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ORIGINAL ARTICLE *Reproductive endocrinology*

Decreased serum anti-Müllerian hormone levels in girls with newly diagnosed cancer

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STUDY QUESTION: Are anti-Müllerian hormone (AMH) levels reduced in girls with newly diagnosed cancer before the start of treatment?

SUMMARY ANSWER: AMH levels are already compromised in girls at the time of cancer diagnosis compared with healthy girls.

WHAT IS KNOWN ALREADY: In women diagnosed with cancer, evidence of reduced ovarian function has been described even before treatment has started. In girls with newly diagnosed cancer, no data are available.

STUDY DESIGN, SIZE, DURATION: We performed an age-matched case–control study in girls with newly diagnosed cancer.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We determined serum AMH levels in a cohort of 208 girls with newly diagnosed cancer, up to 18 years of age at diagnosis, and compared them with AMH levels of 250 age-matched healthy girls. The diagnoses included were acute lymphoblastic leukaemia, acute myeloid leukaemia, Hodgkin lymphoma, non-Hodgkin lymphoma, nephroblastoma, sarcoma and neuroblastoma.

MAIN RESULTS AND THE ROLE OF CHANCE: The median age was 6.6 years (range 0.0–17.4), comparable with that in the control group (median 6.3 years, range 0.3–18.0). Girls with childhood cancer presented with significantly lower serum AMH levels compared with healthy age-matched controls (standard deviation scores (SDS) −0.8, $P < 0.001$). Median AMH level in patients was 1.4 μg/l (0.1–10.2) versus 3.0 μg/l (0.1–18.3) in controls. Specifically, 84% of all patients had AMH levels below the 50th percentile of normal AMH levels, and 19% below the 10th percentile. Surrogate markers of general health status (temperature, C-reactive protein and haemoglobin levels at diagnosis) were significantly correlated with AMH SDS.

LIMITATIONS, REASONS FOR CAUTION: Some caution is warranted because AMH levels increase with age in healthy children but the cases and controls were age-matched in our study. Although our sample size was large, additional studies are still required in an independent cohort.

WIDER IMPLICATIONS OF THE FINDINGS: Our study shows that AMH levels are reduced in girls with newly diagnosed cancer even before the cancer treatment has started. AMH levels correlate with impairment of general health status in girls. Therefore, besides (pre) antral follicle number, other factors may influence serum AMH levels. Longitudinal studies during and after childhood cancer are currently being performed in order to evaluate possible ovarian recovery after discontinuation of treatment.

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Key words: anti-Müllerian hormone / childhood cancer / pretreatment / girls

Introduction

Overall survival of childhood cancer has increased dramatically over the past decades, which has urged clinicians to pay attention to short- and long-term adverse effects of cancer treatment (Howell and Shalet, 1998; McVie, 1999). A well-known long-term side effect of cancer treatment in both adult and childhood cancer survivors is gonadal dysfunction, often resulting in impaired fertility (Lie Fong et al., 2008, 2009). The magnitude of this impairment depends on the treatment modality, the total cumulative dosages, as well as on the genetic susceptibility of a cancer survivor (Sklar et al., 2006; Lie Fong et al., 2009; van Casteren et al., 2009; Van Dorp et al., 2012, 2013). However, data addressing the possible influence of the disease itself on ovarian function are sparse (Brougham et al., 2012).

The assessment of ovarian reserve in adults—including a clinical evaluation of secondary sexual characteristics, menstrual history, transvaginal ultrasound during the early follicular phase including an antral follicle count of the ovaries, and FSH, LH, estradiol and progesterone levels—is not informative in prepubertal girls since the hypothalamic-pituitary-gonadal axis has not been activated yet.

Recently, it has been suggested that anti-Müllerian hormone (AMH) constitutes as a reliable indirect serum marker of ovarian function in healthy prepubertal and postpubertal girls, as well as in girls with childhood cancer during cancer treatment (Hagen et al., 2010; Brougham et al., 2012). AMH is produced by granulosa cells of small growing follicles and indirectly reflects the size of the primordial follicle pool in the ovaries (Visser et al., 2006). In peri-pubertal girls, minor fluctuations in AMH with slightly increasing levels prior to pubertal onset and decreasing levels after onset of puberty have been described (Hagen et al., 2012). After puberty, serum AMH levels are stable within and between menstrual cycles, in contrast to FSH (Hehenkamp et al., 2006; La Marca et al., 2006; Tsepelidis et al., 2007; Streuli et al., 2008). Up to an age of 25 years, AMH levels gradually increase and thereafter a linear decrease occurs with low or undetectable levels at the time of menopause (Lie Fong et al., 2012).

In adult women with Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) ($n = 64$), it has been shown that AMH levels are already reduced before the start of cytotoxic chemotherapy (Lawrenz et al., 2012). This indicates that not only therapy but also the disease or subsequent health status may influence ovarian function later in life. Moreover, also in adolescent and young adult women diagnosed with HL significant damage to gametes has been observed before the start of treatment (Fabbri et al., 2011).

Likewise in boys a considerable percentage had oligospermic or even azoospermic counts at the time of cancer diagnosis (van Casteren et al., 2008). The reason for this impaired testicular function is unclear, but a direct effect of the disease and compromised general health status seem to play an important role.

In girls with newly diagnosed cancer, only scarce data on AMH levels before cancer treatment are available. Knowledge regarding this issue is however important because it might relate to potential ovarian function, which in itself may determine the recovery possibilities after treatment. Brougham and colleagues observed slight but not significant lower AMH levels before treatment in a limited number of youngsters with various types of cancer (Brougham et al., 2012). To address this issue in more detail, we studied AMH levels in a large single-centre cohort of girls at the time of diagnosis of childhood cancer, before the start of treatment.

Materials and Methods

Subjects

We included girls up to the age of 18 years with newly diagnosed childhood cancer at our Paediatric Oncology Centre. Patients with brain tumours were excluded due to possible hypothalamic-pituitary-axis dysfunction, and patients with germ cell tumours were excluded because of the localization of the tumour in the ovary and/or direct influence on hormone production.

Details on age, diagnosis and pubertal stage were retrieved from our local database. Pubertal status was assessed clinically at diagnosis and classified as prepubertal (Tanner stage 1), midpubertal (Tanner stage 2–3) or late pubertal (Tanner stage 4–5) (Tanner, 1969). AMH levels were measured in left-over serum after diagnostic work-up. Informed consent was obtained from all included patients and parents who agreed to use of left-over material for this study according to the standards of the Institutional Review Board.

Controls

As a control group, we included healthy girls and adolescents aged up to 18 years ($n = 250$) from our previously published nomogram study (Lie Fong et al., 2012). Detailed information on recruitment strategy and study populations is available in the original paper.

Laboratory measurements

Serum AMH levels in both subjects and controls were measured at the diagnostic endocrine laboratory at the Erasmus Medical Centre (Rotterdam, the Netherlands). AMH levels were measured using an in-house double-antibody enzyme-linked immunosorbent assay (commercially available as the GenII Beckman Coulter, Beckman Coulter, Inc., Webster, TX, USA) as previously described (Kevenaar et al., 2006). All samples were stored using the same protocol at $-20°C$ until assayed. AMH immunoreactivity in serum samples was stable after repeated freeze-thaw cycles. Intra- and inter-assay coefficients of variation were <5 and $<10\%$, respectively (de Vet et al., 2002; Kevenaar et al., 2006).

Statistics

AMH levels were normally distributed after log-transformation. AMH levels were analysed as continuous variables, as well as standard deviation scores (SDS) and percentiles. AMH percentiles were based on the percentiles of 250 healthy girls: <10th percentile, 10th percentile–50th percentile, 50th percentile–90th percentile and >90th percentile (Lie Fong et al., 2012). As AMH is age-dependent, SDS were calculated to correct for age, based on previously reported AMH levels of (age-matched) healthy girls from our institute (Lie Fong et al., 2012). Nonparametric and chi-square tests were used to compare included and excluded patients, and to compare AMH levels of patients and controls. SDS of girls with childhood cancer were compared with healthy references using the one sample t-test. Linear regression was used to compare AMH SDS between subsets of diagnoses, and to study the association between the surrogate markers of general health status (temperature at diagnosis, haemoglobin (Hb) levels and C-reactive protein (CRP) levels), and AMH SDS. AMH SDS according to Tanner stages were evaluated using the Mann–Whitney U-test. P-values of <0.05 were considered significant. All statistical analyses were performed using the IBM Statistical Package for Social Sciences version 20 (IBM Corp., Armonk, NY, USA).

Results

In 208 girls with cancer AMH levels were measured at the time of diagnosis at the Erasmus Medical Centre, Sophia's Children's Hospital Rotterdam. Seventy-six girls (36.5%) were diagnosed with

acute lymphoblastic leukaemia, 19 (9.1%) with acute myeloid leukaemia, 32 (15.4%) with HL, 18 (8.7%) with NHL, 22 (10.6%) with nephroblastoma, 24 (11.5%) with sarcoma and 17 (8.2%) with neuroblastoma.

The median age was 6.6 years (range 0.0–17.4) and comparable with the median age of girls in the control group (median 6.3 years, range 0.3–18.0, $P = 0.44$). Girls diagnosed with cancer had significantly lower pretreatment AMH levels when compared with the age-matched controls (SDS −0.8, $P < 0.001$) (Fig. 1). Median AMH level in patients was 1.4 μg/l (0.1–10.2) versus 3.0 μg/l (0.1–18.3) in the controls. In all tumour types median AMH SDS were below zero, but no significant differences were observed between the malignancies (Fig. 2).

Of all girls with newly diagnosed cancer, 83.7% had AMH levels below the 50th percentile, and 19.2% had AMH levels below the 10th percentile (Table I).

Tanner stages were reliably documented in 161 patients. Fourteen girls (8.7%) were classified as being late pubertal, 15 as being midpubertal (9.3%) and 132 girls (82.0%) were prepubertal. AMH SDS were not significantly different between the Tanner categorical subgroups ($P = 0.15$).

Temperature at diagnosis, a surrogate marker of general health status, was inversely correlated with AMH SDS (β −0.119, 95% confidence interval (CI) −0.235; −0.003, $P = 0.044$) as was CRP with AMH SDS (β −0.002; 95% CI −0.004; −0.000, $P = 0.016$), while Hb levels and AMH SDS were positively correlated (β 0.064, 95% CI 0.005; 0.123, $P = 0.033$).

Discussion

In the present study, we have shown that AMH levels in girls with newly diagnosed cancer are reduced compared with healthy controls, even before chemotherapy or radiotherapy has started.

The rationale for these reduced levels is unclear. Previous studies in adult women with HL or NHL, but also in adults with systemic diseases, such as diabetes mellitus, systemic lupus erythematosus and cardiovascular diseases, suggest that the compromised general health status due to the consumptive character of the disease decreases AMH levels. In these patients, a decreased AMH production in the small growing follicles inflicted by the chronic disease, an increased metabolism of AMH in cancer patients, or a more rapid decline of the primordial follicle pool might be present (Dorman *et al.*, 2001; Gast *et al.*, 2011; Lawrenz *et al.*, 2011; Isik *et al.*, 2012). In order to gain at least some insight into the influence of a compromised health status, we studied some indirect markers of general illness, i.e. temperature, CRP and Hb levels. Indeed, we found a negative association between temperature, CRP and AMH SDS, while a positive association was observed between Hb levels and AMH SDS. These findings indicate that the pretreatment general health status might contribute to the decreased AMH levels, suggesting that factors other than (pre)antral follicle number may influence serum AMH levels.

Another potential cause of the decreased AMH SDS independently of childhood cancer subtype might be impaired granulosa cell function due to an impaired DNA repair mechanism. Genetic variation mapping to DNA repair genes has been associated with both cancer and ovarian
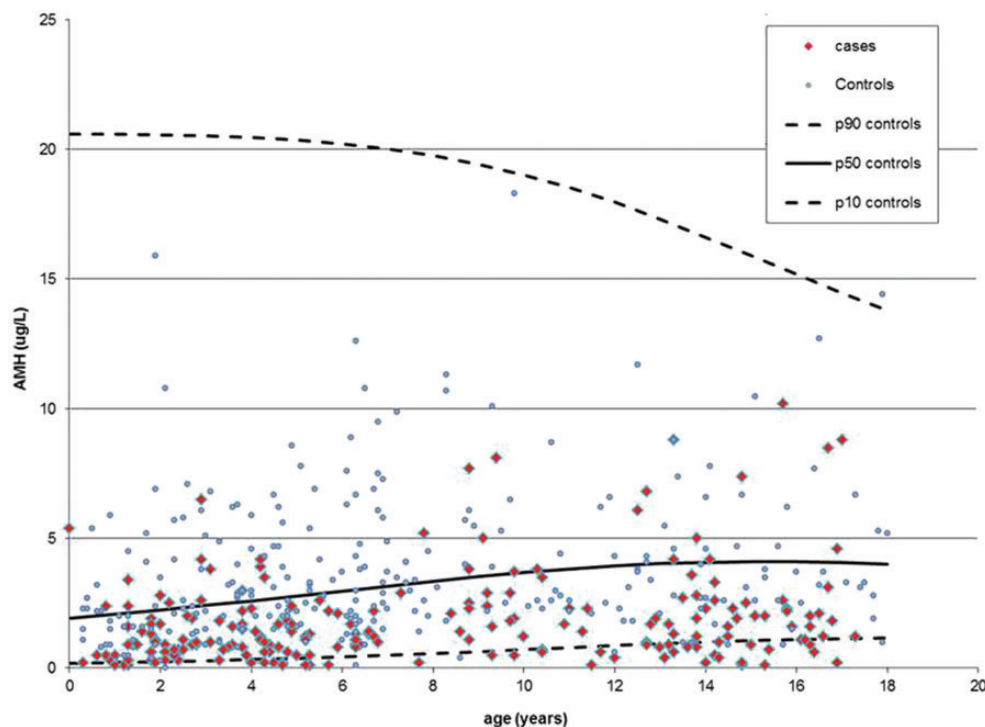
Figure 1 Pretreatment anti-Müllerian hormone (AMH) levels in girls with newly diagnosed childhood cancer ($n = 208$) when compared with AMH levels in a cohort of 250 age-matched healthy girls ($P < 0.001$, one sample *t*-test). p90, p50 and p10 refer to 90th, 50th and 10th percentiles, respectively.
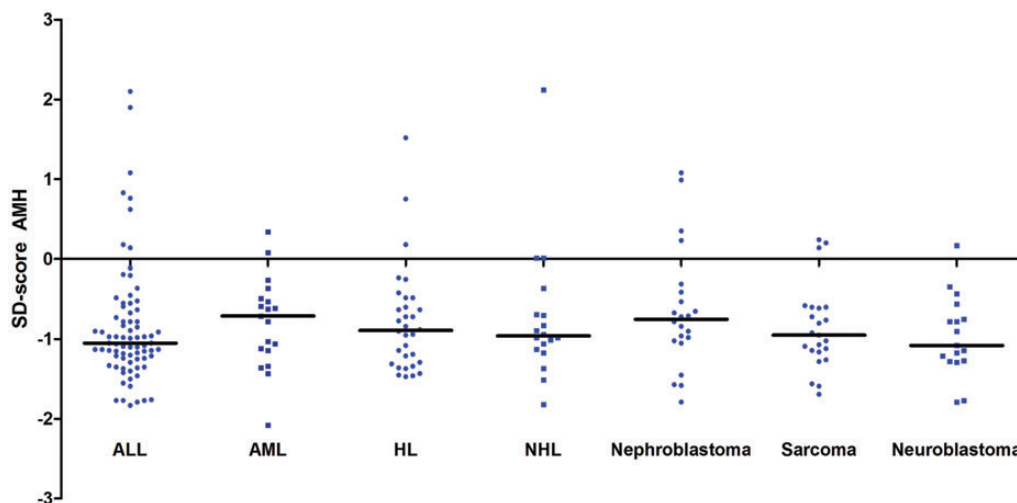
Figure 2 Pretreatment AMH standard deviation scores (SDS) by diagnosis for girls diagnosed with childhood cancer ($n = 208$). ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma.

Table I Anti-Müllerian hormone levels in girls with newly diagnosed cancer when compared with the 10th, 50th and 90th percentiles in healthy age-matched controls.

	N	<p10	%	p10–<p50	%	<p50	%	p50–p90	%
ALL	77	14	18.2	49	63.6	63	81.8	14	18.2
AML	19	4	21.1	14	73.7	18	94.7	1	5.3
HL	32	9	28.1	18	56.3	27	84.4	5	15.6
NHL	18	4	22.2	10	55.6	14	77.8	4	22.2
Nephroblastoma	22	1	4.5	17	77.3	18	81.8	4	18.2
Sarcoma	23	3	13.0	15	65.2	18	78.3	5	21.7
Neuroblastoma	17	5	29.4	11	64.7	16	94.1	1	5.9
Total	208	40	19.2	134	64.4	174	83.7	34	16.3

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma.

ageing (Hoeijmakers, 2009; Stolk *et al.*, 2012). Other factors, such as stress, cannot be excluded. It would be interesting to compare AMH levels in different groups of young girls including those with other severe illnesses in order to study the effect of illness and stress. Whether the reduced AMH levels are reversible and what the mechanism of this accelerated reduction of AMH levels is, are currently unknown. In the normal healthy situation AMH levels reflect the number of growing follicles, and hence AMH has been suggested to be a marker of the quantitative aspect of ovarian reserve. However, when health status is compromised, this may affect granulosa cell function, leading to decreased serum AMH levels without affecting the follicle count. Hence, AMH might be a marker of ovarian function, a reduced primordial follicle pool, or a combination thereof in girls with newly diagnosed cancer.

We analysed AMH levels in a substantial sample of girls with newly diagnosed cancer. Other ovarian function markers, such as FSH and inhibin B, were not measured as it has been reported that FSH and inhibin B are no reliable markers in prepubertal girls with cancer (Brougham *et al.*, 2012). Inhibin B levels were undetectable in the majority of prepubertal female patients with cancer, and FSH levels did not increase during cancer treatment illustrating the quiescence of the hypothalamic-pituitary axis during prepuberty. In adults, AMH is strongly correlated with the antral follicle count and is relatively constant during and between menstrual cycles (Hehenkamp *et al.*, 2006; La Marca *et al.*, 2006; Tsepelidis *et al.*, 2007; Streuli *et al.*, 2008). Nevertheless, some caution is warranted since AMH levels increase with age in healthy children and thereafter remain stable until early adulthood (Kelsey *et al.*, 2011; Nelson *et al.*, 2011; Lie Fong *et al.*, 2012). Moreover, it is important to recall that AMH is an indirect marker for the number of primordial follicles in the ovary. Nelson *et al.* (2011) showed variation in AMH levels in the first 2–3 years of life before an accelerated increase from 4 years onwards. However, further data show that AMH rises until the age of 25 years, after which AMH decreases until the age of menopause (Lie Fong *et al.*, 2012). Hagen *et al.* (2010) showed the same increase, but

all three models do show considerable variability during infancy (Kelsey et al., 2011; Nelson et al., 2011; Lie Fong et al., 2012). It has been suggested that the increase and the variability during infancy might be explained by an increase of the number of granulosa cells producing AMH or that the number of small growing follicles, in which AMH expression is strongest, increases (Weenen et al., 2004). Hagen et al. (2010) observed a post-natal peak in AMH levels, which was also reported by others (Kuiri-Hanninen et al., 2011). A suggested explanation for this increase is shown by the temporal relationship between increased follicular growth and post-natal AMH levels, probably in response to the post-natal FSH surge stimulating ovarian folliculogenesis and AMH transcription (Kuiri-Hanninen et al., 2011). The variation in the timing of this process might at least partly explain the AMH variability. In order to minimize the influence of age and variation during the early years, we compared AMH levels in girls with childhood cancer to age-matched controls. However, the effect of variability cannot be completely ruled out, and therefore caution is warranted when extrapolating these results to other populations. Lastly, although the number of patients analysed in this study is larger than in previous studies, the number is still relatively small. Therefore, we recommend to replicate our study in a larger independent cohort of survivors of childhood cancer.

In adults, pretreatment AMH levels seem to be important for the interpretation of treatment-related ovarian damage during follow-up. It has been shown that baseline AMH levels affect the magnitude of acute changes in ovarian reserve from chemotherapy, and that the rate of recovery of AMH is determined by pretreatment levels (Dillon et al., 2012). The present study is the first large study on AMH levels in girls with newly diagnosed cancer. However, data on recovery are not yet available. Longitudinal studies during and after childhood cancer are currently being performed to determine whether AMH levels recover (i.e. increase) with improved health status.

In conclusion, we show that AMH levels in girls with newly diagnosed cancer are already compromised before treatment starts, suggesting that the disease itself and/or the subsequent health status affects AMH levels. Therefore, besides (pre) antral follicle number, other factors may influence serum AMH levels.

Authors' roles

W.v.D. acquired, analysed and interpreted data, drafted the manuscript and designed the study. M.M.v.d.H.-E. designed the study, drafted the manuscript, and critically revised the manuscript for intellectual content and data interpretation. A.C.H.d.V. critically revised the manuscript for intellectual content. S.M.F.P. analysed and interpreted data, critically revised the manuscript for intellectual content and data interpretation. R.P. critically revised the manuscript for intellectual content and data interpretation. J.A.V. interpreted data, and critically revised the manuscript for intellectual content and data interpretation. J.S.E.L. drafted the manuscript, interpreted data, critically revised the manuscript for intellectual content and designed the study.

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Conflict of interest

None declared.

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