Assessment of ovarian reserve in adult childhood cancer survivors using anti-Müllerian hormone

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BACKGROUND: The aim was to assess possible treatment-induced gonadal damage in a cohort of adult female childhood cancer survivors (CCS) using anti-Müllerian hormone (AMH), the most sensitive marker of ovarian reserve.

METHODS: A total cohort of 185 survivors was compared with 42 control subjects. The median follow-up time was 18.1 years (range 4.1–43.2 year).

RESULTS: Median AMH concentrations in the analysed cohort were not different from controls (median 1.7 versus 2.1 μg/l; P = 0.57). However, AMH levels were lower than the 10th percentile of normal values in 27% (49/182) of our survivors. In addition, 43% (79/182) had AMH levels lower than 1.4 μg/l, a previously established cut-off value which predicts ongoing pregnancy after assisted reproduction. There were no differences in AMH levels in subgroups classified according to disease. However, survivors treated with three or more procarbazine containing chemotherapy cycles had significantly lower AMH levels than controls (median 0.5 μg/l; P = 0.004). Also survivors treated with abdominal or total body irradiation had significantly lower AMH levels than controls (median < 0.1 μg/l; P < 0.001).

CONCLUSIONS: AMH can be used to identify subgroups of CCS at risk for decreased fertility or premature ovarian failure. In these survivors, options for fertility preservation should be considered prior to starting treatment since they may be at risk for poor chances of pregnancy after assisted reproductive treatment.

Key words: anti-Müllerian hormone / childhood cancer / cancer survivor / premature ovarian failure / ovarian reserve / pregnancy

Introduction

Gonadotoxicity is a well known side effect of cancer therapy in adult survivors. Alkylating agents, such as cyclophosphamide and procarbazine, are known to be highly gonadotoxic. In addition, multi-drug regimens may have a cumulative toxic effect on reproductive function (Wallace et al., 2005). Damage to the ovaries causes follicle loss and may result in premature ovarian failure (POF). Indeed, impaired fertility in childhood cancer survivors (CCS) has been described (Byrne, 1999). In several studies, survivors of childhood haematological and solid tumours had high follicle stimulating hormone (FSH) levels and amenorrhoea, indicative of ovarian failure (Couto-Silva et al., 2001; Chemaillily et al., 2006; Lantinga et al., 2006; Rosa E Silva et al., 2007). However, most studies have described ovarian function in small groups, in which the extent of damage to the ovarian reserve was not predictable (Couto-Silva et al., 2001; Lantinga et al., 2006; Rosa E Silva et al., 2007).

Despite the presence of high FSH levels and amenorrhoea after chemotherapy, pregnancies in CCS have been reported as well (Green et al., 2002; Sklar, 2005). Hence, FSH levels or menstrual cyclicity after cancer treatment do not seem to be predictive of the chance to conceive (Couto-Silva et al., 2001; Sklar, 2005).

Recently, serum anti-Müllerian hormone (AMH) was established as a novel marker of ovarian reserve (de Vet et al., 2002). Results from pre-clinical studies confirmed the role of AMH in human folliculogenesis, i.e. inhibition of initial recruitment and attenuation of FSH sensitivity (Visser et al., 2006). Clinical studies showed that the number of antral follicles and age correlate well with serum AMH levels. Moreover, serum AMH levels seem to be related to the onset of menopause (van D Disseldorp et al., 2008). Furthermore, decrease in AMH levels seems to precede changes in conventional parameters associated with peri-menopausal status, such as FSH, inhibit B and estradiol (de Vet et al., 2002; van Rooij et al., 2002). Therefore, serum AMH
levels are the most reliable marker of ovarian ageing. Furthermore, several studies have described AMH as a predictor of ongoing pregnancy after assisted reproductive treatment (Eidar-Geva et al., 2005; Silberstein et al., 2006; Freour et al., 2007; Elgindy et al., 2008). Finally, in contrast to gonadotrophins, AMH levels were unaffected by cyclic variations or the use of oral contraceptive pills (OCPs) (Hehenkamp et al., 2006; Somunkiran et al., 2007).

Although AMH has been described as a useful marker of ovarian reserve in survivors of cancer diagnosed at adult age, data on the value of AMH in adult survivors of childhood cancer are scarce (Anderson et al., 2006; Lutchman et al., 2007; Lie Fong et al., 2008). Recently, in two studies AMH was measured in survivors of childhood Hodgkin lymphoma (HL) (n = 32), haematological malignancies and solid tumours (n = 21) (Bath et al., 2003; van Beek et al., 2007). These studies confirmed that, in CCS with regular menstrual cycles, serum AMH levels were indicative of limited ovarian reserve, whereas FSH, estradiol and inhibin B levels were not different from healthy controls (van Beek et al., 2007). So far, no studies are available on AMH levels in large cohorts of CCS. In the current study, ovarian reserve was assessed in a large single centre cohort of 185 CCS by measuring serum AMH levels and comparing them with controls.

Materials and Methods

Subjects

The study was approved by the local medical ethical review board. Written informed consent was obtained from all participants. These adult female CCS had been treated at the Erasmus MC–Sophia Children’s Hospital from October 1958 until December 2000. Participants were 18 years and younger at the time of diagnosis and had finished treatment at least 5 years earlier. At the time of follow-up they were 17–50 years old and in complete remission. Participants were recruited at the outpatient clinic during follow-up of long-term effects of cancer treatment. Because of possible hypothalamic–pituitary axis dysfunction, survivors of brain tumours were excluded from this study. Details on cancer treatment were recorded, including type and cumulative doses of chemotherapy, type, field, cumulative dose of radiotherapy, type of surgery and/or conditioning regimen prior to stem-cell transplantation, as well as complications and relapse. Subsequently, a general health screening, including extensive history taking and physical examination, was performed. Serum samples were taken randomly during the menstrual cycle, in pregnant survivors as well as in survivors taking OCPs.

A cohort of 42 women without a history of cancer and with a regular menstrual cycle was recruited as controls, as described previously (de Vet et al., 2002). Briefly, inclusion criteria were age between 20 and 35 years, normal body mass index (between 19 and 26 kg/m²) and regular menstrual cycle length between 26 and 31 days. In addition, these women were healthy and were all proven fertile. They did not have any endocrine disease and did not use any medication, OCPs or hormonal treatment. Blood samples were drawn during the early follicular phase.

Hormone assays

AMH was measured in controls using the Immunotech-Coulter assay and in survivors using an in-house double-antibody enzyme-linked immunosorbent assay (ELISA) (de Vet et al., 2002; Kevenaar et al., 2007a). The values from the in-house ELISA were adjusted (2.145) for comparison with the Immunotech-Coulter assay. Intra- and inter-assay coefficients of variance were <10 and <5% in the in-house ELISA and <5 and 8% in the Immunotech-Coulter assay, respectively (de Vet et al., 2002; Kevenaar et al., 2006). Fluorescence-based immunoassays were used to measure FSH levels (Immuno, Diagnostic Products Corp., Los Angeles, CA, USA). A radioimmunoassay was used to assess estradiol serum levels (Diagnostic Products Corp.). Intra- and inter-assay coefficients of variance were <3 and 8% for FSH and <3 and 7% for estradiol, respectively. Serum inhibin B levels were determined using an enzyme-linked immunoassay (Oxford Bio Innovation, Oxford, UK). Intra- and inter-assay coefficients of variation were <9 and 15%.

Data analysis

Non-parametric tests were used to compare general characteristics between survivors and controls. \( \chi^2 \) tests and non-parametric tests were used to compare participants and non-participants. Univariate analysis of covariance (ANCOVA) was performed to compare AMH in survivors and controls. Likewise, subgroup analysis was performed. Survivors were grouped according to their diagnosis or treatment received. Multi-drug chemotherapy regimens containing cyclophosphamide, procarbazine or ifosfamide were referred to as ‘chemotherapy with alkylating properties’. Correlations between AMH and total cumulative dose of chemotherapy were determined using ANCOVA for categorical parameters (more or less than three mechlorethamine, vincristine, procarbazine and prednisone (MOPP) cycles) and partial correlation analysis for continuous variables (total cumulative dose of CY or ifosfamide). Similarly, correlation between AMH and total dose of radiation (Gray (Gy)) was assessed using partial correlation analysis. In addition, subanalysis was performed using ANCOVA in subgroups according to the treatment before or after menarche, cyclicity and pregnancy rate. Oligomenorrhea was defined as a cycle interval longer than 35 days, but shorter than 6 months. Amenorrhea was defined as an interval between cycles of longer than 6 months. All outcome parameters were adjusted for age differences. Survivors with AMH < 0.1 \( \mu g/l \) were analysed separately and their characteristics were compared with survivors with AMH > 0.1 \( \mu g/l \). Data were presented as the median and range.

For the 42 control women, linear regression of age on log-transformed AMH values was performed and 90% prediction limits were calculated. The formula: AMH \( \times \exp(3.47 - 0.102 \times \text{age}) \) was used to calculate the mean decline in AMH levels in controls, adjusting for increasing age. The 10th and 90th prediction intervals for decline of AMH levels were calculated using the formula: AMH \( \times \exp(3.47 - 0.102 \times \text{age}) \pm 1.23 \times \sqrt{1.013 + ((\text{age} - 30.48)/38.45)^2} \), with adjustment for increasing age. For graphical display, results of the linear regression were back-transformed. Statistical analysis was performed using Statistical Package for Social Sciences 12.0 (SPSS Inc., Chicago, IL, USA). A P-value of <0.05 indicates statistical significance.

Results

Survivors

Our full cohort of CCS included 238 women, of whom 25 withdrew from participation. Out of 213 eligible subjects, serum AMH levels were measured in 185 (185/238; 78%) survivors (Fig. 1). In 21% (n = 38) of the 185 CCS samples for the measurement of FSH, estradiol and inhibin B levels were drawn during the early follicular phase of the menstrual cycle and thus were useful for analysis. Median age at diagnosis was 8.2 year (range 0.3–15.8 year) in survivors who discontinued follow-up and those in whom serum AMH levels were not available, and was similar in participants (median 5.8 year; range 0.1–16.8 year; P = 0.82). Neither the type of malignancies (P = 0.14), nor
the frequency of relapse (13 versus 12%; \( P = 0.54 \)), the number of survivors receiving chemotherapy (93 versus 87%; \( P = 0.22 \)) or radiotherapy (36 versus 32%; \( P = 0.73 \)) were different between participants and non-participants.

### Diagnosis and treatment

In participants, median age at the time of treatment was 5.8 years (range 0.1–16.8 year) and median age at the time of AMH assessment was 25.5 year (range 17.0–47.4 year). Twenty-four (13%) survivors were in second complete remission after relapse. Seventy-seven survivors had been treated for acute lymphoblastic leukaemia (ALL) or non-HL. These survivors were assigned to the ‘leukaemia group’. Eight survivors were diagnosed with acute myeloid leukaemia (AML). Fifteen survivors with HL were included, of whom 14 have been described earlier as part of a multi-centre study from our group (van Beek et al., 2007). Since our objective was to analyse our total cohort of female survivors, these women were also included in the present study. The remaining subgroups consisted of 17 survivors of neuroblastoma (NBL), 25 survivors of sarcoma, 28 with a nephroblastoma, 9 with Langerhans cell histiocytosis (LCH), 3 with a germ cell tumour, 1 with a hepatoblastoma.

**Figure 1** Flow diagram of subjects. CT, chemotherapy; RT, radiotherapy; USO, unilateral salpingo-oophorectomy; BSO, bilateral salpingo-oophorectomy; MOPP, mechlorethamine, vincristine, procarbazine and prednisone; EBVD, epirubicin, bleomycin, vinblastine and dacarbazine; TBI, total body irradiation; CY, cyclofosfamide; Ifos, Ifosfamide. *Not included in the analysis.
and 1 with a carcinoid tumour. Three survivors diagnosed with Burkitt lymphoma (n = 1) and germ cell tumours (n = 2) were treated with chemotherapy and an unilateral salpingoophorectomy or bilateral salpingoophorectomy. Since their ovarian reserve was reduced by surgery, these three survivors were analysed separately. Finally, excluding these three subjects, 182 survivors were analysed (Table I).

Sixty-three survivors (63/182; 35%) received chemotherapy and radiotherapy. Fourteen of these 63 women (22%) received alkylating chemotherapy as well as radiotherapy on their whole body or abdomen (Fig. I). Two of the nine survivors of LCH had received radiotherapy on the neck or face and eight were treated with chemotherapy. Survivors of AML were analysed separately from the ‘leukaemia’ group, since their treatment is radically different: five of eight AML survivors received a stem-cell transplantation after a conditioning regimen of alkylating chemotherapy and total body irradiation (TBI). Most survivors (63%) had been treated before they had reached menarche, 17% were treated after menarche and in 20% of cases the age of menarche was unknown. At the time of inclusion, 4 of 182 survivors (2%) were pregnant. Thirty-three survivors (33/182; 18%) had regular menstrual cycles, whereas 28 of 182 (15%) survivors suffered from oligo- or amenorrhoea. In 14 of 182 survivors (8%), data on menstrual cycle at the time of screening were not available. All other survivors used OCPs (98/182; 54%) or hormonal replacement therapy (5/182; 3%) at the time of follow-up (Table I). Median time of follow-up was 18.1 years (4.1–43.2 years).

### Endocrine profiles

Median serum AMH levels in the 182 survivors were within the normal range after adjustment for age (P = 0.57). There were no differences in AMH levels between subgroups classified according to malignancy and controls, except for survivors of LCH in whom serum AMH was higher than in controls (P = 0.011) (Table I). AMH concentrations in survivors treated before menarche were not different from those in controls (P = 0.72). Similarly, AMH levels in survivors treated after menarche were within the normal range (P = 0.94) (Fig. 2A). In addition, after excluding survivors treated with TBI or abdominal radiotherapy and survivors treated with three or more procarbazine containing cycles, serum AMH concentrations were not different in survivors treated prior to or after menarche (2.0 versus 2.1 μg/l; P = 0.88). AMH levels were not different in survivors with regular cycles, oligo- or amenorrhoea or in those taking OCPs and controls (2.3, 1.2, 1.8 versus 2.1 μg/l, respectively). In the five survivors taking hormonal replacement therapy, which was prescribed because of secondary amenorrhoea, median AMH level was <0.1 (<0.1–1.0 μg/l). Moreover, no differences were observed in AMH

### Table 1 Serum AMH levels in childhood cancer survivors (CCS) when compared with 42 control subjects

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>CCS</th>
<th>Serum AMH (μg/l)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects included in analysis</td>
<td>n = 182 (%)</td>
<td>1.7 (&lt;0.1–19.9)</td>
<td>0.57</td>
</tr>
<tr>
<td>Time of treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated prior to menarche</td>
<td>114 (63%)</td>
<td>1.7 (&lt;0.1–19.9)</td>
<td>0.72</td>
</tr>
<tr>
<td>Treated after menarche</td>
<td>31 (17%)</td>
<td>1.4 (&lt;0.1–12.1)</td>
<td>0.94</td>
</tr>
<tr>
<td>Age of menarche unknown</td>
<td>37 (20%)</td>
<td>1.9 (&lt;0.1–12.6)</td>
<td>—</td>
</tr>
<tr>
<td>Menstrual cycles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular cycles</td>
<td>33 (18%)</td>
<td>2.3 (&lt;0.1–13.1)</td>
<td>0.37</td>
</tr>
<tr>
<td>Oligo- or amenorrhoea</td>
<td>28 (15%)</td>
<td>1.2 (&lt;0.1–14.1)</td>
<td>0.13</td>
</tr>
<tr>
<td>OCPs</td>
<td>98 (54%)</td>
<td>1.8 (&lt;0.1–19.9)</td>
<td>0.72</td>
</tr>
<tr>
<td>HRT</td>
<td>5 (3%)</td>
<td>&lt;0.1 (&lt;0.1–1.0)</td>
<td>—</td>
</tr>
<tr>
<td>Gravida</td>
<td>4 (2%)</td>
<td>0.9 (0.4–3.7)</td>
<td>—</td>
</tr>
<tr>
<td>Data on menstrual cycles missing</td>
<td>14 (8%)</td>
<td>1.8 (&lt;0.1–6.7)</td>
<td>—</td>
</tr>
</tbody>
</table>

*p*-values indicate differences in serum AMH levels (in microgram per litre) between survivors and controls by ANCOVA, adjusting for age differences. Median AMH level in controls was 2.1 μg/l, range (0.1–7.4 μg/l). Values are absolute numbers (proportions %) or medians (range). ALL, acute lymphoblastic leukaemia; NHL, non-Hodgkin lymphoma; AML, acute myeloid leukaemia; LCH, Langerhans cell histiocytosis; OCPs, oral contraceptive pills; HRT, hormonal replacement therapy.

*Analysis was not performed because data were missing.

*Analysis was not performed because of small number of patients.
concentrations between survivors with regular cycles and oligo- or amenorrhoea (2.3 versus 1.2 μg/l; P = 0.43) (Fig. 2B) (Table I). Finally, no differences were observed in AMH levels in multipara survivors or nullipara survivors versus controls (1.5, 1.7 versus 2.1 μg/l; P = 0.51; P = 0.78). Unfortunately, in survivors who were pregnant at the time of inclusion, cycle information or gonadotrophin levels prior to their pregnancy were not available. No correlation was observed between the duration of follow-up and serum AMH concentrations (P = 0.69).

Serum AMH levels were significantly lower in survivors who received radiotherapy on the abdomen, pelvis, sacrum or their total body (<0.1 versus 2.1 μg/l; P < 0.001) than in controls, whereas survivors irradiated on other parts of their body had normal AMH levels (1.5 μg/l (<0.1–11.4); P = 0.52) (Fig. 2C). In 25 survivors of ALL, irradiated on the cranium, AMH levels were similar to controls (P = 0.96) and to ALL-survivors without cranial radiotherapy (2.3 versus 1.5 μg/l; P = 0.87) (Table II). The cumulative radiation dose administered on the cranium varied between 18 and 49 Gy. It varied from 10 to 70 Gy when administered on the abdomen and was not correlated with serum AMH concentrations (P = 0.54).

In survivors treated with chemotherapy, AMH concentrations were comparable with those in controls (1.8 versus 2.2 μg/l; P = 0.38). Although median AMH levels in the whole group of HL-survivors were normal (median 0.8 μg/l; P = 0.32), they were significantly lower after three or more MOPP cycles when compared with controls (0.5 versus 2.1 μg/l; P = 0.004) and compared with HL-survivors treated with epirubicin, bleomycin, vinblastine and dacarbazine (EBVD) (0.5 versus 3.2 μg/l; P = 0.04) (Fig. 2C). No significant correlations were observed between serum AMH levels and total cumulative dose cyclophosphamide or ifosfamide (P = 0.63; P = 0.08, respectively). In the only patient treated with an unilateral salpingo-ophorectomy, serum AMH was 8.9 μg/l. In both survivors who had a unilateral salpingoophorectomy, serum AMH levels were undetectable. Survivors treated with non-gynaecological surgery without any chemotherapy or radiotherapy (n = 10) had higher AMH levels than controls (P = 0.029) (Table II).

In 27% (49/182) of our survivors, AMH levels were lower than the 10th percentile of normal values. In only 11 of these 49 survivors, FSH, estradiol and inhibin B levels were evaluable: FSH levels were within normal ranges (5.0 versus 6.2 U/l; P = 0.17), whereas inhibin B levels were lower than in controls (9 versus 113 ng/l; P < 0.001). Even after excluding survivors older than 35 years, 41 (23%) survivors had AMH levels below the 10th percentile. Recently, serum AMH levels ≥1.4 μg/l were described as predictive for ongoing pregnancy after in vitro fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI) treatment cycles in healthy, but subfertile women (Freour et al., 2007). Since this cut-off value was assessed with an assay comparable to the Immunotech-Coulter assay used in our study, the cut-off value was applied to our cohort of survivors. In 79 of 182 (44%) women, serum AMH was lower than this threshold value. These 79 survivors were significantly older than the group survivors with AMH levels ≥1.4 μg/l (median 27 versus 25 years; P = 0.02). However, their age ranged between 17 and 47 years, which was similar to the range in the younger group (16–46 years).

The group of women with AMH levels <0.1 μg/l was compared with the remaining group of survivors (Table III). Women with undetectable AMH levels were significantly older at diagnosis (P = 0.03). At follow-up, they had significantly more irregular menstrual cycles and received more hormonal replacement therapy because of secondary amenorrhea (P < 0.001). In addition, in this group, more women had received radiotherapy, especially on their whole body or abdomen, than survivors with AMH levels >0.1 μg/l (P < 0.001) (Table III).

**Discussion**

Due to increased survival rates, more CCS may tend to delay child-bearing, as observed in the general population (Meadows, 2006). In healthy women older than 35 years, depletion of the primordial follicle pool is accelerated and the chances of conceiving spontaneously are decreased (Macklon and Fauser, 2005). In CCS, follicle loss may be even more accelerated due to gonadal damage caused by cancer treatment. Consequently, in some cancer survivors, reproductive function will be more compromised when compared with controls. Although AMH concentrations in the whole cohort of survivors were similar to those in controls, in 27% of our survivors AMH levels were lower than the 10th percentile of controls. Serum AMH levels are the most sensitive marker of ovarian reserve. Low AMH levels are predictive for poor ovarian response in IVF treatment cycles (van Rooij et al., 2002). Thus, it may be concluded that low AMH levels and low ovarian response to ovarian stimulation are correlated with ovarian ageing and, hence, with decreased fertility (Beckers et al., 2002; de Vet et al., 2002; van Rooij et al., 2005). Therefore, we postulated that women are at risk of decreased fertility, based on low ovarian reserve, and consequently, these women may have low chances of pregnancy after assisted reproductive techniques. Accordingly, their chances of spontaneous pregnancy may be even lower. Moreover, taking into account the previously described threshold value, indicating poor outcome of IVF treatment, only 56% of our survivors would be likely to have an ongoing pregnancy after IVF or ICSI (Freour et al., 2007). Furthermore, not only the elder, but also survivors younger than 20 years had AMH levels below this threshold. Hence, despite normal AMH levels, chances of both future spontaneous pregnancy and pregnancy after ART may be impaired in the majority of our CCS. It may be premature to apply a cut-off value, since this has not been validated in the population under study. However, counselling on fertility is an important topic, especially for the youngest survivors with low AMH levels. Although these young women may not yet think about their future, in terms of reproduction, they should be discouraged from postponing their first pregnancy until an age at which their ovarian reserve is depleted. Until now, cancer survivors can only be counselled based on a limited amount of data from those currently available reports which include cut-off values (Eldar-Geva et al., 2005; Silberstein et al., 2006; Freour et al., 2007; Elgindy et al., 2008; Gnoth et al., 2008). Most studies used a discriminating cut-off value twice as high than the one applied to our cohort (Eldar-Geva et al., 2005; Silberstein et al., 2006; Elgindy et al., 2008). A higher cut-off value would result in an even worse outcome for our survivors. However, results of different studies should be interpreted with care, since international standards for an AMH assay are lacking. In addition, these cut-off levels have been described in women with subfertility and the number of normal women, who may have AMH levels lower than this cut-off level is unknown. Unfortunately, the number of our control subjects was limited to 42 and 11
Figure 2 Serum AMH levels in subgroups of survivors versus 10th, 50th and 90th percentile of AMH levels in controls (——). (A) Survivors treated prior (●) to or after (□) menarche. (B) Survivors with regular menstrual cycles (●) or oligo- or amenorrhoea (□). (C) Survivors treated total body irradiation or abdominal radiotherapy (●), more than three MOPP cycles (□) or other treatment regimen (×).
Table II Serum AMH levels in childhood cancer survivors (CCS) according to treatment when compared with 42 control subjects

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CCS</th>
<th>Serum AMH (μg/l)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects included in analysis</td>
<td>n = 182 (%)</td>
<td>1.7 (&lt;0.1–19.9)</td>
<td>0.57</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient with USO&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1*</td>
<td>8.9*</td>
<td>—</td>
</tr>
<tr>
<td>Patient with BSO&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2*</td>
<td>&lt;0.1*</td>
<td>—</td>
</tr>
<tr>
<td>Surgery (no USO/BSO), no CT, no RT</td>
<td>10 (5%)</td>
<td>3.3 (0.1–11.9)</td>
<td>0.029</td>
</tr>
<tr>
<td>RT</td>
<td>3 (2%)</td>
<td>0.2 (&lt;0.1–9.0)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>CT without alkylating agents</td>
<td>69 (38%)</td>
<td>1.7 (&lt;0.1–14.1)</td>
<td>0.30</td>
</tr>
<tr>
<td>CT containing alkylating agents</td>
<td>100 (55%)</td>
<td>1.8 (&lt;0.1–19.9)</td>
<td>0.95</td>
</tr>
<tr>
<td>Type of alkylating CT and/or RT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Three or more MOPP cycles</td>
<td>9 (5%)</td>
<td>0.5 (&lt;0.1–3.6)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.004</td>
</tr>
<tr>
<td>Less than three MOPP cycles or EBVD cycles</td>
<td>6 (3%)</td>
<td>3.2 (&lt;0.1–12.1)</td>
<td>0.30</td>
</tr>
<tr>
<td>Cyclophosphamide cycles</td>
<td>67 (37%)</td>
<td>1.5 (&lt;0.1–12.7)</td>
<td>0.63</td>
</tr>
<tr>
<td>(Cyclophosphamide+Ifosfamide cycles</td>
<td>18 (10%)</td>
<td>3.2 (&lt;0.1–19.9)</td>
<td>0.08</td>
</tr>
<tr>
<td>CT + RT on abdomen or TBI</td>
<td>14 (8%)</td>
<td>&lt;0.1 (&lt;0.1–2.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CT + RT other sites</td>
<td>49 (27%)</td>
<td>1.5 (&lt;0.1–11.4)</td>
<td>0.52</td>
</tr>
<tr>
<td>ALL</td>
<td>64</td>
<td>1.5 (&lt;0.1–14.2)</td>
<td>0.62</td>
</tr>
<tr>
<td>CT + cranial RT</td>
<td>25</td>
<td>2.3 (&lt;0.1–7.3)</td>
<td>0.96</td>
</tr>
<tr>
<td>CT − cranial RT</td>
<td>39</td>
<td>1.5 (&lt;0.1–14.2)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

<sup>a</sup>P-values indicate differences in serum AMH levels (in microgram per litre) in survivors and controls, by ANCOVA, adjusting for age differences. Median AMH level in controls was 2.1 μg/l, range (0.1–7.4 μg/l). Values are absolute numbers (proportions %) or medians (range). USO, unilateral salpingo-oophorectomy; BSO, bilateral salpingo-oophorectomy; CT, chemotherapy; RT, radiotherapy; MOPP, mechlorethamine, vincristine, procarbazine and prednisone; EBVD, epiduribin, bleomycin, vinblastine and dacarbazine; TBI, total body irradiation.

<sup>b</sup>Patients were not included in analysis because ovarian reserve was compromised by ovarian surgery.

<sup>c</sup>Analysis was not performed because of small number of patients.

<sup>d</sup>ANCOVA between CCS treated with three or more MOPP cycles versus CCS treated with less than three MOPP cycles or EBVD cycles: P = 0.04.
factor for residual ovarian function after treatment, rather than age at treatment. Neither the menarchal state nor the total cumulative dose of cyclophosphamide seemed to influence the extent of damage to the ovaries found in our study. Many studies have shown that administration of alkylating agents was an independent risk factor for POF (Sklar, 2005; Chemaitilly et al., 2006). Indeed, in our study, the total cumulative dose of cyclophosphamide did not correlate with AMH levels. Presumably, the gonadotoxic effect of cancer treatment is determined by different agents (Wallace et al., 2005). Furthermore, it could be hypothesized that other factors, such as genetic predisposition may be involved. The presence of a single nucleotide polymorphism in the AMH gene or the AMH type II receptor gene may result in a slightly faster depletion of the ovarian reserve (Kevenaar et al., 2007b). Cancer treatment may be an additional risk factor for POF in these survivors. Options for fertility preservation should be considered before starting therapy. A substantial part of our survivors had AMH levels below the 10th percentile and may have POF. In addition, a high proportion of CCS is at risk for poor chances of pregnancy after ART. Obviously, chances of spontaneous pregnancy will be even lower, especially if survivors postpone their first pregnancy until the third decade.

### References


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