ORIGINAL ARTICLE: ENDOMETRIOSIS

Infiltrating T lymphocytes and programmed cell death protein-1/programmed death-ligand 1 expression in endometriosis-associated ovarian cancer

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Objective: To characterize T lymphocyte infiltration and programmed cell death protein-1 (PD-1)/programmed death-ligand 1 (PD-L1) expression in early-stage endometriosis-associated ovarian cancer (EAOC), ovarian endometriosis (OE), atypical endometriosis (AE), and deep endometriosis (DE).
Design: Case-control, retrospective study.
Setting: Research University Hospital.
Patient(s): A total of 362 patients with a histologic diagnosis of EAOC, OE, AE, or DE were identified between 2000 and 2019 from Fondazione Policlinico Universitario Agostino Gemelli IRCCS and Gemelli Molise SpA tissue data banks. A 1:1 propensity score-matched method yielded matched pairs of 55 subjects with EAOC, 55 patients with OE, 12 patients with AE, and 42 patients with DE, resulting in no differences in family history of cancer, parity, and use of oral contraceptives.
Intervention(s): Immunohistochemistry assays using the following primary antibodies: CD3+; CD4+; CD8+; PD-1; and PD-L1.
Main Outcome Measure(s): To characterize T lymphocyte infiltration and PD-1/PD-L1 expression in 4 different endometriosis-related diseases.
Result(s): Endometriosis-associated ovarian cancer cases displayed significantly higher levels of PD-1/PD-L1 expression compared with all other endometriosis-related diseases (vs. OE vs. AE vs. DE). Moreover, a significantly lower count of infiltrating T lymphocytes was observed in EAOC cases compared with OE ones. Finally, one-third of OE cases showed a cancer-like PD-1/PD-L1 expression profile.
Conclusion(s): Endometriosis-associated ovarian cancer is characterized by higher levels of PD-1/PD-L1 expression compared with benign endometriosis-related diseases. This profile was found in one-third of clinically benign cases, suggesting that it develops early in the carcinogenesis process. (Fertil Steril® 2021;■:■–■. ©2021 by American Society for Reproductive Medicine.)
Key Words: Endometriosis, endometriosis-associated ovarian cancer, infiltrating lymphocytes, PD-1, PD-L1

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Endometriosis is an estrogen-dependent chronic benign inflammatory disease that affects up to 10% of women in reproductive age. It consists of endometrial-like tissue (involving glandular epithelia and stromal cells) outside the uterine cavity (1).

Several epidemiological, histopathological, and molecular data suggest that this disease predisposes to endometrioid, clear-cell, and low-grade serous ovarian cancers, also termed endometriosis-associated ovarian cancer (EAOC) (2–6). Endometriosis-associated ovarian carcinogenesis is believed to be the result of multifaceted complex pathogenic factors such as endocrine imbalance, oxidative stress, and immune dysregulation (7–9). It is estimated that in 60%–80% of all EAOCs, atypical endometriosis (AE) is the transitioning entity from benign lesions to malignant variants where complex changes in immune surveillance lead to chronic inflammation (10, 11).

When exactly these changes in the immune system take place in the carcinogenesis process is unclear. However, one-third of patients with endometriosis were found to display an EAOC-like immune transcriptome profile, suggesting that changes in immune surveillance herald cancer, developing earlier, in clinically benign conditions (12).

On the other hand, infiltrating T lymphocytes (ITLs), including CD8+ T cells, regulatory T cells, regulatory B cells, type II natural killer T cells, and Th2 CD4+ cells, are known to play a role in remodeling and may promote tumor through an immunosuppressive action (13). In particular, these cells can suppress host antitumor responses and stimulate tumor angiogenesis.

It is well known that under pathological conditions, including cancer and chronic infections, an abnormal expression of programmed cell death protein-1 (PD-1)/programmed death-ligand 1 (PD-L1) can induce immune cell dysfunction suppressing T cell responses (13–15). Programmed cell death protein-1 is usually expressed on the surface of activated T cells, B cells, monocytes, dendritic cells, and, at low levels, natural killer cells, whereas PD-L1 is expressed on a wide variety of cells.

Although the expression and functions of the ITLs and PD-1/PD-L1 pathway have been intensively studied in several cancers including ovarian cancer, few studies described the expression and regulation of PD-1/PD-L1 in samples of endometriosis-related conditions (16, 17).

This study aimed to assess possible differences among EAOC and various types of endometriosis-related disease in terms of ITLs and PD-1/PD-L1 expression profiles. In particular, we hypothesize that ITL count and subtypes as well as PD-1/PD-L1 expression could be determinant to the EAOC phenotype.

MATERIALS AND METHODS

Study Design and Participants

All women with a histologic diagnosis of early EAOC (International Federation of Gynecology & Obstetrics [FIGO] stages IA–IIB) associated with endometriosis, ovarian endometriosis (OE), AE, and deep endometriosis (DE) between January 1, 2000, and December 31, 2019, were identified from Fondazione Policlinico Universitario Agostino Gemelli IRCCS and Gemelli Molise SpA registries of histopathology.

Patients were excluded in case of EAOC without associated endometriotic foci, non-EAOC according to the Sampson and/or Scott criteria, borderline ovarian tumor, advanced-stage EAOC (FIGO stages III–IV), any type of autoimmune disease, any type of immunotherapy, signs of infection occurring at least 4 weeks before surgery, blood transfusion, pregnancy, lactation, and allergies (18, 19). We used a propensity score matching method (nearest neighbor, caliper set to 0.2) to reduce differences among groups on confounding variables (i.e., possible risk factors for ovarian cancer development).

All specimens were obtained from removal of the ovary, and all eligible ones were centrally revised by a dedicated pathologist. The protocol was approved by each institutional review board, and patients enrolled provided their written informed consent for participation. Relevant clinical data collected from every patient were as follows: age; body mass index; family history of cancer; parity; menopause; use of oral contraceptive; use of hormonal replacement therapy; cancer antigen 125; symptoms; and smoking. For EAOC, cancer staging was assessed using the 2014 FIGO staging system (20).

Procedures

From formalin-fixed paraffin-embedded (FFPE) tissue samples, 2-μm sections were obtained and stained with hematoxylin and eosin. The primary antibodies used were as follows: CD3 (clone 2GV6; Ventana, prediluted), CD4 (clone 1F6; Neomarkers, Fremont, CA, prediluted); CD8 (clone SP57; Ventana, prediluted), PD-1(clone Nat105; Ventana, prediluted); and PD-L1 (clone SP263; Ventana, prediluted). The MACH 4 Universal HRP-Polymer detection system (Ventana) was used as the secondary antibody. In a final detection step, 3,3′-diaminobenzidine was applied for visualization. Positive signals were detected using the labeled streptavidin-biotin peroxidase detection system also involving the use of a Ventana automated immunostainer (Ventana Medical Systems, Tucson, AZ).

For lymphocyte subpopulations (CD3+, CD4+, and CD8+), 10 independent areas with the most abundant ITLs were selected at 40× magnification, digitally photographed at a size of 0.0625 mm^2, and counted manually (21). In detail, lymphocyte subpopulations as well as PD-1 and PD-L1 immunostaining were evaluated inside boundaries of the ovarian tumor and endometriotic foci; therefore, only intratumoral and stromal lymphocytes were include in the analysis. The count was performed 3 times in different moments for each photograph by the same investigator without knowing earlier results. Recommendations provided by the International TILs Working Group 2014 for breast cancer were adopted (22).

Staining patterns were analyzed for each antibody. For PD-1 and PD-L1, the percentages of positive staining were determined at ×40 magnification; PD-L1 was assessed using both the combined positive score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells,

multiplied by 100, and tumor proportion score, which is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity. For subsequent statistical analyses, PD-1 and PD-L1 were considered "positive" when more than 1%. Additional sections were prepared for use as negative controls and stained with Rabbit Monoclonal Negative Control Ig, according to the manufacturer protocol. Moreover, we used tonsillar tissue samples as positive controls for each run. Regarding endometriosis specimens, müllerian-type epithelium and endometrial-type stroma were considered as "PD-L1 staining tumor" cells for calculating the CPS and tumor proportion score.

Data Collection and Management

A customized electronic case report form was created for the study. Study data were collected retrospectively and managed using REDCap electronic data capture tools hosted at Fondazione Policlinico Universitario Agostino Gemelli IRCCS (https://redcap.policlinicogemelli.it) (23).

Access to the system was restricted to the study personnel by username and password.

Ethical Issues

This trial was performed in compliance with the protocol, designed to ensure adherence to Good Clinical Practice, as described in the following:

- ICH Harmonized Tripartite Guidelines for Good Clinical Practice, 1996. Note for Guidance on Good Clinical Practice CPMP/ICH/135/95
- EU Directive 2001/20/EC, 2005/28/EC
- Declaration of Helsinki (1964 and its amendments and subsequent clarifications)

Statistical Analysis

The sample was described in its clinical and demographic characteristics applying descriptive statistics techniques: continuous variables were summarized by median and interquartile range, and differences between variables were analyzed using the Mann-Whitney *U* test or *t* test, according to the normality of data. The normality of data was assessed using the Kolmogorov–Smirnov test. Categorical variables were represented by absolute frequencies and percentages (%). These variables were compared using the χ^2 test or Fisher's exact test, as appropriate. The level of significance was set at $\alpha = 0.05$.

A propensity score matching was performed to match patients with OE with patients with EAOC, patients with AE with patients with EAOC, and patients with DE with patients with EAOC. The matching variables were family history of cancer, use of oral contraceptive, and parity. In detail, the nearest neighbor matching with optimal matching (caliper 0.2) was considered.

Infiltrating T lymphocyte distribution was divided into 4 categories (0%, 0%–25%, 25%–50%, and >50%), and comparison between groups was performed using the χ^2 test. To identify characteristics that may distinguish EAOC from OE, multivariate logistic regression analysis was performed with EAOC category as the dependent variable and ITLs as the independent variable (categorized as less than 50%). Other candidate variables were age, body mass index, menopausal status, and high PD-1 expression (identified as more than 1%). The likelihood of EAOC diagnosis is presented as an odds ratio (OR) with standard error and 95% confidence interval (CI). All statistical tests were two-tailed. All analyses were performed using SPSS version 24.0.

Sample Size

To detect a difference in PD-1/PD-L1 proportions in the 2 groups of 0.36 (EAOC_Prop=68% and OE_Prop=32%), setting a 2-side alpha of 0.05 and a power of 95%, sample sizes of 55 in the EAOC group and 55 the in OE group were required.

RESULTS

Patient Selection and Characteristics

As a result of matching technique, 55 subjects with EAOC, 55 patients with OE, 12 patients with AE, and 44 patients with DE were selected. Each group was matched with EAOC, thus resulting in different numbers of patients with EAOC. In each matching, the patients displayed no differences in family history of cancer, parity, and use of oral contraceptives (Supplemental Table 1, available online).

All DE samples showed the following histopathological features: müllerian-type epithelium; endometrial-type stroma; and evidence of chronic hemorrhage. No significant longstanding fibrotic changes were observed in the stroma.

A flow diagram describing the subject recruitment and exclusions is shown in Figure 1.

Table 1 summarizes the baseline characteristics of EAOC, OE, DE, and AE cases, along with *P* values from group comparisons. Supplemental Table 2 (available online) presents the surgical, pathological, and medical characteristics of patients with EAOC.

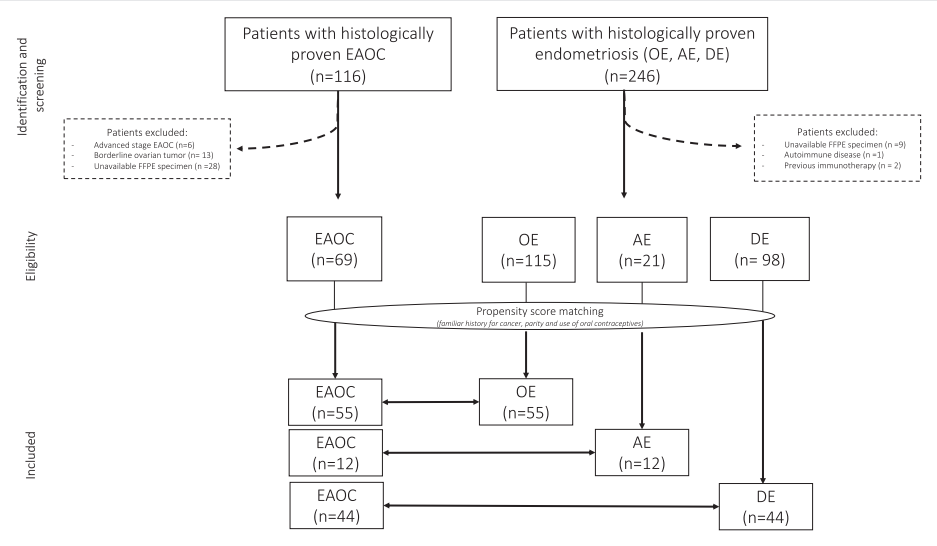
Thus, we analyzed a total of 166 FFPE tissue samples. In detail, FFPE tissue blocks encompassed EAOC clear-cell histotype (n = 14), EAOC endometrioid histotype (n = 36), EAOC serous low grade (n = 1), EAOC mixed (n = 4), OE (n = 55), AE (n = 12), and DE (n = 44). Second, 8 cases of EAOC presenting AE on the contralateral ovary were selected.

ITL, PD-1, and PD-L1 Expression Analysis

Supplemental Figure 1A (available online) shows representative images for immunohistochemical staining in patients with OE, AE, and EAOC, whereas Supplemental Figure 1B shows the DE group. Infiltrating T lymphocyte and PD-1/PD-L1 expression in EAOC compared with OE is presented in Table 2; ITL and PD-1/PD-L1 expression in EAOC compared with AE is presented in Table 3.

A significantly higher frequency of PD-1 and PD-L1 CPS positive cases and a lower number of ITLs were found in EAOC cases compared with OE cases ($P=.001$, $P=.006$, and $P=.01$, respectively). In particular, CD4+ T lymphocytes were lower in EAOC ($P=.01$).

FIGURE 1



Flowchart of the study.

Nero. Immunologic profile in endometriosis. Fertil Steril 2021.

Similarly, a significantly higher frequency of PD-1/PD-L1 CPS positive cases was observed in EAOC cases compared with AE cases ($P=.047$ and $P=.018$, respectively). No difference was found in terms of ITL count or subtypes.

Moreover, we selected 8 EAOC cases having AE on the contralateral ovary (Supplemental Table 3, available online). No differences were observed neither in terms of ITL count nor PD-1/PD-L1 expression between the 2 different lesions within the same patient. Nevertheless, a significantly higher number of CD8+ lymphocytes were observed in the AE group ($P=.05$).

Finally, when comparing EAOC with DE (Supplemental Table 4, available online), as for the other groups, the frequencies of PD-1 and PD-L1 CPS and tumor proportion score were significantly higher ($P<.001$). No differences in terms of ITL count was observed, but a significantly lower number of CD8+ lymphocytes were observed in the DE group ($P=.002$).

TABLE 1

Baseline characteristics of EAOC, OE, AE, and DE.

Parameter	EAOC (n = 55)	OE (n = 55)	DE (n = 44)	AE (n = 12)	*P* value EAOC vs. OE	*P* value[a] EAOC vs. DE	*P* value[b] EAOC vs. AE
Age (years), median (IQR)	50 (46–56)	38 (31–45)	35 (29–43)	47 (42–55)	<.001[c]	<.001[c]	>.99
BMI (kg/m^2), median (IQR)	24 (22–28)	22 (20–25)	22 (20–24)	24 (20–25)	<.001[c]	.002[c]	>.99
Family history of cancer (%)	33 (60)	29 (53)	26 (57)	6 (50)	.282	.665	.340
Parity (%)	26 (47)	18 (41)	20 (44)	5 (42)	.334	.517	.660
Menopause (%)	18 (33)	3 (6)	1 (2)	3 (25)	<.001[c]	<.001[c]	.109
OC (%)	29 (53)	31 (56)	29 (63)	8 (67)	.424	>.99	.667
HRT (%)	3 (6)	0 (0)	0 (0)	0 (0)	.122	.078	…
CA125, median (IQR)	55 (29–359)	69 (37–135)	35 (23–51)	5 (0–43)	.821	.308	.039
Symptoms (%)							
Abdominal pain (%)	15 (27)	8 (15)	3 (7)	1 (8)	.079	.011[c]	.500
Dysmenorrhea (%)	1 (2)	10 (18)	33 (72)	5 (42)	.004[c]	<.001[c]	.019[c]
Dyschezia (%)	0 (0)	2(4)	22 (48)	1 (8)	.248	<.001[c]	.500
Dysuria (%)	0 (0)	1 (2)	5 (11)	1 (8)	.500	.028[c]	.500
Dyspareunia (%)	0 (0)	6 (11)	26 (57)	3 (25)	.014[c]	<.001[c]	.109
Chronic pelvic pain (%)	0 (0)	5 (9)	22 (48)	2 (17)	.028[c]	<.001[c]	.239
Metrorrhagia (%)	2 (4)	7 (13)	3 (7)	0 (0)	.081	.500	.500
Others (%)	6 (11)	0 (0)	1 (2)		.050		

Note: AE = atypical endometriosis; BMI = body mass index; CA125 = cancer antigen 125; DE = deep endometriosis; EAOC = endometriosis-associated ovarian cancer; HRT = hormonal replacement therapy; IQR = interquartile range; OC = oral contraceptives; OE = ovarian endometriosis.

[a] Comparison between 44 patients with EAOC and 44 patients with DE.

[b] Comparison between 12 patients with EAOC and 12 patients with AE.

[c] Statistically significant values.

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TABLE 2

ITL distribution according to clinical groups (EAOC and OE).

Parameter	EAOC (n = 55)	OE (n = 55)	*P* value
ITL count[a]			
0	5 (9)	0 (0)	.01
0 < ITL ≤ 25%	28 (51)	23 (42)	
25% < ITL ≤ 50%	14 (25)	15 (27)	
ITL > 50%	8 (15)	17 (31)	
ITL subtypes[b]			
CD4+	1 (0.1–10)	4 (1.5–12)	.010
CD8+	18 (10–40)	29 (10–48)	.154
CD4+/CD8+ ratio	0.11 (0.01–0.25)	0.25 (0.05–0.42)	.019
PD-1/PD-L1 assessment[a]			
PD-1 ≥ 1%	19 (35)	4 (7)	.001
PD-L1 TPS ≥ 1%	14 (25)	9 (16)	.174
PD-L1 CPS ≥ 1%	30 (54)	16 (29)	.006

Note: CPS = combined positive score; EAOC = endometriosis-associated ovarian cancer; ITL = infiltrating T lymphocyte; OE = ovarian endometriosis; PD-1 = programmed cell death-1; PD-L1 = programmed death-ligand 1; TPS = tumor proportion score.

[a] χ^2 test.

[b] Mann-Whitney *U* test.

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The multidimensional scaling plot in Supplemental Figure 2 (available online) shows the separation of disease categories for ITL percentage and PD-1 expression. Supplemental Table 5 (available online) presents the variables associated EAOC diagnosis, their estimated ORs, standard errors, and CIs. Multivariable logistic regression analysis showed that high PD-1 expression (OR, 22.673 [95% CI, 3.474–147.953; *P*=.008]), low ITL count (OR, 9.667 [95% CI, 1.831–51.044; *P*=0.001]), and age (OR, 1.128 [95% CI, 1.047–1.215; *P*=.002]) were the parameters associated with EAOC diagnosis.

DISCUSSION

Principal Findings

This retrospective case-control study showed that stromal ITLs (CD3+, CD4+, and CD8+) and PD-1/PD-L1 are differently expressed in a population of EAOC, OE, AE, and DE cases previously matched for main known ovarian cancer risk factors. In particular, an increased level of PD-1/PD-L1 expression characterizes EAOC cases compared with all other benign endometriosis-related conditions analyzed. Moreover, a lower ITL count was observed comparing EAOC with OE.

We hypothesized that decreasing ITLs and increasing PD-1/PD-L1 could be significant steps into the path from a benign condition (such as endometriosis) to EAOC. The mechanism of this behavior could be envisaged in the creation of an immune-tolerant environment by a growing tumor within an inflammatory disease. Whether these phenotypic and functional immune adaptations facilitate ovarian cancer establishment and progression or whether they are merely bystander effects is still unclear.

Two findings in particular deserve attention. First, 36% of patients with OE (20 of 55) revealed a PD-1 (4 of 55)/PD-L1

TABLE 3

ITL distribution according to clinical groups (EAOC and AE).

Parameter	EAOC (n = 12)	AE (n = 12)	*P* value
ITL count[a]			.402
0	0 (0)	0 (0)	
0 < ITL ≤ 25%	7 (58)	4 (33)	
25 % < ITL ≤ 50%	2 (17)	5 (42)	
ITL > 50%	3 (25)	3 (25)	
ITL subtypes[b]			
CD4+	0.9 (0.9–9.5)	4 (1.3–11)	.128
CD8+	18.9 (9.9–55)	29.5 (18–45)	.590
CD4+/CD8+ ratio	0.06 (0.01–0.21)	0.11 (0.11–0.25)	.101
PD-1/PD-L1 assessment[b]			
PD-1 ≥ 1%	4 (33)	0 (0)	.047
PD-L1 TPS ≥ 1%	5 (42)	0 (0)	.019
PD-L1 CPS ≥ 1%	8 (67)	2 (17)	.018

Note: AE = atypical endometriosis; CPS = combined positive score; EAOC = endometriosis-associated ovarian cancer; ITL = infiltrating T lymphocyte; PD-1 = programmed cell death-1; PD-L1 = programmed death-ligand 1; TPS = tumor proportion score.

[a] χ^2 test.

[b] Mann-Whitney *U* test.

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(16 of 55) expression profile similar to EAOC cases, suggesting that changes in the immune tolerance develop earlier in patients classified as clinically benign. Second, the differential PD-1/PD-L1 expression observed comparing EAOC cases with AE cases is no more detected when analyzing EAOC and AE samples within the same patient; thus, it could be hypothesized that the establishment of ovarian cancer in AE lesions of women having EAOC on the contralateral ovary is only a matter of time. Nevertheless, it could also be possible that identifying in one-third of the benign endometriotic lesions similar immune cell infiltrate may not mean an early diagnosis of a potential cancer but a false positive of the biomarker. Only further studies will clarify this aspect.

Findings of This Study in the Context of Other Observations

Two previous studies in particular focused on endometriosis-related lesions analyzing directly tissue samples. Scheerer et al. (24) identified and characterized endometriosis-associated immune cell infiltrate in 60 premenopausal women with histologically proven endometriosis. The immunohistochemicalstaining performed revealed several distinct immunologic reactions within the microenvironment of different endometriotic lesions. In particular, significantly more numerous and larger-in-size subgroups of ITLs (CD3+, CD4+, and CD8+ phenotypes) were described in peritoneal endometriosis and OE. These findings cannot be compared with ours because different clinical settings were included in the 2 studies.

Suryawanshi et al. (12) performed a comprehensive immune gene expression analysis of 120 FFPE samples comprising normal controls and benign endometriosis, AE, and EAOC cases. The results showed that immune gene profiles were able to differentiate endometriosis from the other clinical settings. However, some benign endometriosis cases (33%) displayed an immune profile similar to EAOC cases. Similarly, most AE cases (85%) showed an EAOC-like immune environment. These data suggest that those patients with benign clinical conditions (benign endometriosis or AE) who have a cancer-like inflammation profile are at higher risk of developing EAOC. The cancer-like immune signatures observed may in fact anticipate morphological changes in the endometriosis-associated carcinogenesis process. These data are consistent with our findings. The tumor-like inflammation profile described in Suryawanshi et al. (12) was mainly related to complement pathway. Complement signaling is well known to modulate innate immune cell activities, but studies on complement signaling-deficient models suggest that it induces multiple immunosuppressive mechanisms including effector CD4+ and CD8+ T lymphocytes (25, 26).

Moreover, in vivo studies combining complement component fragment 5a receptor signaling blockade and PD-L1 antibody documented a synergistic antitumor action that was CD8+ T cell-dependent (27). These data may partially explain why our results are in line with findings concerning immune genes encoding for complement factors.

To our knowledge, ITL assessment in EAOC has not yet been described. High-grade serous and endometrioid ovarian cancer histotypes were shown to be the ones with the higher ITL levels (28). Accumulating evidence suggest that ovarian cancer cells have the ability to escape from the immune system creating a highly immunosuppressive network in the peritoneal cavity, through interactions between tumor and host immune cells in the tumor microenvironment. As a consequence, a high frequency of CD8+ cytotoxic T cells, which are known to be the forefront in the battle against tumors, was shown to be a positive prognostic factor in several tumors including ovarian cancer (29, 30).

Endometriosis being a chronic inflammatory disease, the PD-1/PD-L1 pathway was supposed to be particularly relevant in the pathogenesis of the disease itself (31). The PD-1/PD-L1 expression in blood of patients affected by endometriosis has been proven to be elevated (32, 33). Other studies showed that the PD-1/PD-L1 expression is up-regulated in eutopic and ectopic human endometrial tissue in patients with endometriosis compared with healthy people (34).

Moreover, patients with endometriosis show high levels of proinflammatory mediators like tumor necrosis factor-α, interleukin-1β, interleukin-6, dysfunctional macrophages, depressed killing capacity of natural killer cells, reduced activity of cytotoxic T cells, and increased accumulation of regulatory T suppressor cells, all of which may favor chronic inflammation and promote the initiation and progression of EAOC (35–38). However, most of these findings were shown in studies focused primarily on cellular phenotypes or soluble mediators in the peritoneal fluid (macrophages, natural killer cells) or peripheral blood (T lymphocytes, natural killer cells) (39, 40).

Clinical and Research Implications

Both our study and the previous study by Suryawanshi et al. (12) highlighted that a cancer-like immune signature is present in approximately one-third of patients with OE. Our study used a reproducible and affordable method to identify such patients who may develop EAOC in the next years. Currently, however, data are not sufficient to ensure clinical usage. In the first place, it would be necessary to validate our findings in a larger subset of patients. Moreover, it would be useful to acquire information regarding the expression of the analyzed markers in multiple areas within the same patient (e.g., contralateral ovary, and peritoneum). With more robust data, in the future, appropriate counseling for specific surveillance programs or risk-reducing surgical options or acceleration of their reproductive program could be offered.

In terms of research implications, the development of a radiogenomic model correlating this immunologic profile with preoperative ultrasound and/or computed tomography scan images of ovarian masses could represent a cost-effective, highly reproducible, large-scale extendable, and time-saving tool to early identify patients at higher risk of developing EAOC, thus addressing them to referral centers. Finally, future studies should clarify what role different environmental contaminants play on the immune system in the process that leads to EAOC from a benign lesion.

Strengths and Limitations

The main limitation of our study is the low number of subjects alongside with its retrospective nature. Although we pursued matching among the patients through propensity score, we cannot exclude potential biases in the comparison.

The absence of standardized immunohistochemical protocols to score ITL and PD-1/PD-L1 in neither ovarian cancer nor endometriosis may reduce the reproducibility of the results, although a dedicated expert pathologist in a gynecological oncology referral center performed the analysis. On the contrary, the novelty of the analysis in the context of such a hot topic makes these findings worthy of further reflections.

In conclusion, ITLs and PD-1/PD-L1 are differentially expressed in various endometriosis-related diseases. In particular, higher PD-1/PD-L1 expression levels and a lower ITL count characterized EAOC cases compared with OE. Nearly one-third of OE cases showed an EAOC-like PD-1/PD-L1 expression profile, but it cannot be clarified whether this activated pathway plays a role in EAOC carcinogenesis or it is caused by it.

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