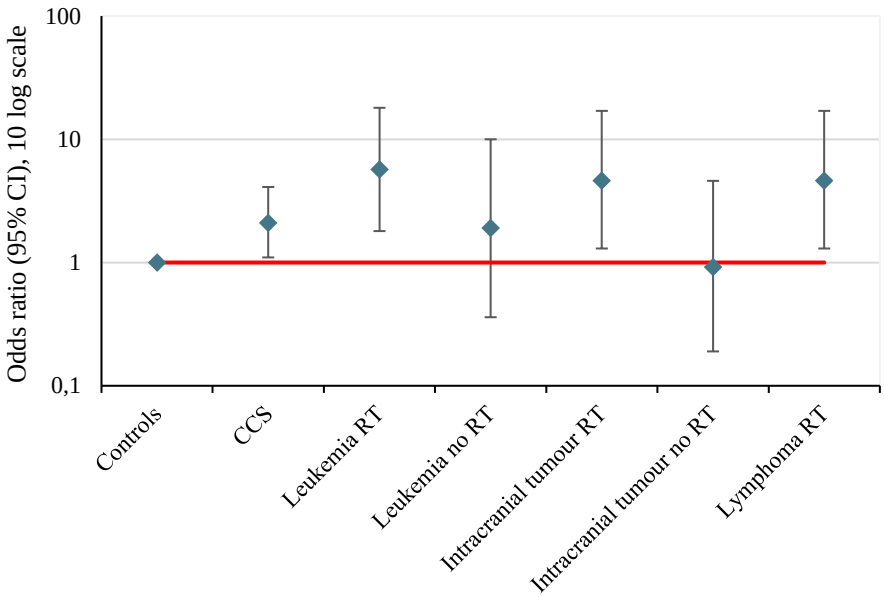

Figure 14. Odds ratios (95% CI) for hypogonadism in childhood cancer survivors (CCS), and CCS treated for leukemia, intracranial tumour or lymphoma, with or withot radiotherapy (RT), compared to controls. No hypogonadal subjects were found among CCS treated for lymphoma without radiotherapy. Adjusted for age at inclusion and current smoking.

The ORs for hypogonadism in CCS, and therapeutic subgroups of CCS, are presented in Figure 15 and Paper II.

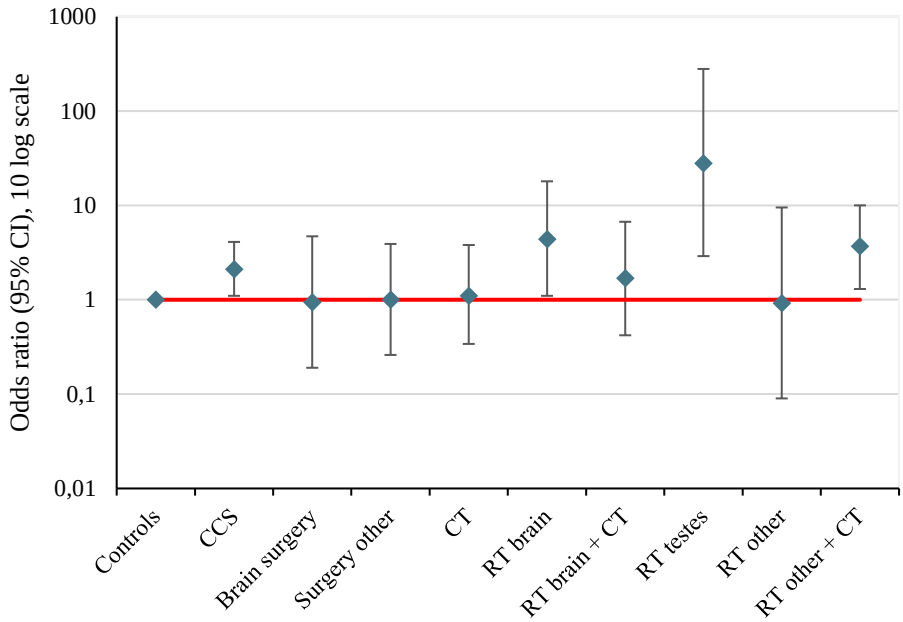

Figure 15. Odds ratios (95% CI) for hypogonadism in childhood cancer survivors (CCS), and therapeutic subgroups of CCS, compared to controls. Surgery other = surgery other than brain surgery; CT = chemotherapy; RT = radiotherapy; RT other = RT to targets other than brain and/or testes. Adjusted for age at inclusion and current smoking.

Testicular cancer survivors

Thirty-six percent of TCS-B and 19% of their controls were classified as hypogonadal. Frequencies of TCS-B and controls with primary, secondary or compensated hypogonadism, or TRT, are presented in Figure 16 and Paper II.

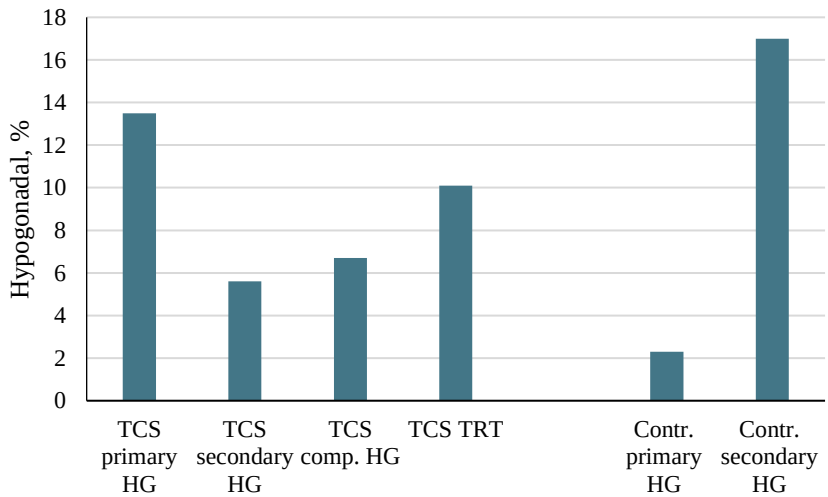

Figure 16. The frequencies of testicular cancer survivors (TCS) and controls (contr.) with primary hypgonadism (HG), secondary HG, compensated (comp.) HG or testosterone replacement therapy (TRT).

The OR for hypogonadism in TCS-B compared to controls was 2.3 (95% CI: 1.1; 4.7, p=0.021). The ORs for hypogonadism in TCS-B, and therapeutic subgroups of TCS-B, are presented in Figure 17 and Paper II. The OR for hypogonadism was not increased for the remaining group of TCS after the exclusion of the ETC subgroup (OR: 1.9, 95% CI: 0.90; 4.0, p=0.093).

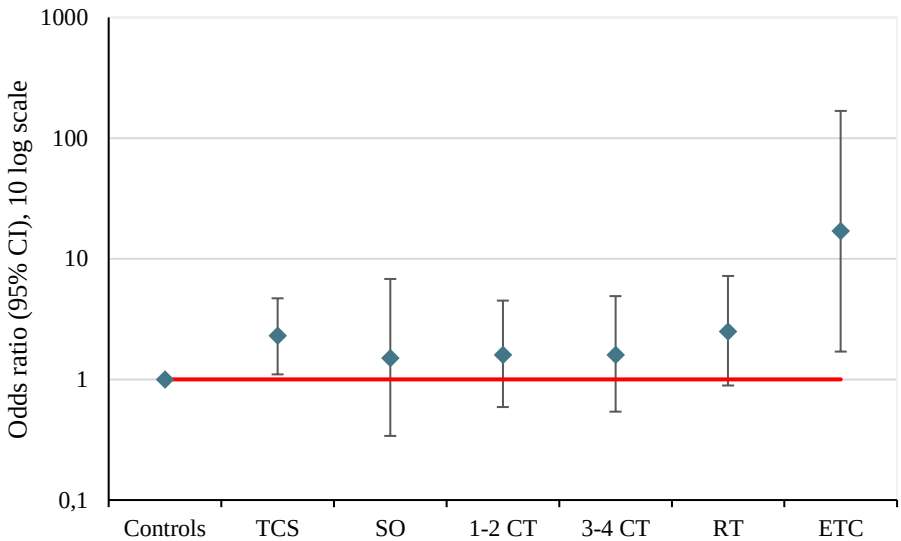

Figure 17. Odds ratios (95% CI) for hypogonadism in testicular cancer survivors (TCS), and therapeutic subgroups of TCS, compared to controls.
SO = Surveillance only
1-2 CT = 1-2 cycles of adjuvant cisplatin-based chemotherapy (CBC) or carboplatin
3-4 CT = 3-4 cycles of adjuvant CBC
RT = Adjuvant radiotherapy administrered to the paraaortic and ipsilateral iliac lymph nodes
ETC = extensive treatment with chemotherapy; >4 cycles of CBC or ≥4 cycles of CBC + radiotherapy at targets other than the remaining testicle
Adjusted for age at inclusion and current smoking

Bone mineral density in cancer survivors and controls

Background characteristics regarding BMD in TCS and CCS are presented in Tables 10 and 11.

Table 10. Bone mineral density (BMD) in testicular cancer survivors, all and divided into subgroups based on gonadal status*, and age-matched controls

	Testicular cancer survivors	Eugonadal	Hypogonadal untreated†	Testosterone replacement therapy	**Controls**
Number	91	58	22	9	91
BMD (g/cm²)					
Total hip	1.073 (0.129)	1.082 (0.120)	1.066 (0.167)	1.044 (0.084)	1.082 (0.125)
LS L1-L4	1.248 (0.162)	1.268 (0.152)	1.207 (0.198)	1.206 (0.125)	1.206 (0.159)
Z-score					
Total hip	-0.12 (0.93)	-0.02 (0.86)	-0.20 (1.18)	-0.45 (0.71)	0.04 (0.87)
LS L1-L4	-0.03 (1.29)	0.19 (1.20)	-0.43 (1.48)	-0.57 (1.20)	-0.23 (1.24)
LBD (Z-score <-1)					
Total hip	17 (19)	8 (14)	6 (27)	2 (22)	11 (12)
LS L1-L4	19 (21)	8 (14)	9 (41)	2 (22)	24 (26)

BMD data missing for 1 testicular cancer survivor, and corresponding control excluded
* Hormone data missing for 2 testicular cancer survivors, of which one with LBD total hip
Hypogonadal untreated = S-testosterone < 10 nmol/L and/or S-LH >10 IU/L
† 5 cases (5.5% of testicular cancer survivors) had isolated high S-LH
BMD and Z-score are reported as means (SD)
LS = lumbar spine
LBD = low BMD
LBD is reported as number (%)

Table 11. Bone mineral density (BMD) in childhood cancer survivors, all and divided into subgroups based on gonadal status*, and age-matched controls

	Childhood cancer survivors	Eugonadal	Hypogonadal untreated†	TRT	**Controls**
Number	125	93	18	13	125
BMD (g/cm²)					
Total Hip§	1.060 (0.150)	1.070 (0.139)	0.98 (0.174)	1.068 (0.165)	1.065 (0.156)
LS L1-L4¤	1.198 (0.148)	1.202 (0.129)	1.143 (0.203)	1.225 (0.164)	1.184 (0.139)
Z-score					
Total Hip§	-0.17 (1.06)	-0.05 (1.0)	-0.8 (1.2)	-0.2 (0.92)	-0.13 (1.09)
LS L1-4¤	-0.25 (1.11)	-0.16 (0.98)	-0.84 (1.5)	-0.26 (1.1)	-0.36 (1.10)
LBD (Z-score <-1)					
Total Hip§	26 (21)	15 (16)	7 (39)	4 (31)	27 (22)
LS L1-4¤	27 (22)	18 (20)	5 (28)	4 (31)	35 (28)

* Hormone data missing for 1 childhood cancer survivor
Hypogonadal untreated = S-testosterone < 10 nmol/L and/or S-LH >10 IU/L
† 2 cases (1.6% of childhood cancer survivors) had isolated high S-LH
TRT = testosterone replacement therapy
BMD and Z-score are reported as means (SD)
§ Mean of right and left side, except for 2 childhood cancer survivors and 1 control with unilateral values.
LS = lumbar spine
¤ Data missing for 1 childhood cancer survivor and 1 control
LBD = low BMD
LBD is reported as number (%)

Total cohorts of cancer survivors

Data on BMD in the total hip and lumbar spine L1-L4, and the ORs for LBD at the total hip and lumbar spine L1-L4, in TCS compared to controls, are presented in Table 12. All estimates were robust for the exclusion of men on oral corticosteroids or TRT (Paper III).

Table 12. Mean differences in bone mineral density (BMD) and odds ratios (ORs) for low BMD (LBD, defined as Z-score <-1): testicular cancer survivors (TCS) vs. controls. Adjusted for age, body mass index and current smoking*

Area	Group	N	BMD, (g/cm^2)#	LBD, n (%)	Mean difference (95% CI), g/cm^2	p	OR for LBD (95% CI)	p
Total hip	TCS	90	1.072 (0.130)	17 (19)	-0.027 (-0.062; 0.009)	0.14	1.6 (0.69; 3.8)	0.27
	Controls	87	1.081 (0.126)	11 (12)			Ref	
LS L1-L4	TCS	90	1.248 (0.163)	19 (21)	0.027 (-0.021; 0.075)	0.27	0.64 (0.31; 1.3)	0.22
	Controls	87	1.205 (0.162)	24 (26)			Ref	

* Smoking data missing for 1 testicular cancer survivor and 4 controls
N = Number participating in analyses
# Unadjusted mean (SD)
Ref = Reference group
LS = lumbar spine

Bone mineral density in the total hip and lumbar spine L1-L4, and ORs for LBD at the total hip and lumbar spine L1-L4, in CCS compared to controls, are presented in Table 13. All results were robust for the exclusion of cases on TRT, GHRT, immunosuppressive oral glucocorticoids or treatment with calcium + vitamin D (Paper IV), and did not change significantly when one CCS on calcium + vitamin D treatment without GHRT was included in the estimation (supplementary information to Paper IV).

Table 13. Mean differences in bone mineral density (BMD) and odds ratios (ORs) of a low BMD (LBD, defined as Z –score <-1) in childhood cancer survivors (CCS) and controls. Adjusted for age, body mass index and current smoking*

Area	Group	N	BMD (g/cm^2)#	LBD, n (%)	Mean difference (95% CI), g/cm^2	p	OR for LBD (95% CI)	p
Total hip	CCS	122	1.060 (0.151)	26 (21)	-0.014 (-0.052; 0.023)	0.44	0.95 (0.51; 1.8)	0.86
	Controls	122	1.065 (0.158)	27 (22)			Ref	
LS L1-L4	CCS	121	1.199 (0.150)	27 (22)	0.006 (-0.030; 0.041)	0.76	0.70 (0.38; 1.3)	0.24
	Controls	121	1.186 (0.141)	35 (29)			Ref	

* Smoking data missing for 3 CCS and 3 controls.
N = Number participating in analyses
# Unadjusted mean (SD)
Ref = Reference group
LS = lumbar spine
Data on lumbar spine L1-L4 missing for 1 childhood cancer survivor and 1 control.

Hypogonadal vs. eugonadal cancer survivors

Both untreated hypogonadal TCS and TCS on TRT exhibited statistically significantly lower total hip BMD than eugonadal TCS. Statistical significance was reached for lumbar spine L1-L4 BMD in TCS with untreated hypogonadism but not in TCS on TRT (Table 14). Correspondingly, the ORs for total hip LBD were increased in both treated and untreated hypogonadal TCS, but reached borderline statistical significance only in the latter subgroup. Hypogonadal untreated TCS had statistically significantly increased OR for lumbar spine L1-L4 LBD, whereas TCS on TRT did not (Table 14, Figure 18).

Table 14. Mean differences in bone mineral density (BMD) and odds ratios (ORs) for low BMD (LBD, defined as Z-score <-1) in testicular cancer survivors: untreated hypogonadal (HG) and hypogonadal on testosterone replacement therapy (TRT) vs. eugonadal. Adjusted for age, body mass index and current smoking*

Area	Group	N	BMD (g/cm^2)#	LBD, n (%)	Mean difference (95% CI), g/cm^2	p	OR for LBD (95% CI)	p
Total hip	HG	22	1.066 (0.167)	6 (27)	-0.063 (-0.122; -0.004)	0.04	3.7 (1.0; 14)	0.05
	TRT	9	1.044 (0.084)	2 (22)	-0.085 (-0.168; -0.003)	0.04	3.1 (0.48; 20)	0.23
	Eugonadal	56	1.081 (0.121)	7 (13)	Ref	Ref	Ref	Ref
LS L1-L4	HG	22	1.207 (0.198)	9 (41)	-0.097 (-0.179; -0.014)	0.02	4.1 (1.3; 13)	0.02
	TRT	9	1.206 (0.125)	2 (22)	-0.092 (-0.206; 0.023)	0.12	1.7 (0.28; 9.9)	0.57
	Eugonadal	56	1.268 (0.154)	8 (13)	Ref	Ref	Ref	Ref

* Smoking or hormone data missing for 3 TCS, and one testicular cancer survivor on oral corticosteroids excluded from the analysis
N = Number participating in analyses
# Unadjusted mean (SD)
Hypogonadal untreated = S-testosterone < 10 nmol/L and/or S-LH >10 IU/L
Ref = Reference group
LS = Lumbar spine

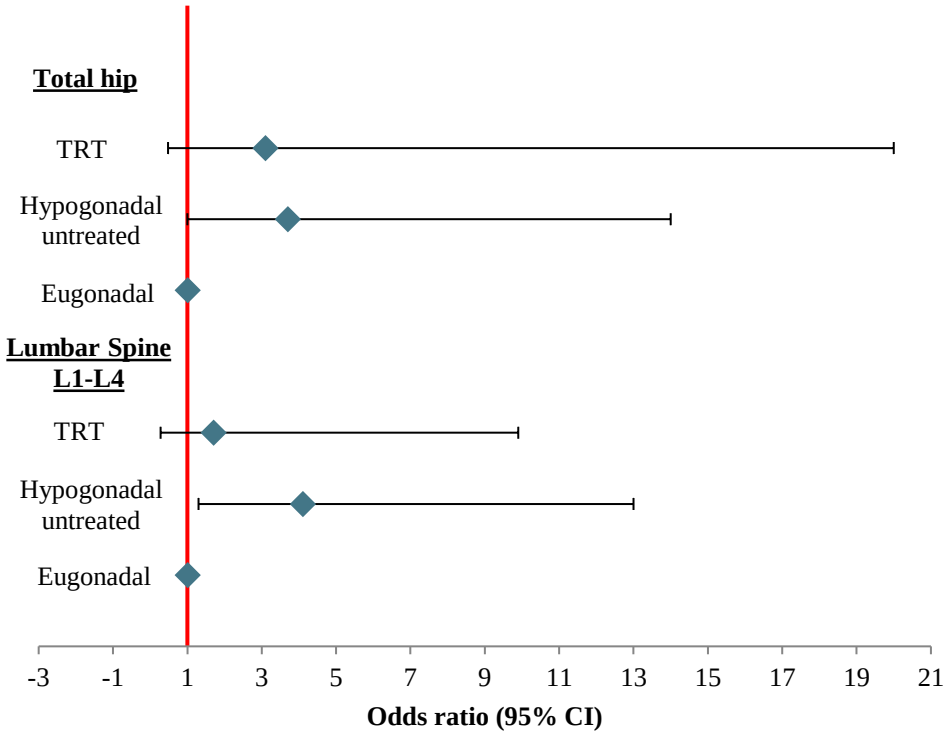

Figure 18. Odds ratios of low bone mineral density in hypogonadal untreated testicular cancer survivors (TCS) and TCS on testosterone replacement therapy (TRT), after exclusion of one testicular cancer survivor on oral corticosteroids. Eugonadal TCS served as the reference group. Untreated hypogonadism was defined as S-testosterone < 10 nmol/L and/or S-LH >10 IU/L.

Untreated hypogonadal CCS exhibited statistically significantly lower total hip and lumbar spine L1-L4 BMD than eugonadal CCS. Childhood cancer survivors with untreated hypogonadism also had a significantly increased OR for total hip LBD, but not for lumbar spine L1-L4 LBD. No statistically significant difference was found in BMD or the OR for LBD between CCS receiving TRT and eugonadal CCS (Table 15, Figure 19). All estimates were robust for the exclusion of patients on immunosuppressive oral glucocorticoids or treatment with calcium + vitamin D, and adjustment for GHRT (Paper IV), and for the exclusion of the 2 cases with isolated high LH (supplementary information to Paper IV).

Table 15. Mean differences in bone mineral density (BMD) and odds ratios (ORs) for low BMD (LBD, defined as Z-score <-1) in childhood cancer survivors: untreated hypogonadal and hypogonadal on testosterone replacement therapy (TRT) vs. eugonadal. Adjusted for age, body mass index and current smoking*

Area	Group	N	BMD (g/cm^2)#	LBD, n (%)	Mean difference (95% CI), g/cm^2	p	OR for LBD (95% CI)	p
TH	Hypogonadal untreated	18	0.985 (0.174)	7 (39)	-0.139 (-0.210; -0.067)	<0.001	4.3 (1.3; 14)	0.02
	TRT	13	1.068 (0.165)	4 (31)	-0.063 (-0.145; 0.019)	0.13	3.1 (0.76; 13)	0.11
	Eugonadal	90	1.072 (0.140)	15 (17)	Ref	Ref	Ref	Ref
LS L1-L4	Hypogonadal untreated	18	1.143 (0.203)	5 (28)	-0.102 (-0.174; -0.030)	0.006	1.7 (0.51; 5.8)	0.38
	TRT	13	1.225 (0.164)	4 (31)	-0.032 (-0.115; 0.051)	0.44	2.0 (0.51; 8.1)	0.32
	Eugonadal	89	1.203 (0.131)	18 (20)	Ref	Ref	Ref	Ref

* Smoking or hormone data missing for 4 CCS, and data on LS L1-L4 missing for 1 childhood cancer survivor
N = Number participating in analyses
# Unadjusted mean (SD)
Hypogonadal untreated = S-testosterone < 10 nmol/L and/or S-LH >10 IU/L
TH = total hip
Ref = Reference group
LS = Lumbar spine

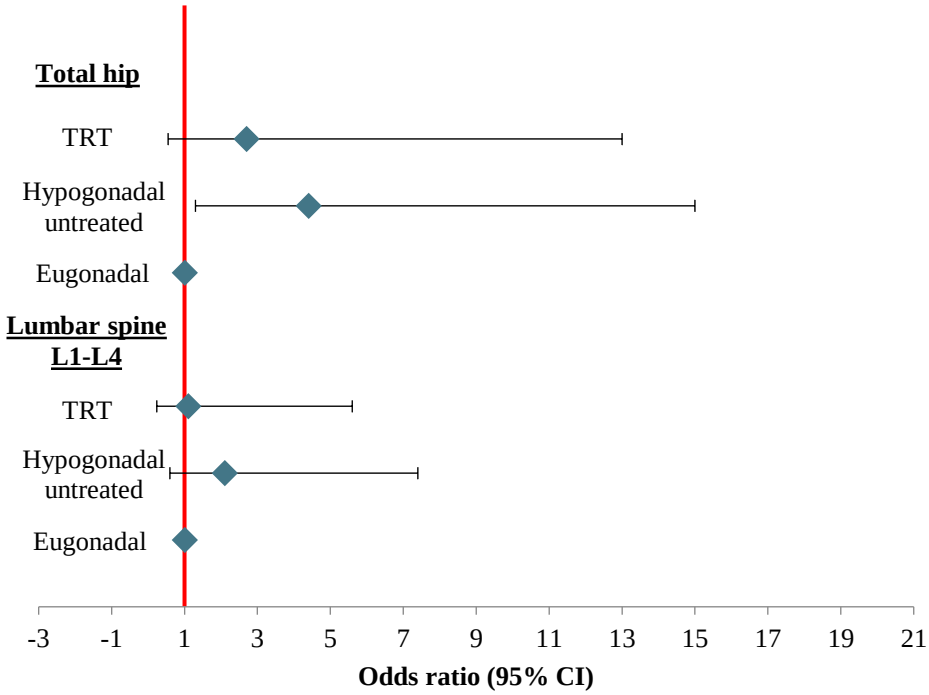

Figure 19. Odds ratios of low bone mineral density in hypogonadal untreated childhood cancer survivors (CCS) and CCS on testosterone replacement therapy (TRT), after exclusion of 3 CCS on immunosuppressive oral corticosteroids and 2 CCS on calcium + vitamin D treatment, with adjustment for growth hormone replacement. Eugonadal CCS served as the reference group. Untreated hypogonadism was defined as S-testosterone < 10 nmol/L and/or S-LH >10 IU/L.

Cancer treatment for hypogonadal untreated CCS and CCS on TRT is presented in Table 16.

Table 16. Cancer treatment for hypogonadal untreated childhood cancer survivors (CCS) and CCS on testosterone replacement therapy.

	Brain surgery[a]	Surgery other than brain surgery[a]	CT[b]	Cranial irradiation[c]	RT other than brain and/or testes[d]
Hypogonadal untreated	1	3	3	4[e]	7
Testosterone replacement therapy	1	-	1	9[f]	2

RT = radiotherapy
CT = chemotherapy
[a] Excluding RT to any target, excluding CT
[b] Excluding RT to any target. In 1 case combined with surgery other than brain surgery.
[c] Median irradiation dose 25 Gy. 1 case received TBI followed by bone marrow transplantation. Another 3 cases also received RT to testes, to a median irradiation dose of 24 Gy. In 7 cases combined with chemotherapy, and in the remaining 6 cases combined with brain surgery
[d] Combined with chemotherapy in 8 cases. One case received high dose chemotherapy followed by bone marrow transplantation. In 6 cases combined with surgery other than brain surgery.
Hypogonadal untreated = S-testosterone < 10 nmol/L and/or S-LH >10 IU/L
[e] Median irradiation dose 33 Gy
[f] Median irradiation dose 25 Gy

Therapeutic subgroups of cancer survivors vs. controls

Data on BMD in the total hip and lumbar spine L1-L4, and the ORs for LBD at the total hip and lumbar spine L1-L4, in therapeutic subgroups of TCS compared to controls are presented in Table 17.

Table 17. Mean differences in bone mineral density (BMD) and odds ratios (ORs) for low BMD (LBD, defined as Z –score <-1), in testicular cancer survivors: therapeutic subgroups vs. controls. Adjusted for age, body mass index and current smoking*

Area	Group	N	BMD, (g/cm^2)$^{\#}$	LBD, n (%)	Mean difference (95% CI), g/cm^2	p	OR for LBD (95% CI)	p
Total hip	SO	11	1.127 (0.119)	-	0.026 (-0.048; 0.100)	0.49	n.d.	-
	ACT	28	1.084 (0.145)	6 (21)	-0.017 (-0.068; 0.034)	0.51	1.8 (0.58; 5.7)	0.31
	SCT	23	1.022 (0.079)	5 (22)	-0.051 (-0.105; 0.003)	0.066	1.7 (0.50; 5.5)	0.40
	RT	21	1.089 (0.158)	4 (19)	-0.034 (-0.093; 0.024)	0.25	2.2 (0.57; 8.5)	0.25
	ETC	5	1.012 (0.071)	2 (40)	-0.098 (-0.203; 0.008)	0.070	5.3 (0.76; 37)	0.092
	Controls	87	1.081 (0.126)	11 (12)	Ref	Ref	Ref	Ref
Lumbar spine L1-L4	SO	11	1.275 (0.137)	1 (9.1)	0.046 (-0.056; 0.147)	0.38	0.24 (0.028; 2.0)	0.19
	ACT	28	1.241 (0.173)	7 (25)	0.015 (-0.055; 0.084)	0.67	0.81 (0.29; 2.2)	0.68
	SCT	23	1.233 (0.121)	3 (13)	0.027 (-0.047; 0.101)	0.47	0.40 (0.11; 1.5)	0.18
	RT	21	1.276 (0.213)	5 (24)	0.047 (-0.033; 0.127)	0.24	0.65 (0.20; 2.1)	0.48
	ETC	5	1.139 (0.060)	3 (60)	-0.089 (-0.233; 0.056)	0.23	3.6 (0.55; 23)	0.18
	Controls	87	1.205 (0.162)	24 (26)	Ref	Ref	Ref	Ref

* Smoking or hormone data missing for 3 TCS and 4 controls, and 2 TCS receiving other type of treatment
N = Number participating in analyses
# Unadjusted mean (SD)
SO = Surveillance only (no further treatment after orchidectomy)
n.d. = not determined because of no subjects with Z-score < -1 in this treatment group
ACT = Adjuvant chemotherapy; 1-2 cycles of cisplatin-based chemotherapy (CBC) or carboplatin
SCT = Standard dose chemotherapy; 3-4 cycles of CBC
RT = Adjuvant radiotherapy administrered to the paraaortic and ipsilateral iliac lymph nodes. Irradiation dose was 25.2 Gray (Gy) in 14 fractions for all but one patient, who received 24 Gy in 16 fractions
ETC = extensive treatment with chemotherapy; >4 cycles of CBC or ≥4 cycles of CBC + radiotherapy at targets other than the remaining testicle
Ref = Reference group

For the two groups of TCS receiving ≥3 cycles of CBC, there was a borderline statistically significant decrease in total hip BMD. These associations were not robust for adjustment for hypogonadism (p=0.17 and p=0.65 after adjustment, respectively). Similarly, the OR for total hip LBD in TCS receiving ETC was 4.1, p=0.25 after adjustment for hypogonadism (Paper III). Absolute BMD and Z-scores for total hip did not differ between the irradiated and the non-irradiated hip in TCS treated with adjuvant radiotherapy (both p-values: 0.37; data not shown).

Childhood cancer survivors treated with cranial irradiation ± chemotherapy exhibited significantly lower total hip and lumbar spine BMD than their controls, but showed no corresponding increase in the OR for LBD (Table 18). These estimates were robust for the exclusion of patients on immunosuppressive oral glucocorticoids or treatment with calcium + vitamin D, and adjustment for hypogonadism and GHRT (Paper IV).

Table 18. Mean differences in bone mineral density (BMD) and odds ratios (ORs) for low BMD (LBD, defined as Z –score <-1), in childhood cancer survivors: therapeutic subgroups vs. controls. Adjusted for age, body mass index and current smoking*

Area	Group	N	BMD, (g/cm^2)#	LBD, n (%)	Mean difference (95% CI), g/cm^2	p	OR for LBD (95% CI)	p
Total hip	Brain surgery‡	14	1.099 (0.110)	2 (14)	0.025 (-0.056; 0.106)	0.54	0.61 (0.13; 2.9)	0.54
	Surgery other than brain surgery‡	19	1.051 (0.155)	6 (32)	-0.022 (-0.093; 0.048)	0.53	1.6 (0.55; 4.8)	0.39
	CT§	29	1.102 (0.140)	4 (14)	0.011 (-0.049; 0.072)	0.72	0.57 (0.18; 1.8)	0.35
	Cranial irradiation¤	33	1.012 (0.166)	10 (30)	-0.076 (-0.133; -0.019)	0.009	1.6 (0.67; 3.9)	0.28
	RT other than brain and/or testes†	27	1.057 (0.147)	4 (15)	0.015 (-0.046; 0.077)	0.62	0.56 (0.17; 1.8)	0.33
	Controls	122	1.065 (0.158)	27 (22)	Ref	Ref	Ref	Ref

Lumbar spine L1-L4	Brain surgery‡	14	1.199 (0.094)	3 (21)	0.004 (-0.071; 0.079)	0.92	0.56 (0.14; 2.2)	0.41
	Surgery other than brain surgery‡	19	1.215 (0.134)	6 (32)	0.028 (-0.038; 0.095)	0.40	0.92 (0.31; 2.8)	0.88
	CT§	28	1.228 (0.116)	2 (7.1)	0.017 (-0.041; 0.074)	0.57	0.25 (0.06; 1.2)	0.076
	Cranial irradiation¤	33	1.137 (0.176)	13 (39)	-0.071 (-0.124; -0.018)	0.009	2.1 (0.90; 4.9)	0.088
	RT other than brain and/or testes†	27	1.230 (0.161)	3 (11)	0.068 (0.010; 0.125)	0.021	0.21 (0.06; 0.79)	0.021
	Controls	121	1.186 (0.141)	35 (29)	Ref	Ref	Ref	Ref

* Smoking or hormone data missing for 4 CCS and 3 controls, and data on lumbar spine L1-L4 missing for 1 childhood cancer survivor and 1 control.
N = Number participating in analyses
# Unadjusted mean (SD)
RT = radiotherapy
CT=chemotherapy
‡ Excluding RT to any target, excluding CT
§ Excluding RT to any target. In 11 cases combined with surgery other than brain surgery.
¤ Median irradiation dose 30 Gy. 2 cases received total TBI followed by bone marrow transplantation. Another 3 cases also received RT to testes, to a median irradiation dose to of 20 Gy. In 21 cases combined with chemotherapy. In 14 cases combined with brain surgery, and in 3 cases combined with surgery other than brain surgery.
† In 22 cases combined with chemotherapy. One case received high dose chemotherapy followed by bone marrow transplantation. In 22 cases combined with surgery other than brain surgery.
Ref = Reference group

In CCS treated with chemotherapy without radiotherapy, no significant difference was found in total hip or lumbar spine L1-L4 BMD, or the OR for total hip or lumbar spine L1-L4 LBD, in those receiving alkylating agents, methotrexate or glucocorticoids compared to controls, before or after adjustment for hypogonadism and GHRT (supplementary information to Paper IV).

Treatment with radiotherapy to targets other than the brain and/or testes ± chemotherapy resulted in increased lumbar spine L1-L4 BMD before, but not after, the exclusion of patients on immunosuppressive oral glucocorticoids or treatment with calcium + vitamin D, and adjustment for hypogonadism and GHRT. This was also expressed as a statistically significantly decreased OR for lumbar spine L1-L4 LBD compared to controls, which was robust for the exclusion of cases on immunosuppressive oral glucocorticoids or treatment with calcium + vitamin D, and adjustment for hypogonadism and GHRT (Paper IV).

Diagnostic subgroups of cancer survivors vs. controls

No statistically significant differences were found in total hip or lumbar spine L1-L4 BMD in the diagnostic subgroups of CCS compared to controls (Table 19). However, CCS treated for lymphoma showed a reduced OR for lumbar spine L1-L4 LBD (OR=0.092; 95% CI: 0.011; 0.76; p=0.027). All estimates were robust for the exclusion of patients on immunosuppressive oral glucocorticoids or treatment with calcium + vitamin D, and adjustment for hypogonadism and GHRT (supplementary information to Paper IV).

Table 19. Mean differences in bone mineral density (BMD) and odds ratios (ORs) of low BMD (LBD, defined as Z –score <-1), in childhood cancer survivors: diagnostic subgroups vs. controls. Adjusted for age, body mass index and current smoking*

Area	Group	N	BMD (g/cm^2)$^{\#}$	LBD, n (%)	Mean difference (95% CI), g/cm^2	p	OR for LBD (95% CI)	p
Total Hip	Leukaemia	27	1.065 (0.148)	6 (22)	-0.029 (-0.092; 0.033)	0.36	1.1 (0.40; 3.2)	0.80
	Intracranial tumour	27	1.051 (0.176)	6 (22)	-0.030 (-0.092; 0.031)	0.33	1.0 (0.38; 2.9)	0.94
	Lymphoma	20	1.088 (0.124)	2 (10)	0.034 (-0.036; 0.104)	0.33	0.34 (0.072; 1.6)	0.17
	Testicular cancer	6	0.993 (0.099)	3 (50)	-0.090 (-0.211; 0.031)	0.15	3.6 (0.67; 19)	0.14
	Wilms' tumour (nephroblastoma)	8	1.069 (0.151)	1 (13)	-0.001 (-0.106; 0.104)	0.99	0.56 (0.065; 4.8)	0.60
	Bone tumour	6	1.090 (0.108)	-	0.039 (-0.082; 0.160)	0.53	n.d.	-
	Other tumours	28	1.050 (0.170)	8 (29)	-0.022 (-0.083; 0.039)	0.47	1.4 (0.53; 3.5)	0.52
	Controls	122	1.065 (0.158)	27 (22)	Ref	Ref	Ref	Ref

Lumbar spine L1-L4	Leukaemia	26	1.189 (0.132)	7 (27)	-0.026 (-0.086; 0.033)	0.39	1.3 (0.48; 3.6)	0.60
	Intracranial tumour	27	1.152 (0.181)	9 (33)	-0.047 (-0.105; 0.010)	0.11	1.3 (0.52; 3.4)	0.56
	Lymphoma	20	1.234 (0.150)	1 (5)	0.059 (-0.006; 0.124)	0.075	0.092 (0.011; 0.76)	0.027
	Testicular cancer	6	1.155 (0.054)	1 (17)	-0.046 (-0.159; 0.067)	0.42	0.59 (0.07; 5.4)	0.64
	Wilms' tumour (nephroblastoma)	8	1.212 (0.213)	2 (25)	0.021 (-0.078; 0.119)	0.68	0.88 (0.16; 4.8)	0.88
	Bone tumour	6	1.269 (0.138)	1 (17)	0.099 (-0.014; 0.212)	0.09	0.35 (0.04; 3.3)	0.36
	Other tumours	28	1.218 (0.122)	6 (21)	0.030 (-0.027; 0.087)	0.30	0.55 (0.20; 1.5)	0.26
	Controls	121	1.186 (0.141)	35 (29)	Ref	Ref	Ref	Ref

* Smoking or hormone data missing for 4 CCS and 3 controls, and data on lumbar spine L1-L4 missing for 1 childhood cancer survivor and 1 control
N = Number participating in analyses
[#] Unadjusted mean (SD)
n.d. – not determined because of no subjects with Z-score <-1 in this treatment group..

Discussion

Azoospermia in testicular cancer survivors

Treatment modality and risk of azoospermia

The risk of azoospermia was significantly increased in TCS treated with ETC in the total cohort. A similar frequency of azoospermia was seen in patients with longitudinal data receiving ETC, the lack of statistical significance probably being due to the small number of patients included in the analysis. This finding is supported by previous studies, showing an increased frequency of azoospermia following treatment with CBC equivalent to >4 cycles of standard chemotherapy [161,223,224].

The finding of no increased risk of azoospermia in men treated with ≤4 cycles of CBC is also consistent with previous studies [225,226]. However, Brydoy et al. found an increased risk of impaired spermatogenesis, expressed as higher FSH levels, lower inhibin B levels and lower sperm counts, in TCS receiving ≤ 4 cycles of CBC or carboplatin compared to TCS subjected to surveillance only after unilateral orchidectomy [224]. This inconsistency may be due to the different outcomes considered in the two studies: azoospermia vs. sperm counts. It should also be noted that the study performed by Brydoy et al. included a larger number of patients, and therefore had higher statistical power. In addition, 10% of TCS receiving ≤ 4 cycles of CBC or carboplatin had also received radiotherapy, and the negative impact on sperm production may be partially due to the more extensive treatment.

Inhibin B as predictor of azoospermia

Inhibin B values obtained 6, 12 and 24 months after treatment were predictive of the risk of azoospermia 3 years after treatment, the inhibin B level 12 months after treatment being the best predictor.

It has been reported previously that the level of inhibin B is correlated to azoospermia in cancer survivors. In a study on CCS, a similar cut-off level of inhibin B, 50 ng/L, was found to be predictive of azoospermia after a mean follow-up period of 19 years [227]. In a Norwegian study on TCS, low or undetectable levels of inhibin B were reported to be associated with azoospermia at a median of 11 years follow-up [224]. Azoospermia was seen despite inhibin B values >80 ng/L in five men in the Norwegian study, which is in contrast to the present findings. However, no information was available on pre-treatment sperm production in these Norwegian men, whereas the longitudinal analysis in the present work was carried out on men with confirmed sperm production before cancer treatment. It cannot be excluded that azoospermic TCS with normal inhibin B levels had obstructive azoospermia, as inhibin B levels in men with obstructive azoospermia are similar to those in fertile men [228]. The Norwegian study also included older men, in whom the causes of azoospermia may differ from those in younger men.

Cryptorchidism as predictor of azoospermia

In the present work, cryptorchidism was reported by 6.9% of TCS-A, and by 5.1% of subjects for whom longitudinal data were available. Cryptorchidism has previously been reported in 11-12% of men with TC [140,173,229], and the lower frequency of cryptorchidism in our longitudinal cohort might explain why cryptorchidism was not predictive of long-term azoospermia in the present work.

Relevance of 36 months after therapy as the endpoint for azoospermia

Thirty-six months after therapy was used as the end-point for predictive factors of azoospermia in the present work. The mean age of fathers in Sweden at the birth of their first child was 32 years in 2015 [230]. As 75% of patients with testicular cancer are between 29 and 43 years of age at diagnosis [231], it is reasonable to assume that many TCS may want to start a family during the first few years after cancer treatment. In a Dutch study of male cancer survivors with a median age of 27 years at semen cryopreservation, semen was used for artificial reproductive techniques after a mean of 57 months (range 15-130 months) [232]. In a Czech study on TCS with a mean age of 28.5 years at semen cryopreservation, the use of cryopreserved semen was reported after a median of 18 months (range 7-70 months) [233]. The time elapsed from the end of cancer treatment to the use of cryopreserved semen varied considerably, but some cancer survivors used their cryopreserved semen within one year. Three years after testicular cancer treatment was therefore deemed to be a clinically relevant endpoint for the assessment of potential fertility.

Strengths and weaknesses of the study

Weaknesses include the timing of blood sample collection and the categorization of TCS as azoospermic based on only one semen sample. Blood samples were collected between 8.00 a.m. and 3 p.m. Inhibin B has a diurnal variation, with a maximum value in the morning and a minimum in the late afternoon. The median decrease from the highest to the lowest level has been reported to be 37% [234]. The categorization of TCS as azoospermic was based on one semen sample due to practical reasons. Sperm count varies significantly within each subject [235], and in the clinical setting, the diagnosis of azoospermia requires the absence of spermatozoa in at least 2 separate centrifuged ejaculates [54]. It can be assumed that these shortcomings tend to reduce the calculated predictive value of Inhibin B measurements. Another limitation is the relatively low number of azoospermic subjects at each time-point evaluated.

The strengths of the study are the relatively large number of TCS included, and the longitudinal analyses performed on subjects with confirmed sperm production after orchidectomy but before further treatment.

Hypogonadism and bone mineral density in young male cancer survivors

Hypogonadism in young male cancer survivors

Childhood cancer survivors

Hypogonadism in CCS was found to be associated with both testicular and cranial irradiation, as well as with the combination of chemotherapy and radiotherapy to targets other than the cranium or testes.

The present study indicates a distinct link between cranial and/or testicular irradiation and subsequent risk of hypogonadism, as illustrated by the increased risk of hypogonadism in leukaemia survivors treated with, but not without, radiotherapy. For CCS receiving cranial irradiation, the median dose was 54 Gy for subjects receiving radiotherapy only, and 24 Gy for subjects receiving cranial irradiation combined with chemotherapy. Radiation to the hypothalamic-pituitary area with doses ≥18 Gy has been reported to be a risk factor for secondary hypogonadism [17]. The difference in irradiation dose is probably the reason why CCS treated with cranial irradiation alone showed an increased OR for hypogonadism, while no increase in risk was observed in CCS receiving cranial irradiation plus chemotherapy.

The risk of hypogonadism was also increased in patients treated with radiotherapy, with targets other than the cranium or testes, combined with chemotherapy. This increased risk may be the result of scattered irradiation to the testes, the risk probably being greater in smaller children than in adults.

Testicular cancer survivors

Among TCS, the risk of hypogonadism was highly increased in those given more than 4 cycles of CBC (in 2 cases combined with non-testicular irradiation), while no other treatment subgroup showed a statistically significantly elevated risk.

The finding of an increased risk of biochemical hypogonadism only in TCS treated with >4 cycles of CBC ± radiotherapy is supported by Gerl et al. [171]. In the latter study, 20% of patients treated with cisplatin >400 mg/m^2 (corresponding to >4 cycles of standard CBC) had low testosterone levels, compared to 4 out of 5 hypogonadal patients in our ETC subgroup. However, patients treated with cisplatin >400 mg/m^2 in the study by Gerl et al. received a median of 6 cycles of chemotherapy, whereas subjects in our ETC subgroup received 8 or 9 cycles. *In vitro* studies have shown that cisplatin impairs testosterone production in a dose-dependent manner; the impairment of Leydig cell function being mediated through increased production of reactive oxygen species [236].

The ORs for hypogonadism were of approximately the same magnitude for the other therapeutic subgroups (~2), but not statistically significantly different from controls, possibly due to limited sample sizes. In contrast, Nord et al. found slightly higher (3.6-4.4) and statistically significant ORs for TCS treated with radiotherapy only and those receiving cisplatin ≤850 mg, compared to controls, probably due to the higher statistical power [180]. Furthermore, the frequency of hypogonadism among controls in their study was 5%, making statistical significance easier to attain. A statistically significant increase in the risk of testosterone deficiency in TCS treated with orchidectomy plus ≤4 cycles of CBC, infradiaphragmatic radiotherapy or more extensive treatment, compared to TCS treated with orchidectomy alone, was also reported in a recent meta-analysis [237]. The higher number of subjects included in this meta-analysis explains the higher statistical power.

Bone mineral density in young male cancer survivors

Hypogonadal vs. eugonadal cancer survivors

One of the main findings of this work was the association between untreated hypogonadism and lower BMD and increased risk of LBD in cancer survivors compared to controls.

The finding of an association between hypogonadism and LBD in TCS is supported by a study carried out by Ondrusova et al., in which low testosterone and high LH levels were seen more frequently in TCS with low BMD, compared to TCS with normal BMD [210]. However, they did not compare BMD in eugonadal and hypogonadal TCS.

Lower total hip BMD was also found in TCS on TRT in the present study. Testicular function is already decreased in men with testicular cancer before cancer diagnosis [238]. It cannot be excluded that BMD in TCS is affected by long-standing hypogonadism before cancer diagnosis, and not fully compensated by a number of years of TRT.

To the best of our knowledge, only two studies have been performed on the association between gonadal function and BMD in adult male CCS [204,205]. Both studies indicate, in line with our findings, that mild hypogonadism can negatively affect BMD.

In a placebo-controlled clinical trial by Finkelstein et al., endogenous gonadal steroid production was suppressed by goserelin acetate in healthy men aged 20-50 years [114]. The subjects were than randomized to different doses of TRT ± anastrozole, which suppresses the conversion of androgens to oestrogens. The authors concluded that oestrogens were the primary regulators of bone homeostasis in men, and that bone loss was unlikely when testosterone levels were above 6.9 nmol/L and/or oestradiol levels above 36.7 pmol/L. The duration of hormonal suppression in their study was 16 weeks. However, it cannot be excluded that bone loss may occur when testosterone levels are above 6.9 nmol/L for more than 16 weeks.

The Endocrine Society's Clinical Guidelines regarding osteoporosis in men suggests DXA in hypogonadal men aged 50-69, and TRT for men with borderline high risk of fracture and symptomatic testosterone deficiency (defined as S-testosterone <6.9 nmol/L), or men with high fracture risk, S-testosterone <6.9 nmol/L, and contraindications to approved pharmacological agents for osteoporosis [126]. Over 75% of our untreated hypogonadal TCS and CCS had S-testosterone levels above 6.9 nmol/L, suggesting that BMD is also affected in younger hypogonadal men with only moderately lowered S-testosterone levels.

Therapeutic subgroups of cancer survivors

Adjuvant irradiation of the retroperitoneal lymph nodes had no impact on the lumbar spine L1-L4 BMD compared to controls, and no difference was seen in total hip BMD between the irradiated and the non-irradiated hip in TCS treated with adjuvant radiotherapy. This is supported by a previous study, in which no difference was found in hip BMD between the irradiated and the non-irradiated side in TCS at long-term follow-up after adjuvant radiotherapy [211]. However, our findings do not

exclude an increased risk of fracture in areas subject to direct or scattered irradiation. As no data on BMD were given in previous studies on radiation-induced fractures, it is not known whether these fractures were associated with a local decrease in BMD [186,187].

Childhood cancer survivors treated with radiotherapy to the brain had significantly lower BMD both in the hip and lumbar spine L1-L4 compared to controls, but no increased OR for LBD. Cranial irradiation has previously been reported to be associated with LBD in CCS [196,199,200]. Cranial irradiation in children is a risk factor for later pituitary malfunction, such as secondary hypogonadism and GHD [191]. Adults with untreated adult-onset GHD have decreased BMD, and adults with childhood-onset GHD have been shown in some studies to have lower BMD than controls [189]. Growth hormone replacement therapy increases BMD in adults with GHD after more than 1 year of treatment [190].

As cranial irradiation can cause both GHD and secondary hypogonadism, it could be argued that the reduced BMD found in our hypogonadal subjects was in fact due to radiation-induced GHD. In our analysis on CCS based on gonadal status, GHRT was used as a proxy for GHD, as the study was not designed to evaluate growth hormone status (e.g. no provocative testing for GHD was performed). It is therefore possible that the real impact of GHD could not be adjusted for, due to potential undiagnosed and untreated GHD in our CCS cohort. However, the median height was almost identical in untreated hypogonadal CCS and Swedish men from the general population, indicating no symptomatic childhood-onset GHD [239].

Diagnostic subgroups of cancer survivors

The decreased risk of lumbar spine L1-L4 LBD in CCS treated for lymphoma was a surprising finding. Reduced BMD already at diagnosis, with a further decrease during cancer therapy, has been reported in children with ALL [197,198]. Reduced BMD has also been found in survivors of ALL after median follow-up periods of 8 [240] and 11.5 years [241]. In contrast, recovery of BMD was reported in a longitudinal study on ALL survivors, where 67% of the subjects with previous BMD Z-scores ≤-2 showed improvements in their Z-score by ≥1 category after a median follow-up of 8.5 years [199]. As our CCS were evaluated a mean of 24 years after cancer treatment, our results could hypothetically reflect some recovery of BMD. Alternatively, the lower LBD risk observed could be a chance finding.

Representativeness of the study participants

The participation rate among CCS was 31%. The CCS invited to take part in the study were living in the region of Skåne at the time of their cancer diagnosis, but due to the long follow-up period, many of them may have moved to other parts of

Sweden, and were thus unwilling or unable to participate in the study for practical reasons. Furthermore, these men had been asked to take part in other clinical studies previously, and may have been unwilling to participate in yet another study, and to be reminded, again, about a life-threatening disease earlier in their life.

Regarding the controls, 25% of those invited participated in the study. Sixteen percent of these controls presented with biochemical hypogonadism, and it is plausible that men with symptoms of hypogonadism might be more prone to participate in such a study. However, the frequency of biochemical hypogonadism has been reported to be 24% in men aged 30-79 years [242] and 14% in men aged 40-49 years [69] in large epidemiological studies. There is, therefore, no reason to believe that biochemical hypogonadism was overrepresented in the control group, compared to the general population.

Finally, comparison of the number of children among participants and non-participants indicated no selection bias due to reproductive problems among CCS, TCS or controls.

Strengths and weaknesses of the studies

Weaknesses in the studies presented in Papers II-IV include the definition of participants as hypo- or eugonadal based on single measurements of testosterone levels, lack of data on the length of TRT, possible selection bias due to incomplete participation, and lack of information on risk factors for osteoporosis.

Patients were defined as hypo- or eugonadal based on single measurements of S-testosterone and S-LH for practical reasons. While testosterone levels show intra-individual variation [243], single measurements of testosterone can provide reliable data, at least in middle-aged and elderly men [244]. The diagnosis of hypogonadism should be based on low morning testosterone on at least two occasions together with symptoms of androgen deficiency in the clinical setting [245]. Therefore, the proportion of subjects with biochemical hypogonadism in our studies does not exactly reflect the proportion of cancer survivors requiring TRT.

Another weakness of these studies is the lack of data on the duration of TRT. The effect of TRT for longer than 36 months on BMD has not been evaluated in clinical studies. Thus, it cannot be excluded that long-time TRT restores normal levels of BMD. Furthermore, the low participation rate among the controls might have led to selection bias, with potential overrepresentation of controls with a family history of osteoporosis or previous fractures. If present, such a selection bias would tend to decrease the difference in BMD between patients and controls. However, the within-patient group comparisons such as hypogonadal vs. eugonadal, are not dependent on the selection of controls.

Finally, an additional limitation is the lack of information regarding lifestyle factors other than smoking, such as physical activity and dietary intake of calcium and vitamin D, which are known to affect BMD [97].

The strengths of these studies are the inclusion of age-matched controls from the general population, and the measurement of testosterone levels between 8 and 10 a.m. Testosterone concentrations follow a circadian rhythm; the highest levels being seen in the morning [79-81] and in the fasting state [82]. Hence, testosterone levels should be determined in fasting morning blood samples, as was done in the current studies.

Regarding studies on hypogonadism in male cancer survivors, there is only one study on TCS that included controls from the general population [144]. The inclusion of age-matched controls from the general population in the present work enabled more valid conclusions to be drawn regarding hypogonadism and the bone mineral status of young male cancer survivors, compared to the general population.

Summary and Conclusions

The overall aim of the work presented in this thesis was to improve the follow-up and counselling of young male cancer survivors, by investigating the prevalence and evaluating potential risk factors of gonadal dysfunction and decreased bone mineral density. In this work, inhibin B was identified as a marker of post-treatment azoospermia in testicular cancer survivors, and hypogonadism was found to be a risk factor for reduced bone mineral density in young male cancer survivors.

- The level of inhibin B 12 months after completion of testicular cancer treatment can be used to identify men at risk of azoospermia up to 3 years after treatment, provided these results can be confirmed in additional studies.
- Biochemical hypogonadism was present in 36% of testicular cancer survivors and 26% of childhood cancer survivors after mean follow-up times of 9 and 24 years, respectively, with odds ratios being statistically significantly increased compared to controls from the general population. This finding highlights the need for long-term follow-up for young male cancer survivors.
- Untreated biochemical hypogonadism was associated with decreased bone mineral density and increased risk of low bone mineral density in both testicular cancer survivors and childhood cancer survivors, already at moderately lowered testosterone levels. Prevention of osteoporosis should be considered an important part in future follow-up of young male cancer survivors, and the assessment of bone mineral density should be considered already at moderately reduced testosterone levels.

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Future Perspectives

The finding that inhibin B is a predictor of long-term azoospermia after cancer treatment must be confirmed in future studies involving larger study populations, with confirmed sperm production before cancer treatment. Such studies will hopefully provide a more exactly defined cut-off value for inhibin B, and the answer to the question of whether treatment-induced azoospermia can arise despite high post-treatment inhibin B levels.

High frequencies of hypogonadism were found in young male cancer survivors at long-term follow-up. Additional longitudinal studies of male cancer survivors are needed to investigate how long after cancer treatment hypogonadism develops, and whether hypogonadism persists, or if Leydig cell function is recovered after cancer treatment. Such longitudinal studies could also reveal whether cancer survivors with compensated hypogonadism subsequently develop primary hypogonadism.

An important finding of this work was the association between untreated hypogonadism and increased risk of low bone mineral density in young males. As little is known about the skeletal effects of hypogonadism in young men, a randomized placebo-controlled trial should be carried out to evaluate testosterone replacement therapy in young hypogonadal males, with bone mineral density as one of the endpoints. Such a trial would render further information on the role of testosterone, and the effect of testosterone replacement therapy, on bone mineral density in young males. Furthermore, longitudinal studies on bone mineral density in male cancer survivors are desirable to further evaluate possible changes in bone mineral density during the years after cancer treatment.

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References

1. Socialstyrelsen. Cancerincidens i Sverige 2013. (http://www.socialstyrelsen.se/Lists/Artikelkatalog/Attachments/19613/2014-12-10.pdf, 2014).
2. O'Leary, M., Krailo, M., Anderson, J.R., Reaman, G.H. & Children's Oncology Group. Progress in childhood cancer: 50 years of research collaboration, a report from the Children's Oncology Group. *Seminars in oncology* **35**, 484-493 (2008).
3. Jessop, E. The History of Childhood Cancer Research. (https://www.stbaldricks.org/blog/post/the-history-of-childhood-cancer-research, 2015).
4. Gustafsson, G., Kogner, P. & Heyman, M. Childhood Cancer Incidence and Survival in Sweden 1984-2010. Barncancerepidemiologiska Forskningsgruppen, Karolinska Institutet, Stockholm, (http://www.forskasverige.se/wp-content/uploads/ChildhoodCancerIncidenceandSurvivalinSweden1984_2010.pdf, 2013).
5. Regionala cancercentrum i samverkan. Långtidsuppföljning efter barncancer (Regionalt cancercentrum, (https://www.cancercentrum.se/globalassets/cancerdiagnoser/barn/vardprogram/vp-langtidsuppfoljning-barncancer.pdf, 2016).
6. Hanna, N. & Einhorn, L.H. Testicular cancer: a reflection on 50 years of discovery. *J Clin Oncol* **32**, 3085-3092 (2014).
7. Verdecchia, A., *et al.* Recent cancer survival in Europe: a 2000-02 period analysis of EUROCARE-4 data. *The Lancet. Oncology* **8**, 784-796 (2007).
8. Ferlay, J., *et al.* Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International journal of cancer. Journal international du cancer* **136**, E359-386 (2015).
9. NORDCAN, The Association of the Nordic Cancer Registries. Testikelcancer män 15-44 år. Data available at: http://www-dep.iarc.fr/NORDCAN/SW/Table3.asp?registry=752&sex=1&type=0&age_from=4&age_to=9&sort=3&period=2015&submit=Execute
10. NORDCAN, The Association of the Nordic Cancer Registries. Incidence of testicular cancer in the nordic countries. Data available at: http://www-dep.iarc.fr/NORDCAN/SW/table2.asp?cancer=271&period=2015&sex=1&type=0&age_from=1&age_to=18&sort=2&registry=1&submit=%A0%A0Utf%F6r%A0%A0

11. NORDCAN, The Association of the Nordic Cancer Registries. Five-year survival after testicular cancer in Sweden. Data available at: http://www-dep.iarc.fr/NORDCAN/SW/Graph24l.asp?cancer=271&male=1&country%5B%5D=752&prevalence=5&orientation=2&grid=1&line=2&submit=%A0%A0%A0Utf%F6r%A0%A0%A0
12. Monje, M. & Fisher, P.G. Neurological complications following treatment of children with brain tumors. *J Pediatr Rehabil Med* **4**, 31-36 (2011).
13. SWENOTECA, Swedish and Norwegian Testicular Cancer Group. SWENOTECA VIII and SWENOTECA IX. (https://www.swenoteca.org/procedures, 2014 and 2016).
14. Armstrong, G.T., *et al.* Late mortality among 5-year survivors of childhood cancer: a summary from the Childhood Cancer Survivor Study. *J Clin Oncol* **27**, 2328-2338 (2009).
15. Armstrong, G.T., *et al.* Reduction in Late Mortality among 5-Year Survivors of Childhood Cancer. *N Engl J Med* **374**, 833-842 (2016).
16. Oeffinger, K.C., *et al.* Chronic health conditions in adult survivors of childhood cancer. *N Engl J Med* **355**, 1572-1582 (2006).
17. Hudson, M.M., *et al.* Clinical ascertainment of health outcomes among adults treated for childhood cancer. *Jama* **309**, 2371-2381 (2013).
18. Maroto, P., Anguera, G. & Martin, C. Long-term toxicity of the treatment for germ cell-cancer. A review. *Crit Rev Oncol Hematol* **121**, 62-67 (2018).
19. Chovanec, M., *et al.* Long-term toxicity of cisplatin in germ-cell tumor survivors. *Ann Oncol* **28**, 2670-2679 (2017).
20. Kvammen, O., *et al.* Long-term Relative Survival after Diagnosis of Testicular Germ Cell Tumor. *Cancer Epidemiol Biomarkers Prev* **25**, 773-779 (2016).
21. Travis, L.B., *et al.* Second cancers among 40,576 testicular cancer patients: focus on long-term survivors. *J Natl Cancer Inst* **97**, 1354-1365 (2005).
22. Richiardi, L., *et al.* Second malignancies among survivors of germ-cell testicular cancer: a pooled analysis between 13 cancer registries. *International journal of cancer. Journal international du cancer* **120**, 623-631 (2007).
23. Fung, C., Fossa, S.D., Milano, M.T., Oldenburg, J. & Travis, L.B. Solid tumors after chemotherapy or surgery for testicular nonseminoma: a population-based study. *J Clin Oncol* **31**, 3807-3814 (2013).
24. van den Belt-Dusebout, A.W., *et al.* Treatment-specific risks of second malignancies and cardiovascular disease in 5-year survivors of testicular cancer. *J Clin Oncol* **25**, 4370-4378 (2007).
25. Travis, L.B., *et al.* Second malignant neoplasms and cardiovascular disease following radiotherapy. *J Natl Cancer Inst* **104**, 357-370 (2012).
26. Meinardi, M.T., *et al.* Cardiovascular morbidity in long-term survivors of metastatic testicular cancer. *J Clin Oncol* **18**, 1725-1732 (2000).

27. van den Belt-Dusebout, A.W., *et al.* Long-term risk of cardiovascular disease in 5-year survivors of testicular cancer. *J Clin Oncol* **24**, 467-475 (2006).
28. Haugnes, H.S., *et al.* Cardiovascular risk factors and morbidity in long-term survivors of testicular cancer: a 20-year follow-up study. *J Clin Oncol* **28**, 4649-4657 (2010).
29. Vogelzang, N.J., Bosl, G.J., Johnson, K. & Kennedy, B.J. Raynaud's phenomenon: a common toxicity after combination chemotherapy for testicular cancer. *Ann Intern Med* **95**, 288-292 (1981).
30. de Haas, E.C., *et al.* Early development of the metabolic syndrome after chemotherapy for testicular cancer. *Ann Oncol* **24**, 749-755 (2013).
31. Bogefors, C., *et al.* Hypogonadism in testicular cancer patients is associated with risk factors of cardiovascular disease and the metabolic syndrome. *Andrology* **5**, 711-717 (2017).
32. OpenStax CNX, Anatomy & Physiology of the Male Reproductive System. Available at https://cnx.org/contents/FPtK1zmh@8.24:Nw1tEY4R@6/Anatomy-and-Physiology-of-the-
33. Corradi, P.F., Corradi, R.B. & Greene, L.W. Physiology of the Hypothalamic Pituitary Gonadal Axis in the Male. *Urol Clin North Am* **43**, 151-162 (2016).
34. Belchetz, P.E., Plant, T.M., Nakai, Y., Keogh, E.J. & Knobil, E. Hypophysial responses to continuous and intermittent delivery of hypopthalamic gonadotropin-releasing hormone. *Science* **202**, 631-633 (1978).
35. Skorupskaite, K., George, J.T. & Anderson, R.A. The kisspeptin-GnRH pathway in human reproductive health and disease. *Hum Reprod Update* **20**, 485-500 (2014).
36. Nieschlag, E., Behre, H.M. & Nieschlag, S. *Andrology, Male Reproductive Health and Dysfunction*. 26 (Springer, New York, 3rd ed. 2010).
37. Pitteloud, N., *et al.* Inhibition of luteinizing hormone secretion by testosterone in men requires aromatization for its pituitary but not its hypothalamic effects: evidence from the tandem study of normal and gonadotropin-releasing hormone-deficient men. *J Clin Endocrinol Metab* **93**, 784-791 (2008).
38. de Kretser, D.M., Hedger, M.P., Loveland, K.L. & Phillips, D.J. Inhibins, activins and follistatin in reproduction. *Hum Reprod Update* **8**, 529-541 (2002).
39. Meachem, S.J., Nieschlag, E. & Simoni, M. Inhibin B in male reproduction: pathophysiology and clinical relevance. *European journal of endocrinology / European Federation of Endocrine Societies* **145**, 561-571 (2001).

40. Nieschlag, E., Behre, H.M. & Nieschlag, S. *Andrology, Male Reproductive Health and Dysfunction*. 15-16 (Springer, New York, 3rd ed. 2010).
41. O'Donnell, L., Stanton P. & de Kretser M. Endocrinology of the Male Reproductive System and Spermatogenesis. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, Koch C, Korbonits M, McLachlan R, New M, Purnell J, Rebar R, Singer F, Vinik A eds. *Endotext.* (South Darmouth (MA). Available at https://www.ncbi.nlm.nih.gov/books/NBK279031/#endocrin-male-reprod.toc-an-overview-of-spermatogenesis, 2000-).
42. Iliadou, P.K., Tsametis, C., Kaprara, A., Papadimas, I. & Goulis, D.G. The Sertoli cell: Novel clinical potentiality. *Hormones* **14**, 504-514 (2015).
43. de Kretser, D.M., Loveland, K.L., Meinhardt, A., Simorangkir, D. & Wreford, N. Spermatogenesis. *Hum Reprod* **13 Suppl 1**, 1-8 (1998).
44. Smith, L.B. & Walker, W.H. The regulation of spermatogenesis by androgens. *Semin Cell Dev Biol* **30**, 2-13 (2014).
45. Nieschlag, E., Behre, H.M. & Nieschlag, S. *Andrology, Male Reproductive Health and Dysfunction*. 42 (Springer, New York, 3rd ed. 2010).
46. Coviello, A.D., *et al.* Low-dose human chorionic gonadotropin maintains intratesticular testosterone in normal men with testosterone-induced gonadotropin suppression. *J Clin Endocrinol Metab* **90**, 2595-2602 (2005).
47. Kumanov, P., Nandipati, K.C., Tomova, A., Robeva, R. & Agarwal, A. Significance of inhibin in reproductive pathophysiology and current clinical applications. *Reprod Biomed Online* **10**, 786-812 (2005).
48. Jensen, T.K., *et al.* Inhibin B as a serum marker of spermatogenesis: correlation to differences in sperm concentration and follicle-stimulating hormone levels. A study of 349 Danish men. *J Clin Endocrinol Metab* **82**, 4059-4063 (1997).
49. Klingmuller, D. & Haidl, G. Inhibin B in men with normal and disturbed spermatogenesis. *Hum Reprod* **12**, 2376-2378 (1997).
50. Pierik, F.H., Vreeburg, J.T., Stijnen, T., De Jong, F.H. & Weber, R.F. Serum inhibin B as a marker of spermatogenesis. *J Clin Endocrinol Metab* **83**, 3110-3114 (1998).
51. von Eckardstein, S., *et al.* Serum inhibin B in combination with serum follicle-stimulating hormone (FSH) is a more sensitive marker than serum FSH alone for impaired spermatogenesis in men, but cannot predict the presence of sperm in testicular tissue samples. *J Clin Endocrinol Metab* **84**, 2496-2501 (1999).
52. Myers, G.M., Lambert-Messerlian, G.M. & Sigman, M. Inhibin B reference data for fertile and infertile men in Northeast America. *Fertil Steril* **92**, 1920-1923 (2009).

53. Petersen, P.M., Andersson, A.M., Rorth, M., Daugaard, G. & Skakkebaek, N.E. Undetectable inhibin B serum levels in men after testicular irradiation. *J Clin Endocrinol Metab* **84**, 213-215 (1999).
54. Male Infertility Best Practice Policy Committee of the American Urological, A. & Practice Committee of the American Society for Reproductive, M. Report on evaluation of the azoospermic male. *Fertil Steril* **82 Suppl 1**, S131-136 (2004).
55. Berookhim, B.M. & Schlegel, P.N. Azoospermia due to spermatogenic failure. *Urol Clin North Am* **41**, 97-113 (2014).
56. Willott, G.M. Frequency of azoospermia. *Forensic Sci Int* **20**, 9-10 (1982).
57. Ashok, S. & Sigman, M. Bioavailable testosterone should be used for the determination of androgen levels in infertile men. *J Urol* **177**, 1443-1446; quiz 1591 (2007).
58. Nieschlag, E., Behre, H.M. & Nieschlag, S. *Andrology, Male Reproductive Health and Dysfunction*. 49-52 (Springer, New York, 3rd ed. 2010).
59. Ito, T. & Horton, R. The source of plasma dihydrotestosterone in man. *J Clin Invest* **50**, 1621-1627 (1971).
60. Grino, P.B., Griffin, J.E. & Wilson, J.D. Testosterone at high concentrations interacts with the human androgen receptor similarly to dihydrotestosterone. *Endocrinology* **126**, 1165-1172 (1990).
61. Amory, J.K., *et al.* The effect of 5alpha-reductase inhibition with dutasteride and finasteride on bone mineral density, serum lipoproteins, hemoglobin, prostate specific antigen and sexual function in healthy young men. *J Urol* **179**, 2333-2338 (2008).
62. Stanik, S., Dornfeld, L.P., Maxwell, M.H., Viosca, S.P. & Korenman, S.G. The effect of weight loss on reproductive hormones in obese men. *J Clin Endocrinol Metab* **53**, 828-832 (1981).
63. de Ronde, W., Pols, H.A., van Leeuwen, J.P. & de Jong, F.H. The importance of oestrogens in males. *Clinical endocrinology* **58**, 529-542 (2003).
64. Finkelstein, J.S., *et al.* Gonadal steroids and body composition, strength, and sexual function in men. *N Engl J Med* **369**, 1011-1022 (2013).
65. Baird, D.T., Uno, A. & Melby, J.C. Adrenal secretion of androgens and oestrogens. *The Journal of endocrinology* **45**, 135-136 (1969).
66. Baird, D.T., Horton, R., Longcope, C. & Tait, J.F. Steroid dynamics under steady-state conditions. *Recent Prog Horm Res* **25**, 611-664 (1969).
67. Bhasin, S., *et al.* Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* **95**, 2536-2559 (2010).
68. Dohle, G.R., *et al.* European Association of Urology. Guidelines on Male Hypogonadism. (https://uroweb.org/wp-content/uploads/18-Male-Hypogonadism_LR.pdf, 2014).

69. Tajar, A., *et al.* Characteristics of Secondary, Primary, and Compensated Hypogonadism in Aging Men: Evidence from the European Male Ageing Study. *J Clin Endocr Metab* **95**, 1810-1818 (2010).
70. Petak, S.M., *et al.* American Association of Clinical Endocrinologists Medical Guidelines for clinical practice for the evaluation and treatment of hypogonadism in adult male patients--2002 update. *Endocr Pract* **8**, 440-456 (2002).
71. Nieschlag, E., *et al.* Testosterone replacement therapy: current trends and future directions. *Hum Reprod Update* **10**, 409-419 (2004).
72. Bhasin, S. & Basaria, S. Diagnosis and treatment of hypogonadism in men. *Best Pract Res Clin Endocrinol Metab* **25**, 251-270 (2011).
73. Corona, G., *et al.* Hypogonadism and metabolic syndrome. *J Endocrinol Invest* **34**, 557-567 (2011).
74. Traish, A.M., Saad, F., Feeley, R.J. & Guay, A. The dark side of testosterone deficiency: III. Cardiovascular disease. *J Androl* **30**, 477-494 (2009).
75. Ding, E.L., Song, Y., Malik, V.S. & Liu, S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. *Jama* **295**, 1288-1299 (2006).
76. Bentmar Holgersson, M., Landgren, F., Rylander, L. & Lundberg Giwercman, Y. Mortality Is Linked to Low Serum Testosterone Levels in Younger and Middle-aged Men. *European urology* **71**, 991-992 (2017).
77. Araujo, A.B., *et al.* Clinical review: Endogenous testosterone and mortality in men: a systematic review and meta-analysis. *J Clin Endocrinol Metab* **96**, 3007-3019 (2011).
78. Holmboe, S.A., *et al.* The Association of Reproductive Hormone Levels and All-Cause, Cancer, and Cardiovascular Disease Mortality in Men. *J Clin Endocrinol Metab* **100**, 4472-4480 (2015).
79. Dabbs, J.M., Jr. Salivary testosterone measurements: reliability across hours, days, and weeks. *Physiol Behav* **48**, 83-86 (1990).
80. Gupta, S.K., Lindemulder, E.A. & Sathyan, G. Modeling of circadian testosterone in healthy men and hypogonadal men. *J Clin Pharmacol* **40**, 731-738 (2000).
81. Cooke, R.R., McIntosh, J.E. & McIntosh, R.P. Circadian variation in serum free and non-SHBG-bound testosterone in normal men: measurements, and simulation using a mass action model. *Clinical endocrinology* **39**, 163-171 (1993).
82. Caronia, L.M., *et al.* Abrupt decrease in serum testosterone levels after an oral glucose load in men: implications for screening for hypogonadism. *Clinical endocrinology* **78**, 291-296 (2013).
83. Vermeulen, A., Kaufman, J.M. & Giagulli, V.A. Influence of some biological indexes on sex hormone-binding globulin and androgen levels in aging or obese males. *J Clin Endocrinol Metab* **81**, 1821-1826 (1996).
84. Field, A.E., Colditz, G.A., Willett, W.C., Longcope, C. & McKinlay, J.B. The relation of smoking, age, relative weight, and dietary intake to serum

adrenal steroids, sex hormones, and sex hormone-binding globulin in middle-aged men. *J Clin Endocrinol Metab* **79**, 1310-1316 (1994).
85. Christiansen K. Behavioral correlates of testosterone. in *Testosterone: action, deficiency, substitution* (ed. Nieschlag, E., Behre, HM.) 132-133 (CambridgeUniversity Press, Cambridge, 2004).
86. Basaria, S., *et al.* Adverse events associated with testosterone administration. *N Engl J Med* **363**, 109-122 (2010).
87. Vigen, R., *et al.* Association of testosterone therapy with mortality, myocardial infarction, and stroke in men with low testosterone levels. *Jama* **310**, 1829-1836 (2013).
88. Finkle, W.D., *et al.* Increased risk of non-fatal myocardial infarction following testosterone therapy prescription in men. *PLoS One* **9**, e85805 (2014).
89. Xu, L., Freeman, G., Cowling, B.J. & Schooling, C.M. Testosterone therapy and cardiovascular events among men: a systematic review and meta-analysis of placebo-controlled randomized trials. *BMC Med* **11**, 108 (2013).
90. Haddad, R.M., *et al.* Testosterone and cardiovascular risk in men: a systematic review and meta-analysis of randomized placebo-controlled trials. *Mayo Clin Proc* **82**, 29-39 (2007).
91. Calof, O.M., *et al.* Adverse events associated with testosterone replacement in middle-aged and older men: a meta-analysis of randomized, placebo-controlled trials. *J Gerontol A Biol Sci Med Sci* **60**, 1451-1457 (2005).
92. Fernandez-Balsells, M.M., *et al.* Clinical review 1: Adverse effects of testosterone therapy in adult men: a systematic review and meta-analysis. *J Clin Endocrinol Metab* **95**, 2560-2575 (2010).
93. Elliott, J., *et al.* Testosterone therapy in hypogonadal men: a systematic review and network meta-analysis. *BMJ Open* **7**, e015284 (2017).
94. Traish, A.M., Haider, A., Haider, K.S., Doros, G. & Saad, F. Long-Term Testosterone Therapy Improves Cardiometabolic Function and Reduces Risk of Cardiovascular Disease in Men with Hypogonadism: A Real-Life Observational Registry Study Setting Comparing Treated and Untreated (Control) Groups. *J Cardiovasc Pharmacol Ther* **22**, 414-433 (2017).
95. Cheetham, T.C., *et al.* Association of Testosterone Replacement With Cardiovascular Outcomes Among Men With Androgen Deficiency. *JAMA Intern Med* **177**, 491-499 (2017).
96. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. *World Health Organ Tech Rep Ser* **843**, 1-129 (1994).
97. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis and Therapy. Osteoporosis prevention, diagnosis, and therapy. *Jama* **285**, 785-795 (2001).
98. Ribot, C., Tremollieres, F. & Pouilles, J.M. Late consequences of a low peak bone mass. *Acta Paediatr Suppl* **411**, 31-35; discussion 36 (1995).

99. Johnell, O., *et al.* Predictive value of BMD for hip and other fractures. *J Bone Miner Res* **20**, 1185-1194 (2005).
100. Kanis, J.A., *et al.* The components of excess mortality after hip fracture. *Bone* **32**, 468-473 (2003).
101. The International Society For Clinical Densitometry. 2015 ISCD Official Positions - Adult. (https://www.iscd.org/official-positions/2015-iscd-official-positions-adult/, 2015).
102. Bailey, D.A., Faulkner, R.A. & McKay, H.A. Growth, physical activity, and bone mineral acquisition. *Exerc Sport Sci Rev* **24**, 233-266 (1996).
103. Weaver, C.M., *et al.* The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. *Osteoporos Int* **27**, 1281-1386 (2016).
104. Gilsanz, V., *et al.* Age at onset of puberty predicts bone mass in young adulthood. *The Journal of pediatrics* **158**, 100-105, 105 e101-102 (2011).
105. Darelid, A., *et al.* Catch up in bone acquisition in young adult men with late normal puberty. *J Bone Miner Res* **27**, 2198-2207 (2012).
106. Gordon, C.M., *et al.* The Determinants of Peak Bone Mass. *The Journal of pediatrics* **180**, 261-269 (2017).
107. Baxter-Jones, A.D., Faulkner, R.A., Forwood, M.R., Mirwald, R.L. & Bailey, D.A. Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. *J Bone Miner Res* **26**, 1729-1739 (2011).
108. Bachrach, L.K. Acquisition of optimal bone mass in childhood and adolescence. *Trends in endocrinology and metabolism: TEM* **12**, 22-28 (2001).
109. Wren, T.A., *et al.* Longitudinal tracking of dual-energy X-ray absorptiometry bone measures over 6 years in children and adolescents: persistence of low bone mass to maturity. *The Journal of pediatrics* **164**, 1280-1285 e1282 (2014).
110. Harman, S.M., *et al.* Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *J Clin Endocrinol Metab* **86**, 724-731 (2001).
111. Kaufman, J.M. & Vermeulen, A. The decline of androgen levels in elderly men and its clinical and therapeutic implications. *Endocr Rev* **26**, 833-876 (2005).
112. Golds, G., Houdek, D. & Arnason, T. Male Hypogonadism and Osteoporosis: The Effects, Clinical Consequences, and Treatment of Testosterone Deficiency in Bone Health. *Int J Endocrinol* **2017**, 4602129 (2017).
113. Rochira, V., Kara, E. & Carani, C. The endocrine role of estrogens on human male skeleton. *Int J Endocrinol* **2015**, 165215 (2015).
114. Finkelstein, J.S., *et al.* Gonadal steroid-dependent effects on bone turnover and bone mineral density in men. *J Clin Invest* **126**, 1114-1125 (2016).

115. Gaffney, C.D., Pagano, M.J., Kuker, A.P., Stember, D.S. & Stahl, P.J. Osteoporosis and Low Bone Mineral Density in Men with Testosterone Deficiency Syndrome. *Sexual medicine reviews* **3**, 298-315 (2015).
116. Auyeung, T.W., *et al.* Testosterone but not estradiol level is positively related to muscle strength and physical performance independent of muscle mass: a cross-sectional study in 1489 older men. *European journal of endocrinology / European Federation of Endocrine Societies* **164**, 811-817 (2011).
117. Vandenput, L., *et al.* Low Testosterone, but Not Estradiol, Is Associated With Incident Falls in Older Men: The International MrOS Study. *J Bone Miner Res* **32**, 1174-1181 (2017).
118. Greenspan, S.L., *et al.* Bone loss after initiation of androgen deprivation therapy in patients with prostate cancer. *J Clin Endocrinol Metab* **90**, 6410-6417 (2005).
119. Kacker, R., Conners, W., Zade, J. & Morgentaler, A. Bone mineral density and response to treatment in men younger than 50 years with testosterone deficiency and sexual dysfunction or infertility. *J Urol* **191**, 1072-1076 (2014).
120. Bobjer, J., *et al.* High prevalence of hypogonadism and associated impaired metabolic and bone mineral status in subfertile men. *Clinical endocrinology* **85**, 189-195 (2016).
121. Isidori, A.M., *et al.* Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. *Clinical endocrinology* **63**, 280-293 (2005).
122. Tracz, M.J., *et al.* Testosterone use in men and its effects on bone health. A systematic review and meta-analysis of randomized placebo-controlled trials. *J Clin Endocrinol Metab* **91**, 2011-2016 (2006).
123. Svartberg, J., *et al.* Testosterone treatment in elderly men with subnormal testosterone levels improves body composition and BMD in the hip. *Int J Impot Res* **20**, 378-387 (2008).
124. Kenny, A.M., *et al.* Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels, low bone mass, and physical frailty. *J Am Geriatr Soc* **58**, 1134-1143 (2010).
125. Aversa, A., *et al.* Effects of long-acting testosterone undecanoate on bone mineral density in middle-aged men with late-onset hypogonadism and metabolic syndrome: results from a 36 months controlled study. *The aging male : the official journal of the International Society for the Study of the Aging Male* **15**, 96-102 (2012).
126. Watts, N.B., *et al.* Osteoporosis in men: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* **97**, 1802-1822 (2012).
127. Walsh, T.J., Croughan, M.S., Schembri, M., Chan, J.M. & Turek, P.J. Increased risk of testicular germ cell cancer among infertile men. *Arch Intern Med* **169**, 351-356 (2009).

128. Jacobsen, R., *et al.* Risk of testicular cancer in men with abnormal semen characteristics: cohort study. *BMJ (Clinical research ed.)* **321**, 789-792 (2000).
129. Petersen, P.M., Skakkebaek, N.E., Rorth, M. & Giwercman, A. Semen quality and reproductive hormones before and after orchiectomy in men with testicular cancer. *J Urol* **161**, 822-826 (1999).
130. Jacobsen, K.D., Theodorsen, L. & Fossa, S.D. Spermatogenesis after unilateral orchiectomy for testicular cancer in patients following surveillance policy. *J Urol* **165**, 93-96 (2001).
131. Beck, S.D., Bey, A.L., Bihrle, R. & Foster, R.S. Ejaculatory status and fertility rates after primary retroperitoneal lymph node dissection. *J Urol* **184**, 2078-2080 (2010).
132. Heidenreich, A., Pfister, D., Witthuhn, R., Thuer, D. & Albers, P. Postchemotherapy retroperitoneal lymph node dissection in advanced testicular cancer: radical or modified template resection. *European urology* **55**, 217-224 (2009).
133. Willemse, P.H., Sleijfer, D.T., Sluiter, W.J., Schraffordt Koops, H. & Doorenbos, H. Altered Leydig cell function in patients with testicular cancer: evidence for bilateral testicular defect. *Acta Endocrinol (Copenh)* **102**, 616-624 (1983).
134. Howell, S.J. & Shalet, S.M. Spermatogenesis after cancer treatment: damage and recovery. *J Natl Cancer Inst Monogr*, 12-17 (2005).
135. Anserini, P., *et al.* Semen analysis following allogeneic bone marrow transplantation. Additional data for evidence-based counselling. *Bone Marrow Transplant* **30**, 447-451 (2002).
136. Children's Oncology Group. Long-Term Follow-Up Guidelines for Survivors of Childhood, Adolescent, and Young Adult Cancers, Version 4.0. (www.survivorshipguidelines.org, 2013).
137. Centola, G.M., Keller, J.W., Henzler, M. & Rubin, P. Effect of low-dose testicular irradiation on sperm count and fertility in patients with testicular seminoma. *J Androl* **15**, 608-613 (1994).
138. Eberhard, J., *et al.* Impact of therapy and androgen receptor polymorphism on sperm concentration in men treated for testicular germ cell cancer: a longitudinal study. *Hum Reprod* **19**, 1418-1425 (2004).
139. Gandini, L., *et al.* Effect of chemo- or radiotherapy on sperm parameters of testicular cancer patients. *Hum Reprod* **21**, 2882-2889 (2006).
140. Huyghe, E., *et al.* Fertility after testicular cancer treatments: results of a large multicenter study. *Cancer* **100**, 732-737 (2004).
141. Green, D.M., *et al.* Fertility of male survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. *J Clin Oncol* **28**, 332-339 (2010).
142. Shalet, S.M., Tsatsoulis, A., Whitehead, E. & Read, G. Vulnerability of the human Leydig cell to radiation damage is dependent upon age. *The Journal of endocrinology* **120**, 161-165 (1989).

143. Bang, A.K., *et al.* Testosterone production is better preserved after 16 than 20 Gray irradiation treatment against testicular carcinoma in situ cells. *Int J Radiat Oncol Biol Phys* **75**, 672-676 (2009).
144. Sprauten, M., *et al.* Longitudinal serum testosterone, luteinizing hormone, and follicle-stimulating hormone levels in a population-based sample of long-term testicular cancer survivors. *J Clin Oncol* **32**, 571-578 (2014).
145. Skinner, R., *et al.* Recommendations for gonadotoxicity surveillance in male childhood, adolescent, and young adult cancer survivors: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group in collaboration with the PanCareSurFup Consortium. *The Lancet. Oncology* **18**, e75-e90 (2017).
146. Darzy, K.H. & Shalet, S.M. Hypopituitarism following Radiotherapy Revisited. *Endocr Dev* **15**, 1-24 (2009).
147. Puscheck, E., Philip, P.A. & Jeyendran, R.S. Male fertility preservation and cancer treatment. *Cancer Treat Rev* **30**, 173-180 (2004).
148. Lopez Andreu, J.A., *et al.* Persistent altered spermatogenesis in long-term childhood cancer survivors. *Pediatr Hematol Oncol* **17**, 21-30 (2000).
149. Kenney, L.B., Laufer, M.R., Grant, F.D., Grier, H. & Diller, L. High risk of infertility and long term gonadal damage in males treated with high dose cyclophosphamide for sarcoma during childhood. *Cancer* **91**, 613-621 (2001).
150. Meistrich, M.L., Wilson, G., Brown, B.W., da Cunha, M.F. & Lipshultz, L.I. Impact of cyclophosphamide on long-term reduction in sperm count in men treated with combination chemotherapy for Ewing and soft tissue sarcomas. *Cancer* **70**, 2703-2712 (1992).
151. Aubier, F., *et al.* Male gonadal function after chemotherapy for solid tumors in childhood. *J Clin Oncol* **7**, 304-309 (1989).
152. van Beek, R.D., *et al.* Inhibin B is superior to FSH as a serum marker for spermatogenesis in men treated for Hodgkin's lymphoma with chemotherapy during childhood. *Hum Reprod* **22**, 3215-3222 (2007).
153. Ghavamzadeh, A., *et al.* Thyroid, parathyroid, gonadal, and pancreatic beta-cell function after bone marrow transplantation with chemotherapy-only conditioning. *Transplantation proceedings* **35**, 3101-3104 (2003).
154. Gundgurthi, A., *et al.* Endocrine complications after busulphan and cyclophosphamide based hematopoietic stem cell transplant: A single tertiary care centre experience. *Indian journal of endocrinology and metabolism* **17**, 855-863 (2013).
155. Panasiuk, A., *et al.* Gonadal function and fertility after stem cell transplantation in childhood: comparison of a reduced intensity conditioning regimen containing melphalan with a myeloablative regimen containing busulfan. *British journal of haematology* **170**, 719-726 (2015).
156. Green, D.M., *et al.* The Cyclophosphamide Equivalent Dose as an Approach for Quantifying Alkylating Agent Exposure: A Report From the Childhood Cancer Survivor Study. *Pediatric blood & cancer* **61**, 53-67 (2014).

157. Green, D.M., *et al.* Cumulative alkylating agent exposure and semen parameters in adult survivors of childhood cancer: a report from the St Jude Lifetime Cohort Study. *The Lancet. Oncology* **15**, 1215-1223 (2014).
158. Bujan, L., *et al.* Impact of chemotherapy and radiotherapy for testicular germ cell tumors on spermatogenesis and sperm DNA: a multicenter prospective study from the CECOS network. *Fertil Steril* **100**, 673-680 (2013).
159. Suzuki, K., *et al.* Regeneration of spermatogenesis after testicular cancer chemotherapy. *Urol Int* **91**, 445-450 (2013).
160. Petersen, P.M. & Hansen, S.W. The course of long-term toxicity in patients treated with cisplatin-based chemotherapy for non-seminomatous germ-cell cancer. *Ann Oncol* **10**, 1475-1483 (1999).
161. Petersen, P.M., Hansen, S.W., Giwercman, A., Rorth, M. & Skakkebaek, N.E. Dose-dependent impairment of testicular function in patients treated with cisplatin-based chemotherapy for germ cell cancer. *Ann Oncol* **5**, 355-358 (1994).
162. Meistrich, M.L., *et al.* Recovery of sperm production after chemotherapy for osteosarcoma. *Cancer* **63**, 2115-2123 (1989).
163. Ghezzi, M., *et al.* Impact of Bep or Carboplatin Chemotherapy on Testicular Function and Sperm Nucleus of Subjects with Testicular Germ Cell Tumor. *Front Pharmacol* **7**, 122 (2016).
164. Lampe, H., Horwich, A., Norman, A., Nicholls, J. & Dearnaley, D.P. Fertility after chemotherapy for testicular germ cell cancers. *J Clin Oncol* **15**, 239-245 (1997).
165. Bajorin, D.F., *et al.* Randomized trial of etoposide and cisplatin versus etoposide and carboplatin in patients with good-risk germ cell tumors: a multiinstitutional study. *J Clin Oncol* **11**, 598-606 (1993).
166. Bokemeyer, C., *et al.* A randomized trial of cisplatin, etoposide and bleomycin (PEB) versus carboplatin, etoposide and bleomycin (CEB) for patients with 'good-risk' metastatic non-seminomatous germ cell tumors. *Ann Oncol* **7**, 1015-1021 (1996).
167. Rivkees, S.A. & Crawford, J.D. The relationship of gonadal activity and chemotherapy-induced gonadal damage. *Jama* **259**, 2123-2125 (1988).
168. Mackie, E.J., Radford, M. & Shalet, S.M. Gonadal function following chemotherapy for childhood Hodgkin's disease. *Medical and pediatric oncology* **27**, 74-78 (1996).
169. Williams, D., Crofton, P.M. & Levitt, G. Does ifosfamide affect gonadal function? *Pediatric blood & cancer* **50**, 347-351 (2008).
170. Ridola, V., *et al.* Testicular function of survivors of childhood cancer: a comparative study between ifosfamide- and cyclophosphamide-based regimens. *European journal of cancer* **45**, 814-818 (2009).
171. Gerl, A., Muhlbayer, D., Hansmann, G., Mraz, W. & Hiddemann, W. The impact of chemotherapy on Leydig cell function in long term survivors of germ cell tumors. *Cancer* **91**, 1297-1303 (2001).

172. Relander, T., Cavallin-Stahl, E., Garwicz, S., Olsson, A.M. & Willen, M. Gonadal and sexual function in men treated for childhood cancer. *Medical and pediatric oncology* **35**, 52-63 (2000).
173. Strader, C.H., Weiss, N.S., Daling, J.R., Karagas, M.R. & McKnight, B. Cryptorchism, orchiopexy, and the risk of testicular cancer. *Am J Epidemiol* **127**, 1013-1018 (1988).
174. Fossa, S.D., Theodorsen, L., Norman, N. & Aabyholm, T. Recovery of impaired pretreatment spermatogenesis in testicular cancer. *Fertil Steril* **54**, 493-496 (1990).
175. Greenfield, D.M., *et al.* Prevalence and consequences of androgen deficiency in young male cancer survivors in a controlled cross-sectional study. *J Clin Endocrinol Metab* **92**, 3476-3482 (2007).
176. Fossa, S.D., Abyholm, T. & Aakvaag, A. Spermatogenesis and hormonal status after orchiectomy for cancer and before supplementary treatment. *European urology* **10**, 173-177 (1984).
177. Tromp, K., *et al.* Reproductive status in adult male long-term survivors of childhood cancer. *Hum Reprod* **26**, 1775-1783 (2011).
178. Huddart, R.A., *et al.* Fertility, gonadal and sexual function in survivors of testicular cancer. *Br J Cancer* **93**, 200-207 (2005).
179. Romerius, P., *et al.* Hypogonadism risk in men treated for childhood cancer. *J Clin Endocrinol Metab* **94**, 4180-4186 (2009).
180. Nord, C., *et al.* Gonadal hormones in long-term survivors 10 years after treatment for unilateral testicular cancer. *European urology* **44**, 322-328 (2003).
181. McBride, J.A. & Coward, R.M. Recovery of spermatogenesis following testosterone replacement therapy or anabolic-androgenic steroid use. *Asian J Androl* **18**, 373-380 (2016).
182. Mehta, A. & Sigman, M. Management of the dry ejaculate: a systematic review of aspermia and retrograde ejaculation. *Fertil Steril* **104**, 1074-1081 (2015).
183. Marmor, D. & Duyck, F. Male reproductive potential after MOPP therapy for Hodgkin's disease: a long-term survey. *Andrologia* **27**, 99-106 (1995).
184. Habbema, J.D., Eijkemans, M.J., Leridon, H. & te Velde, E.R. Realizing a desired family size: when should couples start? *Hum Reprod* **30**, 2215-2221 (2015).
185. Emami, B., *et al.* Tolerance of normal tissue to therapeutic irradiation. *Int J Radiat Oncol Biol Phys* **21**, 109-122 (1991).
186. Schmeler, K.M., *et al.* Pelvic fractures after radiotherapy for cervical cancer: implications for survivors. *Cancer* **116**, 625-630 (2010).
187. Baxter, N.N., Habermann, E.B., Tepper, J.E., Durham, S.B. & Virnig, B.A. Risk of pelvic fractures in older women following pelvic irradiation. *Jama* **294**, 2587-2593 (2005).
188. Wei, R.L., *et al.* Bone mineral density loss in thoracic and lumbar vertebrae following radiation for abdominal cancers. *Radiother Oncol* **118**, 430-436 (2016).

189. Tritos, N.A. Focus on growth hormone deficiency and bone in adults. *Best Pract Res Clin Endocrinol Metab* **31**, 49-57 (2017).
190. Barake, M., Klibanski, A. & Tritos, N.A. Effects of recombinant human growth hormone therapy on bone mineral density in adults with growth hormone deficiency: a meta-analysis. *J Clin Endocrinol Metab* **99**, 852-860 (2014).
191. Chemaitilly, W., *et al.* Anterior hypopituitarism in adult survivors of childhood cancers treated with cranial radiotherapy: a report from the St Jude Lifetime Cohort study. *J Clin Oncol* **33**, 492-500 (2015).
192. Recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis: 2001 update. American College of Rheumatology Ad Hoc Committee on Glucocorticoid-Induced Osteoporosis. *Arthritis Rheum* **44**, 1496-1503 (2001).
193. Hogler, W., *et al.* Incidence of skeletal complications during treatment of childhood acute lymphoblastic leukemia: comparison of fracture risk with the General Practice Research Database. *Pediatric blood & cancer* **48**, 21-27 (2007).
194. Holzer, G., *et al.* Bone mineral density in long-term survivors of highly malignant osteosarcoma. *J Bone Joint Surg Br* **85**, 231-237 (2003).
195. Mandel, K., Atkinson, S., Barr, R.D. & Pencharz, P. Skeletal morbidity in childhood acute lymphoblastic leukemia. *J Clin Oncol* **22**, 1215-1221 (2004).
196. Kang, M.J. & Lim, J.S. Bone mineral density deficits in childhood cancer survivors: Pathophysiology, prevalence, screening, and management. *Korean journal of pediatrics* **56**, 60-67 (2013).
197. Boot, A.M., van den Heuvel-Eibrink, M.M., Hahlen, K., Krenning, E.P. & de Muinck Keizer-Schrama, S.M. Bone mineral density in children with acute lymphoblastic leukaemia. *European journal of cancer* **35**, 1693-1697 (1999).
198. van der Sluis, I.M., van den Heuvel-Eibrink, M.M., Hahlen, K., Krenning, E.P. & de Muinck Keizer-Schrama, S.M. Altered bone mineral density and body composition, and increased fracture risk in childhood acute lymphoblastic leukemia. *The Journal of pediatrics* **141**, 204-210 (2002).
199. Gurney, J.G., *et al.* Bone mineral density among long-term survivors of childhood acute lymphoblastic leukemia: results from the St. Jude Lifetime Cohort Study. *Pediatric blood & cancer* **61**, 1270-1276 (2014).
200. Siegel, D.A., *et al.* Risk factors and surveillance for reduced bone mineral density in pediatric cancer survivors. *Pediatric blood & cancer* **64**(2017).
201. Chaiban, J., *et al.* Modeling pathways for low bone mass in children with malignancies. *Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry* **12**, 441-449 (2009).
202. Aisenberg, J., *et al.* Bone mineral density in young adult survivors of childhood cancer. *Journal of pediatric hematology/oncology* **20**, 241-245 (1998).

203. Polgreen, L.E., *et al.* Modifiable risk factors associated with bone deficits in childhood cancer survivors. *BMC pediatrics* **12**, 40 (2012).
204. Holmes, S.J., *et al.* Reduced bone mineral density in men following chemotherapy for Hodgkin's disease. *Br J Cancer* **70**, 371-375 (1994).
205. Howell, S.J., Radford, J.A., Adams, J.E. & Shalet, S.M. The impact of mild Leydig cell dysfunction following cytotoxic chemotherapy on bone mineral density (BMD) and body composition. *Clinical endocrinology* **52**, 609-616 (2000).
206. Murugaesu, N., Powles, T., Bestwick, J., Oliver, R.T. & Shamash, J. Long-term follow-up of testicular cancer patients shows no predisposition to osteoporosis. *Osteoporos Int* **20**, 1627-1630 (2009).
207. Willemse, P.M., Hamdy, N.A., de Kam, M.L., Burggraaf, J. & Osanto, S. Changes in bone mineral density in newly diagnosed testicular cancer patients after anticancer treatment. *J Clin Endocrinol Metab* **99**, 4101-4108 (2014).
208. Willemse, P.M., *et al.* Prevalence of vertebral fractures independent of BMD and anticancer treatment in patients with testicular germ cell tumors. *J Clin Endocrinol Metab* **95**, 4933-4942 (2010).
209. Foresta, C., *et al.* Altered bone status in unilateral testicular cancer survivors: Role of CYP2R1 and its luteinizing hormone-dependency. *J Endocrinol Invest* **36**, 379-384 (2013).
210. Ondrusova, M., Spanikova, B., Sevcikova, K. & Ondrus, D. Testosterone Deficiency and Bone Metabolism Damage in Testicular Cancer Survivors. *American journal of men's health* (2016).
211. Stutz, J.A., *et al.* Bone density: is it affected by orchidectomy and radiotherapy given for stage I seminoma of the testis? *Clin Oncol (R Coll Radiol)* **10**, 44-49 (1998).
212. SWENOTECA, Swedish and Norwegian Testicular Cancer Group. Follow-up nonseminoma CS1 surveillance, Follow-up seminoma CS1 BEP x 1, Follow-up nonseminoma metastastic, Follow-up seminoma all patients. (https://www.swenoteca.org/follow-up).
213. Eberhard, J., *et al.* Risk factors for post-treatment hypogonadism in testicular cancer patients. *European journal of endocrinology / European Federation of Endocrine Societies* **158**, 561-570 (2008).
214. SWENOTECA, Swedish and Norwegian Testicular Cancer Group. SWENOTECA III, SWENOTECA IV, SWENOTECA V and SWENOTECA VI. (http://www.skane.se/sv/Webbplatser/tumorregistret/Patientprocessarbete/Testikelcancer/Vardprogram/Tidigare-versioner-av-vardprogram/, 1995-2004).
215. Dearnaley, D., Huddart, R. & Horwich, A. Regular review: Managing testicular cancer. *BMJ (Clinical research ed.)* **322**, 1583-1588 (2001).
216. WHO World Health Organization. *WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction* (Cambridge University Press, 1999).

217. Smith, J. & Shoukri, K. Diagnosis of osteoporosis. *Clin Cornerstone* **2**, 22-33 (2000).
218. Callreus, M., McGuigan, F. & Akesson, K. Country-specific young adult dual-energy X-ray absorptiometry reference data are warranted for T-score calculations in women: data from the peak-25 cohort. *Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry* **17**, 129-135 (2014).
219. Groome, N.P., *et al.* Measurement of dimeric inhibin B throughout the human menstrual cycle. *J Clin Endocrinol Metab* **81**, 1401-1405 (1996).
220. Vermeulen, A., Verdonck, L. & Kaufman, J.M. A critical evaluation of simple methods for the estimation of free testosterone in serum. *JCEM* **84**, 3666-3672 (1999).
221. Nieschlag, E., *et al.* Testosterone replacement therapy: current trends and future directions. *Hum Reprod Update* **10**, 409-419 (2004).
222. Ramlau-Hansen, C.H., *et al.* Is smoking a risk factor for decreased semen quality? A cross-sectional analysis. *Hum Reprod* **22**, 188-196 (2007).
223. Hansen, S.W., Berthelsen, J.G. & von der Maase, H. Long-term fertility and Leydig cell function in patients treated for germ cell cancer with cisplatin, vinblastine, and bleomycin versus surveillance. *J Clin Oncol* **8**, 1695-1698 (1990).
224. Brydoy, M., *et al.* Sperm counts and endocrinological markers of spermatogenesis in long-term survivors of testicular cancer. *Br J Cancer* **107**, 1833-1839 (2012).
225. Aass, N., Fossa, S.D., Theodorsen, L. & Norman, N. Prediction of long-term gonadal toxicity after standard treatment for testicular cancer. *European journal of cancer* **27**, 1087-1091 (1991).
226. Pont, J., *et al.* Adjuvant chemotherapy for high-risk clinical stage I nonseminomatous testicular germ cell cancer: long-term results of a prospective trial. *J Clin Oncol* **14**, 441-448 (1996).
227. Romerius, P., *et al.* High risk of azoospermia in men treated for childhood cancer. *International journal of andrology* **34**, 69-76 (2011).
228. Muttukrishna, S., *et al.* Serum anti-Mullerian hormone and inhibin B in disorders of spermatogenesis. *Fertil Steril* **88**, 516-518 (2007).
229. Fraietta, R., Spaine, D.M., Bertolla, R.P., Ortiz, V. & Cedenho, A.P. Individual and seminal characteristics of patients with testicular germ cell tumors. *Fertil Steril* **94**, 2107-2112 (2010).
230. Statistikbanken. Parents age at birth of first child by reporting country and age. Vol. 2018 (http://norden.statbank.dk/chil06).
231. SWENOTECA, Swedish and Norwegian Testicular Cancer Group. Testikelcancer Årsrapport 2013. Vol. 2018 (https://www.cancercentrum.se/globalassets/cancerdiagnoser/testikelcancer-seminom/arsrapport-2013-138-stcr-svenskatestikelcancerregistret-swenoteca.pdf, 2014).
232. van Casteren, N.J., van Santbrink, E.J., van Inzen, W., Romijn, J.C. & Dohle, G.R. Use rate and assisted reproduction technologies outcome of

cryopreserved semen from 629 cancer patients. *Fertil Steril* **90**, 2245-2250 (2008).
233. Zakova, J., *et al.* Sperm cryopreservation before testicular cancer treatment and its subsequent utilization for the treatment of infertility. *ScientificWorldJournal* **2014**, 575978 (2014).
234. Carlsen, E., Olsson, C., Petersen, J.H., Andersson, A.M. & Skakkebaek, N.E. Diurnal rhythm in serum levels of inhibin B in normal men: relation to testicular steroids and gonadotropins. *J Clin Endocrinol Metab* **84**, 1664-1669 (1999).
235. Keel, B.A. Within- and between-subject variation in semen parameters in infertile men and normal semen donors. *Fertil Steril* **85**, 128-134 (2006).
236. Garcia, M.M., *et al.* Cisplatin inhibits testosterone synthesis by a mechanism that includes the action of reactive oxygen species (ROS) at the level of P450scc. *Chem Biol Interact* **199**, 185-191 (2012).
237. Bandak, M., *et al.* Testosterone deficiency in testicular cancer survivors - a systematic review and meta-analysis. *Andrology* **4**, 382-388 (2016).
238. Bandak, M., Aksglaede, L., Juul, A., Rorth, M. & Daugaard, G. The pituitary-Leydig cell axis before and after orchiectomy in patients with stage I testicular cancer. *European journal of cancer* **47**, 2585-2591 (2011).
239. Yeung, S.-C.J. *Internal medical care of cancer patients*. 671 (B C Decker, 2009).
240. Arikoski, P., *et al.* Reduced bone mineral density in long-term survivors of childhood acute lymphoblastic leukemia. *Journal of pediatric hematology/oncology* **20**, 234-240 (1998).
241. Kaste, S.C., *et al.* Bone mineral decrements in survivors of childhood acute lymphoblastic leukemia: frequency of occurrence and risk factors for their development. *Leukemia* **15**, 728-734 (2001).
242. Araujo, A.B., *et al.* Prevalence of symptomatic androgen deficiency in men. *J Clin Endocrinol Metab* **92**, 4241-4247 (2007).
243. Brambilla, D.J., O'Donnell, A.B., Matsumoto, A.M. & McKinlay, J.B. Intraindividual variation in levels of serum testosterone and other reproductive and adrenal hormones in men. *Clinical endocrinology* **67**, 853-862 (2007).
244. Vermeulen, A. & Verdonck, G. Representativeness of a single point plasma testosterone level for the long term hormonal milieu in men. *J Clin Endocrinol Metab* **74**, 939-942 (1992).
245. Basaria, S. Male hypogonadism. *Lancet* **383**, 1250-1263 (2014).

Original publications