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ORIGINAL ARTICLE ***Reproductive endocrinology***

Longitudinal study of AMH variations in 122 Adolescents and Young Adults (AYA) and non-AYA lymphoma patients to evaluate the chemo-induced ovarian toxicity to further personalise fertility preservation counselling

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STUDY QUESTION: What is the influence of age and chemotherapy regimen on the longitudinal blood anti-Müllerian hormone (AMH) variations in a large series of adolescents and young adult (AYA) (15–24 years old) and non-AYA (25–35 years old) lymphoma patients?

SUMMARY ANSWER: In case of alkylating regimen treatment, there was a deep and sustained follicular depletion in AYA as well as non-AYA patients; however in both groups, the ovarian toxicity was extremely low in cases of non-alkylating treatments.

WHAT IS KNOWN ALREADY: AMH is now well-recognised to be a real-time indicator of ovarian follicular depletion and recovery in women treated by chemotherapy. Its longitudinal variations may discriminate between highly and minimally toxic protocols regarding ovarian function. It has been shown, in different cancer types, that age, type of chemotherapy regimen and pre-treatment AMH levels are the main predictors of ovarian recovery. Large studies on longitudinal AMH variations under chemotherapy in lymphoma patients are few but can provide the opportunity to assess the degree of follicle loss at a young age.

STUDY DESIGN, SIZE, DURATION: This prospective cohort study was conducted in the Fertility Observatory of the Lille University Hospital. Data were collected between 2007 and 2016. Non-Hodgkin or Hodgkin lymphoma patients (n = 122) between 15 and 35 years old were prospectively recruited before commencing chemotherapy. Patients were treated either by a non-alkylating protocol (ABVD group; n = 67) or by an alkylating regimen (alkylating group; n = 55).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Serial AMH measurements were performed at baseline (AMH0), 15 days after the start of chemotherapy (AMH1), 15 days before the last chemotherapy cycle (AMH2), and at time 3, 6, 9, 12, 18 and 24 months from the end of chemotherapy. The whole study population was divided into two groups according to age: AYA (15–24; n = 65) and non-AYA (25–35; n = 57). All patients received a once monthly GnRH agonist injection during the whole treatment period. A linear mixed model was used to account for the repeated measures of single patients.

MAIN RESULTS AND THE ROLE OF CHANCE: At baseline, non-AYA patients had higher BMI and lower AMH levels than AYA patients. All AYA and non-AYA patients having received ABVD protocols had regular cycles at 12 months of follow-up. In case of alkylating regimens, amenorrhoea was more frequent in non-AYA patients than in AYA patients at 12 months (37% vs 4%, $P = 0.011$) and at

24 months (24% vs 4%, $P = 0.045$). We distinguished a similar depletion phase from AMH0 to AMH2 between ABVD and alkylating groups but significantly different recovery phases from AMH2 to AMH + 24 months. AMH recovery was fast and complete in case of ABVD protocols whatever the age: AMH reached pre-treatment values as soon as the 6th month of follow-up in the AYA group (mean (95% CI) in log AMH M0 vs M6: 3.07 (2.86 to 3.27) vs 3.05 (2.78 to 3.31), $P = 1.00$) and in the non-AYA group (mean (95% CI) in log AMH M0 vs M6: 2.73 (2.40 to 3.05) vs 2.47 (2.21 to 2.74), $P = 1.00$). In contrast, no patients from the alkylating group returned to pre-treatment AMH values whatever the age of patients (AYA or non-AYA). Moreover, none of the AMH values post-chemotherapy in the non-AYA group were significantly different from AMH2. Conversely in the AYA group, AMH levels from 6 months (mean (95% CI) in log AMH: 1.79 (1.47 to 2.11), $P < 0.001$) to 24 months (mean (95% CI) in log AMH: 2.16 (1.80 to 2.52), $P \leq 0.001$) were significantly higher than AMH2 (mean (95% CI) in log AMH: 1.13 (0.89 to 1.38)). Considering the whole study population (AYA and non-AYA), pre-treatment AMH levels influenced the pattern of the AMH variation both in alkylating and ABVD protocols (interaction *P*-value = 0.005 and 0.043, respectively). Likewise, age was significantly associated with the pattern of the recovery phase but only in the alkylating group (interaction *P*-value =0.001). BMI had no influence on the AMH recovery phase whatever the protocol (interaction *P*-value = 0.98 in alkylating group, 0.72 in ABVD group).

LIMITATIONS, REASONS FOR CAUTION: There was a large disparity in subtypes of protocols in the alkylating group. The average duration of chemotherapy for patients treated with alkylating protocols was longer than that for patients treated with ABVD.

WIDER IMPLICATIONS OF THE FINDINGS: These results make it possible to develop strategies for fertility preservation according to age and type of protocol in a large series of young lymphoma patients. In addition, it was confirmed that young age does not protect against ovarian damage caused by alkylating agents.

STUDY FUNDING/COMPETING INTEREST(S): This work was supported by Agence Régionale de Santé Hauts de France and Agence Onco Hauts-de-France who provided finances for AMH dosages (n° DOS/SDES/AR/FIR/2019/282). There are no competing interests.

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Key word: AMH / chemotherapy / lymphoma / AYA / fertility preservation

Introduction

Adolescents and young adults (AYAs) comprise the largest age group affected by Hodgkin (HL) or non-Hodgkin lymphomas (NHL). Despite excellent overall survival rates, due to advances in treatment regimens, therapy-associated side effects continue to be a major concern especially in young survivors. Many centres establish specific AYA cancer programmes to take into consideration the specificities of this subgroup in terms of information, education and global care. Fertility preservation options and future fertility capacity are obviously highly challenging for these young patients (Lewin *et al.*, 2017). Chemotherapy regimens, particularly those including alkylating agents, are notoriously ovariotoxic as they damage all kinds of ovarian follicles from primordial to antral stages (Morgan *et al.*, 2012). The acute mechanisms of this toxicity are mainly based on granulosa cell apoptotic processes, explaining the follicular depletion (Perez *et al.*, 1997; Oktem and Oktay, 2007; Morgan *et al.*, 2012). But ovarian stromal tissue can also be damaged by chemotherapy which induces cortical fibrosis and blood vessel injury (Meirow *et al.*, 2007). Whatever the mechanisms, the degree of the ovarian toxicity is highly dependent on age at treatment and type/dose of treatment. Ovarian damage can be permanent, especially in the case of alkylating protocols, leading to infertility or worse, to premature ovarian failure (POF).

Anti-Müllerian hormone (AMH) is secreted by granulosa cells of pre- and antral follicles. It is the most specific, accurate, reproducible and non-invasive marker of ovarian follicular content (Dewailly *et al.*, 2014). Its serum levels correlate strongly with the antral follicle count and also the primordial follicle pool (Hansen *et al.*, 2011; Kelsey *et al.*, 2012; Dewailly *et al.*, 2014). In addition, AMH has been shown to be a real-time indicator of follicular depletion and renewal in longitudinal studies performed in breast cancer and in lymphoma patients (Anderson *et al.*, 2006; Decanter *et al.*, 2010; Peigne and Decanter, 2014; Anderson *et al.*, 2018).

We previously investigated the dynamics of blood AMH variations prior to, during and after treatment in a short series of young lymphoma patients, with a mean age of 24 (Decanter *et al.*, 2010). We highlighted two different patterns of the AMH variations especially during the ovarian recovery after the end of treatment according to whether patients received alkylating agents or not (Decanter *et al.*, 2010). Likewise, Anderson *et al.* (2018) confirmed these results in a larger population treated both by ABVD chemotherapy protocols or BEACOPP. Recently, four similar longitudinal studies were conducted in childhood and AYA patients treated for various types of cancer including lymphoma (Brougham *et al.*, 2012; Dillon *et al.*, 2013; Morse *et al.*, 2013; Gupta *et al.*, 2016). All described a prompt drop in AMH levels after starting chemotherapy and lower AMH levels after treatment in comparison with pre-treatment values, especially in the case of alkylating protocols. These variations do not seem to be influenced by the pubertal status (Brougham *et al.*, 2012). Another long-term follow-up study showed that AMH levels are lower than in age-matched controls 10–17 years after Hodgkin disease treatment during childhood (Krawczuk-Rybak *et al.*, 2013). According to the ASCO and ESHRE recommendations, each patient undergoing gonadotoxic treatment should be referred to an oncofertility centre in order to benefit from information on their future fertility but also on fertility preservation techniques (Oktay *et al.*, 2018; ESHRE guideline group on Female Fertility Preservation *et al.*, 2020; Mulder *et al.*, 2021). Techniques such as ovarian tissue and oocyte cryopreservation are progressing

Table I Details of protocols with cumulative Cyclophosphamide equivalent dose (CED mg/m^2).

Protocols (n cycles)	Drugs and doses mg/m^2/cycle	Cumulative dose of cyclophosphamide or equivalent (mg/m^2)
ABVD (4)	Adriamycine 25, Blèomycine 10, Dacarbazine 375, vinblastine	NA
ACVBP+-R (4)	Adriamycine 75, **Cyclophosphamide 1200**, Bleomycine 10, Vindesine 2, Prednisone 40, +- Rituximab 375	4200
CHOP + R (6)	Adriamycine 50, **Cyclophosphamide 750**, Vincristine 1, Prednisone 40 +- Rituximab 375	4500
COPP (4)	**Cydophosphamide 500**, Vincristine 1.4, **Procarbazine** 100, Prednisone 40	2342.8
COPADEM (2)	Acide Folique, **Cyclophosphamide 500**, Doxorubincine 60, Methotrexate 3000, Vincristine 1.5, Prednisone	1000
COPDAC(4)	**Cyclophosphamide 500**, Vincrlstine1,5, Dacarbazine 250, Prednisone 40	2000
OEPA (2)	Adriamycine 40, Vincristine 1,5, Etoposide 100, Prednisone 60	NA
BEACOPP (6)	Adriamycine 25, **Cyclophosphamide 650**, Etoposide, **Procarbazine 100**, Vlncrlstine 1.4	4414
BEACOPP escalated (6)	Adriamycine 25, **Cyclophosphamide 1300**, Doxorubincine 60, methotrexate 3000, Vincristlne 1.4, Prednisone	7800
DHAOX + R	Cytarabine 200, Dexamethasone 40, Oxallplatlne 100, Lenograstim 0.05 + Rituximab 375	NA
DHAP	Cisplatine 100, Cytarabine 4000, Dexamethasone 40	NA
BEAM	**BCNU 300**, Etoposide 100, Cytarabine 200, **Melphalan 140**, **Procarbazine 100**, Prednisone 40	10 100
MINE (3)	Etoposide 150, **Ifosfamide 1500**, Mitoguazone 500, Vinorelbine 15	3294

Alkylating agents are indicated in bold letters. NA, not applicable.

rapidly with an increasing number of healthy babies born afterwards but it remains sometimes difficult to tailor fertility preservation strategies for each patient. Indeed, the respective influence of age at diagnosis and pre-treatment ovarian follicular content remain unclear in young lymphoma patients as most studies have been carried-out in breast cancer patients who are much older.

The purpose of the current longitudinal study was firstly to evaluate the influence of age on the ovarian susceptibility to chemotherapy in young lymphoma patients and secondly to determine the ovarian toxicity of each protocol in order to tailor fertility preservation counselling.

Materials and methods

Subjects

A total of 122 young women (mean age 24 ± 4.7 years; 15–35 years) with a diagnosis of Hodgkin's (n = 93) or non-Hodgkin's lymphoma (n = 29) according to WHO classification were recruited from 2007 to 2016. All were chemotherapy naïve at the inclusion time. All had their two ovaries throughout the follow-up. The study population was divided into two groups according to age: 65 adolescents and young adults (AYA group) aged from 15 to 24 years old and 57 women aged from 25 to 35 (non-AYA group). The upper limit of age 24 for AYA was determined according to the French definition. Of the 122 women, 66 were assigned to a non-alkylating ABVD protocol which combines Adriamycin, Bleomycin, Vinblastine and Dacarbazine (ABVD group) and 56 were assigned to an alkylating protocol that usually includes more ovariotoxic drugs such as cyclophosphamide, ifosfamide or melphalan (CHOP/R-CHOP, ACVBP/R-ACVBP, BEACOPP, COP/COPADEM, DHAP, BEAM and MINE) (alkylating group). Chemotherapy regimens were determined according to the severity and extent of the haematologic disease. Details of the protocols, Cyclophosphamide or Cyclophosphamide equivalent dose (CED)/m^2 and per cycle and cumulative dose per protocol are reported in Table I (Green, 2014). Despite the lack of evidence regarding efficacy for ovarian function preservation (Demeestere *et al.*, 2016; Dolmans *et al.*, 2020), all patients benefitted from once monthly GnRH agonist injection during the whole treatment period to prevent menorrhagia (Meirow *et al.*, 2006). The alkylating-treated patients' group was further divided into three sub-groups called Autograft (n = 13; MINE, BEAM: CED: 7800 to 13 000 mg/m^2), BEACOPP (n = 20; CED: 4400 to 7800 mg/m^2) and others (n = 23; CED: 3300 to 4500 mg/m^2), with the latter including CHOP, ACVBP, COP/COPADEM, OEPA/COPDAC, to evaluate their respective ovarian toxicity potential. Patients were referred to the Fertility Observatory of the Lille University Hospital before the initiation of chemotherapy to be followed-up regarding their menstrual activity and ovarian follicular content through serial blood AMH measurements. This prospective cohort study has been approved by the institutional review board of the Lille University Hospital and all patients gave their informed consent (DC-2008-642; CNIL DEC2015-112). This study is part of the project 'elle va guérir puis voudra un enfant' which is funded by 'Hauts de France' Cancer and Health agencies, respectively.

Study design

Serum samples for AMH measurements were obtained before initiation of chemotherapy (AMH0), 15 days after the first cycle of chemotherapy (AMH1), 15 days before the last cycle of chemotherapy (AMH2) and every 3 months after the end of chemotherapy during the first year and every 6 months during the second year (AMH +3, +6, +9, +12, +18, +24 months) for each patient. At each visit for

Table II Clinical characteristics of the study population with comparison between AYA (adolescent and young adult) and non-AYA groups.

	AYA (n = 65)	Non-AYA (n = 57)	*P*-value
Age (years), mean ± SD	20.7 ± 2.0	29.1 ± 3.2	
Body mass index (kg/m^2), mean ± SD	21.4 ± 2.9	24.1 ± 5.6	0.011
Hodgkin's disease	54 (83%)	39 (68%)	0.058
Taking OCP before chemotherapy	47 (72%)	29 (51%)	0.015
Pre-treatment AMH levels (pmol/L), median (interquartile range)	24 (15–39)	14 (11–25)	0.006
Alkylating protocol	26 (40%)	30 (57%)	0.072
Stem cell transplantation	6 (9%)	6 (11%)	–
Supra-diaphragmatic radiotherapy	35 (53%)	23 (40%)	–
Duration of chemotherapy (months), mean ± SD	5.0 ± 1.6	5.5 ± 2.0	–

AMH, anti-Müllerian hormone; AYA, adolescents and young adults; OCP, oral contraceptive pill.

sample collection, women were asked about their last menstrual bleeding. Amenorrhoea and POF were defined respectively by the absence of menstrual bleeding for at least 3 consecutive months or 12 consecutive months.

AMH assay

Serum AMH levels were measured by ELISA using the second-generation enzyme immunoassay EIA AMH/MIS from Beckman-Coulter-Immunotech (Villepinte, France). This assay has a functional sensitivity of 2.5 pmol/l. The intra- and inter-assay coefficients of variation were <9.5% and <15.0%, respectively (Decanter *et al.*, 2014).

Statistical analysis

Continuous variables are expressed as mean ± SD or median and interquartile range in cases of non-normal distribution. Normality of distribution was checked graphically and according to the Shapiro–Wilk test. Categorical variables are expressed as frequencies and percentages.

Comparison in baseline characteristics between AYA and non-AYA groups was performed using the Student's *t*-test for continuous characteristics (after applying a log-transformation for pre-treatment AMH level) using Chi-square test (or Fisher's exact test when expected cell frequencies < 5) for categorical variables. In AYA and non-AYA groups separately, we compared the evolution of AMH levels (after applying a log-transformation) between the alkylating and non-alkylating protocols by using linear mixed models (an unstructured covariance pattern model) to account for the correlation between repeated measures within the same patients, and by including the time (as a categorical variable), alkylating protocol and an interaction term (alkylating protocol*time) as fixed effects. In case of a significant interaction term, *post hoc* comparisons within (between the baseline and each follow-up measure, and between the end of chemotherapy (AMH2) and each post-chemotherapy measure) and between alkylating and non-alkylating protocols (changes from baseline at each follow-up time points) were performed using linear contrasts corrected for multiple comparisons with the Holm Bonferroni method. The same methodology was used to compare the evolution of AMH between the three subgroups of alkylating regimens, i.e. Autograft, BEACOPP and Others.

Finally, we assessed the factors associated with the evolution of AMH from the end of chemotherapy until the 24th month of follow-up (AMH2 to AMH24) in the whole population in separate linear mixed models (an unstructured covariance pattern model) by including the factor, the time and the interaction term between time and factor as fixed effects.

All statistical tests were performed with a two-tailed alpha risk of 0.05. Statistical analyses were performed using the software SAS (SAS Institute, version 9.4).

Results

Characteristics of the population

Patients' baseline characteristics, type and duration of chemotherapy are summarised according to AYA and non-AYA groups in Table II. The mean serum AMH concentrations before chemotherapy (AMH0) were significantly different between the two groups with a higher level in the AYA group (median (IQR), 24 (15–39)) than in the non-AYA group (14 (11–25)) ($P = 0.006$). AYA and non-AYA patients also significantly differed regarding the body mass index and the use of oral contraceptive pill before chemotherapy. AYA patients more often had Hodgkin's disease (83% vs 68%), but less often had alkylating protocols (40% vs 57%) compared with the non-AYA patients, but the results did not reach the significance level (respectively, $P = 0.058$ and $P = 0.072$). Chemotherapy durations and others baseline characteristics did not differ between the two populations.

Clinical follow-up

All patients were amenorrhoeic during the whole treatment period as they were under GnRH agonist co-treatment.

In the ABVD protocol (n = 66), most patients recovered spontaneous cycles as soon as the third month of follow-up in both groups (79% in AYA group vs 83% in non-AYA; $P = 1.0$). At time + 12 months and + 24 months, 100% of AYA and non-AYA patients

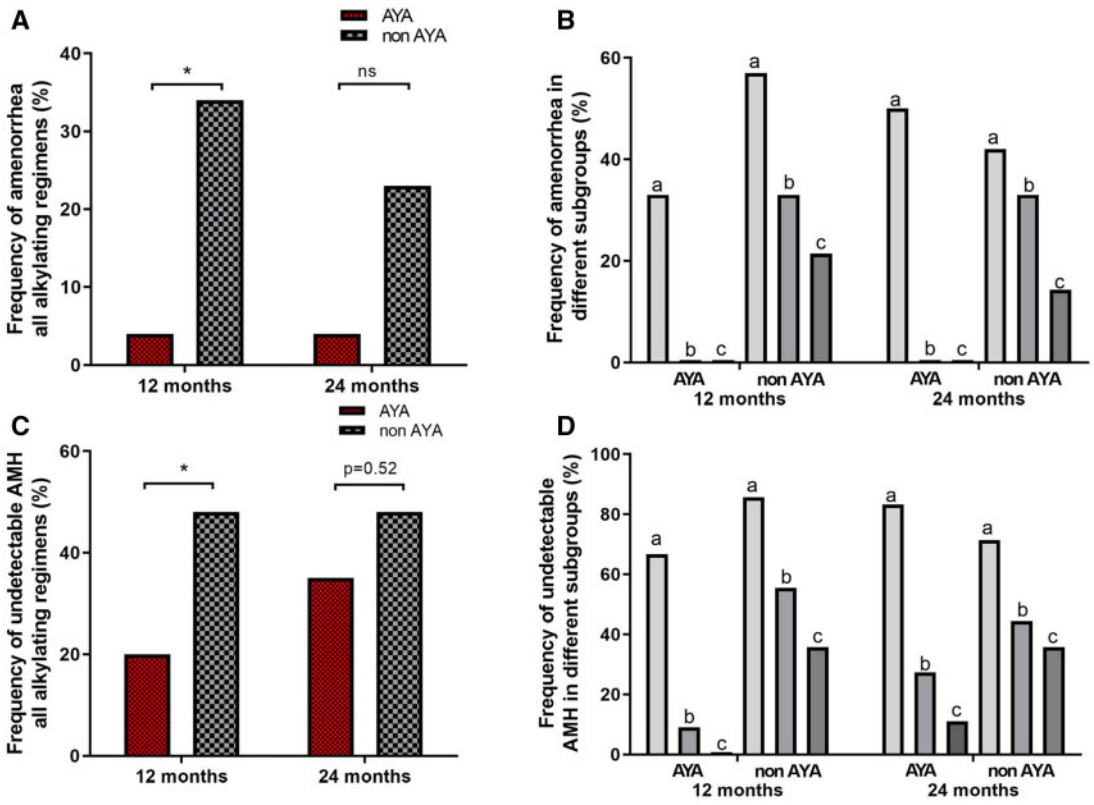

Figure 1. Frequency of amenorrhoea and undetectable AMH levels in AYA vs non-AYA patients treated by alkylating protocols. Panels **A** and **C** show the whole group of patients treated with any alkylating regimen; *$P < 0.05$; ns, non-significant. Panels **B** and **D** illustrate the three different subgroups of alkylating regimen: a = autograft, b = BEACOPP, c = others regimen. Due to the small sample sizes of each subgroup, no statistical tests were performed. AMH, anti-Müllerian hormone; AYA, adolescents and young adults.

from the non-alkylating group returned to spontaneous cycles (data not shown).

In the alkylating protocol (n = 56), the menstrual recovery significantly differed between the two groups. Amenorrhoea was more frequent in non-AYA patients at 12 months (37% vs 4%; $P = 0.011$) and at 24 months (24% vs 4%; $P = 0.045$) (Fig. 1A). Accordingly, the incidence of undetectable AMH value was higher in non-AYA patients at time + 12 months but was not significantly different to that of the AYA group at time + 24 months (Fig. 1C).

AMH follow-up in AYA and non-AYA patients according to protocols

The serum AMH variations before, during and after treatment in AYA and non-AYA patients according to the type of protocol are shown in Fig. 2A and B. One can distinguish a depletion phase from the beginning to the end of chemotherapy (AMH0–AMH2) and a recovery phase from the end of chemotherapy (AMH2) to the time + 24 months. This variation between AMH0 and AMH 24 is influenced by the chemotherapy regimen (protocol*time interaction, P-value < 0.0001) in AYA and non-AYA patients. Regarding the depletion phase, whatever the protocol and the patient group, a similar pattern of variation was observed: mean AMH concentrations fell significantly as soon as 15 days after initiation of chemotherapy (AMH1) and were close to the detection limit of the assay at the end of the chemotherapy (AMH2). However, the depletion phase slope (variation between AMH1 and AMH0) was higher in alkylating regimens than in ABVD group in the AYA group (mean (95% CI) change in log AMH: −1.53 (−1.76 to −1.30) vs −0.70 (−0.89 to −0.52); $P < 0.001$) but not in the non-AYA group (mean (95% CI) change in log AMH: −1.11 (−1.39 to −0.84) vs −0.57 (−0.88 to −0.26); $P = 0.13$).

The influence of the chemotherapy regimen was more obvious during the recovery phase. In the ABVD groups, after the end of treatment, the rise of AMH concentrations occurred earlier and was significantly more pronounced than in the alkylating groups. AMH reached pre-treatment values as soon as the 6th month of follow-up in AYA (mean (95% CI) in log AMH M0 vs M6: 3.07 (2.86 to 3.27) vs 3.05 (2.78 to 3.31), $P = 1.00$) and non-AYA groups (mean (95% CI) in log AMH M0 vs M6: 2.73 (2.40 to 3.05) vs 2.47 (2.21 to 2.74), $P = 1.00$) (Fig. 2A and B).

In contrast, no patients from the alkylating group returned to pre-treatment AMH values regardless of patient's age (AYA or non-AYA) ($P < 0.001$ for all comparisons between baseline and each post-chemotherapy measures). Moreover, none of the AMH values post-

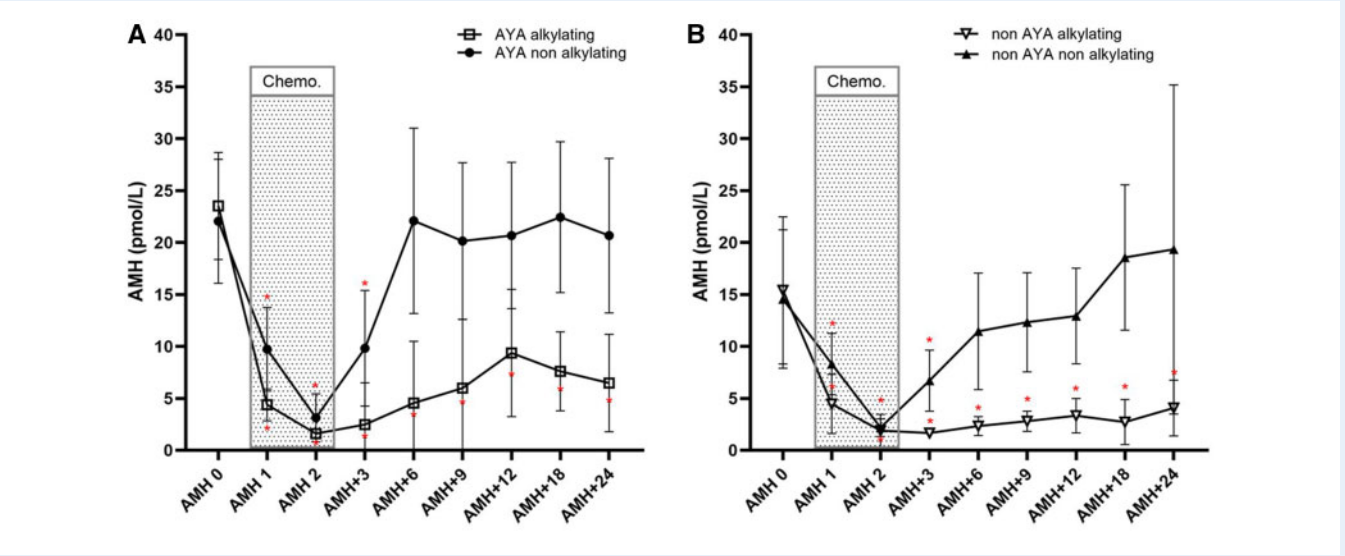

Figure 2. Dynamics of AMH serum levels across time in AYA (A, n = 65) and non-AYA (B, n = 57) patients according to chemotherapy protocols (ABVD vs alkylating). Data are expressed as the geometric mean (±95% CI). *$P < 0.05$ by comparison with the corresponding AMH0. AMH, anti-Müllerian hormone; AYA, adolescents and young adults.

chemotherapy in non-AYA group were significantly different from AMH2. Conversely in the AYA group, AMH levels from 6 months (mean (95% CI) in log AMH: 1.79 (1.47 to 2.11), $P < 0.001$)% CI to 24 months (mean (95% CI) in log AMH: 2.16 (1.80 to 2.52), $P \leq 0.001$) were significantly higher than AMH2 (mean (95% CI) in log AMH: 1.13 (0.89 to 1.38)).

AMH follow-up in the whole population treated by alkylating protocols

Having shown a stronger gonadotoxicity of alkylating regimens both in AYA and in non-AYA patients, we decided to pool these two groups to compare three subtypes of alkylating regimens: Autograft (n = 13; MINE, BEAM), BEACOPP (n = 20) and Others (n = 23; CHOP, ACVBP, COP/COPADEM, OEPA/COPDAC). The corresponding AMH variations are illustrated in Fig. 3. After adjustment for age, the evolution of AMH over time was not significantly influenced by the subtype of alkylating regimens (interaction *P*-value = 0.15). However, when we proceeded to the comparison between Autograft and Others protocols + BEACOPP, after adjustment for age, the profile of AMH variations was significantly different exclusively during the recovery phase, from AMH2 to AMH 12 ($P = 0.028$) and from AMH2 to AMH 18 ($P = 0.017$) (Supplementary Fig. S1).

Factors influencing the pattern of AMH variations during the recovery phase in the whole population

Finally, we investigated the respective influence of age, BMI and AMH0 at treatment initiation on the AMH recovery (from AMH 2 to AMH 24) in the whole population. It appears that AMH0 influenced the pattern of the AMH variation both in alkylating and ABVD groups (interaction *P*-value = 0.005 and 0.043, respectively). The higher the AMH 0 levels, the greater the AMH recovery between AMH 2 to AMH 24, with a mean increase (95% CI) in log-AMH values of 0.22 (−0.38 to 0.82) in lowest AMH0 tertile, 0.84 (0.40 to 1.25) in middle AMH0 tertile and 1.78 (1.43 to 2.13) in the upper AMH0 tertile for the alkylating group, and 1.16 (0.84 to 1.48) in lowest AMH0 tertile, 1.82 (1.31 to2.33) in middle AMH0 tertile and 1.78 (1.43 to 2.13) in the upper AMH0 tertile for the ABVD group. Likewise, age was significantly associated with the pattern of the recovery but only in the alkylating group (interaction *P*-value in alkylating group = 0.001; in ABVD *P*-value = 0.10). BMI had no influence on the AMH recovery curve whatever the protocol (interaction *P*-value = 0.98 in alkylating group, 0.72 in ABVD group).

Discussion

The results of the current study highlight a similar pattern of follicular depletion at the end of chemotherapy both in AYA and non-AYA patients whatever the protocol, but a significantly different recovery phase according to the presence of alkylating agents or not. These results confirm in a much larger population those of our previous short series in lymphoma patients (Decanter *et al.*, 2010). They are also in accordance with studies performed in breast cancer patients (Anderson *et al.*, 2006; Dezellus *et al.*, 2017), recent publications in large populations of AYA cancer survivors (Cameron *et al.*, 2019; Su *et al.*, 2020) and lymphoma patients (Anderson *et al.*, 2018).

We showed that in the ABVD regimen that AMH levels returned very quickly to pre-treatment values in all patients. In AYA patients, AMH levels plateaued at pre-treatment values as soon as 6 months

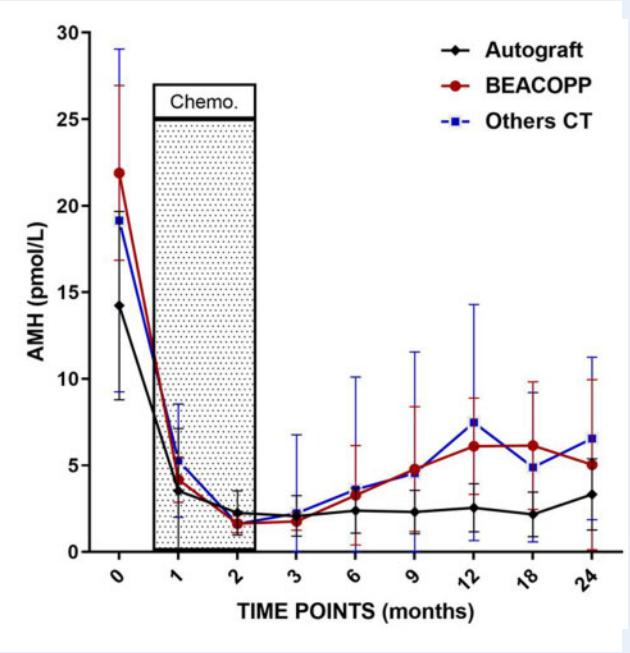

Figure 3. Dynamics of AMH serum levels across time in patients treated with alkylating protocols, either BEACOPP (n = 20), autograft (n = 13) or others (n = 23). Data are expressed as the geometric mean (±95% CI). AMH, anti-Müllerian hormone.

after the end of chemotherapy until 24 months of follow-up, suggesting a complete but also sustained recovery. The AMH recovery was more progressive in non-AYA patients, reaching close to pre-treatment values at time +6 months and continuing to progress until +24 months. The ABVD protocol is considered as a soft chemotherapy protocol regarding the ovarian toxicity despite the presence of Dacarbazine which is a molecule with monofunctional alkylating effect (Decanter *et al.*, 2010; Behringer *et al.*, 2013). Anderson *et al.* recently studied, in an equally sized population to ours, the long-term effect of ABVD on the ovarian reserve indicators (Anderson *et al.*, 2018). The results highlighted a full and extended recovery of AMH levels as high as 125%, in patients younger than 35 years old but not in women aged 35 or older (Anderson *et al.*, 2018). Hence, fertility preservation in ABVD patients should therefore be discussed depending on age but also in the cases of response-adapted therapy with high probability of switching to a more toxic protocol.

In contrast, none of the patients having undergone an alkylating regimen returned to pre-treatment values. In addition, the AMH values plateaued at very low levels suggesting a deep and extended ovarian follicular injury whatever the age, despite a higher degree of AMH re-increase in the AYA group. Likewise, Su *et al.* (2020) in a prospective study enrolling 763 slightly older cancer survivors, including 24 cases of lymphoma, by studying trajectories of AMH variations over two decades following cancer treatment, showed the absence of any protective effect of younger age in case of alkylating regimen. Autologous stem cell transplantation alkylating protocols are well-known to be highly ovariotoxic with a high incidence of POF and infertility (van Dorp *et al.*, 2018). On the other hand, the ovarian toxicity and further fertility in case of others alkylating protocols remains poorly documented making fertility preservation strategies difficult to elaborate. Here we confirmed that aoutologous stem cell transplantation regimens (the Autograft group) are characterised by a strong ovariotoxicity as highlighted by a flat curve of AMH after the end of treatment. Despite slight recovery of AMH levels in the BEACOPP and Others groups, there was no statistical difference between each of these two protocols and the autograft, meaning that they all have strong ovariotoxicity.

These results stress the importance of systematically offering fertility preservation to lymphoma patients who start chemotherapy with alkylating agents, regardless of age. Furthermore, at least in Europe, treatment for Hodgkin disease in adolescent and young adult (before 25 years old) has been switched to the Euro-Net protocol that contains significant doses of Cyclophosphamide. As the autologous stem cell transplantation protocols are usually known to be highly ovariotoxic and associated with the highest rate of POF, ovarian tissue cryopreservation is the first option to discuss as it has demonstrated its efficacy on both endocrine function recovery and fertility (Shapira *et al.*, 2020; Dolmans *et al.*, 2021). It can be combined with oocyte cryopreservation if the time-interval between diagnosis and start of chemotherapy allows it (Delattre *et al.*, 2020). Concerning other alkylating protocols, choosing between oocyte and/or ovarian tissue cryopreservation would depend on the patient condition, the emergency of treatment initiation and the existence or not of a recent exposure to chemotherapy.

During the recovery phase, we showed a high incidence of extremely low or undetectable AMH values in the alkylating group both in non-AYA and AYA patients, including in patients with normal menstrual function. It is now well-recognised that low AMH levels are not predictive of time to pregnancy in the general population (Hagen *et al.*, 2012; Steiner *et al.*, 2017) whereas it is associated with reduced time to menopause (Nair *et al.*, 2015; Depmann *et al.*, 2018; Finkelstein *et al.*, 2020). It remains of course difficult to extrapolate these results from normal populations to women previously exposed to chemotherapy. It has been suggested that very low concentrations of AMH levels may reduce the conception window, especially since mechanisms other than acute follicle apoptosis during chemotherapy may occur, such as accelerated activation of primordial follicles (Kalich-Philosoph *et al.*, 2013) and ovarian stroma and vasculature injury causing fibrosis (Meirow *et al.*, 2007), both inducing a second phase of follicle loss (Spears *et al.*, 2019). Interestingly, Cameron *et al.* (2019) did not show a more rapid AMH decline in cancer survivors versus age-matched controls but highlighted a prolonged plateau of low levels. On the other hand, Swerdlow *et al.* (2014) in a large population of women treated for Hodgkin lymphoma, stress the need to plan intended pregnancies by using individualised risk of early menopause tables including age, treatment type and dose, and pelvic radiotherapy. In addition, large cohort studies performed in lymphoma survivors have highlighted a linear dose relationship between alkylating chemotherapy and POF occurrence (Harel *et al.*, 2011; van der Kaaij *et al.*, 2012). Low birth rates were observed after advanced-stage treatment (Behringer *et al.*, 2013) and the risk of POF occurrence was noted to be increased by 23% per year of age at treatment (van der Kaaij *et al.*, 2012). Hence, in those young lymphoma patients from our current study, mostly treated in their twenties, for whom fertility preservation was not possible or not optimal, a second chance of fertility preservation, 1 or 2 years after completion of treatment, should be offered to increase

the number of cryopreserved oocytes and future pregnancy chances if reutilisation is needed. As post-alkylating regimen AMH levels remain low or extremely low, few oocytes would be collected after ovarian stimulation. Hence, a programme of oocyte accumulation, with the permission of the haematologist, may be proposed.

Whether age at initiation of chemotherapy and pre-treatment AMH levels may influence the AMH recovery phase remains debatable in the literature. We showed in our series that age influenced the AMH recovery phase but only in alkylating protocols whereas pre-treatment AMH had a significant influence in both the alkylating and ABVD protocols, independent of age. These results are in accordance with previous studies in lymphoma (Behringer *et al.*, 2005; Anderson *et al.*, 2018) and in breast cancer patients (Anderson and Cameron, 2011). All patients received a GnRH agonist co-treatment during chemotherapy, and it may have influenced the pattern of depletion phase, especially regarding the value of AMH at the very end of treatment. Whether it may have influenced the recovery phase is more unlikely as the last injection took place 4 weeks before the last chemotherapy cycle.

To the best of our knowledge, this is the largest series of lymphoma patients for whom serial AMH measurements and menstrual function were followed-up until 24 months after the end of chemotherapy. The noticeably young age of patients avoided the age-related bias on AMH variations and allowed us to properly study the respective toxicity of each chemotherapy regimen that is currently used. Previous published longitudinal studies were performed in population with various cancer types at various ages and included small subgroups of lymphoma patients without any details on protocols.

Several limitations of this study warrant discussion. The alkylating group is heterogeneous with various protocol and drug associations that may influence both the speed and the extent of the ovarian recovery. The ELISA assay used to measure AMH levels in our study is not the most sensitive technique. But the key point of this analysis was more to focus on the dynamics of AMH variations reflecting follicular depletion and renewal rather than absolute values of AMH.

To summarise, our study confirms that alkylating protocols used for lymphoma treatment have a strong ovariotoxicity, even in AYA patients who only showed a limited recovery of AMH after the end of treatment. The fact that none of patients treated by alkylating protocols recovered their initial ovarian follicular content advocates for a narrowed conception window and the necessity of fertility preservation counselling before but also post-cancer treatment. Long-term follow-up is clearly needed to confirm that ABVD protocols have no effect on fertility. Likewise, it will enable the evaluation of the clinical relevance of low to extremely low AMH levels on further fertility capacity in the case of alkylating regimens.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article cannot be shared publicly due to the privacy of individuals who participated in the study. Anonymised data will be shared on reasonable request to the corresponding author. ref CNIL.DEC2015-112.

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Authors' roles

Decanter C, Pigny P and Morschhauser F designed the study and recruited patients in the Fertility Observatory. Pigny P performed all of the AMH measurements. Decanter C, Delepine J and Robin C followed-up all patients and participated in data collection in the database. Decanter C wrote the manuscript. Manier S, Bruno B, Barbatti M and Morschhauser F addressed all lymphoma patients to the Fertility Observatory and reviewed and discussed the manuscript. Decanter C, Pigny P, Behal H and Labreuche J participated in the methodology, performed statistical analysis and reviewed or discussed the manuscript.

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Conflict of interest

None declared.

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