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RESEARCH

Correlation Between E-cadherin Immunoexpression and Efficacy of First Line Platinum-Based Chemotherapy in Advanced High Grade Serous Ovarian Cancer

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Abstract To analyze correlation between immunoexpression of E-cadherin and efficacy of first line platinum-based chemotherapy in patients with advanced-stage high-grade serous ovarian carcinoma. The expression of E-cadherin was analyzed immunohistochemically in formalin-fixed, paraffin-embedded samples from 98 patients with advanced-stage high-grade serous ovarian cancer and related to clinical features (stage according to the International Federation of Gynecology and Obstetrics (FIGO) and residual tumors after initial cytoreductive surgery), response to platinum-based chemotherapy (according to Response Evaluation Criteria in Solid tumors (RECIST 1.1 criteria)), platinum sensitivity (according to platinum free interval (PFI) as platinum-refractory, platinum-resistant and platinum-sensitive) and patients progression free survival (PFS) and overall survival (OS). E-cadherin immunostaining was positive in 74 and negative in 24 serous ovarian carcinomas. E-cadherin immunoreactivity was not associated with FIGO stage, residual tumor after initial cytoreductive surgery and number of chemotherapy cycles. Positive E-cadherin expression predict significantly better response to first line platinum-based chemotherapy ($p<0.001$) and platinum sensitivity ($p<0.001$). Moreover, positive E-cadherin expression predict significantly longer PFS ($p<0.001$) and OS ($p<0.001$). The multivariate analysis for OS showed that positive E-cadherin expression is predictor to platinum sensitivity ($p<0.001$) and longer OS ($p=0.01$). Positive E-cadherin expression seems to be a predictor of better response to first line platinum-based chemotherapy, platinum sensitivity and favorable clinical outcome in patients with advanced-stage serous ovarian cancer. Negative E-cadherin expression was shown to be significant, independent predictor of poorer PFS and OS. E-cadherin as a marker has predictive and prognostic value.

Keywords Ovarian cancer · E-cadherin · Immunohistochemistry · Response to platinum-based chemotherapy · Platinum sensitivity · Prognosis

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Introduction

Ovarian cancer is estimated to be diagnosed in more than 238 000 women per year worldwide and remains a significant cause of gynecological cancer mortality [1]. Despite to development in imaging methods and surgical techniques, we fail to make a diagnosis in early stage and take timely intervention [2]. Majority of women continue to present themselves at advanced stages and the overall 5-year survival rate is around 45 % [3].

The current standard of care for newly diagnosed ovarian cancer is a combination of optimal cytoreductive surgery and platinum-based chemotherapy [4]. Although the response of the primary tumor to taxane and platinum-based chemotherapy is high, about 20 % of patients never achieve a clinical response, and majority of other patients relapse and die of

drug-resistant disease [5]. In general, patients who progress or have stable disease during the first-line treatment or who relapse within 1 month are considered to be "platinum-refractory". Patients who respond to primary treatment and relapse within 6 months are considered to be "platinum-resistant" and patients who relapse more than 6 months after completion of initial therapy are characterized as "platinum-sensitive". Longer PFI increases the chances of benefitting by platinum rechallenge, especially if it is longer than 12 months [6].

The drug-resistant nature of epithelial ovarian cancer cells, initial or acquired, defines that effective therapies are lacking, which contributes to the high mortality in patients with ovarian cancer [7]. The identification of the molecular mechanisms underlying chemoresistance is mandatory to achieve advancement in ovarian cancer therapy [8]. Resistance to platinum-based chemotherapy can be intrinsic or acquired. It may be mediated by factors outside or within the cancer cell or its cell membrane [9]. Platinum-resistance is most probably multifactorial and it may be due to excess of a resistance factor, to saturation of factors required for tumor cell killing, or to mutation or alteration of a factor required for tumor cell killing. It could arise from decreased tumor blood flow, extracellular conditions, reduced platinum uptake, increased efflux, intracellular detoxification by glutathione, decreased binding, DNA repair, decreased mismatch repair, defective apoptosis, antiapoptotic factors, effects of several signaling pathways, or presence of quiescent non-cycling cells [10].

Accumulate evidence demonstrates that epithelial-mesenchymal transition (EMT), which modulates cancer progression and metastasis, has also be implicated in the onset of drug resistance and tumor relapses, representing an escape mechanism from apoptosis. The acquisition of mesenchymal phenotypes engenders tumor cells with a multifaceted capacity to proliferate, migrate, and avoid cell death and permanent arrest, as well as protection from extracellular signals and drug effect activities [11].

EMT is a physiological process that occurs during embryonic development and occasionally in adults during wound healing. EMT is a phenomenon during which cells will undergo a transition from an epithelial phenotype to a more motile and invasive mesenchymal phenotype rendering them able to invade tissues and form metastases. The main hallmark of EMT is the loss of E-cadherin [12]. E-cadherin is one of the most important molecules in cell-cell adhesion in epithelial tissues. It is a member of large family calcium-dependent cell adhesion glycoproteins. E-cadherin consists of a large extracellular domain, a single transmembrane segment and a short cytoplasm domain, which interact with actin cytoskeleton through linker molecules, catenins. This connection is important for establishment and maintenance of tissue homeostasis [13]. Reduced expression of E-cadherin has been correlated with poor survival and high invasive capacity in various cancers including prostate, gastric and inflammatory breast cancer [14–17].

Better understanding of the biology of ovarian cancer is of paramount importance. Additional information on the molecular and cellular markers is needed in predicting tumor progression and response to chemotherapy [18]. Knowing the predictors of response to platinum-based chemotherapy can help us to select sensitive patients for chemotherapy and to spare resistant ones from platinum-based chemotherapy toxicity.

The origin and pathogenesis of epithelial ovarian cancer (EOC) have long been investigated but are still poorly understood. Correlated clinicopathologic and molecular genetic studies led to the development of a dualistic model that divides all histological types of EOC into two broad categories designated type I and type II [19]. Low-grade serous carcinomas are a prototypes type I tumor. They are rare and account for about 5 % of all serous carcinomas, clinically indolent and usually present at a low stage. They are genetically stable and are characterized by high frequency of KRAS or BRAF mutations. Low-grade serous carcinomas are initially resistant to chemotherapy and hormonal therapy [19, 20]. High grade serous carcinomas are a prototypes type II tumor, which are characterized by a high level of genetic instability and harbors p53 mutations in nearly all cases. They are highly aggressive and almost all presented in advanced stage. They usually respond to first line platinum-based chemotherapy, but quickly recur [19–22].

The aim of this study was to determine immunohistochemical expression of E-cadherin in high-grade serous ovarian cancer and to assess the correlation of expression of E-cadherin with efficacy to platinum-based chemotherapy.

Methods

Patients

We have analyzed medical history and histological samples of 98 patients with advanced-stage (FIGO III and IV stage) high-grade serous ovarian cancer treated at the Clinical Hospital Centre Split and General Hospital Zadar, Croatia between January 1996 and April 2013.

The inclusion criteria were histologically confirmed advanced-stage high-grade serous ovarian cancer, history of debulking surgery followed by first line platinum-based chemotherapy, accessibility of primary tumor specimens for further pathological analysis and full medical data. All tissue samples used for E-cadherin immunostaining had been taken at initial laparotomy. FIGO stage, tumor grade, residual tumor after primary surgery, age of patients, chemotherapy regimens and number cycles of chemotherapy, response rate, PFS and

OS were obtained from histopathological reports and patient medical records.

The staging was performed in accordance with the standards of the International Federation of Gynecology and Obstetrics (FIGO) [23]. Carcinomas were graded as either low- or high-grade according to the two-tier grading system recommended by Malpica et al. Criteria were based primarily on nuclear variability (>3-fold nuclear atypia) with secondary use of mitotic activity (>12 mitoses) [24]. Residual tumor size was provided by the primary surgeon and postoperative measurement by multi slides computed tomography (MSCT). We classified patients according to residual tumors in three groups: optimal surgery (no visible postoperative residuals), suboptimal surgery (visible residuals) and unknown status of residual tumor [25].

Out of all patients, a great majority 87 (89 %) received paclitaxel plus platinum combinations: 84 (86 %) patients received paclitaxel plus cisplatin/carboplatin (TC), 2 (2 %) patients received cisplatin, gemcitabine and paclitaxel (TCG), 1 (1 %) patient received cisplatin, epirubicin and paclitaxel (TEC). All others, 11 %, received cisplatin based chemotherapy, but without paclitaxel; 7 (7 %) patients received cisplatin, doxorubicin and cyclophosphamide (CAP), 2 (2 %) patients received cisplatin and cyclophosphamide (CC), 1 (1 %) patient received cisplatin and etoposide (PE) and 1 (1 %) patient received cisplatin only. Among all patients, 61 (62 %) received 6 cycles of chemotherapy, 32 (33 %) patients received more than 6 cycles of chemotherapy, and 5 (5 %) patients received less than planned 6 cycles.

Response to platinum-based chemotherapy was defined according to RECIST 1.1 criteria as complete response (CR), partial response (PR), stable disease (SD) and progression of disease (PD). CR was defined as disappearance of all target lesions, PR as 30 % or more decrease in sum of diameters of target lesions, PD as 20 % or more increase in sum of diameters of target lesions and/or appearance of one or more new lesions and SD – which did not qualify for either sufficient shrinkage to qualify for PR or for sufficient increase to qualify for PD [26]. Platinum sensitivity was defined according to platinum free interval as platinum-refractory, platinum-resistant and platinum-sensitive [5]. Patient survivals included PFS and OS. PFS was calculated as the interval from the day of surgery to the first occurrence of any new lesions that could be measured or assessed clinically or patients death whatever the cause. OS was calculated as the interval from the day of surgery to the last visit or death whatever the cause.

The Ethical Committee for Biomedical Research of the Clinical Hospital Split and School of Medicine approved this research to be in compliance with the Helsinki Declaration (reference number 49-1/06).

Immunohistochemical Staining and Analysis

At the time of surgery, tumors were dissected and fixed for 24 h in neutral buffered formalin. After fixation, slices were routinely embedded in paraffin wax. Immunostaining for E-cadherin was performed with monoclonal antibody to human E-cadherin (mouse, clone NCH-38, DAKO, Denmark) at 1:100 dilutions. The 4 μm section were placed on silane-coated slides, deparaffinized and rehydrated in descending concentrations of alcohol, immersed in phosphate-buffered saline (PBS) containing 3 % hydrogen peroxide and then processed in a microwave oven (in 10 mmol/L sodium citrate buffer, pH 6,5, for 15 min at 700 W and additional 10 min at 300 W). The slides were than washed with PBS and incubated overnight with primary antibody. The next day, slides were washed again with PBS, incubate with secondary antibody (EnVision, mouse, DAKO) for 30 min, washed with PBS and visualized with DAB (DAKO) for 10 min. After washing with distilled water counter-staining with Mayer's hematoxylin in duration of 1 min was done. In the end, slides were washed with water, dehydrated in increased concentration of alcohol and xylen, immersed with mounting medium and covered with the covering glass. To ensure accurate and reproducible staining, normal epidermis was used as positive control in which strong and homogenous expression of E-cadherin was observed on the cell membrane.

The E-cadherin expression was scored by two independent observers (ST, DS) without knowledge of the clinical data. When independent scoring of a case was in dispute, the case was rechecked and a conclusive agreement was reached by simultaneously viewing of the section by the two observers using a double-headed microscope. Expression of E-cadherin was assessed using a semiquantitative scoring system, ranging from 0, 1+, 2+, and 3+. E-cadherin expression was scored as follows:

- 0, no immunoreactivity
- 1+, incomplete or dot-like faintly membranous immunoreactivity
- 2+, complete circumferential membranous immunoreactivity of < 10 % of tumour cells
- 3+, complete circumferential membranous immunoreactivity of ≥ 10 % of tumour cells

For statistical purposes, according to literature data, all cases were further summarised into two groups: E-cadherin positive (score 3+) and E-cadherin negative (scores 0, 1+ and 2+). In his meta-analysis, Peng et al. found that cut off ≥ 10 % was used in most of the published studies [27–34].

Positive and negative E-cadherin immunoexpression is shown in Fig. 1a and b.

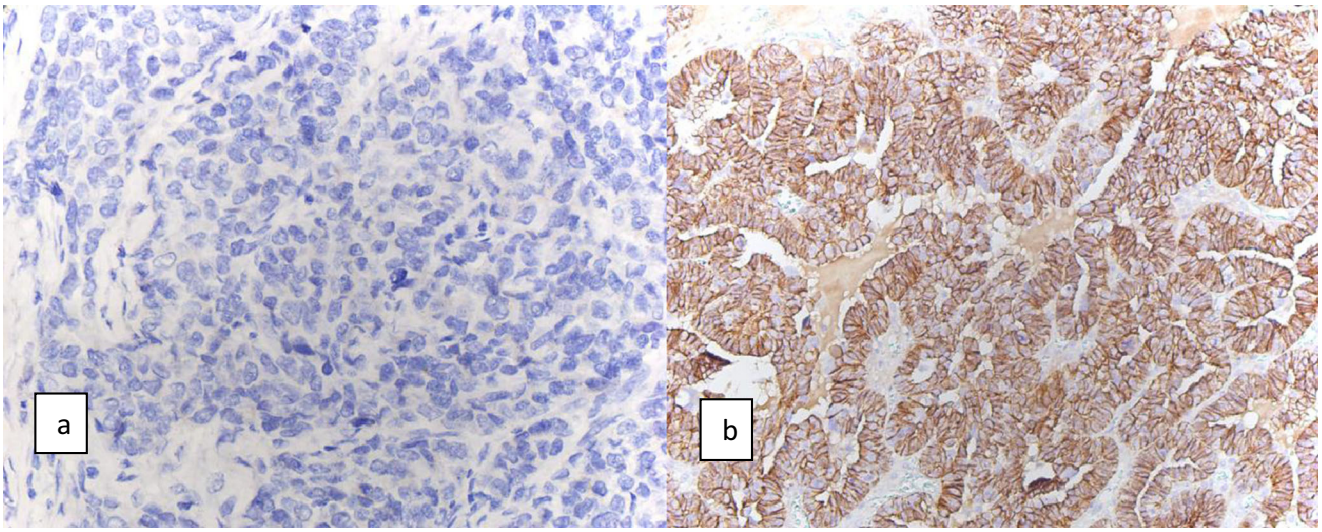

Fig. 1 Immunoreactivity for E-cadherin in high-grade serous ovarian carcinoma. **a** A case of E-cadherin negative serous ovarian carcinoma (x400). **b** A case of E-cadherin positive serous ovarian carcinoma (x400)

Statistical Analysis

Statistical analysis was carried out using the SPSS version 16.0 software package. The association between negative versus positive E-cadherin expression and clinical parameters was evaluated using the χ^2 test. The univariante survival analysis was based on the Kaplan-Meier method. Comparison between the survival curves was analyzed using the Log-rank test. The prognostic and predictive significance of negative E-cadherin expression was assessed using the multivariate Cox's proportional hazard's analysis. P values ≤ 0.05 were considered stastically significant.

Results

Ninety-eight patients with advanced, stage III (80 patients, 82 %) and IV (18 patients, 18 %), high-grade serous ovarian carcinoma were admitted to the Department of Oncology of Split University Hospital and General Hospital Zadar between January 1996 and December 2013. After median follow-up of 27.5 months, out of 98 patients 58 (59 %) died and 40 (41 %) are alive. Out of alive patients, 17 (17 %) developed recurrence with anti cancer treatment ongoing, and 23 (23 %) are without disease recurrence (Table 1).

The median age of patients is 58 years (range 38–79 years). E-cadherin immunostaining was positive in 74 (76 %) and negative in 24 (24 %) tumors. According to RECIST 1.1 criteria 70 (71 %) patients had CR, 8 (9 %) patients had PR, 5 (5 %) patients had SD and 15 (15 %) patients had PD as the best response to chemotherapy. With respect to platinum sensitivity 62 (63 %) patients were sensitive, 24 (25 %) patients were resistant and 12 (12 %) patients were refractory to first line platinum-based chemotherapy. In further analysis we have combined patients with resistant and refractory disease in one group defined as resistant. Median PFS was 16 months (range 4–142 months) and median OS was 31 months (range 7–142 months) (Table 1).

No significant relationship was found between E-cadherin expression and clinical parameters, such as age of patients (≤58 vs. >58) (p=0.729), FIGO stage (III vs. IV) (p=0.720), radicality of surgical treatment (optimal vs. suboptimal) (p<0.091) and the number of cycles of chemotherapy (≤6 vs. > 6) (p=0.675). Positive E-cadherin expression was associated with statistically significantly better response to first-line platinum-based chemotherapy (χ^2_1=17.1, χ^2_2=22.8; p<0.001) and with platinum sensitivity (χ^2=24.6; p<0.001) (Table 2).

Univariate analysis of PFS was shown significant relationship between PFS and FIGO stage (III vs. IV) (p=0.006), objective response (CR + PR vs. SD + PD) (p<0.001), clinical benefit (CR + PR + SD vs. PD) (p<0.001), platinum sensitivity (p<0.001) and E-cadherin expression (positive vs. negative) (p<0.001) (Table 3, Fig. 2).

Cox multivariate analysis of PFS confirmed that increase number of cycles of chemotherapy (>6) and platinum sensitivity were associated with the longer PFS. Objective response on chemotherapy showed 93 % marginal significance with PFS (p=0.066). Contrary, with respect to clinical benefit there were no significant relationship to PFS (p=0.235) (Table 4).

Univariate analysis of OS was found significant relationship between OS and FIGO stage (III vs. IV) (p=0.007), objective response (CR + PR vs. SD + PD) (p<0.001), clinical

Table 1 The association between clinical variables in relation to the patient status at the end of follow-up in 98 patients with advanced-stage high-grade ovarian cancer

N (%)					
		Disease status at the end of follow-up			
		Total (*N*=98)	Died (*N*=58)	Alive with recurrence (*N*=17)	Alive without recurrence (*N*=23)
Age		58(38–79)	56,5(38–75)	62(40–79)	58(38–77)
PFS		16(4–142)	12,5(4–109)	17(5–47)	26(12–142)
OS		31(7–142)	30(7–110)	26(17–111)	36(12–142)
Age	≤58	52(53)	32(55)	6(35)	14(61)
	>58	46(47)	26(45)	11(65)	9(39)
FIGO stage	IIIB	2(2)	1(2)	0	1(4)
	IIIC	78(80)	43(74)	15(88)	20(87)
	IV	18(18)	14(24)	2(12)	2(9)
Surgery	Optimal	15(15)	6(11)	3(18)	6(27)
	Suboptimal	78(80)	48(89)	14(82)	16(73)
	Unknown	5(5)			
Chemotherapy cycles	≤6	66(67)	34(59)	12(71)	20(87)
	>6	32(33)	24(41)	5(29)	3(13)
Response to chemotherapy	CR	70(71)	33(57)	14(82)	23(100)
	PR	8(9)	7(12)	1(6)	0
	SD	5(5)	5(9)	0	0
	PD	15(15)	13(22)	2(12)	0
Platinum resistance	Sensitive	62(63)	25(43)	14(82)	23(100)
	Resistant	24(25)	21(36)	3(18)	0
	Refractory	12(12)	12(21)	0	0
E-cadherin	Positive	74(76)	37(64)	14(82)	23(100)
	Negative	24(24)	21(36)	3(18)	0

N number, *PFS* progression free survival, *OS* overall survival, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progression of disease

Table 2 Correlations between the expression of E-cadherin (positive vs. negative) and clinical variables in 98 patients with advanced-stage high-grade ovarian cancer

N (%)					
		E-cadherin			
		Total	Positive	Negative	*p*
Age	≤ 58	52(53)	40(54)	12(50)	0.729
	> 58	46(47)	34(46)	12(50)	
FIGO stage	III	80(82)	61(83)	19(79)	0.720
	IV	18(18)	13(17)	5(21)	
Surgery	Optimal	15(15)	14(20)	1(5)	0.091
	Suboptimal	78(80)	57(80)	21(95)	
Chemotherapy cycles	≤ 6	66(67)	49(66)	17(71)	0.675
	> 6	32(33)	25(34)	7(29)	
Response to chemotherapy 1	CR + PR(OR)	78(80)	66(89)	12(50)	<0.001
	SD + PD	20(20)	8(11)	12(50)	
Response to chemotherapy 2	CR + PR + SD(CB)	83(85)	70(94)	13(54)	<0.001
	PD	15(15)	4(6)	11(46)	
Chemotherapy sensitivity	Sensitive	62(63)	57(77)	5(21)	<0.001
	Resistant	36(37)	17(23)	19(79)	

N number, χ^2 chi square, *CR* complete response, *PR* partial response, *OR* objective response, *SD* stable disease, *PD* progression of disease, *CB* clinical benefit

Table 3 Univariate analysis of the age of patients, FIGO stage, radicality of surgery treatment, number of chemotherapy cycles, objective response, clinical benefit, chemotherapy sensitivity and expression of E-cadherin on progression free survival in 98 patients with advanced-stage high-grade serous ovarian cancer

		N	HR(95%CI)	*p*
Age	≤ 58[a]	52	1.21(0.77–1.8)	0.408
	> 58	46		
FIGO stage	III[a]	80	2.21(1.3–3.9)	0.006
	IV	18		
Surgery	Optimal[a]	15	1.7(0.85–3.4)	0.134
	Suboptimal	78		
Chemotherapy cycles	≤ 6	66	1.3(0.8–2.0)	0.314
	> 6[a]	32		
Response to chemotherapy 1	CR + PR(OR)[a]	78	11.6(6.2–22)	<0.001
	SD + PD	20		
Response to chemotherapy 2	CR + PR + SD(CB)[a]	83	9.3(4.9–17.7)	<0.001
	PD	15		
Chemotherapy sensitivity	Sensitive[a]	62	19.5(9.7–38.9)	<0.001
	Resistant	36		
E-cadherin	Positive[a]	74	4.3(2.5–7)	<0.001
	Negative	24		

[a] referral level, *N* number, *HR* hazard risk, *CI* confidence interval, *CR* complete response, *PR* partial response, *OR* objective response, *SD* stable disease, *PD* progression of disease, *CB* clinical benefit

benefit (CR + PR + SD vs. PD) ($p<0.001$), platinum sensitivity ($p<0.001$) and E-cadherin expression (positive vs. negative) ($p<0.001$) (Table 5; Fig. 3).

Cox multivariate analysis of OS confirmed that positive E-cadherin expression was statistically significant associated with the longer OS ($p=0.01$) (Table 6).

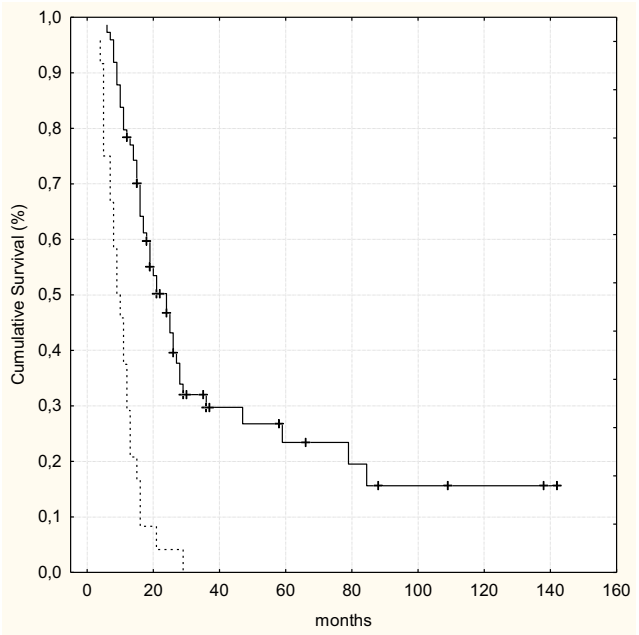

Fig. 2 Progression free survival and E-cadherin expression in 98 patients with advanced-stage high-grade serous ovarian cancer. The *continuous line* represents positive E-cadherin expression ($N=74$). The *dashed line* represents negative E-cadherin expression ($N=24$). Patients with positive E-cadherin expression had median PFS 24 months and patients with negative E-cadherin expression had median PFS 9 months ($p<0.001$)

Discussion

High-grade serous, advanced-stage ovarian carcinoma represents a huge public health problem. Standard approach in treatment of such patient population is platinum based chemotherapy, paclitaxel/carboplatin (TC) as standard [35, 36]. Median overall survival of such patient population has not changed since the introduction of paclitaxel 18 years ago [37]. Unfortunately, during that time we have not witnessed any significant improvement, either in treatment or in selection of patients for optimal therapy. Clearly, there is unmet need for better, more active therapies in future and better selection of patients for therapy than what we have today. We are witnessing tremendous development in the field of breast or lung cancer (Mamma Print, Oncotype DX, ALK and EGFR testing) that are prerequisite for personalized and individualized therapy [38–41]. Similar development in the field of ovarian cancer is needed.

The fact that more than 90 % of ovarian cancers are of epithelial origin defines EMT to play a determining role in ovarian cancer progression. Another important aspect of EMT is its possible involvement in the ability of cancer cells to resist chemotherapeutic agents as well as acquire drug-resistance. Combining these important specificities of EMT, we can make a hypothesis that this phenomenon could be a major factor resulting in the high mortality rates associated with ovarian cancer. Finding new approaches to block EMT induction in cancer may show great promise to improve the outcome for patients [11].

Cell-cell adhesion determines cell polarity and participates in cell differentiation and in establishment and maintenance of tissue homeostasis. During oncogenesis, this organized

Table 4 Multivariate analysis of the age of patients, FIGO stage, radicality of surgical treatment, number of cycles of chemotherapy, objective response, chemotherapy sensitivity and E-cadherin expression on progression free survival in 98 patients with advanced-stage high-grade serous ovarian cancer

		N	HR(95%CI)	*p*
Age	≤ 58[a]	52	1.0(0.6–1.7)	0.907
	> 58	46		
FIGO stage	III[a]	80	0.83(0.4–1.8)	0.633
	IV	18		
Surgery	Optimal[a]	15	1.4(0.6–3.0)	0.405
	Suboptimal	78		
Chemotherapy cycles	≤ 6	66	1.9(1.1–3.5)	0.029
	> 6[a]	32		
Response to chemotherapy 1	CR + PR (OR)[a]	78	2.3(0.95–5.4)	0.066
	SD + PD	20		
Chemotherapy sensitivity	Sensitive[a]	62	17.5(7–44)	<0.001
	Resistant	36		
E-cadherin	Positive[a]	74	1.35(0.7–2.7)	0.4
	Negative	24		

[a] referral level, *N* number, *HR* hazard risk, *CI* confidence interval, *CR* complete response, *PR* partial response, *OR* objective response, *SD* stable disease, *PD* progression of disease, *CB* clinical benefit

adhesion is disturbed by genetic and epigenetic changes, resulting in changes in signaling, loss of contact inhibition, and altered cell migration and stromal interactions. A major member of cell-cell adhesion molecules is E-cadherin. It was characterized as a potent suppressor of cell motility, invasion and metastasis [42, 43].

Epithelial cells transformed by EMT can escape apoptosis and lead to the development of resistance to chemotherapy [6, 10]. The elaboration and understanding of the numerous signaling pathways involved in development of chemotherapy resistance by EMT may help clinicians select an optimal anticancer drug treatment. Based on these findings, it was logical to investigate the association between EMT through E-cadherin expression and chemotherapy resistance in various tumor types.

In our retrospective study, positive E-cadherin expression was statistically significantly associated with better response to first line platinum-based chemotherapy ($p<0.001$) and with platinum sensitivity ($p<0.001$) in patients with advanced-stage high-grade serous ovarian cancer. According to our knowledge, this is the first study to investigate the correlation between E-cadherin expression and efficacy to first line platinum-based chemotherapy in advanced-stage high-grade serous ovarian cancer. Specificity of this study is that our patient population consists only of advanced-stage high-grade serous ovarian cancers which,

Table 5 Univariate analysis of the age of patients, FIGO stage, radicalitiy of surgery treatment, number of chemotherapy cycles, objective response, clinical benefit, chemotherapy sensitivity and expression of E-cadherin on overall survival in 98 patients with advanced-stage high-grade serous ovarian cancer

		N	HR(95%CI)	*p*
Age	≤58[a]	52	1.13(0.67–1.9)	0.647
	>58	46		
FIGO stage	III[a]	80	2.3(1.3–4.3)	0.007
	IV	18		
Surgery	Optimal[a]	15	2.34(0.99–5.5)	0.051
	Suboptimal	78		
Chemotherapy cycles	≤6	66	1.35(0.79–2.3)	0.269
	>6[a]	32		
Response to chemotherapy 1	CR + PR(OR)[a]	78	11.2(5.5–22.6)	<0.001
	SD + PD	20		
Response to chemotherapy 2	CR + PR + SD(CB)[a]	83	12(5.5–26)	<0.001
	PD	15		
Chemotherapy sensitivity	Sensitive[a]	62	11.9(5.9–24)	<0.001
	Resistant	36		
E-cadherin	Positive[a]	74	5.1(2.8–9.3)	<0.001
	Negative	24		

[a] referral level, *N* number, *HR* hazard risk, *CI* confidence interval, *CR* complete response, *PR* partial response, *OR* objective response, *SD* stable disease, *PD* progression of disease, *CB* clinical benefit

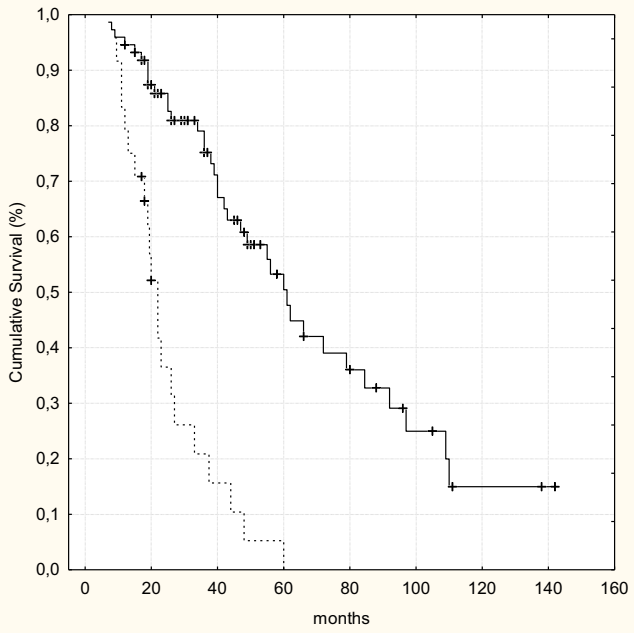

Fig. 3 Overall survival and E-cadherin expression in 98 patients with advanced-stage high-grade serous ovarian cancer. The *continuous line* represents positive E-cadherin expression (N=74). The *dashed line* represents negative E-cadherin expression (N=24). Patients with positive E-cadherin expression had median OS 61 months and patients with negative E-cadherin expression had median OS 22 months (p<0.001)

according to our knowledge, was not the case with other studies in this field.

Similarly to results of our study, Bodnar et al. have examined relationship between β-catenin and E-cadherin expression and response to chemotherapy in 29 patients with advanced-stage, different histological cell types and grades of ovarian cancer. β-catenin forms adherent junction together with E-cadherin. Most of patients (87.5 %) with objective response had decreased membrane β-catenin expression, and that was statistically significant [34].

Predictive and prognostic E-cadherin expression status was investigated in patients with various tumor types [44–51]. Positive correlation between E-cadherin expression and response to chemotherapy was found in breast and colorectal cancer [44–47]. Koo's et al. evaluated the impact of various pathologic and biologic factors in triple negative breast cancer to chemotherapy response using in vitro chemotherapy response assay. In line with our results, positive expression of E-cadherin showed high chemo response for many agents, particularly for vinorelbine [44]. Another in vitro study demonstrated that breast tumor cells with positive E-cadherin expression showed lower sensitivity to cisplatin but no difference to etoposide and 5-FU [45]. Nakamoto et al. studied E-cadherin as potential additional biomarker of response to cetuximab therapy in patients with metastatic colorectal cancer. Expression of E-cadherin was significantly correlated with the efficacy of cetuximab therapy in KRAS wild-type patients. In KRAS mutant-type patients, E-cadherin expression was not significantly correlated with the effect of cetuximab therapy, but all responders with KRAS mutant-type expressed E-cadherin. Therefore, the combination of E-cadherin immunohistochemistry and KRAS analysis may be a more sensitive biomarker than KRAS analysis alone [47]. On the other hand, negative correlation between E-cadherin expression and response to chemotherapy was found in leiomyosarcoma, advanced gastric carcinoma and lung carcinoma [49–51].

Survival is the most wildly used and recognized by clinical scientists as well as medical authorities endpoint in oncology trials. In our study, we correlated E-cadherin expression with OS and PFS, and we confirmed that E-cadherin expression is prognostic factor for patient survival. Multivariate analysis demonstrated no statistically significant difference between the E-

Table 6 Multivariate analysis of the age of patients, FIGO stage, radicality of surgical treatment, number of cycles of chemotherapy, objective response, chemotherapy sensitivity and E-cadherin expression on overall survival in 98 patients with advanced-stage high-grade serous ovarian cancer

		N	HR(95%CI)	*p*
Age	≤ 58[a]	52	1.11(0.62–2.0)	0.718
	> 58	46		
FIGO stage	III[a]	80	1.62(0.79–3.3)	0.190
	IV	18		
Surgery	Optimal[a]	15	1.35(0.53–3.5)	0.525
	Suboptimal	78		
Chemotherapy cycles	≤ 6	66	0.72(0.38–1.4)	0.317
	> 6[a]	32		
Response to chemotherapy 1	CR + PR(OR)[a]	78	1.8(0.73–4.3)	0.209
	SD + PD	20		
Chemotherapy sensitivity	Sensitive[a]	62	9.3(3.7–23)	<0.001
	Resistant	36		
E-cadherin	Positive[a]	74	2.7(1.3–5.9)	0.01
	Negative	24		

[a] referral level, *N* number, *HR* hazard risk, *CI* confidence interval, *CR* complete response, *PR* partial response, *OR* objective response, *SD* stable disease, *PD* progression of disease, *CB* clinical benefit

cadherin expression and PFS (p=0.4). These results are in agreement with those of Dian et al. [31]. They analyzed expression of E-cadherin immunohistochemicaly in 100 serous ovarian cancer tissue samples, all grades and FIGO stages I-IV. Patients with a strong E-cadherin staining intensity had better progression-free and overall survival rate despite the lack of statistical significance [31]. In contrast to the above, Cho et al. published results of study where reduced expression of E-cadherin was correlated with peritoneal metastasis, tumor-related death and overall survival rate [29]. Multivariate analysis for overall survival showed that platinum sensitivity (p<0.001) and positive E-cadherin expression (p=0.01) are associated with longer OS. Numerous other studies have further defined the potential value of E-cadherin as prognostic marker for ovarian cancer [18, 28–30, 32, 33, 52]. Finally, meta-analysis of 9 studies and 915 patients, confirmed that negative expression of E-cadherin was associated with poor overall survival. Majority of population in meta-analysis were in FIGO stages III and IV, therefore the above stated conclusion may be more suitable for advanced ovarian cancer [27]. Negative results have also published making this field more complicated [31, 53, 54].

Potential limitations of our study were a relative small number of patients with ovarian cancer and short median follow-up. Nevertheless, this is according to our knowledge, the largest and the most selective study of E-cadherin predictive and prognostic value in the field of ovarian cancer, including only advanced-stage high-grade serous carcinomas. The short median follow-up period of 27.5 months is also, potentially, one of limitations of the study. In spite of the and following the fact that median PFS in our study was 16 months and median OS was 31 months, we believe that median follow up in our study is sufficient to drown appropriate results.

To conclude, our results indicate that E-cadherin expression via immunohistochemistry is a possible predictive marker to first line platinum-based chemotherapy efficacy in high-grade serous ovarian cancer. Our results confirm prognostic value of E-cadherin in advanced-stage serous ovarian cancer.

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