Chapter 1

General Introduction

GENERAL INTRODUCTION

Epidemiology

Testicular tumours are rare and account for only 1% of cancers in men. These tumours constitute, however, the most common group of malignancies in young men. The vast majority of testicular tumours are germ cell tumours (GCTs). Malignant germ cell tumours originate most frequently in the testis (TGCT), and less commonly in extragonadal sites, in the retroperitoneum or mediastinum.

Two GCT categories are being recognized for treatment purposes: pure seminomas and a heterogeneous group with multivarious histological features, referred to as nonseminomas, which may consist of a combination of non-seminoma and seminoma. Other tumours arising in the testis are the rare sex cord tumours, stromal cell tumours and other tumours such as malignant lymphomas. 95% of all germ cell tumours present between the age of 25 and 45 years, the highest incidence for non-seminoma's being in the second and third decade and for seminomas in the fourth decade.

Testicular cancer is most common in northern European populations with age standardized incidence rates between 4 and 10 per 100,000 males per year.[1] Incidence rates are much lower in Asian, African and African American men, ranging between 0.2 and 1 per 100,000. Worldwide, there is an observed increase in testicular germ cell tumour incidence with an annual increase of 1-6% in the global incidence rate over the last 40 years. This increase is intriguing and remains largely unexplained. A role for environmental factors has been suggested, which is supported by an increasing frequency of other testicular problems such as declining sperm counts and increasing incidence of testicular maldescent.

In The Netherlands, the incidence of malignant germ cell tumours has increased from 4.8 to 6.6 per 100,000 males per year between 1991 and 2002.[2] Annually, more than 500 men are diagnosed with a germ cell tumour. Of all patients with testicular tumours, 47% had a seminoma (SGCT), 42% a non-seminoma (NSGCT), 6% a lymphoma, 4% a Sertoli-Leydig cell tumour and less than 1% had spermatocytic seminoma. The annual mortality of testicular tumours is 0.3 per 100.000 males. About 25 men thus die of this disease each year in The Netherlands. Since the introduction of highly effective cisplatin-based chemotherapy the 10-year survival of men with seminoma has increased from 83% between the years 1970-1977 to 94% between the years 1988-1997. The 10-year survival for men with non-seminoma has increased from 56 to 92% in the same periods.

Clinical presentation

Testicular GCTs often present as a painless, unilateral mass in the scrotum. Scrotal pain is the presenting symptom in approximately 20% of cases whereas in 10% of cases the symptoms are consistent with orchidoepididymitis.

Testicular germ cell tumours are often rapidly growing tumours, which primarily metastasize to the ipsi-lateral para-aortal lymph nodes and via the bloodstream to the lungs and other organs. As prognosis improves with the smaller tumour volumes, awareness and self-examination are important to ensure early diagnosis.

Risk factors

There is a strong association between testicular cancer and an undescended testicle, poor testicular development and function, e.g. testicular atrophy, hypospadias, infertility and subfertility, a family history of testicular cancer among first-degree relatives (father and brother), the presence of a contralateral tumour or testicular intraepithelial tumour, and HIV infection.[3] Studies have led to the formulation of the hypothesis of the existence of a testicular dysgenesis syndrome in patients developing testicular tumours. Regular self-examination in these individuals at increased risk for developing GCT is of more importance.

Approximately 10 % of testicular tumours occur in individuals with an undescended testicle and 25 % of cases occur in the contralateral, normally descended testicle. Orchiopexy decreases the risk for GCT only when performed in early childhood.[4] The intrascrotal position of the testis after orchiopexy facilitates self-examination and early diagnosis of testicular tumours.

About 1.5% of men with testicular cancer present with a synchronous bilateral tumour which are mainly pure seminomas.[5] Up to 5% of GCT patients will have a contralateral (metachronous) testicular germ cell tumour (CTGCT).[6] This incidence is similar to that of carcinoma in situ (CIS) observed in patients with unilateral testicular cancer who undergo biopsy of the contralateral testicle at the time of the orchidectomy.[7] Other disorders such as Klinefelter syndrome, Down syndrome, Peutz-Jeghers syndrome, Carney's complex, androgen insensitivity syndromes and mixed gonadal dysgenesis have also been associated with testicular cancer.

Gonadal function

At the time of presentation with testicular germ cell cancers, the quality of sperm, both cell number and sperm motility, is very low. Almost two thirds of GCT patients present with Sertoli cell dysfunction and impaired spermatogenesis. Infertility is an important issue in most testicular GCT patients, as most of them are relatively young and will display a desire to father children in the years to come. Approximately half of patients with unilateral testicular cancer have oligozoospermia already before therapy because of suppressed spermatogenesis in the affected testis, possibly due to the presence of the malignant tumour and/or due to tumour products, although impaired spermatogenesis in the contralateral unaffected testis has been observed in a significant number of patients.[8]

Leydig cell function is impaired in patients with testicular carcinoma-in-situ. Post unilateral orchidectomy 20% of patients show low serum testosterone concentrations and this may decrease further due to impaired function of the contra-lateral unaffected testis. In long-term survivors partial hypogonadism may persist as evidenced by an increased serum luteinizing hormone (LH) concentrations up to 10 years after orchidectomy.[9]

Diagnosis and treatment

Initial evaluation of men with a testicular mass suspected to be testis cancer consists of physical examination and ultrasound of the scrotum. After the diagnosis is established, the patient should undergo an inguinal orchidectomy with dissection of the spermatic cord at the internal inguinal ring. The tumour markers alpha-fetoprotein (α-FP), the beta unit of human chorionic gonadotrophin (β-HCG) and lactate dehydrogenase (LDH) should be measured in men with suspected testis cancer before orchidectomy. In NSGCT the serum concentrations of α-FP and β-HCG are elevated in over 80% of men. In pure seminomas, α-FP is not elevated and fewer than 20% of men have elevated β-HCG levels. LDH is less specific but has independent prognostic value in patients with advanced GCTs. It is increased in 60% of NSGCTs and in 80% of seminomas. Serum tumour markers alone are not diagnostic of testicular cancer but very high values support a diagnosis of germ cell tumour. CT-scan of the thorax and abdomen is performed for staging purposes once the histopathological diagnosis of a malignant GCT is made.

Tumour markers are measured post orchidectomy to assess whether there is residual tumour. Persistence of elevated serum tumour markers or an increase

in serum tumour markers after initial normalization after unilateral orchidectomy indicates the presence of metastatic disease. Normalization of tumour marker levels after orchidectomy does not, however, rule out the presence of tumour metastases. In case of metastatic disease, the disease should be classified according to the International Germ Cell Cancer Collaborative Group (IGCCCG) risk classification for metastatic GCT patients.

Adjuvant treatment

Non-metastatic Germ Cell Tumours

In case of stage 1 disease, non-seminoma germ cell tumours are often treated with unilateral orchidectomy followed by watchful waiting. Stage 1 seminoma can be treated with either a single intravenous administration of Carboplatin or with iliac and para-aortal lymph node radiation therapy at a dose of 20 Gray. Interestingly, the recurrence rates and survival rates of both treatments are similar.[10] Watchful waiting is an option in stage 1 seminomas with tumours which are < 4 cm in size. Patients with stage 1 seminoma tumours with infiltrative growth into the tunica albuginea or funiculus and patients with perioperative tumour spill may be considered for radiation therapy of the ipsilateral groin region.

Metastatic Germ Cell Tumours

Patients with metastatic disease (stage II and higher) are usually treated with combination chemotherapy. Since the introduction of the cytotoxic agent cisplatin in 1974 as treatment for metastatic germ cell cancer, the survival of patients with carcinoma of the testis has been markedly improved, with cure potentially achieved in the majority of patients.[11-13]

Following the discovery of cisplatin, cisplatin-based chemotherapy consisting of a combination of cisplatin, vinblastin and bleomycin (PVB) has been introduced in the 1970s. Because of the substantial neurotoxicity of PVB-chemotherapy, the efficacy and toxicity of the PVB regimen was later compared to that of the combination of bleomycin, etoposide and cisplatin (BEP). BEP-chemotherapy induced less neurotoxicity and resulted in similar or possibly higher remission rate in patients with advanced disease.[14] Since then, the BEP regimen has been introduced as the standard worldwide chemotherapy regimen of choice for patients with metastatic testicular cancer. An additional advantage of cisplatin-based chemotherapy is the associated significantly 50% reduced risk of developing a CTGCT.[15]

Mean 5 year overall survival for testicular cancer is currently 97.3% (95% CI 96.4-98.2) [16], with 5 year relative survival estimates of 97.6%, 93.3% and 93.6% in respectively classical seminomas, non-seminomas and mixed GCTs.[17] Of the patients with GCT with haematogenous or lymphatic spread of the disease, a high percentage will achieve a complete remission.[18] Eventually, 80% of all patients who present with testicular cancer can be cured and are expected to have a normal life expectancy.

Chemotherapy-induced toxicity

Cytotoxic chemotherapy is associated with acute and long-term co-morbidity. As the prognosis of GCT patients has considerably improved over the last 5 decades, not only acute toxicity but also long-term morbidity related to chemotherapy are of clinical relevance in the management of these patients. The main acute effects of combination chemotherapy are gastro-intestinal disturbance, moderate myelosuppression [19], peripheral vasospastic disorder [20], thrombo-embolic events [21], pulmonary toxicity [22;23], nephrotoxicity [24] and infertility [25], in view of which semen cryopreservation should be offered to all men diagnosed with testis cancer before starting chemotherapy if they so wish. Main long-term co-morbidities are polyneuropathy and ototoxicity [26], partial hypogonadism [9] and cardiovascular complications.[27-29]

Gonadal Function

More than 50% of testicular GCT patients experience significant gonadal dysfunction after curative treatment with cisplatin-based chemotherapy, with Sertoli cells being more sensitive to the deleterious effects of chemotherapy than Leydig cells. Spermatogenesis will recover to some extent within 12 to 36 months after completion of chemotherapy, but 25% of the patients will remain azoospermatic and nearly half of the patients will have persistent sperm abnormalities, such as decreased cell count and decreased mobility of the spermatozoa. After a median follow up of 12.6 years, 68 % of GCT patients present with a functional insufficiency of the Sertoli cells with impaired spermatogenesis and significantly elevated follicle-stimulating hormone (FSH) levels.[30] Brenneman *et al.* found that in 60 % of the patients FSH levels remained elevated up to 8 years after chemotherapy, which reflects a functional insufficiency of the Sertoli cells.[30]

The sperm-producing germinal epithelium of the testes is more vulnerable to chemotherapy than the testosterone-producing Leydig cells, leaving many cancer survivors azoo- or oligozoospermic. The delayed recovery of sperm production is

also influenced by the relative hypo-androgenic intra-testicular milieu in these patients because of Leydig cell dysfunction. The impaired spermatogenesis also seems to be correlated with the patient's pre-treatment gonadal function, with the patient's age at the time of treatment, and with the dose and duration of the chemotherapy.[31] After chemotherapy, the concentration of spermatozoa and the volume of the remaining testis are significantly reduced in comparison to that in patients treated with orchidectomy who did not receive chemotherapy.[32]

Gonadal hormone function is also impaired in GCT patients and especially in patients treated with chemotherapy. Gerl *et al*. showed that high cumulative doses of chemotherapy cause a significant and persistent impairment of Leydig cell function up to 192 months after treatment, with increase of serum LH levels of 50% compared to GCT patients treated only with orchidectomy.[33] Possible explanations for the mild Leydig cell hypofunction observed in patients with unilateral testicular cancer are that chemotherapy can lead to germinal epithelial damage, which results in a reduction in testicular volume and an associated decrease in Leydig cell function and azoospermia. Cisplatin-based chemotherapy may also induce a Raynaud's phenomenon, which causes decreased vascular flow in the contra lateral testis and possible reduction in testosterone production.[34;35] The fact that the mean testosterone level is inversely correlated with the cumulative cisplatin dose applied (> 400 mg/m^2) [36] support the latter hypothesis.

The risk of Metabolic Syndrome and Cardiovascular Disease

The Metabolic Syndrome (MetS) is a cluster of metabolic and interrelated cardiovascular risk factors which has received considerable attention in recent years because of its association with, and predictive value for the development of type 2 diabetes mellitus [37] and cardiovascular disease (CVD).[38] MetS risk factors are believed to directly promote atherosclerotic CVD. The common denominators of MetS are central (visceral) obesity and insulin resistance, which predispose individuals to metabolic risk factors such as elevated serum triglycerides, low high-density lipoprotein (HDL) levels, high fasting glucose levels, and high blood pressure.[39]

Various expert panels have developed classifications of MetS to facilitate screening for cardiovascular risk factors. The World Health Organization (WHO) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults developed a working definition for MetS and the Adult Treatment Panel of the National Cholesterol Education Programme (NCEP-ATP III) modified the definition based on similar characteristics used by the WHO.[40-42] Other classification systems include

the Metabolic Syndrome score developed by the International Diabetes Federation (IDF).[43] A meta-analysis of 21 prospective American and European cohort studies using NCEP-ATPIII or WHO criteria indicated that patients with high MetS prevalence have an increased incidence for CVD and increased associated mortality.[44] A further meta-analysis including 37 studies and 172.573 individuals [45] showed that MetS carried a nearly twofold increased relative risk (RR) of cardiovascular events and deaths of 1.78 (95% CI: 1.58-2.0).

Over the last decade, four cross-sectional studies evaluated MetS prevalence in GCT survivors. Nuver *et al.* observed a higher NCEP-ATPIII MetS prevalence and thereby an increased relative risk for MetS in a smaller cohort of 131 testicular cancer survivors.[46] Interestingly, in this cohort the increased prevalence of MetS in patients treated with chemotherapy (26%) was lower compared to that observed in patients treated with orchidectomy alone (36%). Data from two cross-sectional studies from Norway were reported in 2007 (Table 5).[47;48] One large multicenter study of five University Hospitals [48] reported an unusually high MetS prevalence of 52% in healthy subjects and respectively 45% and 33% in 1135 testicular cancer survivors treated with chemotherapy or orchidectomy alone. The other study [47] reports long term data in 589 testicular cancer survivors who underwent orchidectomy up to 15 years after treatment [47] with MetS incidences of 17.4%, 15.2% and 6.4% in patients respectively treated with combination chemotherapy, radiotherapy or only orchidectomy. Both Norwegian studies used modified NCEP-ATPIII criteria so that the absolute MetS incidence in GCT patients cannot be ascertained.

Haas *et al.* reported the prevalence of MetS and cardiovascular disease, vascular complications and the role of gonadal function in long-term survivors of disseminated non-seminomatous TC.[49] They recorded a 25% MetS incidence in 173 GCT patients a median of 5 years (range 3-20 years) after chemotherapy and calculated a relative risk for MetS of 2.2 (CI 1.5-3.3) compared with the general population. They also found that a total testosterone of <15 nmol/l was associated with an age-adjusted OR of 4.1 (95%CI 1.8-9.3) for the MetS.

Loss of bone mass

Hypogonadism is associated with a decrease in bone mass. As the vast majority of GCT patients are hypogonadal at some stage before or after diagnosis and treatment of TC, they are at increased risk for bone loss. Four published papers have so far addressed this issue. Stutz *et al.* performed bone mineral density (BMD)

measurements using dual-energy X-ray absorptiometry (DXA) in 30 seminoma patients to compare BMD in irradiated and non-irradiated hips and lumbar spine.[50] A positive relationship was only found between lumbar BMD and log transformed time since orchidectomy. No significant relationship was observed between BMD and radiotherapy. Brown al compared BMD of men who had previously received curative treatment with BMD of a healthy age-matched population.[51] They found no statistical difference in BMD between the GCT patients treated with chemotherapy and those treated without chemotherapy at either spine or hip BMD. The mean BMD values in both groups were not lower than the BMD of their control reference group. They also found no difference in gonadal hormone levels between treatment groups. Unfortunately, only half of their patients had GCT (N=64), the other half was treated for non-Hodgkin's lymphoma or Hodgkin's disease (N=61), and their control group consisted of GCT patients treated with orchidectomy only. Murugaesu *et al.* found no difference in BMD after orchidectomy with or without chemotherapy in a cross-sectional study of 39 GCT patients a mean 15.7 years after treatment.[52] Because the number of survivors studied by Murugaesu *et al.* is small and patients with metastasized disease had shorter follow-up (13.1 vs. 17.1 years) slowly evolving changes in BMD may have been missed. This may explain the discrepant findings from the recent study of Ondrusova *et al.* showing an unusual high incidence of osteopenia and osteoporosis of 49.1% in 823 patients with unilateral GCT a median 89 months after primary orchidectomy.[53]

Mechanism of chemotherapy-induced toxicity

Cisplatin-based chemotherapy has been shown to increase the serum plasma concentrations of the highly toxic non-protein bound iron (NPBI), iron, and of ferritin concomitant with a decrease in plasma anti-oxidants as measured 4 days after start of treatment.[54;55] This suggests that chemotherapy-induced oxidative stress may lead to lipid and protein peroxidation which is considered to be one of the most important underlying mechanisms of late cisplatin toxicity. There is substantial evidence from animal studies that experimental iron overload may result in oxidative damage to lipids, proteins, DNA, and cellular organelles including mitochondria [56;57], as manifested by vascular, renal and liver damage.[58] When free iron is not bound to transferrin and enters the blood circulation, free radicals are formed due to the ability of free iron to donate and accept electrons. Secondly, free iron catalyzes the conversion of hydrogen peroxide which also results in toxicity. Under physiological conditions, iron is sequestered in transport proteins, e.g., transferrin and lactoferrin,

and in storage proteins, primarily ferritin in the liver, spleen, and bone marrow. The most important group of iron-binding proteins contains haem molecules to perform redox and electron transport reactions required for oxidative phosphorylation, the principal source of energy for human cells. Normally, only one third of transferrin is saturated with iron. However, when high amounts of the redox reactive non-protein bound iron (NPBI), often referred to as non-transferrin bound iron (NTBI), enter the circulation, transferrin and other iron transport proteins may become oversaturated, leading to a cascade of oxidative damage.[59]

The reported changes in serum lipids early after chemotherapy [60] in combination with the increased prevalence of the MetS documented in TC survivors [46;61] suggest a causal relationship between chemotherapy and the observed long term cardiovascular effects associated with disturbed glucose and lipid metabolism. These effects may be aggravated by direct vascular effects of the chemotherapy. It has indeed been shown *in vitro* that cisplatin inhibits vascular remodelling and regeneration [62] and that bleomycin and cisplatin induce endothelial damage.[63] Electron microscopy studies have also demonstrated bleomycin-induced endothelial damage in capillaries and small arterioles.[64] In the clinic, manifestations of arterial vascular abnormality are also observed, such as the frequently reported development of the Raynaud's syndrome, as side effect of cisplatin and bleomycin-based chemotherapy in GCT patients.[65] Early detection and prevention of vascular events may substantially improve long-term prognosis in patients with GCT. Vascular stiffness has been related to cardiovascular morbidity and mortality [66;67] and changes in vascular stiffness can be reliably measured using magnetic resonance–based pulse wave velocity (MR-PWV) of the aorta.[68]

Various studies suggest that chemotherapy-induced oxidative stress may result in non-alcoholic fatty liver disease [69] and cisplatin-based chemotherapy has shown to be toxic to the liver.[70] Chemotherapy toxicity may also be associated with short term disturbances in liver function which may present early signs of late development of the Metabolic Syndrome (MetS).[61] Finally, visceral fat mass is known to be metabolically active and is identified as a major contributor to adverse effects on glucose and lipid metabolism, and possibly endothelial dysfunction. As such, increases in visceral fat are considered to be important for the development of MetS and for increased cardiovascular risk.[71-73] Measurement of changes in visceral and subcutaneous abdominal fat mass after chemotherapy may be useful in predicting long term risk for MetS and cardiovascular disease in GCT survivors.

OUTLINE OF THE THESIS

In this thesis several comorbidities are described in GCT patients. GCT patients are known to have an increased CVD risk and a number of authors have reported on the increased prevalence of the metabolic syndrome and of cardiovascular risk factors in GCT survivors. However, the reported MetS incidence differs widely between studies and does not correspond with the measured CVD risk in GCT survivors. An important cause for the discrepancy in findings is that the MetS incidence may vary considerably depending on the classification used. In **Chapter 2** we report about the results of a study conducted to both assess MetS prevalence using two different classification systems and to assess the 10-year cardiovascular risk in the same cohort of GCT survivors.

The reported increased CVD prevalence mainly concerns GCT survivors with disseminated disease who received cisplatin-based combination chemotherapy. The cause of the increased CVD prevalence in these survivors is not fully understood. One fifth of GCT patients and especially GCT survivors treated with unilateral orchidectomy and combination chemotherapy, become hypogonadal after anti-cancer treatment.[30] Hypogonadism may result in obesity and insulin resistance, which may in the long run result in increased CVD risk. In **Chapter 3** results of (proton) MRI measurement of abdominal fat volume, hepatic fattening and aortic wall stiffness are reported before, directly after and 6 months after combination chemotherapy and relationship of these data with serum gonadal hormone values was documented.

Cisplatin-based chemotherapy can lead to subacute changes in cardiac function and it is known that bleomycin and cisplatin induce endothelial damage. The direct toxic effect of cisplatin-based chemotherapy on the cardiovascular system could at least partially explain the observed increased CVD risk in GCT survivors. In **Chapter 4** data are presented on cardiac function and myocardial triglycerides measured in fourteen GCT patients before and after cisplatin-based chemotherapy.

The reported vascular toxicity of cisplatin-based chemotherapy is largely due to oxidative stress induced damage to endothelial tissue. Chemotherapy induced iron release in GCT patients may potentially contribute to the oxidative stress. Parameters of iron metabolism may provide an indication for oxidative toxicity during chemotherapy treatments. To better understand the chemotherapy induced damage on various tissues in GCT patients, NPBI and the kinetics of the various related iron parameters were measured in a homogenous group of GCT patients receiving standard first-line chemotherapy for metastatic GCT (**Chapter 5**).

In addition to its association with metabolic changes, hypogonadism may also be associated with bone loss and increased fracture risk in GCT patients. Four studies so far reported contradictory data on bone mineral density (BMD) in GCT patients and none of the studies reported a possible relationship between hypogonadism and BMD. We have observed an unusual high incidence of fractures in some long-term GCT survivors. We therefore performed DXA-scans, vertebral X-rays and serum gonadal hormone analysis in the whole cohort of GCT patients followed up long term in our institution. Data on prevalence of vertebral fractures and of osteoporosis and osteopenia are reported in **Chapter 6**.

As previously mentioned, post-unilateral orchidectomy hypogonadism is more severe and persists longer in GCT patients treated with cisplatin-based chemotherapy. GCT patients treated with chemotherapy are therefore at increased risk for bone loss. In addition to treatment-induced hypogonadism, chemotherapy may also have direct adverse effects on bone cell function, further increasing the risk of bone loss and of poor bone quality. In **Chapter 7** we present the results of a prospective study on natural changes in BMD in GCT patients up to five years after orchidectomy with and without additional cisplatin-based chemotherapy.