Chapter 7

Natural history of changes in bone mineral density in newly diagnosed testicular cancer patients after anti-cancer treatment

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ABSTRACT

Introduction: Patients with germ cell tumours (GCT) have an excellent prognosis but have been shown to be at risk for silent fractures. Data on bone mineral density (BMD) following anti-cancer treatment are scarce. We therefore monitored BMD in newly diagnosed testicular GCT patients treated with or without chemotherapy at one, two and up to five years after anticancer treatment.

Method: We prospectively studied 63 newly diagnosed patients with GCT with a median age of 33 years (range 16-70) within 3 months of unilateral orchidectomy but before anticancer treatment. Twenty-seven patients (42.9%) did not have metastases (stage 1), fifteen of whom received a single dose of adjuvant carboplatin. Thirty-six patients (57.1%) with metastatic disease received combination-chemotherapy.

Results: At baseline, total hip and lumbar BMD were not significantly different between patients with non-metastatic and metastatic tumours. There was no decrease in BMD observed in stage I patients during follow up. A significant loss of lumbar BMD (-0.016 g/cm^2; p=0.004) and total hip BMD (-0.021g/cm^2; p<0.0001) was observed at 1 year and remained stable thereafter 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.

Conclusion: Metastatic GCT survivors suffer significant bone loss within the first year after curative combination-chemotherapy which persisted for up to 5 years after anticancer treatment. Based on these findings we recommend measurements of BMD in GCT patients receiving combination CT to allow timely treatment of bone loss and increased fracture risk.

INTRODUCTION

Testicular germ cell tumours (GCT) are the most common form of cancer in young male adults. The introduction of cisplatin-based combination chemotherapy has led to significant improvement in prognosis, with cure achieved in the vast majority of patients.[11] This translates in an increasing number of long-term survivors of this malignancy who would have undergone unilateral orchidectomy with or without chemotherapy at a relatively young age. Long-term detrimental effects of cancer treatments on the skeleton may be associated with increased morbidity and are potentially important to evaluate and treat as required.

GCT patients may potentially experience accelerated bone loss through the effects of chemotherapy.[200] A high prevalence of osteopenia and osteoporosis has been reported in a large cohort of 823 GCT survivors with unilateral testicular cancer a median 89 months after orchidectomy and anticancer treatment.[53] In contrast, another study reported no deleterious effects of chemotherapy on the skeletal health of long-term survivors of GCT or lymphoma.[51] We have previously shown an increased prevalence of vertebral fragility fractures in cured long-term survivors of GCT. The high prevalence of largely asymptomatic vertebral fractures we observed in GCT patients was independent of tumour stage, chemotherapy or bone mineral density (BMD).[201]

Chemotherapy may be causally related to decreases in BMD because of potential negative effects of chemotherapy on bone remodelling. The widely used high doses of corticosteroid as part of the anti-emetic regimen concomitantly prescribed with chemotherapy may contribute to bone loss [202] as well as to femoral head osteonecrosis.[203]

Cytotoxic chemotherapy is associated with significant gonadal damage, with germ cells being more sensitive to treatment than Leydig cells, with infertility being a more commonly encountered adverse effect than hypogonadism. Whether bone quantity and/or quality are directly affected by chemotherapy or indirectly so by chemotherapy-mediated partial hypogonadism is not known. Various studies reported hypogonadism as evidenced by increased serum LH and low-normal testosterone up to sixty months after chemotherapy in GCT survivors[9;30;204] although the diurnal pulsatile pattern of testosterone secretion and the different parameters used to define hypogonadism preclude comparison between the various studies. Interestingly, partial hypogonadism has shown to be already present before orchidectomy in up to one third of GCT patients.[205]

Although the vast majority of patients remain or become eugonadal in time[9] the sometimes severe bone loss associated with abrupt iatrogenic hypogonadism may not be fully reversible. The aim of our study was to establish whether bone mineral density is affected by chemotherapy in newly diagnosed GCT patients one, two and up to five years after anticancer treatment.

PATIENTS AND METHODS

Patients

We studied 63 newly diagnosed GCT patients aged 16 to 70 years (median 33 years), who were referred to the Department of Clinical Oncology of the Leiden University Medical Center (LUMC) between 2007 and 2009 after unilateral orchidectomy but before anticancer treatment as required. The primary tumour was staged on the basis of tumour histology, CT-scan and serum concentrations of tumour markers, beta unit of human chorionic gonadotrophin (β-HCG), alpha-fetoprotein (α-FP) and lactate dehydrogenase (LDH), measured at the time of diagnosis. Patients were divided into two groups based on diagnosis and anti-cancer treatment; a first group of patients with non-metastatic disease (stage 1) were treated with unilateral orchidectomy only (non-seminomas, n=12) or unilateral orchidectomy plus a single dose of adjuvant carboplatin (AUC7) (pure seminomas, n=15). In our experience carboplatin is not associated with chemotherapy-related complications [201] so that it was decided to include these patients in the non-metastatic group of patients.

A second group of patients had metastatic GCT and had received multiple courses of cisplatin-based combination-chemotherapy. Most GCT patients were asymptomatic, were leading an active life and were in good clinical condition. Only eight of the patients had co-morbidity in the form of diabetes mellitus (n=3), hypertension (n=2), COPD (n=1), ulcerative colitis (n=1) or epilepsy (n=1).

Baseline evaluation included medical history, prior and current medication, prior use of corticosteroids, smoking habits and alcohol use. All patients underwent a full clinical examination including body height, weight, waist and hip circumference, pulse rate and blood pressure.

Laboratory measurements

Blood samples were collected at baseline after orchidectomy and one, two and up to five years thereafter. Serum was measured for creatinine, calcium (corrected for

albumin), phosphate and alkaline phosphatase concentrations using semi-automated techniques. Serum was also measured for the marker of bone resorption beta-carboxyl-terminal cross-linking telopeptide of type I collagen (β-CTX). Serum intact parathyroid hormone (PTH), 25-hydroxy vitamin-D ($25(OH)D_3$) and 1,25-dihydroxy vitamin-D ($1,25(OH)_2D_3$) were measured using standard radioimmunoassay's. Gonadal status was evaluated by measuring luteinising hormone (LH), follicle-stimulating hormone (FSH), total testosterone (TT), estradiol (E_2) and sex hormone binding globulin (SHBG) using standard radioimmunoassay's. Free testosterone (FT) was calculated using a standard formula.[169;170] Hypogonadism was defined as a serum total testosterone concentration of <10.4nmol/l.[84] Vitamin D deficiency was defined as a $25(OH)D_3$ concentrations of <50nmol/L.[171;172] Renal function was calculated using the Cockcroft formula. Serum concentrations of the beta unit of human chorionic gonadotrophin (β-HCG), alpha-fetoprotein (α-FP) and lactate dehydrogenate (LDH) were used for stageing of the disease at the time of diagnosis.

Bone mineral density measurements

Bone mineral density (BMD) was measured at the lumbar spine (L1-L4) and total hip using dual energy X-ray absorptiometry (DXA, Hologic QDR 4500; Waltham, MA, USA). The coefficient of variation of BMD measurements was 1%, and the machine was calibrated at regular intervals. The DXA-scan used in this study was equipped with reference values based on the National Health and Nutrition Examination Surveys (NHANES III) [173], which are compatible with those of a Dutch control population. World Health organization (WHO) criteria were used to define osteopenia (T score between -1 and -2.5) and osteoporosis (T score < -2.5) [174]. Lumbar BMD was assessed from L1 to L4 in posterio-anterior incidence. Total hip BMD was reported as the mean of left and right total hip BMD measurements. Results were also expressed as Z-score, indicating the number of standard deviations below the mean of age- and sex-matched controls.

Statistical analysis

The SPSS for Windows software package (Chicago, IL, USA) and SAS for windows V9.1.3 (SAS Institute, Inc., Cary, NC, USA) were used for statistical analysis. Results are expressed as mean±SD or as median (minimum-maximum). Two-tailed paired sample Student's t-test was used to compare single, normally distributed measurements between study groups. The Wilcoxon signed-rank test was used to compare unevenly distributed measurements between study groups.

The comparability of the study groups at baseline was assessed as appropriate by one-way ANOVA analysis of variance. The serial measurements of the biochemical markers and BMD are analysed with a repeated measures model analysis of variance, with an autoregressive first order covariance structure. The estimated least square means and their p-values for the difference from zero test are used to establish if the patients are deviant from their peers.

Table 1. Clinical characteristics.

	All patients (N=63)	Stage One (N=27)	Disseminated (N=36)	P-value [a]
Demographics (median, min - max)				
Age (years)	33 (16 – 70)	35 (22 – 70)	34 (16 – 59)	NS
Δ Time (years)	0.1 (0.0 – 6.2)	0.2 (0.0 – 0.8)	0.1 (0.0 – 6.2)	NS
Characteristics (mean ± SD)				
Weight (kg)	83 ± 12	82 ± 12	83 ± 11	NS
BMI (kg/m^2)	24.7 ± 3.3	24.3 ± 2.7	25.1 ± 3.7	NS
Waist circumference (cm)	92 ± 10	94 ± 12	90 ± 8	NS
Systolic blood pressure (mmHg)	128 ± 15	126 ± 16	130 ± 15	NS
Renal function (Cockroft clearance ml/min)	135 ± 30	126 ± 25	141 ± 32	NS
Life style; n (%)				
Smoking	24 (38.1)	7 (25.9)	17 (47.2)	NS
Alcohol use (>20 U/week)	9 (14.3)	7 (25.9)	9 (25.0)	NS
Histology; n (%)				NS
Seminoma	27 (42.9)	15 (55.6)	12 (33.3)	
Non-seminoma	22 (34.9)	7 (25.9)	15 (41.7)	
Combined tumor	14 (22.2)	5 (18.5)	9 (25.0)	
TNM Tumor Staging; n (%)				~
Stage I (no metastasis)	27 (42.9)	27 (100.0)	0 (0.0)	
Stage I S (elevated serum tumor markers)	1 (1.6)	0 (0.0)	1 (2.8)	
Stage II (para-aortic lymph node metastasis)	23 (36.5)	0 (0.0)	23 (63.9)	
Stage III (distant metastasis)	12 (19.0)	0 (0.0)	12 (33.3)	
Treatment; n (%)				~
No adjuvant treatment	12 (19.1)	12 (44.4)	0 (0.0)	
Single dose carboplatin	15 (23.8)	15 (55.6)	0 (0.0)	
Combination chemotherapy	36 (57.1)	0 (0.0)	36 (100.0)	

n sample size; NS: non-significant.

[a] p-value for comparison between values of patients treated with and without chemotherapy.

Δ Time: Time between orchidectomy and first measurement (years).

RESULTS

Patients and disease characteristics

Demographic and anthropometric characteristics of the sixty-three consecutive testicular GCT patients included in the study are summarized in Table 1. Twenty-seven patients (42.9%) had stage I disease (no metastases) and thirty-six patients (57.1%) had metastatic disease (Table 1). Of the patients with metastatic disease, one (1.6%) had stage IS (elevated tumour markers but negative CT scan indicating micro metastases), 23 (36.5%) had stage II disease (metastases in one or more loco regional para-aortic lymph nodes) and 12 (19%) had distant metastases, mostly in the lungs (Table 1)**.** Median age was similar in the two patient groups (Table 1).

Patients with metastatic disease received first-line cisplatin-based combination chemotherapy consisting of three (n=22) or four (n=14) cycles of BEP (bleomycin, etoposide and cisplatin) or 4 cycles of EP (etoposide and cisplatin). Each cycle consisted of intravenously administered etoposide (100 mg/m^2 over 1 hr, days 1-5) and cisplatin (20 mg/m^2 over 4 hours, days 1-5), with or without bleomycin (30 IUSP over 30 min) at days 2, 8, and 15. As part of the anti-emetic regimen all 36 patients received intravenously administered high doses of corticosteroids (10 mg dexamethasone intravenously daily during days 1-5 of their 3-weekly chemotherapy courses, with rapidly tapering of oral dexamethasone thereafter: twice daily 3 mg (days 6-7) and twice daily 1.5 mg (days 8-9). Two patients received two extra cycles of VIP (vinblastine, ifosfamide and cisplatin) consolidation chemotherapy and consequently higher administrative doses of corticosteroids.

Two patients aged 53 and 35 years developed avascular necrosis of the hip, respectively one and four years after chemotherapy, started with bisphosphonates, and were excluded from further analysis thereafter. A third patient, aged 21 years, was diagnosed with osteoporosis after one year, started with calcium and vitamin D suppletion, and was also excluded from further analysis.

Laboratory measurements

Laboratory measurements, before, after one and two years and up to five years after treatment, are shown in Table 2A, 2B and 3. Median serum concentrations of measured biochemical parameters were normal at baseline and thereafter and did not significantly differ at any time point between patient groups (Table 2A and 2B).

Table 2A. Laboratory measurements of biochemical in stage 1 GCT patients during five year followup.

Parameters mean±SD median (min-max)	Baseline N=27	Year 1 N=27	Year 2 N=19	Year 3 N=14	Year 4 N=12	Year 5 N=13a	Reference Range
S. creatinine	86±11 85 (70-116)	87±12 87 (62-114)	80±11 81 (56-99)	82±10 83 (62-99)	86±13 84 (73-116)	87±8 87 (74-100)	10 – 130 (µmol/L)
S. calcium *(corr)*	2.28±0.19 2.24 (1.91-3.06)	2.27±0.12 2.26 (2.06-2.54)	2.28±0.14 2.28 (2.08-2.56)	2.30±0.12 2.29 (2.15-2.58)	2.24±0.13 2.24 (2.08-2.54)	2.24±0.12 2.27 (2.06-2.52)	2.15 – 2.55 (mmol/L)
S. phosphate	1.09±0.16 1.10 (0.79-1.44)	1.10±0.18 1.14 (0.79-1.53)	1.11±0.21 1.13 (0.74-1.52)	1.02±0.21 1.07 (0.64-1.29)	1.06±0.14 107 (0.81-1.36)	1.05±0.14 10.7 (0.87-1.28)	0.90 – 1.50 (mmol/L)
S. AP	73±18 73 (26-114)	70±18 59 (41-137)	71±20 69 (36-129)	72±20 72 (37-125)	66±14 67 (42-86)	69±15 70 (39-96)	40 – 120 (U/L)
S. PTH	4.1±2.0 3.1 (1.4-7.7)	4.8±2.4 4.2 (1.7-11.2)	6.1±4.3 5.2 (2.4-21.6)	5.7±4.8 3.8 (1.9-19.2)	6.4±2.7 5.5 (3.4-11.6)	5.3±2.7 4.4 (2.2-12.2)	<10.0 (pmol/L)
S. 25(OH)D	64±29 61 (28-145)	63±24 67 (12-122)	51±23 53 (13-96)	57±26 55 (18-109)	61±9 59 (46-77)	63±31 58 (31-113)	30 – 120 (nmol/L)
S. 1,25(OH)D_3	120±42 109 (69-232)	126±34 128 (64-177)	124±45 129 (62-220)	117±33 122 (66-183)	111±22 114 (84-151)	139±32 144 (99-168)	40-140 (pmol/L)
S. β-CTX	0.52±0.21 0.47 (0.20-0.97)	0.48±0.24 0.45 (0.13-1.13)	0.47±0.19 0.41 (0.20-0.84)	0.42±0.13 0.40 (0.26-0.68)	0.40±0.16 0.37 (0.22-0.72)	0.44±0.18 0.39 (0.18-0.87)	< 0.58 (ng/mL)

[a] One patient who received vitamin D supplementation for hypovitaminose D at 5 years after diagnosis and was excluded.

Table 2B. Laboratory measurements of biochemical in metastatic GCT patients before and after anti-cancer treatment.

Parameters mean±SD median (min-max)	Baseline N=36	Year 1 N=35	Year 2 N=21 ab	Year 3 N=25	Year 4 N=19	Year 5 N=18 b	Reference Range
S. creatinine	77±13 77 (55-130)	85±13 85 (60-112)	83±12 81 (57-116)	87±13 89 (51-111)	82±9 83 (66-100)	84±11 84 (65-107)	10 – 130 (µmol/L)
S. calcium (*corr*)	2.24±0.17 2.28 (1.63-2.46)	2.24±0.09 2.26 (1.97-2.37)	2.23±0.08 2.24 (2.06-2.36)	2.23±0.11 2.25 (1.95-2.41)	2.26±0.08 2.28 (2.12-2.40)	2.25±0.07 2.27 (2.06-2.34)	2.15 – 2.55 (mmol/L)
S. phosphate	1.16±0.20 1.16 (0.80-1.80)	1.09±0.18 1.11 (0.72-1.51)	1.09±0.21 1.09 (0.59-1.66)	1.06±0.17 1.06 (0.72-1.42)	1.04±0.19 1.03 (0.74-1.49)	0.99±0.19 0.99 (0.46-1.27)	0.90 – 1.50 (mmol/L)
S. AP	86±69 77 (19-442)	83±35 77 (49-232)	78±28 72 (51-159)	81±22 79 (51-118)	78±23 73 (48-119)	73±22 70 (40-116)	40 – 120 (U/L)
S. PTH	4.1±2.6 3.5 (1.1-16.0)	5.1±2.7 4.4 (1.1-15.8)	5.2±2.3 5.0 (1.0-10.5)	6.0±3.4 4.3 (2.6-16.3)	5.8±3.8 4.7 (1.1-15.2)	3.8±2.2 3.2 (1.2-9.5)	<10.0 (pmol/L)
S. 25(OH)D	53±24 55 (12-110)	56±22 56 (18-93)	54±27 50 (14-98)	50±23 45 (15-116)	60±24 59 (20-108)	66±25 62 (35-131)	30 – 120 (nmol/L)
S. 1,25(OH)D3	105±40 105 (36-203)	105±40 93 (53-227)	131±46 126 (67-231)	122±55 102 (52-261)	12±45 114 (61-218)	132±71 106 (55-274)	40-140 (pmol/L)
S. β-CTX	0.53±0.36 0.43 (0.18-1.61)	0.53±0.30 0.45 (0.03-1.28)	0.58±0.49 0.43 (0.19-2.15)	0.47±0.26 0.44 (0.05-0.96)	0.48±0.30 0.45 (0.02-1.16)	0.48±0.23 0.46 (0.09-1.01)	< 0.58 (ng/mL)

[a] One patient had osteoporosis one year after initial treatment and started with calcium D3 and was excluded from further analysis.

[b] Two patients had avascular hip necrosas respectively two and four years after start of treatment; both started with bisphosphonates and were excluded from further analysis.

Vitamin D deficiency was documented at baseline in 23 patients (36.5%), was equally distributed between the two patient groups and did not significantly change thereafter. At baseline, β-CTX concentration was increased above the normal laboratory reference range in 17 of the 63 patients (27.0%); in 10 (37.0%) of the stage one patients and in 7 (19.4%) of the patients with metastatic disease. There was no change in β-CTX concentration during follow up.

Figure 1. Time profile graph of LH, FSH, testosterone and estradiol with SD as error bars for both measurement groups (patients with stage one and patients with disseminated disease) at baseline and after anti-cancer treatment.

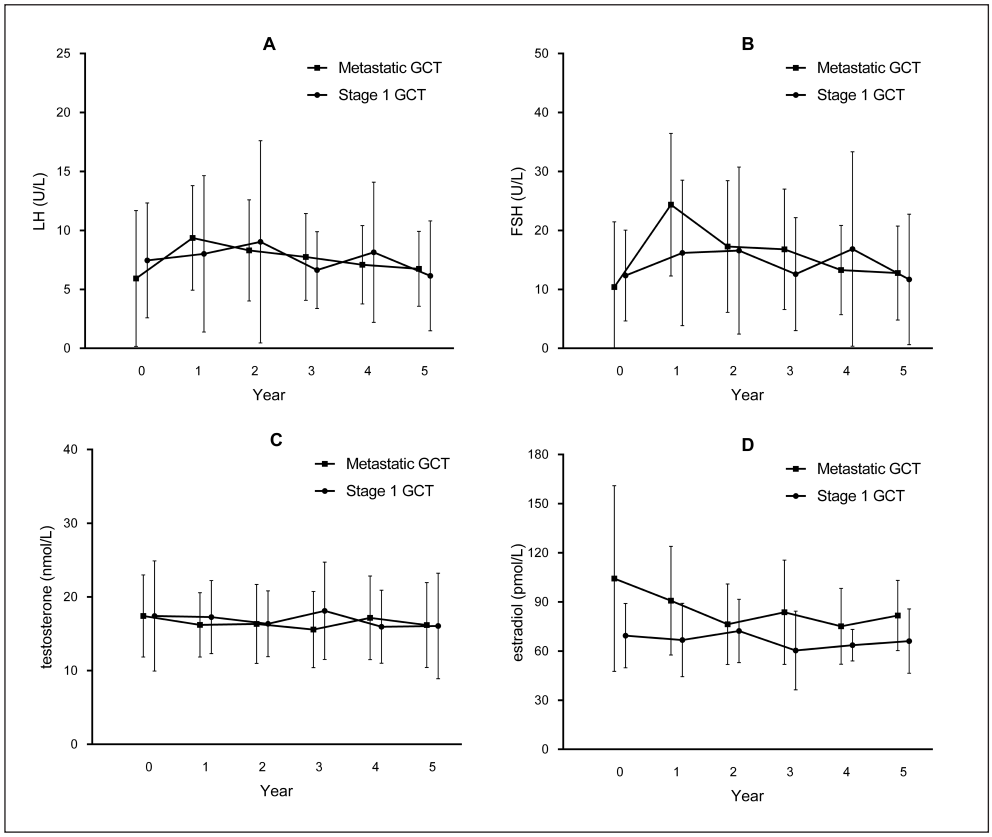


Table 3. Gonadal hormones [mean±SD, median (minimum–maximum)] in testicular cancer patients with stage one and disseminated disease before and after anti-cancer treatment.

Parameters	Baseline	Year 1	Year 2	Year 3	Year 4	Year 5	Reference Range
Stage 1 GCT	**N=27**	**N=27**	**N=19**	**N=14**	**N=12**	**N=14**	
S. TT	17.4±7.5	15.6±4.9	16.3±4.5	18.1±6.6	16.0±5.0	16.1±7.2	8.0 – 35.0
	15.3 (0.2-33.9)	17.1 (9.2-27.4)	15.8 (9.9-25.8)	16.1 (11.4-37.9)	15.6 (9.1-23.4)	16.0 (0.7-29.2)	(nmol/L)
S. estradiol	69±20	66±22	72±19	60±23	63±10	66±20	70 – 200
	75 (28-98)	63 (28-109)	73 (42-118)	59 (26-113)	60 (50-77)	61 (38-99)	(pmol/L)
S. LH	7.5±4.9	8.0±6.6	9.0±8.6	6.6±3.3	8.1±5.9	6.6±4.6	2.0 – 10.0
	6.3 (2.9-25.6)	5.4 (2.5-31.3)	6.8 (2.5-36.0)	5.8 (2.6-13.8)	5.9 (3.3-19.0)	5.0 (3.1-20.1)	(U/L)
S. FSH	12.4±7.7	16.1±12.5	16.6±14.2	12.6±9.6	16.9±16.5	11.7±11.1	2.0 – 10.0
	10.2 (4.6-32.1)	12.3 (3.9-54.1)	12.5 (3.9-50.8)	10.8 (4.0-40.4)	11.5 (4.9-49.1)	10.2 (4.5-48.5)	(U/L)
S. SHBG	30.5±18.3	29.2±9.1	32.4±15.0	28.9±6.4	33.6±6.1	33.1±10.1	20 – 55
	24.9 (3.0-90.0)	26.3 (15.7-48.4)	26.3 (16.1-85.4)	27.4 (16.1-44.8)	34.3 (23.2-42.4)	32.6 (12.5-49.9)	(nmol/L)
Metastatic GCT	**N=36**	**N=35**	**N=23**	**N=26**	**N=20**	**N=21**	
S. TT	17.4±5.6	16.2±4.4	16.3±5.4	15.6±5.2	17.2±5.7	16.2±5.8	8.0 – 35.0
	17.4 (4.7-30.2)	15.3 (9.1-26.5)	14.9 (10.0-30.0)	14.2 (5.4-25.2)	17.6 (6.4-25.1)	15.8 (7.0-29.5)	(nmol/L)
S. estradiol	104±56	91±33	76±25	84±32	75±23	82±21	70 – 200
	91 (31-240)	88 (25-181)	78 (20-123)	74 (36-160)	81 (21-108)	82 (35-118)	(pmol/L)
S. LH	5.9±5.8	9.3±4.4	8.7±4.0	7.7±3.7	7.1±3.3	6.7±3.2	2.0 – 10.0
	4.8 (0.1-24.8)	8.1 (3.4-18.7)	8.1 (2.7-16.4)	6.9 (2.6-14.5)	6.4 (2.9-14.3)	6.1 (2.1-13.5)	(U/L)
S. FSH	10.4±11.1	24.0±12.3	18.1±10.8	16.8±10.2	13.3±4.6	12.8±8.0	2.0 – 10.0
	7.4 (0.1-44.5)	21.6 (5.5-46.0)	14.2 (5.0-39.7)	14.2 (5.0-39.7)	10.8 (3.6-29.1)	10.3 (2.9-29.1)	(U/L)
S. SHBG	31.2±12.0	31.2±10.8	26.4±9.8	29.5±10.2	26.4±6.8	32.8±13.9	20 – 55
	31.9 (6.8-64.0)	30.2 (13.3-52.7)	23.9 (11.0-46.6)	30.5 (14.0-48.4)	28.9 (14.3-35.3)	33.0 (11.7-59.3)	(nmol/L)

Table 4. Total hip and lumbar Bone Mass Density measurements in testicular cancer patients before and after anti-cancer treatment.

Parameters	Baseline	Year 1	Year 2	Year 3	Year 4	Year 5
Stage 1 GCT	N=27	N=27	N=18	N=7	N=5 [a]	N=15
Total hip BMD (g/cm^2)	1.03±0.12	1.03±0.13	1.00±0.11	1.02±0.15	0.99±0.12	0.97±0.13
	1.01 (0.81-1.26)	0.98 (0.80-1.29)	0.98 (0.80-1.19)	0.95 (0.87-1.30)	0.95 (0.86-1.18)	0.98 (0.72-1.21)
Estimate of difference (95%CI)	~	0.000	-0.006	0.005	0.001	-0.011
		(-0.012 / 0.012)	(-0.024 / 0.012)	(-0.022 / 0.032)	(-0.032 / 0.033)	(-0.041 / 0.020)
p-value	~	NS	NS	NS	NS	NS
BMD lumbar vertebrae (g/cm^2)	1.06±0.13	1.05±0.13	1.04±0.12	1.09±0.21	1.07±0.13	1.00±0.12
	1.03 (0.83-1.43)	1.06 (0.80-1.45)	1.01 (0.83-1.22)	1.04 (0.86-1.50)	1.03 (0.93-1.22)	0.97 (0.81-1.20)
Estimate of difference (95%CI)	~	-0.008	-0.010	0.013	0.003	-0.011
		(-0.020 / 0.005)	(-0.029 / 0.009)	(-0.016 / 0.041)	(-0.031 / 0.036)	(-0.043 / 0.021)
p-value	~	NS	NS	NS	NS	NS
Metastatic GCT	N=36	N=36	N=21 [bc]	N=14	N=10 [c]	N=18
Total hip BMD (g/cm^2)	1.03±0.12	1.01±0.12	1.01±0.14	0.99±0.09	1.02±0.10	1.00±0.09
	1.02 (0.79-1.32)	1.00 (0.80-1.31)	1.01 (0.81-1.32)	0.99 (0.82-1.15)	1.00 (0.92-1.25)	0.99 (0.83-1.20)
Estimate of difference (95%CI)	~	-0.021	-0.017	-0.023	-0.027	-0.024
		(-0.031 / -0.011)	(-0.033 / -0.001)	(-0.045 / -0.002)	(-0.052 / -0.001)	(-0.051 / 0.003)
p-value	~	<0.0001	0.04	0.03	0.04	NS
BMD lumbar vertebrae (g/cm^2)	1.05±0.12	1.04±0.12	1.05±0.13	1.05±0.12	1.07±0.11	1.03±0.12
	1.04 (0.77-1.29)	1.02 (0.78-1.33)	1.06 (0.79-1.34)	1.08 (0.85-1.26)	1.05 (0.87-1.26)	105 (0.83-1.27)
Estimate of difference (95%CI)	~	-0.016	0.002	0.006	-0.005	0.008
		(-0.027 / -0.005)	(-0.015 / 0.019)	(-0.017 / 0.028)	(-0.032 / 0.023)	(-0.021 / 0.036)
p-value	~	0.004	NS	NS	NS	NS

Bone Mass Density measurements presented as mean±SD and median (minimum–maximum).
Estimate of difference is compared to baseline values (95% CI).
[a] One patient he received vitamin D supplementation for hypovitaminosis D at 5 years after diagnosis and was excluded.
[b] One patient had osteoporosis one year after initial treatment and started with calcium D3 and was excluded for further analysis.
[c] Two patient had avascular hip necrosis respectively two and four years after start of treatment, both started with bisphosphonates and were excluded from further analysis.

At baseline, four of the 63 patients (6.3%) had plasma testosterone concentrations <10.4nmol/L. Independently of testosterone levels, elevated LH and FSH levels were observed in respectively 11 (17.5%) and 28 (44.4%) patients. Serum estradiol was decreased in 23 patients (36.5%). There was no significant difference in mean levels of serum testosterone, LH and FSH between the two treatment groups (Figure 1A and 1B). Before chemotherapy patients with metastatic disease had significantly higher serum estradiol concentrations compared to patients with stage one disease (91.0 pmol/L; 3.0-240.0 vs. 74.5 pmol/L; 28.0-98.0; p=0.007) (Figure 1D).

Patients with disseminated disease had significantly higher serum FSH (7.4 U/L; 0.1-44.5 vs. 20.8 U/L; 5.5-46.0; p<0.001) and LH (4.8 U/L; 0.1-24.8 vs. 8.2 U/L; 3.4-18.7; p=0.008) concentrations in the absence of a decrease of serum testosterone and estradiol concentrations one years after anti-cancer treatment (Table 3, Figure 1). Gonadal hormone concentrations did not fluctuate significantly during follow-up in stage one patients (Figure 1). No significant changes were observed in free testosterone over time.

BMD measurements

At baseline: Femoral and lumbar BMD did not significantly differ at baseline between patients with stage I non-seminoma, patients with stage I seminoma scheduled to undergo adjuvant single dose carboplatin, or patients with disseminated disease (Table 4).

One year after anti-cancer treatment: There was no significant change in BMD in stage I patients one year after orchidectomy or thereafter. There was no significant difference in BMD changes in patients who did or did not receive one additional dose of carboplatin. In patients with metastatic disease, who were treated with multiple chemotherapy courses, BMD decreased at the lumbar spine (1.05±0.12 vs. 1.03±0.11g/cm^2; p=0.03) and at the hip (1.04±0.12 vs. 1.01±0.12g/cm^2; p<0.001) compared to the pre-treatment values (Figure 2). Two patients with metastatic disease aged 16 and 18 who may not have reached peak bone mass both received cisplatin-based chemotherapy. The youngest patient had normal hip BMD but osteopenia at the lumbar spine which improved at 1 year after anti-cancer treatment and thereafter, the 18 year patient had normal BMD.

Prevalence of low bone mass at baseline: Two of the 63 patients (3.2%) had osteoporosis of the lumbar spine, none at the hip. Eighteen patients (28.6%) had osteopenia; six patients (9.5%) at both the lumbar spine and hip, nine (14.3%) only at the lumbar spine and three (4.8%) only at the total hip. There was no significant difference in the prevalence of osteopenia and osteoporosis between patients with stage one GCT (29.6%) or those with disseminated disease (33.4%). Four of the 63 patients (6.3%) had Z-scores less than -2SD. Twelve patients (19.0%) had Z-scores between -1 and -2 SD at either lumbar spine, total hip or at both sites.

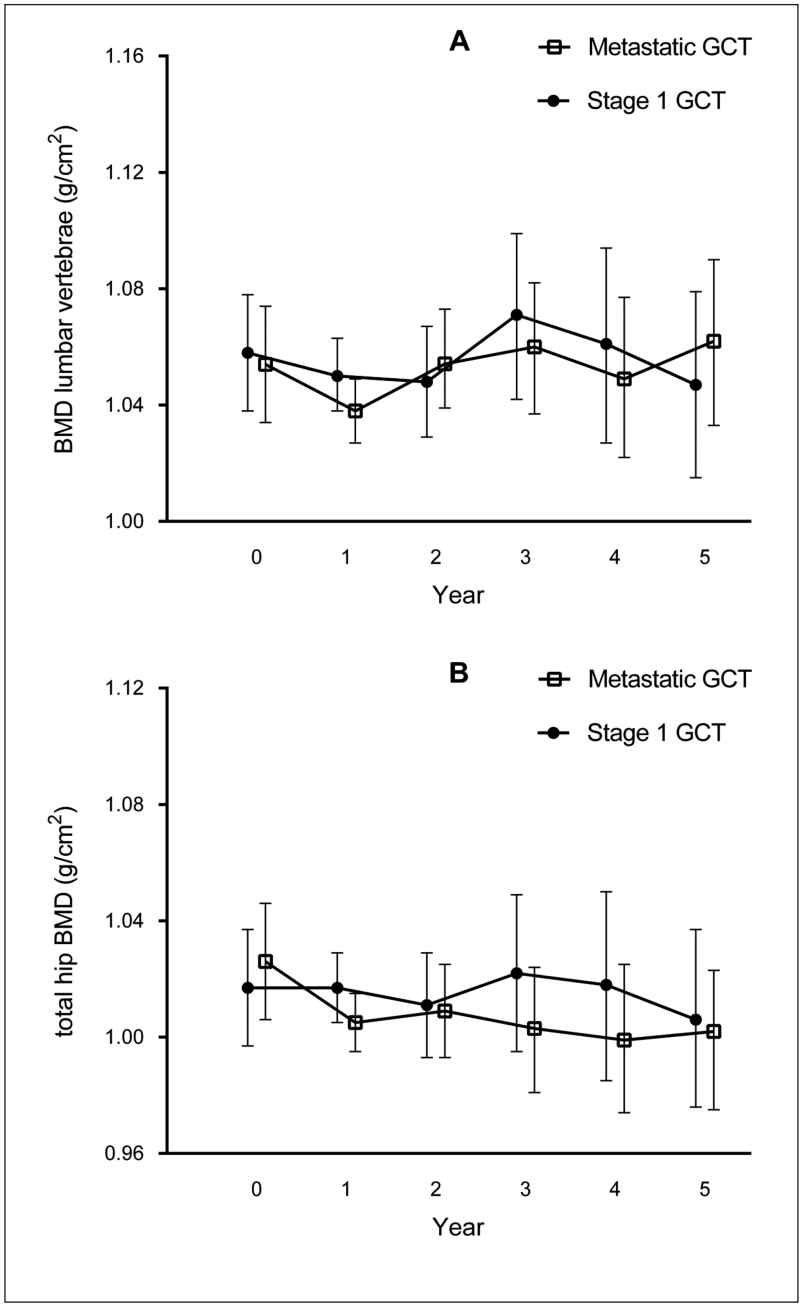
Prevalence of low bone mass status one year after anti-cancer treatment: At one year after anti-cancer treatment osteoporosis persisted in the two patients in whom it was observed at baseline. After one year, we observed no osteoporosis at the hip. Twenty-two patients (34.9%) had osteopenia; eight patients (12.7%) at both lumbar spine and hip, thirteen (20.6%) only at the lumbar spine and one patients (1.6%) had osteopenia at the left hip. There was no significant difference in the prevalence of osteopenia and osteoporosis between patients with stage one GCT (33.3%) and those with disseminated disease (41.7%). Three of the 63 patients (4.8%) had Z-scores less than -2SD. Seventeen patients (27.0%) had Z-scores between -1 and -2 SD at either lumbar spine, hip or both.

Potential risk factors for bone loss in GCT

At baseline, hip BMD was only related to age (ß=-0.31, p=0.01) and weight (ß=0.43, p=0.001). One year after anti-cancer treatment, bone loss was not significantly related to the number of chemotherapy cycles, cumulative dose of individual chemotherapeutic agents or cumulative dose of corticosteroids administered as anti-emetic during chemotherapeutic treatment. The difference in BMD before and after chemotherapy in patients with metastatic disease remained after correction for age and weight.

There was no relationship between BMD and smoking or alcohol use. BMD changes were also independent of gonadal status, vitamin-D deficiency or β-CTX. Decrease of lumbar spine and hip BMD a year after anti-cancer treatment was not related to the number of chemotherapy cycles or dosage of individual chemotherapeutic agents or to the total dose of corticosteroids given as part of the antiemetic regimen. In patients with vitamin D deficiency (34.9%), lumbar spine and hip BMD were not significantly lower compared to BMD in patient with normal serum vitamin D concentrations. There was no relationship between either bone markers and BMD or any biochemical parameters including gonadal hormones and BMD in patients receiving chemotherapy.

Figure 2. Time profile graph of lumbar BMD (g/cm^2) and total hip BMD (g/cm^2) LSMs with 95% CI as error bars for both measurement groups (patients with stage one and patients with disseminated disease) at baseline and after anti-cancer treatment.



DISCUSSION

This is to our knowledge the first study examining the natural course of changes in BMD in GCT patients from baseline and up to five years after anti-cancer treatment. We have previously established in a cross-sectional study that silent vertebral fractures are prevalent in long-term survivors of GCT.[201] It was not clear at what stage of their follow up patients sustained these fractures. Our findings from this study suggest that patients with metastatic GCT experienced bone loss at the lumbar spine and total hip within the first year after chemotherapy and femoral bone loss did not recover up till 5 years after anti-cancer treatment. Bone loss at one year was independent of gonadal status, 25-hydroxy vitamin-D concentrations, smoking habits or changes in bone turnover markers.

In patient with non-metastatic GCT who underwent unilateral orchidectomy, there was no observed bone loss at either lumbar spine or total hip. BMD also remained unchanged in those patients who received adjuvant carboplatin after orchidectomy. Vitamin D levels were low in 23 percent of patients, and despite remaining uncorrected, were not related to the loss in BMD.

We did not measure potential deleterious effects of a single dose of carboplatin on the skeleton, so that it seemed appropriate to include the fifteen patients concerned in the group of patient with non-metastatic GCT who do not require chemotherapy. In keeping with our previous findings from a cross-sectional study in long term survivors of GCT [201] we show that a relatively high percentage of newly diagnosed GCT patients already experience evidence of bone loss suggesting that skeletal fragility may predate anti-cancer treatment. The decrease of in BMD of both femoral and lumbar spine in patients with metastatic GCT treated with chemotherapy is most likely an early direct effect of chemotherapy and/or concomitant corticosteroid treatment on bone remodelling cells or indirectly by deleterious effects on gonadal function. The design of the study did not allow to assess the relative contribution of the transient hypogonadism induced immediately after chemotherapy [9;30;184], which may have -in part- recovered to baseline values at one year.

Consistent with the known detrimental effects of chemotherapy on the germinal epithelium which may be accompanied by an increase in FSH levels and/or reduced sperm counts [8;206] FSH and LH increased significantly in GCT patients with metastatic disease one year after anti-cancer treatment, while serum estradiol and testosterone concentrations decreased albeit not significantly at 1 year as compared to baseline. Little is known about the gonadal status in patients with GCT before

the tumour is diagnosed, although there is some evidence that patients with the testicular dysgenesis syndrome [191] developed partial hypogonadism long before the diagnosis of GCT is established. This could explain why a relatively large percentage of these patients have abnormal BMD already at time of diagnosis of their GCT. These hypotheses need to be confirmed by studies addressing potential correction of bone loss in the long term by adequate correction of gonadal and vitamin D status.

Corticosteroids have been shown to exert multiple effects on osteoblasts as well as osteoclasts [207;208] culminating in bone degradation and a state of low bone turnover as shown in animal models [209] and may have contributed to the observed decline in BMD in survivors who underwent curative combination chemotherapy. Although cumulative doses were not large, treatment was intermittent and only given for 9 to 12 weeks to prevent emesis associated with chemotherapy.

Four other cross-sectional studies have reported BMD data in long-term survivors of GCT treated with and without chemotherapy. Brown *et al*.[51] performed a cross-sectional study in 165 testicular cancer survivors and 51 lymphoma patients and found no difference in BMD in GCT patient treated with or without chemotherapy. Consistent with these data 2 smaller studies in respectively 30 and 39 GCT survivors did not demonstrate deleterious effects of chemotherapy on bone mass of long-term GCT survivors.[50;52] On the other hand, Ondrusova *et al*.[53] reported a high prevalence of osteopenia and osteoporosis in a large cohort of 823 GCT survivors a median 89 months after orchidectomy and anticancer treatment. These last data correspond with our previously published finding in 199 GCT survivors, in which we demonstrate a high prevalence of osteopenia and osteoporosis of 41.7% and 5.5% respectively.[201]

Our study has some strengths as well as limitations. To our knowledge this is the first study addressing long term effects of chemotherapy on skeletal health in GCT survivors. Secondly, we measured BMD in GCT patient with and without distant metastasis, allowing us to measure the possible deleterious effect of intense 9 to 12 weeks of combination chemotherapy in these patients. We also measured the hormonal status and biochemical parameters of bone turnover and vitamin D status at one year allowing us to analyse for potential contributions of these risk factors in skeletal health in the long term, although we did not measure these parameters during the 12 months between baseline and one year. The main limitation of our study is the relatively small size of the cohort studied.

We have only included one bone resorption marker (β-CTX) in our serum analysis. Unfortunately, our laboratory was not able to determine procollagen type 1 N-terminal

propeptide (P1NP) at the start of this study. Serum P1NP values were therefore only available for the last two measurement moments. However, the available serum P1NP concentrations in the fifth year were positively correlated to serum β-CTX values (β=0.53; p=0.006). Thus at least during follow-up, bone resorption was accompanied by bone formation. And this suggests that the loss of BMD after chemotherapy administration is not due to osteoblast inactivity.

CONCLUSION

The prognosis of germ cell tumours is excellent largely due to the high rates of cure obtained with cisplatin-based combination chemotherapy in patients with metastatic disease. The hitherto unsuspected decline in BMD at one year post chemotherapy in metastatic GCT survivors which did not recover in the years thereafter may translate into increased morbidity in these patients, particularly considering their prolonged survival time. Combination-chemotherapy plus high doses of corticosteroids and transient partial hypogonadism may be the cause of the decline in BMD after curative chemotherapy. Pre-existing testicular dysgenesis syndrome might also be at the source of the disturbed bone quality observed in patients with GCT. Whether the observed changes in BMD are a predictor for risk of fractures remains to be established.

Based on our data we advise to screen GCT patients for skeletal abnormalities at diagnosis and to closely monitor their BMD after chemotherapeutic treatment to allow timely therapeutic intervention. Further studies are required to understand the relationship between GCT, intense curative chemotherapy and decline in BMD after chemotherapy.