RESEARCH ARTICLE

Hormone Receptor, HER2/NEU and EGFR Expression in Ovarian Carcinoma - is here a Prognostic Phenotype?

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Abstract

Purpose: We aimed to evaluate the effects of hormone receptor, HER2, and epidermal growth factor receptor (EGFR) expression on epithelial ovarian cancer (EOC) prognosis and investigate whether or not phenotypic subtypes might exist. Materials and Methods: The medical records of 82 patients who were diagnosed with EOC between 2003 and 2012 and treated by platinum-based chemotherapy were retrospectively evaluated. Expression of EGFR, oestrogen (ER), progesterone (PR), and cerbB2 (HER2) receptors were assessed immunohistochemically on paraffin-embedded tissues of these patients. Three phenotypic subtypes were defined according to ER, PR, and HER2 expression and associations of these with EGFR expression, clinicopathologic features, platinum sensitivity, and survival were investigated. Results: When we classified EOC patients into three subtypes, 63.4% had hormone receptor positive (HR(+)) (considering breast cancer subtypes, luminal A), 18.3% had triple negative, and 18.3% had HER2(+) disease. EGFR positivity was observed in 37 patients (45.1%) and was significantly more frequent with advanced disease (p=0.013). However, no significant association with other clinicopathologic features and platinum sensitivity was observed. HER2(+) patients had significantly poorer outcomes than HER2(-) counterparts (triple negative and HR positive patients) (p=0.019). Multivariate analysis demonstrated that the strongest risk factor for death was residual disease after primary surgery. Conclusions: Triple negative EOC may not be an aggressive phenotype as in breast cancer. The HER2 positive EOC has more aggressive behaviour compared to triple negative and HR(+) phenotypes. EGFR expression is more frequent in advanced tumours, but is not related with poorer outcome. Additional ovarian cancer molecular subtyping using gene expression analysis may provide more reliable data.

Keywords: Ovarian carcinoma - receptor expression - phenotypes - prognosis

Asian Pac J Cancer Prev, **15 (22)**, 9739-9745

Introduction

Ovarian cancer is the second most common gynecologic malignancy, the most common cause of gynecologic cancer death, and the fifth leading cause of cancer death in women in developed countries (Siegel et al., 2014). Around 25% of patients present with a tumour confined to the ovary or pelvis (stage I/II) while the other 75 percent present with advanced disease (stage III) or with distant metastasis (stage IV). The standard of care for EOC patients is surgery with a maximal cytoreductive procedure followed by systemic chemotherapy (Young et al., 1983; Boente et al., 1998). However, despite higher tumour response rates, some patients develop distant metastases or relapse locally, while other patients do not respond even to the initial chemotherapy. Therefore, many studies investigate the possible predictive and/or prognostic biomarkers in order to improve survival of EOC patients. These studies identified some major reported factors associated with improved survival: younger age, low volume of residual disease, good performance status, serous histology, and low CA125 level after surgery (Gadducci et al., 1995; Crawford et al., 2005; Winter et al., 2007; Zivanovic et al., 2009; Suprasert and Chalapati, 2013). Moreover, researchers have focused on finding new pathologic, biochemical and molecular markers, as they are becoming important variables in oncology. Some of the important areas recently investigated in several studies include p53, bax bcl-2 expression (Ziolkowska-Seta et al., 2009; Yigit et al., 2011), tumour-infiltrating lymphocytes (Bosmuller et al., 2011), gene signatures (Han et al., 2012), and Wnt-B catenin pathway (Bodnar et al., 2014). Hormone receptor and HER2 expression are well-known prognostic and predictive markers in

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breast cancer, and targeting these receptors with selective agents has yielded a major improvement in breast cancer patient outcome. Moreover, it is well known from neoadjuvant chemotherapy studies that certain breast cancer molecular and/or phenotypic subtypes respond differently to chemotherapy and behave with a different prognosis. In contrast to breast cancer, relatively few reports evaluating the importance of receptor expressions and EOC subtypes exist in the literature and demonstrate contradictory results (Hogdall et al., 2007; Liu et al., 2010; Sinn et al., 2011; Chumworathayi, 2013; Zhao et al., 2013). EGFR expression was also suggested to be related to worse prognosis in a few studies (Skirnisdottir et al., 2001; Noske et al., 2011). In this study, besides traditional clinicopathologic parameters, we evaluated the prognostic and predictive effects of hormone receptor, HER2 and EGFR expressions on EOC prognosis separately, also their correlations and associations with platinum sensitivity. Additionally, we investigated whether or not a prognostic phenotypic EOC subtype, as in breast cancer, may be defined for EOC.

Materials and Methods

Ethics approval

This study has been approved by the local ethics committee of the Ege University, Izmir, (Approval number: 12-1.1/54, Date: 11April 2012) and has been carried out in compliance with the guidelines of the Helsinki Declaration of 1975.

Patients and treatment procedures

Eighty-two patients, diagnosed with EOC and treated in the Department of Medical Oncology, Izmir Ataturk Training and Research Hospital, between 2003 and 2012, were retrospectively evaluated. Standard ovarian carcinoma surgeries were performed by three different departments of gynecology and obstetrics in the same hospital. A suboptimal cytoreduction was defined when residual disease was greater than >1cm. Disease staging was performed using the criteria of the International Federation of Gynecology and Obstetrics (FIGO). Histologic type and tumour differentiation (histologic grade) were assessed on paraffin-embedded tissue specimens by using WHO (World Health Organization) classification (Lee et al., 2003).

The majority of the study group had advanced-stage disease (stage III-IV disease), of whom 52.9% had stage IIIC and IV disease at initial diagnosis. Optimal cytoreduction could be performed in 48.8% of study group. Median age at diagnosis was 54 years (range:24-80). The major histologic type was serous papillary morphology, and 46.3% of the patients had poorly differentiated (grade III) tumours. The follow-up time was defined as the time from diagnosis (operation procedure) to death or last visit, whichever came first. The patients received standard platinum-based combination chemotherapy regimens in each three-week cycle for a minimum of six cycles. A total of 31 patients received chemotherapy through the third line, 13 patients fourth line, 6 patients fifth line, while 2 patients received through the sixth-line and 1 patient received through the seventh-line during the follow-up time.

Treatment response was evaluated by using the WHO response criteria (Miller et al., 1981). Complete response (CR) is defined as the normalization of CA125 levels in patients who had a higher level at baseline and the disappearance of findings in computerized tomography after first-line chemotherapy and confirmation at four weeks. The other responses are defined as partial, stabile, and progressive disease (which is also described as the platinum-resistant group). Progression-free survival (PFS) is defined as the time from diagnosis (operation date) to relapse (or progression) or death (from any cause) or last visit, whichever occurs first. Overall survival (OS) is defined as the time from diagnosis to the last visit or death. The patients were classified into three groups according to relapse patterns: platinum resistant (relapse within 6 months after completion of therapy), platinum sensitive (relapse after longer than 6 months after completion of therapy), and platinum highly sensitive (progression after longer than 24 months after completion of therapy).

Immunohistochemical analysis and evaluation method

Immunohistochemical staining analysis: Immunohistochemical (IHC) staining was performed on formalin-fixed and paraffin-embedded tissue using the LSAB- HRP method. Tissue sections were then incubated for 1.5 hours at room temperature with primary antibodies as follows: rabbit monoclonal (SP2) for progesterone receptor (ab 16661) as 1/100 dilution, rabbit monoclonal (SP1) for oestrogen receptor (ab 16660) as1/100 dilution, rabbit monoclonal (SP3) for ErbB2 (ab 16662) as 1/40 dilution, and anti-EGFR antibody (EP38Y) (ab 52894) as 1/50 dilution. Diaminobenzidine (DAB) was used as a chromogen for reaction visualization. Finally, the sections were counterstained with Mayer's hematoxylin, dehydrated, cleared with xylene, and mounted with cover slips using a permanent mounting medium.

Normal breast tissue was used as a positive control for both oestrogen and progesterone receptor expressions, while cerbB2 3 (+) breast cancer tissue and metastatic lymph node tissue were used as positive controls for cerbB2 and EGFR expressions, respectively.

Assessment of IHC analysis: All sections were examined under light microscopy by a pathologist experienced in gynecologic pathology. Stained tumour cells in each tissue were counted in ten fields at 400 X magnification. For oestrogen receptor (ER) and progesterone receptor (PR) expression, samples in which 1% or more of tumour cells exhibited nuclear staining were judged to be positive. HER2 was scored visually according to the ASCO/CAP 2007 guidelines [0 or 1+ (negative): no staining or incomplete membrane staining in >30% of tumour cells; 2+ (weakly positive, equivocal): strong, complete membranous staining in <30% of tumour cells or weak to moderate heterogeneous staining in >10% of tumour cells); 3+ (strongly positive): strong complete membrane staining in >30% of tumour cells)] (Hammond, 2011). Membranous EGFR expression was scored as positive if tumour cells displayed immunoreactivity in > 1% of cells. (Figure 1a-d)

Statistical analysis

The statistical analyses were performed using SPSS, ver.16 (SPSS Inc., Chicago, IL, USA). Possible associations between protein expressions and survival, as well as other clinicopathologic factors, were investigated. The statistical significance of a difference between two categorized variables was assessed using the x^2 (chi-square) test. Non-parametric tests (Mann-Whitney U and Kruskal-Wallis) were used to compare the relationship between multi-sorted variables (e.g., tumour phenotypic subtype). The survival analyses were performed by using Kaplan-Meier analyses; all of the ranges were described with a 95% confidence interval (CI). A survival comparison according to the different parameters was fulfilled using the log-rank test. Cox regression analysis was used for multivariate analysis to predict the strongest independent prognostic factor. $P<0.05$ were considered as significant for all of the statistical tests.

Results

Immunohistochemical characteristics

ER and PR expressions were negative in 26.8% (n=22) and 48.8% (n=40) of patients, respectively; 42.7% of patients (n=37) were positive for both ER/PR, while 8.5% (n=7) of the patients were positive only for PR and 30.5% of the patients were positive for only ER (n=25). The majority of the patients had cerbB2-negative disease, and cerbB2 3+ expression was observed only in 15 patients (18.3%).When we classified tumours according to receptor expressions into three subtypes, the majority of the patients (63.4%, n=52) were only HR(+) (considering breast cancer subtypes may be called luminal A), while both triple negative and HER2(+) phenotype constituted 18.3% of the patients (n=15, for both). EGFR positive expression was observed in 37 patients (45.1%). The relationship between EGFR expression and other receptors was also examined. EGFR expression did not differ according to hormone receptor, HER2 expression, or phenotypic subtype.

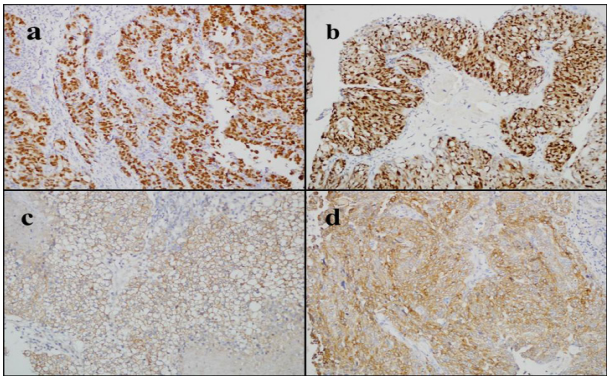

Figure 1. The Immunohistochemical Appearance of Protein Expressions. a) oestrogen receptor; **b)** progesterone receptor; **c)** cerbB2 receptor; **d)** epidermal growth factor receptor (EGFR)

The relationship between receptor expressions and clinicopathologic features

IHC expressions of ER, PR, cerbB2, and EGFR were investigated individually according to clinical parameters (such as age, disease stage, residual disease, distant metastasis and platinum sensitivity). In addition, a non-parametric correlation analysis was performed to investigate whether or not any correlation existed between these parameters. Among these variables, the only significant correlation was observed between EGFR expression and disease stage (Spearman test: r=0.275, p=0.013). EGFR positive expression was significantly more frequent in patients with advanced disease (83.3% of the patients with advanced-stage disease were EGFR positive vs. 16.7% of the patients with early-stage disease were EGFR positive; Chi-square, p=0.013) (Table 1).

Platinum sensitivity and association with clinicopathologic features and receptor expressions

Of the entire study group, 57.3% (n=47) were platinum sensitive. The majority of early-stage patients were also platinum sensitive as expected [88.5% (n= 23/26) of the patients with early-stage disease *vs* 42.9% (n= 24/56) of the patients with advanced-stage disease; p<0.0001]. Consistently, the patients of whom surgery was achieved as an optimal cytoreduction were more platinum sensitive (75% *vs* 40.5%; p=0.002). Platinum sensitivity did not vary according to the other major clinicopathologic features such as age, histologic type and tumour differentiation. Moreover, no significant association was observed between ER, PR, cerbB2, EGFR expressions and phenotypic subtype (Table 2).

Survival analyses

Table 1. The Association of Receptor Expressions and Ovarian Cancer Subtype with Clinical Features.

Variable		Age			Stage			Residual disease			Distant Metastasis		
		≤50 N %	>50 N %	p	Early N %	advanced N %	p	<1cm N %	>1cm N %	p	no N %	yes N %	p
ER	(-)	11 (50%)	11 (50%)		8 (36.4%)	14 (63.6%)		13 (59.1%)	9 (40.9%)		16 (72.7%)	6 (27.3%)	
	(+)	23 (38.3%)	37 (61.7%)	0.34	18 (30%)	42 (70%)	0.58	27 (45%)	33 (55%)	0.25	45 (75%)	15 (25%)	0.83
PR	(-)	13 (32.5%)	27 (67.5%)		11 (27.5%)	29 (72.5%)		18 (45%)	22 (55%)		33 (82.5%)	7 (17.5%)	
	(+)	21 (50%)	21 (50%)	0.1	15 (35.7%)	27 (64.3%)	0.42	22 (52.4%)	20 (47.6%)	0.5	28 (66.7%)	14 (33.3%)	0.1
cerbB2	(-)	28 (41.8%)	39 (58.2%)		21 (31.3%)	46 (68.7%)		35 (52.2%)	32 (47.8%)		49 (73.1%)	18 (26.9%)	
	(+)	6 (40%)	9 (60%)	0.89	5 (33.3%)	10 (66.7%)	0.88	5 (33.3%)	10 (66.7%)	0.18	12 (80%)	3 (20%)	0.58
EGFR	(-)	17 (37.8%)	28 (62.2%)		19 (42.2%)	26 (57.8%)		23 (51.1%)	22 (48.9%)		33 (73.3%)	12 (26.7%)	
	(+)	17 (47.2%)	19 (52.8%)		6 (16.7%)	30 (83.3%)	0.013	16 (44.4%)	20 (55.6%)	0.55	27 (75%)	9 (25%)	0.86
Subtype													
Triple negative		8 (53.3%)	7 (46.7%)		5 (33.3%)	10 (66.7%)		10 (66.7%)	5 (33.3%)		10 (66.7%)	5 (33.3%)	
HR positive		20 (38.5%)	32 (61.5%)		16 (30.8%)	36 (69.2%)		25 (48.1%)	27 (51.9%)		39 (75%)	13 (25%)	
HER2 positive		6 (40%)	9 (60%)	0.58	5 (33.3%)	10 (66.7%)	0.97	5 (33.3%)	10 (66.7%)	0.19	12 (80%)	3 (20%)	0.69

Table 2. The Relationship between Platinum Sensitivity and Clinicopathologic/Immunohistochemical Features

Variable	Platinum sensitive	Platinum resistant	p
Age			
<50	21 (61.8%)	13 (38.2%)	
>50	26 (54.2%)	22 (45.8%)	0.49
Stage			
I-II	23 (88.5%)	3 (11.5%)	
III-IV	24 (42.9%)	32 (57.1%)	<0.0001
Surgery			
Optimal cytoreduction	30 (75%)	10 (25%)	
Suboptimal cytoreduction	17 (40.5%)	25 (59.5%)	0.002
Histologic type			
Serous	27 (54%)	23 (46%)	
Non-serous	20 (64.5%)	11 (35.5%)	0.35
Histologic grade			
well-moderately differentiated	28 (63.6%)	16 (36.4%)	
poorly differentiated	19 (51.4%)	18 (48.6%)	0.26
ER			
Negative	12 (54.5%)	10 (45.5%)	
Positive	35 (58.3%)	25 (41.7%)	0.75
PR			
Negative	20 (50%)	20 (50%)	
Positive	27 (64.3%)	15 (35.7%)	0.19
HER2			
Negative	39 (58.2%)	28 (41.8%)	
Positive	8 (53.3%)	7 (46.7%)	0.73
EGFR			
Negative	29 (64.4%)	16 (35.6%)	
Positive	17 (47.2%)	19 (52.8%)	0.12
Tumour phenotype			
Triple negative	8 (53.3%)	7 (46.7%)	
Hormon receptor positive	31 (59.6%)	21 (40.4%)	
HER2 positive	8 (53.3%)	7 (46.7%)	0.85

Overall survival analysis: Forty-nine deaths were observed during the follow-up period , and the survival portion at 41.7 months of median follow-up time was 40.2% for the study population (Figure 2a). Median estimated overall survival time was 44.9 months (range: 33.5-56.2 months; 95% CI) for the whole group, while for patients with advanced-stage disease it was 34.6 months (range: 30.8-38.4 months; 95% CI) (p<0.0001) (Figure 2b). Cumulative estimated survival proportion at 48 months (4Th year) was 47% for the entire study population, and 21.7% for patients with advanced-stage disease. The patients with residual disease after surgery survived a significantly shorter time period than patients with optimal cytoreduction [median 30.1 months (range: 22.7-37.4) *vs* mean 158 months (range: 126.3-190.6); 95% CI; p<0.0001] (Figure 2c). Patients older than fifty years old had significantly shorter overall survival times than younger patients [median 38.5 months (range: 27.5-49.4) vs. 85.1 months (range: 31.3-139); 95% CI; p=0.033] (Figure 2d). OS times did not differ according to histologic type and grade, despite a tendency toward shorter survival times in patients with pure serous morphology and high grade tumours.

OS times did not differ according to ER (p=0.31), PR (p=0.65), or EGFR expression (p=0.61). HER2(+) patients tended to survive shorter time periods than HER2(-) patients, and this difference was significant in patients with advanced-stage disease [17 months (range: 12.5-21.5) vs. 42.9 months (range: 28-57.8) ; 95% CI; p= 0.015] (Figure 3). When we classified ovarian cancer patients into three phenotypic subtypes as triple negative [or HR(-) HER2(-)], HR+ [or HR+ HER2-] , and HER2(+) [including HR+HER2+ and HR-HER2+], HER2(+) patients survived shorter time periods than other subtypes; and the difference was significant in patients with advanced-stage disease [mean OS: 50.7 months for triple negative vs. median 38.6 months for HR(+) vs. median 17 months for HER2(+); 95% CI; p=0.019]. (Figure 4)

Progression-free survival analysis: Median PFS was 15.7 months (range=13.9-17.5, 95% CI) (Figure 5). No significant difference was observed in the median PFS time according to clinicopathologic features such as age, operation procedure, histologic type, and grade. PFS times were similar in patients with ER(-) /ER(+), PR(-)/PR(+), EGFR(-), and EGFR positive patients. HER2 positive patients had prominently shorter PFS times than HER2(-) patients; however, this difference was not statistically significant [11.7 months for HER2(+) *vs* 16 months for HR(+) *vs* 15.5 months for triple negative patients; 95% CI; p=0.79).

Multivariate analysis: As HER2 positivity and residual disease after surgery were the only significant risk factors for overall survival in advanced-staged patients, we performed a Cox proportional risk model to identify the most important predictor for shorter overall

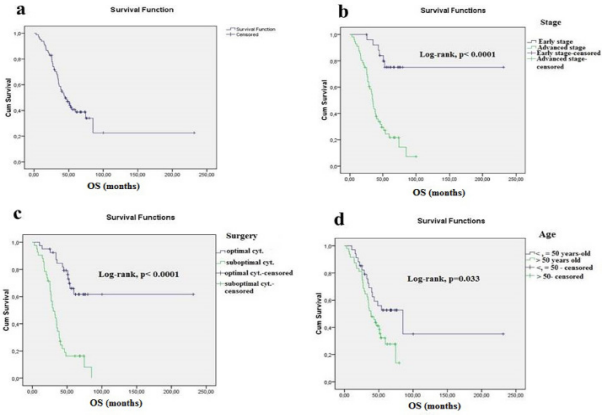

Figure 2. Kaplan Meier Analysis for Overall Survival (OS). a) OS analysis for entire study group; **b)** OS analyses according to disease stage; **c)** OS analyses according to surgical procedure; **d)** OS analyses according to age

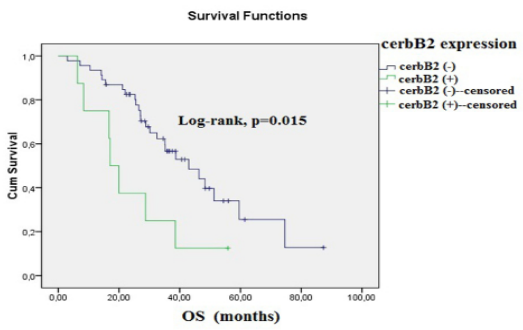

Figure 3. Kaplan Meier Analysis for Overall Survival (OS) According to cerbB2(HER2) Expression in Advanced-staged Patients

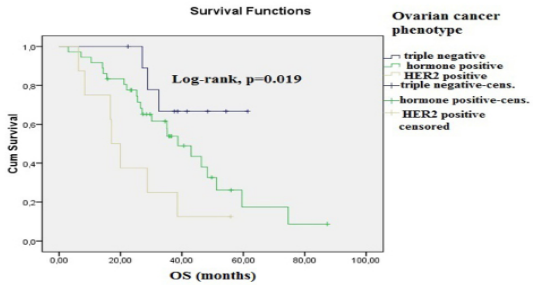

Figure 4. Kaplan Meier Analysis for Overall Survival (OS) According to Ovarian Cancer Subtype

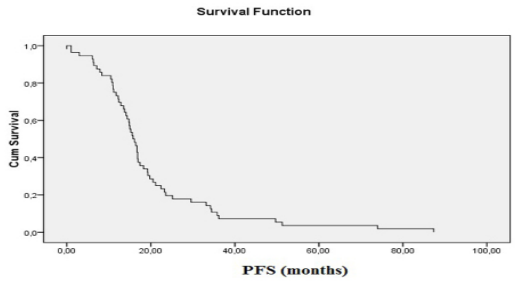

Figure 5. Kaplan Meier Analysis for Progression Free Survival (PFS)

survival. A backward LR model was used, and the levels of significance and hazard risk ratios were given in 95% confidence interval (CI). Of these two factors, residual disease after primary surgery was the strongest predictor for poor survival in advanced-staged patients [HR:3.71(range: 1.29-10.6, 95%CI); p=0.015].

Discussion

In this study, we investigated the effects of the hormone receptor, HER2 and EGFR expression together with clinicopathologic factors on the prognosis of EOC and treatment response in a patient population in which the majority of the patients had advanced-stage disease. Consistent with the literature, our study found older age, advanced-stage disease at diagnosis, and high residual tumour volume after surgery to be important negative prognostic factors. Of the study group, 18.3% of the patients were triple negative, 18.3% were HER2 positive and 63.4% were luminal A (or hormone receptor positive HER2 negative). These expression results were compatible with the results of previous studies (Liu et al., 2010; Lenhard et al., 2012). The results of studies regarding the prognostic role of ER/PR expression in EOC are contradictory, Lenhard et al. (2012) demonstrated that the expressions of PR-β and ER-α were related to a favourable outcome, while ER beta positivity was significantly related to a worse prognosis and survival. Zhao et al. (2013) conducted a meta-analysis to assess the prognostic effect of hormone receptors in EOC; they reviewed 23 studies for ER and 19 for PR expressions. They concluded that, rather than ER expression, PR expression was significantly associated with better OS and PFS times. In our study, we did not observe any association between hormone receptor expressions and clinicopathologic features. Additionally, no prognostic or predictive effects of either receptor could be shown. Perhaps the lack of data about receptor isoforms may be an explanation for non-significant results, or maybe ER/PR has no prognostic value indeed. However, a careful examination of Zhao's meta-analysis (Zhao et al., 2013) demonstrates the heterogeneity of study populations (regarding age, treatment regimens, countries, and analysis techniques, etc.) included in meta-analysis. Therefore, this result may not be an unexpected finding.

HER2, a member of the receptor tyrosine kinase family, plays a crucial role in the growth of both normal tissue and malignant tumours (Saxena and Dwivedi, 2012). HER2 overexpression is observed in 18.9-22.4% of epithelial ovarian carcinomas (Steffensen et al., 2007), and it is reported to be seen more frequently in mucinous ovarian cancers (Yan et al., 2011). The prognostic role of HER2 overexpression in EOC remains unclear. Some studies proposed that an elevated HER2 level was associated with poorer survival (Lassus et al., 2004; Steffensen et al., 2007), whereas other studies could not demonstrate any association (Verri et al., 2005; Tuefferd et al., 2007). In the meta-analysis conducted by Zhao et al. (2013), the prognostic effect of HER2 overexpression was also explored; after reviewing eight studies, they demonstrated that HER2 overexpression was significantly associated with poorer overall survival. In our study, HER2 positivity was observed in 18.3% of the cases, and the majority of HER2 positive tumours were serous tumours (8/16), while only 2/16 cases had mucinous histology. HER2 overexpression was significantly associated with shorter overall survival specifically in patients with advanced-stage disease (stage III-IV). Additionally, HER2 positive patients tended to have shorter PFS times, suggesting its negative predictive effect.

Cancer phenotypic and molecular subtypes have been widely used in predicting the treatment response and survival of breast cancer patients in recent decades. Triple negative and HER2 positive subtypes are well-known subtypes associated with poor clinical outcomes. However, after the development of trastuzumab, a monoclonal antibody against the HER2 receptor, the prognosis of HER2 positive breast cancer has been improved. Due to the lack of any targeted therapy, triple negative breast cancer still remains the most aggressive subtype in breast cancer. In contrast, antiHER2 therapy in HER2(+) EOC is not as effective as in breast cancer. Two previous phase II studies failed to show benefit from trastuzumab in HER2-positive EOC (Bookman et al., 2003; Ray-Coquard et al., 2009). Moreover, cancer subtype has not been widely used for ovarian carcinoma due to the limited number of studies in this area. Two studies on this subject exist in the literature and they demonstrated opposite results. Both of the studies evaluated the prognosis of ovarian cancer according to two subtypes: triple negative and non-triple negative. Liu et al. (2010) suggested that triple negative ovarian cancer was significantly associated with shorter PFS and OS times compared to the non-triple negative subtype. Conversely, a recent study (de Toledo et al., 2013) demonstrated that there was no survival difference between triple negative and non-triple negative EOC

patients. In our study, triple negative and HR positive (or luminal A) EOC subtypes had almost similar survival rates; however, HER2 positive patients had the poorer outcome. Thus, triple negative EOC genuinely may not be an aggressive subtype. Moreover, gene expression profiles are urgently required to demonstrate molecular subtypes and assess their relationships with phenotypic (or IHC) subtypes.

EGFR is one of the crucial targets in cancer treatment, specifically in non-small-cell lung and metastatic colorectal cancers. The expression rates of EGFR in ovarian cancer vary due to different evaluation techniques (gene amplification or protein overexpression by IHC) and reported as 4-70% in the literature (de Graeff et al., 2009). In our study, membranous EGFR positivity was observed in 45.1% of the study group, which was compatible with the results of a previous study performed by Noske et al. (2011). The results of the studies evaluating the prognostic role of EGFR expression are also conflicting. Some studies suggested EGFR positivity as a negative prognostic factor (Skirnisdottir et al., 2001; Psyrri et al., 2005; Lassus et al., 2006; Noske et al., 2011) and demonstrated an association with poorer survival rates, while others reported no relationship between disease outcome and EGFR expression (Nielsen et al., 2004; de Graeff et al., 2008). Variations in IHC procedures, antibodies, and patient selection may provide some explanation for the differing results from these studies. In our study, a positive correlation between EGFR and disease stage was observed: EGFR expression was more frequent in advanced-stage disease. As advanced stage is a well-known negative prognostic factor in ovarian carcinoma, despite a lack of any significant association with survival rates, EGFR may be thought as a negative prognostic factor, however the importance of EGFR expression should be verified in large-scaled studies.

In summary, this study pointed out the following findings: *(1)* Triple negative ovarian cancer may not be as aggressive as in breast cancer; *(2)* HER2 positivity significantly shortens overall survival times in patients with advanced-stage disease, and the HER2 positive phenotype has more aggressive behaviour compared with the triple negative and HR(+) phenotypes; *(3)* ovarian carcinoma phenotype has no relationship to platinum sensitivity; *(4)* EGFR expression is more frequent in advanced tumours; *(5)* residual disease after primary surgery remains the strongest negative predictor for patient outcome.

However, despite these important findings, we have to disclose some limitations with this study. First, our study included a limited number of patients because of some archival problems in the Department of Pathology. Second, we could not assess HER2 gene amplification, and thus could not evaluate the concordance with IHC results. Three patients had cerbB2 2+ disease and, considering breast cancer pathology and HER2 positivity criteria, it would have been helpful to perform in situ hybridization techniques to evaluate HER2 gene amplification at least in these patients. Nevertheless, a previous study demonstrated that 100% of cerbb2 3+ cases were HER2 amplified, while only 25% of cerbB2 2+ cases were also FISH + (Tuefferd et al., 2007). Therefore, the lack of FISH analysis in this study is not a deficiency.

In conclusion, further prospective studies are required to verify the results of this study. Moreover, as with breast cancer studies, ovarian cancer molecular subtyping by using gene expression analysis may be intriguing and may demonstrate more relevant data. Additionally, despite the failure of trastuzumab in EOC, the Zhao meta-analysis (Zhao et al., 2013) and the results of our study may encourage researchers to evaluate the effect of trastuzumab in further randomized multi-centred studies including only the HER2 positive subgroup of EOC patients.

Acknowledgements

This research was supported by grants from the Society of Cancer Research (Izmir, Turkey). We thank the Chairman of this society, Ugur Yilmaz, for his valuable support. We also thank the Department of Pathology, Izmir Ataturk Research and Training Hospital, for their valuable technical assistance.

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