Chapter 8

Summary and General Discussion

THE MAIN FINDINGS PRESENTED IN THIS THESIS

The studies described in this thesis were performed to investigate the short and long-term effects of chemotherapy on bone metabolism, fat metabolism and cardiovascular risk in testicular germ cell tumour (GCT) patients.

In **Chapter 2** we report an increased prevalence of Metabolic Syndrome (MetS) in GCT patients who received chemotherapy. We demonstrate that the two used classification systems (IDF and NCEP-ATPIII) led to discrepancies in reported prevalence of MetS in GCT patients. We found, however, that independently of the classification system used to assess MetS, the relative risk of MetS in GCT patients treated with chemotherapy is twofold increased compared to that in patients with stage 1 disease who did not receive chemotherapy, or to that in healthy controls.

In **Chapter 3** we report the results of magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (^{1}H-MRS) in a small cohort of GCT patients with metastatic disease before and after treatment with combination chemotherapy. Visceral and subcutaneous abdominal fat mass increased significantly concomitant with an increase in serum cholesterol, LDL-cholesterol and insulin concentrations. These early changes in fat metabolism may predict the development of MetS in GCT patients. However, we were unable to demonstrate changes in aortic pulse wave velocity. Hepatic triglyceride content only increased in less than 40% of the patients during follow-up so that we could not demonstrate a conclusive relationship between chemotherapy and changes in hepatic triglyceride concentrations.

Chapter 4 summarizes our findings in the same group of patients regarding a number of aspects of cardiac function. It was observed that left ventricular volume, diastolic volume and left ventricular stroke volume significantly decreased following chemotherapy. Taken together these findings suggest that acute alterations in diastolic heart function in combination with the disadvantageous changes in fat metabolism measured several months after chemotherapy may, at least partially, be responsible for the increased long-term cardiovascular disease (CVD) risk and increased incidence in MetS in GCT patients.

In **Chapter 5** we report on the changes in iron metabolism in GCT patients during standard BEP chemotherapy treatment. Non-protein bound iron (NBPI), latent iron binding capacity (LIBC) and serum iron measurements were measured serially in 26 GCT patients. Within 24 hours of treatment, concentrations of the highly toxic NPBI had increased twofold during treatment and remained elevated up to one week after start of chemotherapy. Serum NPBI concentrations were inversely related to the

latent iron-binding capacity. Similar changes in serum NBPI, LIBC and serum iron were observed during a second chemotherapy cycle. Chemotherapy-associated iron overload may thus play a role in short and long-term chemotherapy induced toxicity in GCT patients.

In **Chapter 6** data on bone mass density (BMD) and morphometric vertebral fractures (VF) from a cross-sectional study in 199 cured long-term survivors of GCT and 45 newly diagnosed patients within 3 months of unilateral orchidectomy but before anti-cancer treatment are reported. Vertebral fractures were observed in 13.6% of cured long-term survivors and in 15.6% of newly diagnosed patients independently of BMD or prior chemotherapy. These VF represent a potential cause of skeletal morbidity in survivors of testicular GCT.

The natural history of changes in BMD in 63 newly diagnosed GCT patients after anti-cancer treatment are reported in **Chapter 7**. Immediately after orchidectomy, hip and lumbar BMD are not significantly different in patients with metastatic GCT compared to patients without metastases. However, after chemotherapy a significant decline of 2% in (age-adjusted) femoral and lumbar BMD was observed in patients with metastatic GCT. This significant loss of lumbar and total hip BMD observed at 1 year remained stable thereafter for up to 5 years of follow up in metastatic GCT patients receiving chemotherapy. BMD decline at 1 year did not relate to gonadal status, vitamin D deficiency or markers of bone turnover, or to cumulative dose of cisplatin or (anti-emetic) corticosteroids. Whether the decline in BMD after curative chemotherapy is directly caused by chemotherapy, or results from the induced transient partial hypogonadism, either alone or in combination with the hormonal disbalance related to a pre-existing testicular dysgenesis syndrome, remains to be established.

GENERAL DISCUSSION

Metabolic Syndrome

Germ cell tumour patients treated with combination chemotherapy have a more than twofold increased prevalence of the metabolic syndrome compared to age-matched healthy subjects.[46-49;210] Especially abdominal obesity, hypertension and hypertriglyceridemia were more frequently observed in this group of patients. We also found an elevated serum fasting LDL-cholesterol in all GCT survivors, which is in agreement with several other published reports.[46;131;210;211]

The exact cause of the increased risk for MetS in GCT survivors is not well established. Some groups report an association between testicular cancer and increased caloric intake in GCT patients [212;213], and increased insulin resistance during follow-up has also been implicated.[46;49] Other reports suggest a causal relationship between the documented hypogonadism in GCT patients and increase in MetS incidence. Chemotherapy, which leads to long-term partial or complete hypogonadism in most GCT survivors [30;36], and low testosterone and sex hormone-binding globulin (SHBG) levels are considered to be risk factors for MetS in otherwise healthy men.[90;91;214] Our data are in keeping with the latter finding, as we found an association between low serum concentrations of testosterone and MetS in all patients, in particular in those treated with chemotherapy.

In keeping with the results from worldwide cohort studies [85], the increase in the absolute prevalence of MetS was strongly dependent on the scoring system used. Indeed, we also found a different MetS prevalence in GCT patients when we used different scoring systems. The prevalence of MetS in GCT patients was almost two fold higher when classified according to the IDF criteria compared with the NCEP-ATP III criteria. However, the relative risk for MetS between the different patient groups or between all patients and healthy subjects remained the same, independent of the scoring system used.

Our data further suggest that the group of GCT survivors who received combination-chemotherapy and who were hypogonadal, had an increased risk for developing MetS compared to the general population. We therefore advocate assessment of gonadal function and correction of androgen deficiency more liberally in this group of patients although the beneficial effect of this approach remains to be established. We also recommend that guidelines for lifestyle counselling and gonadal hormone substitution should be introduced in international urology/oncology guidelines rather than only monitoring the development of potential complications which is the current standard of follow up for the detection of co-morbidities.

GCT patients treated with chemotherapy (CT) or radiotherapy (RT) are reported to have a 25-year risk of long-term cardiovascular complications of approximately 16%.[28;29] Patients who had received chemotherapy were found to be more at risk than those treated with orchidectomy only; Huddart *et al.* reported an odds ratio (OR) for cardiovascular disease (CVD) of 2.6 (95%CI: 1.2-5.8) after a median follow-up of 10.2 years for patients with disseminated disease, treated with combination chemotherapy compared to patients with stage I disease who only underwent an orchidectomy. [27] Van den Belt-Dusebout *et al.* calculated a moderately increased

standardized incidence ratio (SIR) for coronary heart disease of 1.17 (95% CI: 1.04-1.31) at a much longer follow up in a Dutch GCT cohort treated in the period between 1971 and 1985.[28] The increase in cardiovascular events in GCT patients compared to the general population data (SIRs) was especially observed in patients who underwent combination CT more than 25 years previously. They also identified RT as one of the risk factors for CVD. However, high RT doses (40-50 Gy) administered in patients with non-seminoma GCT is no longer standard treatment. Hence, the risk assessment reported for patients with GCT by these authors cannot be generalised to that of currently treated patients. Haugnes *et al.* reported a 2.2% cumulative incidence in their patients 1-14 years after diagnosis [86], an incidence similar to that observed in the general population. They also reported an increased long-term CVD prevalence 13 - 28 years after treatment (OR 3.1; 95%CI: 1.2-7.7).

We analysed our cohort of GCT patients who were treated in the LUMC between 1977 and 2008 for incidence of cardiovascular events.[215] A complete set of data was available for 490 GCT patients. Three patients died of myocardial infarction and one of ischaemic CVA before the start of the study. Of the 425 patients currently alive and under regular follow-up, 5 had a myocardial infarction, 4 had an acute coronary syndrome, and 4 had an ischaemic CVA. Thus a total of 17 out of 490 patients (3.5%) had a cardiovascular event at a median age of 54.4 years (23-76 years), 0.1 to 29 years (median 12.5 years) after their anti-cancer treatment. Fifteen of these 17 patients with a cardiovascular event had been treated with combination-chemotherapy. This low CVD incidence corresponds to the low (3%) 10-year FRS cardiovascular risk estimation in our cohort. However, the risk estimates should be considered cautiously as it is well-known that FRS calculations for persons under the age of forty are less specific.

In GCT patients, the 2.6 to 8.0% prevalence of cardiovascular disease in GCT patients does not correspond with the expected CVD risk based on MetS population measurements. A possible explanation could be that the definition of MetS does not take into account several important risk factors such as age, gender, a history of smoking, or serum total cholesterol concentrations. Furthermore, the association between the severity of risk factors and CVD risk is not considered in the dichotomous nature of the MetS criteria, although other investigators found little or no increase in the predictive power for cardiovascular events by adding abdominal obesity, triglycerides or fasting blood glucose, which are factually more accurate predictors of type II diabetes.[216] These additional factors may add to long-term CVD risk, but this requires validation in prospective studies.

Metabolic changes during chemotherapy

We hypothesized that cisplatin-based chemotherapy would induce metabolic disturbances which may possibly be visualized over time. Our data showed (Chapter 3) that in a cohort of metastasized, but otherwise healthy testicular cancer patients, significant increases of plasma insulin, total cholesterol and LDL-cholesterol, insulin resistance, body mass index (BMI), and a concomitant increase in the volume of the abdominal visceral and subcutaneous fat mass occurred. The changes in lipid and fat distribution in GCT patients occurred concomitantly with changes in serum gonadotrophin concentrations. These changes may be a result of a direct early effect of cytostatics or of the combined result of partial hypogonadism and other metabolic disturbances such as chemotherapy-induced insulin resistance. In *vitro* and in vivo animal studies show that chemotherapy agents such as cisplatin and bleomycin induce endothelial damage [62]. Although we found no difference in aortic pulse wave velocity before and after chemotherapy there was a clear alteration in left ventricular (LV) end diastolic volume and LV stroke volume after treatment. These subclinical changes in cardiac diastolic function may precede the development of clinical dysfunction. Were these early changes to be predictive for later abnormalities in cardiac function, they could be used to monitor GCT patients treated with chemotherapy for cardiovascular risk more specifically, and possibly enable protection of GCT patients against the increased CVD risk of chemotherapy.

Iron parameters

In addition to the associated untoward metabolic effects, treatment of GCT patients with chemotherapy is also associated with acute vascular toxicity.[21;153] We identified a striking increase of NPBI and of total serum iron which exceeded the capacity of iron binding proteins within 24 hours after a single administration of etoposide and cisplatin in 26 GCT patients, as reported in Chapter 5. Interestingly, these changes persisted several days after cytostatic infusions and iron parameters only slowly returned to baseline values. Similar changes in iron parameters were also observed during the following chemotherapy course. There are several explanations for these changes in iron status. Chemotherapy-induced tumour-cell lysis, in particular in chemosensitive tumours, and cellular damage to other tissues, are possible sources of excess iron. Another mechanism is the binding of cisplatin to haemoglobin, leading to dissociation of haem from the haemoglobin molecule.[166] Thirdly, preclinical studies and a few case reports suggest that lysis of iron-rich liver tissue or erythrocytes occur during chemotherapy. Regardless of its source,

the observed release of iron during chemotherapy could be a potential source for vascular cell wall damage. Chemotherapy-associated iron overload and release of potentially harmful catalytic non-protein bound iron in these otherwise healthy young men may thus hold significant clinical implications in cured long-term GCT survivors. Based on these findings we therefore recommend monitoring of the iron status in GCT patients undergoing curative chemotherapy with the perspective that iron-chelating treatment might possibly prevent late unwanted side-effects observed in these otherwise healthy patients.

Vertebral fractures and bone mineral density

In GCT patients, chemotherapy-induced hypogonadism may potentially lead to altered bone metabolism and decreased bone mass density (BMD) in GCT survivors. Although mean BMD appeared to be largely unaffected in our cohort of GCT patients, we measured a high prevalence of osteopenia (41.7%) and osteoporosis (5.5%) in GCT survivors after unilateral orchidectomy with or without additional chemotherapy. These findings are in keeping with the high prevalence of osteopenia and osteoporosis in a large cohort of 823 GCT survivors reported by Ondrusova *et al*.[53] We also reported an incidence of morphometric vertebral deformities in 32.6% of newly diagnosed patients with GCT, within three months of unilateral orchidectomy and in 40.2% of long-term GCT survivors. The vertebral deformities appear to be sustained independently of BMD, age, tumour type or stage, prior chemotherapy, glucocorticoid use and presence of hypogonadism. The underlying mechanism by which these vertebral deformities develop in GCT patients remains unclear. Although we did not find a direct relationship between serum concentrations of gonadal hormones and the number or severity of vertebral fractures (VF), the increased serum FSH and LH concentrations reflect a decrease in germinative cell-function which may lead in the long-term to a disturbed bone metabolism.[25;30;185] Another possible explanation is the still speculative existence of a testicular dysgenesis syndrome in GCT [7;191], which comprises abnormal fertility, androgen insensitivity and/or undescended testis. In GCT patients, this androgen insensitivity may be potentially responsible for altered bone quality leading to increased skeletal fragility.

Pfeilschifter and Diel hypothesized that GCT patients may experience accelerated bone loss through the effects of chemotherapy [200], but three cross-sectional studies addressing this issue have reported no increased incidence of osteoporosis in long-term survivors.[50-52] However, we prospectively measured BMD in a cohort of 63 GCT survivors of whom 36 had received cisplatin-based combination chemotherapy

because of metastasized disease. In our cohort, the patients with metastatic disease who received combination chemotherapy had a significant 2% decrease in femoral and lumbar spine BMD within one year of treatment, which persisted up to five years of follow-up. Low BMD was independent of smoking, gonadal status, vitamin D deficiency or markers of bone turnover. Potential additional risk factors for the decline in BMD were the concomitant administration of corticosteroids and possible malnutrition due to chemotherapy-induced nausea. It is hoped that future long-term studies will be able to clarify the mechanism by which treatment results in deleterious effects on the skeleton in GCT patients. Based on our data demonstrating an increased incidence of silent morphometric vertebral deformities, we recommend that all GCT patients with metastasized disease treated with combination chemotherapy should be evaluated for the presence of osteoporosis and vertebral fractures.

CONSIDERATIONS

We have shown that GCT patients treated with cisplatin-based chemotherapy have a more than twofold increase in risk of acquiring the Metabolic Syndrome. We have also demonstrated a direct relationship between low serum testosterone levels and the development of Metabolic Syndrome in these patients. It has been clearly established that hypogonadism is associated with increased cardiovascular risk [217-219], and several studies have shown a protective effect of testosterone supplementation on CVD risk and risk factors for developing the metabolic Syndrome in hypogonadal men.[220-224] It remains unclear, however, whether in GTC patients chemotherapy- induced hypogonadism is associated with an increased incidence of cardiovascular events. This should be further explored using two possible study designs. A cross-sectional study design in which, all GCT patients who have been treated with cisplatin-based chemotherapy would be studied for incident cardiovascular events during long-term follow-up, relating these events to severity and duration of the post chemotherapy hypogonadism and using as controls GCT patients who did not incur cardiovascular events during follow-up. A second approach would be to prospectively follow all GCT patients treated in our hospital and to examine the incidence of cardiovascular events in patients with low serum testosterone values after chemotherapy compared to those with normal serum testosterone concentrations. Although this approach requires a long-term study, it would also provide the possibility of studying the effect of testosterone supplementation on CVD risk in the long-term by randomly allocating treatment to half of the population studied.

A number of factors other than hypogonadism have been suggested to contribute to the increased cardiovascular risk in GCT patients including increased caloric intake and chemotherapy-induced endothelial damage. We have been able to measure the acute changes in lipid and fat distribution occurring in GCT patient treated with chemotherapy and it would be interesting to see whether these metabolic changes are associated with increased incidence of the MetS and CVD risk in the long term. To this effect it would be necessary to measure body composition and serum lipid spectra in all GCT patients before and immediately after anti-cancer treatment and to identify the patients who would go on to sustain a cardiovascular event. Since the increased cardiovascular risk becomes apparent only twenty to thirty years after initial cancer diagnosis and treatment in GCT patients, it will take decennia before a clear relationship between CVD and chemotherapy-induced metabolic changes could be established. We should also be able to find out which long-term GCT survivors acquired CVD and go on to perform a case-control study of GCT patients with compared to those without CVD, checking in the process the role of treatment related effects, predisposition to CVD and known CVD risk factors.

As suggested in this thesis, the increase in non-protein bound iron in GCT patients could be at the source of the chemotherapy-induced endothelial damage. We have shown that an increase in NPBI is directly related to the increase in serum iron and decrease in LIBC. We were not able, however, to measure an acute effect of cisplatin-based chemotherapy on aortic pulse wave velocity, possibly because more time may be required to develop vascular changes. In GCT patients treated with chemotherapy a prospective analysis of serum iron and LIBC measurement in combination with ultrasound measurements of common carotid artery intima-media thickness might strengthen our hypothesis that free iron may play a major role in the pathogenesis of chemotherapy-induced cardiovascular risk.[225]

We have shown that GCT patients have a high prevalence of osteopenia, osteoporosis and vertebral fractures. The fact that these vertebral fractures are present in the absence of significant decreases in BMD suggests that bone quality is decreased in GCT patients. To determine whether this is the case, we could study components of bone quality by performing thick needle biopsies of the iliac crest and use micro-CT or other newer modalities to study the bone microarchitecture.[226]