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Fertility preservation in patients with haematological malignancies

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Fertility preservation in patients with haematological malignancies

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Abbreviations

ABVD - Doxorubicin, bleomycin, vinblastine, dacarbazine

AMH - Anti-Müllerian hormone

BEACOPP - Bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone

BMI - Body mass index

COPADM - Cyclophosphamide, vincristine, prednisone, doxorubicin, methotrexate

DA-EPOCH-R - Dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, rituximab

DGGG - German Society of Gynaecology and Obstetrics

DGRM - German Society of Reproductive Medicine

DGU - German Society of Urology

ESHAP - Etoposide, methylprednisolone, cisplatin, cytarabine

Linker - Daunorubicin, vincristine, prednisone, L-asparaginase

FSH - Follicle-stimulating hormone

HSCT - Haematopoietic stem cell transplantation

Hyper-CVAD - Cyclophosphamide, vincristine, doxorubicin, dexamethasone

m-BACOD - Methrotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone

OCs - Oral contraceptives

R-CHOP - Cyclophosphamide, doxorubicin, vincristine, prednisone, rituximab

SD - Standard deviation

Resumo

Introdução: A incidência de neoplasias hematológicas tem vindo a aumentar em mulheres de idade fértil. As taxas de sobrevivência acompanham este aumento, sendo fundamental avaliar o impacto dos tratamentos na futura qualidade de vida das doentes. A possível perda de fertilidade causada pelos tratamentos oncológicos é uma das principais preocupações para estas mulheres.

Objetivo: Avaliar o impacto de cada tratamento na reserva ovárica e quais foram as técnicas de preservação da fertilidade mais utilizadas pelas pacientes com neoplasias hematológicas.

Métodos: Foi realizado um estudo retrospetivo com 61 doentes diagnosticadas com neoplasia hematológica acompanhadas num centro de preservação da fertilidade de janeiro de 2008 até junho de 2019.

Resultados: As mulheres mais jovens, nulíparas e solteiras foram as que mais recorreram às técnicas de preservação da fertilidade. Os tratamentos oncológicos provocaram uma diminuição da reserva ovárica, demonstrada por um aumento dos níveis de FSH e pela diminuição dos níveis de AMH. Ao avaliar que tratamentos tiveram maior impacto nos níveis de AMH, constatamos que o regime BEACOOP, os agentes, vincristina, etoposido, procarbazina, prednisona e o transplante de medula óssea foram os principais responsáveis pela sua diminuição. Relativamente à gravidez pós tratamento oncológico, das onze mulheres que engravidaram, dez fizeram-no de forma espontânea, com a grande maioria a ocorrer pelo menos dois anos após o diagnóstico.

Conclusão: Mais estudos são necessários para avaliar o impacto de cada agente quimioterápico na reserva ovárica, assim como a relação entre os níveis de AMH e a ocorrência de gravidez a curto, médio e longo prazo.

Palavras-chave: Preservação da fertilidade, neoplasias hematológicas, reserva ovárica, mulheres em idade reprodutiva, estudo retrospetivo.

Abstract

Introduction: The incidence of haematological malignancies is increasing in women of childbearing age. Survival rates accompany this increase, making it essential to assess the impact of treatments on patients' future quality of life. The potential loss of fertility is a key concern of young women treated for cancer.

Aim: Evaluate the impact of each treatment on ovarian reserve and what were the fertility preservation techniques used by patients with hematologic malignancies.

Methods: A retrospective study was made after data collection of 61 patients diagnosed with haematological malignancies followed in a fertility preservation centre from January 2008 to June 2019.

Results: The youngest, nulliparous and single women were those who most resorted to fertility preservation techniques. Cancer treatments caused a decrease in ovarian reserve, demonstrated by an increase in FSH levels and a decrease in AMH levels. When assessing which treatments have the greatest impact on AMH levels, we found that BEACOPP regimen, the agents vincristine, etoposide, procarbazine, prednisone and the haematopoietic stem cell transplantation were the main responsible for its decrease. Regarding pregnancy after oncological treatments, of the eleven women who became pregnant, ten did so spontaneously, with the vast majority occurring at least two years after diagnosis.

Conclusion: Further studies are needed to assess the impact of each chemotherapeutic agent on the ovarian reserve, as well as the relationship between AMH levels and the occurrence of short, medium and long term pregnancies.

Keywords: Fertility preservation, haematological malignancies, ovarian reserve, women of reproductive age, retrospective study.

Introduction

Hematologic malignancies represent about 17% of cancers in women of childbearing age (15-44 years old) [1]. The incidence has increased in recent decades. Five-year survival rates follow the same rising trajectory, being above 80% for lymphomas and 60% for leukaemias [2]. This increase is due not only to earlier diagnosis but also to improved treatments, such as radiotherapy, chemotherapy and bone marrow transplantation. Despite their development in recent years, these treatments may have a negative impact on ovarian reserve, increasing the risk of infertility and premature ovarian insufficiency. According to the literature, the main gonadotoxic treatments are alkylating agents in chemotherapy, radiotherapy with abdominal or pelvic irradiation and bone marrow transplantation. The gonadotoxic effect of these treatments depends on the patient's age, basal ovarian reserve and the administered dose. [3] Therefore, fertility preservation becomes a key point to ensure the future quality of life of these women.

Fertility preservation can be accomplished through various techniques. These include oocytes, embryos and ovarian tissue cryopreservation. Oocytes cryopreservation requires ovulatory induction that takes 2 weeks. This is done using clomiphene citrate, gonadotropins or aromatase inhibitors [4]. Ovarian tissue cryopreservation is a surgical technique, no longer considered experimental, that allows preserving fertility and restoring endocrine function after ovarian tissue transplantation [5,6,7]. Embryos cryopreservation has been decreasing in its use because it raises ethical issues and does not ensure reproductive autonomy.

The influence of pre-treatment ovarian reserve and patient age on post-treatment ovarian reserve recovery is unclear [8]. The aim of our study was to evaluate the impact of each treatment on ovarian reserve and the fertility preservation techniques used by patients with hematologic malignancies.

Methods

Study design

A retrospective study was made after data collection of 61 patients diagnosed with haematological malignancies referred to a fertility preservation centre from January 2008 to June 2019. Exclusion criteria were age over 40 years old, surgical history of bilateral oophorectomy and premature ovarian insufficiency. Electronic clinical files have been reviewed for personal and family history of gynaecological and oncological diseases. The type of treatment and doses administered, as well as staging at initial diagnosis were also analysed. Ovarian reserve was retrospectively assessed through plasma levels of follicle-stimulating hormone (FSH) and anti-Müllerian hormone (AMH) measured at the first appointment and in the follow-up visit, after oncological treatments.

Since the study involved anonymous data extraction from electronic medical records, patients' consent was not required.

Statistical analysis

Statistical analysis was performed using SPSS Statistics, Version 25.0 (IBM Corp., Armonk NY USA). For quantitative variables, the mean, standard deviation, median, maximum and minimum were calculated. Qualitative variables were expressed as numbers and percentage. For these variables, the statistical test used was Fisher's exact test. For continuous variables, the Kolmogorov-Smirnov test was used if $N > 10$ and the Shapiro-Wilk test if $N < 10$. In these tests, if $p \leq 0.05$ the nonparametric test was performed and if $p > 0.05$ the t-test for paired samples (when comparing FSH and AMH values from the same individuals before and after treatment) or the t-test for independent samples (assessing the impact of treatment on FSH and AMH levels) were used. All tests were 2 tailed and the level of significance was set at $p \leq 0.05$.

Results

Characteristics of the participants

In our study, 61 patients with hematologic malignancies who attended fertility preservation consultation were included. The average age in our sample was 25.97 ± 6.03 [15-36] years old. Of the studied patients, the mean age at menarche was 12.41 ± 1.74 [6-18] years old, 80.3% (n=49) were nulliparous, 52.5% used oral contraception before starting treatment and the mean body mass index (BMI) was 22.54 ± 3.38 [17.80-33.43] kg/m^2.

Most patients were diagnosed with Hodgkin's lymphoma (n=35, 57.4%) or non-Hodgkin's lymphoma (n=20, 32.9%) followed by acute lymphoblastic leukaemia (n=3, 4.9%) and others (n=3, 4.9%), which includes promyelocytic leukaemia and myelodysplastic syndrome.

The Ann Arbor stage at initial diagnosis was mainly stage II in 21 patients (34.4%), followed by stage IV in 9 patients (14.7%) and stage I and III in 2 patients each (3.3%). In 44.3% (n=27) of the patients, it was not possible to determine the patient's stage due to a lack of information.

In our population, the most used chemotherapy regimen was the ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) (n=19, 31.1%), followed by R-CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone, rituximab) (n=10, 16.4%) and BEACOPP regimen (n=8, 13.1%) (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone). The remaining patients (n=21, 34.4%) used other regimens such as m-BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone), Hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, dexamethasone), ESHAP (etoposide, methylprednisolone, cytarabine, cisplatin), Linker (daunorubicin, vincristine, prednisone, L-asparaginase), DA-EPOCH-R (dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, rituximab) and COPADM (cyclophosphamide, vincristine, prednisone, doxorubicin, methotrexate). In addition, among the patients who underwent chemotherapy, 19 (31.1%) also had radiotherapy and 10 (16.4%) bone marrow transplantation.

Most patients chose to preserve fertility (n=35, 57.4%), with oocyte cryopreservation being the most used method (n=18, 29.5%), followed by ovarian tissue cryopreservation (n=14, 23%), embryo and oocyte simultaneously (n=2, 3.3%) and embryo cryopreservation (n=1, 1.6%). For those who did not preserve fertility, the main reason for not doing so was personal choice (n=9, 13.1%) followed by absence of ovarian response (n=3, 4.9%), oncological treatment already started (n=3, 4.9%) and diagnosis of other pathologies (Wilson's disease and ovarian injury) in 2 cases (3.3%).

Moreover, pregnancy after treatment occurred in 11 cases and 10 of them were spontaneous. Among the 4 women who resorted to cryopreserved material, only 1 did become pregnant.

Patients' characteristics are summarized in Table I.

Table I. Patients Characteristics

Variable	*N* = 61	Percentage (%)
Age at initial diagnostic, mean ± standard deviation (SD) [range] (years old)	25.97 ± 6.03 [15-36]	
15-19	12	19.7
20-24	10	16.4
25-30	23	37.7
31-36	16	26.2
Nulliparous	49	80.3
Age of menarche, mean ± SD [range] (years old)	12.41 ± 1.74 [6-18]	
Oral contraception	32	52.5
BMI, mean ± SD [range] (kg/m2)	22.54 ± 3.38 [17.80-33.43]	
14.5-19.9	13	21.3
20-24.9	32	52.4
25-29.9	12	19.7
≥ 30	2	3.3
Missing data	2	3.3
Hematologic malignancies		
Hodgkin lymphoma	35	57.4
Non-Hodgkin lymphoma	20	32.9
Acute lymphoblastic leukaemia	3	4.9
Others*[1]	3	4.9
Ann Arbor stage at initial diagnosis		
I	2	3.3
II	21	34.4
III	2	3.3
IV	9	14.7
Missing data	27	44.3
Chemotherapy regimens		
ABVD	19	31.1
R-CHOP	10	16.4
BEACOPP	8	13.1
Others *[2]	21	34.4
Radiotherapy	19	31.1
Hematopoietic stem cell transplantation	10	16.4
Fertility preservation		
Did not	26	42.6
Personal choice	8	13.1
For having already started treatments	3	4.9
Absence of ovarian response	3	4.9
Other pathology	2	3.3
Missing data	10	16.4
Oocyte cryopreservation	18	29.5
Ovarian tissue cryopreservation	14	23
Embryo and oocyte simultaneously	2	3.3
Embryo cryopreservation	1	1.6
Pregnancy after treatment	11	18.0
Spontaneous pregnancy	10	16.4
Used cryopreserved material	4	6.6

*[1]: promyelocytic leukaemia and myelodysplastic syndrome and one missing
*[2] m-BACOD, Hyper-CVAD, ESHAP, Linker, DA-EPOCH-R and COPADM (missing data 19.7%, n=12)
N: patients' number.
The missing data are explained by the retrospective nature of the study.

Clinical factors that might predict fertility preservation decision

We found that age influenced the decision of whether or not to preserve fertility ($p = 0.048$). Women who chose not to preserve fertility were significantly older than women who preserved fertility (27.730 ± 5.855 [15-36] vs 24.660 ± 5.896 [16-35] years old).

It was also analysed if the stage at initial diagnosis of each patient influenced their option for preserving fertility. There was no relationship between the staging of the disease and the decision to preserve fertility ($p > 0.05$ for all stages).

When analysing women who preserved fertility according to parity, we found a positive correlation with nulliparity. Of the 48 nulliparous, 33 chose to preserve fertility. None of the women who had already a child chose to preserve fertility ($p < 0.001$).

Regarding the marital status of each patient, we found a relationship between being single and preserving fertility ($p = 0.021$). Of the 34 single women, 25 chose to preserve fertility.

Influence of demographic factors on ovarian reserve

The age of diagnosis did not have a negative impact on FSH and AMH levels after treatment. Only women aged between 25 and 30 years had a significant decrease in AMH (2.279 ± 1.775 [0.250-4.900] vs 0.479 ± 0.811 [0.010- 0.510] ng/mL, $p = 0.033$) and an increase in FSH (5.239 ± 3.489 [3.200-24.000] vs 53.263 ± 51.329 [0.200-122.000] mUI/mL, $p = 0.038$) when assessing levels before and after treatment.

Likewise, BMI did not seem to influence the ovarian reserve after treatment.

Finally, the use of oral contraceptives (OCs) during chemotherapy did not demonstrate a protective effect on the ovarian reserve, according the levels of FSH ($p = 0.138$) and AMH ($p = 0.064$).

Impact of haematological diagnosis on the ovarian reserve

In our sample, haematological diagnosis (Hodgkin's or non-Hodgkin's lymphoma) did not negatively influence the ovarian reserve before treatment.

There were no differences neither in FSH nor in AMH levels before treatment in patients diagnosed with Hodgkin's and non-Hodgkin's lymphoma ($p > 0.05$ in both). Therefore, the diagnosis alone had no impact on the ovarian reserve.

Impact of cancer treatments on ovarian reserve

FSH and AMH levels were assessed in the fertility preservation consultation before starting the treatment and later, in the follow-up visit, about two years after the first appointment. When comparing FSH measurements before (9.47 ± 22.87 [0.30 - 156] mUI/mL) and after cancer treatment (37.41 ± 48.22 [0.10-148] mUI/mL), we found a significant increase in FSH levels (p = 0.02; Figure 1.A.). In turn, when we performed the same analysis for AMH levels, it revealed a significant decrease in these levels (0.52 ± 0.06 [0.01-3.70] vs 2.19 ± 1.89 [0.06-7.70] ng/mL, p < 0.001; Figure 1.B.).

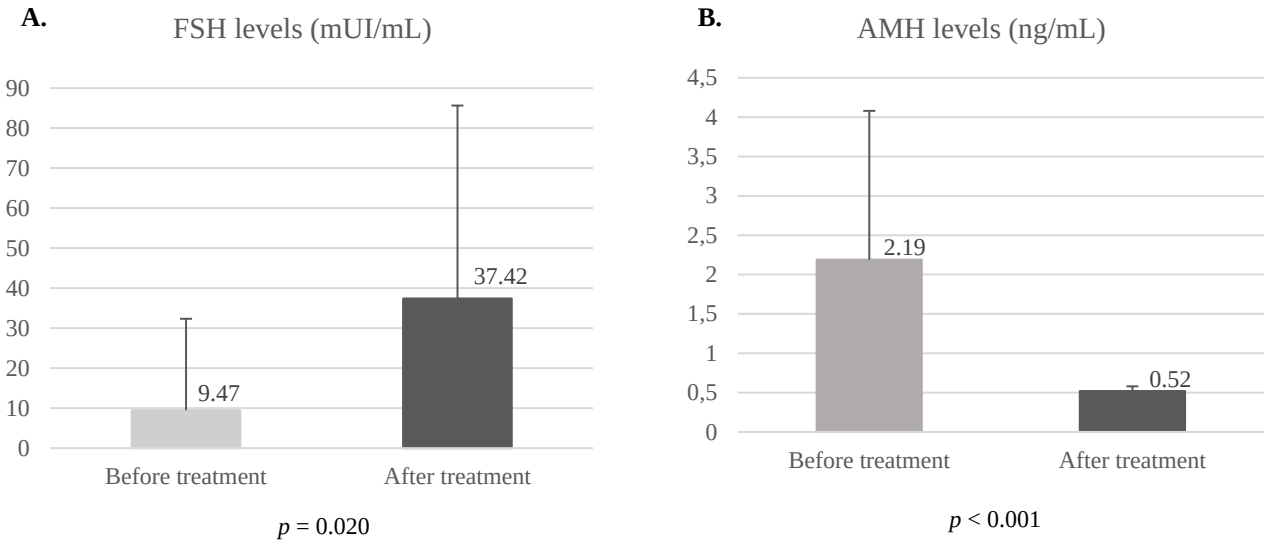

Figure 1. Analysis of the impact of oncological treatments for hematologic malignancies in ovarian reserve. A - FSH levels before and after cancer treatments. B - AMH levels before and after cancer treatments.

In patients who had already initiated cancer treatments at the fertility preservation consultation (n=10, 16.4%), the comparison levels of FSH and AMH with the pre-treatment levels of the remaining patients. We found that patients who had already initiated cancer treatments had lower levels of AMH (0.944 ± 0.907 [0.060-2.500] vs 2.447 ± 1.948 [0.080-7.700] ng/mL, p = 0.055), but no differences were evident in FSH (p = 0.759).

Impact of chemotherapy regimen on ovarian reserve

To assess the impact of each chemotherapy regimen on the ovarian reserve, we compared FSH and AMH levels after treatment in patients submitted to the three most used regimens and in patients that were not submitted to none of those.

In our study, 7 patients (11.5%) have been treated with the BEACOOP regimen. We noticed that AMH levels of patients treated with the BEACOOP regimen were lower than those from the patients submitted to other treatments (0.034 ± 0.025 [0.010-0.060] vs 0.881 ± 1.129 [0.010-3.700] ng/mL, $p < 0{,}001$; Figure 2). However, there are no differences when comparing FSH levels in patients undergoing this chemotherapy regimen.

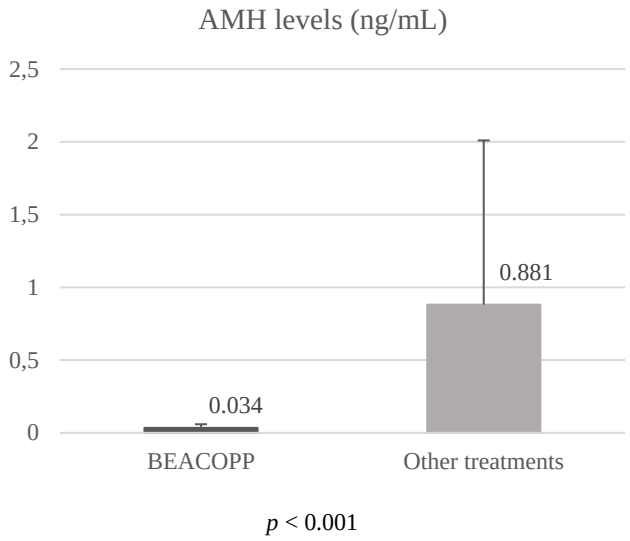

Figure 2. AMH levels in patients undergoing the BEACOOP regimen compared to other treatments.

In the ABVD regimen, we also observed that there were no statistically significant differences in FSH levels ($p = 0.124$). However, a tendency for lower AMH levels was found (1.573 ± 1.385 [0.060-3.700] vs 0.342 ± 0.672 [0.010-3.00] ng/mL, $p = 0.058$).

FSH and AMH levels in women treated with R-CHOP regimen, FSH and AMH levels were similar to those of women undergoing other treatments ($p = 1.000$ and $p = 0.820$, respectively).

Impact of each chemotherapeutic agent on ovarian reserve

We analysed the effect of each chemotherapeutic agent, comparing the levels of FSH and AMH before and after cancer treatment.

Vincristine negatively affects the levels of AMH (after 0.341 ± 0.690 [0.010-3.000] vs before 1.266 ± 1.348 [0.010-3.700] ng/mL, $p = 0.037$), but not those of FSH ($p = 0.124$). Also etoposide (0.034 ± 0.025 [0.010-0.060] vs 0.839 ± 1.117 [0.010-3.700] ng/mL, $p = 0.002$) and procarbazine (0.034 ± 0.025 [0.010-0.060] vs 0.839 ± 1.117 [0.010-3.700] ng/mL, $p = 0.002$) had a negative impact on AMH levels. However, these agents did not have a detrimental effect on FSH levels ($p = 0.241$ for both).

Furthermore, prednisone had a negative impact on the ovarian reserve, translated by the effect on FSH levels (53.240 ± 55.353 [0.200-148.00] vs 6.880 ± 9.824 [0.100-34.000] mUI/mL, $p = 0.041$) and AMH levels (0.311 ± 0.714 [0.010-3.000] vs 1.143 ± 1.248 [0.010-3.700] ng/mL, $p = 0.013$).

Vinblastine and dacarbazine showed a tendency towards less impact on AMH levels (1.573 ± 1.385 [0.060-3.700] vs 0.362 ± 0.659 [0.010-3.000] ng/mL, $p = 0.056$). No impact was seen in FSH levels ($p = 0.124$).

On the contrary, doxorubicin ($p = 0.160$; $p = 0.590$), bleomycin ($p = 0.637$; $p = 0.856$), cyclophosphamide ($p = 0.388$; $p = 0.072$) and rituximab ($p = 0.669$; $p = 0.408$) did not have an effect on ovarian reserve, since no significant alterations on the levels of FSH and AMH were found.

Influence of radiotherapy and haematopoietic stem cell transplantation on ovarian reserve

In patients submitted to radiotherapy, there was no decrease in ovarian reserve ($p > 0.05$). However, patients who underwent haematopoietic stem cell transplantation (HSCT), a statistically significant reduction in ovarian reserve was found (Figure 3). In these patients, the levels of FSH are higher (77.367 ± 59.464 [3.200-135.000] vs 28.846 ± 41.906 [0.100-148.000] mUI/mL, $p = 0.023$) and the AMH levels are lower (0.633 ± 0.980 [0.010-3.700] ng/mL vs 0.039 ± 0.027 [0.010-0.060] ng/mL, $p = 0.015$).

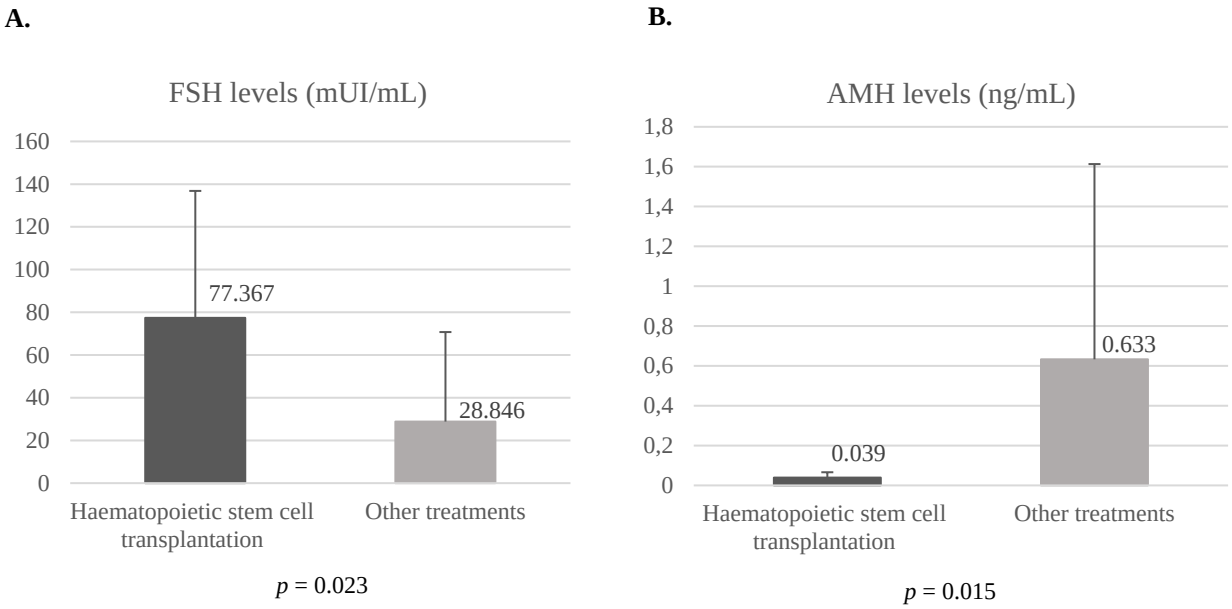

Figure 3. Ovarian reserve assessment in patients undergoing haematopoietic stem cell transplantation compared to patients undergoing other treatments, according FSH (A) and AMH (B) level analysis.

The occurrence of pregnancy according to demographic factors and ovarian reserve after treatment

Of the 11 pregnancies, only one occurred in the period between the end of the treatment and the follow-up consultation. The remaining 10 women became pregnant after the follow-up consultation, that is, most pregnancies occurred at least 2 years after diagnosis.

When we compare the occurrence of pregnancy with age, we found that the patients that got pregnant were significantly older (28.820 ± 4.895 [22-36] vs 24.640 ± 6.031 [15-36] years old, $p = 0.038$).

BMI was not correlated with pregnancy in our study ($p = 0.456$).

In our study, we found no relationship between women who became pregnant after the end of treatment and the post-treatment levels of FSH ($p = 0.592$) and AMH ($p = 0.535$).

Discussion

The impact of each cancer treatment *per si* on the ovarian reserve is still unknown. Some treatments seem to affect the ovarian reserve more, but we do not know how long it takes to recover. Fertility preservation is increasingly a reality for women diagnosed with cancer, therefore, it is essential to analyse which factors influence the decision to preserve fertility in order to guarantee adequate advice to each patient regarding their fertility in the present and in the future.

In our study, in patients diagnosed with Hodgkin's or non-Hodgkin's lymphoma the ovarian reserve was according to the expected for the age prior to starting treatments. Thus, our results are in line with the results obtained by Paradisi *et al.* [9], who demonstrated no differences between Hodgkin's and non-Hodgkin's lymphoma for AMH levels pre-treatment. These results can be explained by the young age of the sample, however, the small sample size and the lack of studies do not allow us to draw this conclusion.

In our sample, age influenced the decision to preserve fertility. Younger, nulliparous and single women tend to undergo fertility preservation techniques more often than older women. This can be explained by the fact that older women already have children or do not wish to become pregnant again. The potential loss of fertility is a key concern of young women treated for cancer [10] so referral to fertility preservation consultations should be done in an appropriate period of time, allowing the patient to choose whether or not to preserve fertility.

The role of OCs in preserving fertility is still controversial. A randomized trial that was prematurely ended by Behringer *et al*. [11] concluded that OCs had no protective effect on gonadal function in patients treated with BEACOPP-escalated for Hodgkin lymphoma. The same authors conducted a retrospective study [12], in which they concluded that women who took OCs during treatments had a lower risk of amenorrhea. In our study sample, OCs did not demonstrate a protective effect on the ovarian reserve. To clarify the effect of OCs on ovarian reserve, further studies are needed.

According to the literature, cancer treatments, such as chemotherapy, radiotherapy and HSCT, have a negative effect on ovarian reserve [13-23]. In our study, treatments caused a decrease in ovarian reserve, as evidenced by the increase in FSH levels and the decrease in AMH levels. In patients who had already started cancer treatment at the first fertility preservation consultation we found that there was a tendency towards a decrease in AMH levels, reinforcing the deleterious effect of treatments on the ovarian reserve in the short and medium-term.

According to the guidelines of the German Society of Gynaecology and Obstetrics (DGGG), coordinated with the German Society of Urology (DGU) and the German Society of Reproductive Medicine (DGRM) [18], ovarian toxicity depends on the type of chemotherapy regimen used, the number of cycles performed and the patient's age. In our study, the BEACOPP regimen demonstrated a negative impact on AMH levels after treatment. Among the patients undergoing this regimen, 4 were between 25-35 years old and 3 were under 25 years old. The same guidelines [18], differentiate the impact of the BEACOPP regimen considering the patient's age. For ages over 35 years old, 6 to 8 cycles have a high risk of causing amenorrhea. In turn, the risk decreases to intermediate, if the patients are between 25-35 years old and low if they are less than 25 years old. Although in our study amenorrhea was not evaluated in these patients, the AMH levels decreased after treatment with this regimen, in accordance with the negative effect of the BEACOPP regimen in these age groups. It is important to consider that 1 patient underwent 4 cycles, 3 patients underwent 6 cycles and in the remaining 3, we were unable to ascertain the number of cycles performed.

Regarding the ABVD regimen, the most recent data indicate that it has a low risk of causing amenorrhea [18]. AMH has a tendency towards higher levels after the treatment when compared to the other treatments. Only 7 patients undergoing this regimen had FSH and LH, consequently the small sample size may skew the results.

Relatively to the R-CHOP regimen, the risk of amenorrhea is intermediate, if the patient is > 35 years old and low if the patient is < 35 years old [18]. This negative impact of the R-CHOP regimen on AMH levels was evidenced by De Bruin et al. [24]. However, in our study, we did not obtain statistically significant changes in the levels of FSH and AMH in patients undergoing R-CHOP regimen.

We analysed the impact of each chemotherapeutic agent on the ovarian reserve. In this field, changes in FSH and AMH levels may be due to the simultaneous use of other drugs, since each agent is included in one or more chemotherapy regimens. According to the literature, alkylating agents have a high risk of gonadal toxicity and amenorrhea [18,19]. In our study population, cyclophosphamide did not have a negative impact on the ovarian reserve. This result can be explained by the small number of patients in our sample, so, in this field, the results should be interpreted with caution.

Contrarily, patients undergoing vincristine experienced a significant decrease in AMH levels which is contradicted by other studies that defined vincristine as an agent with very little or no risk of causing amenorrhea [18,25].

In addition, patients treated with etoposide agent also experienced a decrease in AMH levels. Swerdlow *et al.* [26] found an increased risk of premature ovarian insufficiency in patients undergoing this agent. It should be noted that there are no studies on the effect of etoposide alone on AMH levels, so more studies are needed to confirm its impact on ovarian reserve.

Regarding procarbazine, several studies report a detrimental effect on ovarian reserve, increasing the likelihood of ovarian dysfunction [19,27]. In our sample, the results are in line with other studies, showing a decrease in AMH levels in patients undergoing this regimen.

According to Trull *et al.* [3], vinblastine has a low risk of gonadal toxicity. Our results showed that there was a tendency for levels of AMH to decrease in patients who underwent this chemotherapeutic agent. However, further studies are needed to clarify the impact of vinblastine on ovarian reserve. We also showed a tendency towards a decrease in the levels of AMH in patients submitted to dacarbazine. These results are in line with other studies that have shown that dacarbazine has a high risk of gonadal toxicity [3,27].

There is insufficient data in the literature about the secondary effects of prednisone on the ovarian reserve. Jenny *et al.* [28] found an increase in time to pregnancy in patients taking a daily dose > 7.5mg of prednisone. In an experimental study in rats [29], methylprednisolone had a negative effect on ovarian reserve. In our study, prednisone had a negative impact on ovarian reserve, as shown by an increase in FSH levels and a decrease in AMH levels.

In our study, patients undergoing HSCT had a decrease in ovarian reserve, demonstrated by an increase in FSH levels and a decrease in AMH levels. These results are in agreement with the meta-analysis by Gerstl *et al.* [30], who evaluated 14 studies and showed that there is a lower rate of pregnancy in patients undergoing HSCT. It should be noted that, in our sample, we were not able to differentiate patients who had allogeneic or autologous HSCT. This data is relevant, since the impact on the ovarian reserve seems to be lower in patients undergoing autologous HSCT [30].

Regarding radiotherapy, the current literature is unanimous in terms of its effect on the ovarian reserve, depending on the patient's age, dose and location of irradiation, it has different risks of causing ovarian failure [18,19,31]. In our study population, we did not see changes in FSH and AMH levels after treatment. Most patients underwent cervical and mediastinal irradiation however we were unable to clarify how many underwent total body irradiation. Thus, we cannot conclude whether radiotherapy did not have a negative impact on the ovarian reserve due to the location of the irradiation (cervical and mediastinal) or whether due to recovery over the last 2 years. We also do not know the total amount dose that was administered, so the results in this field should be viewed with some reservations.

Hamy AS *et al.* [32] demonstrated that AMH levels do not have a positive correlation with short-term fertility in women after cancer treatment. Although the concentration of AMH is not related to the occurrence of short-term pregnancy [5], it can help to predict the age of menopause and thus calculate the opportunity to achieve pregnancy in the longer term [33]. In our study, we also found no relationship between women who became pregnant after the end of treatment and the post-treatment levels AMH.

Limitations

There are limitations that need to be addressed when interpreting reproductive outcomes. The best parameter to assess fertility is pregnancy, however the occurrence of this depends on the patients' own will, so we can have patients with normal fertility, but who do not want to become pregnant, thus conditioning the main parameter of fertility assessment. The radiation dose and the use of total body irradiation in radiotherapy were not considered, two important factors to be able to correctly assess the impact of this treatment on the ovarian reserve. In HSCT, we were unable to differentiate which patients were submitted to allogeneic or autologous HSCT, failing to assess the impact of each of these types of HSCT on ovarian reserve. The retrospective design of the study is the main limitation since it does not allow us to fill in the missing data. The sample size and the small number of patients who performed each treatment also contributes to the bias of the results.

Conclusion

In our centre, more than 50 % of the patients referred to consultations chose to preserve fertility. The main reason for not preserving fertility was personal choice. However, about 1 in 6 patients had started oncological treatment before fertility preservation consultation. Patients of childbearing potential should be referred to a fertility preservation centre at an appropriate time, giving them the option to choose to preserve their fertility or not.

Regarding oncological treatment, the ABVD regimen was found to have less impact on AMH levels compared to the other treatments. A significant decrease in ovarian reserve was observed after cancer treatment, namely after BEACOPP regimen and HSCT.

Vincristine, etoposide, procarbazine and prednisone caused a decrease in AMH levels.

Further studies are needed to assess the impact of each chemotherapeutic agent on the ovarian reserve, as well as the relationship between AMH levels and the occurrence of short, medium and long term pregnancies. That will allow to better inform patients on the effect on their fertility and the chances of getting pregnant in the future.

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