Prognostic markers of inflammation in endometrioid and clear cell ovarian cancer

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HIGHLIGHTS

⇒ Intraepithelial CD8+ and CD3+ tumor infiltrating lymphocytes are prognostic markers in endometrioid and clear cell ovarian carcinomas.
⇒ Intratumoral CD3+ and CD8+ tumor infiltrating lymphocytes have prognostic value in endometrioid ovarian cancer, but not in clear cell ovarian cancer.
⇒ Systemic inflammation is not associated with survival in these subtypes of ovarian cancer

ABSTRACT

Objectives Cancer-related systemic inflammation has been associated with prognosis in multiple cancer types. Conversely, local inflammation, which is characterized by dense intratumoral immune infiltrates, is a favorable predictor of survival outcome. However, these survival associations are not well established in ovarian cancer, particularly in the less frequent endometrioid and clear cell endometriosis associated histotypes.

Methods This retrospective study included 119 patients (63 endometrioid and 56 clear cell ovarian carcinomas). We performed a comprehensive survival association analysis of both systemic (neutrophil-to-lymphocyte ratio or presence of endometriosis) and local inflammation markers (CD3+ and CD8+ tumor infiltrating lymphocytes) using multivariate Cox proportional hazards models that account for confounding factors.

Results Medium to high levels of intraepithelial CD8+ tumor infiltrating lymphocytes are associated with longer survival in endometrioid ovarian cancer (p=0.04). In addition, we found that intraepithelial CD8+ tumor infiltrating lymphocytes are prognostic in clear cell ovarian cancer (p=0.02), and that intraepithelial CD3+ tumor infiltrating lymphocytes are also associated with improved outcome (p=0.02). Furthermore, intratumoral CD3+ and CD8+ tumor infiltrating lymphocytes showed improved prognosis in the endometrioid subtype (p<0.1). No prognostic value was observed for systemic immune markers.

Conclusions In this study, patients with endometrioid and clear cell ovarian cancer with moderate to high CD8+ and CD3+ intraepithelial tumor infiltrating lymphocytes had longer overall survival. Higher expression of intratumoral CD3+ and CD8+ tumor infiltrating lymphocytes also showed an improved outcome in endometrioid ovarian cancer. In contrast, systemic inflammation, evaluated by neutrophil-to-lymphocyte ratio or presence of endometriosis, did not have a prognostic impact in these histologic subtypes.

INTRODUCTION

Ovarian cancer is the second most common and the deadliest gynecological tumor in developed countries. It is categorized into five major histologic subtypes: high-grade serous, endometrioid, clear cell, low-grade serous, and mucinous. Despite endometrioid and clear cell ovarian cancer having distinct histopathological, clinical, molecular, and genetic features and, in the case of the clear cell sub-type, relative resistance to chemotherapy, both are currently treated the same way as all other subtypes. Surgery and platinum-based chemotherapy are the cornerstone of treatment and the 10-year overall survival has remained relatively unchanged in decades.[1]

More prognostic and predictive markers are therefore needed to improve clinical management of these subtypes, especially in localized stages where there are doubts about the effectiveness of adjuvant chemotherapy.[2] *BRCA1* and *BRCA2* mutations and general deficiency in the homologous recombination system have been associated with both prognostic and predictive value in ovarian cancer. However, these mutations are mainly observed in high-grade serous ovarian cancer and rarely described in endometrioid and clear cell ovarian cancer.[3] Recent efforts to search for novel features of clinical utility in ovarian cancer have been directed to determining the role of immune response activation, which has been shown to play a role in different tumor types.[4]

The association between survival and systemic inflammatory factors such as the neutrophil-to-lymphocyte ratio or endometriosis is not clear.[5 6] This is particularly relevant to endometrioid and clear cell ovarian carcinomas, since endometriosis is the proposed precursor lesion associated with both histologies. Endometriosis, which is present in 10% of

Table 1 Main clinical and pathological characteristics of the patients

Variables	Localized stages (I–II)			Advanced stages (III–IV)		
	All	EOC	CCOC	All	EOC	CCOC
No of patients	84 (70.6%)	50 (59.5%)	34 (40.5%)	35 (29.4%)	13 (37.1%)	22 (62.9%)
Median age in years (range)	51.4 (29–82)	51.3 (34–82)	52.5 (29–82)	57.2 (33–86)	61.6 (33–76)	56.9 (35–86)
Endometriosis	48 (57.1%)	27 (54%)	21 (61.8%)	16 (45.7%)	4 (30.8%)	12 (54.5%)
Residual disease	0	0	0	10 (28.6%)	3 (23.1%)	7 (31.8%)
<1 cm	0	0	0	2 (5.7%)	1 (7.7%)	1 (4.5%)
>1 cm	0	0	0	8 (22.8%)	2 (15.4%)	6 (27.3%)

CCOC, clear cell ovarian carcinoma; EOC, endometrioid ovarian carcinoma.

women worldwide, seems to activate the immune system due to the inflammatory response led by endometriosis implants.[7] Interestingly, it has recently been observed that endometriosis-associated ovarian cancer has a lower amount of infiltrating T lymphocytes and higher levels of PD-1/PD-L1 expression than benign endometriosis-related diseases, including ovarian endometriosis, which could have a role in the carcinogenesis process.[8] Patients with endometriosis have an increased risk of endometrioid and clear cell ovarian cancer,[9] but endometriosis-associated ovarian cancer may be associated with a better prognosis[10] and could be a separate entity with different histopathological features.[11] However, the association between endometriosis and longer survival remains controversial[6] as it could be the effect of an endometriosis connection with favorable characteristics, such as early stage and low grade. Peripheral blood-derived inflammation-based markers such as the neutrophil-to-lymphocyte ratio have also been related to cancer initiation and progression and high neutrophil-to-lymphocyte ratio values have been linked with worse outcomes in different cancers.[12 13] However, the predictive value of the neutrophil-to-lymphocyte ratio in ovarian cancer remains uncertain.[5]

There is increasing evidence of immune activation in ovarian tumors and the value of immune markers in predicting outcome. High CD3+/CD8+ tumor infiltrating lymphocytes, markers of local inflammation, have been linked to better overall survival in high-grade serous ovarian cancer.[14] The largest study conducted to date by the Ovarian Tumor Tissue Analysis Consortium showed a dose–response association of CD8+ tumor infiltrating lymphocytes and survival time in high-grade serous, endometrioid, and mucinous ovarian cancer but not in the clear cell subtype.[15] A large meta-analysis conducted in 2012 concluded that high levels of intraepithelial CD8+ tumor infiltrating lymphocytes showed a stronger association with a better overall survival than CD3+ tumor infiltrating lymphocytes, with no difference regarding stage, grade, or histologic subtype.[14] However, a gross distinction between stages (III/IV vs mixed stages) and histology (serous vs mixed histology) was made in this study.

In this context, we aimed to define the prognostic role of the immune response activation in patients with endometrioid and clear cell ovarian cancer by comprehensively evaluating markers of systemic (neutrophil-to-lymphocyte ratio and endometriosis) and local (CD8+ and CD3+ tumor infiltrating lymphocytes) inflammation in multivariate models that account for confounding factors.

METHODS

Patient Selection and Clinical Variables

This was a single-institution, retrospective, and observational study. We included all patients with histologically confirmed endometrioid and clear cell ovarian cancer, diagnosed from November 1992 to December 2013 at La Paz University Hospital, Madrid (Spain), with a minimum follow-up of 5 years. Exclusion criteria were patients with unknown stage, mixed histologic subtypes, or doubts about the possibility of ovarian metastasis of a synchronous endometrial cancer diagnosis. In order to control factors that can influence systemic inflammation independently of cancer and avoid possible bias, we confirmed that none of the patients had received previous immunotherapy or suffered an autoimmune disease with immunosuppressive treatment. In addition, we also excluded patients with a recent infection.

Patients were selected using the Pathology Department information management system. A total of 150 patients were initially selected for the study, but 31 patients were excluded (reasons are detailed in Online Supplemental Figure 1). Finally, 119 patients were included in the study. Ninety patients (75.6%) had tumor tissue available. Tissue samples of the tumor were collected at the time of initial debulking surgery. In those cases not candidates for initial surgery, we collected the tumor from the biopsy performed to confirm the diagnosis. This study was approved by the Ethics Committee of La Paz University Hospital (PI-2634) and was conducted in accordance with ethical standards of the Helsinki Declaration of the World Medical Association.

All histopathologic diagnoses were reviewed by an expert gynecological pathologist. The diagnostic test also included the immunohistochemical study of WT1 (clone 6 F-H2, Agilent Technologies #M3561, Glostrup, Denmark), p53 (clone DO-7, Agilent Technologies #GA616), Napsin A (clone TMU-Ad 02, Biocare Medical #CM388 CK, Pacheco, USA), and progesterone receptor (clone PgR1294, Agilent Technologies #GA090), the complete minimum four-marker panel for typing ovarian cancer according to current diagnostic criteria.[13] According to the gynecological pathologist revision, 63 tumors were endometrioid (53.7%) and 56 were clear cell ovarian carcinomas (46.3%). The main clinicopathological characteristics of the patients are summarized in Table 1.

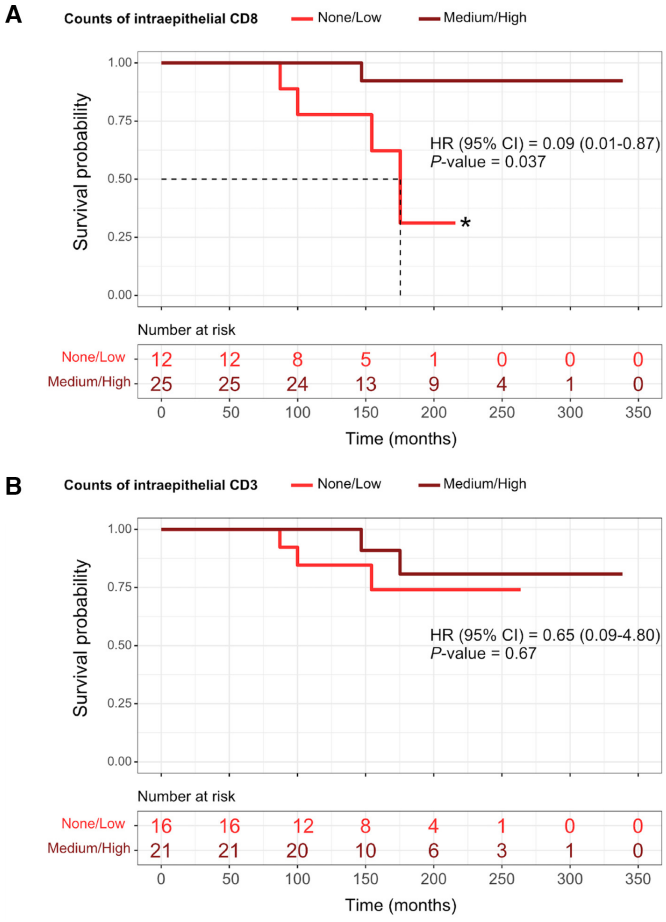

Figure 1 Overall survival curves for endometrioid ovarian cancer according to levels of intraepithelial tumor infiltrating lymphocytes. Kaplan–Meier curves showing the overall survival of patients with endometrioid ovarian cancer split into two groups according to their levels of (A) intraepithelial CD8+ tumor infiltrating lymphocytes and (B) intra-epithelial CD3+ tumor infiltrating lymphocytes. Hazard ratios (HR) were obtained from a Cox proportional hazards model adjusted for age. Asterisks denote significant correlations.

Evaluation of systemic inflammation: assessment of endometriosis and neutrophil-to-lymphocyte ratio

The presence of endometriosis was evaluated by a histological review performed by an expert gynecological pathologist. The peripheral blood-derived inflammation marker neutrophil-to-lymphocyte ratio was calculated before treatment (surgical or neoadjuvant chemotherapy) by dividing the absolute neutrophil count by the absolute lymphocyte count. For each specific histopathological subtype (endometrioid and clear cell ovarian cancer), we used the median neutrophil-to-lymphocyte ratio for stratifying patients as 'low neutrophil-to-lymphocyte ratio' or 'high neutrophil-to-lymphocyte ratio'. The median neutrophil-to-lymphocyte ratio was 3.5 (range 1.1–12.47) and 3.3 (range 1.27–7.5) in endometrioid and clear cell ovarian carcinoma, respectively.

Evaluation of local inflammation: tumor infiltrating lymphocyte immunostaining and scoring

We evaluated local inflammation in 90 patients with available tumor blocks. Local inflammation was evaluated by measuring CD3+ (CD3 Polyclonal Agilent Technologies, #GA503) and CD8+ (cloneC8/144B Agilent Technologies, #IR623) infiltrating lymphocytes at two different locations: intra-tumoral (stromal+intraepithelial) and intraepithelial (within the tumor islet). Hematoxylin and eosin-stained sections were used to guide the selection of a representative region of each area on individual paraffin blocks. Two tissue cores (1 mm in diameter) were obtained from each area and were arrayed into a receptor paraffin block using a tissue microarray workstation (Beecher Instruments, Silver Spring, USA). The tissue microarrays were then used to evaluate all the immunochemistry markers.

Infiltrating lymphocytes were counted as previously described by the Ovarian Tumor Tissue Analysis Consortium,[15] screening each tumor core for a hotspot of the immunostained tumor infiltrating lymphocytes. From each hotspot an area of 0.55 mm was selected and evaluated at 400× magnification to guarantee comparability between samples. CD3+ and CD8+ tumor infiltrating lymphocytes were quantified as the total number of immunolabeled lymphocytes (from 0 to >100) at each core. We then applied the Ovarian Tumor Tissue Analysis criteria[15] for classifying tumor samples in four categories as follows: negative (none), low (1–2 tumor infiltrating lymphocytes), moderate (3–19 tumor infiltrating lymphocytes), and high (≥20 tumor infiltrating lymphocytes). Following the Ovarian Tumor Tissue Analysis procedures, the maximum score of two evaluated cores per tumor was considered for subsequent analysis. For survival analysis we divided the samples into two groups: none or low infiltration of intraepithelial CD8+ tumor infiltrating lymphocytes; and moderate or high infiltration of intraepithelial CD8+ tumor infiltrating lymphocytes.

Statistical analysis

Statistical analyses were performed using the R software and clinical data were managed using Excel (Microsoft, Redmond, USA). Overall survival was defined as the time from the beginning of treatment to death or last follow-up day (January 2021). To describe the data collected we used sample size and frequency (%) for categorical variables. A χ^2 test (function *xtabs* from the *stats* (v.3.6.2) R package) was used for comparison of categorical variables among groups. Kaplan–Meier models were used for visually presenting survival curves. Univariate Cox proportional hazards models were used for assessing the association between overall survival and individual clinicopathological characteristics. Multivariate Cox proportional hazards models were used for assessing the association between overall survival and immune markers. As a standard control to test the robustness of our cohort for overall survival analysis, we assessed the association of well-established variables influencing ovarian overall survival including age, stage (I/II vs III/IV), and residual disease (none/<1 cm vs >1 cm) (Online Supplemental Material). Given the association of these variables with overall survival, we adjusted for these variables in our multivariate analysis. Due to the limited sample size of advanced endometrioid ovarian carcinomas, in particular after stratification of tumor infiltrating lymphocytes category, the numbers were not adequate to perform multivariate Cox proportional hazards survival analysis. Therefore, the prognostic value of immune markers was assessed by including only early-stage endometrioid ovarian carcinomas. Since none of the early-stage endometrioid ovarian carcinomas had residual disease (Table 1), we only adjusted by age in

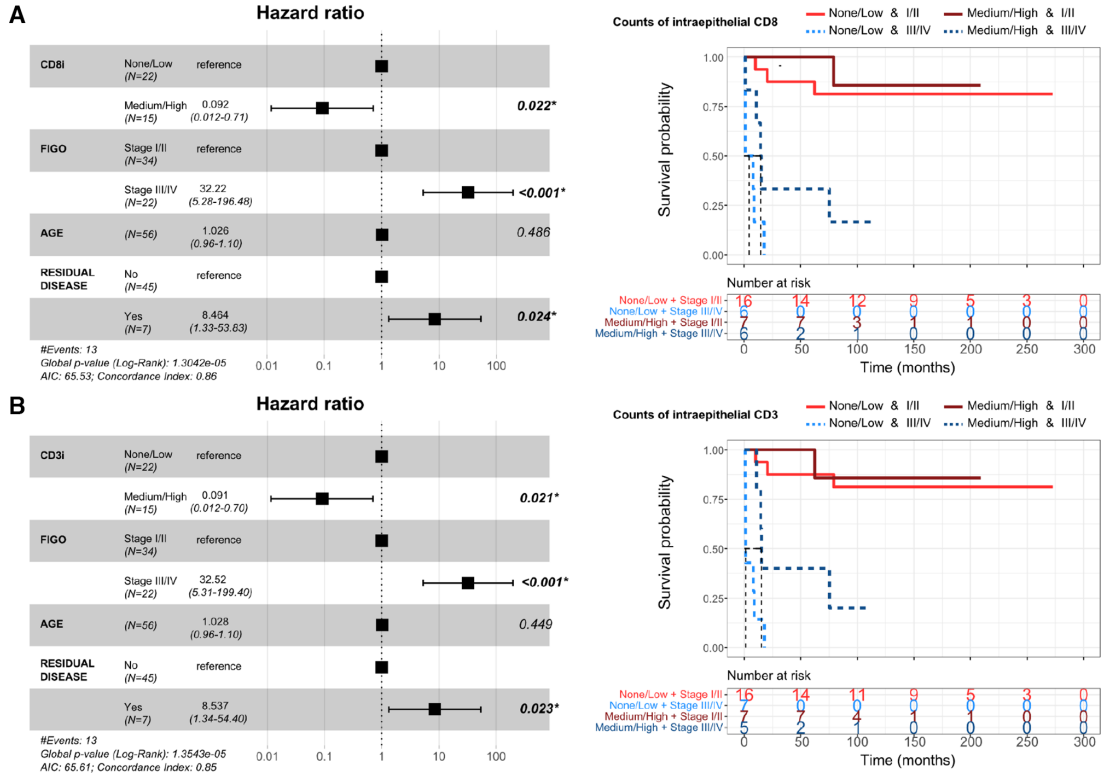

Figure 2 Performance of intraepithelial tumor infiltrating lymphocytes as a prognostic marker in clear cell ovarian cancer. Hazard ratios (HR) obtained from a multivariate Cox proportional hazards model predicting overall survival in patients with clear cell ovarian cancer according to levels of (A) intraepithelial CD8+ tumor infiltrating lymphocytes (CD8i) or) (B) intraepithelial CD3+ tumor infiltrating lymphocytes (CD3i). In both cases models were adjusted for age, residual disease, and disease stage. Asterisks denote significant correlations.

this tumor histotype. For clear cell ovarian carcinomas, the analysis comprised early and advanced cases, 32% with residual disease (Table 1), and we adjusted by age, stage, and residual disease. Kaplan–Meier estimates (function survfit) and Cox proportional hazards models (function coxph) were performed using the survival (v3.1–11) R package. Two-sided p values <0.05 were considered statistically significant for two-sample comparison tests, while a significant threshold of 0.1 was applied for multivariate models. In accordance with the journal's guidelines our data are available in a public, open access repository.[16]

RESULTS

Median age at diagnosis was 54.3 years (range 29–86). Eighty-four patients (70.6%) had stage I–II disease and 35 (29.4%%) stage III–IV. The association of clinical variables with overall survival are shown in the Online Supplemental Material. A median follow-up time of 10.03 years allowed us to perform a robust association analysis between markers of inflammation and overall survival. The cohort also contained sufficient patient numbers to observe robust associations for early-stage disease in endometrioid ovarian cancer and both early-stage and advanced disease in the clear cell subtype (Table 1).

Systemic inflammation is not associated with overall survival

We explored systemic inflammation by examining the presence or absence of endometriosis in tumors. A total of 64 cases (53.8%) were diagnosed as having endometriosis, 31 (49.2%) of which were endometrioid and 33 (58.9%) clear cell ovarian carcinomas (Table 1). We did not find statistical differences in the presence or absence of endometriosis according to histological subtypes (χ^2 test, p=0.29) or stage (χ^2 test, p=0.25). Overall survival analysis indicated that endometriosis did not have a prognostic value for localized endometrioid (Cox proportional hazards model adjusted for age, p=0.68) or clear cell ovarian carcinomas (Cox proportional hazards model adjusted for age, residual disease and stage, p=0.83) (see Online Supplemental Table 1).

Systemic inflammation was also analyzed using the neutrophil-to-lymphocyte ratio. Samples were classified as having a low or high neutrophil-to-lymphocyte ratio according to the median value of the specific histologic type analyzed. A total of 22 (50%) patients with clear cell ovarian cancer and 27 (50%) with endometrioid ovarian cancer were classified as having a high neutrophil-to-lymphocyte ratio, of which 12 (55%) and 6 (22%) were advanced tumors, respectively. We did not find any association between the neutrophil-to-lymphocyte ratio and overall survival for either localized endometrioid ovarian cancer (Cox proportional hazards model adjusted for age, p=0.58) or clear cell ovarian carcinoma (Cox proportional hazards model adjusted for age, residual disease and stage, p=0.50) (see Online Supplemental Table 1).

High intraepithelial lymphocyte counts indicate better outcome

We first assessed the role of intraepithelial CD8+ tumor infiltrating lymphocytes in overall survival across our cohort. Using the same scoring criteria as the Ovarian Tumor Tissue Analysis study, we

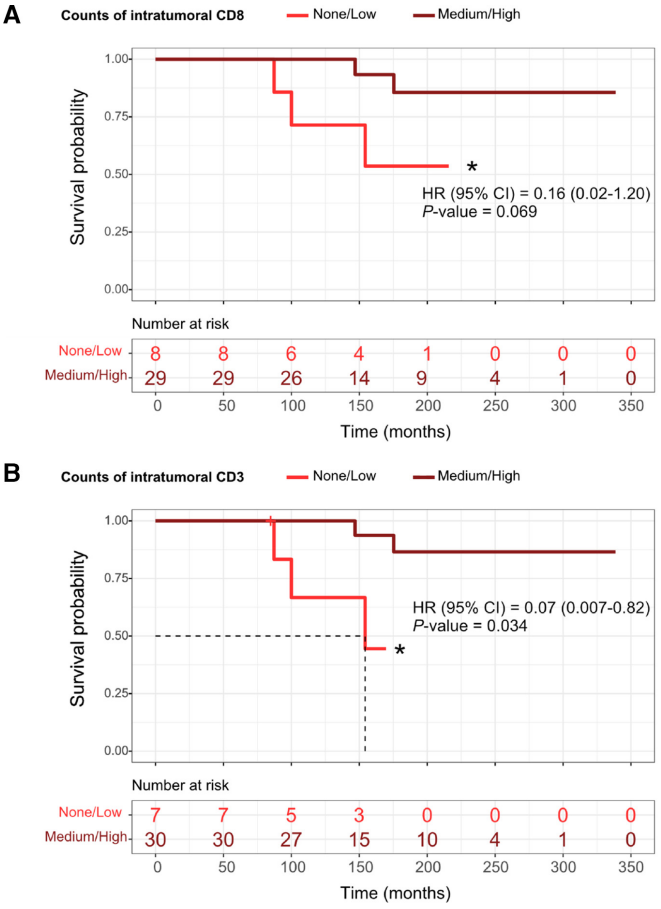

Figure 3 Overall survival curves for endometrioid ovarian cancer according to levels of intratumoral tumor infiltrating lymphocytes. Kaplan–Meier curves showing the overall survival of patients with endometrioid ovarian cancer split into two groups according to their levels of (A) intratumoral CD8+ tumor infiltrating lymphocytes and (B) intratumoral CD3+ tumor infiltrating lymphocytes. Hazard ratios (HR) were obtained from a Cox proportional hazards model adjusted for age. Asterisks denote significant correlations.

found that infiltration levels of intraepithelial CD8+ were significantly higher in endometrioid compared with clear cell ovarian carcinomas (χ^2 test, p=0.018, Online Supplemental Table 2).

We observed that moderate to high levels of intraepithelial CD8+ tumor infiltrating lymphocytes were associated with longer overall survival in localized endometrioid ovarian carcinomas after adjusting for age (p=0.037, Figure 1A). In addition, moderate to high levels of intraepithelial CD8+ tumor infiltrating lymphocytes were also correlated with a good prognosis in clear cell ovarian cancer regardless of stage, age, and residual disease (p=0.022, Figure 2A).

In addition, we evaluated the prognosis value of intraepithelial CD3+ tumor infiltrating lymphocyte levels in endometrioid and clear cell ovarian carcinomas. For scoring infiltration levels and classifying tumors, we also applied the Ovarian Tumor Tissue Analysis criteria. The majority of endometrioid ovarian carcinomas (n=32, 65%) had moderate to high levels of intraepithelial CD3+ tumor infiltrating lymphocytes, significantly higher than the proportion observed in clear cell ovarian cancer cases (n=13 (35%); χ^2 test, p=0.032; see Online Supplemental Table 2).

Survival analysis showed that patients with clear cell ovarian cancer with moderate to high levels of intraepithelial CD3+ tumor infiltrating lymphocytes survived longer regardless of stage, age, and residual disease status (p=0.021, Figure 2B). This association was not observed for localized endometrioid ovarian carcinomas adjusted by age (p=0.67, Figure 1B).

High intratumoral CD3+ lymphocyte counts indicate improved outcomes for endometrioid ovarian cancer

The scoring criteria above only consider tumor infiltrating lymphocytes within the epithelial component of the tumor (tumor islets), not immune cells localized within the stromal component of the tumor or even touching tumor cells.[15] Given the technical difficulty of discriminating tumor infiltrating lymphocytes localized at the tumor edge touching the stroma, we decided to assess the prognostic value of counting all (epithelial and stromal) CD8+ and CD3+ tumor infiltrating lymphocytes present in the selected area (which was delimited in the same way as indicated by the Ovarian Tumor Tissue Analysis Consortium for evaluating intraepithelial tumor infiltrating lymphocytes). If informative, this protocol could be more easily implemented in the clinical setting since, once defined as an immune hotspot in a tumor area, the expert pathologist would not have to carefully differentiate stromal and tumorous tumor infiltrating lymphocytes but rather count them all.

Moderate to high levels of intratumoral CD8+ tumor infiltrating lymphocytes were associated with a good prognosis in endometrioid ovarian carcinomas (Cox proportional hazards model adjusted for age, p=0.069; Figure 3A). However, the levels of intratumoral CD8+ tumor infiltrating lymphocytes were not correlated with overall survival in clear cell ovarian carcinomas (Cox proportional hazards model adjusted for age, residual disease, and stage, p=0.75; Figure 4A). Intratumoral CD3+ tumor infiltrating lymphocytes also had no prognostic value for clear cell ovarian cancer (Cox proportional hazards model adjusted for age, residual disease, and stage, p=0.18; Figure 4B). However, moderate to high levels of intratumoral CD3+ tumor infiltrating lymphocytes were associated with improved overall survival in localized endometrioid ovarian cancer after adjusting for age (p=0.034, Figure 3B).

DISCUSSION

Summary of Main Results

In this study we found that systemic inflammation inferred by the presence of endometriosis or neutrophil-to-lymphocyte ratio is not associated with overall survival in endometrioid and clear cell ovarian cancer. We also found that CD8+ intraepithelial tumor infiltrating lymphocytes and also CD3+ intraepithelial infiltrates are associated with outcome, both in endometrioid and clear cell ovarian carcinomas. Additionally, we found the survival value of tumor infiltrating lymphocytes in different locations including T-cell infiltrates in the intraepithelial and the intratumoral component of tumors. We observed that CD8+ and CD3+ intratumoral tumor infiltrating lymphocytes were prognostic in endometrioid ovarian cancer. However, we did not find an association between CD8+ intratumoral tumor infiltrating lymphocytes and survival in clear cell ovarian cancer.

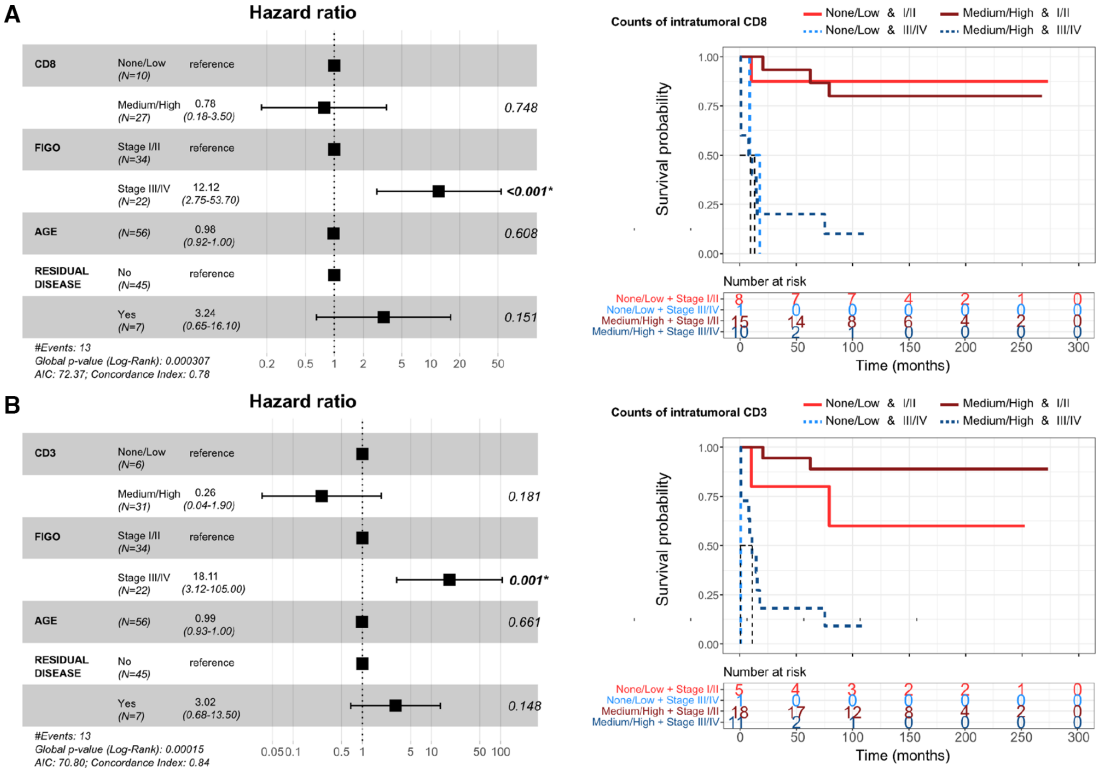

Figure 4 Performance of intratumoral tumor infiltrating lymphocytes as a prognostic marker in clear cell ovarian cancer. Hazard ratios (HR) obtained from a multivariate Cox proportional hazards model predicting overall survival in patients with clear cell ovarian cancer according to levels of (A) intratumoral CD8+ tumor infiltrating lymphocytes and (B) intratumoral CD3+ tumor infiltrating lymphocytes. In both cases, models were adjusted for age, residual disease, and disease stage. Asterisks denote significant correlations.

Results in the Context of Published Literature

The results of our study regarding the association of endometriosis and low neutrophil-to-lymphocyte ratio with better outcome in ovarian cancer may reflect the effects of confounders and are in line with prior reports.[5]

In agreement with the largest study to date conducted by the Ovarian Tumor Tissue Analysis Consortium, we observed that intraepithelial CD8+ tumor infiltrating lymphocytes are associated with longer survival in endometrioid ovarian cancer. In addition, we also found a survival benefit in clear cell ovarian cancer not reported by the Ovarian Tumor Tissue Analysis Consortium. High homogeneity of clinical data and the use of the full minimum four-marker reference immunochemistry panel for differential diagnosis in ovarian cancer,[17] including WT1, TP53, Napsin and progesterone receptor staining, may explain this difference. The prognostic value of CD8+ intraepithelial tumor infiltrating lymphocytes may assist in the stratification of patients with clear cell ovarian cancer and could have clinical implications for treatment decision making. In particular, this prognostic information could give us additional data to decide about adjuvant chemotherapy in early-stage clear cell ovarian cancer, treatment with a controversial benefit in these tumors.[2]

The association of CD8+ and CD3+ intraepithelial tumor infiltrating lymphocytes with a better outcome is in agreement with prior reports indicating prognostic value for both markers in ovarian cancer,[18–20] although there are studies pointing to a stronger survival association with CD8+ intra-epithelial tumor infiltrating lymphocytes.[15] A larger series, with assessment of both infiltrates in parallel, with homogeneous technical procedures and scoring criteria will be needed to robustly determine the most informative marker for clinical implementation.

Strengths and Weaknesses

The strengths of this study include a comprehensive data collection of 119 patients of less frequent ovarian cancer histologies from a single-institution cohort and the review of all histopathologic diagnoses by an expert gynecological pathologist. Furthermore, the recommended four-marker immunohistochemical diagnostic study was carried out in each case. Overall, these strengths ensure the consistency of the results of our study. However, the retrospective design and the modest number of patients are the main weaknesses. Another limitation is the use of tissue microarrays instead of whole slide analysis, as tumor cores may not be representative of the whole specimen. Nevertheless, each core was obtained from areas selected by an expert gynecological pathologist, which included different locations in order to have a better tumor representation. In addition, tissue microarrays were also used in the largest work that evaluated tumor infiltrating lymphocytes in ovarian cancer.[15] On the other hand, there is a limitation regarding the best cut-off point for neutrophil-to-lymphocyte ratio. Published studies have used different cut-off values: median, long-rank test, interquartile level, or the most statistically significant value for their patients. In the meta-analysis by Yin et al involving 10 studies and 2919 patients with ovarian cancer, the authors found statistically significant differences between a higher neutrophil-to-lymphocyte ratio and worse overall survival. However, each study

used a different cut-off value, ranging between 2 and 3.6, and it was pointed out that this variability might reduce the clinical applicability of the neutrophil-to-lymphocyte ratio.[5] Finally, our results have not been validated in an external cohort of patients.

Implications for Practice and Future Research

Given the complexity of scoring tumor infiltrating lymphocytes exclusively within the tumor islet (which involves disregarding tumor infiltrating lymphocytes in the stroma or abutting tumor cells), our finding, if further validated, may help to simplify the reporting of clinical tumor infiltrating lymphocytes, at least for endometrioid ovarian cancer. In this regard, it could be worthwhile to evaluate the assessment of tumor infiltrating lymphocytes by using digital pathology and artificial intelligence algorithms applied to tissue analysis.[21] Furthermore, the subtype-specific survival value of CD3+ intratumoral tumor infiltrating lymphocytes in endometrioid ovarian cancer may reflect the interplay of T-cells infiltrating the stroma and other histotype-specific factors that modulate the local inflammatory response. In this sense, it will be important to define clinical, histopathological, and molecular factors dictating the different abundance of tumor infiltrating lymphocytes across histotypes and also those elements responsible for differences in the immunosuppressive capacity of the tumor microenvironment.[22] This knowledge will be key to understanding and increasing the so far limited success of immunotherapy in ovarian cancer. To date, several phase III trials have published negative results with immune checkpoint inhibitors in front line (JAVELIN 100 and IMAGYN 050)[23 24] and relapse (JAVELIN 200),[25] and various ongoing phase III trials are exploring immune checkpoint inhibitors in front line (ATHENA, FIRST, ENGOT-ov43 and DUO-O) or relapse (ANITA, ATALANTE and AGO-OVAR 2.29), in combination with PARP inhibitors and/or bevacizumab. Globally, it seems that immunotherapy is not going to have a high efficacy in ovarian cancer, but it could perhaps benefit biomarker-selected patients such as those with tumors with a high expression of tumor infiltrating lymphocytes or PD-L1 (as hypothesized in the sub-group analysis of JAVELIN 200 and IMAGYN 050 trials). Larger prospective studies are needed to confirm these results in order to shed light on the clinical, histopathological, and molecular determinants behind tumor infiltrating lymphocyte infiltrates and to establish their additional predictive value in both histotypes.

CONCLUSION

This study confirms the association of moderate to high CD8+ intraepithelial tumor infiltrating lymphocytes with longer survival in endometrioid ovarian carcinomas. In addition, we provide evidence that this is also true for clear cell ovarian cancer and that tumor infiltrating lymphocytes in the epithelial compartment and also in the tumorous stroma are prognostic in endometrioid ovarian carcinomas.

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Competing interests AG reports honoraria and advisory/consultancy (Pharmamar), travel/accommodation/expenses (Roche, Tesaro-GSK, Pierre-Fabre, Pharmamar, MSD), speakers bureau (Roche, Clovis, Astra Zeneca, MSD) outside the submitted work. MM reports having received honoraria (MSD, AstraZeneca and GSK), research grant/funding to her institution (Eisai and PharmaMar), and travel/accommodation/expenses (AstraZeneca, GSK, PharmaMar, Roche and Pfizer) outside the submitted work. GM is a founder, director and shareholder of Tailor Bio Ltd, a genomics company using copy number signatures for precision medicine. AR reports having received honoraria and providing advisory/consultancy services (MSD, AstraZeneca, Roche, GSK, Clovis, PharmaMar, Amgen), research grant/funding to his institution (Eisai, PharmaMar, Roche), travel/accommodation/expenses (AstraZeneca, Tesaro: A GSK Company, PharmaMar, Roche), and participating in a speakers bureau (MSD, AstraZeneca, Roche, GSK, Clovis, PharmaMar) outside the submitted work.

Patient consent for publication Not applicable.

Ethics approval This study was approved by the Ethics Committee of La Paz University Hospital (PI-2634).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository. All code and data to reproduce the analyses found in this paper can be found in the following repository: https://github.com/macintyrelab/InmflammationMarkers.

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