Determinants of ovarian function after response-adapted therapy in patients with advanced Hodgkin’s lymphoma (RATHL): a secondary analysis of a randomised phase 3 trial


Summary

Background Adverse effects on reproductive function are a key concern in young women treated with chemotherapy for advanced Hodgkin’s lymphoma. We aimed to identify risk factors for the extent of ovarian damage in women with Hodgkin’s lymphoma treated with different chemotherapy regimens to inform accurate advice on options for fertility preservation.

Methods We recruited female participants from the randomised phase 3 RATHL trial, aged 18–45 years, based on availability of participants at recruiting sites in the UK. The RATHL trial key inclusion criteria were histologically confirmed classic Hodgkin’s lymphoma, stage IIb–IV or IIA with adverse features (bulky disease or more than two sites of involvement), no previous treatments, and a performance status of 0–3. As part of RATHL, participants were treated with two cycles of doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) or AVD followed by an interim PET-CT scan. Participants who had negative interim scans (PET score of 1 to 3 according to the Lugano classification) were randomly assigned (1:1) by use of minimisation, stratified by interim PET score and study centre, to continue ABVD or AVD for four more cycles. Participants with positive scans (PET score of 4 or 5) were escalated to treatment with bleomycin, etoposide, doxorubicin, cyclophosphamide, vinristine, procarbazine, and prednisolone (BEACOPP-14 or escalated BEACOPP) for four cycles. For the protocol-driven prospective cohort substudy, ovarian function was assessed before treatment, during chemotherapy, and then annually for 3 years by use of serum antimüllerian hormone and follicle-stimulating hormone measurements. The RATHL study is registered with ClinicalTrials.gov, number NCT00678327.

Findings Between Dec 13, 2010, and Dec 19, 2012, 67 eligible participants were recruited for this prospective cohort study; 57 had received ABVD or AVD (ABVD-AVD group) and ten BEACOPP-14 or escalated BEACOPP (BEACOPP group). Follow-up was fixed at 3 years. Antimüllerian hormone concentrations decreased during both chemotherapy regimens. At 1 year after chemotherapy, antimüllerian hormone concentrations recovered to a median of 10·5 pmol/L (IQR 4·3–17·3) in the ABVD-AVD group, but little recovery was seen after BEACOPP (median 0·11 pmol/L [0·07–0·20]). Age also affected the extent of ovarian function recovery, with antimüllerian hormone recovery in participants aged 35 years or older in the ABVD-AVD group to 37% (SD 10) of their before treatment concentrations, compared with full recovery to 127% (SD 12) in those younger than 35 years (p<0·0001). Follicle-stimulating hormone recovery to less than 4·0 license.  

Interpretation Reduced recovery of ovarian function observed in women older than 35 years treated with ABVD or AVD compared with younger women indicates that treatment could reduce their reproductive lifespan and supports discussion of fertility preservation before treatment. Women treated with BEACOPP should be informed of its potential high gonadotoxicity. These findings warrant further investigation in large, prospective studies with fertility and reproductive lifespan as outcomes.

Funding Medical Research Foundation and Cancer Research UK.

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Introduction Hodgkin’s lymphoma is predominantly a cancer of young adult life, occurring at a median age of 35 years. Systemic treatment for this condition has evolved from chemotherapy regimens based on alkylating agents, which had a high risk of infertility and premature ovarian insufficiency in women, to treatment with doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD), which is the current treatment of choice for many adult patients with Hodgkin’s lymphoma. ABVD results in a disease-free survival of 75%, and with less gonadotoxicity than previous treatments. Increasing evidence supports that treatment...
intensification could benefit patients who are at higher risk of relapse, and some clinicians advocate treat-
ment with bleomycin, etoposide, doxorubicin, cyclophos-
phamide, vincristine, procarbazine, and prednisolone
(BEACOPP) for patients with advanced stage or high-risk
early stage disease. However, female gonadal toxicity is
high with dose-escalated BEACOPP resulting in amenor-
rhoea (a surrogate marker of premature ovarian insuf-
ciency) in about 95% of patients older than 30 years.1

Analysis of the effects of cancer therapies on the ovaries
requires consideration of both the degree of loss of
ovarian function during chemotherapy and changes in
ovarian function thereafter. The degree of loss of the
primordial follicle pool (the ovarian reserve) during
chemotherapy is the most important effect since it
determines the potential for any recovery of ovarian
function and fertility, and subsequent age at menopause,
although the degree of loss cannot be assessed in vivo.
Additional effects of chemotherapy might be seen on the
non-follicular components of the ovary, including the
stroma and vasculature.1 Few prospective analyses have
been done of ovarian function from the time of diagnosis
and before chemotherapy.2,4 Most larger studies use
amenorrhoea to indicate loss of ovarian function,1 and
so are unable to differentiate between women who
have largely intact remaining ovarian function and
those who have lost most of their ovarian reserve and
will soon become menopausal and infertile.5,10 Although measurement of follicle-stimulating hormone
concentration is central to the diagnosis of premature
ovarian insufficiency, the hormone has substantial
variation during the menstrual cycle, complicating its use
to measure ovarian reserve in women with ovarian
activity.11 Analysis of concentrations of serum antimül-
lerian hormone has become increasingly valuable for women with
ovarian function was affected by age. We found that among
older women baseline concentrations of antimüllerian
hormone did not affect recovery, so reduced hormone recovery
in older women was not due to the age-related decline in
ovarian reserve. Little recovery in ovarian function was seen
after treatment with BEACOPP in women of all ages, although
a similar number of pregnancies was observed in the two
treatment groups. Due to the small size of the cohorts analysed,
these results warrant further confirmation.

Implication of all the available evidence
The absence of detectable ovarian damage after treatment with
ABVD or AVD in younger women with Hodgkin’s lymphoma is
reassuring, although longer follow-up is needed in future
studies for a full assessment of potential reproductive effects.
Antimüllerian hormone is not a predictor of short-term fertility.
The finding of reduced ovarian recovery in older women with
Hodgkin’s lymphoma treated with chemotherapy indicates that
age-specific discussions and consideration of fertility
preservation procedures should be considered before
treatment. These considerations are of growing importance
given the increasing age at which women are having children in
developed countries.

Methods
Study design and participants
In this prospective cohort study, participants were
recruited from the RATHL trial, which used PET-CT

Research in context
Evidence before this study
Chemotherapy regimens are associated with toxic effects on
human ovaries, manifesting as premature ovarian insufficiency,
infertility, and reduced reproductive lifespan. Therefore,
accurate assessment of the ovarian toxicity of widely used
chemotherapy regimens is needed. We searched PubMed for
articles published in English between inception and
March 12, 2018, using the terms “ovary”, “ovarian reserve”,
“chemotherapy”, and “cancer”. We found little data from
prospective studies that used current biomarkers of ovarian
function. Both age and ovarian reserves before treatment are
predictive of ovarian recovery after chemotherapy with some
regimens, but their importance is unknown for treatments for
patients with Hodgkin’s lymphoma.

Added value of this study
We found that changes in antimüllerian hormone
concentration could distinguish between the effects of
treatment on ovarian function with doxorubicin, bleomycin,
vinblastine, and dacarbazine (ABVD) or AVD, and bleomycin,
etoposide, doxorubicin, cyclophosphamide, vincristine,
procarbazine, and prednisolone (BEACOPP) in patients with
advanced Hodgkin’s lymphoma, particularly from 3 years of
follow-up after chemotherapy. Treatment with ABVD or AVD
was confirmed to have little gonadotoxicity in female patients
with Hodgkin’s lymphoma who were younger than 35 years at
diagnosis; however, in older patients (>35 years) recovery of
ovarian function was affected by age. We found that among
older women baseline concentrations of antimüllerian
hormone did not affect recovery, so reduced hormone recovery
in older women was not due to the age-related decline in
ovarian reserve. Little recovery in ovarian function was seen
after treatment with BEACOPP in women of all ages, although
a similar number of pregnancies was observed in the two
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these results warrant further confirmation.

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www.thelancet.com/oncology Published online September 13, 2018 http://dx.doi.org/10.1016/S1470-2045(18)30500-X
to guide response-adapted chemotherapy, for patients with advanced Hodgkin’s lymphoma (appendix p 1). Recruitment for RATHL was open in hospitals across the UK, Italy, Norway, Sweden, Denmark, Australia, and New Zealand from Aug 29, 2008, to Dec 21, 2012. RATHL inclusion criteria were histologically confirmed classic Hodgkin’s lymphoma according to the WHO classification; age 18 years or older; stage IIIB–IV or IIA with adverse features (such as bulky disease or more than two sites of involvement); no previous treatment with chemotherapy, radiotherapy, or investigational treatments for Hodgkin’s lymphoma; performance status 0–3 (according to WHO criteria); adequate bone marrow function; creatinine less than 150% of the upper limit of normal (ULN); bilirubin concentration less than two times ULN; aminotransferase concentration less than 2.5 times ULN; and life expectancy of more than 3 months. The full eligibility criteria for the RATHL trial are available in the protocol.

For this prospective cohort substudy, women and aged 18–45 years (ie, premenopausal) were actively recruited from RATHL sites in the UK (appendix pp 3–5). The protocol for the substudy is available online.

This cohort substudy received centralised ethical committee approval that covered all sites (National Research Ethics Service, Southampton and South West Hampshire) and all participants gave written informed consent.

Procedures
In the RATHL study, after initial staging and a baseline PET-CT scan, participants were treated with two cycles of ABVD, intravenously, on days 1 and 15 of a 28 days cycle followed by an interim PET-CT scan (doses and schedule in the appendix [p 2]). Participants who had negative interim scans by central review (PET score of 1 to 3 per Lugano criteria) were randomly assigned (1:1) by the Cancer Research UK and University College London Cancer Trials Centre using minimisation, with stratification according to PET score (1, 2, or 3) and centre, to continue ABVD (intravenously, days 1 and 15) or to receive doxorubicin, vinblastine, and dacarbazine (AVD; intravenously, days 1 and 15) for four more cycles. Since randomisation was done centrally, concealment of assignment was not an issue. Participants with positive scans (PET score of 4 or 5) were escalated to either BEACOPP-14 for six cycles of 14 days or escalated BEACOPP for four cycles (doses and schedule in the appendix [p 2]). Neither patients nor study staff were masked to allocation.

Ovarian function was assessed by use of serum concentrations of antimüllerian hormone in participants enrolled into this substudy and follicle-stimulating hormone concentrations from all eligible participants (women and aged 18–45 years) from the RATHL trial. Luteinising hormone and oestradiol measurements were also taken. Oestradiol measurements are necessary for the identification of women with premature ovarian insufficiency. During the RATHL trial, blood samples were taken before any treatment, after two cycles of initial ABVD treatment, at the end of chemotherapy, and at 1, 2, and 3 years after chemotherapy. Samples taken after participants showed disease progression and required additional treatment were excluded from both analyses reported here. Sampling was not timed by the participant’s stage of menstrual cycle. Follicle-stimulating hormone measurements were done locally as part of the RATHL trial; additional samples from participants enrolled in this substudy were taken to measure antimüllerian hormone, follicle-stimulating hormone, luteinising hormone, and oestradiol concentrations by use of Roche Elecsys assays (automated cobas e 411

Figure 1: Study profile
RATHL trial and ovarian function substudy participants who contributed to analyses. ABVD=doxorubicin, bleomycin, vinblastine, and dacarbazine. AVD=doxorubicin, vinblastine, and dacarbazine. BEACOPP=bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone. FSH=follicle-stimulating hormone.

See Online for appendix
Articles

Table: Baseline characteristics

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<th>Ovarian function substudy cohorts</th>
<th>RATHL trial cohorts</th>
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<tr>
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<td>ABVD-AVD group (n=57)</td>
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<td>ABVD group (n=165)</td>
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<td>Age, years</td>
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<td>Hormonal contraception at baseline</td>
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<td>Baseline antimüllerian hormone, pmol/L</td>
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<tr>
<td>Baseline follicle-stimulating hormone concentration, IU/L</td>
<td>4.6 (0.3-12)</td>
</tr>
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</table>

Data are median (range) or n (%) unless otherwise stated. RATHL trial cohort data are for women younger than 45 years included in this study. ABVD=doxorubicin, bleomycin, vinblastine, and dacarbazine. AVD=doxorubicin, vinblastine, and dacarbazine. BEACOPP=bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone. *One patient in this group had a missing performance status.

Outcomes

The primary outcome of this study, specified as an exploratory outcome in the RATHL trial, was assessment of gonadal function. The prespecified outcomes in this substudy were analyses of acute toxic effects of each treatment regimen on the ovaries, and ovarian function after treatment (including progression to ovarian failure and recovery of ovarian function). Other prespecified outcomes were the association between fertility after treatment and ovarian function before treatment, degree of acute ovarian toxic effects, which will be reported separately.

Statistical analysis

As a secondary analysis of the RATHL trial, the prespecified exploratory analyses presented here are not powered; we aimed to recruit as many patients as possible while the RATHL trial was open. We hypothesised that antimüllerian hormone concentrations could show differences between the gonadotoxicity of the chemotherapy regimens in the RATHL trial, both during and after treatment, and that concentrations of antimüllerian hormone before treatment would be predictive of recovery of ovarian function after treatment. We analysed antimüllerian hormone and follicle-stimulating hormone concentrations in the ovarian subgroup after log transformation using ANOVA with Bonferroni’s multiple comparisons test for changes during treatment and recovery. We calculated Pearson correlation coefficients and used multiple linear regression without transformation to analyse age and before treatment concentration of antimüllerian hormone against percentage hormone recovery (calculated as [concentration at 2 years÷concentration before treatment]×100) and against concentration at 2 years. We used the Mann-Whitney U test to compare hormone concentrations between the treatment groups. We analysed proportions of participants using Fisher’s exact test to determine the number of women who had a pregnancy after chemotherapy. We measured follicle-stimulating hormone recovery using Cox regression and Kaplan-Meier survival analysis. We measured time to recovery (follicle-stimulating hormone concentration of ≤25 IU/L) from the end of treatment until the first follicle-stimulating hormone measurement of 25 IU/L or less, or a reported pregnancy (whichever came first). Patients with follicle-stimulating hormone concentrations of more than 25 IU/L at baseline were excluded, and patients who had neither event were censored at the last reported follicle-stimulating hormone measurement. Any measurements recorded after disease progression were excluded from all analyses because patients would have been exposed to further treatment at this point. We tested the assumption of proportional

analysed, Roche, Burgess Hill, UK). The substudy samples were sent post to a central laboratory (MRC Centre for Reproductive Health, Edinburgh), where the serum was stored at −20°C until analysis. The antimüllerian hormone assay has a limit of detection of 0.07 pmol/L, and for all assays the interassay and intra-assay coefficients of variation were below 5%. A follicle-stimulating hormone concentration at 2 years÷concentration before treatment. We used the Mann-Whitney U test to compare hormone concentrations between the treatment groups. We analysed proportions of participants using Fisher’s exact test to determine the number of women who had a pregnancy after chemotherapy. We measured follicle-stimulating hormone recovery using Cox regression and Kaplan-Meier survival analysis. We measured time to recovery (follicle-stimulating hormone concentration of ≤25 IU/L) from the end of treatment until the first follicle-stimulating hormone measurement of 25 IU/L or less, or a reported pregnancy (whichever came first). Patients with follicle-stimulating hormone concentrations of more than 25 IU/L at baseline were excluded, and patients who had neither event were censored at the last reported follicle-stimulating hormone measurement. Any measurements recorded after disease progression were excluded from all analyses because patients would have been exposed to further treatment at this point. We tested the assumption of proportional
hazards using Schoenfeld residuals, and when it did not hold we used restricted mean survival times with a 3 year cutoff. All p values are two-sided and a p value of less than 0·05 was considered statistically significant.

Data analyses were done in STATA (version 15.1) and Graphpad Prism 7. The RATHL trial is registered with ClinicalTrials.gov, number NCT00678327.

Role of the funding source
The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. RAA, AAK, and PWMJ had access to the raw data. The corresponding author had final responsibility for the decision to submit for publication.

Results
Between Dec 13, 2010, and Dec 19, 2012, participants from the RATHL trial were recruited for this ovarian function substudy (figure 1; appendix p 3). 115 (9%) of 1214 participants in the RATHL trial were eligible for the substudy, of whom 74 (69%) registered. Seven (10%) of 74 registered participants were excluded from the substudy and the main trial, six for scan protocol deviations, and one for psychological reasons, leaving 391 (94%) of 415 eligible (18–45 years) female RATHL participants, of whom 321 (82%) were evaluable for follicle-stimulating hormone analyses, and 67 (91%) were evaluable for substudy analyses. Of the 67 evaluable participants for the substudy analyses, 24 (36%) had been treated with ABVD, 33 (49%) with AVD, four (6%) with BEACOPP-14, and six (9%) with escalated BEACOPP. No difference in baseline hormone concentrations were found between participants treated with ABVD versus AVD, or BEACOPP-14 versus escalated BEACOPP (table); thus these subgroups were combined as ABVD-AVD (n=57) and BEACOPP (n=10), respectively, for subsequent analyses. The baseline characteristics of the participants who had follicle-stimulating hormone measurements from the RATHL trial, and the combined ABVD-AVD and BEACOPP subgroups evaluated in this substudy are in the table.

Figure 2: Ovarian function biomarkers at prespecified timepoints per treatment group
(A) Concentrations of antimüllerian hormone and (B) follicle-stimulating hormone. All participants included in the substudy were analysed (ABVD-AVD group, n=57; BEACOPP group, n=10). Boxes are median and IQR, bars are ranges. Panel A is plotted on a log_10 scale to clearly show the low concentrations seen in the BEACOPP group. ABVD=doxorubicin, bleomycin, vinblastine, and dacarbazine. AVD=doxorubicin, vinblastine, and dacarbazine. BEACOPP=bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisolone.

For nine participants in the substudy, data at post-baseline timepoints were not available because of disease progression and further treatment (six in the ABVD-AVD group, three in the BEACOPP group), and 13 blood samples were missing or taken at incorrect timepoints. Eight blood samples taken from eight women during pregnancy in the ovarian subgroup were excluded from analysis.

Antimüllerian hormone concentrations decreased in all participants during chemotherapy treatment with reciprocal increases in follicle-stimulating hormone concentrations (figure 2). In the ABVD-AVD group, concentrations of antimüllerian hormone decreased from a median of 9·8 pmol/L (IQR 5·9–18·1) before treatment to 1·7 pmol/L (IQR 0·4–4·3) at the end of treatment (p<0·0001), with a small rise between cycle two of initial ABVD treatment and the end of treatment (p=0·0001). After chemotherapy in the ABVD-AVD group, concentrations of antimüllerian hormone increased to 10·5 pmol/L (IQR 4·3–17·3) at 1 year, similar to concentrations before treatment, with no change at later timepoints (figure 2). Antimüllerian hormone was undetectable in two (4%) of 56 participants at the end of treatment with follicle-stimulating hormone concentration higher than 25 IU/L and low oestradiol concentrations indicating premature ovarian insufficiency. For one of these participants, antimüllerian hormone became detectable during recovery, and for the other, antimüllerian hormone showed a transient recovery at 1 year after treatment, becoming undetectable thereafter.
In the BEACOPP group, concentrations of antimüllerian hormone decreased from a median of 6.8 pmol/L (IQR 2.2–12.8) before treatment to 0.08 pmol/L (0.07–0.24) at the end of treatment (p<0.0001; figure 2A). After treatment, by contrast with participants in the ABVD-AVD group, concentrations of antimüllerian hormone showed very little recovery (median 0.11 pmol/L [IQR 0.07–0.20] at 1 year) and were undetectable in five (71%) of seven participants at 3 years (figure 2A).

Follicle-stimulating hormone concentrations had generally reciprocal changes compared with concentrations of antimüllerian hormone and increased during treatment in both groups (figure 2B). Subsequently, in the ABVD-AVD group concentrations of follicle-stimulating hormone decreased to levels similar to before treatment at years 1 to 3 after treatment. However, in the BEACOPP group, follicle-stimulating hormone concentrations did not decrease in the follow-up period. Concentrations of luteinising hormone showed the same pattern of changes, increasing from before treatment to the end of treatment in both groups (both p<0.0001) and then recovering after treatment to before-treatment concentrations in the ABVD-AVD group, but remaining significantly increased after treatment in the BEACOPP group (data not shown).

Both antimüllerian hormone concentration 2 years after treatment and follicle-stimulating hormone recovery data provide evidence of the effect of age on ovarian recovery in women treated with ABVD or AVD (figures 3 and 4). We saw a correlation between the concentration of antimüllerian hormone before treatment and at 2 years after treatment (r=0.89; p<0.0001) but not with antimüllerian hormone recovery (r=0.30; p=0.051).

However, significant and similar negative correlations were seen between age and antimüllerian hormone concentrations at 2 years (r=–0.40; p=0.0055) and with antimüllerian hormone recovery (r=–0.40; p=0.0080). This negative effect of age on recovery was supported by mean concentrations of antimüllerian hormone at recovery, with participants younger than 35 years showing complete recovery at 127% (SD 12; median 10.0 pmol/L [IQR 7.5–21.1]) before treatment vs 13.4 pmol/L (7.1–22.3) at 2 years), whereas concentrations in those aged 35 years and older had incomplete recovery (37% [SD 10]; ≤35 years, median 5.7 pmol/L [IQR 2.1–9.7] before treatment vs 1.4 pmol/L [0.2–4.2]) at 2 years; for comparison of age groups p<0.0001). Analysis of antimüllerian hormone concentrations before treatment grouped by whether they were below or above the overall median value (9.77 pmol/L [IQR 5.9–18.1]) showed that recovery was similar between the two groups (below 123% [SD 21] vs above 103% [SD 9]; p=0.85). Multiple linear regression analysis also confirmed that age had a significant effect on antimüllerian hormone recovery (β=–0.43; p=0.004), but that antimüllerian hormone concentrations before treatment did not have an effect on hormone recovery (β=–0.15, p=0.30).

Thus, although overall concentrations of antimüllerian hormone after treatment seem to recover to before treatment concentrations, the degree of recovery is restricted by increasing age.

We also assessed the degree and timecourse of recovery of ovarian function, as indicated by normalisation of follicle-stimulating hormone concentrations, in the evaluable participants from the RATHL trial (figure 4). Follicle-stimulating hormone measurements after treatment were available from 321 women in the RATHL trial, with a median follow-up ( censoring at recovery) of 59.3 months (IQR 41.9–60.9). Follicle-stimulating hormone concentration recovery to 25 IU/L or lower was seen in 270 (96%) of 282 of participants treated with ABVD or AVD, compared with 26 (67%) of 39 treated with BEACOPP-14 or escalated BEACOPP (hazard ratio [HR] 0.37, 95% CI 0.25–0.56; p<0.0001). The assumption of proportional hazards did not hold for this comparison so a better estimate is given by the restricted mean survival (recovery) times (areas under the curves up to 3 years), which are 208.9 days for ABVD or AVD and 529.9 days for BEACOPP (p<0.0001)—ie, patients treated with ABVD or AVD have a mean recovery time of 321.0 days (95% CI

Figure 3: Correlations of antimüllerian hormone recovery and before treatment hormone concentrations or age for the ABVD-AVD group.

Panels show scatter plots of baseline hormone concentration versus concentration at 2 years (A) and versus percentage hormone recovery (B), and age versus hormone concentration at 2 years (C) and percentage hormone recovery (D). All patients in the ABVD-AVD group of the substudy included. Each dot shows antimüllerian hormone concentration or recovery; recovery was calculated for each participant as the concentration at 2 years after treatment as a proportion of the concentration before treatment. In the case of missing data, recovery could not be calculated. Dotted ine shows linear regression. ABVD=doxorubicin, bleomycin, vinblastine, and dacarbazine. A/VD=doxorubicin, vinblastine, and dacarbazine.
Kaplan-Meier estimates for participants treated with ABVD or A VD were 83% (95% CI 77–88) at 1 year and 96% (93–98) at 2 years compared with estimates for those aged 35 years and older of 54% (43–66) at 1 year and 83% (73–91) at 2 years. The proportions were similar between the age groups at 3 years (<35 years 98% [95–99] vs ≥35 years 93% [85–97]). We saw no difference in follicle-stimulating hormone recovery between participants treated with escalated BEACOPP or BEACOPP-14 for all ages (HR 0·72, 95% CI 0·33–1·56).

In the RATHL cohort, 64 (16%) of 391 participants were recorded as having 81 pregnancies. Median follow-up for the 91 patients was 52 months (IQR 42–64). 57 (17%) participants in the ABVD-A VD group had 71 pregnancies and seven (13%) participants in the BEACOPP group had ten pregnancies. These proportions were not substantially different. In the subgroup cohort, antimüllerian hormone measurements were available within the follow-up period for six women who became pregnant in the ABVD-A VD group and two in the BEACOPP group. At 1 year after chemotherapy, concentrations of antimüllerian hormone ranged from 0·74 pmol/L to 26·9 pmol/L in those who became pregnant in the ABVD-A VD group, and were 0·19 pmol/L and 0·20 pmol/L in those in the BEACOPP group. Two women, one in each treatment group, had undetectable antimüllerian hormone, high follicle-stimulating hormone, and low oestradiol concentrations after the recorded pregnancy, consistent with development of premature ovarian insufficiency within 3 years of chemotherapy treatment.

Adverse event data were reported in detail previously,17 but they did not include any data on ovarian function or fertility. The main study16 found an excess of grade 3–4 toxic effects from BEACOPP regimens, specifically febrile neutropenia and thrombocytopenia.

**Discussion**

In this prospective cohort study, we have shown that antimüllerian hormone concentrations decrease in women with Hodgkin’s lymphoma who are treated with ABVD, A VD, or BEACOPP (BEACOPP-14 or escalated BEACOPP), and although antimüllerian hormone concentrations recover to baseline concentrations after treatment with ABVD or A VD, little recovery is seen after treatment with BEACOPP. However, we found...
that recovery of ovarian function after treatment with ABVD or AVD is dependent on age, with full recovery of antimüllerian hormone seen in participants younger than 35 years, but not in women aged 35 years or older. We found that recovery of follicle-stimulating hormone after these two chemotherapy regimens also differed in both speed and extent. Follicle-stimulating hormone recovery after treatment with ABVD or AVD was slower in women aged 35 years or older than in those younger than 35 years, and the extent of recovery was much lower for those treated with either BEACOPP regimen, supporting the increased ovarian toxicity of this drug combination. The greater toxicity to other organ systems is also reflected in the higher incidence of febrile neutropenia and thrombocytopenia with BEACOPP.

The potential adverse effect of chemotherapy on ovarian function in women with Hodgkin’s lymphoma is of major concern, affecting fertility, sexual and bone health, and cardiovascular risk. Potential loss of fertility is a key concern of young women treated for cancer and, when appropriate, options for fertility preservation have been established; thus, the accurate assessment of the effect of different chemotherapy regimens is of substantial importance. Assessment of ovarian function after treatment via menstrual function and traditional biomarkers such as follicle-stimulating hormone are not of value in detecting ovarian damage that has resulted in incomplete loss of ovarian function. Measurement of concentrations of antimüllerian hormone has emerged as a reliable biomarker to detect partial loss of ovarian function in women after cancer therapy, with the additional advantages of showing little variation across the menstrual cycle. Although most data are from women after treatment for breast cancer and childhood cancer, we have shown the merit of antimüllerian hormone measurements in the assessment of ovarian function in adult women with Hodgkin’s lymphoma. Specifically, younger women treated with ABVD or AVD showed full initial recovery of antimüllerian hormone after chemotherapy, consistent with previous data that indicate that this regimen has little effect on age at menopause, but reduced recovery was seen in women aged 35 years or older at diagnosis. By contrast, treatment with BEACOPP resulted in noticeable and irreversible decreases in antimüllerian hormone concentrations, so low in many women that the hormone was undetectable after treatment even with the highly sensitive assay we used. However, the number of participants we analysed was small, which should inform interpretation of tests of significance. Participants received either escalated BEACOPP or BEACOPP-14, a dose-intense version that appears to be similarly gonadotoxic to baseline BEACOPP. Our data support this similarity, although the confidence intervals in our estimates were wide.

Antimüllerian hormone concentrations decreased quickly during treatment with both regimens, consistent with both treatments resulting in loss of the growing follicles that are the source of antimüllerian hormone. After the initial decrease, a small partial recovery in antimüllerian hormone was seen after two cycles in women treated with ABVD or AVD during later treatment cycles. This finding is consistent with increased activation of early follicles during chemotherapy resulting from reduced inhibition of growth initiation, which is proposed to be part of the mechanism for chemotherapy-induced premature ovarian insufficiency. This mechanism has been shown for high toxicity, cyclophosphamide-based regimens, but has not previously been identified for low toxicity regimens such as ABVD or AVD.

The degree of recovery of antimüllerian hormone after treatment with ABVD or AVD in young women could indicate a low toxic effect on the non-growing primordial follicle pool, which constitutes the true ovarian reserve and is the basis for after treatment fertility and the duration of the female reproductive lifespan. Under physiological conditions, antimüllerian hormone concentrations reflect the size of the primordial follicle population, although this population size can be perturbed under circumstances such as a new diagnosis of lymphoma. However, the 3-year follow-up period in this study is likely to have been sufficient for full recovery of ovarian function and therefore restoration of physiological associations between antimüllerian hormone and the ovarian reserve. We saw a prominent effect of age on recovery, with substantially lower recovery in women aged 35 years or older than in those younger than 35 years, independent of their antimüllerian hormone concentration before treatment. This finding indicates an effect either of age alone or of age in addition to the effects of ABVD or AVD that result in a substantial decrease in the size of the growing follicle population, and by implication of the non-growing population, after treatment. Chemotherapy has been shown to affect both the stroma and vasculature of the human ovary, causing fibrosis and hyalinisation of small vessels with loss of primordial follicles within areas of damage. Changes in ovarian stromal function with age include fibrosis and other hallmarks of chronic inflammation, such as the presence of macrophages, and can impair follicle development. These changes could be reversed by gonadotropin suppression, indicating that the increases in concentrations of luteinising hormone and follicle-stimulating hormone during chemotherapy shown here could be of aetiological importance, and the pre-existing more fibrotic stroma in older women could be more susceptible to chemotherapy-induced changes than the stroma in younger women, even with so-called low gonadotoxicity regimens such as ABVD or AVD. These age-associated effects of chemotherapy could have therapeutic implications and be part of the mechanism of action of gonadotropin-releasing hormone agonists to reduce the risk of premature ovarian insufficiency after chemotherapy for breast cancer, although this effect has not been confirmed for women treated for lymphoma.
The effect of age on recovery of ovarian function after both regimens, and the different toxic effects of each regimen on the ovaries, is also supported by our analysis of follicle-stimulating hormone recovery in the whole RATHL study cohort. In this analysis, follicle-stimulating hormone was dichotomised at a value consistent with premature ovarian insufficiency. In addition to confirming the effect of age on recovery from treatment with ABVD or AVD, our analysis showed clear differences in both the speed and extent of recovery between regimens, supporting the antimüllerian hormone data. Follicle-stimulating hormone concentrations are affected to a much greater extent than antimüllerian hormone concentrations by changes across the menstrual cycle and use of hormonal contraception, but our analysis shows measurement of follicle-stimulating hormone concentrations is of value in large cohorts in which more detailed characterisation is difficult.

Previous analyses in women with breast cancer have shown that receiving treatment at a young age and high concentrations of antimüllerian hormone before treatment are predictive of recovery of ovarian function after chemotherapy, albeit with variable relative contributions. Women with breast cancer are generally older (approximate mean age 40 years) than those with Hodgkin’s lymphoma and treatment involves more gonadotoxic alkylating agent-based regimens, resulting in a high probability of premature loss of ovarian function, particularly in women older than 40 years. These differences emphasise the need for defined populations and treatment regimens to analyse the toxic effects of chemotherapeutic regimens on the ovaries and, particularly, recovery.

The data in this study support the paucity of the value of current ovarian reserve markers for the prediction of fertility in the short term, because very low concentrations of antimüllerian hormone did not preclude chances of pregnancy. The lack of predictive value of antimüllerian hormone concentrations for short term fertility has been shown in prospective cohorts of women mostly in their pregnancy. The lack of predictive value of anti-

In conclusion, the results of this secondary analysis of the RATHL trial indicate the value of antimüllerian hormone as a biomarker of toxic effects of chemotherapy on the ovaries during and after different chemotherapy regimens for advanced Hodgkin’s lymphoma. We provide additional evidence that treatment with ABVD or AVD has no detectable effect on gonadal function in young women, although increasing age does restrict ovarian recovery; confirmation in larger studies is needed to confirm and define this interpretation more precisely, and determine its mechanism. By contrast, BEACOPP shows substantial gonadotoxicity in women of all ages, although some patients might have sufficient ovarian function after treatment to achieve pregnancy. Concentrations of antimüllerian hormone after treatment could be of use in advising women treated for Hodgkin’s lymphoma of their probable reproductive lifespan after treatment.

Contributors
RAA and PWMJ designed the study; collected, analysed, and interpreted data; contributed to manuscript writing; and approved the manuscript before submission. RR collected and analysed data and approved the manuscript before submission. AA and LC collected, analysed, and interpreted data; contributed to manuscript writing, and approved the manuscript before submission. CH collected and interpreted data, gave constructive comments on the manuscript, and approved the manuscript before submission. LS and TR collected data, gave constructive comments on the manuscript, and approved the manuscript before submission. LS and TR collected data, gave constructive comments on the manuscript, and approved the manuscript before submission. CH collected and analysed data and approved the manuscript before submission. NK, DWM, PM, and FMS collected data, contributed to manuscript writing, and approved the manuscript before submission. CR recruited patients, collected data, contributed to manuscript writing, and approved the manuscript before submission.

Declaration of interests
RAA reports non-financial support from Roche diagnostics, and a grant from the Medical Research Foundation. All other authors declare no competing interests.

Acknowledgements
The RATHL trial was funded by Cancer Research UK (reference CRUK/07/033), and the ovarian substudy by the Medical Research Foundation (reference 509909). Part of this work was done in the MRC Centre for Reproductive Health, University of Edinburgh, UK, which is funded by MRC Centre grant MR/N022556/1. We thank Roche Diagnostics for the supply of assay reagents, A Forbes Howe for hormone assays, and all investigators in RATHL for their contributions to patient recruitment and management.
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