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Role of inflammatory blood parameters from complete blood count in predicting ovarian follicular density in cancer patients undergoing ovarian tissue cryopreservation

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Abstract

Background Inflammation is a key feature of neoplastic diseases, especially cancer. Predicting follicular density (FD) in ovarian cortical tissue is essential for evaluating ovarian tissue cryopreservation (OTC) outcomes in fertility preservation. However, to date, no studies have explored the role of inflammatory markers in predicting FD in OTC patients. This study aims to investigate the relationship between blood inflammatory parameters and FD in this population.

Methods We conducted a retrospective observational study on 101 OTC patients. The primary goal was to assess whether parameters from Complete Blood Count (CBC) that include White Blood Cells (WBC), absolute neutrophil count, absolute lymphocyte count, Neutrophil/Lymphocyte Ratio (NLR), Mean Platelet Volume (MPV), Platelet Count (PC), MPV/PC and the Platelet/Lymphocyte Ratio (PLR) could predict FD. We also evaluated the impact of factors such as oncological diagnosis, smoking, Body Mass Index (BMI), and germline BRCA mutations. Spearman's correlation coefficient and the Mann-Whitney test were used for analysis.

Results Significant correlations were found in patients aged between 27 and 31. In this group, NLR was inversely correlated with FD (Rho = -0.374, $p = 0.032$), while lymphocyte count (Rho = 0.371, $p = 0.034$) and MPV/PC (Rho = 0.365, $p = 0.037$) were positively correlated with FD. An inverse correlation was also found between PLR and FD (Rho = -0.38, $p = 0.028$).

Conclusions Our findings suggest that NLR, lymphocyte count, MPV/PC and PLR may be useful in predicting FD in a subgroup of OTC patients. Larger studies are needed to confirm these results.

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Keywords Inflammatory parameters, Ovarian reserve, Ovarian follicular density, Ovarian tissue cryopreservation (OTC), Fertility preservation, Cancer

Introduction

Cancer incidence in Adolescents and Young Adults (AYAs), aged between 15 and 39, is approximately 1.3 million cases annually worldwide [1]. In 2020, there were 89,500 new diagnoses in the U.S [2]. Among the 67,692 cancer survivors within this age group in Italy, 40,700 were women [3]. Fertility preservation is a key concern for young women with cancer, as treatments like alkylating agents and pelvic radiotherapy can damage ovarian function [4, 5]. A major consequence of gonadotoxic therapy is Premature Ovarian Insufficiency (POI), which can impair fertility and affect bone and cardiovascular health, leading to osteoporosis and increased thrombotic risk [6, 7]. These issues significantly reduce the quality of life for young cancer survivors, making oncofertility counseling essential [8, 9].

Several options for preserving ovarian function and fertility in cancer patients include GnRH agonists, oocyte cryopreservation (OC), embryo cryopreservation (EC), ovarian transposition, and ovarian tissue cryopreservation (OTC) [10]. OTC, while invasive, is the only method for prepubertal patients, as it doesn't require ovarian stimulation. It preserves both endocrine function and fertility, avoiding delays in treatment for patients needing urgent therapy [11–14]. OTC preserves a significant number of primordial follicles in a small volume of ovarian tissue, and immature oocytes can be matured in vitro for additional fertility preservation [15–18]. In OTC, part or all the ovary is removed via laparoscopy before cancer treatment and stored in liquid nitrogen. After treatment, the tissue can be reimplanted orthotopically to restore ovarian function or heterotopically to another site like the abdominal cavity or under the skin at the arm level [19–22]. Each step of OTC and transplantation impacts follicle survival. Despite advancements in freezing technology that allow for the survival of many follicles during freezing and thawing [23], studies show that transplantation, rather than cryopreservation, causes the greatest loss of follicles due to ischemic injury [23, 24]. Angiogenesis, necessary for revascularization, takes over 48 h, leading to ischemic damage and follicle loss [24]. Additionally, follicle activation and "burnout" occur in OTC grafts, likely due to ovarian homeostasis disruption [25–27]. In this context, to compensate for the effects of follicle loss, it is clear how crucial a high follicular density (FD) related to the patient's ovarian reserve is to the success of the OTC technique, which is why parameters for measuring ovarian reserve are used before performing OTC. Ovarian reserve refers to the quantity and quality of a woman's remaining oocytes in her ovaries, while FD refers to the number of primordial follicles per cubic millimeter (mm^3) counted histologically on the ovarian cortical tissue. Age, blood Anti-Müllerian Hormone (AMH) level and Antral Follicle Count (AFC) performed within the first three days of the menstrual cycle by transvaginal ultrasound are considered the most reliable biomarkers for assessing ovarian reserve [28]. Since the measurement of AMH is reliable on any day of the menstrual cycle and it is very useful in OTC patients, who do not require hormonal stimulation, they can undergo ovarian tissue retrieval just a few days after counseling, provided that the AMH value is adequately high. Regarding the cut-off value used in other centers, an AMH level of at least 1 ng/ml at our Institute is considered indicative of good ovarian reserve for performing OTC, along with patients 39 years or younger. Nevertheless, there are several endogenous and exogenous factors that may influence serum AMH levels hindering the proper interpretation of its values in the clinical setting such as recent (up to three months) use of oral contraceptive pills, smoking habits, obesity, and low-vitamin D levels [29, 30]. Moreover, the absence of an international gold standard for AMH prevents a definite assessment of which patients can be included in the OTC program [31, 32]. Identifying other predictive parameters of FD would be very useful. Recent studies focusing on the correlation between circulating and local inflammation parameters and ovarian response to in vitro fertilization (IVF) techniques, are not absent of controversial results [33–35]. Increasing evidence reports that inflammation is correlated with various ovarian dysfunctions, such as POI, Polycystic Ovary Syndrome (PCOS), and ovarian aging, where it has been suggested as a possible contributing factor to the early decline in women ovarian reserve [36–40]. Particularly, in cancer patients, it is well-established that the level of inflammation correlates with both the presence and the stage of neoplastic disease [41, 42]. In this context, the aim of our study is to investigate whether the inflammatory parameters derived from the Complete Blood Count (CBC), which is routinely performed on cancer patients before OTC, could serve as predictive markers of FD or if any of these parameters are correlated with a decrease in ovarian reserve in these patients. Having easily detectable markers that help determine the actual ovarian reserve status of a patient undergoing OTC would be essential for predicting the outcome of the procedure and for evaluating the feasibility and benefits of the procedure for each patient. To the best of our knowledge, studies have yet to investigate the relationship between blood inflammatory markers and FD in OTC patients and hoping that

our findings will provide new insights and contribute to the evaluation of ovarian reserve in selecting candidates eligible for OTC.

Materials and methods

Study design and participants

This is a retrospective observational study based on data collected from a cohort of oncological patients who underwent OTC for fertility preservation in the period between January 2017 and March 2023 at the Ovarian and Germ Cell Tissue Bank of the IRCCS Regina Elena National Cancer Institute in Rome.101 patients were included in our study. The inclusion criteria involved: having undergone OTC, having a CBC available prior to the procedure, and having FD count performed on fresh cortical tissue. From the CBC performed prior to OTC, we selected parameters of interest that are associated with inflammation: White Blood Cell count (WBC), absolute neutrophil count, absolute lymphocyte count, Neutrophil/Lymphocyte Ratio (NLR), Mean Platelet Volume (MPV), Platelet Count (PC), MPV/PC and the Platelet/Lymphocyte Ratio (PLR). We examined the correlation between these parameters with respect to FD. For each patient, we also collected the AMH value determined at the time of OTC. Data concerning Body Mass Index (BMI), smoking habits, and germline BRCA status were collected when available and their correlation with FD was also evaluated.

Ovarian tissue retrieval

Ovarian tissue harvesting was performed by minimally invasive surgery (laparoscopy or percutaneously assisted laparoscopy) where part of the harvested tissue was histologically examined. All procedures for preparing culture solutions and tissue handling during freezing and thawing were performed under a class A laminar flow hood with a grade D background environment according to the manufacturer's guidelines (Legislative Decree No. 191 of November 6, 2007; Legislative Decree No. 16 of January 25, 2010; and Legislative Decree No. 85 of May 30, 2012.) The harvested tissue was immediately immersed in sterile transport medium at 0–4 °C after surgery (Flushing Medium, Cooper Surgical, Origio) which were then sent directly to the laboratory from the operating theatre for processing. Once the Lab received the ovarian tissue, it was placed in a 90 mm Petri dish containing 10 ml of sterile 0.9% saline solution. The ovarian cortical was then isolated under the flow hood using anatomical forceps and scalpel. The saline was regularly replaced during the procedure. Following removal of the medullary tissue, the tissue was trimmed to 1–2 mm thickness and cut into fragments measuring 5×10 mm. The standardized freezing protocol validated in the Laboratory of Reproductive Biology at "Rigshospitalet" (Copenhagen University Hospital) was used for the OTC procedure [19].

Histological processing and FD assessment

A small piece of ovarian cortex was separated from the fragments for cryopreservation and freshly fixed in Bouin's solution for 10/15 hours and histological processed in a ASP6025 Leica Biosystems automatic tissue processor. Once the paraffin tissue block was obtained, sequential 25-micron thick sections were cut using a Leica Biosystems sliding microtome. The sections obtained were stained with hematoxylin/eosin using an automatic stainer, Histo Core SPECTRA ST (Leica Biosystems). All the slides with stained sections were digitally captured using an Aperio AT2 image scanner (Leica Biosystems). Primordial follicles (oocytes surrounded by a single layer of squamous follicular cells) were counted in every second section to avoid counting the follicles twice. The area was subsequently evaluated once every four sections using the area measurement feature of the Aperio scanner. Any data related to follicle count and area measurements were combined on an Excel software, through which FD was calculated in terms of number of follicles per square millimeter of cortical tissue.

Data collection

Clinical data were obtained from patient electronic medical records stored at the IRCCS Regina Elena National Cancer Institute of Rome and included demographic and clinical information, oncological diagnosis, and CBC values. NLR, PLR, and MPV/PC were calculated as the ratio between the specific parameters. Data related to AMH, weight, and height for BMI calculation were extracted from the medical report written during the first oncological fertility counseling visit. The data regarding the FD was collected from the database of the Pathology Unit.

Statistical analysis

Categorical variables were summarized with frequencies and percentage values while continuous variables were summarized with median values and relative range or tertiles. We decided to divide age into tertiles to find a better compromise between the sample size and a cut-off that made sense with such an age-dependent outcome as FD.

The Mann-Whitney and the Kruskal-Wallis nonparametric tests were used, when appropriate, to compare FD values and AMH values among different groups, which include age tertiles and type of cancer, BMI, smoking status and germline BRCA status for breast cancer patients. Bonferroni's adjustment for multiple comparisons was applied when needed. Furthermore, we stratified the sample by age tertiles and tested the correlation between blood values and FD by using Spearman nonparametric

correlation test. A p-value < 0.05 was considered statistically significant. All statistical analyses were carried out with SPSS (IBM) v.29.0.1. A p-value of < 0.05 was considered statistically significant.

Results

Patients

In this study, 101 patients were included; their demographic, clinical and laboratory data are reported in Table 1.

Our study cohort consists of patients ranging from 10 to 39 years. We stratified the sample by age tertiles: group 1 includes patients up to 27 years old, group 2 up 28 to 31 years old, and group 3 over 31 years old, as shown in Table 1. Regarding the cancer diagnosis, among the 101 patients, 58 (57%) had breast cancer, 16 (16%) had sarcoma, and the remaining 27 (27%) of patients had mixed diagnoses (11 lymphomas, 8 cervical cancer, 1 rectal cancer, 1 anal cancer, 1 vulvar cancer, 1 medulloblastoma, 1 endometrial cancer, 1 yolk sac tumor, 1 desmoid tumor, 1 nasopharynx cancer). The median age at the time of OTC was 29 years (ranging between 10 and 39 years). FD assessed on a fresh ovarian cortical fragment from each patient varied from 0 to 117 follicles/mm^3, median FD resulted 9.4 follicles/mm^3. All median values observed in our patients for the parameters analyzed are reported in Table 1. Germline BRCA testing was available for 52/56 (91%) of breast cancer patients. Of these 51 patients, 12 were mutated for BRCA1/2 and 39 resulted to be wild type (data not shown).

Table 1 Patient characteristics

Characteristics	Number (*N*)	Percentage (%)
N° of patients	101	
Age at diagnosis (years)		
Group 1–10-27 (1st tertile)	34	34
Group 2–28-31 (2nd tertile)	33	32
Group 3–31+ (3rd tertile)	34	34
Type of cancer		
Breast Cancer	58	57
Sarcoma	16	16
Other	27	27
Median age at OTC (years) (range)	29.0 (10–39)	
Median FD (Follicles/mm^3) (range)	9.40 (0-117.0)	
Median AMH Value (ng/ml) (range)	2.48 (1-27.60)	
Median MPV/PC (range)	0.036 (0.020–0.089)	
Median MPV (fL) (range)	9.00 (7.20–12.70)	
Median NLR (range)	2.1 (0.6–20.2)	
Median PLR (range)	129.9 (45.3–570.0)	
Neutrophil count (x10^3/µl)	3.72 (1.80–14.50)	
Median PC (x10^3/µl)	252 (135–437)	
Median Lymphocyte count (x10^3/µl)	1.85 (0.60–6.44)	
Median WBC (x10^3/µl)	6.44 (3.61–17.44)	
BMI*		
Not overweight	79	78
Overweight	21	21

** 1 missing value*

Abbreviations: OTC, ovarian tissue cryopreservation; FD, follicular density; AMH, anti-müllerian hormone; MPV, mean platelet volume; PC, platelet count; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; PC, platelet count; WBC, white blood cells count; BMI, body mass index.

Correlation between blood parameters and FD

No significant differences were observed in group 1 (10–27 years) and group 3 (31–39 years) when blood parameters and FD were compared. In group 2 (28–31 years), no correlation was found between WBC, absolute neutrophil count, absolute lymphocyte count, PC, and the PLR with respect to FD. However, in this group, a significant inverse correlation was noticed between increased NLR and FD (Spearman's Rho = -0.374 $p = 0.032$). Interestingly, a significant inverse correlation was pinpointed between PLR and FD (and Spearman's Rho= -0,38 $p = 0,028$). Moreover, a significant direct correlation was found between lymphocytes level and FD (Spearman's Rho = 0.371 $p = 0.034$) and between MPV/PC and FD (Spearman's Rho = 0.365 $p = 0.037$).

FD and AMH values in relation to patient's age

As expected, FD significantly decreases with age at OTC, both when considering all patients ($p < 0.001$) and when comparing group 1 vs. group 2 and group 1 vs. group 3 ($p < 0.001$). Interestingly, the difference between group 2 and group 3 is no longer significant ($p = 0.367$) (data not shown).

Yet, we did not observe any differences in AMH distribution among age at OTC class neither overall ($p = 0.141$) nor when we conducted sub-comparisons.

Relation between oncological diagnosis and FD, AMH and blood parameters

The median values of FD, AMH, and blood parameters regarding oncological diagnosis are shown in Table 2. We observed statistically higher FD values in patients diagnosed with sarcoma compared to patients harboring with breast cancer or other neoplasms ($p < 0.001$).

Relation between BMI, smoking habits and BRCA status and FD

No significant correlation was observed between BMI, smoking habits and BRCA status and FD.

Discussion

The main aim of our study was to evaluate the relationship between inflammatory CBC-derived parameters and FD in a cohort of OTC oncological patients who were

Table 2 Follicular density and blood parameters by cancer site

	Breast Cancer (BC)	Sarcomas (S)	Other site (O)	Overall *p*-value*	Significant** pairwise comparisons
Median FD (Follicles/mm^3) (range)	6.7 (0.3–71.8)	51.0 (5.9–109.0)	9.7 (0-117.0)	< 0.001	BC vs. S $p < 0.001$ O vs S *p=0.001*
Median AMH Value (ng/ml) (range)	2.4 (1.1–14.4)	3.3 (1.0-6.2)	2.4 (1.2–27.6)	0.656	
Median MPV/PC (range)	0.04 (0.02–0.09)	0.04 (0.02–0.06)	0.03 (0.02–0.71)	0.016	BC vs. O $p = 0.012$
Median MPV (fL) (range)	9.1 (7.6–12.7)	8.8 (7.2–12.2)	9.1 (7.4–11.3)	0.431	
Median NLR (range)	2.0 (0.6–14.8)	2.4 (1.0-4.9)	2.3 (0.9–20.2)	0.258	
Median PLR /range)	120.5 (45.3–41.3)	129.9 (78.9–20.6)	172.7 (49.0-570.0)	0.042	BC vs. O $p = 0.037$
Neutrophil count (x10^3/µl)	3.7 (1.8–11.1)	4.2 (2.1–9.9)	3.9 (2.6–14.5)	0.329	
Median PC (x10^3/µl)	243.0 (135.0-339.0)	246.0 (150.0-369.0)	284.0 (150.0-437.0)	0.016	BC vs. O $p = 0.012$
Median Lymphocyte count (x10^3/µl)	1.9 (0.7–6.4)	1.9 (1.3–2.8)	1.6 (0.6–3.6)	0.195	
Median WBC (x10^3/µl)	6.4 (4.2–13.5)	6.6 (4.1–13.2)	7.4 (4.5–17.4)	0.435	

*Kruskal-Wallis non parametric test; ** Adjusted for multiple comparisons (Bonferroni)

Abbreviations: AMH, anti-müllerian hormone; MPV, mean platelet volume; PC, platelet count; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; PC, platelet count; WBC, white blood cells count.

stratified by age tertiles to identify potential new parameters that are useful for predicting tissue FD. To the best of our knowledge, while recent studies have focused on investigating the relationship between systemic (peripheral blood) and local (ovarian tissue) inflammation markers to determine whether these markers could be predictive of ovarian response during in vitro fertilization (IVF) treatments, ours is the first study that considers the relationship between blood inflammatory markers and tissue FD in cancer patients undergoing OTC.

A strong correlation between inflammation and cancer has been demonstrated. Inflammation has been associated with both the development and progression of cancer, and inflammatory cells and cytokines found in tumors contribute to tumor growth and progression [41, 42]. Since inflammation seems to play a role in both follicular development and ovarian aging [37–40, 43, 44], we wondered whether inflammatory parameters derived from CBC, which are easily measurable, could provide insight into the FD of patients undergoing OTC. As specified in the methods section, the parameters analyzed in our study were WBC, absolute neutrophil count, absolute lymphocyte count, NLR, MPV, PC, MPV/PC and the PLR. No correlations were found in group 1 and group 3. In group 2, we first observed a statistically significant inverse correlation between NLR and FD (Spearman's Rho = -0.374 $p = 0.032$). Consistent with our results, also Ilhan et al. found that NLR values were significantly higher in 37 POI patients in respect to 37 control patients having normal ovarian reserve [36]. The study also reported a direct correlation between NLR and Follicle Stimulating Hormone (FSH), a marker of ovarian insufficiency and an inverse correlation between NLR and AMH, thus highlighting an inverse correlation between NRL and ovarian reserve. NLR is a known biomarker of systemic inflammation that can be readily obtained from a peripheral CBC. An elevated NLR is often associated with an active inflammatory state, as it indicates a relative increase in neutrophils (which reflect an acute inflammatory response or infection) and a decrease in lymphocytes (which may reflect modulation or suppression of the adaptive immune response). In group 2, we also found a weak but statistically significant direct correlation between MPV/PC compared to FD (Spearman's Rho = 0.365 $p = 0.037$). A study by Hong et al. assessed the role of inflammatory markers in predicting ovarian stimulation response, specifically the number of oocytes recruited, in 91 breast cancer patients undergoing IVF. The study found no correlation between NLR and the number of recruited oocytes after ovarian stimulation and oocyte retrieval. Hong et al. reported that the total number of mature oocytes recruited after stimulation was higher in patients with an MPV ≥ 10.15 compared to those with MPV < 10.15, and that a lower MPV/PC was associated with a lower number of oocytes recruited [34]. However, while the data regarding MPV/PC seems to concur with Hong et al., the correlation between MPV and FD in our study was not statistically significant. In contrast, Ozgu-Erdinc et al. investigated the potential role hematological markers play in predicting IVF outcomes, particularly the relationship between WBC, NLR, Monocyte/Lymphocyte Ratio (MLR), PLR, MPV, and Platelet Distribution Width (PDW) in predicting a positive Human Chorionic Gonadotropin test (HCG) after ET, without finding any correlation [35].

Moreover, a weak inverse correlation was finally found between PLR and FD (Rho – 0.38, $p = 0.028$) in group 2 of our study. Platelets are primarily involved in the process of hemostasis, but they also play an important role in the inflammatory response. During inflammation, platelets are activated and release several inflammatory mediators, such as cytokines, which amplify the inflammatory

response. During the development of an acute or chronic inflammatory response, the number of lymphocytes may decrease, while other cells, such as neutrophils, may increase. An increased PLR (i.e., high platelet and low lymphocyte count) has been associated with several inflammatory and chronic pathological conditions, such as cancer, cardiovascular disease, autoimmune disease, and inflammatory disorders [45]. The inverse correlation between PLR and FD could therefore confirm a decrease in ovarian reserve concerning an inflammatory state. Even though there is no literature supporting this association, further studies are needed. A significant direct correlation was found between lymphocytes and FD in group 2 (Spearman's Rho = 0.371 $p = 0.034$), but this result needs to be further investigated with additional studies. For example, an important aspect could also be to characterize the circulating lymphocytes, as Zhao et al. did at the level of the ovarian follicular fluid (FF) of non-oncological patients with diminished ovarian reserve (DOR) [38, 39]. Zhao's studies demonstrated that the local altered proportion of CD8 + T lymphocytes, along with elevated levels of CCL5 and IFN-γ cytokines, may disrupt the immune balance in FF and hinder granulosa cell (GC) growth, thereby contributing to the progression of DOR. Therefore, for future studies, it is important to characterize the lymphocyte subpopulations in our patient population to better understand the mechanisms that may link lymphocytes and ovarian reserve in OTC cancer patients.

Regarding the influence of disease type on FD, a higher FD was found in patients diagnosed with sarcoma compared to patients diagnosed with breast cancer ($p < 0.001$) as well as between sarcoma patients and the rest of the cohort ($p < 0.001$). This result may possibly be brought about by the fact that sarcoma patients are younger than all other patients, and since FD is age-related, they also have a higher ovarian reserve.

With respect to the influence of BMI, smoking habits, and the presence of BRCA 1/2 mutations, we found no statistically significant differences in FD within our patient cohort. Regarding the association between BMI and ovarian reserve, our findings are in line with those of Oladipupo et al., who also found no significant correlation between BMI and decreased ovarian reserve [46]. Similarly, Dolleman et al. did not observe a significant link between BMI and reduced ovarian reserve, as assessed by AMH levels [47]. On the contrary, a recent study by Yuan-Li Li et al. reported significantly lower AMH levels in overweight and obese individuals compared to those who had normal weight ($p < 0.001$) [48]; in this cited study, statistical significance was only found in the 20–30 and 30–35 age groups, while no difference was observed in those aged between 35 and 45 years ($P = 0.430$). As to the relationship between smoking habits and FD, it is important to note that smoking data was incomplete for some patients, and the number of cigarettes smoked per day, or smoking duration was not always reported, which may limit the strength of our results. The relationship between smoking and ovarian reserve has been widely studied, often using AMH levels as a parameter, showing contradictory results [49–52]. The previously cited study by Oladipupo et al. found no association between current smoking (measured by urinary cotinine) and diminished ovarian reserve (DOR), while a history of heavy and prolonged smoking did show a significant impact on ovarian reserve [46]. The same study also highlighted the role of N-acetyltransferase2 (NAT2) polymorphisms, which may increase the damage from smoking and affect ovarian reserve. Dolleman et al. found that current smoking was inversely correlated with age-adjusted AMH levels, regardless of the number of cigarettes smoked [47]. Finally, our study aimed to evaluate the correlation between BRCA status and FD, as several studies over the past decade have highlighted that germline BRCA mutations, particularly BRCA1, may be linked to decreased ovarian reserve, reduced fertility, and POI. Research on the relationship between BRCA1/2 mutations and ovarian reserve has yielded different results [53–56]. Some studies have specifically analyzed the link between germline BRCA mutations and FD. For example, Irit Bene-Ahron et al. compared ovarian tissue from women with BRCA mutations undergoing prophylactic oophorectomy to an age-matched control group undergoing partial oophorectomy for benign conditions, finding a decrease in primordial follicles in BRCA carriers [57]. Lambertini et al. found that, in patients undergoing oocyte or OTC, immature oocytes collected from ovarian tissue fragments before cryopreservation showed a significant decrease in BRCA mutation carriers compared to non-carriers [54]. However, other studies have not shown a significant difference in ovarian reserve between women with and without BRCA mutations [55, 58]. These findings are in line with our study, although the small number of BRCA mutation carriers in our sample warrants further research, given the importance of this topic.

To the best of our knowledge, ours is the first study to explore the relationship between smoking, BMI, and ovarian reserve, specifically assessed by FD in tissue.

In our patient cohort, we also observed how both FD and AMH vary in relation to age. As expected, we found a statistically significant decrease in FD with increasing age. This is true when considering both the entire patient population as well as when dividing the patients into different age groups. We found that this decrease was statistically significant when group 1 (up to 27 years) and group 2 (27–31) were compared, while the decrease observed when comparing group 2 with group 3 (older than 31 years) was not considered statistically significant.

This observation could reflect a further drastic decline in the ovarian reserve in younger patients compared to those of intermediate age, as opposed to the two older patient categories. Compliant with this observation, it is well known that the decline in ovarian reserve does not follow a complete linear pattern, but rather presents intermittent decreases. Interestingly, we did not find any significant decreases between AMH levels and age in our patient cohort. This result may possibly confirm that AMH values can be influenced by various parameters, however further research is needed to explore this area in the near future.

Our study has several limitations. The first limitation of our study is its retrospective observational nature, which, for example, did not allow to include other inflammation markers, both serum and in the follicular fluid, which would have enriched the study. Another significant limitation lies in the variability of the study population, particularly regarding age and cancer pathology. We attempted to minimize the effect of these differences by stratifying patients based on age and histological diagnosis. However, a more homogeneous population would likely yield more consistent results. On the other hand, the heterogeneity of our sample may also be seen as a strength, as it better reflects real-life clinical situations. Finally, the most relevant outcomes for fertility preservation are pregnancy and live birth rates. However, since none of our patients have undergone ovarian tissue autotransplantation thus far, these parameters could not be used as primary outcomes.

Conclusions

Our study suggests that certain inflammation markers, specifically NLR, lymphocytes, MPV/PC and PLR may be correlated with ovarian FD in a subgroup of OTC cancer patients of intermediate age. However, these findings need to be confirmed by future studies. As to the influence of pathology type, BMI, smoking habits, and BRCA mutations, no significant correlation with FD was found in our study.

Abbreviations

ACF	Antral Follicle Count
AMH	Anti-Müllerian Hormone
AYAs	Adolescents and Young Adults
BMI	Body Mass Index
CBC	Complete Blood Count
EC	Embryo Cryopreservation
ET	Embryo Transfer
FD	Follicular Density
FSH	Follicle Stimulating Hormone
GC	Granulosa Cells
MPV	Mean Platelet Volume
NLR	Neutrophils to Lymphocytes Ratio
OC	Oocytes Cryopreservation
PC	Platelet Count
PCOS	Polycystic Ovary Syndrome
PLR	Platelet to Lymphocytes Ratio
POI	Premature Ovarian Insufficiency
OTC	Ovarian Tissue Cryopreservation
WCB	White Blood Cells

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Author contributions
L.R. contributed to data collection, conceptualization, and writing of this manuscript I.T. Provided critical insights into the data interpretation, prepared the tables A.M.L. Contributed to data collection and curation M.I. Contributed to data collection and curation M.F. Contributed with technical support N.S.B. Contributed to the review and editing of the manuscript F.S. Provided critical insights into the data interpretation C.M. Contributed to data collection and curation E.M. Contributed to patient recruitment M.C. Provided critical clinical insights E.V. provided guidance on study design and interpretation of results G.C. provided guidance on study design and interpretation of results.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
The present study was conducted in accordance with the Declaration of Helsinki. All enrolled patients signed an informed consent to the protocol approved by the Comitato Etico Centrale IRCCS Lazio Sezione IRCCS IFO – Fondazione G.B. Bietti (Experimentation Registry number 1514/21).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

Consent to participate
All patients enrolled in the study signed written consent for the collection, storage and use of biological material for research purposes.

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References

1. You L, Lv Z, Li C, Ye W, Zhou Y, Jin J, Han Q. Worldwide cancer statistics of adolescents and young adults in 2019: a systematic analysis of the global burden of disease study 2019. ESMO Open. 2021;6(5):100255.
2. Miller KD, Fidler-Benaoudia M, Keegan TH, Hipp HS, Jemal A, Siegel RL. Cancer statistics for adolescents and young adults, 2020. CA Cancer J Clin. 2020;70(6):443–59.
3. Bernasconi A, Barigelletti G, Tittarelli A, Botta L, Gatta G, Tagliabue G, Contiero P, Guzzinati S, Andreano A, Manneschi G, Falcini F, Castaing M, Filiberti RA, Gasparotti C, Cirilli C, Mazzucco W, Mangone L, Iacovacci S, Vitale MF, Stracci F, Piffer S, Tumino R, Carone S, Sampietro G, Melcarne A, Ballotari P, Boschetti L, Pisani S, Cavalieri D, Cuccaro F, D'Argenzio A, D'Orsi G, Fanetti AC, Ardizzone A, Candela G, Savoia F, Pascucci C, Castelli M, Storchi C, Trama A. Adolescent and young adult Cancer survivors: design and characteristics of the first nationwide Population-Based cohort in Italy. J Adolesc Young Adult Oncol. 2020;9(5):586–93.
4. Familiari G, Caggiati A, Nottola SA, Ermini M, Di Benedetto MR, Motta PM. Ultrastructure of human ovarian primordial follicles after combination chemotherapy for Hodgkin's disease. Hum Reprod (Oxford England). 1993;8(12):2080–7.
5. Wallace WHB, Thomson AB, Kelsey TW. The radiosensitivity of the human oocyte. Hum Reprod (Oxford England). 2003;18(1):117–21.
6. van Dorp W, Mulder RL, Kremer LCM, Hudson MM, van den Heuvel-Eibrink MM, van den Berg MH, Levine JM, van Dulmen-den Broeder E, di Iorgi N, Albanese A, Armenian SH, Bhatia S, Constine LS, Corrias A, Deans R, Dirksen U, Gracia CR, Hjorth L, Kroon L, Lambalk CB, Landier W, Levitt G, Leiper A, Meacham L, Mussa A, Neggers SJ, Oeffinger KC, Revelli A, van Santen HM, Skinner R, Toogood A, Wallace WH, Haupt R. Recommendations for Premature Ovarian Insufficiency Surveillance for Female Survivors of Childhood, Adolescent, and Young Adult Cancer: A Report From the International Late Effects of Childhood Cancer Guideline Harmonization Group in Collaboration With the PanCareSurFup Consortium. J. Clin. Oncol: official journal of the American Society of Clinical Oncology 2016, 34 (28), 3440–3450.
7. The ESHRE Guideline Group on POI, Webber L, Davies M, Anderson R, Bartlett J, Braat D, Cartwright B, Cifkova R, de Keizer-Schrama S, Hogervorst E, Janse F, Liao L, Vlaisavljevic V, Zillikens C, Vermeulen N. ESHRE Guideline: management of women with premature ovarian insufficiency, *Human Reproduction*, Volume 31, Issue 5, May 2016, 926–937.
8. Heritage SR, Feast A, Mourad M, Smith L, Hatcher H, Critoph DJ. Documentation of oncofertility communication in adolescents and young adults with cancer: A retrospective analysis. J Adolesc Young Adult Oncol. 2022;11(3):275–83.
9. Lambertini M, Peccatori FA, Demeestere I, Amant F, Wyns C, Stukenborg JB, Paluch-Shimon S, Halaska MJ, Uzan C, Meissner J, von Wolff M, Anderson RA, Jordan K, ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. Fertility preservation and post-treatment pregnancies in post-pubertal cancer patients: ESMO clinical practice Guidelines†. Ann Oncol. 2020;31(12):1664–78.
10. Donnez J, Dolmans M-M. Fertility preservation in women. Nat Rev Endocrinol. 2013;9(12):735–49.
11. Kristensen SG, Wakimoto Y, Colmorn LB, Dueholm M, Pors SE, Macklon KT, Mamsen LS, Nikiforov D, Cadenas J, Greve VH, Bay Bjørn AM, Rosendahl M, Pedersen AT, Nyboe Andersen A, Fedder J, Ernst E, Andersen CY. Use of cryopreserved ovarian tissue in the Danish fertility preservation cohort. Fertil Steril. 2021;116(4):1098–106.
12. Yding Andersen C, Mamsen LS, Kristensen SG. FERTILITY PRESERVATION: freezing of ovarian tissue and clinical opportunities. Reproduction. 2019;158(5):F27–34.
13. Donnez J, Martinez-Madrid B, Jadoul P, Van Langendonckt A, Demylle D, Dolmans MM. Ovarian tissue cryopreservation and transplantation: a review. Hum Reprod Update. 2006 Sep-Oct;12(5):519–35.
14. Donnez J, Dolmans M-M. Cryopreservation and transplantation of ovarian tissue. Clin Obstet Gynec. 2010;53(4):787–96.
15. Shapira M, Dolmans M-M, Silber S, Meirow D. Evaluation of ovarian tissue transplantation: results from three clinical centers. Fertil Steril. 2020;114(2):388–97.
16. Gellert SE, Pors SE, Kristensen SG, Bay-Bjørn AM, Ernst E, Yding Andersen C. Transplantation of Frozen-Thawed ovarian tissue: an update on worldwide activity published in Peer-Reviewed papers and on the Danish cohort. J Assist Reprod Genet. 2018;35(4):561–70.
17. Kim S, Lee Y, Lee S, Kim T. Ovarian tissue cryopreservation and transplantation in patients with cancer. Obstet Gynecol Sci. 2018;61(4):431–42.
18. Telfer EE, Andersen CY. In vitro growth and maturation of primordial follicles and immature oocytes. Fertil Steril. 2021;115(5):1116–25.
19. Rosendahl M, Schmidt KT, Ernst E, Rasmussen PE, Loft A, Byskov AG, Andersen AN, Andersen CY. Cryopreservation of ovarian tissue for a decade in Denmark: A view of the technique. Reprod Biomed Online. 2011;22(2):162–71.
20. Oktay K, Karlikaya G. Ovarian function after transplantation of frozen, banked autologous ovarian tissue. N Engl J Med. 2000;342(25):1919.
21. Oktay K, Oktem O. Ovarian cryopreservation and transplantation for fertility preservation for medical indications: report of an ongoing experience. Fertil Steril. 2010;93(3):762–8.
22. Aubard Y, Piver P, Cogni Y, Fermeaux V, Poulin N, Driancourt MA. Orthotopic and heterotopic autografts of frozen-thawed ovarian cortex in sheep. Hum Reprod. 1999;14(8):2149–54.
23. Kristensen SG, Liu Q, Mamsen LS, Greve T, Pors SE, Bjørn AB, Ernst E, Macklon KT, Andersen CY. A simple method to quantify follicle survival in cryopreserved human ovarian tissue. Hum Reprod. 2018;33(12):2276–84.
24. Lee J, Kong HS, Kim EJ, Youm HW, Lee JR, Suh CS, Kim SH. Ovarian injury during cryopreservation and transplantation in mice: A comparative study between cryoinjury and ischemic injury. Hum Reprod. 2016;31(8):1827–37.
25. Roness H, Gavish Z, Cohen Y, Meirow D. Ovarian follicle burnout: a universal phenomenon? Cell Cycle. 2013;12(20):3245–6.
26. Gavish Z, Peer G, Roness H, Cohen Y, Meirow DF. Activation and burn-out contribute to Post-Transplantation follicle loss in ovarian tissue grafts: the effect of graft thickness. Hum Reprod (Oxford England). 2014;29(5):989–96.
27. Gavish Z, Spector I, Peer G, Schlatt S, Wistuba J, Roness H, Meirow D. (2018). Follicle activation is a significant and immediate cause of follicle loss after ovarian tissue transplantation. J. Assist. Reprod. Genet 2018, 35(1), 61–69.
28. Practice Committee of the American Society for Reproductive Medicine. Electronic address: Asrm@asrm.org; practice committee of the American society for reproductive medicine. Testing and interpreting measures of ovarian reserve: a committee opinion. Fertil Steril. 2020;114(6):1151–7.
29. Schuh-Huerta SM, Johnson NA, Rosen MP, Sternfeld B, Cedars MI, Reijo Pera RA. Genetic variants and environmental factors associated with hormonal markers of ovarian reserve in Caucasian and African American women. Hum Reprod (Oxford England). 2012;27(2):594–608.
30. Tal R, Seifer DB. Potential Mechanisms for Racial and Ethnic Differences in Antimüllerian Hormone and Ovarian Reserve. Int. J. Endocrinol. 2013, 2013, 818912–818912.
31. Oh SR, Choe SY, Cho YJ. Clinical application of serum anti-Müllerian hormone in women. Clin Exp Reprod Med. 2019;46(2):50–9.
32. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, Griesinger G, Kelsey TW, La Marca A, Lambalk C, Mason H, Nelson SM, Visser JA, Wallace WH, Anderson RA. The physiology and clinical utility of Anti-Mullerian hormone in women. Hum Reprod Update. 2014;20(3):370–85.
33. Huang Y, Cheng Y, Zhang M, Xia Y, Chen X, Xian Y, Lin D, Xie S, Guo X. Oxidative stress and inflammatory markers in ovarian follicular fluid of women with diminished ovarian reserve during in vitro fertilization. J Ovarian Res. 2023;16(1):206.
34. Hong YH, Kim SK, Lee JR, Suh CS. Utility of blood markers for predicting outcomes of fertility preservation in patients with breast Cancer. Front Endocrinol. 2022;13:803803–803803.
35. Ozgu-Erdinc AS, Coskun B, Yorganci A, Hancerliogullari N, Yilmaz N, Engin-Ustun Y. The role of inflammatory hematological markers in predicting IVF success. JBRA Assist Reprod. 2021;25(1):71–5.
36. Ilhan G, Atmaca FFV, Altan E, Zebitay AG, Sozen H, Akyol H, Kurek Eken M. Evaluation of Neutrophil-Lymphocyte ratio, Platelet-Lymphocyte ratio and red blood cell distribution Width-Platelet ratio for diagnosis of premature ovarian insufficiency. J Family Reprod Health. 2016;10(4):211–6.
37. Lliberos C, Liew SH, Mansell A, Hutt KJ. The inflammasome contributes to depletion of the ovarian reserve during aging in mice. Front Cell Dev Biol. 2021;8:628473.
38. Zhao N, Zhang C, Ding J, Wu H, Cheng W, Li M, Zhu R, Li H. Altered T lymphocyte subtypes and cytokine profiles in follicular fluid associated with diminished ovary reserve. Am J Reprod Immunol. 2022;87(4):e13522–13522.
39. Zhao N, Zhang C, Wu Y, Ding J, Wang F, Cheng W, Li H, Zhu R. ROS-CCL5 axis recruits $CD8^+$ T lymphocytes promoting the apoptosis of granulosa cells in diminished ovary reserve. J Reprod Immunol. 2023;155:103789.
40. Boots CE, Jungheim ES. Inflammation and human ovarian follicular dynamics. Semin Reprod Med. 2015;33(4):270–5.
41. Michels N, van Aart C, Morisse J, Mullee A, Huybrechts I. Chronic inflammation towards cancer incidence: A systematic review and meta-analysis of epidemiological studies. Crit Rev Oncol Hematol. 2021;157:103177.

42. Balkwill F, Mantovani A. Inflammation and cancer: back to virchow?? Lancet. 2001;357(9255):539–45.
43. Vital Reyes VS, Téllez Velasco S, Hinojosa Cruz JC, Ortiz Romero Mde J, Chavarría Olarte ME, Reyes Fuentes A. Serum levels of IL-1beta, IL-6 and TNF-alpha in infertile patients with ovarian dysfunction]. Ginecol Obstet Mex. 2005;73(11):604–10.
44. Yang Z, Tang Z, Cao X, Xie Q, Hu C, Zhong Z, Tan J, Zheng Y. Controlling chronic low-grade inflammation to improve follicle development and survival. Am J Reprod Immunol. 2020;84(2):e13265.
45. Templeton AJ, Ace O, McNamara MG, Al-Mubarak M, Vera-Badillo FE, Hermanns T, Seruga B, Ocaña A, Tannock IF, Amir E. Prognostic role of platelet to lymphocyte ratio in solid tumors: a systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev. 2014;23(7):1204–12.
46. Oladipupo I, Ali T, Hein DW, Pagidas K, Bohler H, Doll MA, Mann ML, Gentry A, Chiang JL, Pierson RC, Torres S, Reece E, Taylor KC. Association between cigarette smoking and ovarian reserve among women seeking fertility care. PLoS ONE. 2022;17(12).
47. Dólleman M, Verschuren WM, Eijkemans MJ, Dollé ME, Jansen EH, Broekmans FJ, van der Schouw YT. Reproductive and lifestyle determinants of anti-Müllerian hormone in a large population-based study. J Clin Endocrinol Metab. 2013;98(5):2106–15.
48. Li YL, Yan EQ, Zhao GN, Jin L, Ma BX. Effect of body mass index on ovarian reserve and ART outcomes in infertile women: a large retrospective study. J Ovarian Res. 2024;17(1):195.
49. Freour T, Masson D, Mirallie S, Jean M, Bach K, Dejoie T, Barriere P. Active smoking compromises IVF outcome and affects ovarian reserve. Reprod Biomed Online. 2008;16(1):96–102.
50. Freour T, Masson D, Dessolle L, Allaoua D, Dejoie T, Mirallie S, Jean M, Barriere P. Ovarian reserve and in vitro fertilization cycles outcome according to women smoking status and stimulation regimen. Arch Gynecol Obstet. 2012;285(4):1177–82.
51. Waylen AL, Jones GL, Ledger WL. Effect of cigarette smoking upon reproductive hormones in women of reproductive age: a retrospective analysis. Reprod Biomed Online. 2010;20(6):861–5.
52. Nardo LG, Christodoulou D, Gould D, Roberts SA, Fitzgerald CT, Laing I. Anti-Müllerian hormone levels and antral follicle count in women enrolled in in vitro fertilization cycles: relationship to lifestyle factors, chronological age and reproductive history. Gynecol Endocrinol. 2007;23(8):486–93.
53. Lambertini M, Goldrat O, Toss A, Azim HA Jr, Peccatori FA, Ignatiadis M, Del Mastro L, Demeestere I. Fertility and pregnancy issues in BRCA-mutated breast cancer patients. Cancer Treat Rev. 2017;59:61–70. https://doi.org/10.1016/j.ctrv.2017.07.001.
54. Lambertini M, Goldrat O, Ferreira AR, Dechene J, Azim HA Jr, Desir J, Delbaere A, de t'Kint MD, de Azambuja E, Ignatiadis M, Demeestere I. Reproductive potential and performance of fertility preservation strategies in BRCA-mutated breast cancer patients. Ann Oncol. 2018;29(1):237–43.
55. Michaelson-Cohen R, Mor P, Srebnik N, Beller U, Levy-Lahad E, Eldar-Geva T. BRCA mutation carriers do not have compromised ovarian reserve. Int J Gynecol Cancer. 2014;24(2):233–7.
56. Wang ET, Pisarska MD, Bresee C, Chen YD, Lester J, Afshar Y, Alexander C, Karlan BY. BRCA1 germline mutations May be associated with reduced ovarian reserve. Fertil Steril. 2014;102(6):1723–8.
57. Ben-Aharon I, Levi M, Margel D, Yerushalmi R, Rizel S, Perry S, Sharon E, Hasky N, Abir R, Fisch B, Tobar A, Shalgi R, Stemmer SM. Premature ovarian aging in *BRCA* carriers: a prototype of systemic precocious aging? Oncotarget. 2018;9(22):15931–41.
58. Gunnala V, Fields J, Irani M, D'Angelo D, Xu K, Schattman G, Rosenwaks Z. BRCA carriers have similar reproductive potential at baseline to noncarriers: comparisons in cancer and cancer-free cohorts undergoing fertility preservation. Fertil Steril. 2019;111(2):363–71.

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