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ORIGINAL ARTICLE *Infertility*

Reproductive status in adult male long-term survivors of childhood cancer

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BACKGROUND: This study assessed the long-term effects of cancer therapies on reproductive status in adult male childhood cancer survivors, evaluated the treatment-related risk factors for hypergonadotropic hypogonadism and assessed the association between the FSH levels and the later need for assisted reproductive techniques (ART).

METHODS: The study cohort included adult male 5-year survivors of childhood cancer who were treated in our institution between 1966 and 2003. Data concerning patient and treatment characteristics, FSH, LH and testosterone levels and pregnancy outcome were collected. Multivariate regression analyses were performed to evaluate the treatment-related risk factors for disturbances in reproductive endocrine status. The diagnostic and predictive values of FSH and later need for ART were evaluated.

RESULTS: Data on reproductive endocrine status were available for 488 survivors (86.4%) of the 565 male survivors who visited the outpatient clinic in adulthood. The median follow-up time from initiation of treatment to first visit to the outpatient clinic in adulthood was 15 years. The prevalence rates of elevated FSH levels and decreased testosterone levels were 33 and 12%, respectively. The use of procarbazine, cyclophosphamide, vinca-alkaloids, other alkylating agents, pelvic/abdominal irradiation, total body irradiation and testicular surgery were identified as treatment-related risk factors for elevated FSH levels. During the follow-up period, 73 men reported 120 conceptions, which resulted in 103 live births. Of these men, 56 (77%) were able to achieve conception naturally. All men whose partners conceived by assisted reproductive techniques ($n = 13$) had elevated FSH levels at their first visit after their 18th birthday (sensitivity: 100%; 95% CI: 71–100%) and all male survivors with a normal FSH level did not need assisted reproductive techniques (negative predictive value: 100%; 95% CI: 89–100%).

CONCLUSIONS: One-third of young adult male survivors of childhood cancer has elevated FSH levels. FSH appears to be a very sensitive marker for the need of assisted reproductive techniques in male childhood cancer survivors.

Key words: childhood cancer / cancer survivors / fertility / FSH / males

Introduction

Advances in treatment for childhood cancer have enormously improved survival rates. Between the 1960s and 1990s, 5-year survival increased from 23 to 70% (Heymans and Caron, 2001; Curry *et al.*, 2006). Improved prognosis has, however, been accompanied by the occurrence of late treatment-related morbidity (Oeffinger *et al.*, 2008). Iatrogenic reproductive failure and endocrine disturbances are frequently encountered late effects, which have major impact on quality of life (Geenen *et al.*, 2007).

In males, testicular dysfunction is one of the most important long-term side effects of cancer treatment. Recently, Green *et al.* (2010) evaluated the long-term fertility of 6224 male survivors in the Childhood Cancer Survivor Study through questionnaires, which showed that male survivors were less likely to sire a pregnancy than their siblings who had not undergone cancer treatment. Testicular irradiation,

higher cumulative alkylating agent dose and treatment with procarbazine or cyclophosphamide were identified as risk factors. Earlier studies, concerning gonadotoxic effects of treatment in male childhood cancer survivors, showed an association between testicular, cranial or abdominal irradiation, total body irradiation (TBI), alkylating agents and the increased risk of testicular dysfunction (Viviani et al., 1985; Byrne et al., 1987; Müller et al., 1996; Relander et al., 2000; Van den Berg et al., 2004). However, these studies often described small study populations, included only one type of cancer or had a short follow-up time. The seminiferous epithelium seems to be most sensitive to the detrimental effects of irradiation and cytostatic drugs (Müller et al., 1996; Relander et al., 2000). The degree of impairment can vary from oligospermia to azoospermia and can be reflected by indirect markers such as follicle-stimulating hormone (FSH), inhibin B, luteinizing hormone (LH) or testosterone (van Casteren et al., 2009). However, the predictive values of these markers for achieving a pregnancy are not known.

In this long-term follow-up study, we determined the prevalence of abnormalities in FSH, LH and testosterone levels in a large cohort of more than 500 male survivors of various childhood malignancies treated between 1966 and 2003. Subsequently, we evaluated treatment-related risk factors for the occurrence of disturbances in reproductive endocrinology and the association between the FSH levels and the later need for assisted reproductive techniques.

Materials and methods

Study population

All male patients, diagnosed with and treated for a primary malignancy at 17 years of age or younger in the Emma Kinderziekenhuis/Academic Medical Center (EKZ/AMC) between 1966 and January 2003, who survived more than 5 years and who had reached a minimum age of 18 years at 1 January 2008 were identified using the Childhood Cancer Registry of the EKZ/AMC ($n = 879$). Eighty-three survivors had died before their first visit to the outpatient clinic after their 18th birthday. Therefore, 796 survivors were eligible for this study (Fig. 1).

Data collection and follow-up

Since 1996, 5-year survivors of childhood cancer were traced and invited periodically to visit the outpatient clinic of the EKZ/AMC for the assessment of long-term effects of childhood cancer treatment. At the outpatient clinic a physician performs a full medical assessment according to standardized follow-up protocols including medical history, physical examination, additional radiological and functional investigations and blood analysis. We extracted medical follow-up data of the first visit to the outpatient clinic after the survivors' 18th birthday. FSH, LH and testosterone were determined from blood samples collected at the first visit to the outpatient clinic after 18th birthday. In order to evaluate the predictive value of an abnormal FSH measurement at the first visit we collected follow-up data concerning pregnancy from this registry until 2008.

Of the 796 survivors eligible for study 231 (29%) did not visit the outpatient clinic after their 18th birthday. Figure 1 shows reasons for not visiting the outpatient clinic. Consequently, 565 survivors (71%) were included in this study.

Outcome measures

Endocrine evaluation for male fertility included analysis of serum hormone levels of FSH, LH and testosterone. According to the reference values of the laboratory of the EKZ/AMC for adult males, the following definitions for an abnormal value were used: FSH >10 U/l, LH >15 U/l and testosterone <11.0 nmol/l. This study was not designed to identify childhood cancer survivors with hypogonadotropic hypogonadism due to cranial irradiation because additional tests are needed for this diagnosis. Follow-up data concerning pregnancy outcomes were collected until 1 January 2008 to assess the effects on reproduction. The methods of conception, number of pregnancies and number of life births were determined.

Statistical analysis

Statistical analyses were performed using SPSS version 16.0.01 software. First, descriptive statistics were used to examine patient and treatment characteristics, endocrine status and pregnancy outcome. We planned to perform univariate and multivariate linear regression for FSH, LH and testosterone to evaluate the identified treatment-related risk factors for disturbances in reproductive endocrinology focused on hypergonadotropic hypogonadism. However, due to lack of normality in the residuals of FSH and LH, these analyses could not be performed for FSH and LH. Therefore, treatment-related risk factors for elevated FSH levels were analysed by the use of univariate and multivariate logistic regression. Due to small-event numbers LH could not be analysed by means of logistic regression and was not taken into consideration in the analysis of possible treatment-related risk factors.

Interaction effects between various chemotherapeutic agents were explored, but were not included in the multivariate regression analysis due to non-significance and small-event numbers. To adjust for the effect of age at diagnosis and follow-up time since diagnosis, univariate and multivariate analyses always included these two variables in the model.

Pregnancy data of males whose partners conceived after long-term follow-up were described and method of conception was linked to their FSH level measured at their first visit to the outpatient clinic after their 18th birthday. The sensitivity, specificity and predictive values for FSH level and need for assisted reproductive techniques were calculated.

Results

Patient characteristics

The study population consisted of 565 out of 796 eligible survivors (71%). Patient and treatment characteristics are listed in Table I. The median age at diagnosis was 7.8 years (range: 0.0–17.8 years). Median follow-up time from diagnosis until first visit to outpatient clinic after 18th birthday was 15.0 years (range: 5.0–39.0 years). The median attained age of survivors at first visit to the outpatient clinic after their 18th birthday was 21.0 years (range: 18.0–46.0 years), of whom 24.8% were 18 years of age. Lymphoma was the most common diagnosis in our study population with 154 survivors (27.3%). Treatment consisted of a combination of chemotherapy and surgery for 172 survivors (30.4%). Almost 90% of the population received chemotherapy; only nine survivors (2.4%) were treated with a chemotherapeutic agent other than an alkylating agent, vinca-alkaloid or antimetabolite.

Endocrine status

We succeeded in obtaining FSH levels of 488 (86.4%) survivors, LH levels of 489 (86.5%) survivors and testosterone levels of 460 (81.4%) survivors. FSH and LH were not normally distributed and skewed to the right; testosterone was normally distributed. FSH

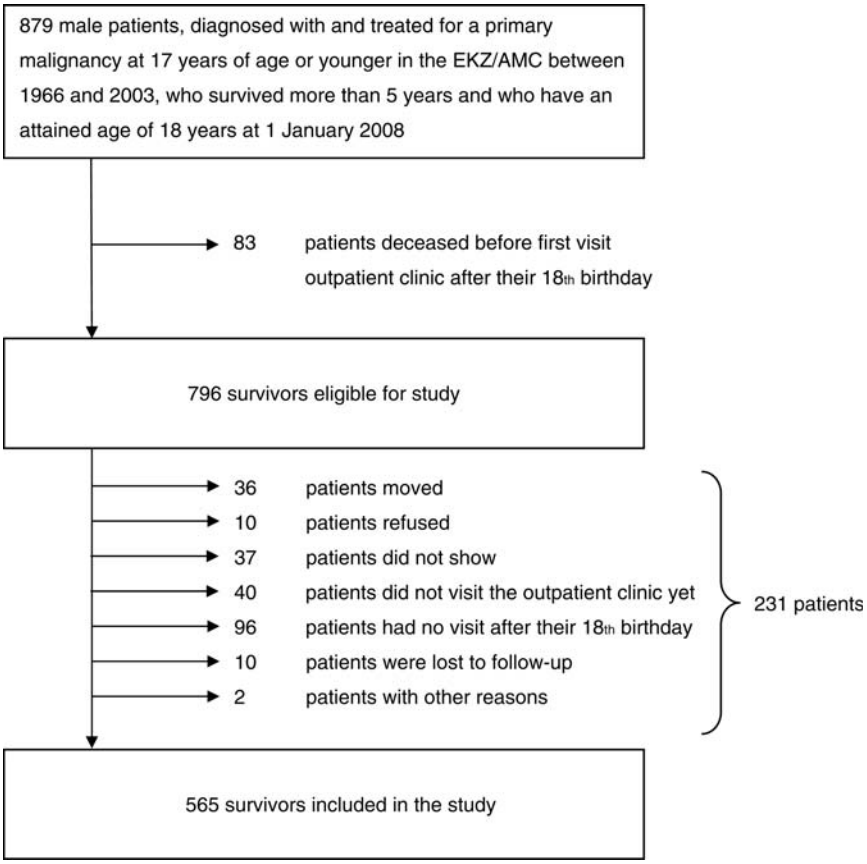

Figure 1 Flow chart of the selection of our study population from the EKZ/AMC.

hormone levels were raised in 161 men (33.0%). Only 14 survivors (2.9%) had elevated LH levels and 57 survivors (12.4%) had decreased testosterone levels (Table II).

The third column of Table I shows a description of the distribution of elevated FSH levels in the study population. Elevated FSH levels were seen in all survivors treated with TBI.

Logistic regression analyses of treatment-related risk factors for elevated FSH

Results of the univariate and multivariate logistic regression analyses performed to evaluate treatment-related risk factors for elevated FSH levels are summarized in Table III. All comparisons were adjusted for age at diagnosis and follow-up time. Because all patients treated with TBI had elevated FSH levels, patients who received TBI were not included in the multivariate model (Table III).

Multivariate logistic regression analysis identified a significantly higher risk of elevated FSH levels after use of procarbazine (OR: 3.8; 95% CI: 1.8–8.2), cyclophosphamide (OR: 4.2; 95% CI: 2.2–8.0), other alkylating agents (OR: 2.1; 95% CI: 1.1–4.0), vinca-alkaloids (OR: 2.8; 95% CI: 1.0–7.3), pelvic/abdomen irradiation (OR: 2.4; 95% CI: 1.0–5.4) and surgery of the testicular region (OR: 2.6; 95% CI: 1.1–6.3). The group of other alkylating agents included busulfan, carmustine, mechlorethamine, ifosfamide, lomustine, melfalan and temozolomide. Due to small-event numbers, we were not able to perform separate analyses for these agents.

Linear regression analyses of treatment-related risk factors for decreasing testosterone

Univariate linear regression evaluating risk factors for decreasing testosterone levels identified treatment with TBI as the only risk factor. All comparisons were adjusted for age at diagnosis and follow-up time. Univariate regression analysis with TBI showed that patients who received TBI had a significant lower mean testosterone level than patients who did not receive TBI ($\beta = -3.53$; $P = 0.036$). However, testosterone levels of both groups were still within the range of the reference values. The multivariate regression model could not identify any risk factors for decreasing testosterone levels in this study.

Pregnancy outcome

During the follow-up period, 73 men reported that their partner had become pregnant: 120 conceptions resulted in 103 live births and 14 miscarriages; for three conceptions follow-up data were not available. Of these men, 56 (77%) were able to achieve conception naturally. Linking the pregnancy data to FSH status showed that FSH levels for

Table I Characteristics of 565 survivors eligible for study related to FSH status.

Characteristics	Number of survivors (%)	Number of elevated measurements of number of total FSH measurements (%)
No. of survivors	565 (100.0)	161/488 (33.0)
Age at diagnosis, years		
0–4	185 (32.7)	39/154 (25.3)
5–9	165 (29.2)	45/149 (30.2)
10–14	172 (30.4)	62/147 (42.2)
15–17	43 (7.6)	15/38 (39.5)
Primary childhood tumour		
Leukaemia	125 (22.1)	30/118 (25.4)
Lymphoma	154 (27.3)	73/143 (51.0)
Kidney tumour	64 (11.3)	5/50 (10.0)
Brain/CNS tumour	47 (8.3)	3/45 (6.7)
Bone tumour	53 (9.4)	21/43 (48.8)
Soft tissue sarcoma	70 (12.3)	17/52 (32.7)
Neuroblastoma	19 (3.3)	6/15 (40.0)
Endocrine tumours[a]	5 (0.9)	0/1 (0.0)
Testicular tumour	9 (1.9)	3/8 (37.5)
Other	19 (3.4)	3/13 (23.1)
Recurrence of primary tumour		
Yes	78 (13.8)	35/69 (50.7)
No	487 (86.2)	126/419 (30.1)
Second tumour		
Yes	21 (3.7)	8/18 (44.4)
No	544 (96.3)	153/470 (32.6)
Follow-up time until first screening after 18th birthday, years		
5–9	130 (23.0)	40/116 (34.5)
10–14	145 (25.7)	39/133 (29.3)
15–19	169 (29.9)	46/142 (32.4)
20–24	71 (12.7)	23/62 (37.1)
25–39	50 (8.8)	13/35 (37.1)
Age at first screening after 18th birthday, years		
18	140 (24.8)	33/121 (27.3)
19–24	250 (44.2)	69/223 (30.9)
25–34	147 (26.0)	50/129 (38.8)
35–46	28 (5.0)	9/15 (60.0)
Treatment modality		
Radiotherapy only	4 (0.7)	0/4 (0.0)
Chemotherapy only	127 (22.5)	38/119 (31.9)
Surgery only	29 (5.1)	2/7 (28.6)
Radiotherapy + chemotherapy	95 (16.8)	41/92 (44.6)
Radiotherapy + surgery	30 (5.3)	2/24 (8.3)
Chemotherapy + surgery	172 (30.4)	48/148 (32.4)
Radiotherapy + chemotherapy + surgery	108 (19.1)	30/94 (31.9)
Type of chemotherapy	502 (88.8)	157/453 (34.7)
Alkylating agents only[b]	34 (6.8)	5/31 (16.1)
Anti-metabolites only[b]	1 (0.2)	0/1 (0.0)
Vinca-alkaloids only[b]	67 (13.3)	5/54 (9.3)

Continued

Table I *Continued*

Characteristics	Number of survivors (%)	Number of elevated measurements of number of total FSH measurements (%)
Alkylating agents + anti-metabolites[b]	8 (1.6)	1/8 (12.5)
Alkylating agents + vinca-alkaloids[b]	170 (33.9)	80/153 (52.3)
Antimetabolites + vinca-alkaloids[b]	86 (17.1)	9/82 (11.0)
Alkylating agents + anti-metabolites + vinca-alkaloids[b]	124 (24.7)	57/116 (49.1)
Other chemotherapeutic agents only	9 (2.4)	0/8 (0.0)
Localization radiotherapy	237 (41.9)	73/214 (34.1)
TBI[c]	11 (4.6)	11/11 (100.0)
Cranial irradiation (including CNS) only[c]	120 (50.6)	19/113 (16.8)
Pelvic/abdomen irradiation only[c]	51 (21.5)	16/44 (36.4)
Cranial + pelvic/abdomen irradiation[c]	4 (1.7)	4/4 (100.0)
Other areas of irradiation only	51 (21.5)	23/42 (54.8)
Localization surgery	339 (60.0)	82/273 (30.0)
Testicular region[d]	38 (11.2)	16/32 (50.0)
Only other areas	279 (88.8)	66/241 (27.4)

CNS, central nervous system.
[a]Including three thyroid tumours and two tumours of the glandula pinealis.
TBI, total body irradiation; CNS, central nervous system.
[b]With or without other chemotherapy.
[c]With or without irradiation to other areas.
[d]With or without surgery of other areas.

Table II Endocrine status: distribution of FSH, LH and testosterone.

Hormone	Number of eligible patients (%)
FSH (median: 6.0; range: 0.1–72.7)	488 (86.4)
≤10.0 U/L[a]	327 (67.0)
>10.0 U/l	161 (33.0)
LH (median: 6.0; range: 1.0–40.0)	489 (86.5)
≤15.0 U/l[a]	475 (97.1)
>15.0 U/l	14 (2.9)
Testosterone (mean: 17.2; sd: 5.5)	460 (81.4)
<11.0 nmol/l	57 (12.4)
≥11.0 nmol/l[a]	403 (87.6)

FSH, follicle-stimulating hormone; LH, luteinizing hormone.
[a]Reference value.

67 of 73 men were available. All men whose partners conceived by artificial insemination with donor semen or *in vitro* fertilization ($n = 13$) had elevated FSH levels at the first visit to the outpatient clinic. However, 11 of the 51 survivors whose partners conceived spontaneously also had elevated FSH levels. Hence, the sensitivity and specificity of an increased FSH level with regard to being unable to conceive naturally were 100% (95% CI: 77–100%) and 78% (95% CI: 65–87%), respectively. Partners of all male survivors with normal FSH levels did not need assisted reproductive techniques to achieve a pregnancy. Hence, the predictive value of a normal FSH level with regard to being able to conceive naturally (negative predictive value) was 100% (95% CI: 89–100%). (Table IV)

Discussion

In this large study of 565 childhood cancer survivors, treated for all types of cancer, we showed that one-third of male 5-year childhood cancer survivors have elevated FSH levels after a median follow-up time of 15 years. Multivariate regression analysis identified the use of procarbazine, cyclophosphamide, vinca-alkaloids, other alkylating agents, pelvic and/or abdominal irradiation, treatment with TBI and surgery of the testicular region as independent treatment-related risk factors for elevated FSH levels. A higher age at diagnosis and longer follow-up duration may also be associated with the risk of elevated FSH levels. This possible association should be evaluated in more detail in new longitudinal studies. The risk factors identified are in line with previously suggested risk factors in other studies (Viviani *et al.*, 1985; Byrne *et al.*, 1987; Müller *et al.*, 1996; Relander *et al.*, 2000; Van den Berg *et al.*, 2004; Oeffinger *et al.*, 2008; Ridola *et al.*, 2009; Green *et al.*, 2010). However, most study groups were small, follow-up time was short, multivariate analysis has not been performed or the studies used different outcome measures for reproductive status. We were not able to investigate the effect of dosage on the outcome because the dosage registration was not complete. Green *et al.* (2010) recently found a linear relationship between an increasing cumulative alkylating agent dose and the inability to sire a pregnancy.

One-third of the childhood cancer survivors had an elevated FSH level at a median age of 21.0 years. This is of great concern for this

Table III Univariate and multivariate logistic regression analysis of predictors of elevated FSH.

Factor	Follicle-stimulating hormone					
	Univariate[a]			Multivariate[a]		
	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*
Age at diagnosis			0.001[†]			0.014[†]
1 year increase	1.09	1.04–1.14		1.08	1.02–1.16	
Follow-up time			0.029[†]			0.020[†]
1 year increase	1.04	1.00–1.08		1.06	1.01–1.12	
Cisplatin and/or carboplatin			0.251			0.085
Yes	0.68	0.35–1.32		2.29	0.89–5.89	
No	1.00			1.00		
Procarbazine			0.000[†]			0.001[†]
Yes	4.38	2.65–7.24		3.79	1.76–8.17	
No	1.00			1.00		
Cyclophosphamide			0.000[†]			0.000[†]
Yes	3.54	2.32–5.38		4.23	2.24–8.00	
No	1.00			1.00		
Other alkylating agents			0.000[†]			0.018[†]
Yes	3.24	2.14–4.89		2.14	1.14–4.00	
No	1.00			1.00		
Vinca alkaloids			0.000[†]			0.036[†]
Yes	5.60	2.74–11.43		2.80	1.07–7.30	
No	1.00			1.00		
Antimetabolites			0.664			0.669
Yes	1.09	0.73–1.63		1.15	0.63–2.07	
No	1.00			1.00		
Anthracyclines			0.000[†]			0.863
Yes	2.46	1.55–3.71		1.06	0.56–2.00	
No	1.00			1.00		
Other chemotherapeutic agents			0.427			0.694
Yes	1.19	0.78–1.83		0.90	0.50–1.59	
No	1.00			1.00		
Cranial irradiation			0.000[†]			0.078
Yes	0.38	0.22–0.63		0.55	0.28–1.07	
No	1.00			1.00		
Pelvic/abdomen irradiation			0.229			0.043[†]
Yes	1.49	0.78–2.87		2.35	1.03–5.37	
No	1.00			1.00		
Total body irradiation[‡]						
Yes						
No	1.00					
Other irradiation			0.211			0.073
Yes	1.394	0.83–2.35		1.78	0.95–3.34	
No	1.00			1.00		
Surgery testicular region			0.020[†]			0.033[†]
Yes	2.433	1.15–5.14		2.61	1.08–6.29	
No	1.00			1.00		

[a]All comparisons adjusted for age at diagnosis and follow-up time.
[†]Significance value < 0.05.
[‡]Not included in the multivariate model due to undetermined OR in univariate analysis.

Table IV FSH status in males achieving conception per method of conception.

Method of conception	Number of males achieving conception (%)	Number of elevated measurements of total FSH measurements (%)
Natural conception	56 (77.0)	11/51 (21.6)
In vitro fertilization	3 (4.1)	3/3 (100.0)
AID	10 (13.5)	10/10 (100.0)
Unknown	4 (5.4)	1/3 (33.3)
Total (% of conceptions)	73 (100.0)	25/67 (37.3)

AID, artificial insemination with donor semen; FSH, follicle-stimulating hormone.
Sensitivity of elevated FSH for the need of assisted reproductive techniques: 100% (95% CI: 71–100%).
Specificity of normal FSH for natural conception: 78% (95% CI: 64–88%).
Predictive value of elevated FSH for the need of assisted reproductive techniques: 54% (positive predictive value; 95% CI: 33–74%).
Predictive value of normal FSH for natural conception: 100% (negative predictive value; 95% CI: 89–100%).

young adult survivor group. Because serum FSH is an important marker of Sertoli cell function and spermatogenesis, we expect a sperm count less than 1 million spermatozoa per ml or azoospermia in majority of men with elevated FSH levels. This assumption is supported by the presence of a negative correlation between sperm concentration and FSH in fertile and infertile men (Uhler *et al*., 2003; Andersson *et al*., 2004; Meeker *et al*., 2007). In a study on 349 Danish men who had not attempted pregnancy yet, positive predictive value (PPV) of an FSH level >10 IU/l for detecting sperm counts <20 million/ml was 85.7% (Jensen *et al*., 1997). Additionally, Mahmoud *et al*. (1998) found a sensitivity and PPV of 74 and 96.3%, respectively, in a cohort of 47 subfertile men Romerius *et al*. (2011) evaluated 129 men treated for malignancies during childhood. Among these survivors, 66% with subnormal inhibin B levels and 50% with elevated FSH levels were azoospermic. The median age of the survivors in the present study is 21.0 years. As the mean age of fatherhood in the Netherlands was 34.2 years in 2006, data concerning quality of semen and continuing follow-up are essential to evaluate the actual fertility after childhood cancer.

A unique feature of our study is that we were able to investigate the association between the FSH level at the first measurement after the age of 18 years and the need for assisted reproductive techniques later during follow-up in males achieving conception. We found that all survivors whose partners conceived with assisted reproductive techniques had elevated FSH levels at their first visit after the age of 18 years (sensitivity of elevated FSH levels for assisted reproductive techniques during follow-up: 100%; 95% CI: 71–100%). Partners of all survivors with a normal FSH level had a natural conception during follow-up (negative predictive value of an FSH test for natural conception: 100%; 95% CI: 89–100%). However, the positive predictive value of an elevated FSH for assisted techniques was lower (54%; 95% CI: 33–77%). Although this study was not designed to identify childhood cancer survivors with hypogonadotropic hypogonadism, we also performed analysis of the predictive values for the later need for assisted reproductive techniques with exclusion of the survivors treated with cranial radiotherapy. These calculations showed similar results. These results suggest that FSH could be used as a first screening marker in childhood cancer survivors to detect male survivors at risk for subfertility. FSH measurement could select a group of survivors in whom further diagnostic tests are needed, such as semen analysis. For the counselling of survivors it is important to realize the implications of the low PPV of FSH testing: 46% of the survivors with an elevated FSH were able to achieve pregnancy naturally.

The present study shows that only 3% (14 of 475) of the survivors have elevated LH levels and 12% (57 of 403) decreased testosterone levels. Raised LH and decreased testosterone levels represent damage to Leydig cells and previous literature shows that irradiation and cytostatic drugs mostly affect the Sertoli cells and not Leydig cell function (Apperley and Reddy, 1995; Relander *et al*., 2000). As a result, the production of LH and testosterone could be normal and secondary sexual characteristics may develop normally in childhood cancer survivors.

A limitation of this study is that we were not able to investigate the whole cohort of male survivors due to missing data. However, missing endocrine measurements were randomly distributed among different treatment groups (data not shown) and no systematic bias could be identified. Furthermore, we were not able to analyse data on inhibin B in our study. Inhibin B has been suggested as a sensitive marker for male fertility status, especially in combination with FSH (Jensen *et al*., 1997; Kumanov *et al*., 2006). Evaluation of gonadal function in childhood cancer survivors with the use of inhibin B has recently been performed by Van Casteren *et al*. (2009), who showed severe gonadal impairment based on several fertility markers, including FSH and inhibin B. The diagnostic value of these tests should be validated in a large cohort of male survivors with sperm count as a control test. Furthermore, we did not perform a longitudinal study and data on the course of FSH levels during a longer follow-up period are not yet available. We analysed the first measurement of FSH, LH and testosterone at our Late Effects Outpatient Clinic after the age of 18 years, with a median follow-up time after diagnosis of 15 years. Based on the knowledge from earlier studies we do not expect normalization of FSH in our survivor group after a longer follow-up time (Viviani *et al*., 1985; Charak *et al*., 1990); this should however be verified in longitudinal studies.

In conclusion, we showed that one-third of young male survivors of childhood cancer had elevated FSH levels and we identified several treatment-related risk factors. Based on studies in other patient groups, male survivors with an increased FSH are at increased risk of subfertility or infertility. We showed a high sensitivity for an increased level of FSH at the first visit after the age of 18 years and the need for assisted reproductive techniques during longer follow-up. Fortunately, we also showed that two-third of the reported conceptions was achieved naturally.

Our results have implications for clinical practice. First, pre-treatment preservation of fertility is essential for special groups of childhood cancer patients, and survivors should receive adequate information about the risks of infertility. Boys who undergo gonadotoxic therapy should be offered cryopreservation of semen as soon as possible after diagnosis and before start of treatment. However, for prepubertal boys lacking in haploid gametes, currently only experimental options are available (Pacey, 2007). Second, because a sperm test might be experienced as

more stressful by survivors than a venapuncture, FSH could be a promising test during the follow-up care to identify patients at highest risk for infertility. As a result a smaller group of survivors could be selected for sperm analysis. The diagnostic and predictive value of FSH and other blood tests for detecting subfertility should be confirmed in a large cohort of male survivors, preferably with longitudinal FSH data and with sperm count as a control test. Until these results are available, the recommendations of the Dutch guidelines for follow-up of childhood cancer survivors do not include screening for male subfertility by evaluation of FSH or other blood tests. If men would like to be informed, sperm analysis is advised. According to these guidelines (SKION LATER, 2010), testosterone should only be measured when clinical symptoms suggest a deficit. Because of the low number of survivors with an abnormal LH level we advise no standard screening of LH in male childhood cancer survivors.

Future research on male fertility in childhood cancer survivors should be performed in large cohorts of childhood cancer survivors with complete follow-up, focusing on the diagnostic values of FSH and other markers, such as inhibin B, in detecting oligospermia (<1 million spermatozoa/ml) or azoospermia and on new pre-treatment preservation techniques of fertility.

Authors' roles

K.T. designed the study and wrote the manuscript. She developed the study protocol and performed the statistical analyses.

J.J.M.C. designed the study. She contributed to the analyses and interpretation of the results and critically reviewed the manuscript. S.L.K. designed the study, contributed to the identification of the cohort, the data extraction and critically reviewed the manuscript. H.J.H.P. was involved as the physician in the outpatient clinic of the EKZ/AMC. She helped design the study and critically reviewed the manuscript. F.E.L. was involved in the set up of the outpatient clinic, the identification of the cohort and the development of the standardized follow-up protocols. She also critically reviewed the manuscript. H.N.C. is head of the department of paediatric oncology in the EKZ/AMC, is involved in the set up of the outpatient clinic and the development of the standardized follow-up protocols. He critically reviewed the manuscript. C.C.M.B. designed the study. She contributed to the analyses and interpretation of the results and critically reviewed the manuscript. L.C.M.K. is head of the EKZ/AMC outpatient clinic. She designed the study, contributed to the identification of the cohort, the analyses and the interpretation of the results. She also critically reviewed the manuscript.

All authors approved the final version of the manuscript.

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