Long-term ovarian function in women treated with CHOP or CHOP plus etoposide for aggressive lymphoma

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Background: Chemotherapy-associated ovarian damage comprises not only infertility, but also premature menopause. The latter has been reported as a consequence of alkylating chemotherapy for breast cancer or Hodgkin’s lymphoma. In this study, we assessed the long-term impact of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone)-like regimens on ovarian function in patients with aggressive non-Hodgkin lymphoma (NHL).

Patients and methods: Long-term survivors after CHOP or CHOP plus etoposide (CHOEP) treatment within the Mabthera International Trial or the NHL-B1 trial of the German NHL Study Group were requested to respond to a questionnaire and to consent to blood sampling for hormone assessment.

Results: A total of 46 of 81 contacted patients with a median age of 32.5 years at the time of enrolment into the aforementioned clinical trials responded to the questionnaire. The median follow-up after completion of treatment was 14 years. Last menstrual bleeding occurred significantly earlier in patients compared with the general population (47 versus 51 years, P < 0.0001). In comparison to the distribution of menopausal symptoms in the general population, the

References: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19

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percentage of women with moderate or severe menopausal symptoms was increased. In 23 patients who agreed to participate in laboratory analyses, anti-Muller hormone as a marker of ovarian reserve was decreased when compared with correspondent age groups of the general population.

**Conclusion:** Although most female patients regain fertility after CHOP-like chemotherapy, late ovarian impairment occurs frequently. Therefore, awareness of such delayed side-effects at the time of counselling is of importance.

**Key words:** lymphoma, chemotherapy, CHOP, gonadotoxicity, ovarian failure, menopause

**introduction**

As a result of the advances in cancer treatment, an increasing number of patients achieve long-term survival. For younger patients, gonadal failure represents a major long-term adverse effect. In addition to infertility, gonadal impairment in women may lead to premature menopause. As menopausal symptoms may considerably impair the quality of life [1], detailed information on the risks associated with specific treatment regimens is essential for counselling of patients, improving quality of survivorship care, and optimizing future treatment strategies. With respect to haematologic malignancies, most of the data published on late ovarian failure have been collected from patients after Hodgkin lymphoma (HL) treatment [2–6] whereas information on patients after treatment of non-Hodgkin lymphoma (NHL) is limited. The goal of our study was to assess the long-term ovarian toxicity of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), a standard treatment regimen in aggressive lymphoma, as well as of CHOP plus etoposide (CHOEP). We retrospectively analysed young female patients treated with these regimens in two large prospective clinical trials, the Mabthera International Trial (MInT) [7], and the German NHL-B1 trial [8]. In the present report, we evaluate the late ovarian function in continuation of the previous report on parenthood [9]. To assess ovarian reserve, the menstrual status, menstrual symptoms, and anti-Muller hormone (AMH) levels were analysed.

**patients and methods**

**patients and treatment**

This study was carried out on German patients with aggressive lymphoma who were enrolled in the MInT and NHL-B1 studies between 1993 and 2003. Patients enrolled in MInT received six cycles of CHOEP given every 21 days with or without rituximab. Patients in the NHL-B1 trial were treated with six cycles of CHOP or CHOEP given every 21 or every 14 days. Overall, 1533 patients were included in both trials with 1415 patients being evaluable for analysis regarding the original study end points. Inclusion criteria for our study on gonadal toxicity were: (i) age 18–40 years at the time of enrolment in the respective trials, (ii) completion of six treatment cycles, (iii) no radiotherapy to gonadal area as part of primary treatment, (iv) ongoing first remission, (v) no chemo- or radiotherapy for secondary neoplasia, (vi) alive at the time of survey, and (vii) valid postal address. Eligible patients were requested to respond to a questionnaire and to consent to blood sampling for hormone assessment.

**questionnaire**

The questionnaire was approved by the local ethics committee in August 2011 and informed consent was obtained from all responders. The following items were addressed: pregnancy and parenthood, use of contraceptives or hormone replacement, gynaecological surgery, menopausal status (with menopause defined as irreversible amenorrhoea lasting more than 12 months), and time of last menstrual bleeding. Menopausal symptoms were assessed using the Menopause Rating Scale (MRS), a standardized self-administered questionnaire for the assessment of menopausal complaints (http://www.menopause-rating-scale.info/). Data were collected between September 2011 and October 2012, and updated in February 2014.

**hormonal analyses**

Blood samples were centrally processed and serum samples were stored in aliquots at −80°C until analysis. AMH enzyme-linked immunosorbent assay (AMH ELISA kit, Beckman Coulter) was used for quantitative measurement of serum AMH with a lower limit of detection of 0.14 ng/ml. All assays were conducted in the Central Laboratory, Heidelberg University Hospital, Germany.

**data on general population**

Data on the age at menopause in the German general population were derived from the Heidelberg cohort [10] of the European Prospective Investigation into Cancer and Nutrition (EPIC-Heidelberg). The analytic cohort comprised 11 612 women born between 1930 and 1963 (aged 35–66 years at the time of data collection in 1993/1994), a random sample of the general population of Heidelberg, Germany, and surrounding communities. Again menopause was defined as irreversible amenorrhoea lasting more than 12 months.

**statistics**

Demographics and disease characteristics were summarized using descriptive statistics. Data were analysed using the statistic environment R, version 2.15.3. Kaplan–Meier plots were carried out to describe the cessation of menstrual bleeding in the study population in contrast to the general population. The log-rank test was used to evaluate differences in age at last menstrual bleeding between the study group and the general population. The $\chi^2$ test was applied to compare the percentages of women with different degrees of menopausal symptoms in the study population with that in the general population. All results were interpreted on a significance level of 5%.

**results**

**Patients’ characteristics**

Of 81 long-term survivors eligible for the present study, a total of 46 women (14 and 32 treated with CHOP and CHOEP, respectively) agreed to participate in this analysis, corresponding to a response rate of 56.8% (supplementary Figure S1, available at Annals of Oncology online). The participating patients were born between 1955 and 1977, the median age at treatment and at time of data collection was 32.5 (range 18–40) years and 47 (range 32–56) years, respectively, with a median follow-up upon completion of therapy of 14 (range 8–19) years. Due to the eligibility criteria of our fertility study, the median age at treatment and also prognostic factors differed from the overall MInT/NHL-
B1 study population. Comparison of participating and non-participating eligible patients showed, however, no major differences in clinical characteristics, demonstrating that the participants were representative for the entire eligible population (data not shown).

**Menopausal status**

In 10 patients, the menstrual status could not be assessed, mostly due to surgical menopause or concurrent exogenous hormone use (oral contraceptives or hormone replacement therapy). Among the remaining 36 patients (Figure 1), 17 reported regular menstrual bleeding and 19 had already experienced menopause. In 8 of these 19 patients, amenorrhoea had persisted after chemotherapy (median age at treatment 39.5 years, range 35–40 years) whereas in 11 patients menopause had occurred after temporary recuperation of regular menstrual cycles (median age at treatment 34 years, range 25–38 years), the latter including 3 patients with childbirth after chemotherapy. In comparison to the German general population, last menstrual bleeding occurred significantly earlier in our patient population (Figure 2). The median age at menopause in the study group versus the control group was 47 versus 51 years, respectively, and the log-rank test showed a significant difference in age at menopause ($P < 0.001$). Only two female patients received a GnRH analogue simultaneously during chemotherapy, and both have ongoing menstrual bleeding (both aged 40 years at the time of analysis).

**Menopausal symptoms**

In 35 patients, the total score for menopausal symptoms according to the MRS was available (supplementary Table S1, available at *Annals of Oncology* online). Among these patients, 25.7%...
experienced no or few, 17.1% mild, 37.1% moderate, and 20% severe menopausal symptoms. This differed significantly \((P = 0.0029)\) from the distribution of menopausal symptoms in the German general population aged 45–60 years \([11]\), where 48% reported no or few, 25% mild, 20% moderate, and 8% severe menopausal symptoms.

### Table 1. Hormonal analyses

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age group (years)</th>
<th>Age (years)</th>
<th>Concurrent hormone use</th>
<th>Menstrual status</th>
<th>Patient serum AMH (ng/ml)</th>
<th>Median serum AMH (ng/ml) in presumably healthy population (10th percentile, 90th percentile) ([12])</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>36</td>
<td>–</td>
<td>Postmenopausal</td>
<td>&lt;0.14</td>
<td>2.62 (0.81, 5.92)</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>37</td>
<td>–</td>
<td>Premenopausal</td>
<td>&lt;0.14</td>
<td>1.72 (0.31, 4.19)</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>38</td>
<td>–</td>
<td>Premenopausal</td>
<td>0.98</td>
<td>2.16 (0.80, 5.90)</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>38</td>
<td>–</td>
<td>Premenopausal</td>
<td>1.38</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>38</td>
<td>–</td>
<td>Premenopausal</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>39</td>
<td>+</td>
<td>–*</td>
<td>0.25</td>
<td>1.74 (0.57, 4.55)</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>42</td>
<td>–</td>
<td>Premenopausal</td>
<td>0.19</td>
<td>1.26 (0.28, 3.07)</td>
</tr>
<tr>
<td>8</td>
<td>≥43</td>
<td>44</td>
<td>–</td>
<td>Premenopausal</td>
<td>&lt;0.14</td>
<td>Not reported</td>
</tr>
<tr>
<td>9</td>
<td>44</td>
<td>44</td>
<td>+</td>
<td>–*</td>
<td>&lt;0.14</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>44</td>
<td>44</td>
<td>–</td>
<td>Premenopausal</td>
<td>&lt;0.14</td>
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</tr>
<tr>
<td>11</td>
<td>46</td>
<td>46</td>
<td>–</td>
<td>Premenopausal</td>
<td>&lt;0.14</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>46</td>
<td>46</td>
<td>–</td>
<td>Postmenopausal</td>
<td>&lt;0.14</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>47</td>
<td>47</td>
<td>–</td>
<td>Postmenopausal</td>
<td>&lt;0.14</td>
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<tr>
<td>14</td>
<td>47</td>
<td>47</td>
<td>–</td>
<td>Premenopausal</td>
<td>&lt;0.14</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>50</td>
<td>50</td>
<td>–</td>
<td>Postmenopausal</td>
<td>&lt;0.14</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>50</td>
<td>50</td>
<td>–</td>
<td>Postmenopausal</td>
<td>&lt;0.14</td>
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<tr>
<td>17</td>
<td>50</td>
<td>50</td>
<td>–</td>
<td>Postmenopausal</td>
<td>&lt;0.14</td>
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<tr>
<td>18</td>
<td>50</td>
<td>50</td>
<td>+</td>
<td>–*</td>
<td>&lt;0.14</td>
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<tr>
<td>19</td>
<td>50</td>
<td>50</td>
<td>+</td>
<td>–*</td>
<td>&lt;0.14</td>
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<tr>
<td>20</td>
<td>52</td>
<td>52</td>
<td>+</td>
<td>–*</td>
<td>&lt;0.14</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>53</td>
<td>53</td>
<td>–</td>
<td>Postmenopausal</td>
<td>&lt;0.14</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>54</td>
<td>54</td>
<td>–</td>
<td>Postmenopausal</td>
<td>&lt;0.14</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>56</td>
<td>56</td>
<td>–</td>
<td>Postmenopausal</td>
<td>&lt;0.14</td>
<td></td>
</tr>
</tbody>
</table>

*aMenstrual status not evaluable due to concurrent hormone use.

AMH, anti-Muller hormone.

hormonal analyses

Twenty-three patients agreed to participate in laboratory analyses (Table 1). AMH was not detectable (<0.14 ng/ml) in patients with absent menstrual bleeding or in patients above age 42. In younger (36–42 years) patients who were still menstruating, AMH levels were low (<0.14 ng/ml in two patients, 0.14–0.3 ng/ml in three patients, >0.3–1.4 ng/ml in two patients) when compared with the correspondent age groups of the general population \([12]\).

### Discussion

To the best of our knowledge, systematic evaluation of late ovarian function after CHOP-like chemotherapy has not been reported so far. Our analyses show that despite a low rate of ovarian failure immediately after chemotherapy as reflected by child birth and recovery of menstrual bleeding shortly after treatment completion, late impairment of ovarian function occurs in a relevant proportion of patients. The most remarkable findings of our study are: (i) a significant decrease in the age at menopause, (ii) a significant increase in the frequency of severe menopausal symptoms, and (iii) low AMH levels in patients who have received CHOP or CHOEP when compared with the general population.

Historically, the main parameter used for evaluation of ovarian toxicity has been persistent amenorrhoea after completion of chemotherapy. Only recently, premature ovarian ageing despite the temporary recovery of cyclic ovarian function has gained attention and was recognized to be a result of a diminished ovarian follicle pool due to gonadotoxic therapy \([13–15]\). Premature menopause as well as decreased AMH levels suggesting follicular depletion have been reported after treatment of childhood cancer \([16–24]\), breast cancer \([25, 26]\) or HL \([2–6]\). AMH, although not secreted by the primordial follicles themselves, is currently considered the most valuable indicator of the primordial follicle pool size \([27]\).

After treatment with CHOP, most female patients initially regain regular menstrual cycling and child-bearing potential \([28–31]\). However, the long-term impact of CHOP-like regimens on ovarian function has not been adequately addressed. Existing information is based on mostly small studies with special emphasis on acute ovarian failure. With respect to hormonal analyses, decreased AMH levels have been described in different heterogeneous cohorts of patients with haematologic malignancies \([32–34]\). In patients after CHOP-like chemotherapy, AMH levels have not been studied in detail.
In the present study, premature ovarian failure in a narrower sense, defined as age at menopause <40 years, was observed only in a minority of women. However, the age at last menstrual bleeding was significantly lower in our CHOP/CHOEP patients in comparison to the German general population. As we relied on the patients’ report on the age at last menstrual bleeding, inaccuracies cannot be excluded. However, the data on the general population are also based on self-reported information. Furthermore, we observed a significantly higher percentage of women with moderate or severe menopausal symptoms compared with the German general population [11] despite the fact that our patients were younger than the women in the study of Potthoff et al. Self-reported data on age at last menstrual bleeding and menopausal symptoms in our patients is substantiated by the results of the hormone analyses. AMH as a marker of ovarian reserve was not detectable in most examined patients aged >40 years at time of analysis and in one-third of the patients aged <40 years at time of analysis. In the remaining patients, AMH was decreased when compared with age-matched controls [12]. Since AMH is reported to become undetectable decreased when compared with age-matched controls [12], early menopause is to be expected in at least some presently menstruating women of our patient cohort.

Our study, albeit limited by its retrospective nature and the number of assessable patients, nevertheless provides novel and clinically important information on the late impairment of ovarian function. Obviously, issues such as age at menopause, severity of menopausal symptoms, or hormone parameters could be better addressed within a large prospective trial. Such prospective trials have not been carried out in NHL patients so far, and might not be feasible as the incidence of NHL in young patients is low when compared with HL or breast cancer. Furthermore, assessment of long-term gonadal function would inevitably require a long-term follow-up for decades. The aforementioned limitations of our study are at least in part outweighed by our investigation of a uniform patient cohort. By examining only patients treated with CHOP or CHOEP within two large multicentre trials, and by focusing on patients who completed the complete six cycles of chemotherapy without radiation therapy to the gonadal area or any kind of salvage chemotherapy we were able to confine our study to a homogeneous patient population.

In summary, our knowledge on CHOP as a regimen with a low risk of infertility has to be complemented by a substantial rate of late ovarian impairment in these patients. Although rarely premature, early cessation of menstrual bleeding occurs frequently in women after treatment with CHOP-like regimens. As more patients with aggressive lymphoma achieve long-term cure, not only survival, but also quality of life after surviving cancer becomes increasingly important. With respect to the variety of adverse health outcomes and the symptom burden associated with menopause, survivorship care should include the detection of ovarian failure, individual discussion of hormone replacement and symptomatic management of menopausal symptoms. Furthermore, awareness of such late effects is essential for optimizing future clinical trials for young lymphoma patients.

**funding**

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**disclosure**

The authors have declared no conflicts of interest.

**references**

Vitamin D and pancreatic cancer: a pooled analysis from the Pancreatic Cancer Case–Control Consortium


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Background: The potential role of vitamin D in the aetiology of pancreatic cancer is unclear, with recent studies suggesting both positive and negative associations.

Patients and methods: We used data from nine case–control studies from the International Pancreatic Cancer Case–Control Consortium (PanC4) to examine associations between pancreatic cancer risk and dietary vitamin D intake. Study-