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ORIGINAL ARTICLE ***Reproductive Endocrinology***

Longitudinal follow-up in female Childhood Cancer Survivors: no signs of accelerated ovarian function loss

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STUDY QUESTION: Is the long-term decline of ovarian function, as reflected by a decrease in serum anti-Müllerian hormone (AMH) concentration, accelerated over time in female childhood cancer survivors (CCS) as compared to healthy women of the same age?

SUMMARY ANSWER: The median decline of AMH levels in long-term female CCS is not accelerated and similar to that observed in healthy controls.

WHAT IS KNOWN ALREADY: Gonadal function is compromised in female CCS treated with chemotherapy and/or radiation therapy. Ovarian function is most compromised in survivors treated with total body irradiation, abdominal or pelvic irradiation, stem cell transplantation or high doses of alkylating agents.

STUDY DESIGN SIZE, DURATION: Longitudinal single-centre cohort study in 192 CCS in Rotterdam, The Netherlands, between 2001 and 2014.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Serum AMH levels of 192 adult female CCS were assessed, at least five years after cessation of treatment and at a follow-up visit with a median of 3.2 years (range: 2.1–6.0) later and were compared to the age-based P_{50} of AMH in healthy controls.

MAIN RESULTS AND THE ROLE OF CHANCE: Median AMH levels were below the P_{50} at both visit 1 (−0.59 μg/L) and at visit 2 (−0.22 μg/L). In women with a sustained ovarian function (AMH > 1.0 μg/L), the decline in AMH is similar to that in the normal population (difference in decline per year: −0.07 μg/L (range: −2.86 to 4.92), $P = 0.75$). None of the treatment modalities was correlated with a significant acceleration of decline of AMH per year.

LIMITATIONS REASONS FOR CAUTION: We selected CCS that visited our late effect outpatient clinic and who had two AMH levels available. It is conceivable that women without any apparent late effects of treatment as well as women with extreme late effects, which might be the ones with the largest impact on ovarian function, could be more likely to be lost to follow-up. However, general characteristics did not differ between the included and excluded patients.

WIDER IMPLICATIONS OF THE FINDINGS: While prospective longitudinal research is required to strengthen our findings, they may help physicians to counsel female CCS about their expected reproductive lifespan.

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Key words: anti-Müllerian hormone / ovarian function/ childhood cancer / females / follow-up studies / longitudinal studies / reproductive health / survivors / neoplasms

Introduction

The number of childhood cancer survivors (CCS) is growing. These increased success rates confront health care providers with new challenges regarding long-term adverse health-related outcomes (Meadows, 2006; Smith *et al.*, 2010; Hjorth *et al.*, 2015).

Potentially affected organ systems in survivors include the reproductive organs (Oeffinger *et al.*, 2006; Hudson *et al.*, 2013; Robison and Hudson, 2014). Gonadotoxicity in adult CCS varies depending on treatment modality and the administered dose (Green *et al.*, 2009; Lie Fong *et al.*, 2009; Thomas-Teinturier *et al.*, 2013). Patients treated with total body irradiation, with or without stem cell transplantation, local irradiation on the gonadal area or high dosage of alkylating agents seem to carry a higher risk for gonadal impairment in the long-term (Sanders *et al.*, 1996; Schimmer *et al.*, 1998; Cheng *et al.*, 2012; Panasiuk *et al.*, 2015). Clinical manifestations of gonadal impairment include irregular menses, infertility or peri-menopausal complaints. Prior to clinical presentation, impaired gonadal function is preceded by low serum anti-Müllerian hormone (AMH) levels (van Beek *et al.*, 2007). AMH is produced solely in the ovary by granulosa cells of small growing follicles and is considered to be a reliable marker of ovarian function (de Vet *et al.*, 2002; Rosen *et al.*, 2012). Despite increasing knowledge from cross-sectional studies of direct treatment-related gonadotoxicity in CCS, little is known about the longitudinal changes in ovarian function over time. For instance, knowledge is lacking as to whether CCS show a more rapid loss of ovarian function compared to the normal population in the long-term. Considering the increase in age at which women have their first child especially in western societies, it is relevant to investigate whether CCS should be counselled about a potentially reduced reproductive lifespan and the individual implications of such shortening.

In adult male CCS the course of Inhibin B levels has been studied. Surprisingly, after initial impairment, recovery of male gonadal function was suggested long after treatment in subsets of male CCS with modest gonadal impairment (Inhibin B levels ≥105 ng/L) (Pedrick and Hoppe, 1986; Marmor and Duyck, 1995; van Dorp *et al.*, 2013b). In female CCS only one 10-year follow-up study is available, showing a seemingly normal ovarian function after an interval of 10 years, but this analysis was performed in a small cohort of 35 woman selected on the basis of having regular cycles which might constitute a group of CCS with a relative good prognosis (Nielsen *et al.*, 2013).

As gonadotoxicity is considerable in CCS, it is conceivable that AMH may show a more rapid decline in female CCS than normally expected. In the current study, we investigated whether the decline in AMH levels in CCS differs from that observed in the healthy normal population.

Materials and Methods

Subjects

This retrospective single centre study was performed in adult female CCS from the Erasmus MC- Sophia Children Hospital in Rotterdam who visited the adult late-effects outpatient clinic. Survivors were diagnosed with a primary tumour between 1960 and 2005 and were in complete remission. Only CCS who visited our late-effects outpatient clinic twice or more between 2001 and 2014 were included. CCS were at least 16 years of age at first measurement of AMH and were at least 5 years after cessation of cancer treatment. A second blood sample was taken at least 2 years after the first sample. If more samples were available we used the two samples with the longest time interval between them. Patients over the age of 50 years and patients who had undergone a bilateral salpingo-oophorectomy were excluded.

Information on patients' characteristics, type of disease and treatment was retrieved from medical records. The alkylating agent dose (AAD) score was calculated to include the effect of high-risk chemotherapy as previously reported (Tucker *et al.*, 1987; Green *et al.*, 2009; van Dorp *et al.*, 2013a, b, van Dorp *et al.*, 2013a, b). Patients not exposed to alkylating agents were assigned an AAD-score of zero.

Hormone assays

Peripheral blood samples were obtained while CCS visited the LATER outpatient clinic for patient care. Serum samples were taken randomly during the menstrual cycle. All serum measurements were performed in one laboratory at the Erasmus MC, Rotterdam, the Netherlands. In cohorts before 2011, AMH was measured with an ultrasensitive ELISA (Immunotech-Coulter, Marseilles, France). These AMH values were adjusted to allow comparison with the currently used ELISA (commercially available as the Gen II Beckman Coulter, Beckman Coulter, Inc., Webster, TX). Intra- and inter-assay variation coefficients were <5 and <10%, respectively (de Vet *et al.*, 2002). The reference data were measured in a similar manner (Lie Fong *et al.*, 2012).

Statistics

To compare the longitudinal AMH levels of CCS with AMH levels of a healthy reference population of the same age, we used the cross-sectional data available from our earlier report (Lie Fong *et al.*, 2012). The original data of this large Dutch reference population was not normally distributed for each age group. We could therefore not calculate *Z*-scores of AMH for our CCS. Moreover, due to the absence of sequential AMH data in a large group of healthy women, we could not compare slopes between CCS and the normal population. Instead, we calculated the difference between the observed AMH level and its specific age-based P_{50} in μg/L (observed AMH−age-based P_{50} AMH) for each measurement, and analysed the difference between visits 1 and 2.

In our full cohort, we analysed the difference between visits 1 and 2 with the related-samples Wilcoxon signed rank test. The Kruskal–Wallis test was used to compare the difference between visit 1 and 2 by age group and by type of irradiation, AAD score, pre- or post-menarche at diagnosis and stem cell transplantation. We identified five age groups in our previously reported nomogram (Lie Fong *et al.*, 2012) based on different slopes in AMH decline. The high AMH levels of women with polycystic ovary syndrome (PCOS) can possible skew our results. We therefore additionally analysed the difference between visits 1 and 2 excluding patients with an initial AMH level >5 μg/L and patients

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with an initial AMH level >10 μg/L since information about follicle count and hyperandrogenism was not available.

Next, we stratified our cohort in two groups depending on baseline AMH levels, based on values considered clinically relevant for fertility. AMH levels below 1.0 μg/L were considered to be low, all other levels are considered not to be low and will be referred to as 'normal'. The group with an initial low AMH and the group with an initial 'normal' AMH were analysed separately, evaluating, e.g. the probability to stay 'normal' if your baseline AMH level was 'normal'. This was done for several patient characteristics and different treatment modalities, using a chi-square goodness-of-fit or Mann–Whitney *U* test for categorical and continuous variables, respectively. *P*-values <0.05 were considered statistically significant. Statistical analyses were performed with the Statistical Package for Social Sciences version 21.0 (SPSS, Chicago, IL, USA), part of the graphics was created with the free software environment R version 3.2.2 and GraphPad Prism version 7 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com.

Ethical approval

Informed consent was obtained from all included patients according to the standards of the Medical Institutional Review Board of Erasmus MC. This study was approved by the latter Review Board.

Results

Initial AMH levels were available from 358 out of 460 female CCS visiting our outpatient clinic. The 192 adult female CCS in whom a second AMH measurement was performed were included in our study. Clinical

Table I Comparison of survivors with a second anti-Müllerian hormone (AMH) measurement and included in the studywith the total group of female adult childhood cancer survivors (CCS). Data are expressed as median (range) or frequencies (%).

	Total group of adult female CCS	Survivors included in this study	*P*-value[a]
Age at diagnosis (yrs)	5.4 (0.1–16.8)	6.1 (0–16.8)	0.04
Age at first visit (yrs)	21.4 (5.9–57.4)	23.6 (17.1–46.2)	<0.01
Age at second visit (yrs)	NA	26.9 (20.0–49.2)	
Interval between stop treatment and first AMH level (yrs)	15.1 (4.0–43.2)	15.8 (5.0–43.2)	0.07
Interval between first and second visit (yrs)	NA	3.2 (2.1–6.0)	
BMI at first visit (kg/m^2)	22.9 (15.3–40.0)	23.0 (16.2–39.6)	0.83
AMH level at first visit (μg/L)	2.50 (0.00–25.90)	2.50 (0.00–21.01)	0.69
AMH level at second visit (μg/L)	NA	2.43 (0.01–24.03)	
Difference in AMH with P_{50}, first visit (μg/L)	NA	−0.59 (−4.07–17.05)	
Difference in AMH with P_{50}, second visit (μg/L)	NA	−0.22 (−3.75–20.50)	
Diagnosis			<0.01
ALL & T-NHL	128 (30)	69 (36)	
Acute myeloid leukemia	9 (2)	8 (4)	
B-cell non Hodgkin lymphoma	25 (6)	7 (4)	
Hodgkin lymphoma	29 (7)	15 (8)	
Sarcoma	57 (13)	15 (8)	
Renal tumour	47 (11)	25 (13)	
Neuroblastoma	32 (8)	24 (13)	
Germ cell tumour	12 (3)	0 (0)	
Brain tumour	52 (12)	10 (5)	
Other	34 (8)	19 (10)	
Radiotherapy			0.48
Abdominal radiotherapy	27 (7)	19 (10)	
Total body irradiation	13 (3)	7 (4)	
Chemotherapy (AAD score)			0.01
0	237 (56)	94 (49)	
1	47 (11)	26 (14)	
2	47 (11)	30 (16)	
3	70 (17)	34 (18)	
≥ 4	24 (6)	8 (4)	

[a]Comparison between groups by Mann–Whitney *U* test for continuous outcome and Chi square test for categorical outcome.
NA = not applicable; AMH = anti-Müllerian hormone; BMI = Body Mass Index; ALL = Acute Lymphoblastic Leukemia, T-NHL = T-cell non Hodgkin lymphoma; AAD score = alkylating agent dose score.

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characteristics and treatment details of the total cohort of female CCS of our centre and the survivors included in this study are shown Table I. The included sample is representative for the total cohort of female CCS of our centre although fewer survivors included in this study had received treatment without alkylating agents (AAD score = 0). A comparable percentage was treated with the higher cumulative AAD scores 3 and 4 which are known to be highly correlelated with gonadotoxicity. Importantly, AMH levels at the first visit of the included women were similar ($P = 0.69$) to the available AMH levels of the full cohort.

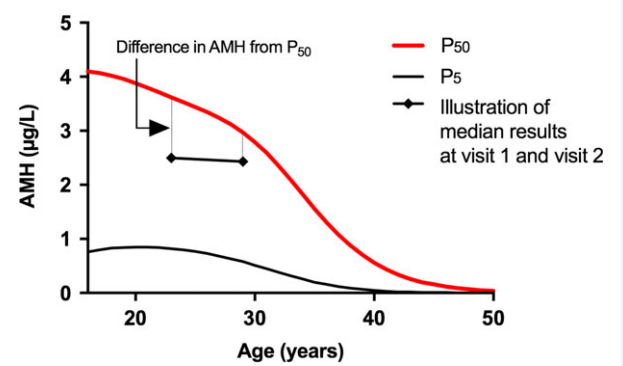

Figure 1 P_{50} and P_5 from healthy females (Lie Fong *et al.*, 2012). Median results from our cohort (median age and median anti-Müllerian hormone (AMH) at visit 1 (minimally 5 years after stop treatment) and visit 2 (after an interval of 3.2 years)) are depicted. P_{50} and P_5 refer to 50th and 5th percentiles, respectively.

The median time since cessation of treatment was 15.8 years (range: 5.0–43.2) at the first visit, the second visit was after a median interval of 3.2 years (range: 2.1–6.0). The median observed AMH level in the included CCS was lower than the age-based P_{50} level, both at the first visit and second visit: −0.59 µg/L (−4.07 to 17.05 µg/L) and −0.22 µg/L (range −3.75 to 20.50 µg/L), respectively (Fig. 1).

Figure 2 represents a Boxplot of the calculated absolute differences between the observed AMH level and the expected age-based AMH level according to the 50th percentile (P_{50}) of the healthy controls at visit 1 and at visit 2, for each age category. This difference did not vary significantly across the age categories (*P*-value 0.16) between visits 1 and 2 and our analysis was not stratified based on age.

Analysis of the AMH levels compared to the P_{50} of the normal healthy controls is presented in Table II. After a median follow-up interval of 3.2 years (range: 2.1–6.0 years), the median AMH levels were still below the P_{50} but the distance to the P_{50} was not increased. This indicates that the AMH levels of CCS remain well below the AMH levels of normal healthy controls, also at very long-term follow-up. However, there is no additional acceleration in the loss of AMH in

Table II Analysis of difference between the observed anti-Müllerian hormone (AMH) value at visits 1 and 2 and the P_{50} of healthy peers stratified by treatment and menarche prior or after diagnosis, in female childhood cancer survivors.

	N	Difference with p50 at T1 (AMH µg/L) median (range)	Difference with p50 at T2 (AMH µg/L) median (range)[a]	*P*-value[b]
Total	192	−0.59 (−4.07–17.05)	−0.22 (−3.75–20.50)	0.04
Radiotherapy				0.68
No irradiation	123	−0.27 (−3.73–17.05)	−0.18 (−3.66–20.50)	
Abdomen/pelvis	7	−1.7 (−3.96–0.56)	−1.26 (−3.69–2.26)	
Half of abdomen	12	−0.54 (−2.39–13.81)	−0.43 (−3.36–16.54)	
Thorax	8	−0.88 (−4.07–2.82)	−0.30 (−2.10–10.63)	
Cranial and nerve system	27	−0.14 (−3.09–11.60)	−0.25 (−2.70–12.33)	
Total body irradiation	7	−3.35 (−4.02–−2.31)	−3.16 (−3.75–−2.30)	
Others	8	−0.88 (−3.23–2.67)	−1.17 (−2.60–7.01)	
Chemotherapy (AAD-score)				0.21
0	94	−0.08 (−3.88–13.81)	0.49 (−3.57–20.50)	
1	26	−1.20 (−3.91–17.05)	−0.72 (−3.16–18.53)	
2	30	−0.82 (−3.96–7.46)	−1.11 (−3.69–7.90)	
3	34	−0.95 (−4.07–14.46)	−0.83 (−3.75 −17.25)	
4	8	−1.18 (−3.73–6.98)	−0.14 (−3.66–4.77)	
Menarche				0.25
Pre-treatment	130	−0.69 (−4.07–17.05)	−0.26 (−3.75–20.50)	
Post-treatment	29	−0.88 (−3.88–4.22)	−0.21 (−3.66–10.63)	
Stem cell transplantation				n.a.
Yes	4	−3.44 (−4.02–2.31)	−3.37 (−3.75–−2.30)	
No	132	−0.50 (−3.88–17.05)	−0.18 (−3.66–18.53)	

[a]After a median interval of 3.2 yr (range 2.1–6.0); T1 = visit 1, minimally 5 years after stop treatment; T2 = visit 2, minimally 2 years after T1; AAD score = Alkylating Agent Dose score, group 4 = 4 or greater than 4.
[b]Kruskal–Wallis test, testing the change in AMH over time; n.a. = not applicable due to too small groups to test.

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CCS as compared to normal healthy controls (Fig. 1). Whether the decrease in difference from the P_{50} is a clinically relevant observation requires more investigation.

Analysis of the cohort excluding women with a possible PCOS (based on AMH levels >5 or >10 μg/L) did not change these results (Supplementary Table SI). In addition, we analysed the effect of the treatment modalities on the distances to the P_{50}. These analyses showed similar results, i.e. no statistically significant association of any treatment modality with change in AMH (Table II).

There were 139 CCS (72%) who had an initial AMH level above 1.0 μg/L. In these women with a supposedly sustained 'normal' ovarian function after treatment, the decline in AMH is not different from what is known in normal healthy controls (difference in decline per year: −0.07 μg/L (range: −2.86 to 4.92), $P = 0.75$). In this group with retained ovarian function, 122 (87.8%, group D) remained above the threshold of 1.0 μg/L at follow-up (Table III, group D). Survivors with an AAD-score of 3 were more likely to show reduced ovarian function i.e. an AMH below 1.0 μg/L at follow-up (28.6% instead of 12.2%, *P*-value of difference 0.02), although this group is too small to draw definitive clinically relevant conclusions. No other risk factors in treatment modality could be determined (Supplementary Table SII)

In our cohort, 53 CCS (28%) had an initial low AMH level (Table III, group A + B). Of these women, 60.4% still had a low AMH level at the second visit (group A), while 39.6%, mainly young CCS, increased towards an AMH level above 1.0 μg/L (group B).

Figure 3 shows the sequential AMH measurements of CCS in each group according to their AMH level at T1 and T2 in comparison to the AMH nomogram previously reported by Lie Fong *et al.* (2012). This indicates that while the variation in AMH over time within the groups is substantial, most AMH levels remain within the normal range (P_5–P_{95}) during follow-up with the exception of Group A: remaining low AMH levels. Group C: from 'normal' to low AMH levels, also fell below the fifth percentile at T2 but most began at relatively low serum levels.

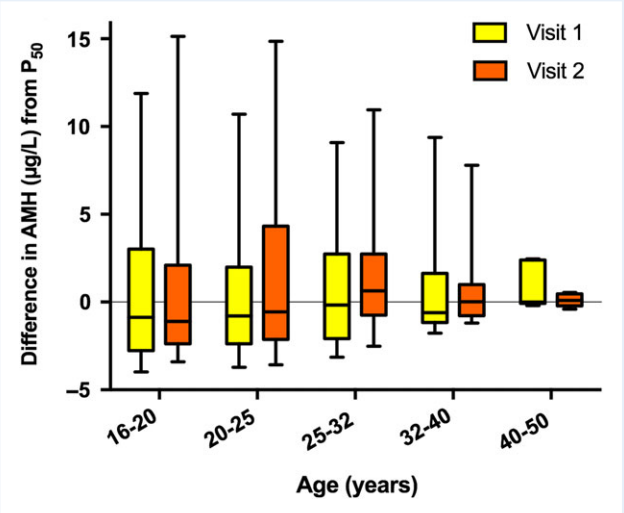

Figure 2 Difference in anti-Müllerian hormone (AMH) (in μg/L) from the observed AMH value of childhood cancer survivors with the P_{50} of healthy women of same age at visit 1 and 2 per age category. Horizontal small bars represent the 5–95th percentile range, and the boxes indicate the 25–75th percentile range. The horizontal line in each box corresponds to the median.

Discussion

The current study indicates that CCS seem to experience a single assault on their ovarian function caused by their disease and/or treatment. While this initial impairment is still evident after long-term follow-up, the decay of ovarian function seems not to be accelerated after the initial impairment has occurred. Given the gonadal impairment, CCS follow a similar rate of decline over time as compared to normal healthy controls.

While this study was not designed to detect early onset of menopause, our data do not give reason to assume CCS have an earlier onset of menopause other than would already be expected based on their, on average low, age-specific AMH levels. Long-term follow-up

Table III Low or normal anti-Müllerian hormone (AMH) at second visit, groups stratified based on low or normal AMH at first visit. Data are presented as median (range) or *N* (%).

	AMH first visit <1.0 μg/L, *N* = 53 (28%)			**AMH first visit >1.0 μg/L, *N* = 139 (72%)**		
Change per year of AMH compared to P_{50} (slope)	0.22 (−0.05– 2.40)*			−0.07 (−2.86–4.92)***		
	N	**Group A: AMH at T2 < 1.0 μg/L**	**Group B: AMH at T2 > 1.0 μg/L**	***N***	**Group C: AMH at T2 < 1.0 μg/L**	**Group D: AMH at T2 > 1.0 μg/L**
Age at T1	53	33.9 (17.1–45.6)	24.0 (17.9–37.5)++	139	22.3 (18.3–46.2)	23.0 (17.6–36.7)
BMI at T1	48	23.3 (16.2–39.6)	23.1 (16.6–34.7)	114	23.6 (18.8–39.4)	22.9 (17.1–37.2)
Interval T1-T2	53	3.2 (2.3–4.3)	3.0 (2.1–3.8)	139	3.0 (2.4–3.9)	3.2 (2.1–6.0)
AMH at T1	53	0.24 (0.00–0.94)	0.63 (0.00–0.94)*	139	1.57 (1.04–5.00)	4.93 (1.09–21.01)*
Overall incidence	**53**	**32 (60.4)**	**21 (39.6)**	**139**	**17 (12.2)**	**122 (87.8)**

Mann–Whitney *U* test for change per year of AMH compared to P_{50} (slope), Age at T1, BMI at T1, Interval T1-T2, AMH at T1.
**P*-value <0.001.
***P*-value = 0.02.
****P*-value = 0.75.
++*P*-value = 0.02.

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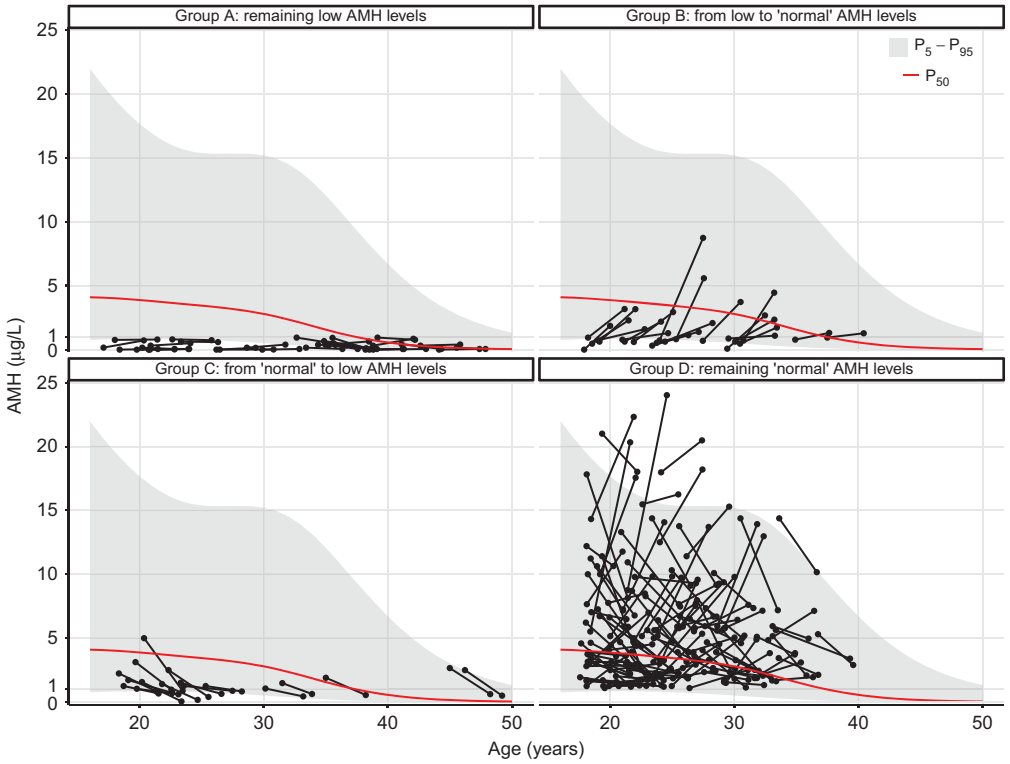

Figure 3 Individual longitudinal anti-Müllerian hormone (AMH) levels of childhood cancer survivors in each group according to their AMH level at visit 1 (minimally 5 years after stop treatment) and visit 2 (after an median interval of 3.2 years) in comparison to the AMH nomogram previously reported by (Lie Fong *et al.*, 2012). Low AMH = <1.0 µg/L; Normal AMH = >1.0 µg/L; P_{50} and P_5–P_{95} refer to 50th and 5th until 95th percentiles, respectively, of healthy females.

studies assessing ovarian function over time in female CCS are scarce. Shorter follow-up has been done quite extensively (Schimmer *et al.*, 1998; Larsen *et al.*, 2003; Green *et al.*, 2009). To our best knowledge only one previous study has examined ovarian function in CCS after 10 years of follow-up (Nielsen *et al.*, 2013). This study reported the comparison of ovarian function only of the 30 women with regular menstrual cycles, which might represent a relatively healthy cohort of CCS. Our study confirmed these findings, in a larger cohort, indicating that no further deterioration of ovarian function is to be expected after the initial impairment (Nielsen *et al.*, 2013).

Chemotherapy has been identified as a key risk factor for ovarian impairment, and the extent of gonadotoxicity is related to cumulative dose (Sanders *et al.*, 1996; Elisabeth *et al.*, 2003; Green *et al.*, 2009; Lie Fong *et al.*, 2009; Thomas-Teinturier *et al.*, 2013). However, neither chemotherapy, nor any treatment modality, was found to be associated with a change in AMH levels at long-term follow-up in our cohort.

AMH represents the activity of small antral follicles and is the best measure of ovarian function for different clinical conditions currently available (Kristensen *et al.*, 2012; Birch Petersen *et al.*, 2015). AMH levels are influenced by several factors that have an impact on ovarian follicles. For instance, women with PCOS are known to reveal higher AMH levels due to a surplus of antral follicles while women with endometriosis and/or medical history of surgery of the ovaria generally have lower AMH levels. The variation of AMH levels throughout the menstrual cycle is generally believed to be limited (de Vet *et al.*, 2002; Streuli *et al.*, 2008). The influence of oral contraceptives on AMH levels is still debated, with studies showing decreased AMH levels (van Beek *et al.*, 2007; Streuli *et al.*, 2008; Deb *et al.*, 2012) in contrast to others indicating no effect at all of hormonal contraceptives (van den Berg *et al.*, 2010; Bentzen *et al.*, 2012; Kristensen *et al.*, 2012; Johnson *et al.*, 2014; Birch Petersen *et al.*, 2015). In this study, we did not adjust for oral contraceptive usage due to missing data.

It has been established that even in CCS with low circulating AMH levels pregnancies can occur (Dillon *et al.*, 2013; Nielsen *et al.*, 2013). This suggests that a low AMH value in young CCS may still be accompanied by a relatively good oocyte quality in contrast to older healthy women with a similar low AMH value and poor oocyte quality as a result of cumulative acquired damage during most of their reproductive period. Therefore, despite a low AMH, oocyte quality seems not to be compromised as in older women. Indeed, data, albeit scarce, on pregnancy rates in these CCS do indicate virtually normal chances for successful conception (Chow *et al.*, 2016). In addition, our study suggests a possible clinical relevant recovery of ovarian function in almost 40% of the CCS initially showing signs of gonadal impairment. Such a recovery is seen mainly in young women under the age of 32 years. Even though this finding could be partly attributed to varying storage or assay conditions (Rustamov *et al.*, 2013) and regression towards the mean, this observed phenomenon underlines the importance of counselling patients with low AMH levels about their fertility and the risk of (unintended) pregnancy. This study suggests that in

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CCS, after initial impairment of ovarian function, the decline in ovarian function is not accelerated compared to normal healthy fertile women. We hypothesize that the stabilization of ovarian function as measured by serum AMH levels is due to impairment of only part of the ovarian reserve. The remaining smaller yet undamaged ovarian pool may develop and decrease similar to that observed in normal healthy women.

There are certain limitations that must be taken into account when interpreting these data. We selected CCS that visited our late effect outpatient clinic and had two AMH levels available. It is conceivable that women without any apparent late effects of treatment could be more prone to become lost to follow-up as well as woman with extreme late effects (such as secondary neoplasms) and accompanying morbidity or even mortality. The latter group might constitute the one with the largest impact on ovarian function. However, general characteristics did not differ between the excluded patients with only one AMH level and the included patients with multiple available AMH levels. Due to the absence of normal sequential data on AMH during a woman's reproductive lifespan, we used the cross-sectional data available from our earlier reports (Lie Fong *et al.*, 2012). We could therefore not compare the slopes between CCS and the normal population, but we were able to assess the change in distance to the P_{50} at each time point. Based on our sample size of $n = 192$ and assuming a normal distribution with a mean difference of 0.55, a standard deviation of 3.30 and an alpha of 0.05, the beta (type II error) would be 0.7 (power 30%). This indicates that the current study lacked power to completely rule out a false negative conclusion, i.e. that there is an acceleration of ovarian function loss while we did not find one. We feel our data nevertheless conveys an important message: in our cohort, we observed no additional decline in in AMH in CCS compared to healthy women. We recommend confirming our data in larger prospective cohort studies with a healthy reference group. This would also enable to include various treatment modalities as independent variables.

The presented study was conducted to investigate the longitudinal decline of ovarian function in female CCS at very long-term follow-up. Our data showed that after initial impairment due to cancer treatment, the further decline of AMH levels in long-term female CCS is not accelerated. Moreover, no treatment modality caused an increased extra risk for accelerated depletion of the primordial follicle pool. Of CCS with an initial AMH level in the clinically normal range, 88% were still within the normal range at long-term follow-up. The results from this study can provide improvement of the counselling patients must receive before their treatment starts and at long-term follow-up, regarding their expected fertile lifespan.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Author's roles

A.L.F.v.d.K. acquired, analysed and interpreted data, wrote the manuscript and designed the study. M.M.v.d.H.-E. designed the study, interpreted data and critically revised the manuscript for intellectual content. S.M.F. analysed data and revised the manuscript for intellectual content. E.v.D.-d.B., A.N. and S.N. revised the manuscript for intellectual content. W.D. acquired data and revised the manuscript for intellectual content. J.S.E.L. designed the study, interpreted data and critically revised the manuscript for intellectual content.

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Conflict of interest

J.S.E.L. has received grants from the following companies (in alphabetical order): Ferring, Genovum, Merck Serono, Merck Sharp and Dome, Organon, Serono, Shering Plough and Shering. The other authors have no conflicts of interest to declare.

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