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Original Paper

Low Serum Inhibin B Concentrations in Male Survivors of Childhood Malignancy

P.M. Lähteenmäki,[1] J. Toppari,[1,2] A. Ruokonen,[3] P. Laitinen[3] and T.T. Salmi[1]

[1]Department of Paediatrics; [2]Department of Physiology, University of Turku, FIN-20520 Turku, Finland; and [3]Department of Clinical Chemistry, University of Oulu, Oulu, Finland

The aim of this study was to assess the value of serum inhibin B in detecting male gonadal dysfunction in childhood cancer survivors. 27 male postpubertal (Tanner's pubertal stage G5 or P6) and 12 pubertal (≥G2) patients were drawn from the endocrine follow-up protocol of childhood cancer patients at the Paediatric Clinic of Turku University Hospital, Turku, Finland. The average time (mean ± S.D.) between the diagnosis and this study was 11.7 ± 4.5 years in the postpubertal and 7.0 ± 3.9 years in the pubertal group. Serum samples for the determination of follicle-stimulating hormone (FSH), luteinising hormone (LH), oestradiol, testosterone, and inhibin A and B dimers were collected. The demographic factors, pubertal stage and testicular size of the patient were measured at the same routine outpatient visit. Serum inhibin concentrations were correlated to testicular volume and gonadotrophin concentrations. Strong correlations were observed between testicular size ($r = 0.80$, $P < 0.001$) or FSH ($r = -0.58$, $P = 0.002$) and inhibin B concentration in the postpubertal group. Inhibin A was not detectable (< 2 pg/ml). Testicular volume measurement was accurately documented in 21 postpubertal subjects. Patients with small testicles (< 10 ml) had inhibin B concentrations under 42 pg/ml and those whose testicular size was over 13 ml had inhibin B concentrations exceeding 100 pg/ml. In all 12 pubertal survivors, serum inhibin B levels were ≥94 pg/ml, except in one case of testicular cancer where inhibin B was 8.1 pg/ml and the FSH concentration was elevated. Inhibin B seems to be an indicator of male gonadal function in postpubertal childhood cancer survivors and could be used in the estimation of gonadal function of male survivors earlier than testicular volume or semen analyses would be routinely possible. However, the correct cut-off level of serum inhibin B, as well as the details of inhibin B physiology during puberty, remain to be determined before semen analysis can be replaced by the measurement of inhibin B. © **1999 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

THE DEVELOPMENT of intensified chemotherapy has greatly improved the outcome of children with acute lymphoblastic leukaemia (ALL) and other malignancies. Currently, nearly 80% of new ALL patients will survive and the survival rate of patients with solid tumours is over 50%. However, cancer treatment may have important effects on multiple organ systems and the follow-up of childhood cancer patients should include the evaluation of gonadal function [1].

Correspondence to P. Lähteenmäki, e-mail: paivi.lahteenmaki@utu.fi
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Ovarian cells in young women and Leydig cells in men are relatively resistant to the effects of chemotherapy, whereas male germ cells are extremely sensitive to several classes of chemotherapeutic agents [2–5]. However, radiation can result in germ cell damage in both men and women, as well as in Leydig cell damage if given in sufficient doses [6–10]. Infertility from no other cause than childhood cancer treatment was observed in 15% of patients out of 2238 former cancer patients [11], but chemotherapy using alkylating agents, with or without radiation to sites below the diaphragm, was in men associated with a fertility deficit of approximately 60%. A Scandinavian follow-up study of 299

childhood leukaemia survivors reported female patients having nearly normal reproductive patterns during young adulthood [12]. The study also indicated that only 1% of male survivors of ALL, compared with 23% of female survivors, had children. Approximately 16% of male survivors of childhood ALL have been shown to have poor fertility as assessed either directly from semen samples or indirectly from increased levels of gonadotrophins and reduced testicular volume [13, 14]. Testicular histology, studied during or just after ALL chemotherapy, showed a severely depressed tubular fertility index in 41% of patients [15]. However, with increasing time from completion of chemotherapy the tubular fertility index seemed to improve.

Inhibin is a glycoprotein hormone and growth factor that suppresses follicle-stimulating hormone (FSH) secretion and was isolated for the first time in 1985 from bovine and porcine follicular fluid [16, 17]. The principal source of inhibin secretion in the testis is Sertoli cells, although Leydig cells also secrete inhibin [18]. Mature inhibin is a 31–32 kDa heterodimeric glycoprotein composed of an α-subunit and one of two possible β-subunits, β_A or β_B, joined by disulphide bridges [19]. Only the dimeric species are biologically active (inhibin A and inhibin B). FSH upregulates inhibin α-subunit production in Sertoli cells and luteinising hormone (LH) in Leydig cells [20, 21]. In the rat, inhibin α- and β_B-subunit production is maximal at the stages of spermatogenesis that are most sensitive to FSH, suggesting that inhibin B is the predominant dimer, regulated by FSH [22, 23]. This is supported by the clinical findings that in men dimeric inhibin B is the dominant form present in serum [24]. Furthermore, inhibin B is undetectable in the sera of orchiectomised men and inhibin B levels will increase following FSH administration in healthy men [25] and prepubertal hypogonadotrophic boys [26]. Thus, inhibin B plays a central role in feedback regulation between the testis and the pituitary. FSH stimulates the production of inhibin B in the testis and inhibin B inhibits the secretion of FSH.

The recognition that inhibin B is the dominant form of dimeric inhibin in men and that serum levels of inhibin B correlate with FSH and possibly with sperm density offers hope that inhibin may be a direct marker of seminiferous tubular damage and may aid in the prediction of fertility [27–30]. In order to assess the status of inhibin B in detecting male gonadal dysfunction compared with indirect clinical methods, gonadotrophin concentration and testicular volume, inhibin B was studied in postpubertal survivors of childhood cancer. Furthermore, a smaller population of pubertal (G2 to G4) boys was studied to determine the level of inhibin B in relation to gonadotrophins.

PATIENTS AND METHODS

The study subjects were boys who, since January 1994, had participated in the endocrine follow-up protocol for childhood cancer patients at the Paediatric Clinic of Turku University Hospital, Turku, Finland. Up to the end of May 1996, a total of 100 survivors (50 boys) had entered the follow-up protocol. There were 27 boys with Tanner's pubertal stage G5 or P6 and 12 boys with stage G2 to G4. At the regular outpatient visit, a blood sample was driven from an antecubital vein between 08.00 and 13.00 h. Serum samples for determination of FSH, LH, oestradiol, testosterone, and inhibin A and B dimers were collected. At the same visit the height, weight, sitting height, pubertal stage according to Tanner [31] and testicular size (in mm) of the patient were measured. In order to improve the compliance of the sexually inactive subjects, no semen samples were requested. The mean time between the diagnosis and this study was 11.7 years (S.D. 4.5) in the postpubertal group and 7.0 years (3.9) in the pubertal group.

Testicular size was measured by a ruler and volume calculated using the formula: volume = 0.52 × longitudinal axis × squared transverse axis [32]. Both testicles were measured and the resulting testicular volume represents a mean of the two calculated values. A Finnish mean value (90% range) for testicular volume is 28 ml (20–40 ml) [33, 34]. Serum FSH and LH were measured by solid-phase two-site time-resolved fluoroimmunometric assay (AutoDELFIA™, Wallac, Turku, Finland). Testosterone and oestradiol were measured by coated tube radioimmunoassay (SPECTRIA™, Orion Diagnostica, Turku, Finland). In 1996 it became possible to analyse inhibin A and B dimers separately by commercial enzyme-linked immunosorbent assays (ELISA) using monoclonal antibodies raised against the inhibin β_A and β_B-subunits in combination with an enzyme-labelled antibody raised against the inhibin α-subunit, as described previously [35, 36] (Serotec, Oxford, U.K.). The previously collected and frozen serum samples were analysed by both assays.

Results are reported as means and standard deviations (S.D.). Correlation was assessed by the Pearson correlation coefficient or by Spearman correlation coefficient in cases where the values were not normally distributed. Statistical significance was defined as a P-value < 0.05. Computations were performed with the SAS system for Windows, release 6.12. The Joint Commission on Ethics of Turku University and Turku University Central Hospital approved the study protocol.

RESULTS

The characteristics of the postpubertal subjects are shown in Table 1 and those of the pubertal subjects in Table 2.

Postpubertal survivors

Testicular volume and the serum concentrations of FSH, LH, testosterone, oestradiol and inhibin B are presented in Table 3. Abnormal FSH and LH concentrations were detected in 9 and in 4 out of 27 postpubertal survivors, respectively. One postpubertal survivor had testosterone substitution and another two survivors had subnormal testosterone concentrations, all having normal LH concentrations. Inhibin-A was not detectable (< 2 pg/ml) in the study subjects.

Testicular volume measurement was accurately (both length and width) documented for 21 postpubertal survivors and the mean (± S.D.) value was 11.3 ± 6.3 ml in this population, 11.9 ± 7.2 ml in ALL survivors and 10.4 ± 4.9 ml in the others. 17 were either married or had a regular girlfriend but none had yet fathered a child.

All postpubertal survivors with small testicles (< 10 ml) had inhibin-B concentrations of < 42 pg/ml and 6/7 had abnormal FSH (Table 3). In survivors with a testicular volume > 13 ml, the inhibin-B level was > 100 pg/ml and the FSH level was normal. All postpubertal survivors with a testicular volume < 10 ml had been treated with alkylating agents and two had also received testicular irradiation. In the group of men with a testicular size of 10–13 ml, there was one survivor (pt 20) who had an inhibin B level < 42 pg/ml. He was postpubertal at the time of diagnosis but had been treated with a MOPP regimen (nitrogen mustard, vincristine,

Table 1. *Characteristics of the postpubertal subjects*

Patient	Diagnosis	Year of diagnosis	Age (years) At diagnosis	Age (years) At study	CNSI	TESI	TBI	Other local irradiation	Alkylating agent	BMI	Height (SDS)
1	ALL	1979	9.2	24.3	No	Yes	Yes	No	Cycloph.	20	−1.0
2	ALL	1979	2.5	18.0	Yes	No	No	No	Cycloph.	24	1.2
3	ALL	1984	4.0	14.3	No	No	No	No	No	21	1.1
4	ALL	1980	4.4	18.4	No	No	No	No	No	23	1.8
5	ALL	1989	15.0	19.8	Yes	No	No	No	Cycloph.	19	0.4
6	ALL	1976	7.5	26.0	Yes	No	No	No	Cycloph.	18	1.2
7	ALL	1979	8.8	23.4	No	No	No	No	No	22	1.3
8	ALL	1985	11.3	20.5	No	No	No	No	No	21	2.3
9	ALL	1980	2.1	16.4	No	No	No	No	No	20	1.4
10	ALL	1981	7.6	20.7	No	No	No	No	No	21	−0.5
11	ALL	1975	3.1	21.8	Yes	No	No	No	Cycloph.	17	−1.9
12	ALL	1982	6.7	18.7	No	No	No	No	No	20	0.6
13	ALL	1981	4.5	17.7	Yes	No	Yes	No	Cycloph.	16	−2.4
14	ALL	1983	11.2	22.3	Yes	Yes	No	No	Cycloph.	25	−0.5
15	ALL	1985	10.8	19.2	No	No	No	No	No	23	−1.0
16	HD	1983	7.3	19.0	No	No	No	Neck/mediast.	MOPP	21	−1.5
17	HD	1982	7.3	19.7	No	No	No	Upper chest	No	25	−2.2
18	HD	1994	15.2	15.8	No	No	No	Upper chest	No	17	0.4
19	HD	1987	12.4	19.4	No	No	No	No	MOPP	20	−2.0
20	HD	1987	15.9	23.0	No	No	No	Neck/mediast.	MOPP	22	0.0
21	HD	1990	15.3	20.5	No	No	No	No	No	23	0.1
22	HD	1985	11.7	20.1	No	No	No	No	MOPP	23	3.2
23	NHL	1987	11.4	18.8	No	No	No	No	COMP	18	−1.0
24	NHL	1978	3.9	19.6	No	No	No	Vertebra LII	Cycloph.	22	0.6
25	NHL	1982	6.7	18.5	No	No	No	No	LSA2-L2	18	0.0
26	NHL	1975	1.5	20.5	No	No	No	No	COAP	22	−0.1
27	NHL	1977	4.8	20.5	No	No	No	Abdomen	LSA2-L2	25	−1.4

Mean ± S.D. for age at diagnosis was 8.2 ± 4.4 years, for age at study 19.9 ± 2.5 years, for body mass index (BMI) 20.9 ± 2.5 and for standard deviation score (SDS) of height 0.0 ± 1.4. CNSI, central nervous system irradiation; TESI, testicular irradiation; TBI, total body irradiation; ALL, acute lymphoblastic leukaemia; HD, Hodgkin's disease; NHL, non-Hodgkin's lymphoma; mediast., mediastinum; cycloph., cyclophosphamide; MOPP, nitrogen mustard, vincristine, prednisone and procarbazine; COMP, cyclophosphamide, vincristine, methotrexate and prednisone; COAP, cyclophosphamide, vincristine, doxorubicin and prednisone; LSA2-L2, a 12-drug regimen containing cyclophosphamide.

Table 2. *Characteristics of the pubertal subjects*

Patient	Diagnosis	Age at study (years)	Time since diagnosis (years)	CNSI	Other local irradiation	Alkylating agent	BMI	Height (SDS)	Testicular volume (ml)	S-Inhibin B (pg/ml)	S-FSH	S-LH	S-oest.	S-test.
1	ALL	11.5	5.1	Yes	No	Cycloph.	20	2.0	5.3	174.6	0.91*	0.78	40	0.7
2	ALL	14.1	12.9	No	No	No	19	0.0	i.d.	137.3	1.3	0.82	40	1.2
3	ALL	13.2	8.4	No	No	No	26	−0.5	6.9	176.3	3.3	1.2	40	1.5
4	ALL	12.2	7.9	No	No	No	19	1.4	5.4	94.0	2.8	0.73	40	1.9
5	HD	13.1	4.0	No	No	MOPP/ABVD	19	−0.2	4.2	118.0	9.8*	3.9	40	12.0*
6	NHL	12.0	3.9	Yes	No	LSA2-L2	18	1.0	5.6	149.9	5.7	2.2	40	7.5
7	NHL	12.2	4.0	No	No	COMP	27	1.0	i.d.	129.4	1.6	0.29	38	5.0
8	Wilms'	14.3	13.4	No	Yes	No	17	−1.0	6.1	195.7	2.5	2.1	40	5.9
9	Wilms'	16.0	11.7	No	Yes	No	19	−0.5	12.6	129.5	3.8	1.7	43	16.0*
10	Wilms'	14.5	7.9	No	Yes	No	17	−1.0	6.2	149.8	3.3	2.8	40	14.0*
11	Embr.ca (tes)	14.0	2.2	No	No	Cycloph.	22	0.0	6.6	8.1	16.0*	5.9*	40	2.3
12	Astrocytoma	11.5	3.0	Yes	No	No	22	1.0	11.4	213.4	4.7	1.4	40	9.0

Mean ± S.D. for age at diagnosis was 6.2 ± 3.2 years, for age at study 13.2 ± 1.4 years, for body mass index (BMI) 20 ± 3.3, for standard deviation score (SDS) of height 0.3 ± 0.9, and for testicular volume 7.0 ± 2.7 ml. Embr.ca (tes), embryonal carcinoma (testes); ABVD, doxorubicin, bleomycin, vinblastine and dacarbazine. Reference ranges: serum follicle-stimulating hormone (S-FSH), 1–7 IU/l; serum luteinising hormone (S-LH), 0.2–5.0 IU/l; serum oestradiol (S-oest.), < 114 pmol/l; serum testosterone (S-test), 0.6–11 nmol/l. i.d., inaccurately documented. For other abbreviations see Table 1. *Abnormal levels.

Table 3. Serum hormone concentrations and testicular size of the postpubertal subjects

Patient	Diagnosis	Testicular volume (ml)	S-oest.	S-test.	S-LH	S-FSH	S-Inhibin-B (pg/ml)
Testicular volume < 10 ml							
1	ALL	0.1	123	44.0†	0.1†	0.2†	3.2
6	ALL	8.3	77	13.0	12.0†	21.0†	7.8
13	ALL	1.5	87	10.0	5.4	13.0†	23.8
14	ALL	1.4	51	11.0*	0.9	2.2	14.9
16	HD	3.5	40	6.4†	6.9	16.0†	5.7
19	HD	6.1	40	16.0	2.9	16.0†	36.8
23	NHL	7.7	55	14.0	7.2†	20.0†	41.9
Testicular volume 10–13 ml							
5	ALL	12.9	70	22.0	2.1	2.6	70.4
10	ALL	11.0	40	14.0	3.3	1.8	145.2
15	ALL	11.6	52	18.0	3.4	13.0†	62.6
17	HD	13.0	40	11.0	3.0	4.4	162.2
20	HD	13.0	99	20.0	2.3	5.9	16.4
25	NHL	10.6	80	23.0	1.5	4.7	43.5
26	NHL	10.1	53	12.0	3.0	3.4	106.4
Testicular volume > 13 ml							
3	ALL	17.1	40	13.0	2.0	4.8	135.2
4	ALL	21.0	54	19.0	2.7	3.5	227.4
7	ALL	19.4	114	34.0	4.1	4.1	238.1
8	ALL	16.3	40	14.0	6.4	2.4	208.4
9	ALL	14.6	63	14.0	2.1	3.9	201.5
12	ALL	19.0	60	12.0	5.2	3.5	111.0
21	HD	19.6	65	23.0	2.0	2.2	146.5
Testicular volume i.d.							
2	ALL	i.d.	78	27.0	3.4	1.8	146.1
11	ALL	i.d.	60	17.0	3.6	7.0	35.8
18	HD	i.d.	40	8.4†	1.7	1.6	235.9
22	HD	i.d.	83	23.0	7.9†	18.0†	2.3
24	NHL	i.d.	55	10.0	5.7	16.0†	36.5
27	NHL	i.d.	94	13.0	5.7	8.9	75.6
Mean		11.3	65	17.1	3.9	7.5	94.1
S.D.		6.3	24	8.2	2.6	6.5	79.5

*A survivor with testosterone substitution (included because testicular volume was the main determinant in comparisons with serum inhibin B). i.d., inaccurately documented (missing width, length or both). For other abbreviations see Tables 1 and 2. Reference ranges: S-oestradiol, < 114 pmol/l; S-testosterone, 10–33 nmol/l; S-LH, 0.4–7.0 IU/l; S-FSH, 1.1–10.5 IU/l. †Abnormal levels.

prednisone and procarbazine). In the group of 6 men with inaccurately documented testicular size there were 3 survivors with an inhibin B level < 42 pg/ml, 2 of whom also had high FSH levels. There were only 2 postpubertal survivors treated with alkylating agents having inhibin B levels > 100 pg/ml (pt 26 106.4 pg/ml, pt 2 146.1 pg/ml). The lowest inhibin B value (pt 22 2.3 pg/ml) was measured in a man treated with a MOPP regimen. The 7 survivors with the highest inhibin B levels (≥ 146.5 pg/ml) had not been treated with alkylating agents or irradiation other than in the upper chest region (pts 4, 7, 8, 9, 17, 18 and 21).

There was a strong positive correlation between testicular size and inhibin B concentration (Pearson's $r = 0.80$, $P < 0.001$; Figure 1a). Inhibin B was negatively correlated to FSH values ($r = -0.58$, $P = 0.002$; Figure 1c), whereas correlation to LH ($r = -0.27$, $P = 0.12$) or testosterone ($r = 0.00$, $P = 0.99$) was not found (data not shown). Also in the group of ALL survivors, the correlation between inhibin B and FSH (Figure 1d) was statistically significant ($r = -0.70$, $P < 0.05$) if the men (pts 1 and 14) with clear testicular irradiation-induced gonadal atrophy but low gonadotrophin values were excluded. Testicular size was not correlated to testosterone values ($r = 0.06$, $P = 0.80$; data not shown) and the negative correlation to FSH concentration did not reach statistical significance ($r = -0.39$, $P = 0.08$; Figure 1f) except for solid tumour survivors ($r = -0.78$, $P = 0.02$; data not shown). If the patients who received testicular irradiation (1 and 14) were excluded, the correlation between testicular volume and FSH was statistically significant, both overall ($r = -0.71$, $P < 0.05$) and in the ALL group ($r = -0.64$, $P < 0.05$).

Pubertal survivors

Testicular volume and the serum concentrations of FSH, LH, testosterone, oestradiol and inhibin-B are presented in Table 2. Abnormal FSH and LH concentrations were detected in 3 and in 1 out of 12 pubertal survivors, respectively. The testosterone concentration was low for the pubertal stage in the patient who had an elevated serum LH concentration (pt 11). Serum FSH concentration was abnormal in 3 out of 5 survivors who had been treated with alkylating agents. Only one of them had a clearly low concentration of serum inhibin B (pt 11). All other pubertal study subjects had serum inhibin B concentrations ≥ 94.0 pg/ml. Inhibin-A was not detectable (< 2 pg/ml) in the study subjects. As expected, there was no significant correlation either between testicular size and inhibin B concentration ($r = 0.25$, $P = 0.45$; data not shown) or

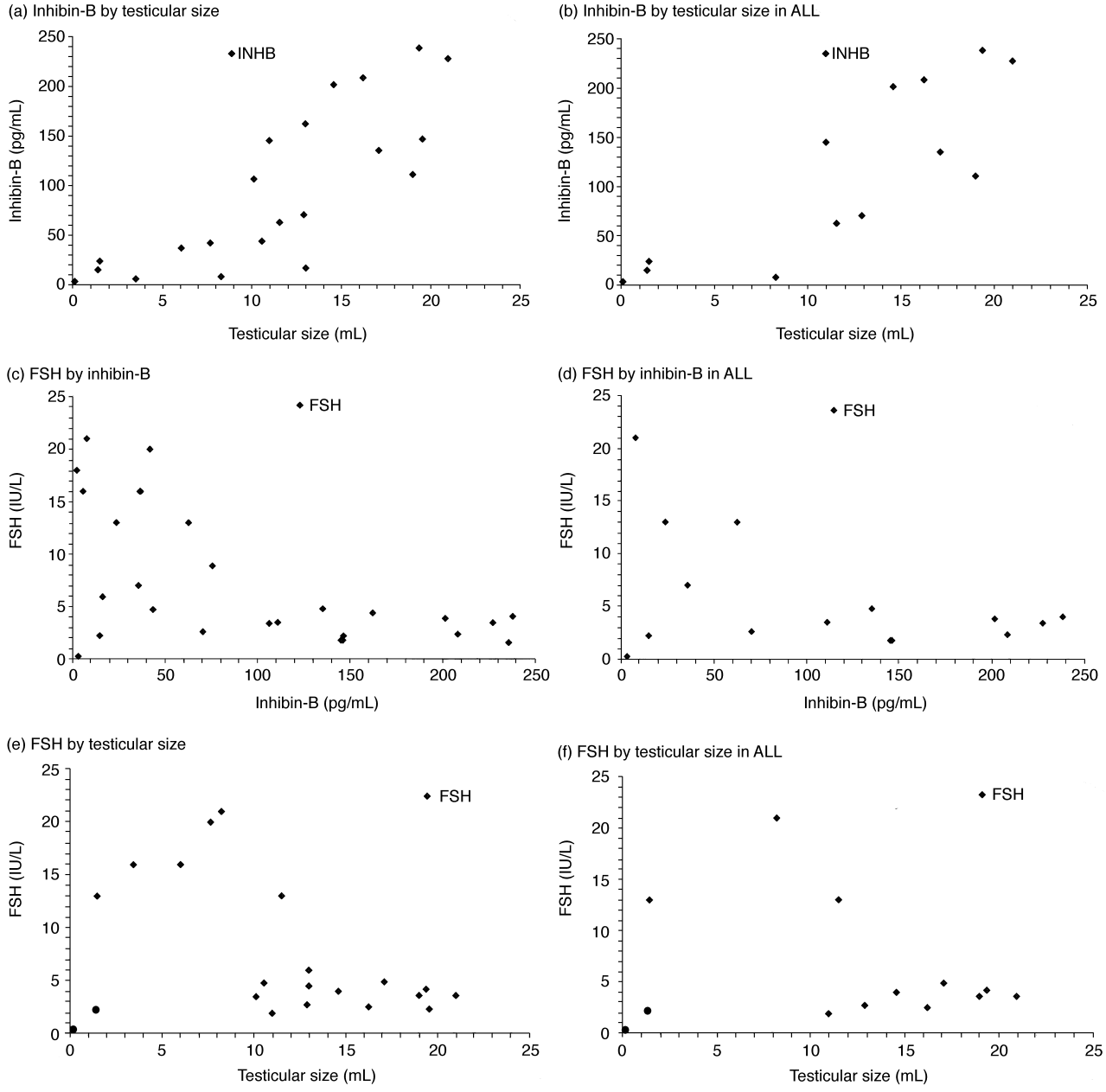

Figure 1. Serum concentrations of inhibin B (INHB), follicle-stimulating hormone (FSH) and testicular volume for all postpubertal survivors of childhood cancer (a, c, e) and for those with acute lymphoblastic leukaemia (ALL) (b, d, f). (a) and (b) demonstrate the correlation between testicular volume and inhibin B. An inverse correlation between FSH and inhibin B is evident in survivors with low inhibin B concentrations (c, d). FSH concentrations were high for survivors with small testicles (e, f), except for two gonadotrophin-deficient subjects (pts 1 and 14).

between inhibin B and FSH concentration ($r = -0.26$, $P = 0.42$; data not shown). Testicular size had some correlation to testosterone values but this was not significant ($r = 0.32$, $P = 0.36$).

DISCUSSION

This is the first report on inhibin B in childhood cancer survivors. Strong correlations between testicular size or FSH and inhibin B concentration were observed in the postpubertal survivors. The postpubertal patients with small testicles (< 10 ml) had inhibin B concentrations under 42 pg/ml and when testicular size was over 13 ml the inhibin B concentration exceeded 100 pg/ml. In a previous study the men with elevated FSH concentrations had a mean inhibin B concentration of 21.1 pg/ml [95% confidence interval (CI) 13.6–33.5] [24]. In that study for healthy men the inhibin B concentration was, on average, 135.6 pg/ml (95% CI 108.4–169.4) and the mean concentration of inhibin B in infertile men was 66.0 pg/ml (95% CI 45.8–95.3). In Finland inhibin B levels for 16 postpubertal healthy men (aged from 16 to 36 years) were studied using the same commercial assay (Serotec) as in the present study (Jäösteläinen, 1998, Kuopio, Finland). In that study group the mean ± S.D. inhibin B level was 153.2 ± 39.5 ng/l (range 75–232 ng/l). Another study followed-up 12 adult male patients with haematological malignancies during treatment [37]. It was found that inhibin B levels decreased significantly by 1 month from a mean ± S.E. baseline level of 273.2 ± 32.8 pg/ml, reaching a nadir of 52.6 ± 15.3 pg/ml at 4 months ($P < 0.0001$). FSH

levels increased within the first month from a baseline level of 3.9 ± 0.6 IU/l, reaching a peak level of 22.4 ± 3.3 IU/l at 4 months ($P < 0.0001$). FSH and inhibin B were significantly and inversely correlated ($r = 0.69$, $P < 0.0001$). The present data concur with these findings, suggesting that men with small testicles and low inhibin B levels will most probably remain infertile.

The control of gonadotrophin secretion is mediated by neuroendocrinological mechanisms in childhood, but at the onset of puberty the inhibitory mechanisms mediated by the gonad become more important [38]. The golden standard of fertility studies in men is semen analysis, but in larger population studies semen samples are difficult to obtain, at least within sampling frames that allow comparisons. Indirect evaluation for gonadal dysfunction can be made by assessing pituitary production of LH and FSH, both of which will be elevated in complete gonadal failure. However, when both hypothalamic/pituitary function and gonadal function are disturbed, this indirect indication of gonadal failure cannot be used. It is also known that histologically defined hypospermatogenesis [39] or even azoospermia [40] can be present when normal FSH levels are documented. In the postpubertal stage, testicular size correlates well with both higher LH and FSH concentrations and sperm production [33, 41]. In a sample of 33 male long-term survivors of childhood cancer, it was found that men with abnormally small (< 13 ml) testicular volume and supranormal FSH concentration (> 10 IU/l) were azoospermic [42], whereas patients with normal testicular volume and FSH concentration were normospermic. Siimes and coworkers [34] reported that the probability of normospermia is 50% in patients with normal testicular volume (> 20 ml) and FSH concentration (< 10 IU/l) and 0% if testicular volume is < 10 ml and serum FSH is > 10 IU/l. The probability of normospermia was 11% in patients outside these categories. The abnormal histology of the germinal epithelium has been correlated with elevated serum FSH concentration and small testicular volume after leukaemia treatment [43]. In the present study postpubertal survivors with small testicles had high FSH levels, with the exception of two survivors with hypogonadotrophic hypogonadism and one with testosterone substitution after testicular and central nervous system (CNS) irradiation. The data confirm previous findings that postpubertal males with testicular volume < 10 ml often have abnormal serum FSH concentrations.

However, direct serum biomarkers of spermatogenesis are of interest. After the isolation and purification of inhibin in 1985, radioimmunoassays displaced *in vitro* bioassays in inhibin measurement [44]. Later, it became apparent that free α-subunits exist in serum without biological activity and the interpretation of results obtained with α-subunit assays had to be cautious and limited [45]. Recently, a new generation of two-site assays has been developed, including assays specific for inhibin A and inhibin B [24, 35, 36, 46]. These new ELISA formats have shown that inhibin A is generally undetectable in men and that the patterns of circulating inhibin A and inhibin B differ from each other during the human menstrual cycle [24, 25, 36, 47]. In healthy boys serum inhibin B levels increase early in puberty, and late puberty (from stage III) is characterised by a negative correlation between inhibin B and FSH [48]. Raivio and coworkers reported serum inhibin B and gonadotrophin concentrations in 38 healthy boys during their progression through puberty [49]. In that study, there was a plateau in serum inhibin B concentrations after pubertal stage G2 and the inverse correlation between inhibin B and FSH was seen at stage G4 ($r = -0.57$, $P < 0.001$). According to a new Danish study, inhibin B secretion is independent of the presence of spermatocytes before puberty, but becomes dependent on these cells during puberty [50]. The authors have suggested that inhibin β_B expression occurs in pachytene spermatocytes after puberty, which would explain the finding. The present findings are also in accordance with this notion and, thus, inhibin B could be a direct indicator of the presence and quantity of advanced spermatogenic cells (meiosis) from Tanner stage 3 of puberty onwards.

Several studies have suggested impaired fertility for 51–67% of childhood solid tumour survivors [4, 10, 11, 51, 52]. In a study of survivors of childhood Hodgkin's disease (HD), depending on the stage of disease and associated with the intensity of chemotherapy, 28.9%, 50.0% or 62.5% of the patients had elevated basal FSH levels [2]. Irreversible azoospermia or oligospermia were reported in 63% and 32% of survivors of childhood HD, respectively [5]. Extrapolation with serum inhibin B below 42 pg/ml gives an estimate of impaired fertility for 50% of HD or non-Hodgkin lymphoma (NHL) survivors. If the cut-off level was 75 pg/ml, the lowest normal in a sample of Finnish healthy men, 67% of HD or NHL survivors would have had impaired fertility. These estimates on male fertility are in the same order of magnitude as previous studies have suggested. For ALL survivors, the same extrapolations gave increased values of subfertility, 33 or 47%, compared with previous reports. These ALL survivors were followed up for ≥ 11 years or ≥ 4.8 years after diagnosis, respectively.

The follow-up period of pubertal survivors was shorter than in the postpubertal group and this may have contributed to the finding of well-preserved serum inhibin B concentrations in the pubertal study group. However, among this population there were no survivors of high-risk ALL or survivors of HD treated with MOPP for more than three cycles. Therefore, also the risk of gonadal failure was lower for the study group of pubertal childhood cancer survivors. However, it has not yet been definitely proven whether inhibin B reflects the function of germ cells already at pubertal stages G2 and G3 [50].

According to the findings of this study, combined with reports on serum inhibin B in Finnish healthy men as well as with reports on correlations between testicular size, sperm quality and gonadotrophins, it can be concluded that serum inhibin B seems to be a direct indicator of male gonadal failure in postpubertal childhood cancer survivors. The cellular origin of inhibin B secretion during puberty is still unconfirmed, but previous evidence on the inverse correlation of inhibin B and FSH after stage G3 suggests that inhibin B determination might provide an opportunity to make direct estimations of a survivor's fertility, even before adulthood and sexual activity (i.e. before requests for sperm samples are appropriate). Serum inhibin B measurement could also be useful in cases where sperm samples are not available, e.g. for cultural or ethical reasons. However, the correct cut-off level of serum inhibin B remains to be determined in a study of adult survivors with sperm samples as a reference.

1. Meister LA, Meadows AT. Late effects of childhood cancer therapy. *Curr Probl Pediatr* 1993, **23**, 102–131.

2. Brämswig JH, Heimes U, Heiermann E, Schlegel W, Nieschlag E, Schellong G. The effects of different cumulative doses of chemotherapy on testicular function. *Cancer* 1990, **65**, 1298–1302.
3. Ortin TT, Shostak CA, Donaldson SS. Gonadal status and reproductive function following treatment for Hodgkin's disease in childhood: the Stanford experience. *Int J Radiat Oncol Biol Phys* 1990, **19**, 873–880.
4. Rautonen J, Koskimies AI, Siimes MA. Vincristine is associated with the risk of azoospermia in adult male survivors of childhood malignancies. *Eur J Cancer* 1992, **28A**, 1837–1841.
5. Heikens J, Behrendt H, Adriaanse R, Berghout A. Irreversible gonadal damage in male survivors of pediatric Hodgkin's disease. *Cancer* 1996, **78**, 2020–2024.
6. Lushbaugh CC, Casarett GW. The effects of gonadal irradiation in clinical radiation therapy: a review. *Cancer* 1976, **37**, 1111–1125.
7. Brauner R, Czernichow P, Cramer P, Schaison G, Rappaport R. Leydig-cell function in children after direct testicular irradiation for acute lymphoblastic leukemia. *N Engl J Med* 1983, **309**, 25–28.
8. Matus Ridley M, Nicosia SV, Meadows AT. Gonadal effects of cancer therapy in boys. *Cancer* 1985, **55**, 2353–2363.
9. Sklar CA, Robison LL, Nesbit ME, *et al*. Effects of radiation on testicular function in long-term survivors of childhood acute lymphoblastic leukemia: a report from the Children Cancer Study Group. *J Clin Oncol* 1990, **8**, 1981–1987.
10. Meistrich ML, Wilson G, Brown BW, Da Cunha MF, Lipshultz LI. Impact of cyclophosphamide on long-term reduction in sperm count in men treated with combination chemotherapy for Ewing and soft tissue sarcomas. *Cancer* 1992, **70**, 2703–2712.
11. Byrne J, Mulvihill JJ, Myers MH, *et al*. Effects of treatment on fertility in long-term survivors of childhood or adolescent cancer. *N Engl J Med* 1987, **317**, 1315–1321.
12. Nygaard R, Clausen N, Siimes MA, *et al*. Reproduction following treatment for childhood leukemia: a population-based prospective cohort study of fertility and offspring. *Med Pediatr Oncol* 1991, **19**, 459–466.
13. Blatt J, Poplack DG, Sherins RJ. Testicular function in boys after chemotherapy for acute lymphoblastic leukemia. *N Engl J Med* 1981, **304**, 1121–1124.
14. Wallace WH, Shalet SM, Lendon M, Morris Jones PH. Male fertility in long-term survivors of childhood acute lymphoblastic leukaemia. *Int J Androl* 1991, **14**, 312–319.
15. Lendon M, Hann IM, Palmer MK, Shalet SM, Jones PH. Testicular histology after combination chemotherapy in childhood for acute lymphoblastic leukaemia. *Lancet* 1978, **ii**, 439–441.
16. Robertson DM, Foulds LM, Leversha L, *et al*. Isolation of inhibin from bovine follicular fluid. *Biochem Biophys Res Commun* 1985, **126**, 220–226.
17. Ling N, Ying SY, Ueno N, Esch F, Denoroy L, Guillemin R. Isolation and partial characterization of a M_r 32,000 protein with inhibin activity from porcine follicular fluid. *Proc Natl Acad Sci USA* 1985, **82**, 7217–7221.
18. Maddocks S, Sharpe RM. Assessment of the contribution of Leydig cells to the secretion of inhibin by the rat testis. *Mol Cell Endocrinol* 1989, **67**, 113–118.
19. Burger H, Igarashi M. Inhibin: definition and nomenclature, including related substances. *Endocrinology* 1988, **122**, 1701–1702.
20. Krummen LA, Toppari J, Kim WH, *et al*. Regulation of testicular inhibin subunit messenger ribonucleic acid levels *in vivo*: effects of hypophysectomy and selective follicle-stimulating hormone replacement. *Endocrinology* 1989, **125**, 1630–1637.
21. Roberts V, Meunier H, Sawchenko PE, Vale W. Differential production and regulation of inhibin subunits in rat testicular cell types. *Endocrinology* 1989, **125**, 2350–2359.
22. Bhasin S, Krummen LA, Swerdloff RS, *et al*. Stage specific expression of inhibin alfa and beta-B subunits during the cycle of the rat seminiferous epithelium. *Endocrinology* 1989, **124**, 987–991.
23. Kaipia A, Penttila TL, Shimasaki S, *et al*. Expression of inhibin beta-A and beta-B, follistatin and activin-A receptor mRNAs in the rat seminiferous epithelium. *Endocrinology* 1992, **131**, 2703–2710.
24. Illingworth PJ, Groome NP, Byrd W, *et al*. Inhibin-B: a likely candidate for the physiologically important form of inhibin in men. *J Clin Endocrinol Metab* 1996, **81**, 1321–1325.
25. Anawalt BD, Bebb RA, Matsumoto AM, *et al*. Serum inhibin B levels reflect Sertoli cell function in normal men and men with testicular dysfunction. *J Clin Endocrinol Metab* 1996, **81**, 3341–3345.
26. Raivio T, Toppari J, Perheentupa A, McNeilly AS, Dunkel L. Treatment of prepubertal gonadotrophin-deficient boys with recombinant human follicle-stimulating hormone. *Lancet* 1997, **350**, 263–264.
27. Wallace EM, Healy DL. Inhibins and activins: roles in clinical practice. *Br J Obstet Gynaecol* 1996, **103**, 945–956.
28. Bhasin S, De Kretser DM, Drew CR. Editorial: Measurement of circulating inhibin levels: revisiting the inhibin hypothesis. *J Clin Endocrinol Metab* 1996, **81**, 1318–1320.
29. Anderson RA, Irvine DS, Balfour C, Groome NP, Riley SC. Inhibin B in seminal plasma: testicular origin and relationship to spermatogenesis. *Hum Reprod* 1998, **13**, 920–926.
30. Jensen TK, Andersson AM, Hjollund NH, *et al*. Inhibin B as a serum marker of spermatogenesis: correlation to differences in sperm concentration and follicle-stimulating hormone levels. A study of 349 Danish men. *J Clin Endocrinol Metab* 1997, **82**, 4059–4063.
31. Tanner JM. *Growth at Adolescence*. Oxford, Blackwell Scientific Publications, 1962.
32. Hansen PF, With TK. Clinical measurement of the testes in boys and men. *Acta Med Scand* 1952, **142**, 457–465.
33. Siimes MA, Elomaa I, Koskimies A. Testicular function after chemotherapy for osteosarcoma. *Eur J Cancer* 1990, **26**, 973–975.
34. Siimes MA, Rautonen J, Makipernaa A, Sipila I. Testicular function in adult males surviving childhood malignancy. *Pediatr Hematol Oncol* 1995, **12**, 231–241.
35. Groome NP, O'Brian M. Immunoassays for inhibin and its subunits. Further applications of the synthetic peptide approach. *J Immunol Methods* 1993, **165**, 167–176.
36. Groome NP, Illingworth PJ, O'Brien M, *et al*. Measurement of dimeric inhibin B throughout the human menstrual cycle. *J Clin Endocrinol Metab* 1996, **81**, 1401–1405.
37. Wallace EM, Groome NP, Riley SC, Parker AC, Wu FC. Effects of chemotherapy-induced testicular damage on inhibin, gonadotropin, and testosterone secretion: a prospective longitudinal study. *J Clin Endocrinol Metab* 1997, **82**, 3111–3115.
38. Conte FA, Grumbach MM, Kaplan SL. A diphasic pattern of gonadotropin secretion in patients with the syndrome of gonadal dysgenesis. *J Clin Endocrinol Metab* 1975, **40**, 670–674.
39. De Kretser DM, Burger HG, Fortune D, *et al*. Hormonal, histological and chromosomal studies in adult males with testicular disorders. *J Clin Endocrinol Metab* 1972, **35**, 392–401.
40. Waxman JHX, Terry YA, Wrigley PFM, *et al*. Gonadal function in Hodgkin's disease: long-term follow-up of chemotherapy. *Br Med J* 1982, **285**, 1612–1613.
41. Takihara H, Cosentino MJ, Sakatoku J, Cockett ATK. Significance of testicular size measurement in urology: II. Correlation of testicular size with testicular function. *J Urol* 1987, **137**, 416–419.
42. Muller HL, Klinkhammer Schalke M, Seelbach Gobel B, Hartmann AA, Kuhl J. Gonadal function of young adults after therapy of malignancies during childhood or adolescence. *Eur J Pediatr* 1996, **155**, 763–769.
43. Quigley C, Cowell C, Jimenez M, *et al*. Normal or early development of puberty despite gonadal damage in children treated for acute lymphoblastic leukemia. *N Engl J Med* 1989, **321**, 143–151.
44. McLachlan RI, Robertson DM, Healy DL, Burger HG, De Kretser DM. Circulating immunoreactive inhibin levels during the normal human menstrual cycle. *J Clin Endocrinol Metab* 1987, **65**, 954–961.
45. Burger HG. Clinical review 46: Clinical utility of inhibin measurements. *J Clin Endocrinol Metab* 1993, **76**, 1391–1396.
46. Groome NP, Illingworth PJ, O'Brien M, *et al*. Detection of dimeric inhibin throughout the human menstrual cycle by two-site enzyme immunoassay. *Clin Endocrinol (Oxf)* 1994, **40**, 717–723.
47. Byrd W, Bennett MJ, Carr BR, Dong Y, Wians F, Rainey W. Regulation of biologically active dimeric inhibin A and B from infancy to adulthood in the male. *J Clin Endocrinol Metab* 1998, **83**, 2849–2854.

48. Andersson AM, Juul A, Petersen JH, Muller J, Groome NP, Skakkebaek NE. Serum inhibin B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol levels. *J Clin Endocrinol Metab* 1997, **82**, 3976–3981.
49. Raivio T, Perheentupa A, McNeilly AS, *et al.* Biphasic increase in serum inhibin B during puberty: a longitudinal study of healthy Finnish boys. *Pediatr Res* 1998, **44**, 552–556.
50. Andersson AM, Müller J, Groome NP, Skakkebaek NE. Serum inhibin B levels in prepubertal boys are germ cell independent. *Horm Res* 1998, **50**, 12 (Abstract).
51. Aubier F, Flamant F, Brauner R, Caillaud JM, Chaussain JM, Lemerle J. Male gonadal function after chemotherapy for solid tumors in childhood. *J Clin Oncol* 1989, **7**, 304–309.
52. Jaffe N, Sullivan MP, Ried H, *et al*. Male reproductive function in long-term survivors of childhood cancer. *Med Pediatr Oncol* 1988, **16**, 241–247.