RESEARCH ARTICLE

Lack of Prognostic Value of Blood Parameters in Patients Receiving Adjuvant Radiotherapy for Breast Cancer

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Abstract

Aim: To determine prognostic value of blood parameters on overall and progression-free survival in cases received adjuvant radiotherapy and chemotherapy with diagnosis of stage I-III breast cancer. Materials and Methods: We retrospectively reviewed files of 350 patients with non-metastatic breast cancer who were treated in the Radiation Oncology Department of Kayseri Teaching Hospital between 2005 and 2010. Pretreatment white blood cell (WBC), neutrophil, monocyte, basophil and eosinophil counts, and the neutrophil/lymphocyte ratio (NLR) and platelet lymphocyte ratio (PLR) were recorded. The relationship between clinicopathological findings and blood parameters was assessed. Results: Overall, 344 women and 6 men were recruited. Median age was 55.3±0.3 years (range: 22-86). Of the cases, 243 (61.4%) received radiotherapy while 329 (94.3%), received chemotherapy and 215 (61.4%) received hormone therapy. Mean overall survival (OS) and progression-free survival (PFS) was 84.4 and 78.8 months, respectively. During follow-up, 48 patients died due to either disease-related or non-related causes. Local recurrence was detected in 14 cases, while distant metastasis was noted in 45 cases. In univariate analysis, age, pathology, perinodal invasion were significantly associated with overall survival, whereas gender, stage and hormone therapy were significantly associated with progression-free survival. In multivariate analysis, histopathological diagnosis (OR: 0.3; 95%: 0.1-0.7; p=0.006) and perinodal invasion (OR: 0.1; 95% CI: 0.1-1.3; p=0.026) were significantly associated with overall survival, whereas tumor stage (OR: 2.1; 95% CI: 0.0-0.7; p=0.014) and hormone therapy (OR: 2.1; 95%: 1.2-3.8; p=0.010) were significantly associated with progression-free survival. Conclusions: It was found that serum inflammatory markers including WBC, neutrophil, lymphocyte and monocyte counts, and NLR and PLR had no effect on prognosis in patients with breast cancer who underwent surgery and received adjuvant radiotherapy and chemotherapy.

Keywords: Breast cancer - blood parameters - prognosis

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Introduction

Breast cancer is most frequently encountered cancer in women. It comprises 18% of all cancers in women. Risk for incident breast cancer is increased by advancing age (Siegel et al., 2012). Clinical behavior is characterized with a prolonged natural course and heterogeneity. Patients diagnosed as breast cancer carry metastasis risk for long periods and definition of cure is problematic. Treatment decisions depend on tumor characteristics and treatment response (Sotiriou et al., 2003; Sezer et al., 2011). Adjuvant therapy reduces mortality risk by 20-25% in pre- and post-menopausal cases (Aggarwal and Gehlot 2009; Azab et al., 2012; Siegel et al., 2012).

Diagnosis of breast cancer raises several questions for both patient and clinician. The most important problems include which treatments will be employed, and in which combinations and order, particularly to which cases. To answer these questions, likelihood of recurrence should be determined first when no treatment is given, and benefit and potential adverse effects of treatment used should be assessed before making a decision. However, biological markers are needed to estimate recurrence risk and to identify patients groups with poor prognosis in breast cancer which has highly variable natural course. In previous studies, it was suggested that many tumor-, patient- and treatment-related biological markers may have prognostic value (Sotiriou et al., 2003; Lyon et al., 2008; Aggarwal and Gehlot 2009; Azab et al., 2013). Currently, benefit is anticipated from these prognostic factors in certain clinical conditions and it is emphasized that blood parameters may also have prognostic values (Lyon et al., 2008; Pierce et al., 2009; Azab et al., 2013; Engin et al., 2013).

In prior two decades, novel evidence has introduced indicating that breast cancer results from a dysregulated inflammatory response. Although cause and underlying mechanisms aren't fully elucidated, a molecular basis

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regarding role of inflammation in breast cancer is provided by the identification of transcription factors such as NF-kB, AP-1 and STAT3 and their gene products such as tumor necrosis factor, interleukin-1, interleukin-6, chemokines, matrix metalloproteases, and vascular endothelial growth factor (Bachelot et al., 2003; Lyon et al., 2008; Aggarwal and Gehlot 2009; Azab et al., 2012; Ceber et al., 2013; Hu et al., 2013). It has been suggested that risk factors considered being important in the etiology such as tobacco, stress, dietary agents, obesity, alcohol, infectious agents, irradiation, and environmental stimuli contribute to pathogenesis by facilitating underlying inflammatory process. In recent years, molecular mechanisms by which these risk factors induce cancer have been partially elucidated and inflammation seems to be the major shared process by all these risk factors. It has been thought that activated inflammatory process is also involved in tumor growth, invasion, angiogenesis and metastasis (Aggarwal and Gehlot 2009; Pierce et al., 2009). In breast cancer, there is limited number of publications about relationship between inflammation and white blood cells or their subsets.

In this retrospective study, we assessed effects of blood parameters on local recurrence and survivals in 350 patients with non-metastatic breast cancer who received adjuvant radiotherapy and chemotherapy after surgery, and investigated whether clinicopathological and laboratory parameters have effects on survivals. The results were discussed in the context of literature.

Materials and Methods

Patient group and demographic characteristics

In this retrospective study, we reviewed 350 patients with breast cancer who were treated in Radiation Oncology Department of Kayseri Teaching Hospital between 2005 and 2010. In all patients recruited, age, gender, menopausal status, parameters of complete blood count measured before treatment, adjuvant therapies employed, histopathological findings, and data regarding recurrence, metastasis, and overall and progression-free survival times were evaluated. Patients with missing data and those lost in follow-up were excluded from analyses. The study was planned in accordance to local ethics regulations and Helsinki Declaration.

Treatment modalities

All patients underwent abdominal sonography, mammography, complete blood count, biochemical evaluations and posterioanterior chest radiography before surgery. Modified radical mastectomy (MRM) and breast-conserving surgery (BCS) were performed as surgical therapy. After surgery, staging was performed based on histopathology results by using American Joint Committee on Cancer (AJCC) 2002 staging system. Chemotherapy was given to patients with tumor diameter≥1 cm and axillary lymph node ≥1 positive, ECOG (Eastern Cooperative Oncology Group) performance status 0-2, normal renal and bone marrow functions but having no severe cardiac problem. Chemotherapy schedules included one of the following: CMF (Cyclophosphamide, Methotrexate, 5-Flurouracyl), CAF (Cyclophosphamide, Doxorubicin, 5-Flurouracyl), CEF (Cyclophosphamide, Epirubicin, 5-Flurouracyl), AC (Doxorubicin, Cyclophosphamide) or docitaxel.

Adjuvant hormone therapy was initiated after chemotherapy and/or radiotherapy in cases which one or both hormone receptors were positive. Tamoxifen and/or LHRH analogs were given to premenopausal patients while tamoxifen or aromatase inhibitors were given to postmenopausal patients over 5 years.

Radiotherapy was given after chemotherapy. Radiotherapy was used in patients underwent BCS with tumor diameter >2 cm and axillary lymph node ≥3 positive. Radiotherapy was delivered by conventional fractionation (200 cGy fx/5 days) via Co-60 (gamma beam) or linear accelerator device. Total dose was 50-60 Gy in patients underwent MRM, while 60-66 Gy in those underwent BCS. Firstly, radiotherapy was delivered to breast and/or peripheral lymphatics with a dose of 46-50 Gy in all patients. Then, overall radiotherapy dose at tumor bed was completed to 60-66 Gy by delivering additional electron doses of 10-16 Gy with appropriate energy levels to metallic clips, incision scar and excision pouch detected by sonography with a margin of 1 cm. Radiotherapy field in chest wall included the area limited by mid-sternal line at medial, mid-axillary line at lateral, clavicle at superior (if no supra area) and line that passes 2 cm below inframammarian sulcus. After delivering doses of 50 Gy to chest wall, additional dose of 10 Gy was delivered to scar tissue in patients with skin invasion and to axilla in patients with extra-capsular invasion at axillary region.

Blood samples

Pretreatment hemoglobin, hematocrit, white blood cell, neutrophil, lymphocyte, monocyte, eosinophil, basophil values, and NLR and PLR were included to analysis. NLR and PLR were calculated as the ratio of the neutrophils and platelets to lymphocytes. Median value was used for NLR and PLR due to lack of normal distribution. The patients were stratified into two groups according to median value of NLR and PLR (low: <3 or high: ≥3 and low: <160 or high ≥160, respectively).

Follow-up

Treatment response was assessed according to WHO criteria. Follow-up visits were scheduled by 3-months interval within first year; biannually until end of year 5; and annually thereafter. Complete blood count, biochemical parameters, Ca 15-3 and CEA levels were measured biannually, while chest radiographs, mammography, abdominal sonography and bone scintigrapy were obtained annually.

Statistical analysis

SPSS for Windows version 15.0 (SPSS Inc., Chicago, Illinos, USA) was used for data analyses. For each data, normality was tested by using Kolmogrov-Smirnov method. Numeric data were expressed as median (min-max), while categorical variables were expressed as percentage. Overall survival time was calculated as time from diagnosis to time of death due to any reason

while disease-free survival was calculated as time from diagnosis to recurrence. Survival analysis was performed by using Kaplan-Meier curves. Univariate analysis was performed by using log-rank test, while multivariate analysis was performed by using Cox regression test. $p<0.05$ was considered as statistically significant.

Results

Table 1 presents distribution of demographic characteristics as well as tumor- and treatment-related features. Of the patients included, 344 were women while 229 were younger than 60 years. Median age was 55.2±0.3 years (range: 26-86 years). Of the patients, 58.9% was postmenopausal women; 60.6% had ECOG performance status 0, 53.1% had tumor localized at left breast; and 50.6% had stage II disease. Regarding surgery, 260 patients (96.3%) underwent MRM, while 13 patients (3.7%) underwent BCS. In histopathological evaluation, there was invasive ductal carcinoma in 323 cases, while there was T2 tumor in 60%, negative lymph node in 33.7%, grade 2 tumor in 47.1%, perinodal invasion in 64.3%, and lymphovascular invasion in 58.3%. In immunohistochemical evaluation, estrogen receptor was positive in 55.4%, while progesterone receptor was positive in 52.3%. In addition, HER2 was positive in 31.4%. Postoperative chemotherapy was given to 329 cases, while adjuvant radiotherapy was given to 243 cases. In addition, hormone therapy was given to 215 cases. CEF was most commonly used regimens in chemotherapy. Hormone therapy was given to 285 cases (76.8%) with positive hormone receptor for 5 years, including tamoxifen in 97 cases, aromatase inhibitory in 122 cases. Hemoglobin value was <12 g/dL in 66 cases, while PLR was <160 in 300 cases and NLR was <3 in 228 cases.

Mean follow-up was range 10 days-112 months. Mean OS was 84.4 months (range: 75.5-93.4) while mean PFS was 78.8 months (range: 71.5-86.0). Five-years and 9-years OSs were 76% and 54%, while PFSs were 61% and 57%, respectively. During follow-up, 48 patients died due to either disease-related or non-related causes. Local recurrence was detected in 14 cases, while distant metastasis in 45 cases. When cases with metastasis were assessed, there was multiple-organ metastasis in 17 cases (38%), bone metastasis in 15 cases (33%), pulmonary metastasis in 7 cases (15%), brain metastasis in 2 cases (4.4%) and other organ metastasis in 8 cases (8.8%).

Table 2 presents mean OS and PFS according to histopathological and clinical findings. Table 3 presents patient-, tumor- and treatment-related prognostic factors affecting survival. Histopathological diagnosis ($p=0.05$) and perinodal invasion ($p=0.05$) were significantly associated with OS whereas tumor stage ($p=0.026$), HER2 positivity ($p=0.006$), surgery type ($p=0.046$) and hormone therapy ($p=0.016$) were significantly associated with PFS. Although OS and PFS were better in female patients, those younger than 60 years, those with ECOG performance of 0, those with tumors localized at right, those with smaller tumor diameter, those without lymph node involvement, those with grade 1 disease, those with positive ER or PR, those without lymphovascular invasion, those with

Table 1. Baseline Characteristics of the Breast Cancer Patients

Characteristic		Patients, n (%)
Gender	Male	6 (1.7)
	Female	344 (98.3)
Age (years) mean (range)		55.3±0.3 (26-86)
	<60	229 (65.4)
	≥60	121 (34.5)
Menopausal status	Premenopausal	135 (38.6)
	Postmenopausal	206 (58.9)
Performance status	ECOG 0	212 (60.6)
	ECOG 1	138 (39.4)
Tumor localization	Right	161 (46)
	Left	186 (53.1)
	Bilateral	3 (0.9)
Tumor stage	I	41 (11.7)
	II	177 (50.6)
	III	132 (37.7)
Pathology	Invasive ductal	323 (92.3)
	Inflematuar	13 (3.7)
	The other	14 (4.0)
Tumor size	I	72 (20.6)
	II	210 (60.0)
	III	52 (14.5)
	IV	16 (4.6)
Lymph node status	0	118 (33.7)
	I	111 (31.7)
	II	70 (20.0)
	III	47 (13.4)
Histologic grade	I	71 (20.3)
	II	165 (47.1)
	III	91 (26)
	Unknown	23 (6.6)
ER status	Positive	194 (55.4)
	Negative	132 (37.7)
	Unknown	24 (6.9)
PR status	Negative	159 (45.4)
	Positive	183 (52.3)
	Unknown	8 (2.3)
HER2 immunohistochemistry	Negative	217 (62.0)
	Positive	110 (31.4)
	Unknown	23 (6.6)
Perinodal involvement	No	125 (35.7)
	Yes	225 (64.3)
Lymphovascular invasion	No	146 (41.7)
	Yes	204 (58.3)
Surgery	Mastectomy	337 (96.3)
	Lumpectomy	13 (3.7)
Chemotherapy	Yes	329 (94.3)
	No	20 (5.7)
Chemotherapy regime	CEF	108 (31.8)
	CAF	80 (23.6)
	AC	57 (16.3)
Radiotherapy	Yes	243 (69.4)
	No	107 (30.6)
Hormon replacement therapy	Yes	215 (61.4)
	No	135 (38.6)
Hemoglobin (g/dl)	<12	66 (18.9)
	≥12	284 (81.1)
Hemoglobin (g/dl)		14.1±1.9 (7.1-18.2)
Hematocrit (%)		42.6±5.1 (22.7-66.7)
White blood cell ($x10^3\ \mu l^{-1}$)		8.01±2.6 (1.5-21.7)
Neutrophil ($x10^3 \mu l^{-1}$)		5.4±2.4 (1.4-5.4)
Lymphocyte ($x10^3\ \mu l^{-1}$)		2.1±1.0 (0.4-7.3)
Monocyte (μl^{-1})		0.6±0.4 (0.2-3.0)
Eosinophil (μl^{-1})		0.2±0.2 (0.0-1.0)
Basophil (μl^{-1})		0.0±0.0 (0.0-0.6)
Platetelet ($x10^3\ \mu l^{-1}$)		240.0±75.0 (40.0-536.0)
PLR	132.7±66.2	(17.3-476.0)
NLR	3.0±2.2	(0.6-16.7)
PLR	<160	300 (85.7)
	≥160	50 (14.3)
NLR	<3	228 (65.1)
	≥3	122 (34.9)

Table 2. Overall and Progression-free Survival and *p value*

Variable		No. of patients	Mean OS (95% CI)	p value	Mean PFS (95% CI)	p value
Gender	Male	6	38.6 (18.1-59.1)	0.16	36.0 (19.0-52.0)	0.76
	Female	344	84.9 (75.9-93.9)		79.3 (72.0-86.6)	
Age (years)	<60	221	83.8 (72.1-95.4)	0.844	78.1 (68.9-87.3)	
	≥60	129	80.4 (66.6-94.2)		75.1 (62.8-87.5)	
Menopausal status	Premenopausal	135	64.4 (57.7-71.2)	0.656	58.3 (51.5-65.2)	0.831
	Postmenopausal	206	85.7 (75.4-95.8)		82.6 (73.9-91.3)	
Performance status	ECOG 0	212	86.5 (74.0-98.9)	0.49	80.7 (72.2-89.3)	0.927
	ECOG 1	138	79.2 (67.3-91.1)		75.1 (64.5-85.6)	
Tumor localization	Right	160	84.8 (72.2-97.3)	0.34	86.7 (78.0-95.5)	0.526
	Left	186	77.8 (69.6-85.9)		67.0 (58.1-75.9)	
Tumor stage	I	41	85.0 (65.7-102.7)	0.152	99.2 (87.3-111)	0.026
	II	177	84.2 (74.5-95.4)		74.0 (64.1-83.8)	
	III	132	68.8 (60.0-77.6)		59.5 (49.9-69.2)	
Pathology	Invasive ductal	323	90.7 (83.1-98.2)	0.05	79.5 (71.8-87.3)	0.302
	Inflematuar	13	58 (36.7-80.7)		80.2 (64.0-96.4)	
	The other	14	49.7 (21.7-77.5)		62.0 (42.9-81.1)	
Tumor size	I	72	80.8 (67.3-94.4)	0.341	86.4 (73.9-98.8)	0.476
	II	210	77.3 (68.3-86.2)		70.7 (62.8-78.6)	
	III	52	69.6 (64.8-74.3)		53.1 (42.3-63.8)	
	IV	16	47.5 (35.1-59.8)		46.3 (35.0-57.1)	
Lymph node status	0	119	85.5 (70.1-100.9)	0.332	80.3 (69.0-91.6)	0.237
	I	112	80.6 (67.6-93.6)		79.3 (67.4-91.2)	
	II	71	72.0 (61.7-82.4)		67.8 (56.6-79.0)	
	III	48	49.2 (40.0-58.5)		46.5 (37.6-55.3)	
Histologic grade	I	71	84.4 (73.4-95.3)	0.735	76.8 (65.0-86.7)	0.604
	II	165	77.0 (63.0-91.1)		76.2 (65.8-86.7)	
	III	91	67.5 (63.0-74.9)		61.3 (51.8-70.8)	
	Unknown	23	47.2 (38.9-55.4)		43.9 (36.1-51.8)	
ER status	Positive	194	87.9 (78.9-97.0)	0.888	84.8 (75.7-93.8)	0.348
	Negative	132	74.0 (62.3-85.6)		70.0 (39.9-82.5)	
	Unknown	24	82.3 (55.1-109.5)		61.2 (71.5-86.0)	
PR status	Positive	183	78.4 (65.7-91.0)	0.344	78.4 (69.6-87.2)	0.78
	Negative	159	70.4 (62.8-78.0)		68.6 (60.0-77.1	
HER2	Negative	217	91.9 (83.8-100.0)	0.607	84.3 (75.8-92.9)	0.006
	Positive	110	51.9 (43.3-60.5)		45.5 (38.7-52.3)	
	Unknown	23	64.8 (52.8-76.89		68.7 (51.6-85.9)	
Perinodal involvement	No	125	97.7 (89.1-106.3)	0.05	78.9 (67.5-90.39	0.886
	Yes	225	75.3 (64.8-85.7)		75.7 (67.0-84.4)	
Lymphovascular invasion	No	146	92.5 (82.7-102.3)	0.144	80.7 (70.4-90.9)	0.61
	Yes	204	74.3 (62.2-86.4)		74.3 (64.8-83.8)	
Surgery	Mastectomy	337	82.5 (72.2-92.9)	0.127	93.9 (81.6-106.2)	0.046
	Lumpectomy	13	93.3 (80.6-105.9)		75.9 (67.8-84.0)	
Chemotherapy	Yes	329	88.3 (80.5-96.1)	0.57	78.2 (70.5-86.0)	0.892
	No	30	67.4 (53.3-81.5)		63.6 (43.2-84.0)	
Radiotherapy	Yes	243	78.3 (68.7-87.9)	0.208	73.8 (60.1-87.6)	0.733
	No	107	95.0 (83.8-106.0)		77.3 (69.3-85.4)	
Hormon0therapy	Yes	215	82.6 (70.6-94.7)	0.89	73.2 (64.1-82.3)	0.016
	No	135	72.0 (75.5-93.4)		73.5 (65.8-81.1)	
Hemoglobin (g/dl)	<12	66	80.0 (67.9-92.0)	0.838	78.3 (65.8-90.7)	0.535
	≥12	284	82.4 (71.6-93.2)		77.0 (68.9-85.2)	
PLR	<160	258	84.0 (73.2-95.0)	0.412	79.0 (70.5-87.6)	0.928
	≥160	92	82.2 (70.2-94.3)		75.4 (63.1-87.8)	
NLR	<3	228	85.6 (74.6-96.6)	0.43	80.2 (71.0-89.5)	0.409
	≥3	122	78.8 (65.4-92.2)		73.2 (71.5-86.0)	

*Abbreviations: CI: Confidence Interval; ECOG: Eastern Cooperative Oncology Group; N: Node; OS: Overall Survival; PFS: Progression-free Survival; T: Tumor; NLR: Neutrophil/Lymphocyte Ratio; PLR: Platelet Lymphocyte Ratio

hemoglobin value>12, those with NLR<3 and those with PLR<160, the differences didn't reach statistical significance (p>0.05).

Table 4 and 5 presents results of univariate and multivariate analysis of risk factors for OS and PFS. In univariate analysis, age (p=0.05), histopathological diagnosis (p=0.025), and perinodal invasion (p=0.05) were significantly associated with OS, whereas age (p=0.004), tumor stage (p=0.011) and hormone therapy (p=0.018) were significantly associated with PFS. In multivariate analysis, histopathological diagnosis (OR: 0.3; 95%: 0.1-0.7; p=0.006) and perinodal invasion (OR: 0.1; 95% CI: 0.1-1.3; p=0.026) were significantly associated with OS, whereas tumor stage (OR: 2.1; 95% CI: 0.0-0.7; p=0.014) and hormone therapy (OR: 2.1; 95%: 1.2-3.8; p=0.010) were significantly associated with PFS.

Discussion

In the treatment of breast cancer, goal is to reduce mortality and morbidity and to prolong life expectancy with preserved quality of life (Azab et al., 2012; Azab et al., 2013). There are prognostic factors which were confirmed to increase loco-regional recurrence in

Table 3. Univariate Analysis of Risk Factors for the Overall and Disease-free Survival

Risk factors		Overall survive multivariate analysis		Disease-free survive multivariate analyis	
		OR (95% CI)	p value	OR (95% CI)	p value
Gender	Male	Ref		Ref	
	Female	0.2 (0.0-1.0)	0.05	0.1 (0.0-0.6)	0.004
Age	<60	Ref		Ref	
(years)	≥60	0.9 (0.5-1.7)	0.844	1.0 (0.6-1.8)	0.76
Menopausal status					
Premenopausal		Ref		Ref	
Postmenopausal		1.1 (0.6-2.0)	0.657	1.0 (0.6-1.8)	0.832
Performance status					
ECOG 0		Ref		Ref	
ECOG 1		0.8 (0.4-1.5)	0.492	1.0 (0.6-1.7)	0.927
Tumor localization					
Right		Ref		Ref	
Left		0.7 (0.4-1.3)	0.343	0.8 (0.5-1.4)	0.527
Tumor stage	I	Ref		Ref	
	II	0.5 (0.2-1.2)	0.501	0.2 (0.0-0.7)	0.011
	III	0.6 (0.3-1.1)	0.603	0.8 (0.4-1.4)	0.447
Pathology					
Invasive Ductal		Ref		Ref	
Inflematuar		0.3 (0.1-0.8)	0.025	0.8 (0.3-2.0)	0.655
The Other		0.5 (0.1-2.1)	0.394	0.2 (0.0-1.8)	0.208
Tumor size					
I		Ref		Ref	
II		0.8 (0.1-3.7)	0.83	0.6 (0.1-2.9)	0.585
III		0.7 (0.1-3.1)	0.707	1.1 (0.2-4.5)	0.891
IV		0.3 (0.0-1.7)	0.175	0.9 (0.2-4.3)	0.95
Lymph node status					
0		Ref		Ref	
I		0.5 (0.2-1.1)	0.096	0.5 (0.2-1.0)	0.072
II		0.7 (0.3-1.7)	0.447	0.4 (0.2-1.0)	0.062
III		0.8 (0.3-2.0)	0.664	0.5 (0.2-1.3)	0.183
Histologic grade					
I		Ref		Ref	
II		0.6 (0.1-2.4)	0.525	0.6 (0.2-1.89	0.399
III		1.0 (0.3-3.3)	0.979	0.9 (0.4-2.5)	0.996
Unknown		0.8 (0.2-3.2)	0.862	0.8 (0.3-2.3)	0.753
ER status					
Positive		Ref		Ref	
Negative		1.1 (0.3-3.9)	0.765	0.5 (0.2-1.2)	0.155
Unknown		1.0 (0.3-3.5)	0.938	0.6 (0.3-1.5)	0.676
PR status					
Negative		Ref		Ref	
Positive		1.3 (0.7-2.3)	0.347	1.0 (0.6-1.8)	0.78
HER2 immunohistochemistry					
Negative		Ref		Ref	
Positive		0.7 (0.3-2.0)	0.622	1.1 (0.3-3.1)	0.833
Unknown		1.0 (0.3-3.0)	0.899	2.5 (0.8-7.1)	0.094
Perinodal involvement					
No		Ref		Ref	
Yes		0.5 (0.2-1.0)	0.05	0.9 (0.5-1.6)	0.885
Lymphovascular invasion					
No		Ref		Ref	
Yes		0.6 (0.3-1.1)	0.148	0.8 (0.5-1.4)	0.61
Surgery					
Mastectomy		Ref		Ref	
Lumpectomy		0.2 (0.0-1.7)	0.161	0.1 (0.0-1.2)	0.169
Chemotherapy					
Yes		Ref		Ref	
No		0.7 (0.2-2.1)	0.574	0.9 (0.3-2.5)	0.932
Radiotherapy					
Yes		Ref	0.212	Ref	0.733
No		0.6 80.3-1.2)		1.0 (0.6-1.89	
Hormon replacement therapy					
Yes		Ref		Ref	
No		0.9 (0.5-1.7)	0.89	1.9 (1.1-3.4)	0.018
Hemoglobin (g/dl)					
<12		Ref		Ref	
≥12		1.0 (0.5-2.1)	0.838	0.8 (0.4-1.5)	0.536
Hemoglobin (g/dl)		1.0 (0.9-1.2)	0.353	1.0 (0.9-1.1)	0.455
Hematocrit (%)		0.9 (0.9-1.0)	0.678	0.9 (0.9-1.0)	0.33
White blood cell (x10^3 μl^{-1})					
		0.9 (0.8-1.0)	0.236	1.0 (0.9-1.1)	0.968
Neutrophil(x$10^3\mu l^{-1}$)		0.9 (0.8-1.0)	0.433	0.9 (0.8-1.0)	0.638
Lymphocyte (x10^3 μl^{-1})					
		0.9 (0.7-1.2)	0.793	1.0 (0.8-1.2)	0.959
Monocyte(μl^{-1})		0.7 (0.3-1.5)	0.414	0.9 (0.5-1.6)	0.821
Eosinophil (μl^{-1})		1.9 (0.5-7.1)	0.305	0.6 (0.1-2.6)	0.578
Basophil (μl^{-1})		1.3 (0.0-21.9)	0.833	0.0 (0.0-1.7)	0.089
Platetelet (x10^3 μl^{-1})		1.0 (0.9-1.0)	0.81	0.9 (0.9-1.0)	0.539
PLR					
<160		Ref		Ref	
≥160		0.7 (0.4-1.4)	0.414	0.9 (0.5-1.7)	0.928
NLR					
<3		Ref		Ref	
≥3		0.7 (0.4-1.4)	0.432	0.8 (0.5-1.3)	0.41

Table 4. Multivariate Analysis of Risk Factors for the Overall and Disease-free Survival

Risk factors	Overall survive multivariate analysis		Disease-free survive multivariate analyis	
	OR (95% CI)	p value	OR (95% CI)	p value
Tumor stage				
I	-	-	Ref	
II			0.2 (0.0-0.7)	0.014
III			0.8 (0.5-1.4)	0.555
Pathology			-	-
Invasive ductal	Ref			
Inflematuar	0.3 (0.1-0.7)	0.006		
The other	0.1 (0.0-1.3)	0.136		
Perinodal involvement				
No	Ref		-	-
Yes	0.4 (0.2-0.9)	0.026		
Hormon replacement therapy		-		
Yes	-	-	Ref	
No			2.1 (1.2-3.8)	0.01

patients with breast cancer. These factors are classified as tumor-, patient- and treatment-related characteristics. Tumor-related factors included tumor localization, tumor diameter, axillary metastasis, histological grade, presence of extensive intraductal component, multicentricity and biological markers (estrogen and progesterone receptors, Her2/neu status, BRCA-1 and BRCA-2). Patient-related factors included age, menopausal status, family history, age at menarche, age at menopauses, lactation and parity. Treatment-related factors included type of surgery, surgical margin, quality of radiotherapy and systemic treatment (Sotiriou et al., 2003; Lyon et al., 2008; Sezer et al., 2011; Azab et al., 2012; Afsharfard et al., 2013; Ceber et al., 2013; Hu et al., 2013). In agreement to literature, OS and PFS were found to be better in female patients, those younger than 60 years, those with ECOG performance of 0, those with tumors localized at right, those with tumors at early stage, those with smaller tumor diameter, those without lymph node involvement, those with grade 1 disease, those with positive ER or PR, those without lymphovascular invasion.

Besides these known prognostic factors, there are ongoing attempts to identify novel biological markers. There are limited numbers of studies published in the literature about predictive value of white blood cell subtypes for determining prognostic value of chronic

inflammation in breast cancer (Bachelot et al., 2003; Pierce et al., 2009; Azab et al., 2013). In this study, it was aimed to evaluate effects of inflammatory markers on prognosis in breast cancer.

In our study, it was found that platelet, white blood cell, neutrophil, lymphocyte, monocyte, eosinophil and basophil counts had no effect on OS and PFS. Rudolf Virchow was the first who proposed a role for chronic inflammation in cancer in 1863 based on observation of leukocytes in neoplastic tissues. Virchow postulated that inflammatory milieu is involved in initiation and development of carcinogenesis by promoting a cellular environment (Harada et al., 1994; Aggarwal and Gehlot 2009; Pierce et al., 2009). In the previous studies, it has been shown that there is an association between inflammatory markers such as peripheral neutrophil, lymphocyte and platelet counts and adverse outcomes in both breast cancers and other cancers. Although underlying mechanism is unclear, increased pretreatment peripheral blood neutrophil, and platelet counts have been associated with poor survival in patients with several cancers (Kusumanto et al., 2003; Pierce et al., 2009; Proctor et al., 2011). However, it was proposed that lower lymphocyte count is associated with poor outcomes in patients with advanced cancer, which is attributed to immunity with destruction of host cancer cells. Platelets have an important and versatile role in the progression of cancer. Platelets can play role in the promotion of tumor growth by inducing angiogenesis via vascular endothelial growth factor (VGEF). A direct correlation was shown between circulating platelets and serum VGEF levels (Kusumanto et al., 2003; Kassim et al., 2004). In studies by Pierce et al., and Bachelot et al., it was shown that there was an association with higher inflammatory markers (C-reactive protein, serum amyloid A and serum interleukin-6 etc.) and reduced survival in patients with breast cancer (Bachelot et al., 2003; Pierce et al., 2009). In our study, survival was shorter in patients with higher neutrophil and platelet counts, but the difference didn't reach statistical significance. These finding are in agreement with literature.

In our study, OS and PFS were found to be better in white blood cell, those with hemoglobin >12, those with NLR<3 and those with PLR<160, but the difference didn't reach statistical significance. As there is substantial evidence indicating that neutrophil has been involved in the pathophysiology of cancer, it becomes more attractive to understand available roles of neutrophil more comprehensively. In cancer, increased neutrophil count drives several biochemical mechanisms leading tissue damage. These biochemical mechanisms include release of arachidonic acid metabolites, platelet aggregation factors, cytotoxic free oxygen radicals, myeloperoxidase, elastase, and hydrolytic enzymes. Relative lymphopenia observed in cancer patients results from damage in immune system caused by cancer cells (Bachelot et al., 2003; Pusztai et al., 2004; Azab et al., 2012; 2013). It was shown that both neutrophil and lymphocyte are important inflammatory markers for the prediction of survival in cancer. Today, NLR is considered as a parameter which indicates increased neutrophil count representing inflammation and negative effects of decreased lymphocyte count representing immune system together. As an independent predictor, NLR combines predictive risks of these two WBC subtype in one risk factor (Bachelot et al., 2003). It has been suggested that NLR, derived from circulating neutrophil and lymphocyte count, is associated with survival in several types of cancer including lung, bladder, stomach, pancreatic, colorectal and ovarian cancers (Gorelik et al., 2005; Tenesa et al., 2010; Azab et al., 2013). In a study, Proctor et al. found that NLR predicts mortality better than total WBC count in patient with cancer. In addition it is known that platelets lead tumor progression by enhancing angiogenesis. Thus, it has been also suggested that PLR, derived from circulating platelet and lymphocyte counts, is also associated with survival in patients with pancreas, lung or breast cancer (Proctor et al., 2011). In many studies, both NLR and PLR have been demonstrated as prognostic markers in several cancers. In addition, negative effects of increased NLR and PLR on survival have been demonstrated in many previous studies (Azab et al., 2012; 2013). In a study on 27,031 patients with cancer, Proctor et al. investigated prognostic values of C-reactive protein, albumin, white cell, neutrophil, lymphocyte and platelet counts, and NLR and PLR at presentation (Proctor et al., 2011). Authors found that NLR and PLR had predictive value for cancer specific survival in bladder, breast, colorectal, gastroesophageal, gynecological, prostatic, pulmonary and renal cancer. In that study, authors reported that increased NLR and PLR were predictor for reduced cancer specific survival independent of age, sex and deprivation and tumor site (Azab et al., 2013). In a study on 437 patients with breast cancer, Azab et al reported that pretreatment NLR was an independent predictor of long-term mortality, whereas pretreatment PLR was not better than absolute lymphocyte count alone regarding prediction of long-term mortality (Azab et al., 2012). In another study on 316 patients with breast cancer, the same group investigated whether NLR is predictive for short- and long-term mortality in patients with breast cancer. Authors concluded that NLR is an independent predictor for short- and long-term mortality in breast cancer. However, prospective studies are needed to investigate NLR as a simple prognostic test in breast cancer.

In conclusion, in our study, it was found that WBC, neutrophil, lymphocyte and monocyte counts as well as, NLR and PLR had no effect on prognosis. We think that these results may not be representative due to early stage disease and shorter follow-up in our study. However, these data should have to be supported by results of comprehensive, prospective studies and systematic reviews that combines results of these studies.

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