Gonadal function in boys with newly diagnosed cancer before the start of treatment

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ABSTRACT

Study question: Are inhibin B and testosterone levels reduced in boys with newly diagnosed cancer prior to therapy?

Summary answer: Pretreatment serum levels of inhibin B and testosterone are significantly reduced in boys with newly diagnosed cancer, compared to reference values.

What is already known: Disease-related gonadal impairment has been demonstrated in girls and young women diagnosed with cancer, prior to therapy.

Study design, size, duration: We conducted a descriptive study in boys newly diagnosed with cancer between January 2006 and February 2014.

Participants/materials, setting, methods: Serum inhibin B and testosterone levels were determined in 224 boys, up to the age of 18 years, with newly diagnosed cancer prior to therapy. Hormone levels were compared with age-matched reference values. The cohort consisted of patients with acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), nephroblastoma, neuroblastoma and sarcoma.

Main results and the role of chance: This study demonstrates reduced serum levels of inhibin B in boys with newly diagnosed cancer, compared to reference values (standard deviation score (SDS) -0.9, $P < 0.001$). Median inhibin B level in patients was 103.5 ng/l (range 20-422). Of all patients, 78.6% showed inhibin B levels below the 50th percentile, and 58.5% had inhibin B levels below the 25th percentile. Serum testosterone levels were significantly lower than the reference range population (SDS -1.2, $P < 0.001$). Median testosterone level in pubertal patients was 7.3 nmol/l (range 0.1-23.6). No correlation with clinical signs of general illness and hormone levels were observed.

Limitations, reasons for caution: In this study, reproductive hormone levels were compared with age-matched reference values. Future studies may compare reproductive hormone levels with case controls.

Wider implications of the findings: Future longitudinal studies are necessary to determine whether pretreatment impaired gonadal function at the time of cancer diagnosis is an important determinant of ultimate recovery of spermatogenesis after treatment and later on in adulthood.

INTRODUCTION

Long-term survival of childhood cancer has steadily increased following optimized treatment regimens over the past decades[1]. As a result, the absolute number of survivors of childhood cancer is increasing[2]. Consequently, awareness of direct and long-term side effects after treatment of pediatric cancer is growing[3]. Gonadal dysfunction with the risk of impaired fertility is one of these long-term side effects in both childhood and adult cancer survivors[4,5]. Gonadal dysfunction depends on administered treatment modality as well as the total cumulative dosages[4]. Recently, we reported significantly reduced anti-Müllerian hormone (AMH) levels in girls with newly diagnosed cancer prior to treatment, indicating that not only gonadotoxic treatment but also the disease itself and the concomitant health status affects gonadal reserve in girls with cancer[6]. Currently, only scarce information is available about gonadal function in boys with newly diagnosed cancer before the start of treatment[7].

To assess gonadal function in men, semen analysis is considered to be the gold standard[4]. In addition, inhibin B and follicle-stimulating hormone (FSH) have been identified as reliable serum markers of spermatogenesis during adulthood, as significant associations between Inhibin B, as well as between FSH, and sperm concentrations have been reported[4,8-13].

Inhibin B is a dimeric hormone produced by Sertoli cells, which provides negative feedback on FSH secretion[8]. During the first months after birth, inhibin B levels are elevated as a result of transient activation of the hypothalamic-pituitary-testicular axis. After the postnatal peak, inhibin B levels gradually decline until the age of 2 years to a constant level during childhood. At the start of puberty, inhibin B levels increase until adult levels are reached. Both periods of high inhibin B production are associated with the presence as well as the proliferative activity of Sertoli cells[14-17]. It has been suggested that the number of Sertoli cells determines the spermatogenic potential later in life[14,15]. Therefore, serum inhibin B levels may provide a reflection of gonadal function even in young boys[14,16].

Sperm production requires testosterone production. This process of testosterone synthesis and secretion by Leydig cells in the testis, is stimulated by luteinizing hormone (LH)[18]. Testosterone levels show a similar increase as inhibin B following birth. Unlike inhibin B, testosterone levels rise to a peak at 1-3 months of age and then decline to barely detectable levels at 1 year of age till puberty, after which a second peak occurs during puberty. The postnatal as well as the pubertal peaks of testosterone levels follow the proliferation and maturation of Leydig cells[14].

Based on reports on disease-related gonadal impairment at time of diagnosis before cancer treatment in girls and young women, we hypothesized that similarly compromised gonadal dysfunction may occur in boys with newly diagnosed cancer. This would be similar to adult cancer patients where oligozoospermia is observed at time of diagnosis before any treatment has started[19-21]. The exact underlying mechanism is unknown yet. In boys

with cancer, such data on the effect of disease on gonadal hormone production, based on a substantial number of cases, is not available.

Knowledge of pretreatment gonadal function in boys with newly diagnosed cancer is of interest, as this may be the baseline for potential recovery of fertility after childhood cancer. Therefore, we evaluated gonadal function and disease-related determinants in boys with newly diagnosed cancer, using inhibin B and testosterone as markers of gonadal function.

MATERIALS AND METHODS

Study population

We included boys up to the age of 18 years with newly diagnosed cancer at our Paediatric Oncology Centre between January 2006 and February 2014. Patients with brain tumours were excluded due to potential hypothalamic-pituitary-axis dysfunction, and patients with germ cell tumours were excluded because of the localization of the tumour in the testes and/or direct influence on hormone production. We only included groups of tumour subtype with at least nine patients. Details on age, diagnosis, pubertal stage and clinical parameters were retrieved from patient record files. Pubertal status at diagnosis was assessed clinically and classified as prepubertal (Tanner stage 1), midpubertal (Tanner stage 2 – 3) and postpubertal (Tanner stage 4 – 5) as previously described[22]. Baseline inhibin B levels were measured in all subjects and testosterone, FSH and LH levels at time of presentation with cancer were measured in boys from Tanner stage 2 onwards, since these hormones are barely measurable before puberty[14,16]. Because of the small number of pubertal boys, testosterone levels were evaluated only in patients with acute lymphoblastic leukaemia (ALL), Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). To identify the determinants of the pretreatment hormonal deviations at diagnosis, we used the surrogate markers, body temperature, C-reactive protein (CRP) and haemoglobin (Hb) levels at diagnosis, as proxy for general health status. CRP has been shown to be sufficiently reliable in cohort studies as an acute phase protein and provides together with Hb a reflection of being chronically unwell[23]. Increased CRP as acute phase protein and Hb as a reflection of being chronically unwell are indeed not very hard indicators for disease as they are not specific. However, as anaemia and enhanced inflammation can be signs of an unhealthy status, and as these markers were available in the majority of all patients, we decided to include them as surrogate markers of being unwell. Using these surrogate markers as reflection of chronic well-being has been recently applied in a similar study with female childhood cancer patients[6]. Informed consent was obtained from all included patients and parents to use left-over material for additional research including this study, according to the standards of the Institutional Review Board.

Laboratory measurements

Serum samples were stored at -20° C until analysis. Inhibin B was used as a surrogate marker for gonadal function[4,8]. Inhibin B levels were measured using an enzyme-linked immunosorbent assay (inhibin B Gen II ELISA kit, Beckman Coulter, Inc. Brea, CA). Within-assay coefficients of variation (CVs) were 4.4% at 11.6 ng/L and 1.9% at 146.3 ng/L, respectively. Between-assay CVs for inhibin B were 14.3% at 15 ng/L and 11.4% at 162 ng/L. In addition, serum FSH and LH were determined with the Immulite assay (Siemens DPC, Los Angeles, CA, USA). Within-assay and between-assay CV were <6% and <9% and <5% and 11% for FSH and LH respectively. Serum total testosterone levels were determined using standard coated-tube radioimmunoassays (Siemens DPC) and liquid chromatography tandem mass spectrometry (LC-MS-MS) methods. Within-assay and between-assay variation coefficients were 3% and 4.5% for radioimmunoassays. For LC-MS, within-assay CVs were 6.1% at 0.029 nmol/L, 3.5% at 0.0073 nmol/L and 7.1% at 1.127 nmol/L. Between-assay CVs were 9.2% at 0.038 nmol/L, 4.2% at 0.08 nmol/L and 6.8% at 1.04 nmol/L. Hormone levels were compared with age-matched reference values from previously published studies[16,24-26].

Statistics

After log-transformation, inhibin B levels turned out to be normally distributed. Inhibin B and testosterone levels were analyzed as continuous variables. Standard deviation scores (SDS) were used to be able to adjust for age, using reference values of inhibin B and testosterone, which is important as these reproductive hormones vary with age and development stage[16,25,26]. The one-sample Wilcoxon signed rank test was performed to compare inhibin B, testosterone, FSH and LH SDS in boys with newly diagnosed cancer with reference values. The Kruskal-Wallis test was used to compare inhibin B and testosterone levels of cases in various diagnostic subgroups. Both the Kruskal-Wallis test and the Mann-Whitney *U*-test were used to explore the association between inhibin B SDS, respectively, testosterone SDS and Tanner stage. The correlation between SDS of reproductive hormones and the indirect markers of general health condition (body temperature, CRP levels and Hb levels at diagnosis) was studied using the Spearman rank correlation test. These analyses have been performed in both the entire cohort, as divided by Tanner stages. *P*-values <0.05 were considered significant. Statistical analyses were performed using the IBM Statistical Package for Social Sciences version 20 (IBM Corp., Armonk, NY, USA).

RESULTS

Reproductive hormone levels were analyzed in 224 boys with newly diagnosed cancer. The cohort consisted of patients with ALL, acute myeloid leukaemia (AML), HL, NHL, nephro-

blastoma, neuroblastoma and sarcoma. The median age of the boys was 5.7 years (range 0.1 – 17.7) (Supplementary data, Table S1).

The median pretreatment inhibin B level in boys with newly diagnosed cancer was 103.5 ng/l (range 20 – 422) (SDS -0.9), which was overall significantly low as compared to reference values ($P < 0.001$) (Table 1). Inhibin B levels were below the 50th percentile in 78.6% of all boys with childhood cancer, and below the 25th percentile in 58.5% (Fig 1). Twenty-eight (12.5%) had inhibin B levels of -2 SDS or lower at time of cancer diagnosis. Inhibin B SDS levels were low in all tumour types, with the exception of nephroblastoma patients (Fig. 2). No significant differences in inhibin B SDS levels were observed between cancer types (Table 1).

Table 1. Univariate analysis of pretreatment absolute Inhibin B levels and Inhibin B standard deviation scores (SDS) by diagnosis in boys presenting with cancer ($n = 224$).

Diagnosis (n)	Inhibin B (ng/l), median [range]	Inhibin B SDS, median [range]	p-value [a,b]
ALL (92)	96 [34-376]	-0.9 [-2.7 to 1.0]	<0.001
AML (31)	92 [20-273]	-1.2 [-3.7 to 0.7]	<0.001
HL (24)	135 [30-318]	-1.2 [-2.9 to 0.7]	<0.001
NHL (28)	124.5 [26-299]	-0.6 [-3.4 to 0.7]	0.003
Nephroblastoma (9)	103 [57-182]	-0.3 [-1.6 to 1.1]	0.26
Neuroblastoma (20)	99 [27-422]	-0.8 [-2.9 to 1.4]	0.01
Sarcoma (20)	109 [29-299]	-0.6 [-3.0 to 2.0]	0.02
Total group (224)	103.5 [20-422]	-0.9 [-3.7 to 2.0]	<0.001

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma. [a] *P*-values were calculated using the one-sample Wilcoxon signed rank test. [b] Inhibin B levels did not differ between diagnostic subgroups ($P = 0.29$: Kruskal-Wallis test).

Testosterone levels were studied in 38 pubertal boys diagnosed with ALL (n = 13), AML (n = 2), HL (n = 10), NHL (n = 10) and sarcoma (n = 3). The median pretreatment testosterone level in patients was 7.3 nmol/l (range 0.1 – 23.6), i.e., significantly lower compared to reference values (SDS -1.2, $P < 0.001$) (Supplementary data, Table SII). Only three subsets of tumour types (ALL, HL, NHL) showed appropirate numbers in order to analyse testosterone levels by diagnosis, separately (Supplementary data, Table SIII). In these three tumour types, we found relatively low testosterone SDS levels in all cases. In addition pretreatment testosterone SDS were reduced in all boys diagnosed with AML (range -0.92 to -1.21) and sarcoma (range -0.74 to -2.44), as well. Of the 38 pubertal boys with available testosterone levels, 4 (10.5%) boys showed testosterone levels of -2 SDS or lower before the start of treatment. Testosterone SDS levels showed no significant differences between the three malignancies (ALL, HL, NHL).

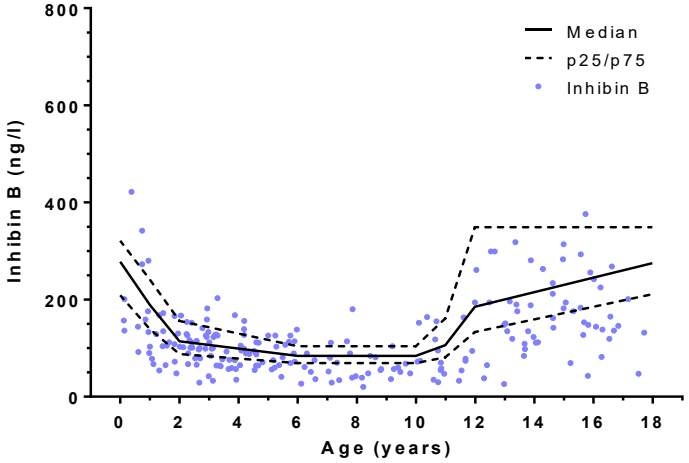

Figure 1. Pretreatment inhibin B levels in boys with newly-diagnosed cancer (n = 224) as compared to reference values (P < 0.001, one-sample Wilcoxon signed rank test). p75, p50 and p25 refer to 75th, 50th and 10th percentiles, respectively. Of all boys, 78.6% had inhibin B levels below the 50th percentile, and 58.5% had inhibin B levels below the 25th percentile.

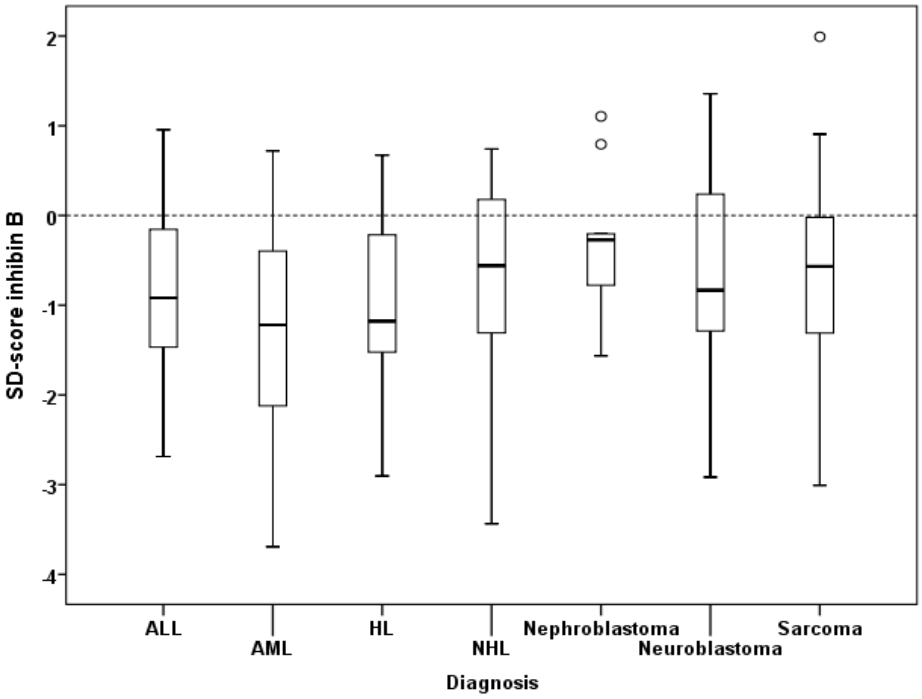

Figure 2. Pretreatment inhibin B standard deviation scores (SDS) by diagnosis in boys with cancer (n = 224). ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma.

Serum levels of FSH SDS at diagnosis did not differ significantly from reference values (SDS – 0.04, $P = 0.9$) (Supplementary data, Table SIV). LH SDS serum levels were significantly higher as compared to reference values (SDS 0.2, $P = 0.01$) (Supplementary data, Table SV).

Data on pubertal status were available in 200/224 boys. Of those, 162 were prepubertal (72.3%) and 38 boys (17%) were mid- and postpubertal (Supplementary data, Table SVI). At diagnosis, inhibin B and testosterone levels were significantly different ($P < 0.001$, $P = 0.03$) between the subcategories of pubertal status.

Median body temperature was 37.2 °C (range 35.0 – 39.9). At diagnosis median CRP level was 13.0 mg/l (range 1.0 – 296) and Hb level was 5.9 mmol/l (range 2.2 – 9.8) (Supplementary data, Table S1). None of the variables was significantly associated with inhibin B nor testosterone SDS levels (Supplementary data, Tble SVII). Also, when we stratified the analyses by pubertal status, none of the correlations were significant.

DISCUSSION

Over the past decades, optimization of paediatric cancer therapy has improved long-term survival of childhood cancer tremendously. Consequently, post-treatment quality of life, including fertility, is a critical issue for childhood cancer patients[4]. Pretreatment testicular function is of interest as this is the baseline for the gonadotoxicity and potential recovery to be monitored after childhood cancer treatment[19]. Also, in pubertal boys, it may reflect the feasibility and success rate of common but also novel preservation options, such as sperm cryopreservation or testicular sperm extraction (TESE). In adult males with cancer, the disease status was shown to hamper such options[4,27]. As in boys, this has not been systematically investigated. We studied baseline gonadal function in boys with newly diagnosed cancer before the start of treatment by hormonal evaluation.

This study shows for the first time that serum inhibin B and testosterone levels are reduced in young boys with newly diagnosed cancer prior to therapy. The cause of these reduced reproductive hormone levels is unclear. We anticipated that hormone alterations in boys with newly diagnosed cancer may be negatively affected by stress, downregulation by endocrine substances or cytokines produced by some tumours and metabolic conditions or malnutrition[28]. Such a phenomenon was shown to occur in girls with cancer that demonstrated reduced AMH levels before the start of treatment, and we could show that general health status accompanying the de novo cancer determined gonadal impairment[6]. Here, we show that in boys a correlation with disease state (as reflected by body temperature, CRP and Hb) does not seem to influence gonadal status, defined as reduced inhibin B and/or testosterone levels. The reason for this difference between boys and girls with this respect remains unclear. Previously, histological abnormalities such as Leydig cell atrophy and loss of Sertoli cells were observed in adult male HL patients at diagnosis[29]. Despite

these histological abnormalities, it might be that male gonads are less susceptible than female gonads for the impact of cancer by overall health status, but the exact mechanism is unclear. In adult male patients with HL, a high erythrocyte sedimentation rate (ESR) at diagnosis was associated with low semen quality[30]. It should also be considered that for male gonadal impairment testing, body temperature, CRP and Hb may not be the most relevant surrogate markers for general health status. We suggest that other possible markers, e.g., ESR, are potentially more relevant surrogate markers of general health in boys diagnosed with cancer before the onset of treatment[31,32].

Nevertheless, this study does show reduced pretreatment inhibin B levels in paediatric patients with HL, thereby confirming observations in adult studies[20,29,30,33], which revealed impaired gonadal function in male patients with HL before therapy, using testosterone[20,29,30,33]. Several other authors suggested that there may be an association between gonadal impairment and patients with HL; however, other types of disease are hardly studied[20,29]. In children, we show here that inhibin B levels are statistically signifcantly decreased in all tumour subtypes with the exception of nephroblastoma. Interestingly, in our group of pubertal boys, testosterone levels were low in ALL and NHL but not in HL patients.

In order to obtain more insight in the reason for impaired inhibin B and testosterone production, serum levels of FSH and LH were also measured in the subset pubertal boys at diagnosis. Although we expected increased pretreatment FSH levels, our results show that FSH levels did not differ significantly from reference values, thereby insinuating that the feedback mechanism (central axis) may be less sufficient. Hence, future studies are needed to further study this phenomenon. In contrast, we demonstrate elevated pretreatment LH levels, illustrating that reduced testosterone levels are indeed due to primary testicular failure, while Leydig cell function tends to compensate the relative testicular insufficiency. Previously, experimental studies in rats have suggested that inflammatory cytokines, such as interluekin-1, may play a role[34,35]. Also, in adult male patients, it has been suggested that Leydig cell suppression in case of acute stress may be considered as a protective mechanism for temporarily less vital functions, in order to preserve energy and metabolic substrates[36].

Apart from critical ilness, reduced inhibin B and testosterone levels could conceivably be due to stress. In adult males, it has been suggested that stress associated with the cancer process reduces reproductive function by the effect of stress hormones, which eventually suppresses the secretion of GnRH[37]. This may induce a disturbance of the hypothalamic-pituitary axis and therefore secondary testicular failure. This hypothesis might explain why FSH at diagnosis is not significantly higher compared to reference values. Though, this hypothesis is inconsistent with the demonstrated elevated levels of LH at diagnosis. Previously, experimental studies on the effect of acute stress in baboons and rats have suggested that glucocorticoids might have a suppressive effect on the steroidogenesis resulting in declined testosterone levels[38,39]. Therefore, acute stress may also affect gonadal function at testicular level. Measuring early morning stress hormone cortisol levels could provide

insight in the influence of stress on gonadal function in future studies. Unfortunately, fasting cortisol levels at diagnosis were not available in our patients.

A previous study showed normal inhibin B levels in 16 boys with childhood cancer, diagnosed with ALL, NHL, neuroblastoma, sarcoma and Wilms tumor, before the start of treatment[7]. We here show in a larger cohort of paediatric patients that pretreatment inhibin B levels are low as compared to normal controls. The discrepancy between these findings may be influenced by the previously limited sample size or a difference in tumor subgroups. As we present the first large series here, on pretreatment male gonadal function in children, we appreciate the fact that confirmation of our data in even larger cohorts of paediatric oncologic patients is important.

In this study, reproductive hormone levels were compared with age-matched reference values. The hormone levels of the reference serum used in our assays for calibration were within the range of the normal controls described in the literature, nevertheless measurement bias cannot be totally excluded. We suggest that future studies should compare reproductive hormone levels with case controls. Also we recommend to assess gonadal function in larger cohorts of pubertal patients for replication.

In summary, we show reduced inhibin B and testosterone levels in boys with newly diagnosed cancer already before starting treatment. The reason for the reduced levels of these reproductive hormones remains unclear. Future longitudinal studies are necessary to determine whether pretreatment impaired gonadal function at the time of cancer diagnosis is an important determinant of ultimate recovery of spermatogenesis after treatment and later on in adulthood.

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SUPPLEMENTAL DATA

Table SI. Characteristics of study population.

	n	Percentage (%)	median, [range]
Age (y)			5.7 [0.1-17.7]
Tumour subtype	224	100	
ALL	92	41.1	
AML	31	13.8	
HL	24	10.7	
NHL	28	12.5	
Nephroblastoma	9	4.0	
Neuroblastoma	20	8.9	
Sarcoma	20	8.9	
Pubertal stage	200	89.3	
Tanner 1	162	72.3	
Tanner 2-3	18	8.0	
Tanner 4-5	20	8.9	
Markers of general health			
Body temperature (°C)	130		37.2 [35.0-39.9]
CRP (mg/l)	123		13.0 [1.0-296]
Hb (mg/l)	215		5.9 [2.2-9.8]

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; Tanner 1, prepubertal; Tanner 2-3, midpubertal; Tanner 4-5, postpubertal; CRP, C-reactive protein; Hb, haemoglobin.

Table SII. Univariate analysis of pretreatment testosterone standard deviation scores (SDS) and absolute testosterone levels by diagnosis in boys with cancer (*n* = 33).

Diagnosis	Testosterone (nmol/l), median [range]	Testosterone SDS, median [range]	p-value[a,b]
ALL (13)	6.3 [0.1-16.4]	-1.6 [-2.0-0.2]	0.002
HL (10)	6.1 [0.3-23.6]	-1.2 [-2.0-1.0]	0.05
NHL (10)	10.8 [0.5-20.1]	-0.6 [-2.0-0.4]	0.01
Total group (33)	7.3 [0.1-23.6]	-1.2 [-2.0-1.0]	<0.001

ALL, acute lymphoblastic leukaemia; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma. [a]: *P*-values were calculated using the one-sample Wilcoxon signed rank test. [b]: Testosterone levels did not differ between diagnostic subgroups (*P* = 0.39: Kruskal-Wallis test).

Table SIII Univariate analysis of pretreatment testosterone standard deviation scores (SDS) and absolute testosterone levels according to pubertal stage in boys with cancer (*n* = 38).

Stage of puberty, (n)	Testosterone (nmol/l), median [range]	Reference value (nmol/l), median [range]	Testosterone SDS, median [range]	p-value [a]
P1G1 (162)	n.a.	n.a.	n.a.	n.a.
P2G2 (11)	1.7 [0.1-16.4]	5.3 [0.1-10.5]	-1.82 [-2.03-0.20]	0.004
P3G3 (7)	9.6 [1.4-23.6]	14.95 [0.4-29.5]	-0.74 [-1.86-1.03]	0.13
P4-5G4-5 (20)	8.0 [2.74-20.1]	17.5 [5.6-29.4]	-1.10 [-2.44-0.44]	<0.001
Total group (38)	7.34 [0.10-23.6]	n.a.	-1.21 [-2.44-1.03]	<0.001

P2G2, Tanner stage 2; P3G3, Tanner stage 3; P4-5G4-5, Tanner stage 4-5. n.a., not applicable. [a]: *P*-values were calculated using one-sample Wilcoxon signed rank test.

Table SIV. Univariate analysis of pretreatment absolute Inhibin B and testosterone levels by pubertal stage in boys with cancer.

Pubertal stage (n)	Inhibin B (ng/l)		Pubertal stage (n)	Testosterone (nmol/l)	
	median, [range]	p-value[a]		median, [range]	p-value[a]
Tanner 1 (162)	91 [20-422]	<0.001	**Tanner 1 (n.a.)**	n.a.	0.03
Tanner 2-3 (18)	152.5 [26-299]		**Tanner 2-3 (18)**	2.4 [0.1-23.6]	
Tanner 4-5 (20)	164.5 [47-314]		**Tanner 4-5 (20)**	8.0 [2.7-20.1]	

Tanner 1, prepubertal; Tanner 2-3, midpubertal; Tanner 4-5, postpubertal; [a]: *P*-value was calculated using the Kruskal-Wallis test. [b]: *P*-value was calculated using the Mann-Whitney *U*-test.

Table SV. Univariate analysis of pretreatment FSH standard deviation scores (SDS) and absolute FSH levels by diagnosis in pubertal boys with cancer (*n*=37).

Stage of puberty	FSH (nmol/l) median [range]	FSH SDS, median [range]	p-value[a]
P1G1 (162)	n.a.	n.a.	n.a.
P2G2 (10)	3.2 [0.9-5.2]	-0.1 [-0.5-0.5]	0.48
P3G3 (7)	3.5 [1.1-15.9]	0.2 [-0.4-3.4]	0.74
P4-5G4-5 (20)	3.6 [1.5-11.3]	-0.03 [-0.8-1.6]	0.90
Total group (37)	3.4 [0.9-15.9]	-0.04 [-0.8-3.4]	0.93

P2G2, Tanner stage 2; P3G3, Tanner stage 3; P4-5G4-5, Tanner stage 4-5 [a]: *P*-values were calculated using one-sample Wilcoxon signed rank test.

Table SVI. Univariate analysis of pretreatment LH standard deviation scores (SDS) and absolute LH levels by diagnosis in pubertal boys with cancer (*n*=38).

Stage of puberty	LH (nmol/l) median [range]	LH SDS, median [range]	p-value[a]
P1G1 (162)	n.a.	n.a.	n.a.
P2G2 (11)	1.1 [0.2-2.9]	-0.02 [-0.7-0.8]	0.48
P3G3 (7)	2.4 [1.1-9.7]	0.1 [-0.2-1.7]	0.24
P4-5G4-5 (20)	3.0 [1.1-7.7]	0.3 [-0.2-2.7]	0.001
Total group (38)	2.2 [0.2-9.7]	0.2 [-0.7-2.7]	0.01

P2G2, Tanner stage 2; P3G3, Tanner stage 3; P4-5G4-5, Tanner stage 4-5 [a]: *P*-values were calculated using one-sample Wilcoxon signed rank test.

Table SVII. Correlation between pretreatment Inhibin B and testosterone standard deviation scores (SDS) and markers of general health in boys with cancer (*n* = 224).

Pubertal Stage	Markers of general health	Inhibin B SDS, *R* (*p*-value)[a]	Pubertal Stage	Markers of general health	Testosterone SDS, *R* (*p*-value)[a]
Tanner 1 (162)	Body temperature (100)	-0.13 (0.22)	**Tanner 1 (162)**	Body temperature	**n.a.**
	CRP (91)	-0.08 (0.48)		CRP	
	Hb (157)	-0.04 (0.59)		Hb	
Tanner 2 (18)	Body temperature (7)	-0.57 (0.18)	**Tanner 2 (18)**	Body temperature (7)	-0.61 (0.15)
	CRP (10)	-0.38 (0.28)		CRP (10)	-0.02 (0.96)
	Hb (17)	0.49 (0.05)		Hb (17)	0.37 (0.15)
Tanner 3 (20)	Body temperature (13)	-0.16 (0.61)	**Tanner 3 (20)**	Body temperature (13)	0.30 (0.33)
	CRP (10)	0.25 (0.49)		CRP (10)	-0.12 (0.75)
	Hb (19)	0.07 (0.77)		Hb (19)	0.35 (0.14)
Total group (224)	Body temperature (130)	-0.10 (0.26)	**Total group (38)**	Body temperature (20)	-0.09 (0.70)
	CRP (123)	-0.10 (0.26)		CRP (20)	0.003 (0.99)
	Hb (215)	-0.06 (0.38)		Hb (36)	0.22 (0.21)

Tanner 1, prepubertal; Tanner 2, midpubertal; Tanner 3, postpubertal; CRP, C-reactive protein; Hb, haemoglobin, *R*, Correlation coefficient. [a]: Correlation coefficients and *p*-values were calculated using the Spearman rank correlation test.