

## RESEARCH

# Childhood reproductive hormone levels after pediatric hematopoietic stem cell transplantation in relation to adult testicular function

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## Abstract

**Objectives:** Longitudinal assessment of testicular function after pediatric hematopoietic stem cell transplantation (HSCT) is needed to guide clinical follow-up. We investigated dynamics in male reproductive hormones after pediatric HSCT, focusing on pubertal timing and associations with testosterone deficiency and azoospermia in adulthood.

**Methods:** This retrospective, longitudinal study included 39 survivors median 19 years after pediatric HSCT. Serum concentrations of LH, testosterone, FSH, and inhibin B from the time of HSCT, during puberty, and into adulthood were analyzed. Pubertal timing (rise in LH and testosterone) was compared to a reference cohort of 112 healthy boys. Associations between reproductive hormone levels during puberty and adult testicular function (including semen quality) were investigated.

**Results:** Pubertal induction with testosterone was needed in 6/26 patients who were prepubertal at HSCT. In the remaining patients, pubertal timing was comparable to the reference cohort. However, 9/33 patients (without pubertal induction) developed testosterone deficiency in early adulthood, which was associated with higher LH levels from age 14 to 16 years. Azoospermia in adulthood was found in 18/26 patients without testosterone substitution. Higher FSH and lower inhibin B levels from mid-pubertal age were associated with azoospermia in adulthood, in patients being prepubertal at HSCT.

**Conclusion:** Our results indicate a substantial risk of deterioration in testicular function after pediatric HSCT, despite normal pubertal timing. Although reproductive hormone levels from mid-puberty indicated adult testicular function, prolonged follow-up into adulthood is needed in these patients, including clinical examination, reproductive hormone analysis, and semen sample for patients interested in their fertility potential.

## Key Words

- ▶ pediatrics
- ▶ hematopoietic stem cell transplantation
- ▶ late effects
- ▶ gonadal function

*Endocrine Connections*  
(2021) **10**, 1352–1365

## Introduction

Hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for children with

life-threatening hematological and immunological diseases, including high-risk leukemias, but late effects are becoming prevalent due to increasing survival rates and longer follow-up time (1). HSCT interferes with the

male reproductive axis, potentially causing testosterone deficiency and impaired spermatogenesis due to the detrimental effects of high-dose chemotherapy and total body irradiation (TBI) (2). Studies addressing gonadal function in pediatric HSCT survivors are highly needed, as these patients experience an insult to the reproductive axis during the critical developmental stages of life, compromising direct comparisons with adult HSCT patients (3). However, only a few older time-course studies have investigated the dynamics in circulating levels of reproductive hormones after pediatric HSCT (4, 5, 6, 7). These studies were rather small (15–41 patients of which some were prepubertal at last follow-up), the follow-up times were limited (median age at last follow-up 14–19 years), and importantly, none of the studies included semen quality data. Moreover, no previous studies have investigated pubertal timing after pediatric HSCT compared to the general population, or whether reproductive hormone levels during puberty are associated with adult testicular dysfunction – information which is needed in clinical follow-up.

In a recent cross-sectional, long-term follow-up study, we reported a high risk of testosterone deficiency and azoospermia in adult survivors of pediatric allogeneic HSCT (8). In the present study, we aimed at combining these data with a retrospective analysis of reproductive hormones and semen samples in order to (i) characterize the development of testicular failure from the time of transplant, during puberty, and into adulthood, (ii) evaluate pubertal timing compared to the general population, and (iii) investigate

the associations between post-transplant reproductive hormone levels during the pubertal years and testosterone deficiency and azoospermia in adulthood.

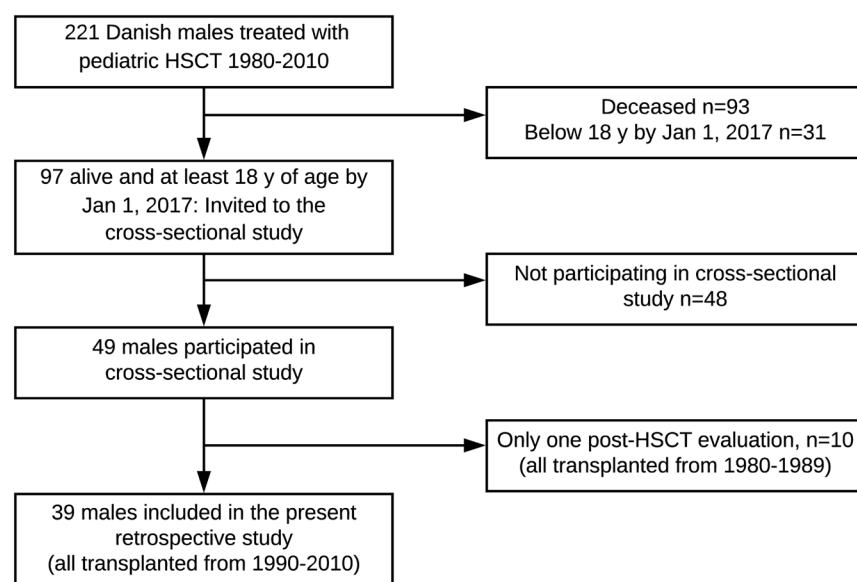
## Materials and methods

### Design and patient population

This study is an extension of our cross-sectional study conducted in 2017 (8). The cohort included male survivors treated with allogeneic HSCT before age 17 from January 1, 1980 to January 1, 2010, who were at least 18 years of age by January 1, 2017, and eligible independent of diagnosis. Patients treated with more than one HSCT were excluded. In this longitudinal study, we retrospectively analyzed the reproductive hormone levels before and after HSCT in the Danish patients who had data available from a minimum of two examinations after HSCT. Patient in- and exclusion flow is presented in Fig. 1.

### Treatment characteristics

Information on pre-HSCT treatment and transplant characteristics was obtained from patient files, treatment protocols, and records reported to the Center for International Blood and Marrow Transplant Research, as previously described (8). TBI was given in three fractions to a total dose of 11.3–12.0 Gy, except for two patients treated with low-dose TBI (2 Gy, single dose) for severe aplastic anemia (SAA). Cumulative treatment doses (pre-HSCT plus



**Figure 1**  
Study population inclusion and exclusion flow.

HSCT doses) of testicular irradiation (Gy), CNS irradiation (Gy), and cyclophosphamide equivalents (CED) (g/m<sup>2</sup>) were calculated (9).

### Pubertal development

Pubertal stage at HSCT was obtained from patients' files (prepubertal vs pubertal/postpubertal). Patients were regarded as prepubertal if testis volume was  $\leq 3$  mL, and no other puberty signs had emerged. If clinical data were missing ( $n = 4$ ), total testosterone levels below the lower detection limit at several timepoints before and after HSCT indicated prepubertal stage. In patients being prepubertal at HSCT, the onset of puberty was categorized as either spontaneous or induced by testosterone substitution. Pubertal timing was assessed by an increase in serum total testosterone and luteinizing hormone (LH). Further, bone age (BA), assessed by the method of Greulich and Pyle (10), was evaluated in relation to chronological age (CA).

### Testosterone substitution

All patients completed a detailed questionnaire on their health since HSCT, including information on the use of testosterone substitution (any use and if relevant, year of initiation). In patients treated with testosterone substitution, reproductive hormone measurements and semen quality data during testosterone therapy were excluded from the analyses to avoid reflecting the possible effects of the substitution therapy (lower gonadotropin levels, higher testosterone levels, and risk of azoospermia).

### Reproductive hormones and semen samples

From a clinical database, we obtained registered daytime circulating levels of follicle-stimulating hormone (FSH), LH, total testosterone, and inhibin B as well as registered semen quality data (semen volume, sperm concentration, motility, and morphology) analyzed according to the World Health Organization's guidelines, as previously described in detail (11).

Reproductive hormone levels from a total of 532 examinations were included in the analyses (median 13 examinations per patient, interquartile range 9–17). FSH and LH were measured by a time-resolved fluoroimmunoassay (DELFI, Perkin Elmer) during the total follow-up period. The lower limit of detection (LoD) was 0.05 IU/L for both FSH and LH, and the interassay coefficient of variation (CV) was below 5% in both assays. Total testosterone was measured by three

different methods due to changes in the laboratory's practice: (i) from January 1990 to August 2014, a RIA was applied (Coat-A-Count total testosterone assay, Siemens Healthcare Diagnostics) with an LoD of 0.23 nmol/L and an interassay CV below 10%; (ii) From September 2014 to October 2016, a chemiluminescence immunoassay was applied (Access2 Immunoassay System, Beckman Coulter) with an LoD of 0.35 nmol/L and an interassay CV below 6%; and (iii) From October 2016 and onwards, an isotope diluted Turboflow liquid chromatography-tandem mass spectrometry (LC-MS/MS) was applied, except for 11 samples analyzed by the Access2 assay. The LC-MS/MS had a limit of quantification (LoQ) of 0.10 nmol/L and an interassay CV below 10% (12, 13). Likewise, inhibin B was measured by two different double-antibody immunometric assays: (i) Before June 2010 by Serotec (Oxford, UK; previously named Oxford Bio-Innovation); and (ii) From June 2010 and onwards by GenII (Beckman Coulter), with LoDs of 20 and 3 pg/mL, and interassay CVs below 18 and 11%, respectively. All hormone analyses were conducted in the same laboratory, and a comprehensive internal validation was conducted for every change in assays to standardize the analyses.

### Reference material

Hormone levels during childhood and puberty were compared to a prospectively followed reference cohort of 112 boys from the Danish general population (aged 5.8–16.4 years) participating in the COPENHAGEN Puberty Study (14). Hormone levels in adulthood (age  $\geq 18$  years) were compared to local reference ranges generated from the Danish general population with upper FSH and LH limits of 15 and 8 IU/L, respectively, and lower total testosterone and inhibin B limits of 10 nmol/L and 50 pg/mL, respectively (15, 16, 17). All hormone analyses were conducted in the same laboratory as in the patient population.

### Classification of Leydig cell function

Testosterone deficiency was defined as the use of testosterone substitution or a minimum of three consecutive serum testosterone measurements below 10 nmol/L after the age of 18 years without returning to levels within the reference range. Compensated Leydig cell dysfunction was defined as LH  $> 8$  IU/L at minimum one timepoint after HSCT and testosterone levels within the reference range during follow-up (no more than two consecutive measurements below 10 nmol/L after the age of 18 years). Normal Leydig cell function was defined as

LH  $\leq$  8 IU/L at all timepoints after HSCT and testosterone levels within the reference range (no more than two consecutive measurements below 10 nmol/L after the age of 18 years).

## Statistics

Comparison of the patient group and the reference cohort regarding pubertal development included three steps. First, we applied a locally estimated scatterplot smoothing (LOESS) for modeling the hormone levels for a given age in each group (18). Secondly, we compared the mean age of each group at which a certain hormone level was crossed (pubertal timing) using Tobit regression accounting for the irregularly spaced measurements and using interval-, right-, and left-censoring (19). Thirdly, we compared the two groups' hormone levels at age 15–16 years using a linear regression model containing the covariates *group* and *age-16 years* (age at last measurement minus age 16 years). By this, we standardized the group comparison to the age of 16 years to avoid confounding by age at last measurement.

Regarding delay in BA, a mean BA-CA difference was calculated for each patient across ages of 6 to 16.5 years and tested with a one sample *t*-test against a mean difference of 0.

Comparison of inhibin B levels at pre-HSCT (the evaluation closest to HSCT), early post-HSCT (first evaluation post-HSCT), and 2 years post-HSCT (evaluation closest to two years post-HSCT) was performed by Wilcoxon signed-rank test for patients with available data at all three timepoints ( $n = 15$ ).

The associations between the patient's mean reproductive hormone levels at specific age intervals and the risk of testosterone deficiency and azoospermia in adulthood, respectively, were evaluated by simple logistic regression with estimates reported as odds ratio (OR) with 95% CI. As testosterone substitution may cause azoospermia, only patients without testosterone substitution therapy were included in the analysis of risk of azoospermia. Two-sided  $P$ -value  $\leq 0.05$  were considered statistically significant. Statistical analyses were performed using R (<http://www.R-project.org>).

## Ethics

The initial cross-sectional study was approved by The Regional Committee of Health Research Ethics, Denmark (H-16043059), and the Danish Data Protection Agency, and informed consent was obtained from all participants.

In addition, this study was approved by the Danish Patient Safety Authority.

## Results

### Study population

We included 39 males, all transplanted from 1990 to 2010 and postpubertal at last follow-up (median age 27.6 years (range 18.5–40.4)). These participants were comparable to the non-participating males transplanted in the same time period ( $n = 37$ ) regarding key patient and transplant characteristics (Table 1). The distribution of the included patients according to the pubertal stage at HSCT, onset of puberty, use of testosterone substitution, and spermatogenic status in adulthood is presented in Fig. 2.

### Patients being prepubertal at HSCT

#### Pubertal development

Of patients being prepubertal at HSCT, 6/26 (23%) needed induction of puberty. These patients were all treated for acute lymphoblastic leukemia (ALL) with TBI-based conditioning. In addition, one had undergone bilateral orchiectomy, two had undergone unilateral orchiectomy with additional testicular irradiation of the contralateral testicle, two had received a 4 Gy testicular boost in addition to TBI, and one had received additional CNS irradiation prior to HSCT. These patients were excluded from further analysis.

Onset of puberty was spontaneous in the remaining 20 males (77%). In these patients, the timing of rise in LH and testosterone corresponded to the reference population (Fig. 3A and B); however, at age 15–16 years, the testosterone levels were lower in the patient group ( $P < 0.001$ ), and LH levels appeared to be higher, although not significantly ( $P = 0.061$ ). Likewise, FSH levels were higher and inhibin B levels were lower in the patient group at age 15–16 years ( $P < 0.001$ ) (Fig. 3C and D). Despite a normal timing of puberty, BA was delayed compared to CA (mean BA-CA difference:  $-0.84$  years, 95% CI ( $-1.34$  to  $-0.35$ ),  $P = 0.002$ ) (Fig. 4).

### Leydig cell function in adulthood

The six males that needed induction of puberty continued testosterone substitution into adulthood. For the 20 males with spontaneous onset of puberty after HSCT, the patterns of LH and testosterone levels during the total follow-up

**Table 1** Patient and treatment characteristics for participants vs non-participants and for patients who were prepubertal at transplant vs pubertal/postpubertal at transplant.

Patient characteristics	Participants, <i>n</i> = 39	Non-participants, <i>n</i> = 37	Participants prepubertal at HSCT, <i>n</i> = 26	Participants pubertal/postpubertal at HSCT, <i>n</i> = 13
Age at HSCT, median (range)	9.4 (0.4–16.9)	9.7 (3.1–17.0)	8.1 (0.4–13.8)	15.1 (13.0–16.9)
Prepubertal stage at HSCT, <i>n</i> (%)	26 (67)	NA	26 (100)	0 (0)
Age at last follow-up, median (range)	27.6 (18.5–40.4)	25.6 (18.4–38.5)	26.1 (18.5–38.0)	29.5 (24.8–40.4)
Years from HSCT at last follow-up, median (range)	19.0 (7.7–27.3)	15.4 (8.1–26.3)	20.1 (7.7–27.3)	15.8 (8.6–25.1)
<b>Diagnosis (malignant vs non-malignant)</b>				
Malignancies, <i>n</i> (%)	29 (74)	22 (59)	19 (73)	10 (77)
Acute lymphoblastic leukemia	12	13	9	3
Acute myeloid leukemia	6	2	2	4
Myelodysplastic syndrome	5	2	5	0
Non-Hodgkin lymphoma	4	1	1	3
Other leukemia (biphenotypic, JMML, CML)	2	4	2	0
Non-malignant diseases, <i>n</i> (%)	10 (26)	15 (41)	7 (27)	3 (23)
Severe aplastic anemia	5	4	3	2
Immune deficiency syndromes	2	2	2	0
Other non-malignant diseases	3	9	2	1
<b>Donor match, <i>n</i> (%)</b>				
Matched sibling donor	14 (36)	15 (41)	9 (35)	5 (38)
Matched unrelated donor	19 (49)	15 (41)	12 (46)	7 (54)
Alternative donor	6 (15)	7 (19)	5 (19)	1 (8)
<b>Stem cell source, <i>n</i> (%)</b>				
Bone marrow	38 (97)	33 (92)	26 (100)	12 (92)
Peripheral blood	1 (3)	4 (11) <sup>a</sup>	0	1 (8)
<b>Conditioning regimens</b>				
BU + CY (+ MEL/VP16/THIO), <i>n</i> (%)	14 (36)	16 (43) <sup>b</sup>	12 (46)	2 (15)
TBI 2 Gy + CY, <i>n</i> (%)	2 (5)	0	1 (4)	1 (8)
TBI 11.3–12 Gy + CY/BU-CY/VP16, <i>n</i> (%)	23 (59)	21 (57)	13 (50)	10 (77)
CNS shielding, <i>n</i>	0	0	0	0
Gonadal shielding, <i>n</i>	2 <sup>c</sup>	1	1	1
<b>Additional irradiation at conditioning</b>				
Testicular irradiation 4 Gy, <i>n</i>	11	9	5	6
CNS irradiation 10 Gy, <i>n</i>	1	3	1	0
<b>Cumulative treatment (pre-HSCT plus conditioning)</b>				
CED (g/m <sup>2</sup> ), median (range)	5.31 (0–28.74)	NA	5.43 (0–23.46)	4.48 (2.00–28.74)
Testicular irradiation (Gy), median (range)	11.3 (0–36)	NA	3.4 (0–36)	12.0 (0–16)
CNS irradiation (Gy), median (range)	12.0 (0–36)	NA	11.3 (0–36)	12.0 (0–12)
<b>Treatment groups (cumulative treatment)</b>				
Chemotherapy only, <i>n</i>	13	NA	11	2
Low-dose testicular irradiation <sup>d</sup> , <i>n</i>	4	NA	2	2
TBI 11.3–12 Gy, no add. irradiation, <i>n</i>	7	NA	4	3
TBI 11.3–12 Gy plus add. CNS irradiation, <i>n</i>	3	NA	3	0
TBI 11.3–12 Gy plus add. testis irradiation, <i>n</i>	10	NA	4	6
TBI 11.3–12 Gy plus add. testis and add. CNS irradiation, <i>n</i>	2	NA	2	0
<b>Orchiectomy</b>				
Unilateral, <i>n</i>	4	NA	3	1
Bilateral, <i>n</i>	1	NA	1	0
<b>Graft vs host disease</b>				
Acute graft vs host disease, <i>n</i> (%)	23 (67)	24 (65)	18 (69)	5 (38)
Grade I	6	8	4	2
Grade II	13	14	10	3
Grade III+IV	4	2	4	0

<sup>a</sup>One non-participant received both bone marrow and peripheral blood but was only counted in as receiving peripheral blood; <sup>b</sup>Three non-participants treated for severe aplastic anemia received conditioning with CY only, and one treated for Fanconi anemia received 4.5 Gy thoracoabdominal irradiation in addition to CY; <sup>c</sup>Testicular irradiation dose estimated to be 40% of the TBI dose; <sup>d</sup>Low-dose testicular irradiation included patients treated with TBI 2 Gy or TBI 11.3–12 Gy with gonadal shielding.

add., additional (to TBI); BU, busulfan; CED, cyclophosphamide equivalent dose; CML, chronic myeloid leukemia; CY, cyclophosphamide; HSCT, hematopoietic stem cell transplantation; JMML, juvenile myelomonocytic leukemia; MEL, melphalan; TBI, total body irradiation; THIO, thiotepea; VP16, etoposide.



period are presented in Fig. 5A and B. Five of these 20 males (25%) eventually needed testosterone substitution (median age at initiation 17.6 years, range 16.1–21.0) (two were treated with chemotherapy for SAA and myelodysplastic syndrome (MDS), respectively; one with chemotherapy and CNS irradiation for ALL; and two with TBI ± testicular boost of 4 Gy for MDS and biphenotypic leukemia, respectively). Of the remaining 15 patients, 6 had compensated Leydig cell dysfunction.

### Spermatogenic capacity in adulthood

Of the 15 patients who were prepubertal at HSCT and not receiving testosterone substitution in adulthood, 14 delivered a semen sample at the last follow-up. All 6 males with detectable sperm had normal FSH levels at all timepoints after HSCT, while 5/8 males with azoospermia had elevated FSH at a minimum one timepoint (Fig. 6A).

Although the inhibin B levels were significantly decreased at first evaluation after HSCT, the levels returned to pre-HSCT levels within 2 years after transplant (Fig. 7A). Then, during puberty, the inhibin B levels eventually declined to below lower reference limit in 7/8 males presenting with azoospermia in adulthood, while 6/6 males with detectable sperm in adulthood maintained inhibin B levels above lower reference limit during follow-up (Fig. 6B).

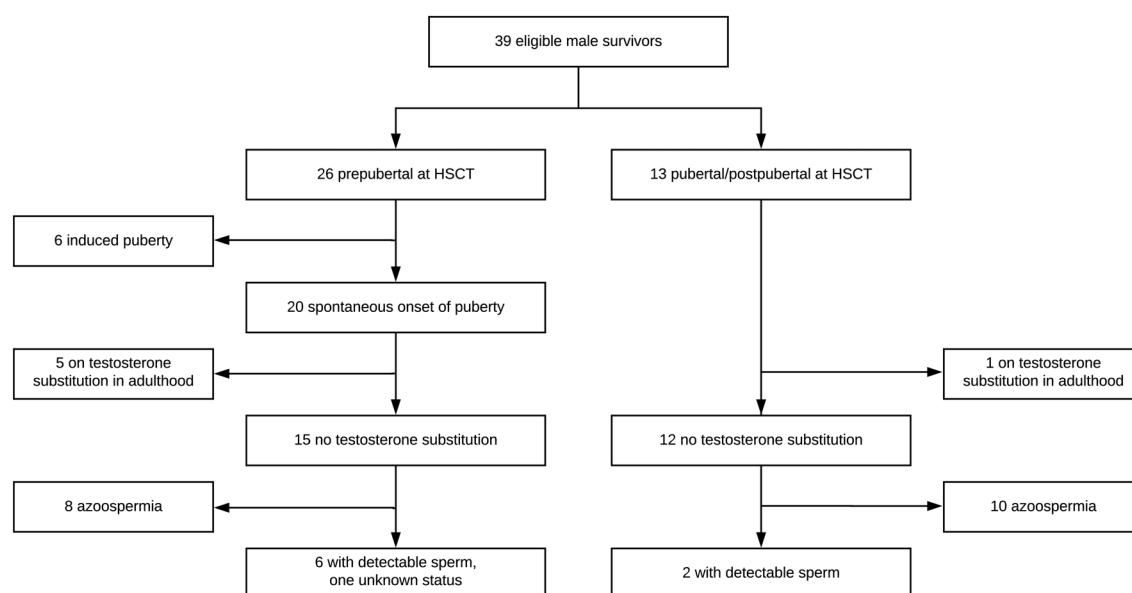
### Patients being pubertal/postpubertal at HSCT

#### Leydig cell function in adulthood

Thirteen males were pubertal/postpubertal at HSCT (Fig. 5C and D) of which one had started testosterone substitution at the age of 23 years, about 8 years after transplant (treated for ALL with TBI plus testicular boost of 4 Gy). Of the twelve patients without testosterone substitution, three eventually developed biochemical testosterone deficiency (Fig. 5D) (one treated with chemotherapy for thalassemia; two treated with TBI plus a testicular boost of 4 Gy for ALL and non-Hodgkin lymphoma, respectively). Of the remaining nine patients, six had compensated Leydig cell dysfunction.

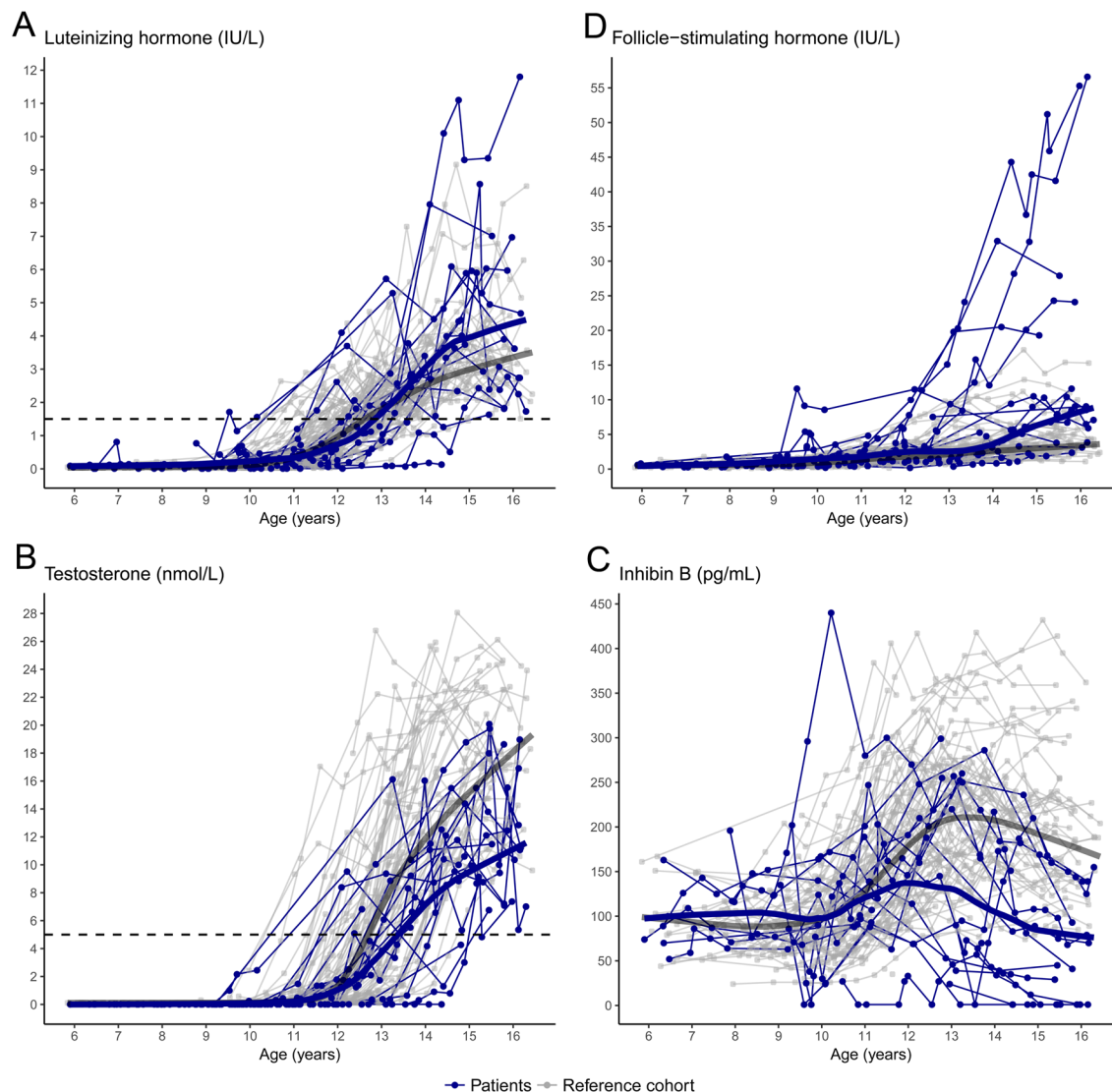
#### Spermatogenic capacity in adulthood

Of the 12 patients who were pubertal/postpubertal at HSCT and not receiving testosterone substitution, ten had azoospermia in adulthood. The two males with detectable sperm in their ejaculate presented with persistently *high* FSH levels and persistently *low* inhibin B levels after HSCT (Fig. 6C and D); one had six consecutive inhibin B measurements below the detection limit of the assay, and despite this, he presented with a total sperm count of 1.04 million and reported to have fathered two children. The other male had a total sperm count of 1.89 million and had experienced one unexpected conception. Of the



**Figure 2**

Distribution of the 39 male survivors of pediatric HSCT according to the pubertal stage at transplant, spontaneous vs induced onset of puberty, use of testosterone substitution, and spermatogenic status in adulthood. HSCT, hematopoietic stem cell transplantation.



**Figure 3**

Pubertal timing in boys with spontaneous onset of puberty after pediatric HSCT ( $n = 20$ ) compared to boys from the general population ( $n = 112$ ). Dynamics of LH (panel A), testosterone (panel B), FSH (panel C), and inhibin B (panel D) during childhood and puberty are presented. Bold curves represent the estimated curves for the two populations. Dashed lines indicate the hormone levels at which age at sample was compared between the two groups to evaluate delay in puberty. There was no significant difference in age at crossing the LH level of 1.5 IU/L between patients and reference population (mean group difference 0.27 years, 95% CI  $(-0.34$  to  $0.89)$ ,  $P = 0.38$ ) and no significant difference in LH levels at age 15–16 years (estimated group difference 1.1 IU/L, 95% CI  $(-0.03$  to  $2.2)$ ,  $P = 0.06$ ) (panel A). Likewise, there was no significant difference in age at crossing the testosterone level of 5 nmol/L between patients and reference population (mean group difference 0.29 years, 95% CI  $(-0.21$  to  $0.78)$ ,  $P = 0.25$ ), but testosterone levels at age 15–16 years were significantly reduced in the patient group (estimated group difference  $-5.9$  nmol/L, 95% CI  $(-8.6$  to  $-3.3)$ ,  $P < 0.001$ ) (panel B). Further, the patients' FSH levels at age 15–16 years were higher compared to the reference population (estimated group difference 13.4 IU/L, 95% CI  $(8.0$  to  $18.7)$ ,  $P < 0.001$ ) (panel C) and inhibin B levels lower (estimated group difference  $-146$  pg/mL, 95% CI  $(-193$  to  $-100)$ ,  $P < 0.001$ ) (panel D). *Statistics:* Tobit regression and linear regression. FSH, follicle-stimulating hormone; HSCT, hematopoietic stem cell transplantation; LH, luteinizing hormone.

males presenting with azoospermia in adulthood, 7/10 had elevated FSH levels at minimum one timepoint after HSCT and 9/10 eventually had inhibin B levels below lower reference limit (Fig. 6C and D). Regarding dynamics of inhibin B around the time of HSCT, inhibin B was significantly decreased at first evaluation after transplant, and no recovery was observed (Fig. 7B).

### Associations between reproductive hormone levels during puberty and adult testicular function

For all patients with spontaneous onset of puberty ( $n = 33$ ), we analyzed whether post-HSCT LH levels during the pubertal years were associated with the risk of testosterone deficiency in adulthood. Higher LH levels at age 14–16 years were

associated with a higher risk of testosterone deficiency, in contrast to LH levels at age 9–12 and 12–14 years (Table 2).

Associations between post-HSCT levels of FSH and inhibin B levels during the pubertal years and spermatogenic capacity in adulthood appeared to differ according to the pubertal stage at transplant (as shown in Fig. 6). In the patients who were prepubertal at HSCT ( $n = 14$ ), we analyzed whether post-HSCT FSH and inhibin B levels during the pubertal years were associated to the risk of azoospermia in adulthood. Higher FSH levels from the age of 12 years were associated with a higher risk of azoospermia in adulthood, while lower inhibin B levels from the age of 14 years were associated with a higher risk of azoospermia in adulthood (Table 2). Of note, higher inhibin B levels in the age interval of 9–12 years appeared to be associated with a higher risk of azoospermia in adulthood. Males who were pubertal/postpubertal at HSCT had too few data to be analyzed as a separate group.

### Repeated semen samples

Repeated semen sample analyses were available in 9 of the 39 survivors. Five males (3 prepubertal, 1 pubertal, and

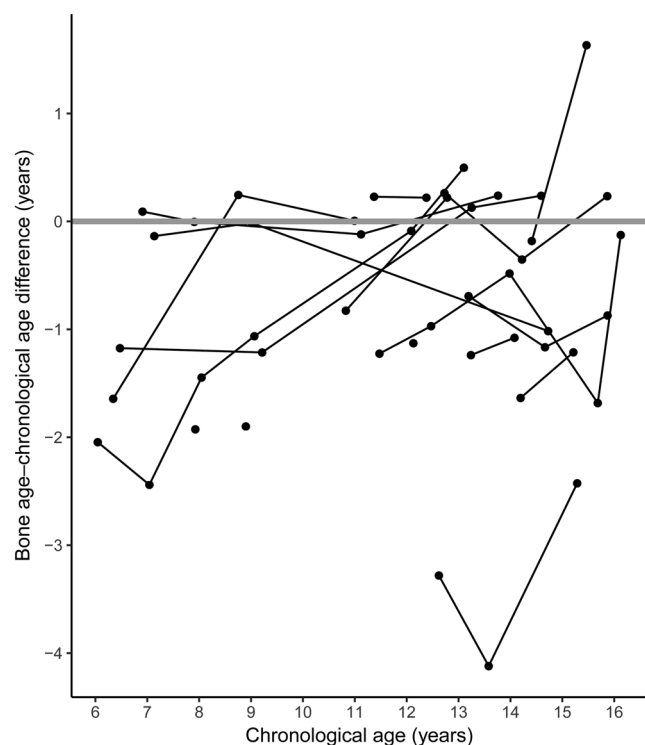
1 postpubertal at HSCT) had consistent azoospermia (at two to four timepoints) over a time period of 4 months to 16 years. Three patients (1 prepubertal and 2 postpubertal at HSCT) had few detectable spermatozoa at minimum one timepoint with azoospermia in the remaining samples (total 3–4 samples over 5–10 years). One of those males was referred to a testicular biopsy in which spermatozoa were detected and cryopreserved for use in assisted reproduction. Finally, one male (pubertal at HSCT) revealed a steady increase in total sperm count over a period of 2 years; moving from 0.06 to 0.13 to 1.89 million sperm.

### Discussion

This longitudinal study describes the dynamics in male reproductive hormone levels after pediatric allogeneic HSCT. For the first time, pubertal timing after pediatric HSCT was compared with the timing in the general population, and we assessed whether reproductive hormone levels during puberty were associated with testosterone deficiency and azoospermia in adulthood. These new insights are of clinical importance in the endocrinological follow-up and prognostic assessment after pediatric HSCT.

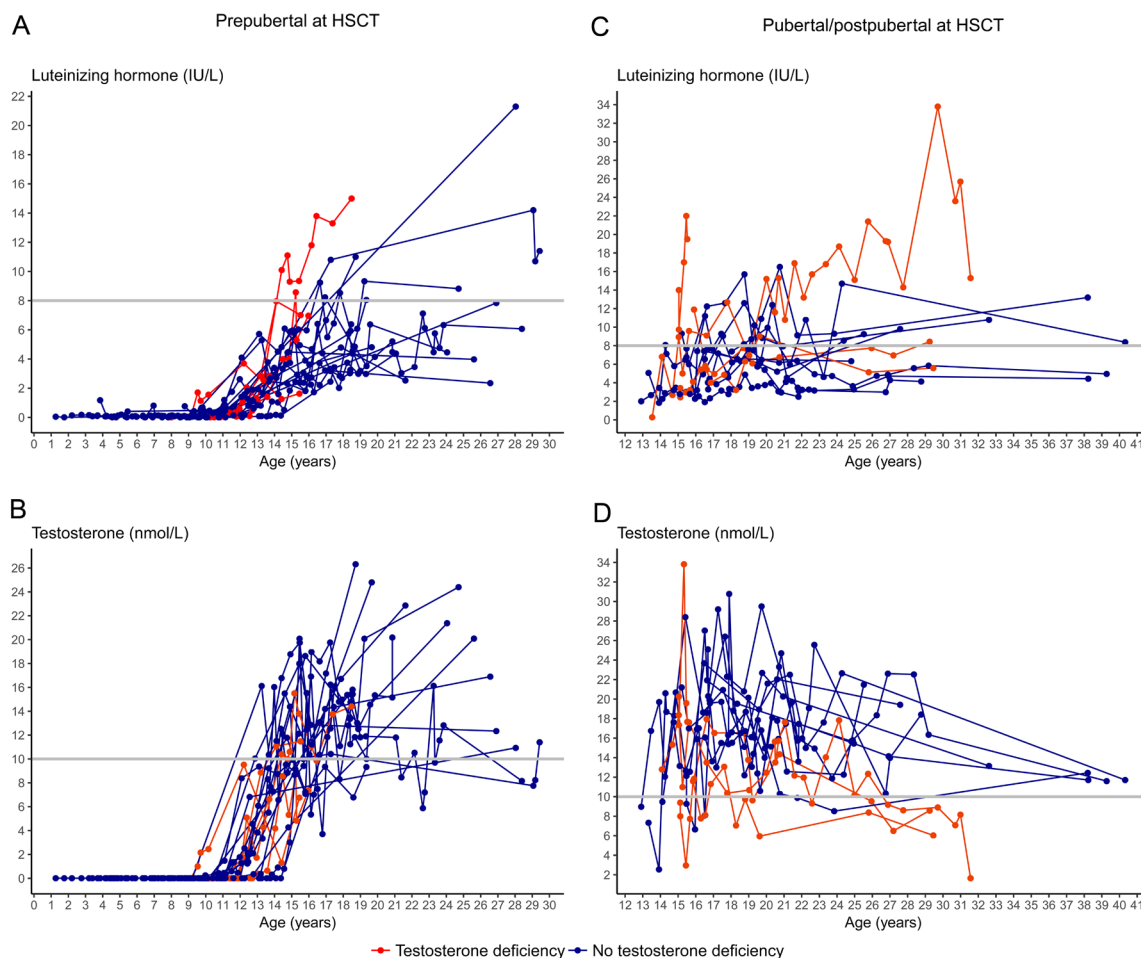
Induction of puberty was needed in one-fourth of patients who were prepubertal at transplant and all continued testosterone substitution into adulthood, indicating complete and permanent Leydig cell failure. These patients were all treated for ALL and had undergone orchiectomy or received testicular or CNS irradiation in addition to TBI. Thus, these patients represent the most heavily treated pediatric HSCT survivors, exposed to treatment modalities that are abandoned nowadays. The need for pubertal induction may therefore be expected to be a rare phenomenon in boys not exposed to orchiectomy or irradiation in addition to TBI, as suggested by others (4, 7, 20, 21).

For patients with spontaneous onset of puberty, the pubertal timing corresponded to the general population. Nevertheless, we observed an impaired increase in circulating testosterone levels during the pubertal years, and testosterone substitution was eventually started in one-fourth of these patients, indicating an impaired Leydig cell response to LH stimulation during puberty leading to decompensation in early adulthood. These patients differed regarding diagnosis and treatment regimens, implying a possible genetic component in the risk of Leydig cell failure, which should be addressed in future studies. Another three patients, transplanted after the onset of



**Figure 4**  
The difference between bone age and chronological age (CA) in relation to CA in boys with spontaneous onset of puberty after pediatric HSCT ( $n = 17$ , three patients had no available data).



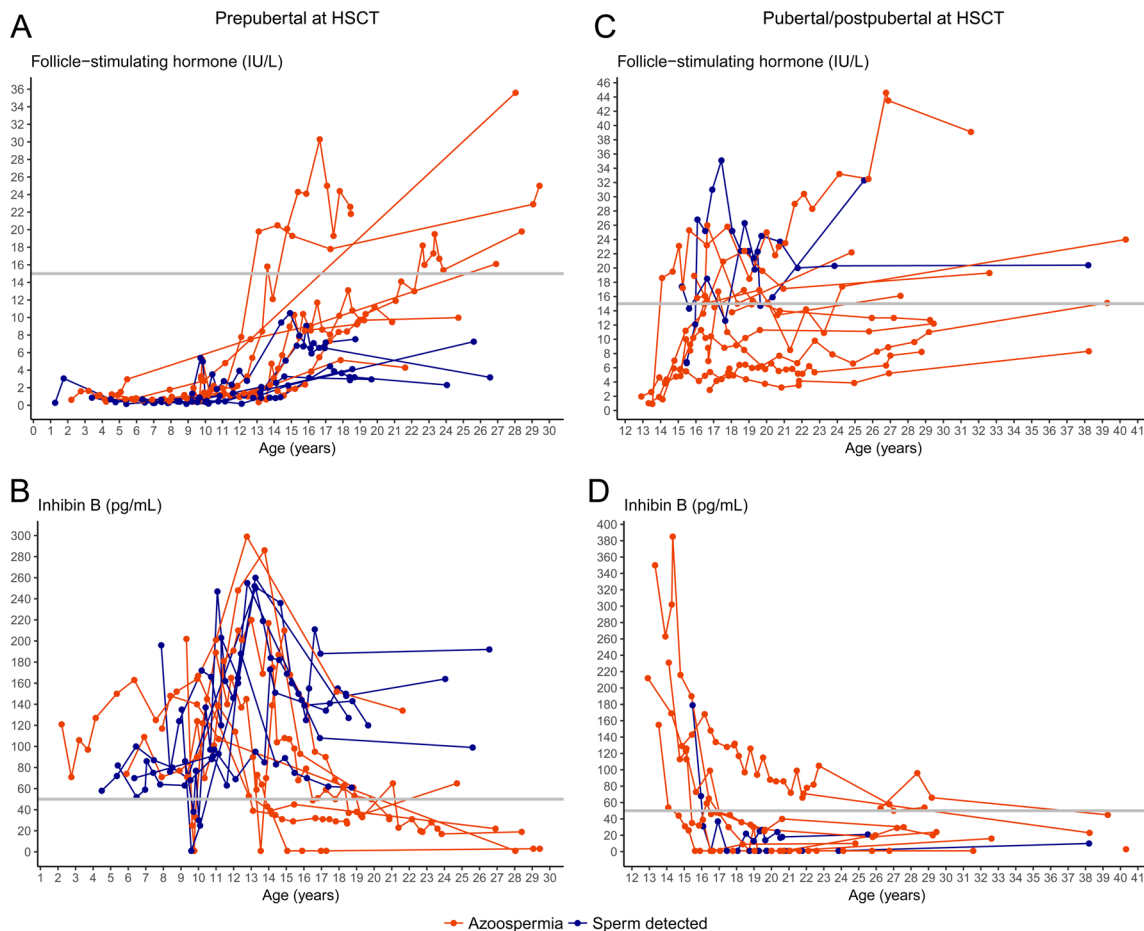


**Figure 5**

Testosterone and LH patterns in patients who were prepubertal at HSCT and had spontaneous onset of puberty ( $n = 20$ , panel A and B), and patients who were pubertal/postpubertal at HSCT ( $n = 13$ , panel C and D). Red curves represent patients with testosterone deficiency with or without testosterone substitution at last follow-up; measurements at timepoints from initiation of substitution therapy and onwards were excluded. Blue curves represent patients with normal or compensated Leydig cell function, not treated with testosterone substitution at any timepoint. Horizontal gray lines indicate the upper limit of normal for LH (8 IU/L) and lower limit of normal for testosterone (10 nmol/L) in adulthood. LH levels during the early pubertal years did not seem to clearly indicate risk of testosterone deficiency (panel A and B), while higher LH levels from the late pubertal years and onwards seemed to indicate an increased risk of testosterone deficiency in adulthood (panel A, B and C, D). Nevertheless, few males developed testosterone deficiency despite normal LH levels, indicating a potential hypothalamic-pituitary component of the hypogonadism (panel A and C). HSCT, hematopoietic stem cell transplantation; LH, luteinizing hormone.

puberty, developed biochemical testosterone deficiency over time, lending support to Taneja *et al.* that recently reported a substantial risk of deterioration in Leydig cell function in adulthood after pediatric HSCT (22). Taken together, these findings emphasize the need for systematic evaluation of Leydig cell function during puberty and into adulthood to allow timely treatment of testosterone deficiency. This may in turn lower the risk of other late effects potentially linked to testosterone deficiency such as altered body composition, metabolic syndrome, low bone mineral density as well as fatigue and depression (23) – conditions that are frequently reported in these patients (24, 25, 26).

The gonadotropin levels remained within normal limits until the onset of puberty, as previously shown by others (4, 5, 7). Moreover, we here report that signs of testicular failure were not evident at early puberty, whereas higher LH levels from mid to late puberty (age 14–16 years) indicated an increased risk of later testosterone deficiency. Although this finding suggests a predictive value of LH during the pubertal years, it is important to note that some males developed testosterone deficiency despite normal LH levels, possibly indicating a blunted LH response due to a hypothalamic/pituitary component of the hypogonadism, and prolonged follow-up should, therefore, be recommended for all patients.



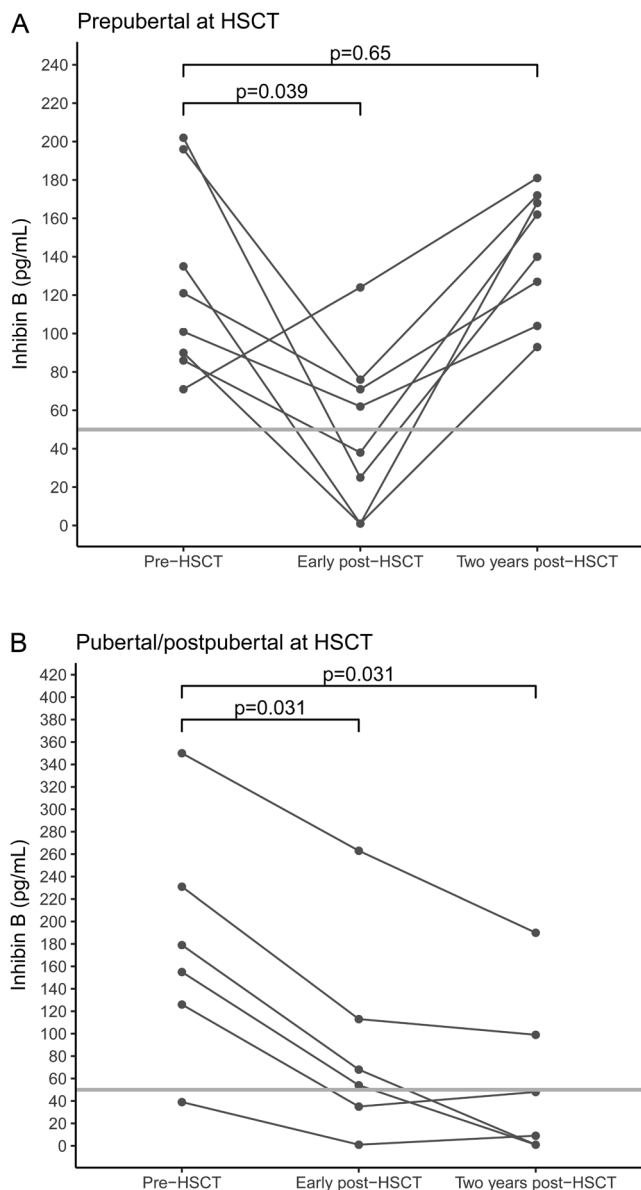
**Figure 6**

FSH and inhibin B patterns in patients who were prepubertal at HSCT and had spontaneous onset of puberty (panel A and B), and patients who were pubertal/postpubertal at transplant (panel C and D). Red curves represent patients with azoospermia at last follow-up, whereas blue curves represent patients with sperm in the ejaculate. Patients on testosterone substitution therapy and patients without semen sample data were excluded in the plots; thus,  $n = 14$  for the prepubertal group and  $n = 12$  for pubertal/postpubertal group. Horizontal gray lines indicate the upper limit of normal for FSH (15 IU/L) and lower limit of normal for inhibin B (50 pg/mL) in adulthood. In patients being prepubertal at HSCT, FSH levels and inhibin B levels from mid to late puberty seemed to indicate spermatogenic status in adulthood (panel A and B). This pattern was not as clear in patients being pubertal/postpubertal at HSCT, as the only two males with detectable sperm presented high FSH levels and very low inhibin B levels over time, and several males with azoospermia had normal FSH levels (panel C and D). FSH, follicle-stimulating hormone; HSCT, hematopoietic stem cell transplantation.

Prediction of fertility is an even more critical issue after pediatric HSCT. For patients being prepubertal at HSCT, higher FSH levels and lower inhibin B levels were associated with increased risk of azoospermia in adulthood from the age of 12 and 14 years, respectively, and at post-puberty, both FSH and inhibin B were clearly indicative of spermatogenic capacity. Unexpectedly, we did not find a similar pattern in the patients transplanted after the onset of puberty, as the only two males with detectable sperm presented with persistently high FSH levels and low inhibin B levels, and several males with azoospermia had FSH levels within normal limits. Accordingly, the predictive value of these markers may be questioned in this subgroup of

patients, lending support to Green *et al.*, who reported a poor specificity of FSH and inhibin B in determining spermatogenic capacity in adult male childhood cancer survivors (27). Therefore, we conclude that although FSH and inhibin B to some degree indicate spermatogenic capacity, direct evaluation by semen samples should be recommended for all patients interested in their fertility potential.

Regarding dynamics of inhibin B, we observed a significant drop in inhibin B levels immediately after HSCT. In prepubertal patients, the inhibin B levels then recovered to pre-transplant levels within 2 years, followed by a decrease in inhibin B levels during puberty in patients with



**Figure 7**  
 Inhibin B levels pre-HSCT, early post-HSCT, and 2 years post-HSCT for patients who were prepubertal at HSCT (panel A) and patients who were pubertal/postpubertal at HSCT (panel B). Only patients with available data at all three time points were included in analyses; thus,  $n = 9$  for prepubertal patients and  $n = 6$  for pubertal/postpubertal patients. Pre-HSCT evaluation was median 2.9 weeks prior to HSCT (range 1.0–8.4), early post-HSCT evaluation (first evaluation after transplant) was median 5.4 months post-HSCT (range 3.3–7.0), and 2 years post-HSCT evaluation was median 2.0 years post-HSCT (range 1.6–2.3). Horizontal gray lines indicate the lower limit of normal for inhibin B (50 pg/mL) in adulthood. *Statistics:* Wilcoxon signed-rank test. HSCT, hematopoietic stem cell transplantation.

azoospermia. These dynamics are most likely explained by the shift in inhibin B subunit production after the onset of puberty. In the prepubertal years, both subunits of inhibin B ( $\alpha$  subunit and  $\beta$ B subunit) are produced by the Sertoli

cells, whereas after the onset of puberty, the  $\beta$ B subunit is solely produced by early precursors of sperm cells (28, 29). Accordingly, when spermatogenesis is affected by chemotherapy and irradiation, the production of the  $\beta$ B subunit during puberty is missing, leading to an abrupt decline in inhibin B, a pattern also observed in patients with Klinefelter syndrome (47,XXY) (30). Due to these biological processes, inhibin B indicates spermatogenic status only from mid-puberty and onwards, which is critical to remember in research and clinical follow-up. Surprisingly, we found that *higher* inhibin B levels at age 9–12 years were associated with a higher risk of azoospermia in adulthood, which may be a by chance finding or explained by excessive stimulation of the intact Sertoli cells by gonadotropins in patients with testicular failure, already in these prepubertal/early pubertal years (31).

Repeated semen samples revealed consistent azoospermia in most of the nine survivors with available data. Nevertheless, three males had few detectable spermatozoa at some point after HSCT despite azoospermia in the remaining samples. This indicates some degree of spermatogenesis, and these men may have a chance of retrieving sperm by testicular sperm extraction for use in assisted reproduction (32). This was done successfully in one of the survivors, and together with two other case reports (33, 34), we find this a proof of concept.

To our knowledge, this is the first study of male HSCT survivors to evaluate pubertal timing compared to the general population and to investigate associations between reproductive hormone levels during puberty and the risk of testicular failure in adulthood. In contrast to previous longitudinal studies of male gonadal function after pediatric HSCT (4, 5, 6, 7), we included semen quality data and follow-up into adulthood. In addition, the study population appeared representative of the total cohort of male HSCT survivors transplanted in the same time period regarding transplant characteristics such as age at HSCT, diagnoses, and conditioning regimens. Although this is the most extensive longitudinal study of male gonadal function after pediatric HSCT, some limitations exist, primarily linked to the retrospective design. We could not ascertain that all reproductive hormones were analyzed from morning samples, and we acknowledge that the shift in testosterone assays may have added to the variability in testosterone levels. These limitations are impossible to overcome in a retrospective study with a median follow-up time of 19 years. However, most samples were measured between 1990 and 2014 using one RIA, and to meet the risk of variability in testosterone levels, we chose to define biochemical testosterone deficiency as three consecutive testosterone

**Table 2** Associations between reproductive hormone levels during the pubertal years and risk of testosterone deficiency and risk of azoospermia in adulthood. Only post-transplant hormone measurements were included in the analyses, and for inhibin B, only measurements more than 2 years after transplant were included due to a decrease in inhibin B levels immediately after transplant and recovery within 2 years. Thus, to be eligible for analyses, the patients should be transplanted before or during the specific age interval, and for inhibin B analyses, 2 years should have passed from transplant. Associations between LH levels and risk of testosterone deficiency were analyzed only for the first three age intervals, as some patients started testosterone substitution therapy during the last age interval (16–20 years) (median age at last follow-up 27.4 years (range 18.5–40.4)). Associations between FSH or inhibin B levels and risk of azoospermia were analyzed only in patients who were prepubertal at HSCT and without testosterone substitution (median age at last follow-up 24.4 years (range 18.5–29.5)). *Statistics:* Simple logistic regression, estimates reported for 1 IU/L increase in FSH and LH and for 10 pg/mL increase in inhibin B.

Patients with spontaneous onset of puberty ( <i>n</i> = 33)				
Mean LH level	Eligible, <i>n</i>	Number of eligible patients with available measurements, <i>n</i>	Testosterone deficiency	
			OR (95% CI)	<i>P</i>
Age ≥ 9 to < 12 years	19	19 Missing data: 0/5 with testosterone deficiency 0/14 with normal testosterone	3.76 (0.19–111.19)	0.36
Age ≥ 12 to < 14 years	23	20 Missing data: 1/6 with testosterone deficiency 2/17 with normal testosterone	1.28 (0.56–3.00)	0.54
Age ≥ 14 to < 16 years	30	24 Missing data: 1/9 with testosterone deficiency 5/21 with normal testosterone	1.54 (1.10–2.59)	<b>0.006</b>
Patients who were prepubertal at HSCT and without testosterone substitution at last follow-up ( <i>n</i> = 14)				
Mean FSH level	Eligible, <i>n</i>	Number of eligible patients with available measurements, <i>n</i>	Azoospermia	
			OR (95% CI)	<i>P</i>
Age ≥ 9 to < 12 years	13	12 Missing data: 1/7 with azoospermia 0/6 with sperm	2.34 (0.74–14.71)	0.22
Age ≥ 12 to < 14 years	14	12 Missing data: 1/8 with azoospermia 1/6 with sperm	2.70 (1.06–33.66)	<b>0.028</b>
Age ≥ 14 to < 16 years	14	10 Missing data: 3/8 with azoospermia /6 with sperm	1.26 (0.99–2.07)	0.061
Age ≥ 16 to < 20 years	14	12 Missing data: 1/8 with azoospermia 1/6 with sperm	2.55 (1.20–13.91)	<b>0.003</b>
Mean inhibin B level	Eligible, <i>n</i>	Number of eligible patients with available measurements, <i>n</i>	Azoospermia	
			OR (95% CI)	<i>P</i>
Age ≥ 9 to < 12 years	13	13 Missing data: 0/7 with azoospermia 0/6 with sperm	1.44 (1.03–2.36)	<b>0.029</b>
Age ≥ 12 to < 14 years	13	12 Missing data: 1/7 with azoospermia 0/6 with sperm	0.95 (0.81–1.10)	0.49
Age ≥ 14 to < 16 years	14	10 Missing data: 2/8 with azoospermia 2/6 with sperm	0.76 (0.49–0.99)	<b>0.044</b>
Age ≥ 16 to < 20 years	14	12 Missing data: 2/8 with azoospermia 0/6 with sperm	0.73 (0.47–0.96)	<b>0.021</b>

FSH, follicle-stimulating hormone; HSCT, hematopoietic stem cell transplantation; LH, luteinizing hormone. Bold indicates statistical significance, *P* < 0.05.



measurements under the reference limit without returning to normal values. The study is also limited by small patient numbers and diverse diagnoses and treatment regimens; therefore, the estimates (OR and their CIs) should be interpreted cautiously, and we may have missed associations due to lack of statistical power. Nevertheless, the results are supported by previous evidence and current biological insights; thus, we feel confident in our interpretation.

In conclusion, induction of puberty with testosterone was needed in one-fourth of the male survivors of pediatric HSCT and was related to orchiectomy and irradiation in addition to TBI. The timing of puberty appeared to be normal for the remaining survivors; nevertheless, the reproductive hormone levels were affected at end of puberty, and several patients developed testosterone deficiency with time. Although the reproductive hormone levels from mid-puberty to some degree indicated adult testicular function, systematic prolonged clinical and biochemical follow-up is needed for all male pediatric HSCT survivors, including semen samples for patients interested in their fertility potential.

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

#### Funding

This work was supported by the Danish Childhood Cancer Foundation (grant no. 2015-15 and 2016-0261), Rigshospitalet's Research Foundation (Denmark), the Danish Cancer Society (grant no. R165-A10527), the Danish Cancer Research Foundation, Dagmar Marshall's Fund (Denmark), and the Hartmann Brothers' Fund (Denmark) (grant no. A30640). COPENHAGEN Puberty Study was funded by Kirsten and Freddy Johansen's Foundation.

#### Author contribution statement

S M, K S, K M, and A J contributed to the study design. S M, K S, C P H, and A J collected data. S M and J H P conducted the statistical analyses. S M, K S, M I, C P H, A J, and K M contributed to the data analyses and interpretation. S M, K S, K M, and A J drafted the manuscript. All authors critically revised the manuscript and approved the final version.

#### Acknowledgements

The authors thank the patients for participating in the initial cross-sectional study. In addition, the authors thank Lisbeth Nørgaard Møller, biostatistician at The Pediatric Oncology Research Laboratory, Rigshospitalet, University of Copenhagen, for statistical advice. Anders Juul and Klaus Müller: co-senior authors.

## References

- Majhail NS & Rizzo JD. Surviving the cure: long term follow up of hematopoietic cell transplant recipients. *Bone Marrow Transplantation* 2013 **48** 1145–1151. (<https://doi.org/10.1038/bmt.2012.258>)
- Tichelli A & Rovó A. Fertility issues following hematopoietic stem cell transplantation. *Expert Review of Hematology* 2013 **6** 375–388. (<https://doi.org/10.1586/17474086.2013.816507>)
- Pulsipher MA, Skinner R, McDonald GB, Hingorani S, Armenian SH, Cooke KR, Gracia C, Petryk A, Bhatia S, Bunin N, *et al.* National Cancer Institute, National Heart, Lung and Blood Institute/ Pediatric Blood and Marrow Transplantation Consortium First International Consensus Conference on late effects after pediatric hematopoietic cell transplantation: the need for pediatric-specific long-term follow-up guidelines. *Biology of Blood and Marrow Transplantation* 2012 **18** 334–347. (<https://doi.org/10.1016/j.bbmt.2012.01.003>)
- Sarafoglou K, Boulad F, Gillio A & Sklar C. Gonadal function after bone marrow transplantation for acute leukemia during childhood. *Journal of Pediatrics* 1997 **130** 210–216. ([https://doi.org/10.1016/s0022-3476\(97\)70345-7](https://doi.org/10.1016/s0022-3476(97)70345-7))
- Mayer EI, Dopfer RE, Klingebiel T, Scheel-Walter H, Ranke MB & Niethammer D. Longitudinal gonadal function after bone marrow transplantation for acute lymphoblastic leukemia during childhood. *Pediatric Transplantation* 1999 **3** 38–44. (<https://doi.org/10.1034/j.1399-3046.1999.00006.x>)
- Couto-Silva AC, Trivin C, Thibaud E, Esperou H, Michon J & Brauner R. Factors affecting gonadal function after bone marrow transplantation during childhood. *Bone Marrow Transplantation* 2001 **28** 67–75. (<https://doi.org/10.1038/sj.bmt.1703089>)
- Bakker B, Massa GG, Oostdijk W, Weel-Sipman MH Van, Vossen JM & Wit JM. Pubertal development and growth after total-body irradiation and bone marrow transplantation for haematological malignancies. *European Journal of Pediatrics* 2000 **159** 31–37. (<https://doi.org/10.1007/s004310050006>)
- Mathiesen S, Sørensen K, Nielsen MM, Suominen A, Ifversen M, Grell K, Lähteenmäki P, Frederiksen H, Juul A, Müller K, *et al.* Male gonadal function after allogeneic hematopoietic stem cell transplantation in childhood: a cross-sectional, population-based study. *Biology of Blood and Marrow Transplantation* 2020 **26** 1635–1645. (<https://doi.org/10.1016/j.bbmt.2020.05.009>)
- Green DM, Nolan VG, Goodman PJ, Whitton JA, Srivastava D, Leisenring WM, Neglia JP, Sklar CA, Kaste SC, Hudson MM, *et al.* The cyclophosphamide equivalent dose as an approach for quantifying alkylating agent exposure: a report from the Childhood Cancer Survivor Study. *Pediatric Blood and Cancer* 2014 **61** 53–67. (<https://doi.org/10.1002/pbc.24679>)
- Greulich WW & Pyle SI. *Radiographic Atlas of Skeletal Development of the Hand and Wrist*, 2nd ed. Stanford University Press, 1959.
- Jørgensen N, Joensen UN, Jensen TK, Jensen MB, Almstrup K, Olesen IA, Juul A, Andersson AM, Carlsen E, Petersen JH, *et al.* Human semen quality in the new millennium: a prospective cross-sectional population-based study of 4867 men. *BMJ Open* 2012 **2** e000990. (<https://doi.org/10.1136/bmjopen-2012-000990>)
- Søeborg T, Frederiksen H, Fruekilde P, Johannsen TH, Juul A & Andersson AM. Serum concentrations of DHEA, DHEAS, 17 $\alpha$ -hydroxyprogesterone,  $\delta$ 4-androstenedione and testosterone in children determined by TurboFlow-LC-MS/MS. *Clinica Chimica Acta* 2013 **419** 95–101. (<https://doi.org/10.1016/j.cca.2013.01.019>)
- Søeborg T, Frederiksen H, Johannsen TH, Andersson AM & Juul A. Isotope-dilution TurboFlow-LC-MS/MS method for simultaneous quantification of ten steroid metabolites in serum. *Clinica Chimica Acta* 2017 **468** 180–186. (<https://doi.org/10.1016/j.cca.2017.03.002>)
- Mieritz MG, Rakét LL, Hagen CP, Nielsen JE, Talman MLM, Petersen JH, Sommer SH, Main KM, Jørgensen N & Juul A. A longitudinal study of growth, sex steroids, and IGF-1 in boys with physiological gynecomastia. *Journal of Clinical Endocrinology and Metabolism* 2015 **100** 3752–3759. (<https://doi.org/10.1210/jc.2015-2836>)
- Aksglaede L, Andersson AM, Jørgensen N, Jensen TK, Carlsen E, McLachlan RI, Skakkebaek NE, Petersen JH & Juul A. Primary testicular failure in Klinefelter's syndrome: the use of bivariate luteinizing



- hormone-testosterone reference charts. *Clinical Endocrinology* 2007 **66** 276–281. (<https://doi.org/10.1111/j.1365-2265.2006.02722.x>)
- 16 Andersson AM, Petersen JH, Jørgensen N, Jensen TK & Skakkebaek NE. Serum inhibin B and follicle-stimulating hormone levels as tools in the evaluation of infertile men: significance of adequate reference values from proven fertile men. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 2873–2879. (<https://doi.org/10.1210/jc.2003-032148>)
- 17 Damgaard-Olesen A, Johannsen TH, Holmboe SA, Søbørg T, Petersen JH, Andersson AM, Aadahl M, Linneberg A & Juul A. Reference ranges of 17-hydroxyprogesterone, DHEA, DHEAS, androstenedione, total and free testosterone determined by TurboFlow-LC-MS/MS and associations to health markers in 304 men. *Clinica Chimica Acta: International Journal of Clinical Chemistry* 2016 **454** 82–88. (<https://doi.org/10.1016/j.cca.2015.12.042>)
- 18 Cleveland WS. Robust locally weighted regression and smoothing scatterplots. *Journal of the American Statistical Association* 1979 **74** 829–836. (<https://doi.org/10.1080/01621459.1979.10481038>)
- 19 Long J. *Regression Models for Categorical and Limited Dependent Variables*. Thousand Oaks: SAGE Publications, Inc., 1997.
- 20 Frisk P, Arvidson J, Gustafsson J & Lönnnerholm G. Pubertal development and final height after autologous bone marrow transplantation for acute lymphoblastic leukemia. *Bone Marrow Transplantation* 2004 **33** 205–210. (<https://doi.org/10.1038/sj.bmt.1704324>)
- 21 Steffens M, Beauloye V, Brichard B, Robert A, Alexopoulou O, Vermeylen Ch & Maiter D. Endocrine and metabolic disorders in young adult survivors of childhood acute lymphoblastic leukaemia (ALL) or non-Hodgkin lymphoma (NHL). *Clinical Endocrinology* 2008 **69** 819–827. (<https://doi.org/10.1111/j.1365-2265.2008.03283.x>)
- 22 Taneja S, Rao A, Nussey S & Leiper A. Leydig cell failure with testicular radiation doses <20Gy: the clinical effects of total body irradiation conditioned haematopoietic stem cell transplantation for childhood leukaemia during long-term follow-up. *Clinical Endocrinology* 2019 **91** 624–632. (<https://doi.org/10.1111/cen.14059>)
- 23 Bhasin S, Brito JP, Cunningham GR, Hayes FJ, Hodis HN, Matsumoto AM, Snyder PJ, Swerdloff RS, Wu FC & Yialamas MA. Testosterone therapy in men with hypogonadism: an Endocrine Society Clinical Practice Guideline. *Journal of Clinical Endocrinology and Metabolism* 2018 **103** 1715–1744. (<https://doi.org/10.1210/jc.2018-00229>)
- 24 Baker KS, Bresters D & Sande JE. The burden of cure: long-term side effects following hematopoietic stem cell transplantation (HSCT) in children. *Pediatric Clinics of North America* 2010 **57** 323–342. (<https://doi.org/10.1016/j.pcl.2009.11.008>)
- 25 Di GG, Thacker N, Schechter T & Pole JD. Anxiety, depression, and mental health-related quality of life in survivors of pediatric allogeneic hematopoietic stem cell transplantation: a systematic review. *Bone Marrow Transplantation* 2020 **55** 1240–1254. (<https://doi.org/10.1038/s41409-020-0782-z>)
- 26 Graef DM, Phipps S, Parris KR, Martin-Elbaresh K, Huang L, Zhang H & Crabtree VML. Sleepiness, fatigue, behavioral functioning, and quality of life in survivors of childhood hematopoietic stem cell transplant. *Journal of Pediatric Psychology* 2016 **41** 600–609. (<https://doi.org/10.1093/jpepsy/jsw011>)
- 27 Green DM, Zhu L, Zhang N, Sklar CA, Ke RW, Kutteh WH, Klosky JL, Spunt SL, Metzger ML, Navid F, *et al.* Lack of specificity of plasma concentrations of inhibin B and follicle-stimulating hormone for identification of azoospermic survivors of childhood cancer: a report from the St Jude lifetime cohort study. *Journal of Clinical Oncology* 2013 **31** 1324–1328. (<https://doi.org/10.1200/JCO.2012.43.7038>)
- 28 Andersson AM, Müller J & Skakkebaek NE. Different roles of prepubertal and postpubertal germ cells and Sertoli cells in the regulation of serum inhibin B levels. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 4451–4458. (<https://doi.org/10.1210/jcem.83.12.5360>)
- 29 Andersson AM & Skakkebaek NE. Serum inhibin B levels during male childhood and puberty. *Molecular and Cellular Endocrinology* 2001 **180** 103–107. ([https://doi.org/10.1016/s0303-7207\(01\)00520-2](https://doi.org/10.1016/s0303-7207(01)00520-2))
- 30 Christiansen P, Andersson AM & Skakkebaek NE. Longitudinal studies of inhibin B levels in boys and young adults with Klinefelter syndrome. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 888–891. (<https://doi.org/10.1210/jc.2002-021379>)
- 31 Andersson AM, Juul A, Petersen JH, Müller 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. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 3976–3981. (<https://doi.org/10.1210/jcem.82.12.4449>)
- 32 Bernie AM, Mata DA, Ramasamy R & Schlegel PN. Comparison of microdissection testicular sperm extraction, conventional testicular sperm extraction, and testicular sperm aspiration for nonobstructive azoospermia: a systematic review and meta-analysis. *Fertility and Sterility* 2015 **104** 1099.e1–1103.e1. (<https://doi.org/10.1016/j.fertnstert.2015.07.1136>)
- 33 Ignatov AP, Eisenberg MS & Turek PJ. Paternity after directed collection of testicular sperm for in vitro fertilization after BMT for hematological malignancies. *Bone Marrow Transplantation* 2010 **45** 1474–1476. (<https://doi.org/10.1038/bmt.2009.372>)
- 34 Jacobsen FM, Fode M, Sønksen J, Ohl DA & Jensen CFS. Successful extraction of sperm cells after autologous bone marrow transplant: a case report. *Scandinavian Journal of Urology* 2019 **53** 174–175. (<https://doi.org/10.1080/21681805.2019.1570329>)

Received in final form 14 September 2021

Accepted 17 September 2021

Accepted Manuscript published online 17 September 2021