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ORIGINAL ARTICLE

The role of CD146 in serous ovarian carcinoma

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Summary

Purpose: *The heterogeneous phenotype of epithelial ovarian cancer (EOC) explains the unpredictable behaviour in terms of response to therapy, time to progression and survival. In this context, CD146, a cell adhesion molecule, has been focused on as a marker of poor prognosis in various solid cancers, being also capable to modulate the activity of endothelial cells. Therefore, we proposed to investigate its role in serous ovarian carcinoma.*

Methods: *The study included 101 patients diagnosed with EOC and treated within "Ion Chiricuta" Oncology Institute by optimal surgical debulking followed by platinum-based chemotherapy. Clinico-pathological characteristics were collected from patient files. CD146 expression was assessed by immunohistochemistry in serous ovarian carcinoma primary tumours, taking into account both staining intensity and the percentage of positive tumor cells. Expression of CD146 in endothelial cells of tumour microvessels was also evaluated. CD34 immunostaining was used for intratumoral microvessel density estimation.*

Results: *CD146 positivity in tumor cells was objectified in 49.5% of samples and 37.1% presented a high CD146 endothelial expression. Our analysis showed that CD146 was as reliable as CD34 for microvascular density estimation. The distribution of cases according to CD146 tumor expression was similar regardless of age, initial serum CA125 level, FIGO stage, presence/absence of malignant ascites. Multivariate analysis confirmed that expression of CD146 in tumor cells was a negative prognostic factor for overall survival, significantly asociated with a higher risk of chemotherapy resistance.*

Conclusions: *Although CD146 immunoreactivity in tumor cells did not correlate with the routinely used clinico-pathological parameters, expression of CD146 in tumor cells was an independent pronostic factor for survival in serous ovarian carcinomas. Moreover, CD146 might be regarded as a novel biomarker of tumor neovasculature.*

Key words: *angiogenesis, CD146, ovarian cancer, prognosis*

Introduction

Angiogenesis is a key process for tumor development and metastasis, becoming an essential target of current therapeutic strategies in ovarian cancer. A large part of recent research has focused on the identification of specific markers for tumor neovascularization. Of these, VEGF-A and VEGFR-2 have been used as the main therapeutic antiangiogenic targets. In the study of the tumor neoangiogenesis process, it has been observed that the cell adhesion molecule CD146 acts as a VEGFR-2 co-receptor [1].

CD146 (MCAM, MET-CAM, MUC18) is a Ca-independent membrane glycoprotein formed by 5 extracellular Ig-like domains, a transmembrane region and an intracellular domain involved in the recognition of some protein kinases, thus be-

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ing able to influence certain signaling pathways, both intracellular and extracellular [2]. Initially described as a cell adhesion molecule (CAM) highly expressed in malignant melanocytes, it was subsequently studied as a marker for neoangiogenesis. CD146 is a cell adhesion molecule concentrated in interendothelial junctions, where through the mediation of cell-cell interactions it influences the migration of endothelial cells and angiogenesis [1]. Thus, endothelial cells migrate to the perivascular space, where they proliferate, adhere to each other and to the extracellular matrix, forming capillaries and neovessels [3].

High CD146 expression has been associated with tumor progression and development of distant metastases in various malignant tumors such as malignant melanoma [4-6], breast cancer [7,8], prostate cancer [9,10] or NSCLC [11]. In malignant melanoma, the hypothesis according to which CD146 represents a molecular target, potentially inhibited by an anti-CD146 monoclonal antibody, has been confirmed in experimental models, with a lung metastasis suppression effect, through a reduction of *in vivo* invasion and control of angiogenesis [12]. In breast cancer, it was initially considered that high CD146 expression was associated with a protective rather than prometastatic effect, but subsequent studies conducted on animal models showed a correlation of CD146 overexpression with a poorly differentiated phenotype and the triple negative subtype, characterized by increased aggressiveness, high metastatic capacity and poorer prognosis regarding long-term survival [9,10,13]. Studies performed in breast cancer cell lines have associated CD146 overexpresssion with phenotypic changes observed in EMT, suppression of epithelial markers and induction of mesenchymal markers, favoring in this way tumor cell migration and invasion [14]. It is known that EMT is regulated by transcription factors (SIP1, Snail, Slug, Twist) through signaling pathways, of which RhoA is known for its implication in actin reorganization processes in the cytoskeleton. It has been observed that increased CD146 expression induces EMT and promotes breast cancer progression by activation of the RhoA pathway and stimulation of Slug transcription factor [15,16].

CD146 expression has also been analyzed in gynecological cancers. CD146 positivity has been found in endometrial and cervical cancer, unlike normal tissues corresponding to these locations. In cervical carcinoma, CD146 overexpression has been significantly associated with the squamous histological subtype, and in endometrial carcinoma, with the degree of malignancy and the depth of myometrial invasion, respectively. In both locations, CD146 immunoreactivity in the tumor cell membrane and in the majority of tumor vessels has been confirmed. Consequently, the active implication of CD146 in tumor development processes through angiogenesis is re-suggested in these cancers [16].

In the case of patients with advanced epithelial ovarian cancers (EOC), the clinicopathological characteristics routinely used are not sufficient to predict long-term prognosis. For this reason, many researchers have aimed to identify new prognostic factors in relation to tumor biology. It has been observed that many complex events responsible for tumor progression occur at the tumor-stroma interface, through processes partially mediated by cell adhesion molecules (CAM) at this level, which modulate signal transduction for various pathways involved in extracellular matrix remodeling [15]. Recent studies have found that high CD146 expression levels differentiate between epithelial ovarian cancer and borderline tumors, benign tumors or normal ovarian epithelium [2,17]. There are few literature studies assessing the prognostic value of CD146 overexpression in EOC, which is why the role of this biomarker is not yet clearly defined [2,15].

In this study, we aimed to investigate the prognostic role of CD146 and its potential predictive role, by evaluating its impact on survival and response to platinum-based chemotherapy, in relation to other clinicopathological characteristics.

Methods

Patients

611 ovarian carcinoma cases diagnosed at "Prof. Dr. Ion Chiricuță" Oncology Institute in Cluj-Napoca (IOCN) in the period January 2006 – November 2011 were retrospectively evaluated. Of these, 102 patients were selected, who met the following study inclusion criteria: histopathological diagnosis of ovarian serous carcinoma, primary standard treatment performed at IOCN: primary cytoreductive surgery (either optimal resection to no residual or suboptimal resection with a residual tumor size ≤10 mm), and platinum-based adjuvant chemotherapy. The exclusion criteria were: histology other than serous, suboptimal surgery, with a postoperative residual tumor size greater than 10 mm [18], neoadjuvant chemotherapy administered prior to cytoreductive surgery, absence of periodic follow-up at IOCN, presence of other synchronous or metachronous neoplasms.

By reviewing the files of the patients, data related to their clinicopathological characteristics were retrospectively collected, including: diagnosis, FIGO stage, degree of malignancy, presence of ascites and/or peritoneal carcinomatosis, initial serum level of CA125 antigen, and type of progression (pelvic recurrence/peritoneal or distant recurrence). For the preoperative serum CA125 level,

a value 10 times higher than the upper normal limit (>10 x 35 U/ml) was considered as a significant increase. After reviewing a number of literature studies, we chose this threshold value for serum CA125, which was also used in the German study DESKTOP I [19]. The response to platinum-based chemotherapy was established depending on the time period elapsed from completion of treatment to disease progression (progression-free interval – PFI). As such, patients were considered responsive/sensitive to chemotherapy in the case of a PFI longer than 6 months, and non-responsive/resistant to chemotherapy in the case of disease progression during or within 6 months of completion of chemotherapy [20].

The follow-up period was calculated from the date of initiation of treatment (date of surgery) to the date of the last follow-up visit to IOCN. Progression-free survival (PFS) was expressed as the time period from diagnosis (date of surgery) to the first evidence of disease progression. Overall survival (OS) was calculated from the time of diagnosis to the date of death or the date of completion of the study. The date of completion of the study was 31 January 2015. Information regarding mortality was obtained from the Population Register. All deaths of the patients included in the study were due to cancer, so that cancer-specific survival could be calculated.

Immunohistochemical analysis using the tissue microarray (TMA) technique

The hematoxylin-eosin (HE) slides pertaining to the selected cases were identified in the archive of the "Prof. Dr. Ion Chiricuță" Oncology Institute Cluj-Napoca and were reassessed by the same anatomopathologist. The cases were restaged according to the 2014 FIGO staging system and the degree of tumor differentiation was re-expressed according to the 2014 WHO classification as high-grade serous carcinoma (HGSC) and low-grade serous carcinoma (LGSC) of the ovary.

The ovarian tumor tissue samples fixed in formaldehyde solution and embedded in paraffin were processed using the TMA technology. In the paraffin blocks obtained by this technique, immunohistochemical staining was performed in order to determine CD146 expression. Cone samples 2 mm in diameter were extracted from representative tumor areas identified microscopically on the hematoxylin-eosin (HE) slides. From the paraffin blocks newly formed using the TMA technique, 4 µm thick sections were cut, which were mounted on silanized slides and were incubated overnight at 37°C. The sections were subsequently deparaffinized in xylene, rehydrated through a graded ethanol series and then washed. Antigen recovery was performed by heating in 10 mM citrate buffer solution (pH 6.0) for 20 min. After cooling at room temperature, the slides were rinsed in tris-buffered saline (TBS), and endogenous peroxidase activity was inhibited by incubation in 3% hydrogen peroxide for 5 min. Then, each slide was incubated with mouse anti-human CD146 primary monoclonal antibody (clone N 1238, NCL-CD146, MCAM, Novocastra Lyophilized, Leica Biosystems) for 60 min, at a 1:50 dilution. After washing in TBS, the slides were incubated with Post Primary Block (Novocastra) for 25 min and with anti-mouse/rabbit IgG-Poly-HRP secondary antibody (Novolink Polymer Novocastra, Leica Biosystems). Peroxidase activity was carried out with diaminobenzidine solution (DAB) (Novocastra), and the sections were subsequently processed by counterstaining with Mayer's hematoxylin, rinsing in saturated lithium carbonate solution, dehydration, immersed in xylene, and mounting in Faramount Mounting Medium (S3025; Dako).

The control was considered immunohistochemical staining for CD146 in a healthy human appendix tissue slide, evidencing intense staining of the cytoplasm and membrane of endothelial cells and smooth muscle cells in the muscularis propria. The negative control was achieved by omission of primary antibodies. In the ovarian serous carcinoma tissues, CD146 immunoreactivity in the tumor cell membrane and tumor stroma, predominantly in tumor microvessel endothelial cells, was observed. In order to quantify CD146 expression in the tumor cells of the studied ovarian serous carcinomas, a semiquantitative system comparable to the Allred system was used, taking into consideration the proportion of positive cells and the intensity of immunohistochemical staining. Cell membrane staining was classified depending on intensity as follows: negative, weak, moderate, strong. Corroborated with the percentage of immunoreactive tumor cells, CD146 expression in tumor cells was considered positive if more than 10% of tumor cells showed moderate or intense staining.

Intratumoral microvessel density (MVD) was quantified based on the number of CD34-positive vessels. Endothelial CD146 expression was assessed by identifying hypervascularized tumor areas and by counting immunoreactive vessels for CD146, at increased magnification (400x, field size 3.14 mm^2), in TMA sections.

Statistics

In this study, statistical analysis included descriptive statistical elements for survival analysis, with the identification of independent prognostic factors and the testing of associations and concordances between different variables. Quantitative variables were expressed as mean ± standard deviation, while qualitative variables were formulated as absolute and relative frequencies (%). For bivariate analysis of associations between different qualitative nominal variables, Fisher's exact test or the Chi-square test was used. The magnitude of the effect of the analyzed correlations was expressed by odds ratio (OR) and 95% confidence interval for OR. Univariate analysis of potential predictors for PFS and OS was performed with the Log-rank test, and survival curves were designed using the Kaplan-Meier method. In multivariate analysis, Cox regression was used to identify independent prognostic factors for survival time. The multivariate regression model was developed based on independent variables with a significance level estimated by univariate analysis $p<0.05$. The final model was obtained by comparing several overlapping models using the Deviance (Likelihood Ratio) test. The final model included all covariates whose exclusion would have caused a change of more than 20% in the coefficients of the remaining variables. To quantify the significance of these

predictors for progression-free survival (PFS) and overall survival (OS), the adjusted hazard ratio (HR) with its 95% confidence intervals was also calculated. We used McNemar's test to evidence that CD146 can be considered an endothelial marker for tumor microvascularization in comparison to microvessel density determined by CD34. Given that the two parameters were defined as two dichotomous nominal variables, a significant result of the test would indicate a significant difference between the proportions of positive results measured by CD146 and CD34, respectively, while an insignificant result would involve the absence of a significant difference between the two proportions.

The level of significance (α) for all two-way tests used was equal to 0.05, and statistical significance was met in case of a significance level $p<0.05$. Statistical analysis was carried out by means of IBM SPSS v.19 (Armonk, NY: IBM Corp), Statistica, v.6.0. (StatSoft) and R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The age of the patients included in the study ranged between 35 and 79 years, and the median age was 57 years. From the date of diagnosis, the patients were followed up for a median time period of 54 months. The clinicopathological characteristics of the patients included in the study group are shown in Table 1. More than half of the patients (60.78%) were diagnosed with ovarian cancer at less than 60 years of age, and advanced stages FIGO III/IV represented the majority (83.33% of cases). Diagnosis at early diseases stages was established only in 16.66% of cases. The serum CA125 level was increased (>350 U/mL) in 54.9% of cases. In 70.58% of cases, optimal debulking surgery was performed, without residual macroscopic tumors in the peritoneal cavity. 29.41% of patients were

Table 1. Characteristics of patients in the study group

Characteristics	*No. of patients*	*%*
Age, years		
<60	62	60.78
≥60	40	39.21
FIGO stage		
I/II	17	16.66
III/IV	85	83.33
CA125, U/ml		
<350	46	45.09
≥350	56	54.90
Ascites		
No	30	29.41
Yes	72	70.58
Peritoneal carcinomatosis		
No	28	72.54
Yes	74	27.45
Histologic grade		
Low	8	7.84
High	94	92.15
Cytoreductive surgery		
Optimal resection (0 cm)	72	70.58
Suboptimal (<1cm)	30	29.41
Type of recurrence		
Absent	24	23.52
Pelvic/abdominal	58	56.86
Distant metastases	8	7.84
Both	12	11.76
Progression-free interval, months		
< 6	20	19.60
> 6	82	80.39

Table 2. Distribution of patients depending on CD146 expression and age at the time of diagnosis

Variables	*CD146 expression*		*OR (95% CI)*	*p value*
	Negative n (%)	*Positive n (%)*		
Age at diagnosis, years			1.12 (0.50-2.50)	0.777
≤ 60	32 (51.6)	30 (48.4)		
> 60	19 (48.7)	20 (51.3)		
Total	51 (50.5)	50 (49.5)		

Table 3. Distribution of patients depending on CD146 expression and preoperative serum CA125 level

Variables	*CD146 expression*		*OR (95% CI)*	*p value*
	Negative n (%)	*Positive n (%)*		
Preoperative CA125 (U/ml)			1.42 (0.65-3.13)	0.427
< 350	21 (45.7)	25 (54.3)		
≥ 350	30 (54.5)	25 (45.5)		
Total	51 (50.5)	50 (49.5)		

Table 4. Distribution of patients depending on CD146 expression and FIGO stage

Variables	*CD146 expression*		*OR (95% CI)*	*p value*
	Negative n (%)	*Positive n (%)*		
FIGO stage			1.49 (0.52-4.30)	0.451
I-II	10 (58.8)	7 (41.2)		
III-IV	41 (48.8)	43 (51.2)		
Total	51 (50.5)	50 (49.5)		

treated with suboptimal surgery, with the persistence of tumors less than 10 mm in size in the abdominopelvic cavity. The presence of malignant ascites and peritoneal carcinomatosis was found in about 70% of cases. 92.15% of the diagnosed ovarian tumors were classified as high-grade ovarian serous carcinoma subtype (Table 1). Following immunohistochemical staining performed in ovarian tumor samples, positive CD146 expression was detected in the tumor cell membrane in 50.98% (52/102) of cases. Among these, 19.23% showed intense staining (Figure 1D), 46.15% moderate staining (Figure 1C), and 34.61% moderately reduced staining (Figure 1B), while the remaining cases showed completely negative tumor cell staining (Figure 1A).

The association between CD146 expression in tumor cells and the clinicopathological characteristics of patients included in the study was investigated using the x^2 test. Regarding these characteristics: age (Table 2), initial serum CA125 level (Table 3), FIGO stage (Table 4), presence of malignant ascites (Table 5), a quasi-similar distribution of the cases depending on CD146 expression was found, without the identification of statistically significant correlations (p>0.05). In patients without peritoneal carcinomatosis, CD146 expression was negative in 64.3% of cases, and among patients with peritoneal carcinomatosis, CD146 expression was positive in 54.8% of cases. A tendency to statistical significance was observed in the case of this association (OR=2.18, p=0.086) (Table 6).

Table 5. Distribution of patients depending on CD146 expression and the presence/absence of malignant ascites

Variables	*CD146 expression*		*OR (95% CI)*	*p value*
	Negative n (%)	*Positive n (%)*		
Ascites			1.42 (0.60-3.36)	0.42
Absent	17 (56.7)	13 (43.3)		
Present	34 (47.9)	37 (52.1)		
Total	51 (50.5)	50 (49.5)		

Table 6. Distribution of patients depending on CD146 expression and peritoneal carcinomatosis

Variables	*CD146 expression*		*OR (95% CI)*	*p value*
	Negative n (%)	*Positive n (%)*		
Peritoneal carcinomatosis			2.18 (0.88-5.36)	0.086
Absent	18 (64.3)	10 (35.7)		
Present	33 (45.2)	40 (54.8)		
Total	51 (50.5)	50 (49.5)		

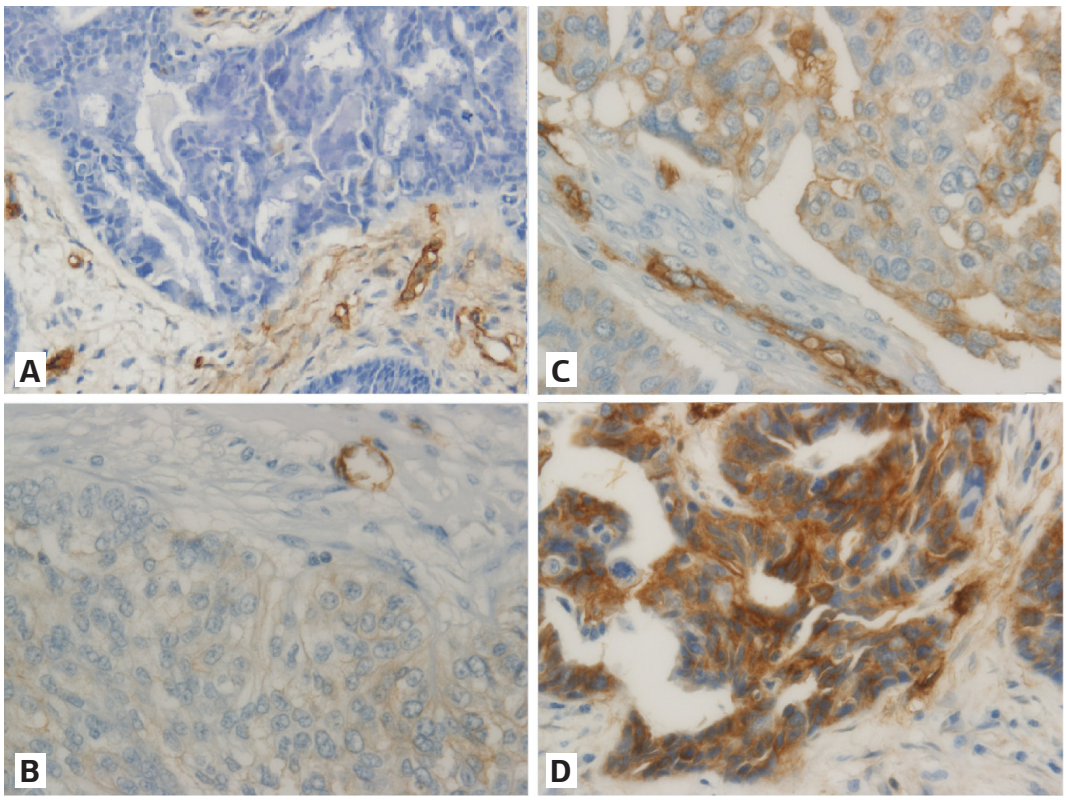

Figure 1. CD146 expression in ovarian tumor cells. **A:** negative tumor cell staining, positive stroma staining; **B:** weak intensity; **C:** moderate intensity; **D:** strong intensity of staining for CD146 in tumor cells. Magnification ×400.

Table 7. Distribution of cases depending on CD146 expression and location of disease recurrence

Variables	*CD146 expression*		*OR (95% CI)*	*p value*
	Negative n (%)	*Positive n (%)*		
Type of recurrence			4.27	0.118
Absent	16 (66.7)	8 (33.3)		
Locoregional	24 (42.1)	33 (57.9)		
Distant ± locoregional metastases	11(55.0)	9 (45.0)		
Total	51 (50.5)	50 (49.5)		

Table 8. Results of multivariate analysis using the risk of recurrence as a dependent variable

Outcome	*Risk of recurrence*		
Factors	*OR*	*95% CI*	*p value**
CD146 expression (positive vs. negative)	2.38	0.90-6.25	0.077
FIGO stage (III/IV vs. I/II)	1.29	0.39-4.24	0.674
Age, years (> 60 vs. ≤ 60)	0.83	0.32-2.18	0.718

* estimated p-values obtained from Wald test

Table 9. Distribution of cases depending on CD146 expression and response to chemotherapy

Variables	*CD146 expression*	
	Negative n (%)	*Positive n (%)*
Response to chemotherapy, months (Progression free interval)		
< 6	6 (30.00)	14 (70.00)
≥ 6	45 (55.55)	36 (44.44)
Total	51 (50.5)	50 (49.5)

Table 10. Results of multivariate analysis using resistance to chemotherapy as a dependent variable

Outcome	*Resistance to chemotherapy*[a]		
Factors	*OR*	*95% CI*	*p value**
CD146 expression (positive vs. negative)	3.01	1.01-8.93	0.048
FIGO stage (III/IV vs. I/II)	4.82	2.83-42.26	0.156
Age, years (>60 vs. ≤60)	2.83	0.98-8.11	0.054

[a]Progression-free interval <6 months; *estimated p values obtained from Wald test

The median follow-up period was 54 months, during which disease recurrences were registered in 78 (76.47%) patients. Of these, 58 had abdominopelvic recurrences, and 20 developed distant metastases with/without locoregional recurrences (Table 1). Among patients without disease progression after primary treatment (23.52% of cases), the majority (66.7%) had negative CD146 expression in tumor cells in the analyzed samples. 57.9% of cases with locoregional recurrences showed positive CD146 expression. In contrast, in the group with distant metastases, CD146 positivity in tumor cells was detected in 45% of cases (Table 7). The association between CD146 expression and the location of the disease recurrence was not statistically significant (OR=4.27, p=0.118).

Regardless of the location of the recurrence, we analyzed the risk of recurrence depending on

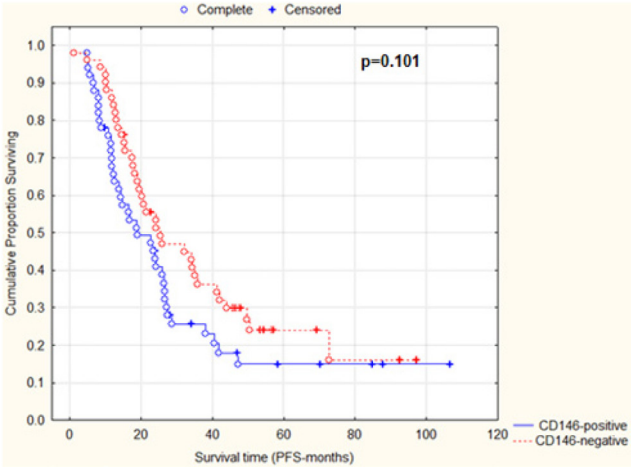

Figure 2. Kaplan-Meier survival curve for progression-free survival (PFS) in patients diagnosed with ovarian serous carcinoma, depending on CD146 expression in tumor cells.

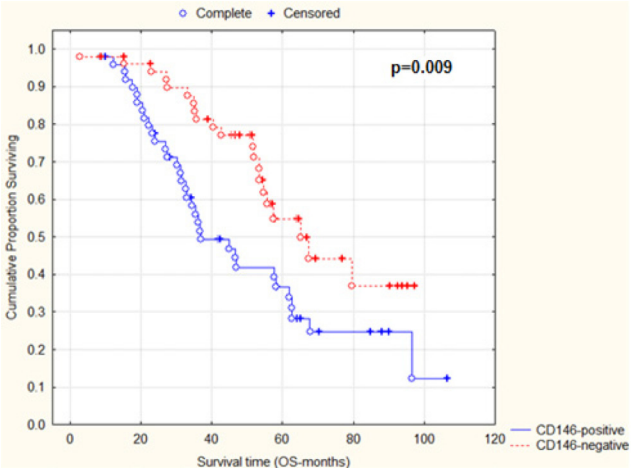

Figure 3. Kaplan-Meier survival curve for overall survival (OS) in patients with ovarian serous carcinomas, depending on CD146 expression in tumor cells.

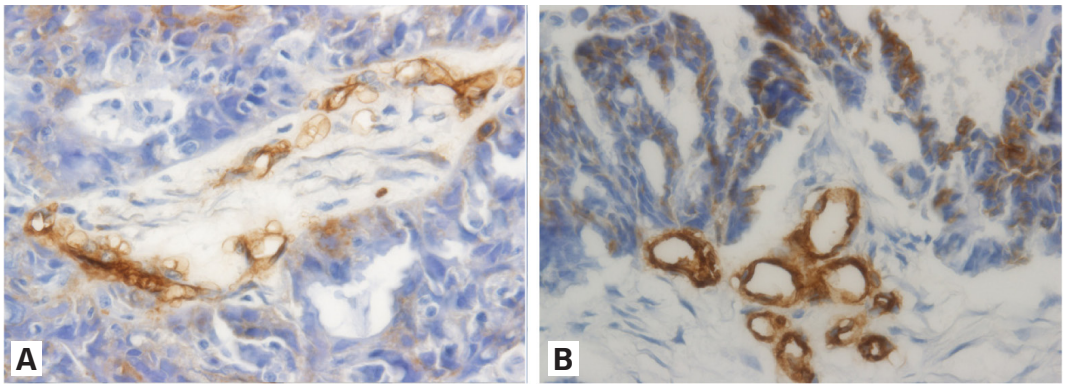

Figure 4. Positive CD146 expression in the tumor microvessel endothelial cells at the tumor-stroma interface. **A, B**: representative images from two different cases showing a uniform staining pattern of tumor microvessel endothelial cells. Magnification ×400.

Table 11. Progression-free survival depending on CD146 expression in tumor cells

CD146 expression	*No. of patients*	*No. of recurrences*	*Estimated median PFS (months)*	*95% CI*	*p value*
Negative	51	37	25.40	11.49-39.30	
Positive	50	40	19.00	10.54-27.45	0.101
Overall	101	77	24.00	18.62-29.37	

Table 12. Results of multivariate analysis using PFS as a dependent variable

Outcome	*Progression-free survival (PFS)*		
Factors	*OR*	*95% CI*	*p value**
Age, years (> 60 vs. ≤ 60)	1.71	0.73-1.87	0.509
FIGO stage (III/IV vs. I/II)	3.10	1.46-6.59	0.003
CD146 expression (positive vs. negative)	1.56	0.99-2.46	0.054

* estimated p values obtained from Wald test

CD146 expression in tumor cells. In multivariate analysis, the Cox regression model evidenced an OR=2.38 (p=0.077), independent of age at diagnosis and FIGO stage. The results of multivariate analysis are shown in Table 8.

Depending on the progresion-free interval (PFI), 19.5% of patients were considered resistant, non-responsive to platinum-based chemotherapy. The rest of patients (80.39%), with a PFI longer than 6 months, were considered sensitive to chemotherapy (Table 1). 70% of chemotherapy-resistant cases (a PFI shorter than 6 months) had positive CD146 expression. Among chemotherapy-sensitive patients (a PFI longer than 6 months), more than half (55.55%) had no CD146 expression in primary ovarian tumors (Table 9).

By applying Cox regression, the risk of resistance to chemotherapy associated with positive CD146 expression, a risk adjusted depending on age and the disease stage, was estimated. An OR=3.01 was calculated, the value of p=0.048 being statistically significant. In multivariate analysis, the age of patients over 60 years old had a tendency to associate with resistance to chemotherapy (OR=2.83, p=0.054), as shown in Table 10.

Subsequently, we analyzed the impact of CD146 expression on PFS and OS in patients included in the study. In the CD146-positive group, there were 40 recurrences, and the median progression-free time was 19 months in this group, compared to 25.4 months in the group with negative expression (Table 11). The association of CD146 positivity with a shorter PFS had a tendency to statistical significance (p=0.101) and is illustrated in Figure 2. In multivariate analysis, following adjustment depending on the FIGO stage and age of patients, this association maintained its tendency to statistical significance (p=0.054), with a HR of 1.56 (Table 12). For the FIGO stage, as part of the same Cox regression model, a HR of 3.1 was calculated, which was statistically significant (p=0.003).

Regarding OS relative to CD146 expression in ovarian tumors, there were 34 deaths in the CD146-positive group versus 21 in the CD146-negative group. The median survival time of patients in this group was 36 months, compared to 65 months in

Table 13. Survival time of patients depending on CD146 expression in tumor cells

CD146 expression	*No. of patients*	*No. of deaths*	*Estimated median OS (months)*	*95% CI*	*p value*
Negative	51	21	65.16	50.94-79.38	
Positive	50	34	36.83	23.10-50.55	0.009
Overall	101	55	57.40	49.11-65.68	

Table 14. Results of multivariate analysis using overall survival as a dependent variable

Outcome	*Overall survival*		
Factors	*OR*	*95% CI*	*p value**
CD146 expression (positive vs. negative)	2.31	1.33-4.02	0.003
Age, years (>60 vs. ≤60)	1.62	0.93-2.82	0.085
FIGO stage (III/IV vs. I/II)	5.65	1.72-18.58	0.004

* estimated p values obtained from Wald test

Table 15. Distribution of cases depending on microvessel density (MVD) determined by CD34 and CD146

Variables	*CD146 - MVD*		*p value*
	Low n (%)	*High n (%)*	
CD34-MVD			0.607
Low	52 (89.7)	6 (10.3)	
High	9 (23.1)	30 (76.9)	
Total	61 (62.9)	36 (37.1)	

the group with negative CD146 expression (Table 13). Following application of the Log-rank test, positive CD146 expression was statistically significantly associated (p=0.009) with low OS, an association illustrated in Figure 3.

The impact on OS was maintained following multivariate analysis, with an adjusted HR depending on age and FIGO stage of 2.31 and a statistically significant p (p=0.003). Following application of Cox regression, FIGO III/IV stage of disease was negatively associated with OS, with a HR of 5.65, p=0.004. In the case of age over 60 years, a tendency to statistical significance was observed (HR=1.62, p=0.085) (Table 14).

Intratumoral microvessel density (MVD) was assessed by CD34 immunostaining in the tumor microvessel endothelial cells, with a mean value of 46.72±22.29, quantified based on the number of CD34-positive microvessels. Regarding CD146 expression in capillary endothelial cells (Figure 4), the mean number of immunoreactive tumor microvessels was 44.73±21.44. In 60 (58.82%) cases, a concentration of CD146-positive microvessels at the tumor-stroma interface was observed. This microvascular growth pattern at the surface of the tumor was focal or extended depending on the density of the microvessel network.

Tumor microvessel density, determined by either CD34 or CD146, was considered increased in case of a number of positive microvessels situated above these mean values. 4 cases were excluded from statistical analysis because of missing data. The distribution of cases depending on MVD levels determined by each of the two endothelial markers is shown in Table 15. The application of McNemar's test resulted in no statistically significant differences regarding microvessel density measured by CD146 and CD34 (p=0.607), which demonstrates the concordance between the two methods for the quantification of MVD.

Discussion

In this study, we evaluated CD146 expression in ovarian serous carcinoma, in parallel to other known clinicopathological factors such as: age, FIGO stage, initial serum CA125 level, presence of malignant ascites and peritoneal carcinomatosis, degree of malignancy. The study group included patients diagnosed both at early and advanced stages, treated at IOCN by debulking surgery followed by standard platinum-based chemotherapy. The majority of the patients were aged less than 60 years and had advanced disease stages, with evidence of malignant ascites and peritoneal carcinomatosis in more than 70% of the cases. Early disease stages, FIGO I/II, accounted only for 16.6% of the cases. About 70% of patients had optimal surgery, without residual macroscopic tumors in the abdominopelvic cavity.

Following the immunohistochemical study performed, CD146 positivity was evidenced both in the tumor cell membrane and in the endothelial cell cytoplasm of microvessels in the tumor stroma. CD146 expression in the tumor cells and stroma opens multiple perspectives regarding the possible implications of this cell adhesion molecule in tumor development and progression.

In the first part of the study, we analyzed the potential associations between CD146 expression in tumor cells and the mentioned clinicopathological parameters, with a known prognostic role in ovarian carcinoma. The distribution of cases depending on CD146 expression was similar regardless of age, initial serum CA125 level, FIGO stage, presence/absence of malignant ascites, without statistically significant associations. In the presence of peritoneal carcinomatosis, CD146 positivity was more frequently observed, unlike the group without peritoneal carcinomatosis, an association with a tendency to statistical significance. An Italian study conducted an immunohistochemical analysis of CD146 expression in a similar manner, using the tissue microarray (TMA) technique in 133 tumor samples from patients with epithelial ovarian cancer [15]. According to the results of this study, positive CD146 expression in tumor cells was significantly associated with more advanced disease stages (FIGO III/IV), with the serous histological subtype, with a low grade of tumor differentiation, as well as with the accumulation of protein p53. Given that all these elements are characteristic of type II EOC, CD146 was proposed as a new marker for this histological subtype. In our study, no correlation between CD146 expression in tumor cells and the FIGO stage or the degree of tumor differentiation was observed.

Cell adhesion molecules (CAM) play an important role in migration and attachment to the omentum, pelvic peritoneum, intestinal or liver serosa, diaphragm, thus participating in the development of metastases. A previous study demonstrated a higher CD146 expression in metastatic ovarian cancer lesions [2]. In this study, we analyzed CD146 expression in primary ovarian tumors. Depending on this expression, we aimed to test the association with the locoregional or distant location of the disease recurrence. No statistically significant correlation between disease progression through distant metastases and CD146 overexpression in primary ovarian tumors was demonstrated. Independently of the type of recurrence (locoregional and/or distant), of the initial stage of the disease and the patients' age, positive CD146 expression was associated with a 2.38-fold higher risk of recurrence in multivariate analysis, with a tendency to statistical significance. The FIGO stage and the age of patients at the time of diagnosis were not statistically significantly associated with the risk of recurrence.

Following a literature review, we found no studies proposing the hypothesis of a link between CD146 expression in ovarian carcinoma and resistance to chemotherapy. In the current study, we observed a higher frequency of cases with positive CD146 expression in the group of patients considered resistant to chemotherapy, with progression of the disease within less than 6 months of completion of platinum-based chemotherapy. In contrast, patients considered sensitive to platinum-based chemotherapy had negative CD146 expression more frequently. Multivariate analysis confirmed a statistically significant association of CD146 expression in tumor cells with a risk of resistance to chemotherapy, independent of the patients' age and the initial FIGO stage. CD146 was studied in relation to resistance to platinum and taxanes in EOC through the mediation of hospicells, a type of stromal fibroblast-like cells, identified by surface markers such as CD146, but also CD9, CD10, CD29, CD166. Isolated from the ascites fluid of patients with ovarian cancer, hospicells interact with epithelial ovarian carcinoma cells, conferring them chemoresistance through the implication of multidrug resistance proteins [21]. In the absence of sufficient evidence, further studies are required to clarify the underlying mechanism of the association between CD146 expression and resistance to chemotherapy in ovarian cancer.

During the follow-up period of the 102 patients in whom CD146 expression was determined, 77 disease recurrences after primary treatment and 55 deaths were registered. In the subgroup of patients wih positive CD146 expression in tumor cells, a relatively similar number of recurrences was found compared to the subgroup without CD146 expression (41 vs. 37 relapses), but the number of deaths from ovarian cancer (34 vs. 21) was higher. Regarding the median disease progression time depending on CD146 expression, a difference of 6.4 months to the disadvantage of the subgroup with positive CD146 expression was seen, with a tendency to statistical significance. However, in multivariate analysis, after adjustment depending on the FIGO stage and age of patients, CD146 expression proved to be a prognostic factor for PFS, through a HR of 1.56, with a tendency to statistical significance (p=0.054). Nevertheless, the FIGO stage was a stronger prognostic factor than CD146 expression for PFS, considering a HR of 3.1 and a p value equal to 0.003.

The median survival time for patients with CD146-positive ovarian tumor cells was 29 months shorter compared to that of patients without CD146 expression in tumor cells (36 months vs. 65 months). Following survival analysis with the Log-rank test, this difference in OS between the two subgroups demonstrated statistical significance. Similar results were reported by Aldovini et al. in their study, in which CD146 overexpression was associated with a shorter PFS and a lower OS [15].

Compared to our study, significantly greater differences were observed between the two subgroups – CD146-positive vs. CD146-negative – regarding the median PFS (22 months vs. 79 months), as well as OS (42 months vs. 131 months). However, the longer follow-up period of patients in the Italian study should be mentioned. Furthermore, in this study, CD146 expression in tumor cells proved to be a stronger independent prognostic factor than the postsurgical residual tumor size, given the higher HR (5.25 vs. 3.77) for the prediction of disease progression. The prognostic value of CD146 was observed including patients with complete response after primary treatment, who presented early recurrences and a rapid evolution towards death. In the case of these patients, treated by optimal debulking (no residual) and having a complete response to adjuvant chemotherapy, CD146 might indicate the presence of microscopic disease, with the identification of a subgroup of patients potential candidates for a more intensive follow-up, a maintenance treatment or new therapeutic strategies [15,22]. The prognostic role of CD146 corroborated with its potential predictive role for response to chemotherapy might support its status as a therapeutic target in ovarian serous carcinoma. Experimental studies have demonstrated that a reduction of CD146 expression is responsible for an increase in malignant cell apoptosis and a reduction of the invasion capacity beyond the extracellular matrix [2,17].

CD146 expression was initially studied in endothelial cells in the context of tumor neoangiogenesis. Besides its role as a cell adhesion molecule, mainly expressed at the level of interendothelial junctions, the VEGFR2 co-receptor function of CD146 is an additional argument regarding its role in the control of angiogenesis. In the current study, CD146 expression in the endothelial cell cytoplasm was quantified by counting the number of CD146-positive intratumoral microvessels, with a calculated mean of 44.73±21.44 microvessels. Above this mean value, the CD146-positive microvessel density (MVD) was considered increased. In relation to MVD determined by the expression of CD34, a well-established endothelial marker, no statistically significant differences were found regarding MVD measured by CD146 and CD34 in primary ovarian tumors. The absence of statistical significance confirms the concordance between the two MVD quantification modalities, which validates the role of CD146 as a reliable endothelial biomarker and the possibility of its use for assessing tumor neoangiogenesis. In more than half of the analyzed cases, a more or less extensive concentration of CD146-positive microvessels at the tumor-stroma interface was observed. This area is described as a growth front, which favors tumor invasion and progression through complex processes that occur at this level, including as part of epithelial-mesenchymal transition (EMT) [17]. Consequently, this microvascularization pattern, evidenced by CD146 expression, can represent another argument in favor of a more aggressive tumor behavior.

Immunohistochemical studies conducted so far have highlighted a pronounced immunoreactivity in tumor tissue vessels compared to normal tissue vessels by using anti-CD146 monoclonal antibodies, as well as the possibility of an effective inhibition of proliferation and angiogenesis by means of these antibodies [23]. Practically, a reduction of CD146 expression can contribute to the suppression of VEGF-mediated signaling pathways, such as p38 MAPK and Akt [2].

In support of this idea, other studies have shown that CD146 inhibition determines the suppression of endothelial cell proliferation, adhesion and migration [24]. A study conducted on pancreatic carcinoma cell lines evidenced the additive effect of an anti-CD146 monoclonal antibody used in combination with bevacizumab in inhibiting tumor angiogenesis [1]. Given the relatively reduced efficiency of bevacizumab in ovarian cancer, the double inhibition of VEGF and CD146 could bring a therapeutic benefit and further studies are warranted to test this hypothesis.

Conflict of interests

The authors declare no conflict of interests.

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