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ORIGINAL ARTICLE

High expression of mesothelin in advanced serous ovarian cancer is associated with poor prognosis

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Summary

Purpose: *Mesothelin is a cell surface glycoprotein which is highly expressed in various types of epithelial cancers. Its expression level is associated with poor prognosis in many cancer types. The aim this study was to evaluate the association of the level of mesothelin expression with clinicopathological characteristics and its prognostic significance in patients with advanced serous ovarian cancer (SOC).*

Methods: *Tissue blocks from a total 42 patients with advanced SOC treated at the medical oncology clinic of Izmir Katip Celebi University Ataturk Training and Research Hospital between 2006 and 2013 were evaluated. Immunohistochemical staining for mesothelin was performed. Clinical characteristics, optimal or suboptimal operation, response to platinum-based chemotherapy, and overall survival (OS) were analyzed.*

Results: *The cut-off value of 45 for mesothelin H-score determined by ROC analysis predicted survival with 86% sensitivity and 75% specificity (p=0.020). We found a notable negative correlation between mesothelin H-score and OS (r= -0.570, p=0.0001). The median OS was 67 months (95%CI, 36.114 to 97.886) in the low-staining mesothelin H-score group and 27 months (95%CI, 22.238 to 31.762) in the high-staining mesothelin H-score group (p=0.002). Univariate analysis showed that the clinical stage IV disease (p=0.023), platinum chemoresistance (p=0.001), higher mesothelin H-score (p=0.002), and suboptimal surgery (p=0.024) were associated with worse OS. In the multivariate Cox regression model, mesothelin H-score (B=1.15, 95%CI=1.016 to 9.850, p=0.047) and the status of platinum sensitivity (B=-.916, 95%CI=.185 to -.864, p=0.020 were statistically significant predictors for OS.*

Conclusion: *These results indicated that high mesothelin H-scores were significantly associated with poor prognosis in patients with advanced SOC.*

Key words: *mesothelin, serous ovarian cancer, overall survival, prognosis*

Introduction

Ovarian cancers (OC) can be composed of epithelial cells, germ cell or sex-cord stromal cells. About 90% of OC is derived from malignant transformation of the ovarian surface epithelium. It is the leading cause of gynecologic cancer mortality, which is expected to occur in 22,240 women, with 14,070 deaths in the United States in 2018 [1]. The median age at the time of diagnosis is 63 years [2]. Unfortunately, less than 40% of women with OC can be cured [1], with a five-year survival rate of 44% [2]. Serous ovarian cancer (SOC) is clinically the most common subtype and accounts for 68% of all epithelial OC, with more than 80% of them being in stage III/IV disease at diagnosis [3]. This

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disease is lethal due to lack of reliable screening test, robust biomarkers for early detection, and vague symptoms [2,4]. Survival outcome primarily depends on disease stage at diagnosis and, to a lesser extent, the age of patient, tumor grade, and histological type. Although the prognostic value of clinicopathologic factors is well-established in SOC, the role of molecular markers is still unclear and it is worth discussing their usefulness in predicting prognosis and estimating therapeutic response.

Mesothelin is a cell surface glycoprotein and already structurally present in mesothelial cells lining the peritoneum, pericardium and pleura [5,6]. The primary product is encoded by the mesothelin gene, which is the 71 kDa precursor protein. This protein is partitioned by some proteases into a 40-kDa C-terminal fragment (C-ERC/mesothelin) that remains membrane-bound, and a 31-kDa N-terminal fragment (N-ERC/mesothelin), which is secreted into the blood [5]. C-ERC/mesothelin binds to the cell membrane via a glycosyl-phosphatidylinositol [7]. Mesothelin can be detected in plasma, tissues, peritoneal fluid or urine.

The biological function of mesothelin is not well-understood. Many researchers have investigated the role of mesothelin expression in tumor biology and have shown the importance of mesothelin expression for cell survival, proliferation, invasion, migration, tumor progression, and resistance to chemotherapy [8-11]. Bharadwaj et al. showed that the overexpression of mesothelin can induce Nuclear factor kappa-B (NF-kB) signal transduction and IL-6 production. Similarly, it can activate the signal transducer and activator of transcription 3 (STAT-3), inhibit apoptotic signaling and TNF-alpha-induced apoptosis, and accelerate the G1-S transition [10].

Overexpression of this plasma membrane differentiation antigen has been described as a new marker associated with a variety of human cancers including mesothelioma, ovarian, gastric, colorectal, breast, lung, cholangiocarcinoma and gastrointestinal carcinomas [5,12-18]. In contrast, low expression levels of mesothelin were detected in some other tumors such as breast, thyroid, kidney and prostate cancers [19]. Clinical impacts of mesothelin expression have also been evaluated in these studies. High expression of mesothelin was generally found to be related to poor survival [11]. This protein has very high specificity especially for serous type of OC [20].

The aim of this study was to evaluate the association of the levels of mesothelin expression with clinicopathological characteristics and its prognostic significance in patients with advanced SOC.

Methods

Patient selection

The medical records of patients with SOC, who were admitted to the Medical Oncology Clinic of Izmir Katip Celebi University Ataturk Training and Research Hospital between 2006 and 2013, were retrospectively reviewed. The cut-off date for data collection for this analysis was February 2018. Staging was performed according to the International Federation of Gynecology and Obstetrics (FIGO 2014) classification. The grade and histological type were classified according to the World Health Organization (WHO-2014) criteria. Patients with stage I/II disease and non-serous type ovarian carcinoma were excluded. The local Institutional Review Board approved the study.

Pathological assessment

Formalin-fixed, paraffin-embedded sections (4 μm) from representative tissue blocks were used. The ex-

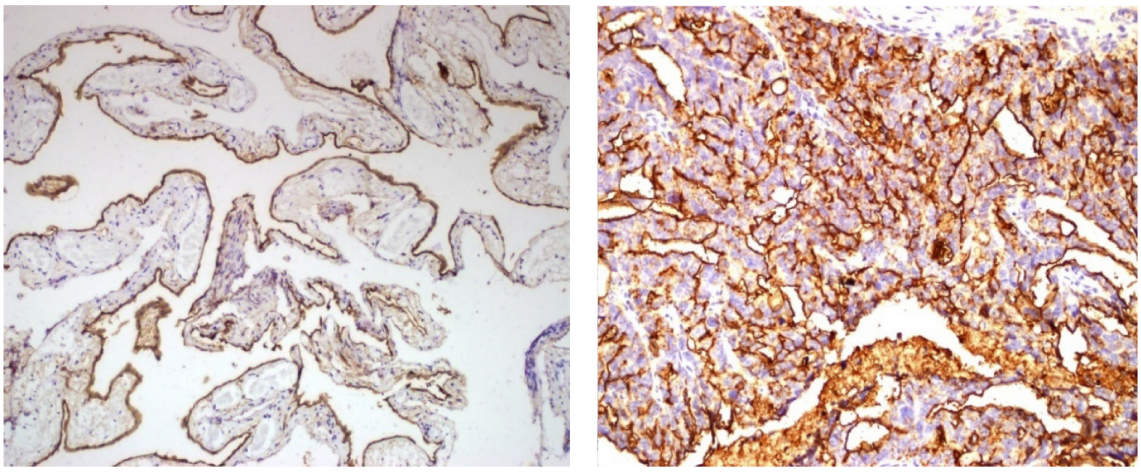

Figure 1. Immunohistochemical staining of mesothelin; positive control (left) and high expression of mesothelin (right) in serous ovarian cancer (x10).

pression of mesothelin was evaluated by immunohistochemically using a 'Leica BOND-MAX' automatic immunostaining device. Normal peritoneal mesothelial cells were used as a positive control for mesothelin staining (Figure 1). For immunohistochemical (IHC) evaluation of mesothelin expression, sections were incubated with anti-mesothelin antibody (Dako, Hamburg, Germany) at a 1:30 dilution. Reactions were visualized using DAB (Diaminoaminobenzidine) choromogen. The staining was evaluated using cytoplasmic expression with apical membranous accentuation as the pattern for mesothelin-specific labeling. Mesothelin staining was quantified with the H-score, which assesses the proportion of tumor cells with positive staining and the intensity of staining (1+, 2+, 3+), with a maximal score of 300(13) (Figure 1).

Treatment endpoints

Overall survival (OS) was defined as the time from the date of pathological diagnosis to the date of final follow-up or death. Optimal debulking was defined if the maximum diameter of residual tumor nodules was less than 1 cm [21].

Statistics

The Kaplan–Meier method was performed to estimate survival outcomes and groups were compared with log-rank test. Cox proportional hazards models were fit to determine the association between mesothelin and survival outcomes after adjustment for patient and disease characteristics. The 95% confidence interval (CI) was used to quantify the relationship between survival time and each independent factor. All statistical tests carried out were two-sided, and a p value ≤0.05 was considered statistically significant. To test for correlations between mesothelin H-scores and OS, Pearson's correlation analysis was performed. All statistical analyses were made using the Statistical Package of Social Science (SPSS) version 16.0 software (Chicago, IL).

Table 1. Clinical characteristics of the patients with serous ovarian cancer

Patient characteristics	*n (%)*
Age (years), median (range)	55 (36-80)
Disease stage at initial diagnosis	
IIIA	2 (4.8)
IIIC	18 (42.9)
IV	22 (52.4)
Grade	
1-2	13 (31)
3	29 (69)
Debulking status (< 1cm)	
Optimal resection	17 (40.5)
Suboptimal resection	25 (59.5)
Clinical response to chemotherapy	
Chemosensitive	27 (64.3)
Chemoresistant	15 (35.7)
Disease status at last follow-up	
No evidence of disease	3 (7.1)
Evidence of persistent disease	1 (2.4)
Dead	38 (90.5)
Initial serum CA-125 level (U/ml; median, range)	535 (12-6942)

Results

Forty-two consecutive patients with advanced SOC were investigated. The median follow-up time was 30.5 months (range 7-107). Thirty-eight (90.5%) of the patients who exhibited tumor progression dieddue to disease-related factors. The median age was 55 years (range 36-80) . Nearly half of the patients (52.4%) had FIGO stage IV disease All cases had serous adenocarcinoma histology, with most of them having poor differentiation (69.0%). The characteristics of the 42 patients are shown in Table 1.

Fourteen patients (33.3%) underwent an initial optimal cytoreduction. There were only three patients (7.1%), in whom the clinical stage was IV, who received neoadjuvant chemotherapy (CT) followed by surgery. Twenty-five patients (59.5%) underwent suboptimal debulking surgery.

Patients were divided into two groups according to the effectiveness of the operation as follows: optimally debulked (OD) vs. suboptimally debulked (SD). Median OS was 41 months (95%CI, 9.73 to 72.26) for the OD patients and 29 months (95%CI, 22.88 to 35.12) (p=0.024) for SD patients.

At the end of 6 or 8 cycles of postoperative standard CT, the patients were divided into two groups based on the criteria described previously as follows: A) chemosensitive group (n=27, 64.3 %), if there was a regression in tumor size as determined clinically or radiologically after completion of the first-line platinum-based combination CT, with no evidence of relapse or progression. B) chemoresistant group (n=15, 35.7 %), if the disease persisted or progressed after completion of the first line platinum-based combination CT [1,22].

The median OS for the platinum-sensitive and platinum-resistant patients was 39 months and 24 months, respectively (p=0.01) (Figure 2).

The data regarding the stages of the patients were as follows: stage IIIA (n=2;4.8%), stage IIIC (n=18;42.9%), stage IV (n=22;52.4%) (Table1). Patients were grouped as stage III or IV disease. There was a statistically significant difference between the groups in terms of OS [stage III (34 months) vs. stage IV patients (25 months), log rank p=0.006].

Mesothelin H-score cut-off level was determined as 45, using ROC analysis. Sensitivity and

specifity of mesothelin H-score for this value was 86% and 75%, respectively. Among the 42 patients, 8 cases (19.0%) had low staining scores (H-score; 0-45) and 34 cases (81.0%) had high staining scores (H-score; 45-270) for mesothelin expression staining. The median OS was 67 months (95%CI, 36.114 to 97.886) in the low-staining mesothelin H-score group and 27 months (95%CI, 22.238 to 31.762) in the high-staining mesothelin H-score group (p=0.02; Figure 3). A notable negative correlation was found between mesothelin H-scores and OS (r= -0.570, p=0.0001, Pearson's correlation test).

Probable clinicopathological parameters and biomarkers were evaluated to predict OS for the SOC. Univariate analysis showed that the clinical stage (III/IV), the status of platinum sensitivity (sensitive vs. resistant), mesothelin H-scores (low vs. high), and type of surgery (OD vs. SD) were associated with OS. In the multivariate Cox regression model, mesothelin H-scores (B=1.15, 95%CI=1.016 to 9.850, p=0.047) and the status of platinum sensitivity (B=-.916, 95%CI=.185 to -.864, p=0.020) were statistically significant predictors for OS (Table 2).

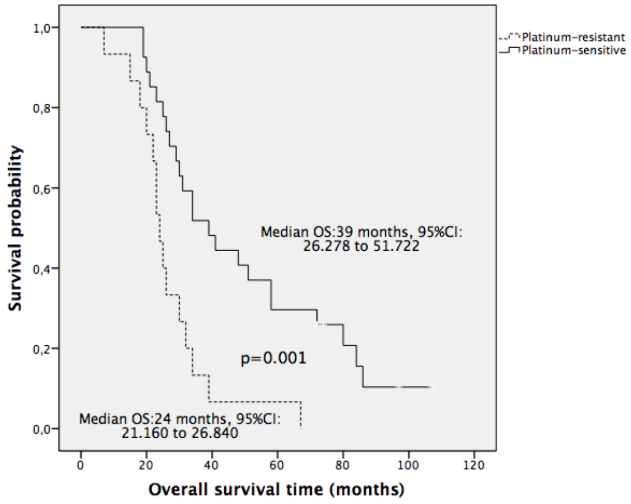

Figure 2. Overall survival according to platinum sensitivity.

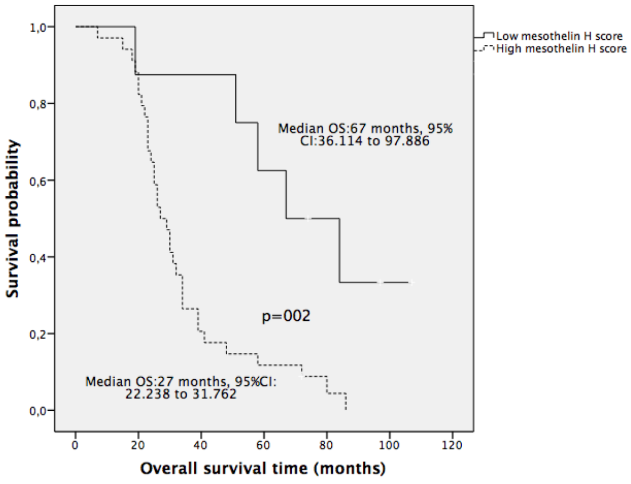

Figure 3. Overall survival according to mesothelin H-scores.

Discussion

In the present study, mesothelin was stained in all patients with advanced SOC and the median OS was found to be shorter in the high-staining mesothelin H-score group than that in the low-staining mesothelin H-score group, with a statistically significant difference.

There has been great effort over the last few years to identify more promising biomarkers. Mesothelin is a novel biomarker which is expressed in SOC and can be measured in clinical practice by its expression with ICH staining, in serum or other body fluids including urine [12,13,17,23]. Mesothelin is strongly expressed in normal mesothelial cells and ovarian tumors. Mesothelin expression was first evaluated in ovarian tumors in 1992 by Chang et al. [24]. They reported mesothelin expression in 10 of 15 (66%) non-mucinous OC using the K1 antibody. Hassan and colleagues found the rate of mesothelin expression in SOC as 82% [25], whereas this rate was was 100% in our patients. The staining in the OC and primary peritoneal serous carcinoma was seen in 14 of 14 (100%) patients and 5 of 5 (100%) patients, respectively [26]. Thus, Cheng and co-workers reported that tumors with advanced stages had higher mesothelin than those with early disease stages [11].

Table 2. Univariate analysis of factors associated with overall survival and Cox proportional hazards regression model of clinical factors predicting overall survival in patients with serous ovarian cancer

	Log Rank (Mantel Cox)		*Cox-proportional Hazard*	
	Chi-square	*p*	*B (95%CI)*	*p*
Mesothelin H-scores (low vs. high)	9.192	0.002	1.151 (1.016-9.850)	0.047
Status of platinum-sensitivity (sensitive vs. resistant)	10.372	0.001	-0.916 (0.185–0.864)	0.020
Type of surgery (optimally debulked vs. suboptimally debulked)	5.109	0.024	0.205 (0.559-2.696)	0.610
Clinical stage (III vs. IV)	5.135	0.023	0.053 (0.476-2.336)	0.896

Its serum levels have many beneficial characteristics that may assist in earlier diagnosis of high risk patients [23]. When combined with CA-125, serum mesothelin has a greater sensitivity for cancer diagnosis [27]. Mesothelin has also similar specificity and sensitivity to CA-125 for OC diagnosis [27], providing a potential supplemental role in combination with CA-125 during the monitoring and diagnosis of OC patients [28]. The relationship between serum mesothelin level and prognosis has been evaluated in several articles. Serum mesothelin levels were significantly increased at advanced stages and elevated mesothelin levels before therapy showed worse OS for the epithelial OC patients(p=0.042) [29]. Our first significant finding was that mesothelin H-scores was an independent predictor of OS. There are two studies reporting that mesothelin expression by IHC staining is in association with survival in OC. Similarly, Chang et al. analyzed the levels of mesothelin expression and survival outcomes in OC patients. They divided cancer patients into two groups according to the expression levels of mesothelin as high- and low-expressed and found statically significantly worse OS for high-expressed group (p< 0.001) [11]. By contrast, Yen et al. [30] reported positive correlation between OS and the level of mesothelin staining. They separated high-grade cases into two groups: group A with diffuse immunoreactivity (immune-staining score: 2-4) and group B with negligible or focal immunoreactivity (immune-staining score: 0-1). Patients in group A had a significantly better OS than patients in group B (p=0.023). This result is not what we expected, because high expression of mesothelin expression has been found to be associated with poor survival outcomes in other tumors. Tomas et al. reported that patients with high-mesothelin expressing tumors had significantly shorter OS compared with patients with low- or no-mesothelin expressing tumors (p=0.014) [31]. Similarly, mesothelin expression in patients with early stage lung adenocarcinoma was also associated with increased risk of recurrence and reduced OS [16]. Mesothelin was implicated as a molecular marker of tumor aggressiveness in that trial [16]. Yun et al. investigated the mesothelin expression in breast cancer [32]. They included 141 breast cancer patients, with most of them having triple negative pattern and mesothelin staining was found to be an independent poor prognostic factor in patients with breast cancer [32]. The expression of mesothelin was associated with an unfavorable outcome in patients with pancreatic ductal adenocarcinoma [33].

The second significant finding of this study was that mesothelin expression conferred a worse platinum sensitivity in advanced SOC patients. As the effect of cytotoxic drugs is influenced by tumor volume and stage, we analyzed whether the expression level of mesothelin correlated with response to CT in SOC patients with different disease stages or surgery status (optimal or suboptimal). All of these prognostic factors were associated with OS in the univariate analysis. But in the multivariate Cox regression model, platinum resistance and high mesothelin H-scores were the only two prognostic factors related to OS in our study. High mesothelin levels were correlated with platinum chemoresistance in our study, as well as in the study of Chang et al [11].

Our study has some limitations. Despite the small sample size providing the association between mesothelin expression and worse outcome in the advanced-stage patients, we couldn't demonstrate this relation in the early-stage disease or ovarian benign lesions.

In summary, mesothelin expression can be stained immunohistochemically in advanced SOC. It may provide knowledge regarding whether the disease is chemosensitive or chemoresistant. It is an independent prognostic factor and high mesothelin H-score is associated with the worse OS in advanced SOC.

Conflict of interests

The authors declare no conflict of interests.

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