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ORIGINAL ARTICLE

Prognostic significance of EGFR, MUC1 and PD-L1 expressions in cases with triple negative breast cancer

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Summary

Purpose: *Twenty percent of the breast cancers are triple negative (TNBC). Despite the impressive progression in the biology of this subgroup, data is limited as compared to hormone and/or HER2 positive cases. Thus, the aim of this study was to detect the expression levels and to identify the prognostic values of MUC1, EGFR and PD-L1 in TNBC.*

Methods: *MUC1, EGFR and PD-L1 expressions were detected by immunohistochemistry in 97 cases with TNBC. Associations between clinical and histopathological parameters with overall survival (OS) and progression-free survival (PFS) were analyzed using the Kaplan-Meier method and compared by the log-rank test. Prognostic effects were analyzed by Cox proportional hazard models.*

Results: *During a median follow-up of 93 months (0.6-168.7) the mean PFS was 110.1 and OS was 121.8 months. Tumor diameter (T), involved lymph node status (N) and TNM stage were found to be prognostic for PFS and OS. PD-L1 in microenvironment (PD-L1 ME) and EGFR expression were found to be associated with longer PFS and OS, but MUC1 and tumor PD-L1 (PD-L1 TM) expressions were not. All combined analyses showed that in the subgroups of MUC1, PD-L1 TM or ME positive, EGFR expression was correlated with longer PFS and OS than those who were not. Older age (≥70 years), T and N status and also EGFR expression were found to be independent prognostic factors for OS in Cox regression analysis.*

Conclusion: *EGFR expression was found to be one of the most important prognostic factors in addition to T and N status in cases with TNBC.*

Key words: *EGFR, MUC1, PD-L1, triple negative breast cancer, prognosis*

Introduction

Breast cancer (BC) is the most common cancer in women and 20% of these cases are triple negative breast cancer (TNBC) [1,2]. In TNBC estrogen and progesterone receptors are negative or show less than 1% expression by IHC and also HER2 is less than (++) expression by IHC and HER2 FISH (-) according to the American Society of Clinical Oncology and American College of Pathology (ASCO-CAP) [3]. Patients with TNBC have higher disease grade, larger and less differentiated tumors and more common lymph node involvement as compared with other subtypes of BC. In the first 3 and 5 years, the risk of recurrence, metastasis and death is higher than in other breast cancer subtypes [4]. Visceral metastases are more common than bone, and this is also related with poor prognosis [5]. Although apart the PARP inhibitors in patients with BRCA mutation (which are detected in 10-20% of

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the cases) there is no well established targeted therapy in neoadjuvant, adjuvant and metastatic setting [6], so there are many ongoing studies to find new targets for new drugs [7].

Mucins are high molecular weight glycoproteins and have important activity at protection of epithelial cells and lubrication. However, with changes of their expressions they cause progression in many cancers including pancreas, ovary, breast, colon and prostate [8]. Mucin 1 (MUC1) is a heterodimeric protein and is a member of this family. MUC1 overexpression has been reported in 90% of the cases with TNBC. With the loss of transmembrane subunit and apical polarization MUC1 gains oncoprotein function [9-11]. Besides, MUC1 directly activates MUC1-B-catenin→TCF4→MYC and NF-kB/p65 pathways and causes increase in programmed cell death protein 1 ligand (PD-L1) expression [12,13]. PD-L1 intearcts with PD-1 expression on lymphocytes and inhibits death function of T cells. Although PD-L1 expression is a poor prognostic factor in most tumors, its role in breast cancer is unclear [14]. In addition to this data, the expression of cell surface MUC1 is a critical enhancer of epidermal growth factor (EGF)-induced epidermal growth factor receptor (EGFR) activation in human breast and colon cancer cells [15]. MUC1 avtivates and interacts with many of these pathways and receptors at cell level and contributes to tumor growth, metastasis, tumor escape from immune system and resistance to chemotherapy [16]. However, the clinical significance of these interactions in TNBC patients is unclear. Thus, the aim of this study was to detect the expression levels MUC1, EGFR and PD-L1 in TNBC and to identify the prognostic values of individual or combined expressions using multivariate models.

Methods

Between December 2004 and August 2012, 97 adjuvantly treated cases with TNBC were included in this study. Clinical and demographic findings were obtained from the patient archive files and the hospital information operating system and tumor characteristics re-evaluated. Patients were accepted as TNBC according to the 2007 American Society of Clinical Oncology/College of American Pathologist guidelines [3].

Immunohistochemical analyses and scoring

Immunohistochemical (IHC) method was used to detect the expression of MUC1, EGFR and PDL1. IHC staining: IHC was performed on 5-mm sections of formalin-fixed, paraffin-embedded tissues. Monoclonal antibody PDL-1 (E1L3N,Cell Signaling,USA) was used to detect PD-L1, anti-EGFR (ab320077, Abcam, USA) and MUC1 (ab15481, Abcam,USA). The visualization system used was the BenchMark XT with enzymatic digestion (ISH protease 2, Ventana) and the iView Blue Detection Kit (Ventana). Specimens stained with PD-L1 were scored according to intensity of cytoplasmic and/or membranous positivity as follows: 0 (no staining), (+): weak or equivocal staining, (++) (moderate staining) or (+++) (strong staining). MUC1 was scored as 0 (no staining), +1 (weak staining), 2+ (moderate staining), 3+ (strong staining). Complete and incomplete membranous staining were accepted positive for EGFR and scored as follows: 0, no staining, or weak membranous staining in <10% of the tumour cells; 1+, weak membranous staining in ≥10% of the tumour cells; 2+, moderate, membranous staining in ≥10% of the tumour cells; 3+, strong membranous staining in ≥10% of the tumour cells. Immunohistochemical scores of HER2 0 or (+) were regarded as negative (24 cases), and the rest of equivocal (+ +) 14 cases were confirmed by fluorescence *in situ* hybridization (FISH).

Statistics

Overall survival (OS) was calculated from the date of diagnosis to the date of death, and censored at the date of last follow-up for survivors. Progression-free survival (PFS) was calculated from the date of diagnosis to the date of recurrence or death and censored at the date of last follow-up for survivors without recurrence. Kaplan–Meier method and log-rank test were used to determine the association among clinical characteristics and survival times (OS and PFS). For MUC1, EGFR and PD-L1 evaluation, we grouped negative (0, no staining) and positive (1+, 2+, 3+ staining) cases for the purpose of statistical analysis. Univariate and multivariate Cox regression analyses were used to evaluate the prognostic value of MUC1, EGFR and PD-L1. Tumor tissue/microenvironment stainings were adjusted by age, grade, T and N status, which were found to be prognostic in univariate analysis. Hazard ratios (HR) and 95% confidence intervals (CIs) were estimated using Cox regression analysis. Statistical analyses were performed with SPSS software version 21 (SPSS IBM Corp., Armonk, NY, USA), and differences were considered statistically significant at $p<0.05$.

Results

All of our patients were female. Median age was 49 years (28-84). Eighty nine of our cases (91.8%) had invasive ductal carcinoma, 8 (8.2%) had medullary carcinoma. There was no grade I disease, 29 cases had grade II and 68 cases had grade III disease. HER2 by IHC was (++) in 14 cases and FISH negativity was confirmed in all of these cases. TNM staging: 17 (17.5%), 47 (48.5%), 25 (25.8%) and 8 (8.2%) cases had stage I, II, III and IV, respectively. Tumor diameter was T1 in 19 (19.6%), T2 in 66 (66%), T3 in 8 (8.2%) and T4 in 6 (6.2%) cases. According to the lymph node involvement 42 cases (43.3%) had N0, 28 (28.9%) had N1, 18 (18.6%) had N2 and 9 (9.2%) had N3 disease. All of our cases had been treated by anthracycline and taxane-

containing regimens for adjuvant therapy. Median follow up was 93.6 months (0.6-168.7). Metastatic disease developed in 38 cases and 66 cases were alive during this analysis. Local recurrence developed in 8 cases, bone and visceral metastases were detected in 5 and 27 cases, respectively.

Mean OS and PFS were 110.1 and 121.8 months, respectively. PFS and OS times according to the clinical and histopathological variables is shown in Table 1. There were no differences for PFS and OS according to older age (≥70), histologic subtype and histologic grade. Median PFS was longer in cases with T1-T2 disease than cases with T3-T4 disease; mean PFS was 124.9 vs 22.3 months (p=0.000) and OS 134.6 vs 41.5 months (p=0.000). Mean PFS and OS were longer in cases with N0-1 disease as compared with N2-3 disease: 133.4 vs 42.2 months (p=0.000) and 147.8 vs 47.1 months

Table 1. Overall and progression-free survival according to clinical parameters (n=97)

	Total	*PFS*			*OS*		
	n (%)	*Mean*	*Median*	*p**	*Mean*	*Median*	*p*
Age, years							
<70	92 (94.2)	111.4		0.416	123.6		0.192
≥70	5 (5.2)	61.6			64		
Subtype							
Ductal	89 (91.7)	111.1		0.651	122.3		0.870
Medullary	8 (8.3)	88.4			106.6		
Histologic grade							
2	29 (29.9)	108.2		0.821	122.6		0.889
3	68 (70.1)	108.1			117.9		
HER 2				0.970			0.626
0	60 (61.9)	108.1			116.8		
1	23 (23.7)	91.4			115.4		
2	14 (14.4)	107.4			114.8		
T stage				**0.000**			**0.000**
1-2	83 (85.5)	124.9			134.6		
3-4	14 (14.5)	22.3	23		41.5	31	
N stage				**0.000**			**0.000**
0-1	70 (72.2)	133.4			147.8		
2-3	27 (27.8)	42.2	23		47.1	31	
Stage TNM (AJCC)							
1	17 (17.5)	137.1		**0.000**	138.6		**0.000**
2	47 (48.5)	140.4			155.2		
3	25 (25.8)	50.3	29		61.5	41	
4	8 (8.2)	10.9	2		16.6	3	
Overall	97 (100)	110.1			121.8		

AJCC: American Joint Committee on Cancer. Bold numbers denote statistical significance

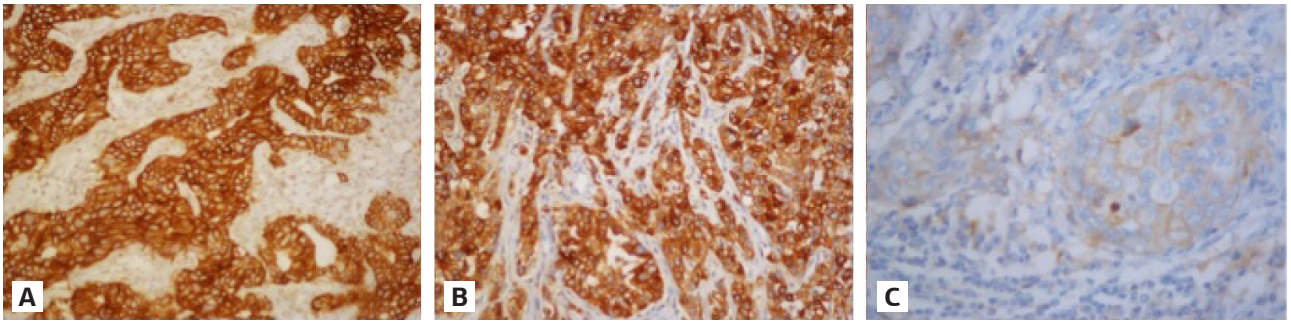

Figure 1. Immunohistochemistry. **A:** EGFR positivity (IHCx100). **B:** MUC1 positivity (IHCx40). **C:** PD-L1 positivity (IHC×400).

Table 2. Overall and progression-free survival according to MUC1, EGFR and PD-L1

	Total	*PFS*			*OS*		
	n (%)	*Mean*	*Median*	*p*	*Mean*	*Median*	*p*
MUC 1							
Negative	17 (17.5)	89.5	67	0.261	104.9		0.329
Positive	80 (82.5)	113.4			124.7		
MUC 1 expression				0.315			0.424
0	17 (17.5)	89.5			104.9		
1	16 (16.5)	113.5			129.2		
2	33 (34)	106.7			116.1		
3	31 (32)	111.6			120.8		
EGFR							
Negative	14 (14.4)	47.4		**0.000**	53.7		**0.000**
Positive	83 (85.6)	119.3			132.7		
EGFR expression				**0.023**			**0.002**
0	14 (14.4)	47.4			53.7		
1	25 (25.8)	119.3			130.1		
2	39 (40.2)	116.8			129.2		
3	19 (19.6)	112.9			129.5		
PD-L1-TM							
Negative	80 (82.5)	105.7		0.206	117.2		0.149
Positive	17 (17.5)	125.9			138.1		
PD-L1-ME							
Negative	76 (79.4)	102.5		**0.049**	114.6		**0.049**
Positive	21 (20.6)	133.9			141.5		
MUC1-EGFR				**0.001**			**0.011**
Both (-)	4 (24.7)	56.5	29		57.3	31	
EGFR (+), MUC_1 (-)	13 (40.2)	98.2			117.6		
EGFR (-), MUC_1 (+)	10 (12.3)	39.5	29		50.2	33	
Both (+)	70 (22.8)	122.2			134.8		
MUC1-PD-L1				0.116			0.106
Both (-)	16 (56.7)	91.6	67		101.6		
At least one +	64 (8.3)	107.1			121.1		
Both (+)	17 (10.3)	130			136.8		
MUC1-PDL1-ME				0.088			0.137
Both (-)	16 (16.5)	91.6	67		101.6		
At least one +	61 (62.8)	103.1			118.2		
Both (+)	20 (20.7)	137.8			141		
EGFR-PDL1 TM				**0.002**			**0.000**
Both (-)	14 (14.4)	47.4	29		53.7	33	
At least one +	65 (67.1)	116.5			129.9		
Both (+)	18 (18.5)	125.9			138.1		
EGFR-PDL1-ME				**0.000**			**0.000**
Both (-)	13 (13.4)	37.9	29		42.3	31	
At least one +	66 (68.1)	113.2			126.4		
Both (+)	18 (18.5)	143.2			154.6		
Overall	97 (100)	110.1			121.8		

PD-L1-TM, programmed death ligand 1 tumoral; PD-L1-ME, programmed death ligand 1 microenvironment. Bold numbers denote statistical significance

Table 3. Results of Cox regression analyses

	B	*SE*	*df*	*Sig.*	*OR*	*95.0% CI for OR*	
						min	*max*
Age	0.054	0.018	1	0.002	1.056	1.020	1.093
Grade	-0.273	0.497	1	0.582	0.761	0.288	2.014
T stage (1-2 VS 3-4)	-1.503	0.456	1	0.001	0.222	0.091	0.543
N stage (0-1 VS 2-3)	-2.708	0.482	1	0.000	0.067	0.026	0.171
MUC1	0.611	0.622	1	0.326	1.842	0.544	6.233
EGFR	-2.102	0.536	1	0.000	0.122	0.043	0.349
PD-L1 TM	0.777	1.139	1	0.495	2.176	0.233	20.274
PD-L1 ME	-0.424	1.182	1	0.720	0.655	0.065	6.641

PD-L1-TM: programmed death ligand 1 tumoral; PD-L1-ME, programmed death ligand 1 microenvironment

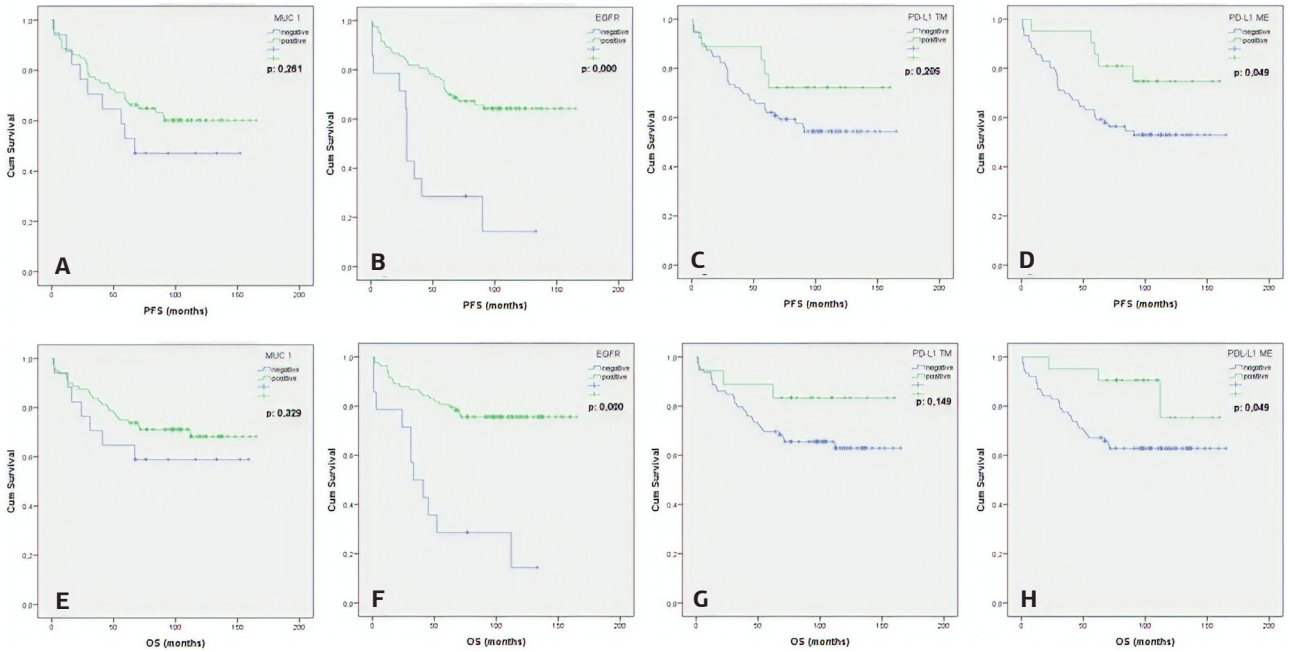

Figure 2. Kaplan-Meier survival curves for PFS and OS. **A-E:** MUC 1. **B-F:** EGFR, **C-G:** PD-L1 TM, **D-H:** PD-L1 ME.

(p=0.000), respectively. PFS and OS were longer in cases with early-stage disease as compared with advanced disease. Table 1 shows significant differences in survival according to stage.

IHC analyses: MUC1 was found positive in 80 cases; EGFR was positive in 83 cases, PD-L1 was positive in 17 cases in tumor and PD-L1 also positive in 21 cases in microenvironment (Figure 1). PFS and OS according to MUC1, EGFR and PD-L1 expressions are shown in Table 2. Although PFS and OS were longer in cases with MUC1 expression as compared with those without, the difference was not significant: PFS was 113.4 vs 89.5 months (p=0.261), and OS was 124.7 vs 104.9 months (p=0.329). Also PFS and OS were not different according to MUC1 staining pattern. PFS and OS were found to be longer in cases with positive EGFR expression as compared with negative EGFR expression: PFS 119.3 vs 47.4 months (p=0.000), OS 132.7 vs 53.7 months (p=0.000). PFS and OS were different also according to EGFR staining pattern (Table 3). PFS and OS were not found different according to PD-L1 expression in tumor tissue (p=0.206, p=0.149, respectively). However, PFS and OS were longer in cases with PD-L1 expression in microenvironment (133.9 vs 102.5 months and 141.5 vs 114.6 months, respectively; p=0.049 and p=0.049).

PFS and OS and statistical differences according to MUC1, EGFR and PD-L1 in tumor and microenvironment in all cases are shown in Table 3 according to the single or double staining for MUC1, EGFR and PD-L1 (Figure 2). Double expressor cases for MUC1 and EGFR showed longer PFS and OS as compared to double negative and/or single expressors (PFS, p=0.000, p=0.007, OS, p=0.000, p=0.042, respectively) Similarly, cases with double expression for MUC1 and PD-L1 (tumor and micro-

environment) had longer PFS and OS but without significance (PFS, p=0.116, p=0.088, OS, p=0.106, p=0.137, respectively). PFS and OS times were different according to combined EGFR and PD-L1 TM expression (p=0.002, p=0.000). PFS and OS were longer in cases with double expression for EGFR and PD-L1 TM compared to double negative expressor (p=0.002, p=0.000) but there was no significant different to single expressor group (p=0.480, p=0.419). There were also significant differences between PFS and OS in the combined evaluation of EGFR and PD-L1 microenvironment expression (p=0.000, p=0.000). Both double expressor groups had longer survival than double negative and/or single expressors (Table 2).

Cox regression analysis showed that age, T stage (1-2 vs 3-4), N stage (0-1 vs 2-3), and EGFR expression were independent risk factors for OS (Table 3). The odds ratio (OR) for age was 1.05 (95%CI: 1.02-1.09, p=0.002), 0.222 (95%CI: 0.091-0.543, p=0.001) for T stage, 0.067 (95%CI: 0.026-0.171, p=0.000) for N stage and 0.122 (95%CI: 0.043-0.349, p=0.000) for EGFR. Grade, MUC1, PD-L1 TM and PD-L1 ME were not found to be associated with good prognosis in multivariate analysis.

Discussion

Breast cancer is a heterogeneous disease characterized by different clinical outcomes according to the different subtypes [17]. TNBC is the most aggressive subtype of breast cancer, the risk of relapse is high and PFS and OS are shorter in this unique type and there are limited therapeutic options due to lack of druggable target [18]. Age, grade, tumor size, lymph node involvement, stage, hormone receptor, HER2 status, Ki-67 index and lymphovascular invasion are well defined prognostic factors in breast cancer. However, these factors are not clear, giving conflicting results in cases with TNBC [19]. Age, nodal status and tumor size have been found to be prognostic for OS in univariate analysis, while only nodal status has been found to be prognostic in multivariate analysis in a study covering 267 cases [20]. In another study covering 841 cases Ki-67 has been found as the single prognostic indicator for OS [21]. On the other hand, lymph node involvement and grade were found to be prognostic in multivariate analysis by Asaga et al [22]. In our study, age and grade were not found to be prognostic in univariate analysis, while older age (>70 years), tumor size and lymph node involvement were found to be prognostic in multivariate analysis. The low number of patients older than 70 years (5.2%) and the lack of grade I disease in our study group may be the reason of the lack of prognostic importance of these parameters in univariate analysis. Larger tumor size and high number of axillary lymph node involvement are suggestive for the aggressive behaviour for malignant tumors including TNBCs. We found shorter survival in larger tumors and more lymph node involvements re-confirming the poorer biology in TNBC.

In this study we tried to explore the prognostic significance of EGFR, MUC1 and PD-L1 expression in TNBC and found longer survival in cases with positive expression of EGFR and PD-L1 ME. How can we define these findings? MUC1 is overexpressed in epthelial tumors and regulates metabolic genes in cancer cells as transcriptional co-activator, support the biosynthetic genes via kinase signalling necessary for cell growth, regulates metabolic functions by interacting with various enzymes (ATP, TCA cycle) and also contributes to tumor growth, metastasis, and resistance to drugs at different steps [9,23]. MUC1 is expressed in more than 90% of breast cancer cases and it has been detected in 94% of the cases with TNBC [24,25]. Similarly, we found 82.5% expression in our study group. The prognostic significance of MUC1 expression in breast cancer is controversial. There was no PFS or OS difference in cases with or without MUC1 expression in the study published by Siroy et al, while MUC1 expression has been found to be related with longer OS in another study covering 243 cases [25,26]. However, MUC1 expression has been found to be associated with shorter OS in another study [27]. In our study PFS and OS were longer in cases with MUC1 expression but without significant difference which may be due to the relatively low number of the cases. In summary, prognostic significance of MUC1 in breast cancer and also in TNBC is not clear and must be evaluated in larger populations.

EGFR family consists of ErbB1 (HER1), ErbB2 (HER2/cNeu), ErbB3 (HER3) and ErbB4 (HER4) having tyrosine kinase activity [28]. Among these, EGFR (HER1) and HER2 are the most active in oncogenic processes and are targeted with various agents. In normal conditions EGFR is inactive and it is activated by binding to extracellular domain with EGF and tyrosine is phosphorylated. This phosphorylation activates Janus kinase/Signal transducer and activator of transcription (JAK/STAT) pathways which have active role in tumor proliferation, migration and tumorigenesis with RAS, MEK, ERK, PI3K and AKT activation [29]. EGFR overexpression is highly variable in breast cancer and this expression has been reported in 13-52% of the cases with TNBC [30,31]. Poor prognostic significance of EGFR both for PFS and OS

in early breast cancer especially in TNBC has been determined in systematic reviews [31,32]. In another study EGFR expression has been found to be independent poor prognostic factor for shorter OS [33]. However, there is no clear consensus about the prognostic significance of EGFR expression in breast cancer. Another important point on this issue is the different cut offs for evaluation of EGFR expression by IHC because there is no consensus for IHC evaluation. We used different cut offs and our analyses showed that increased expression was found to be associated with longer PFS and OS and additionally EGFR expression was found to be an independent good prognostic factor for OS. It is well known that TNBC is heterogeneous and 7 subtypes with different clinical outcomes have been defined by gene expression profiles while EGFR expression patterns are not clear in these genetic subtypes [34]. Because of this situation, so far no beneficial effects of EGFR targeting therapy in TNBC were reported [35], and more detailed studies may show a specific subtype about EGFR expression and the importance of targeting this pathway. One of the most important mechanisms for tumor growth and metastasis is escape from tumor surveillance [36]. Well established pathways for immune escape are programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). T cell killer function is inhibited with the interaction between PD-1 expressed on lymphocytes and its ligand-PD-L1 [14]. PD-L1 expression has been found to be increased in various malignant tumors including melanoma, lung, bladder, colon, liver and head-neck cancers and it is thought that, at least in some cancers, PD-L1 expression is predictive for response to anti-PD-1/PD-L1 treatments [37-40]. PD-L1 has been found to be increased in cases with TNBC as compared with other subtypes of breast cancer according to the TCGA and this expression was as high as 20-30% of these cases and related with high grade tumor [41]. We found PD-L1 expression by IHC in 17.5 and 20.6% in tumor and microenvironment, respectively. In some tumors PD-L1 expression was associated with poor prognosis but the prognostic significance of PD-L1 expression in breast cancer was not clear. In melanoma PD-L1 was associated with aggressive tumor behaviour, loco-regional recurrence and shorter melanoma-specific survival [42,43] and shorter OS related to PD-L1 expression has been found in a lung cancer meta-analysis [44]. Baptista et al found that 50% of patients had breast cancer and those with PD-L1 expression had longer OS, however in this cohort it has not been mentioned the rate of TNBC subtype [45]. Sabatier et al found an association between PD-L1 expression and longer metastasis-free survival in basal breast cancers [46]. Beckers et al reported lower breast cancer-specific death rate in cases with tumor PD-L1 expression and lower death rate was associated with all-cause death rates in cases with stromal PD-L1 expression [47]. Li et al found longer DFS in cases with stromal PD-L1 expression in multivariate analysis [48]. We did not find an associaton between tumor PD-L1 exression and PFS and/or OS while PD-L1 expression at ME was associated with longer PFS and OS. So, PD-L1 expression in TNBC has some prognostic and predictive value and firstly PD-L1 targeted treatment showed longer PFS in cases with IMpassion 130 in TNBC presented at last ESMO 2018 meeting [49]. This finding suggests that PD-L1 expression in ME will be more important and predictive for targeted treatment in TNBC.

MUC1 interacts with EGFR and it activates the promoter region [15]. There is no clear data about the importance of EGFR expression in cases with MUC1 expression positive subgroup despite this interaction at cellular level. In the MUC1 positive group, we determined that patients with EGFR expression had longer PFS and OS than those who had not (p=0.000, p=0.000). This interaction, which was first detected, will be more informative in case of confirmation of this association in other studies. Although increased expression of PD-L1 in cases with MUC1 positivity has been shown in cell lines [50], prognostic significance of this interaction has not been shown so far in clinical samples. We found longer survival in cases with combined MUC1 and PD-L1 expression as compared with double negative cases, but the difference was non-significant (Table 2). This finding needs confirmation with larger studies. All these results show that in the subgroups of MUC1, PD-L1 TM or ME positive, where EGFR was expressed, had longer PFS and OS than those who had not. Also, among these we found only EGFR expression as unique independent risk factor with Cox regression analyses. Therefore, this study is the first to show the prognostic significance of combined expressions of MUC1, EGFR and PD-L1 in breast cancer and also in TNBC subtype. There are two important limiting points in this study: one is its retrospective nature and the other is the lack of information about the subgroup of TNBC. We need more informative studies covering more detailed information with TNBC.

Conclusion

TNBC is the most aggressive and most commonly investigated subtype of breast cancer for targeted therapy. Among clinical and demographic

variables T and N status are independent risk factors for OS. Alhough there is some prognostic significance of MUC1, EGFR and PD-L1 determined in previous studies, we found only PD-L1 ME expression at univariate analysis and EGFR expression both in univariate and multivariate analyses as independent risk factors for OS in clinical practice for the first time. EGFR expression has been found to be most important factor for longer OS in TNBC.

Ethical approval

Ethical approval may not required dependent on the law and the national ethical guidelines of our country and written informed consent was not required for individual patient because of retrosprective nature. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Conflict of interests

The authors declare no conflict of interests.

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