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Original Research Article

Clinicopathological association of CD44 and PD-L1 expression in epithelial ovarian carcinoma: a hospital-based cross-sectional study from AIIMS Patna

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ABSTRACT

Background: Epithelial ovarian carcinoma (EOC) is the most lethal gynecological malignancy, often presenting at advanced stages. Molecular markers such as CD44, a stem cell-associated adhesion molecule, and PD-L1, an immune checkpoint regulator, have been implicated in tumor progression, chemoresistance, and immune evasion. Understanding their expression patterns and correlation with clinicopathological features may aid prognostic stratification and therapeutic decision-making. Aim was to assess CD44 and PD-L1 expression in EOC and analyze their association with clinicopathological parameters.
Methods: A hospital-based cross-sectional study was conducted over 5 years (2018-2023) in the department of pathology, AIIMS Patna. A total of 132 histologically confirmed cases of EOC were included. Immunohistochemistry (IHC) for CD44 and PD-L1 was performed, and expression was scored semi-quantitatively. Associations with age, histological subtype, tumor grade, stage, and lymph node involvement were evaluated. Statistical analysis was performed using chi-square and logistic regression.
Results: CD44 positivity was observed in 79/132 cases (59.8%), while PD-L1 was expressed in 62/132 cases (47.0%). Co-expression of both markers was found in 38 cases (28.8%). High CD44 expression was significantly associated with high-grade serous carcinoma (p=0.01), advanced FIGO stage (p=0.03), and lymph node metastasis (p=0.04). PD-L1 positivity correlated with advanced stage (p=0.02) and presence of ascites (p=0.03). Co-expression was linked to poor differentiation and advanced disease. Multivariate analysis showed CD44+/PD-L1+ tumors had 2.6-fold higher odds of lymph node metastasis.
Conclusions: CD44 and PD-L1 are frequently expressed in EOC and show significant association with adverse pathological features. Their combined expression may serve as a prognostic biomarker and highlight potential candidates for targeted and immune checkpoint therapies in ovarian carcinoma.

Keywords: Epithelial ovarian carcinoma, CD44, PD-L1, Immunohistochemistry, Prognostic markers

INTRODUCTION

Epithelial ovarian carcinoma, or EOC, is the most deadly gynecological cancer and accounts for an overwhelming number of cancer-related deaths yet is still one of the rarest types of gynecological tumors. After 14 years of research, no advancements are made. More than 70% of the cases at the 3 most advanced stages are due to the lack of research.

The 70% of cases are advanced stages. The lack of advanced research has contributed to widespread ovarian tumors due to poor prognosis and high mortality.[1-3] Specially in India, we see a rising incidence of dying of EOC and often see EOC in the most Advanced stages. This highlights the value of identifying risk factors to predict and assess prognosis value of EOC to plan therapy.[4,5] One of the most important markers of cancer and advanced ovarian tumors is CD44. CD44 is an adhesion molecule that assists in the escape and dissemination of tumors and is one of the most important advanced markers of ovarian tumors.[6,7] CD44 has been seen to be positively associated with high grade tumors, aggressive behavior and recurrences especially in high grade EOC.[8,10]

Programmed cell death-ligand 1 (PD-L1) is an immune checkpoint marker that is expressed in tumors that is capable of binding to the PD-1 receptor on activated T-lymphocytes leading to immune suppression. In EOC, PD-L1 expression has been shown to correlate with an increase in disease stage, decrease in tumor infiltrating lymphocytes, lesser tumor differentiation, and poorer clinical outcomes.[11-13] Immunotherapy clinical trials that featured the use of PD-1/PD-L1 blockers in the treatment of ovarian cancer have reported varying outcomes. This is, to some extent, the result of the tumor heterogeneity with regard to PD-L1 expression.[14] Recent reports have proposed that the combined assessment of tumor-initiating cell (TIC) markers such as CD44 and immune checkpoint-inhibiting targets such as PD-L1 is a superior predictor of aggressive biological behavior of the associated tumor. The presence of tumors that co-expressed these markers may possess the dual characteristics of immune evasion and cancer cell stemness. This would contribute to a greater treatment resistance and increase the likelihood of metastatic spread.[15-17] However, there is a critical shortage of evidence emanating from Indian tertiary care centers which have assessed the combined prognostic significance of CD44 and PD-L1. The current study was aimed with the purpose of assessing the expression of CD44 and PD-L1 in EOC and determining the relationship with some important clinicopathological characteristics. Profiling of co-expression of these features may help shed some light on the tumor characteristics and help the identification of suitable targets for immune and tumor-directed therapy.

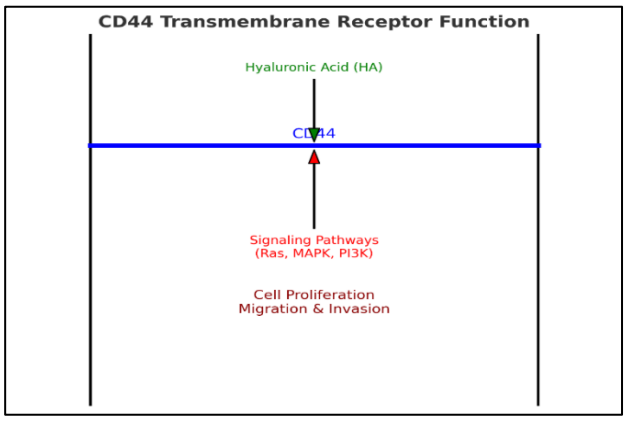

Figure 1: CD44 transmembrane receptor function.

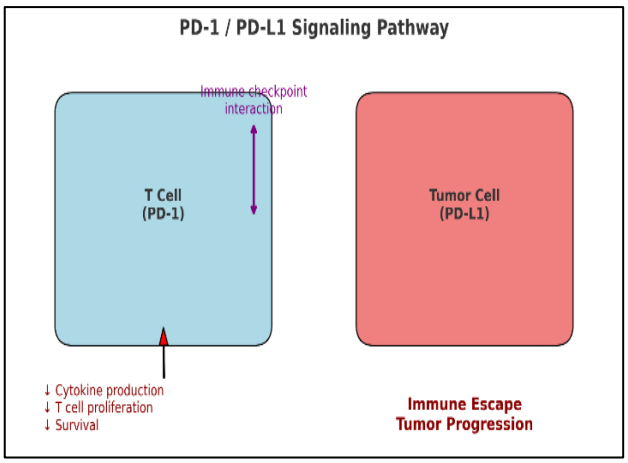

Figure 2: PD-1/PD-L1 signaling pathway.

METHODS

Study design and setting

This hospital-based cross-sectional study was done in the Department of Pathology, All India Institute of Medical Sciences (AIIMS), Patna, Bihar, India, within the timeline of five years between January 2018 and December 2023. All cases of EOC that were histologically confirmed during this period were analyzed for the expression of CD44 and PD-L1.

Selection criteria

The study included any patients that had been given a confirmed histopathological diagnosis of EOC and had sufficient formalin-fixed paraffin embedded (FFPE) tissue blocks that were available for analysis. The exclusion criteria consisted of non-epithelial ovarian tumors, cases of recurrent tumors that were missing a significant amount of clinical information, and patients that underwent neoadjuvant chemotherapy. From the medical records, the demographic and clinicopathological information was collected, which included age, menopausal status, histological subtype, tumor grade, FIGO stage, and whether there were lymph node metastasis and ascites.

Procedure

FFPE tissue blocks were sectioned to 4 µm thickness using a rotary microtome. Prior to conducting IHC, H and E-stained sections were confirmed by a pair of pathologists. Subsequently, IHC was completed using both CD44 (clone BSB-12) and PD-L1 (clone SP263) monoclonal antibodies. Using the manufacturer's directions on antigen retrieval and chromogen development, IHC staining was automated on the VENTANA BenchMark GX. CD44 was considered positive if more than 10% of the tumor cells stained the membrane. Using the tumor proportion score (TPS), PD-L1 expression was considered positive if at least 1% of the viable tumor cells stained positively. All

the slides were independently evaluated and any differences were negotiated by consensus.

Ethical approval

Ethical approval for the study, we received ethical clearance from the institutional ethics committee of AIIMS Patna (IEC No.: AIIMS/Pat/PGTh/Jan19/21). Owing to the fact the study included the anonymized archival tissue samples, the informed consent requirement was waived according to the institutional guidelines.

Statistical analysis

All of the analyses were performed using IBM SPSS statistics, version 25. Descriptive and frequency statistics were applied to demographics and pathology data. Using the chi-square or Fisher's exact test as appropriate, the association of the expression of CD44 and PD-L1 with the clinical and pathological variables was examined. Logistic regression was performed to determine the independent variables predicting the presence of lymph node metastasis. A $p<0.05$ was considered statistically significant.

RESULTS

A total of 132 patients with EOC were included in the study. The mean age of the study population was 51.4±10.6 years, with the majority belonging to the postmenopausal age group. Table 1 presents the demographic characteristics of the patients. Most patients (62%) were aged ≥50 years, reflecting the known age distribution of ovarian carcinoma, which commonly affects women in the peri-and postmenopausal periods. Similarly, 62% of the study population were postmenopausal, suggesting a strong association between menopausal status and the onset of epithelial ovarian malignancies. The data show that older, postmenopausal women formed the predominant group, consistent with the epidemiological trend of increasing ovarian cancer risk with advancing age.

Table 1: Demographic characteristics of patients with EOC.

Parameters	Value
Mean age (in years)	51.4±10.6
Age group <50 years	50 (38%)
Age group ≥50 years	82 (62%)
Menopausal status: Premenopausal	50 (38%)
Menopausal status: Postmenopausal	82 (62%)

Table 2 displays the distribution of histological subtypes. High-grade serous carcinoma constituted the largest proportion of cases (56%), followed by endometrioid carcinoma (18%), mucinous carcinoma (15%), and clear cell carcinoma (11%). This distribution aligns with global patterns, where high-grade serous carcinoma remains the most common and clinically aggressive subtype.

Table 2: Distribution of histological subtypes.

Subtypes	Cases, (n=132)	Percentage (%)
High-grade serous carcinoma	74	56
Endometrioid carcinoma	24	18
Clear cell carcinoma	14	11
Mucinous carcinoma	20	15

The predominance of high-grade serous carcinoma in more than half of the cases highlights the aggressive nature of ovarian cancer in the present cohort.

Table 3 summarizes the expression levels of CD44 and PD-L1. CD44 positivity was observed in 59.8% of cases, while PD-L1 positivity was noted in 47.0%. Co-expression of both markers was present in 28.8% of tumors. These findings indicate that a substantial proportion of EOC cases demonstrate activation of both stemness-related and immune-evasion pathways.

Table 3: CD44 and PD-L1 expression.

Markers	Positive cases, (n=132)	Percentage (%)
CD44	79	59.8
PD-L1	62	47.0
Co-expression	38	28.8

The co-expression rate of almost 29% suggests a biologically aggressive subgroup with dual pathway activation.

Table 4 shows the association between marker expression and clinicopathological variables. CD44 expression showed significant correlation with high-grade serous carcinoma, advanced FIGO stage, lymph node metastasis, and poor tumor differentiation ($p<0.05$). PD-L1 expression demonstrated significant association with advanced FIGO stage, poor differentiation, and the presence of ascites. The co-expression subgroup was characterized by advanced disease and a higher likelihood of metastatic involvement. The consistent correlation of both markers with adverse pathological features highlights their potential utility as prognostic biomarkers.

The serous carcinoma (Figure 3 A) typically appears as a unilocular or multilocular cyst with delicate papillary excrescences projecting into the lumen. These papillary structures often correspond to areas of high-grade malignant proliferation observed microscopically. In contrast, the mucinous carcinoma (Figure 3 B) presents as a large multiloculated cystic mass with a smooth external capsule. The locules are filled with thick, gelatinous mucinous material, reflecting the abundant mucin production characteristic of this subtype. These gross features aid in differentiating serous from mucinous tumors even before histological evaluation.

Table 4: CD44 and PD-L1 expression in EOC and their clinicopathological associations

Clinicopathological parameters	CD44 positive, (n=79)	CD44 negative, (n=53)	P value	PD-L1 positive, (n=62)	PD-L1 negative, (n=70)	P value
High-grade serous carcinoma	52	22	0.01	41	33	0.12
Endometrioid carcinoma	11	13	0.19	8	16	0.08
Clear cell carcinoma	7	7	0.97	6	8	0.67
Mucinous carcinoma	9	11	0.55	7	13	0.22
FIGO Stage III/IV	49	25	0.03	40	34	0.02
Lymph node metastasis	38	19	0.04	30	27	0.09
Poor differentiation	44	20	0.02	35	27	0.03
Presence of ascites	31	17	0.06	29	17	0.03
Co-expression (CD44+/PD-L1+)	38	-	-	38	-	-

The high-grade serous carcinoma (Figure 4 A) shows complex branching papillae lined by markedly atypical epithelial cells with prominent nucleoli, nuclear pleomorphism, and increased mitotic activity. These features account for the aggressive clinical behavior of this subtype.

The mucinous carcinoma (Figure 4 B) demonstrates tall columnar epithelial cells arranged in glandular and papillary structures surrounded by mucin pools. Areas of nuclear stratification and focal necrosis may be present, particularly in higher-grade lesions.

Figure 4 C exhibits the architecture of endometrioid carcinoma, characterized by back-to-back glandular formations resembling endometrial glands. Tumor cells show moderate-to-marked atypia with cribriform or confluent glandular patterns. Recognition of these microscopic differences is crucial for accurate histological subtyping and prognostication.

High-grade serous carcinoma (Figure 5 A) exhibits diffuse, strong membranous CD44 positivity, consistent with its known association with stemness and aggressive tumor biology. Mucinous carcinoma (Figure 5 B) also shows intense CD44 staining, indicating its potential involvement in tumor invasion and chemoresistance. Figure 5 C demonstrates PD-L1 immunopositivity, typically seen as focal to patchy membranous staining on tumor cells. The moderate expression in mucinous carcinoma reflects the heterogeneity of immune escape pathways among different subtypes.

Figure 5 D shows CD44 positivity in endometrioid carcinoma, confirming that CD44 expression is not restricted to serous carcinomas but may also contribute to tumor progression in other EOC subtypes. Overall, IHC profiles in these figures visually support the quantitative results, illustrating how CD44 and PD-L1 expression patterns correlate with tumor aggressiveness and histological subtype.

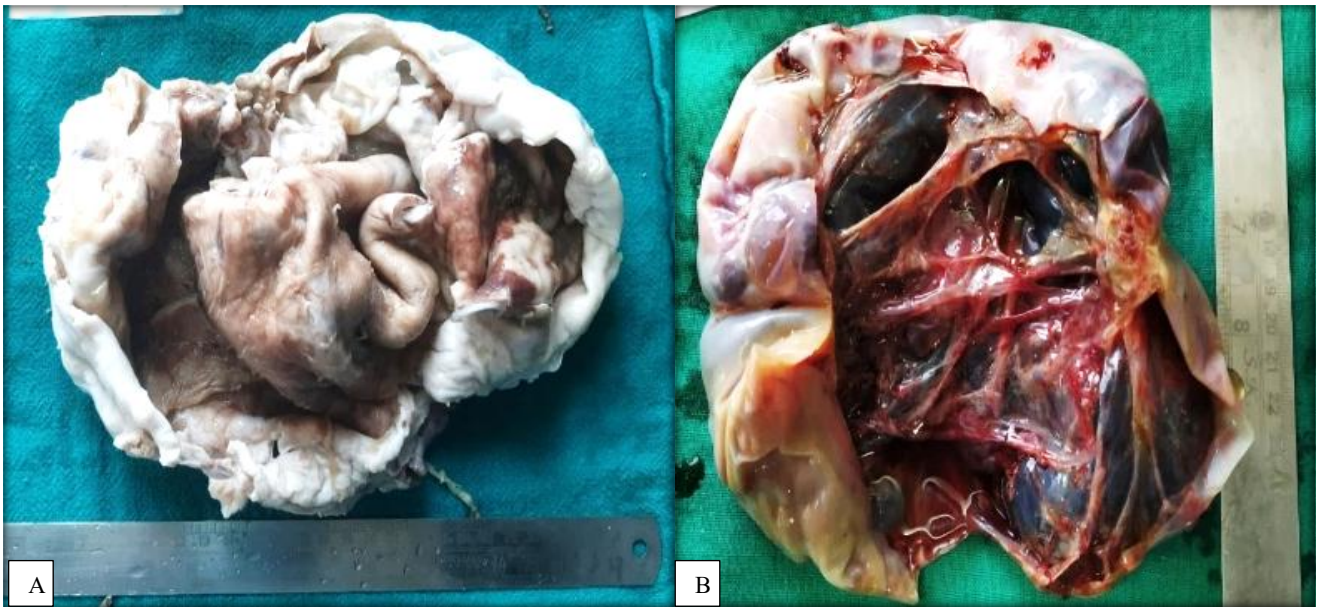

Figure 3 (A and B): A-Serous carcinoma. Unilocular cyst with papillary projection. B-Mucinous carcinoma. Smooth capsule, multilocular cyst filled with gelatinous material.

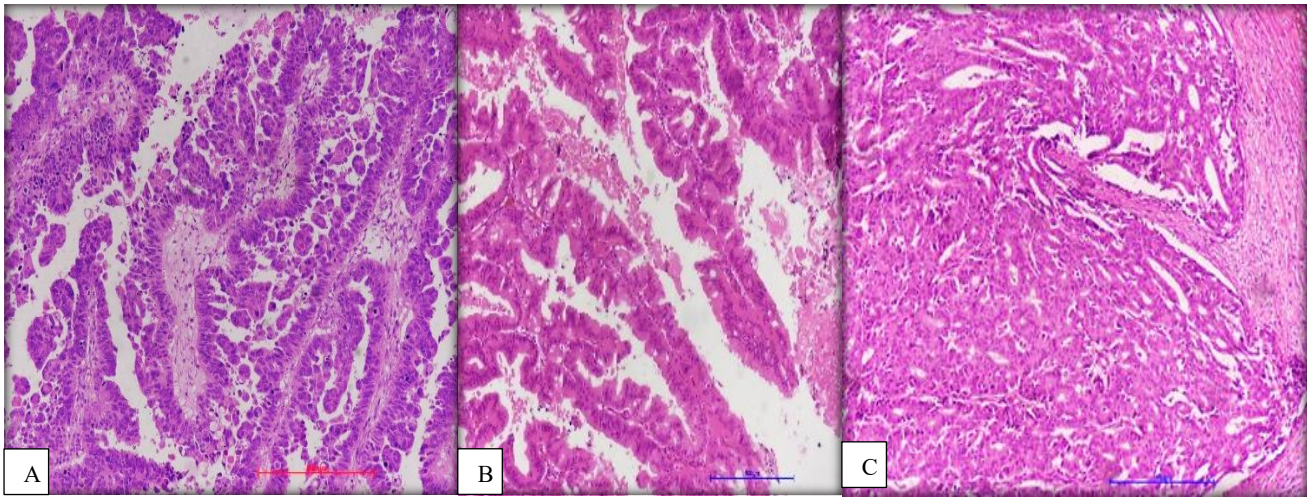

Figure 4 (A-C): A-High grade serous carcinoma. Tumour cell arranged in papillary pattern (H and E ×100), B-Mucinous carcinoma showing papillary fronds along with necrosis (H and E ×100) and C-high grade endometroid carcinoma showing glandular architecture (H and E ×100).

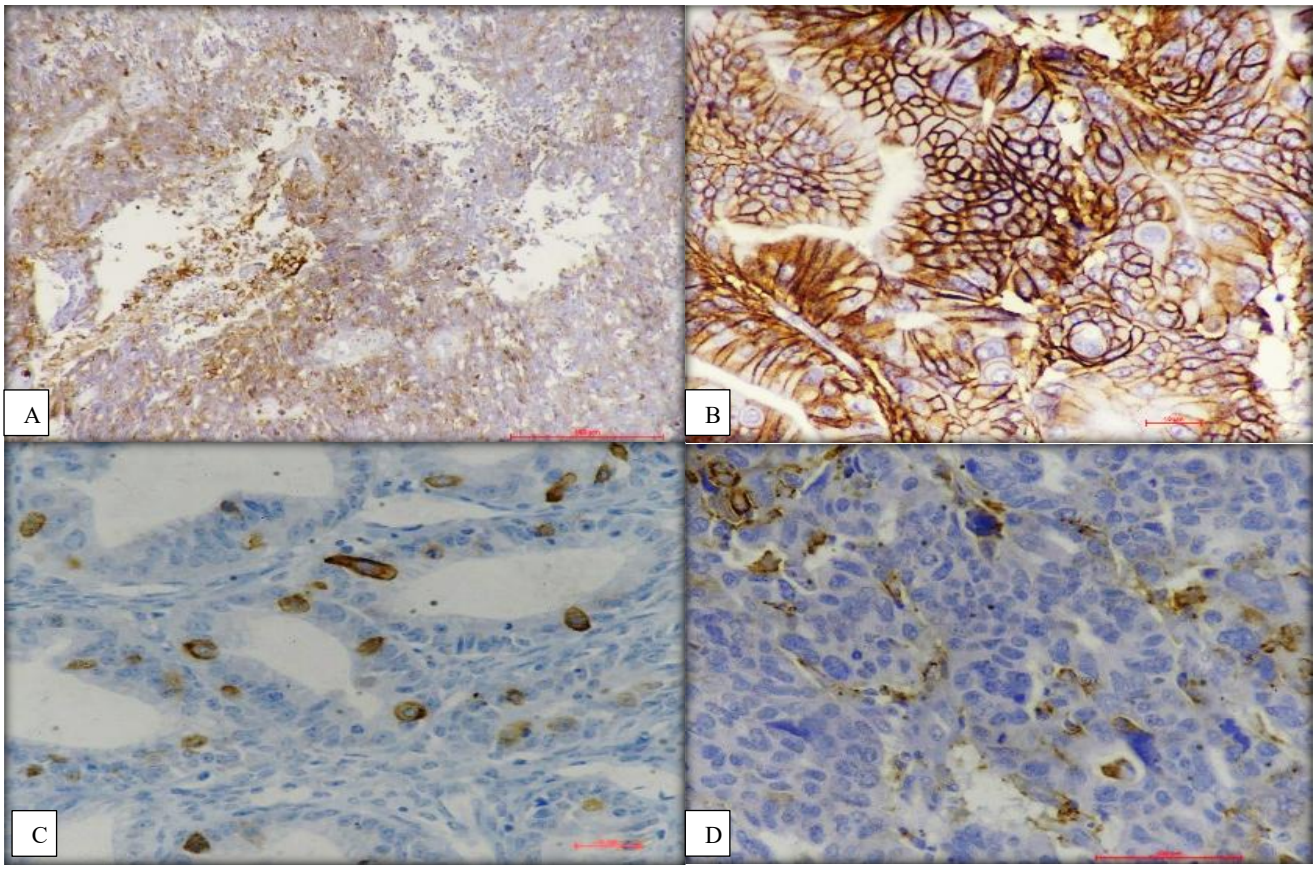

Figure 5 (A-D): A-High grade serous carcinoma. Diffuse membranous positivity (IHC, CD44, ×100), B-Mucinous carcinoma. Diffuse and strong membranous immunopositivity for CD 44 (IHC, ×400), C-Mucinous carcinoma showing focal and patchy membranous immunopositivity for PD-L1 (IHC, ×400) and D-Endometroid carcinoma. Membranous immunopositivity for CD44 (IHC, ×100).

DISCUSSION

The current study illustrates the case that PD-L1 and CD44 are often found together in those suffering from EOC and their presence is associated with different and concerning clinicopathological parameters. Throughout the study, 60% of participants displayed CD44 positivity. This supports the idea that CD44 is associated with the advancement of the tumor through the provision of stemness, epithelial/mesenchymal transition, and an

increased ability to metastasize. Our studies replicate that of others in indicating the presence of advanced stage and poorly differentiated EOC coupled with the overexpression of CD44 and all that comes with it as the disease progresses and the patient experiences greater therapeutic resistance.[18,19] Most notable is the overexpression of CD44 in high grade serous carcinoma as it supports the idea that this overexpression is at least in part responsible for the aggressive biology associated with these tumors that are the most dangerous subtype of ovarian cancer. PD-L1 positivity was also displayed by 47% of participants and the advanced stage of FIGO and presence of ascites coupled with PD-L1 positivity supports that the participant's disease had experienced a stage of progression that activated immune evading tactics. Additionally, many have also mentioned the overexpression of PD-L1 in advanced stages of the disease that is associated with high-grade ovarian tumors in an attempt to explain the insufficient cytotoxic T cell response to the tumor. This implies the presence of a tumor that is able to survive immune surveillance.[20,21] The observed relationship between PD-L1 positivity and poor differentiation in our study is particularly noteworthy, as poorly differentiated tumors frequently harbor a highly suppressive tumor microenvironment characterized by reduced T-cell infiltration and increased regulatory immune cells.[22]

A key finding of this research study is the CD44 and PD-L1 co-expression of 28.8%, which further characterizes EOC into biologically aggressive entities. Tumors with dual positive expression were significantly more likely to exhibit poor differentiation and metastasize to lymph nodes. CD44 positive tumors are believed to promote immune checkpoint molecules into less-differentiated aggressive tumors likely to metastasize, as this may achieve immune evasion and elicit immune escape responses to promote the tumors persistence and metastasis.[23] CD44 high ovarian cancer cells have been shown to self-renew and activate and/or upregulate mechanisms of immunosuppression and PD-1/PD-L1 signaling, which may promote resistance to therapy and enhanced metastasis.[24,25] Our results, to the best of our knowledge, are the first to demonstrate that the antagonistic activation of cancer stem cell and immune evasion mechanisms may represent important factors influencing the aggressiveness of the tumors and this is important clinically. The co-expression of CD44 and PD-L1 demonstrates to be of great clinical utility in identifying patients who may benefit from the dual therapies of cancer stem cell targeting and immune checkpoint blockade.[26] Early-phase trials have suggested that dual-target approaches may enhance treatment response by overcoming resistance mediated by cancer stem-like cells and restoring antitumor immunity.[27] Our observation that CD44+/PD-L1+ tumors were strongly associated with advanced pathological features lends further support to the potential utility of combined biomarker-based risk stratification in routine pathology practice.

Several limitations still need to be addressed. The fact that this is individual one-site cross-sectional research with no follow-up data means we cannot evaluate survival outcomes. The lack of molecular profiling means we cannot understand more about the regulatory pathways that drive CD44 and PD-L1 expression. Also, PD-L1 scoring methodological heterogeneity between studies may make the comparison more limited, even though the studies themselves will be less limited. There is an urgent need to conduct more extensive multicenter investigations with considerable attention to genomic data, immune microenvironment profiling, and progression-free and total survival statistics to fully understand the expression of CD44 and PD-L1 and prognostication and prediction of survival to details that are still lacking.

Fortunately, this research study adds on to the still increasing pool of studies that are directed more to research on the growing importance of the markers involved in CD44 and PD-L1 in the case of EOC. There is evident association of stem cell and immune evasion around tumors that will make it possible to discover more about the importance and predictive capabilities of personalized management and combinatorial treatment strategies. The importance of interplay stealth objectives is also evident around the immune evasion and perhaps the stem cell themselves. Moreover, the present study is advancing the ambitions concerning CD44 and PD-L1 and the ovarian carcinoma case greatly. The present study is advancing a great deal and especially concerning the predictive capabilities surrounding CD44 and PD-L1 vision. The more studied, the more their value can be fully presented.

CONCLUSION

CD44 and PD-L1 are frequently expressed in EOC and show strong associations with adverse pathological features. Their co-expression identifies tumors with aggressive potential and highlights the possibility of combining targeted and immunotherapeutic strategies. Routine evaluation of these markers may provide valuable insights for personalized management of EOC.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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