

RESEARCH ARTICLE

Diagnostic and Prognostic Importance of the Neutrophil Lymphocyte Ratio in Breast Cancer

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Abstract

Background: The aim of this study was to determine diagnostic and prognostic roles of the neutrophil to lymphocyte ratio (NLR) in breast cancer patients. To date, data are limited on associations of primary breast carcinoma (PBC) and benign proliferative breast disease (BPBD) with preoperative NLR values. **Materials and Methods:** Our study covered of 120 female patients with PBC and 50 with BPBD. Diagnostic values of NLR were estimated using sensitivity, specificity and areas under receiver operating characteristic curves (AUC). **Results:** NLR values were significantly higher in the PBC patients than in those with BPBD, with an AUC of 0.668 in the PBC case. The optimal cut-off for NLR was 2.96 and this was validated in the testing set, giving a sensitivity and a specificity of 79.7% and 76.2%, respectively, in PBC patients. **Conclusions:** Preoperative high NLR is a significant diagnostic predictor of distinction of breast cancer from BPBD and elevated NLR is also an important prognostic marker for primary invasive breast cancer.

Keywords: Breast carcinoma - neutrophil - lymphocyte - ration - diagnosis - prognosis

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Introductions

Breast cancer is the most common type of cancer in women and the risk increases with age. As in other tumors the development of breast carcinoma is associated with systemic inflammation. The systemic inflammatory response can change with tumor growth, invasion, angiogenesis and metastasis. Inflammatory markers such as c reactive protein (CRP) and interleukin 6 (IL 6) are increased in breast cancer patients and a have adverse effect on survey (Balta et al., 2013).

Benign proliferative breast disease (BPBD) is most common in women who are in their thirties to fifties. Various sizes of cysts, adenosis, hyperplasia with atypia and/or without atypia and apocrine metaplasia can be seen in BPBD on microscopic examination. Despite the strong association between BPBD and breast cancer development, the exact causes of BPBD are still unknown. Hormonal imbalance especially increased estrogen via to progestin is thought to play a role in development of BPBD. Breast cancer risk related with proliferative breast disease. Several studies showed that inflammation plays an important role in the development of BPBD and early breast carcinogenesis (Catsburg et al., 2014).

Complete blood count and its subtype neutrophil count and neutrophil lymphocyte ratio (NLR) are an indicator of systemic inflammation (Guthrie et al., 2013).

NLR is an inexpensive and simple parameter of systemic inflammation. It is associated with prognosis in various types of cancers including gastrointestinal tract cancers, hepatocellular carcinoma, non-small cell carcinoma and cervical carcinoma (Walsh SR et al., 2005; Guthrie et al., 2013; Unal et al., 2013; Eryilmaz MK et al., 2014; Kemal et al., 2014) Preoperative NLR values can be contribute clinicians as well as diagnosis and prognosis in BPBD and breast cancer which associated with inflammation. The purpose of present study was to assess the association between primary breast carcinoma and BPBD which are related with inflammatory processes.

Materials and Methods

Cases

The data were retrospectively collected The data were retrospectively collected between February 2005 and June 2014) between February 2005 and June 2014. The study includes 120 female patients with primary invasive breast carcinoma and 50 female patients with BPBD.

Operation specimens consist of 69.2% (n=83) radical mastectomy, 15.8% (n=19) excision, 7.5%(n=9) incision and 7.5%(n=9) needle biopsy. The materials were fixed in 10% formalin solution. 3-5µm thick, formalin fixed and paraffin embedded sections were stained with the haematoxylin and eosin (H/E) stain. H/E stained sections

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from all cases were reviewed by two authors (G.Ö. and A.K.) and various pathological assessment such as lymph node status (excisional, incisional and needle biopsies were excluded) tumor diameter (needle biopsies and incisional biopsies were excluded), perinoral-angiolymphatic invasion (needle biopsies were excluded) and histologic type of tumor were evaluated. The tumors classified (invasive ductal, invasive lobular and mixed invasive and ductal carcinoma) and graded (1, 2, 3) according to the WHO histological classification of breast tumors and Nottingham modification of the Scarff-Bloom- Richardson system. Based on immunohistochemical profile (estrogen (ER), progesterone (PR), human epidermal growth factor (HER2) and cytokeratin 5/6 (CK 5/6) all tumors also divided molecular subtypes as Luminal A (ER+ and/or PR+, HER2-), Luminal B (ER+ and/or PR+, HER2+), HER2 (HER2+, ER- and PR-) and basal like (triple-negative)(ER-, PR-, HER2-) type.

Immunohistochemical staining

Immunohistochemical analyses were performed in all tumor cases, using the following antibodies: ER (Clone 6F11, Leica), PR (Clone 16, Leica), HER2 (Clone CB11, Leica), CK 5/6 (Clone EP24/EP67, 1:100 dilution, Emergo Europe). Immunohistochemical studies were performed with the compact polimary method according to standard procedures in Bond fully integrated IHC and ISH system.

While ER and PR scores were assessed; only nuclear expression was concerned (Lester et al., 2009; Hammond et al., 2010) and for ER staining of >10% of tumor nuclei was considered positive. PR expression was considered to be positive if the nuclei of more than 1% of cells were stained positive (Diaz et al., 2004; Fisher et al., 2005). HER2 score assessment was made according to intensities and the proportions of the cells which showed membrane staining (Lester et al., 2009; Gutierrez and Schiff, 2011). The cases with the triple negative phenotype (14.4%) were assessed for the expression of CK 5/6. Tumor cells with weak or strong cytoplasmic and/or membranous positivity CK 5/6 was scored as positive (Choccalingam et al., 2012).

Peripheral blood analysis

Preoperative complete blood counts (leukocytes, neutrophil, and lymphocytes) of the patients were analyzed and then neutrophil/lymphocyte ratio (NLR) was calculated. Patients with active infection, active bleeding and hematological disorders, acute-chronic inflammatory or autoimmune disease, splenectomy and steroid therapy excluded from the study.

Statistical analysis

Data analysis was performed by using SPSS for Windows, version 17.0 (SPSS Inc., Chicago, IL, United States). While, the continuous and ordinal data were shown as mean±standard deviation, otherwise, number of cases or percentages was used for categorical variables. Whether the differences among groups regarding for continuous and ordinal variables were statistically significant or not was evaluated by Kruskal Wallis test. When the p value from the Kruskal Wallis test statistics are statistically significant Conover's non-parametric multiple comparison test was

used to know which group differ from which others. Categorical data were analyzed by Pearson's Chi-square or Fisher's exact test, where applicable. The diagnostic value of NLR was assessed using receiver operating characteristic (ROC) curve analysis. Logistic regression models were fitted to calculate the risk (odds ratio [OR] and 95% confidence interval [CI]) A p value less than 0.05 was considered statistically significant.

Results

The data of 120 cases (70.6%) of infiltrating breast carcinomas and 50 cases (29.4%) of BPBD were evaluated. The clinicopathological characteristics of primary breast tumors are summarized in Table 1. The mean age of infiltrating breast carcinoma and BPBD were; 54.02±13.45 and 51.90±10.26 (p>0.05). Infiltrating ductal carcinoma (58,8%) was the predominant histological type of all carcinomas. According to histological grading of invasive carcinomas were found; grade 1) 24.6%, grade 2) 57% and grade 3) 1.,4%. Lymph node metastases were noted in 54.4% of the patients. The tumours size classified into three groups as ≤ 2 cm (21.2%), 2.1-5cm (65.9%) and >5 cm (12.9%). According to molecular subtypes

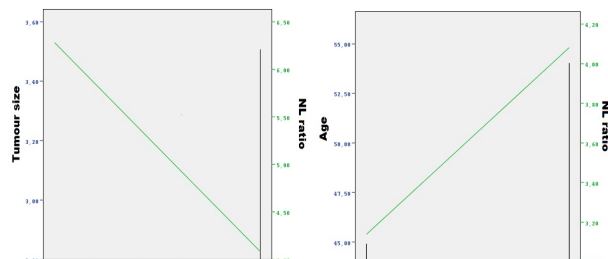
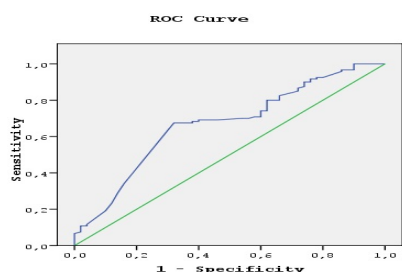
Table 1. Patients' Characteristics of Tumour Group (n=120)

Characteristics	
Age (mean±SD, year)	54.02±13.45
Histological type (%)	
Ductal	83.3
Lobular	15.0
Mix	1.7
Tumor size (cm, %)	
≤2 cm	21.2
2.1-5 cm	65.9
>5 cm	12.9
Histologic grade (%)	
I	24.8
II	56.6
III	18.6
Perinoral invasion (%)	
Negative	77.6
Positive	22.4
Angiolymphatic invasion (%)	
Negative	76.5
Positive	23.5
Lymph node status (%)	
Negative	28.6
Positive	71.4
ER (%)	
Negative	23.3
Positive	76.7
PR (%)	
Negative	20.8
Positive	79.2
HER2 (%)	
Negative	75.6
Positive	24.4
Molecular subtype (%)	
Luminal A	37.8
Luminal B	23.3
HER2	24.4
Basal like (Triple negative)	14.4

Table 2. r and p Values of Correlation Analyses between NLR and Other Parameters

	Age	Grade	Lymph-Node Metastasis	Tumor Size
NLR	r: 0.217 p: 0.005	r: 0.282 p: 0.0001	r: 0.282 p: 0.003	r: 0.332 p: 0.001

*NLR: Neutrophil-Lymphocyte Ratio; r: r value, p: p value

**Figure 1. The Relationship between Age, Tumor Size and NLR with Breast Cancer****Figure 2. Assessment of Cut Off Value of NLR for Prediction of Cancer Diagnosis**

38.5% of patients were luminal A, 23,1% of patients were luminal B, 24.2% of patients were HER2 and 14.4% of patients were basal type (triple negative). There were not statistically significant differences between the molecular subtypes ($p > 0.05$).

In the group with the breast tumors and BPBD the value of NLR was 4.08 ± 1.54 and 3.13 ± 1.27 . There were statistically significant differences between these two groups according to NLR ($p < 0.001$). There were a significant positive correlation between NLR and age, grade, lymph node metastasis and tumor size (Table 2) (Figure 1). In the tumors with lymphovascular invasion the value of NLR was significantly higher ($p < 0.05$).

ROC analysis showed that if the chosen cut-off point for NLR is 2.96 the specificity and sensitivity are 79.7%, 76.2%, respectively. These were statistically significant. ($p < 0.001$ AUC=0.668, %95CI=0.579-0.757) (Figure 2).

Discussion

In this current study, we have demonstrated that elevated preoperative NLR is a significant factor predicting breast cancer diagnosis. Preoperative NLR was significantly higher in patients with breast cancer than in BPBD. Our study showed that high values of preoperative NLR in patients with suspicious breast mass can be used as a predictor for malignancy and it can be useful for clinicians and pathologist for management of breast masses.

Increasing evidence supports the inflammation plays

a major role in the development and progression of cancer. Absolute counts of neutrophil and lymphocyte could be altered by various physiological, pathological and physical events but NLR is not affected by these factors (Proctor et al., 2012). Tumors are infiltrated by leucocytes and produce cytokines and chemokines. These cytokines and chemokines have the potential to stimulate tumor cell proliferation and may contribute directly malignant progression. Many cytokines and chemokines are alterable by hypoxia and oxidative stress, which is a most important physiological difference between tumor and normal tissue (Balkwill and Mantovani, 2001). TNF is a major mediator of inflammation and can be detected in malignant or stromal cells in human cancers especially in breast, ovarian, prostate, bladder and colorectal cancer (Naylor et al., 1993; Burke et al., 1996). In breast cancer infiltrating leucocytes are a major source of TNF (Leek et al., 1998). On the other hand lymphocytic response is the main component in the control of cancer progression. Tumor infiltrating lymphocytes especially natural killer and T helper type 1 which produce interferon gamma are effective against cancer growth and/or metastasis in several cancers (Ohashi et al., 2006). Cellular immune response decreased as a consequence of lymphocytopenia. Recent studies showed that decreased tumor infiltrating T cells have been associated with poor prognosis in some cancers (Walsh et al., 2005; Fogar et al., 2006; Bhatti et al., 2010; Dou et al., 2013). Due to all these effects of inflammation lead to the NLR increase in peripheral blood. NLR is convenient, inexpensive and reproducible method which could be show association with inflammation and tumour. Previous studies clearly suggested the diagnostic and prognostic importance of NLR on different types of cancer patient (Guthrie et al., 2013). Highlights of these studies we want to determine the sensitivity and specificity power of NLR on breast cancer. The cut of value for NLR was determined as 2.96 in this study and the specificity and sensitivity are 79.7%, 76.2%, respectively. To our knowledge in the literature this is the first study evaluating the preoperative NLR for distinguishing breast cancers from fibrocystic disease. Similar this study Kemal et al showed that lung cancer had high levels of NLR compared to the healthy control group and they found that NLR could be useful in lung cancer diagnosis (Kemal et al., 2014). Additionally older age, high histological grade, nodal involvement and larger tumors were shown to be associated with high NLR. These results are consistent with the results of previous studies (Azab et al., 2012; Dirican et al., 2014).

Breast cancer is considered a highly heterogeneous disease. Different types of this cancer exhibit variable histopathological features and different outcome. Based on a high degree of heterogeneity, recently these tumors classify according to molecular characteristics (Perou et al., 2000; Sorlie et al., 2003; Viale, 2012). In the literature several studies have shown that triple negative (ER, PR and HER2 negative; basal like type) and HER2 subtypes were associated with poor prognosis compared with the luminal A subtype (Parise et al., 2009; Zhao et al., 2009; Su et al., 2011; Lv et al., 2011; Rao et al., 2013). In the present study, positive HER 2 (IHC 3+) and negative ER,

PR and HER2 (positive with CK 5/6, basal like type) were associated with high NLR while positive ER and PR related with lower NLR. Similar to the current study reported that positive HER2 associated with high NLR and negative ER and PR related with lower NLR (Dirican et al., 2014). Differently from this study, we found that both positive HER2 and triple negative type associated with high NLR. Due to higher NLR values in basal like and HER2 types in this study NLR could be an independent significant predictor of prognosis in breast cancer patients.

In conclusion, we thought that preoperative higher NLR may be used a distinctive predictor of breast cancer diagnosis and prognosis. Also, preoperative NLR could be a predictive marker for clinicians and pathologist before evaluating of histopathological slides.

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Contribution of Authors

- A-Research concept and design; GO, CY
- B-Collection and/or assembly of data; GO, EK, AK
- C-Data analysis and interpretation; GO, BK, FY
- D-Writing the article; GO, CY
- E-Critical revision of the article; EK, BK
- F-Final approval of article; GO, CY

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