JBUON 2015; 20(4): 994-1000
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Prognostic impact of tumor lymphocytic infiltrates in patients with breast cancer undergoing neoadjuvant chemotherapy

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Summary

Purpose: *The presence of a pronounced tumor lymphocytic infiltrate (TLI) is deemed to reflect the presence of an immunoinflammatory response against the tumor and may thus have prognostic significance. We investigated the prognostic value of TLI detected in pathological specimens collected following neoadjuvant chemotherapy (NACT) in patients with breast cancer.*

Methods: *100 consecutive patients with breast cancer (mean age 47.8±11.4 years) who were scheduled to undergo anthracycline- and/or taxane-containing NACT were enrolled. Specimens collected after NACT were scored with the 4-point Klintrup scoring criteria for the presence of TLI.*

Results: *60 patients had low-grade TLI and 40 high-grade TLI. Comparison of the patient population according to low-grade vs high-grade TLI revealed statistically significant difference both in terms of disease-free survival (DFS) (log rank=4.28, p<0.05) and overall survival (OS) (log rank=3.96, p<0.05), with high-grade TLI patients showing a better prognosis. Multivariate Cox regression analysis identified postoperative tumor size and low-grade TLI as the two main independent adverse prognostic factors.*

Conclusion: *High-grade TLI may interfer with tumor growth and can represent a favorable prognostic factor in women with breast cancer undergoing NACT.*

Key words: *breast cancer, neoadjuvant chemotherapy, prognosis, tumor lymphocytic infiltrates*

Introduction

Breast cancer continues to represent the most common cancer in women worldwide, comprising 23% of all malignancies [1]. According to the American Cancer Society, approximately 1.3 million women are diagnosed with breast cancer annually around the world and approximately 465,000 cases will die from this neoplasm [2]. NACT is defined by the administration of chemotherapy before locoregional treatment (with surgery and/or irradiation) [3]. NACT followed by cytoreduction has currently become a part of standard care for patients with locally advanced breast cancer [4]. There is now convincing evidence that NACT can offer several advantages, including downstaging of large tumors, providing information on tumor response to a specific chemotherapeutic agent, and improving clinical outcomes (presumably through early clearance of systemic micrometastates) [5-7]. Despite being associated with significant clinical benefits, breast cancer patients undergoing NACT continue to have a wide range of clinical outcomes [6,7]. In this context, the identification of clinically applicable prognostic features that may allow an improved prognostic

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Received: 16/02/2015; Accepted: 14/03/2015

stratification is eagerly awaited.

The presence of a pronounced TLI in pathological specimens has also repeatedly associated with favorable clinical outcomes in a variety of solid malignancies [8-11]. Because the type and density of immune cells in and around the tumor reflects the presence of an immunoinflammatory response against the neoplasm [12], TLI could be a significant determinant of tumor progression. In order to provide an objective assessment of TLI, Klintrup and coworkers [13] have proposed a simplified method for structured scoring of the inflammatory reaction at the tumor invasive edge. The Klintrup classification includes all white cell types and results in a binary score of low-grade or high-grade TLI [13].

The prognostic value of TLI in patients with breast cancer remains controversial. Mohammed et al. [14] have previously shown that a high lymphocytic infiltrate was associated with improved survival, independent of clinicopathological characteristics including ER status, in primary operable ductal invasive breast cancer. However, a recent meta-analysis involving a total of 66 independent studies (totalling 34,086 patients) provided no definite evidence of an association between tumor inflammatory cell infiltrates and clinical outcomes in primary operable breast cancer [15]. Inconsistencies between studies have been attributed to lack of methodological validation for determing the presence of TLI, small sample sizes, and patient heterogeneity [15]. Another potential source of confounding is the use of chemotherapy, which may have a significant impact on anticancer immune responses [16].

In order to shed more light on the clinical significance of TLI in breast cancer patients, we designed the current study to examine the prognostic value of TLI (as assessed using the Klintrup score) in pathological specimens collected following NACT in a sample of patients with breast cancer.

Methods

Study participants

The current investigation was designed as a retrospective, observational study of 100 consecutive patients with breast cancer (mean age 47.8 ± 11.4 years) who were scheduled to undergo NACT before attempting cytoreductive surgery at the Department of Oncology of the Uludag University Medical Center, Bursa, Turkey. Enrollment took place between September 2006 and August 2011. All patients were of Turkish descent. The clinicopathological characteristics of the study participants were collected from pathological reports and medical charts. Lesion staging was performed according to the sixth edition of the American Joint Committee on Cancer (AJCC) staging manual for breast cancer. All participants received anthracycline- and/or taxane-containing NACT before surgery. The study protocol conformed to the tenets of the Declaration of Helsinki and was approved by the local ethics committee.

All patients gave oral informed consent.

Assessment of TLI

The presence of TLI in tumor specimens collected after NACT was assessed using the Klintrup scoring system [13]. Briefly, specimens were scored according to a 4-point scale. Scores were based on appearances at the deepest area of tumor invasion. A score of 0 indicated no increase in inflammatory cells at the deepest point of the tumor's invasive margin; a score of 1 denoted a mild and patchy increase in inflammatory cells; a score of 2 indicated a prominent inflammatory reaction forming a band at the invasive margin with some evidence of destruction of cancer cell islands; finally, a score of 3 denoted a florid cup-like inflammatory infiltrate at the invasive edge with frequent destruction of cancer cell islands. For the purpose of analysis, these scores were then divided into two subgroups, i.e. low-grade TLI (scores 0 and 1) and high-grade TLI (scores 2 and 3) [13].

Study endpoints

Disease-free survival (DFS) was defined as time from surgery to the date of first relapse, second primary malignancy, or death resulting from any cause (whichever occurred first). Overall survival (OS) was defined as time from surgery to the date of death resulting from any cause. Patients who were alive (for OS) and disease-free (for DFS) were censored at the date of the last follow-up.

Statistics

The data were checked for normality using the Kolmogorov-Smirnov test for continuous variables. Variables were expressed as means ± standard deviation, medians (lower quartile-upper quartile), or as counts (percentages) if categorical. Correlations were tested using the Spearman's correlation coefficient. The association of each risk factor with DFS and OS was assessed by multivariate Cox proportional hazard regression analysis. The multivariate Cox model included all the characteristics listed in Table 1. The appropriateness of the proportional hazards assumption was verified using graphical methods and tested as described previously [17]. The assumption of linearity for the Cox models was examined through visual inspection, and no violation was found. Hazard ratios (HRs) and their

Table 1. General patient, disease and chemotherapy characteristics (patients, N=100)

Characteristics	*N (%)*
Age (years) , mean±SD	47.8 ± 11.4
Postmenopausal status (yes/no)	55/45
Preoperative primary tumor stage	
T1	16 (16)
T2	64 (64)
T3	12 (12)
T4	8 (8)
Preoperative axillary lymph node stage	
N0	63 (63)
N1	35 (35)
N2	1 (1)
N3	1 (1)
Number of nodal metastases	
No metastases	77 (77)
1-3	18 (18)
4-9	1 (1)
≥ 10	1 (1)
Unknown	3 (3)
Clinical TNM stage	
1A	13 (13)
1B	0 (0)
2A	48 (48)
2B	29 (29)
3A	5 (5)
3B	4 (4)
3C	1 (1)
Histology	
Invasive ductal carcinoma	90 (90)
Invasive lobular carcinoma	6 (6)
Mucinous carcinoma	2 (2)
Tubulolobular carcinoma	1 (1)
Unknown	1 (1)
Neoadjuvant chemotherapy	
Anthracycline combinations	40 (40)
Taxane/anthracycline combinations	48 (48)
Unknown	12 (12)
Number of chemotherapy cycles, mean±SD	5.7 ± 1.0
Postoperative tumor size (mm) median (range)	18 (5-30)
Postoperative primary tumor stage	
T1	62 (62)
T2	28 (28)
T3	10 (10)
T4	0 (0)
Pathological TNM stage	
0	13 (13)
1A	33 (33)
1B	1 (1)
2A	33 (33)
2B	17 (17)
3A	2 (2)
3B	0 (0)
3C	1 (1)
TLI (Klintrup score)	
0	7 (7)
1	53 (53)
2	15 (15)
3	25 (25)

Data are expressed as counts, mean±standard deviation, or median (range), as appropriate

95% confidence intervals (CIs) were calculated with the estimated regression coefficients and their standard errors in the Cox models. DFS and OS curves were plotted using the Kaplan-Meier method, and the differences were compared using the log-rank test. All calculations were performed using SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA). All tests were two-sided, and $p<0.05$ was considered as statistically significant.

Results

The general characteristics of patients with breast cancer undergoing NACT are summarized in Table 1. Of the 100 study participants, 90 had invasive ductal carcinoma, 6 invasive lobular carcinoma, and the remaining 4 other forms of carcinoma. The primary tumor status was T1 in 16 patients, T2 in 64 patients, T3 in 12 patients, and T4 in 8 patients. The lymph node status was as follows: N0 in 63 patients, N1 in 35 patients, N2 in 1 patient and N3 in 1 patient. The mean number of NACT cycles was 5.7±1.0. The patients were followed up for a mean of 38.2±11.4 months.

TLI and clinical outcomes

The distribution of the Klintrup scores in the 100 breast cancer specimens collected after NACT was as follows: 7 patients had a score of 0, 53 had a score of 1, 15 had a score of 2, and 25 had a score of 3 (Table 1). Therefore, there were 60 patients with low-grade TLI and 40 with high-grade TLI. We then examined the associations between the extent of TLI and the general characteristics of the study participants (Table 2). Interesting-

Table 2. Correlation analysis of TLI scores (Klintrup scoring system) with the general characteristics of breast cancer patients undergoing neoadjuvant chemotherapy (N = 100)

Parameters	*Spearman's correlation coefficient*	*p value*
Age	0.018	0.441
Postmenopausal status	0.049	0.628
Preoperative primary tumor stage	-0.388	< 0.001
Preoperative axillary lymph node stage	-0.128	0.205
Number of nodal metastases	-0.176	0.081
Clinical TNM stage	-0.388	< 0.001
Histology	0.131	0.192
Neoadjuvant chemotherapy	0.065	0.528
Number of chemotherapy cycles	0.095	0.347
Postoperative tumor size	-0.388	< 0.001
Postoperative primary tumor stage	-0.114	0.258
Pathological TNM stage	-0.169	0.094

Table 3. Results of multivariate regression analysis for disease-free survival and overall survival

Parameters	*Disease-free survival, HR (95% CI), p value*	*Overall survival, HR (95% CI), p value*
Age	HR=1.1 (0.9–1.7), 0.85	HR=1.2 (0.9–1.5), 0.74
Postmenopausal status	HR=1.5 (0.8–1.9), 0.56	HR=1.4 (0.7–1.8), 0.67
Preoperative primary tumor stage	HR=2.7 (0.9–2.8), 0.78	HR=2.4, (0.9–2.3), 0.45
Preoperative axillary lymph node stage	HR=1.8 (0.8–1.9), 0.49	HR=1.5 (0.7–1.5), 0.67
Number of nodal metastases	HR=5.9 (0.8–6.4), 0.24	HR=5.5 (0.9–5.8), 0.18
Clinical TNM stage	HR=2.3 (0.9–2.5) 0.70	HR=2.4 (0.8–2.6), 0.73
Histology	HR=1.3 (0.4–2.5), 0.87	HR=1.4 (0.5–2.4), 0.90
Neoadjuvant chemotherapy	HR=2.5 (0.8–2.8), 0.35	HR=2.6 (0.7–2.5), 0.24
Number of chemotherapy cycles	HR=1.3 (0.4–1.5), 0.59	HR=1.5 (0.6–1.8), 0.68
Postoperative tumor size	HR=4.2 (1.1–10.4), <0.05	HR=4.4 (1.3–11.6), <0.05
Postoperative primary tumor stage	HR=1.1 (0.5–1.2), 0.56	HR=1.2 (0.7–1.3), 0.67
Pathological TNM stage	HR=2.4 (0.9–2.9), 0.81	HR=2.3 (0.8–2.7), 0.74
Low-grade TLI	HR=1.2 (1.1–2.4), p<0.05	HR=1.5 (1.1–3.6), <0.05

HR: hazards ratio, CI: confidence interval, TLI: tumor lymphocytic infiltrate

ly, we found significant associations of TLI with preoperative primary tumor stage, clinical TNM stage, and postoperative tumor size. These results suggested that higher TLI scores were associated with less severe preoperative primary tumor and clinical TNM stage as well as a lower postoperative tumor size. In the entire study cohort, the mean DFS was 36.5 ± 12.1 months, whereas the mean OS was 37.1±11.3 months (p=0.63). Categorization of the patient population according to low-grade vs high-grade TLI revealed statistically significant difference both in terms of DFS (log rank = 4.28, p<0.05, Figure 1) and OS (log rank = 3.96, p<0.04, Figure 2), with patients with high-grade TLI showing better prognosis. After allowance for potential confounders, the results of multivariate Cox regression analysis indicated that postoperative tumor size (DFS, HR=4.2, 95% CI = 1.1–10.4, p<0.05; OS, HR=4.4, 95% CI = 1.3–11.6, p<0.05) and low-grade TLI (DFS, HR=1.2 , 95% CI = 1.1–2.4, p<0.05; OS, HR=1.5, 95% CI = 1.1–3.6, p<0.05) were the two main independent adverse prognostic factors in our patients undergoing NACT (Table 3).

Discussion

This prospective investigation was designed to assess the association between the presence of TLI and prognosis in a series of breast cancer

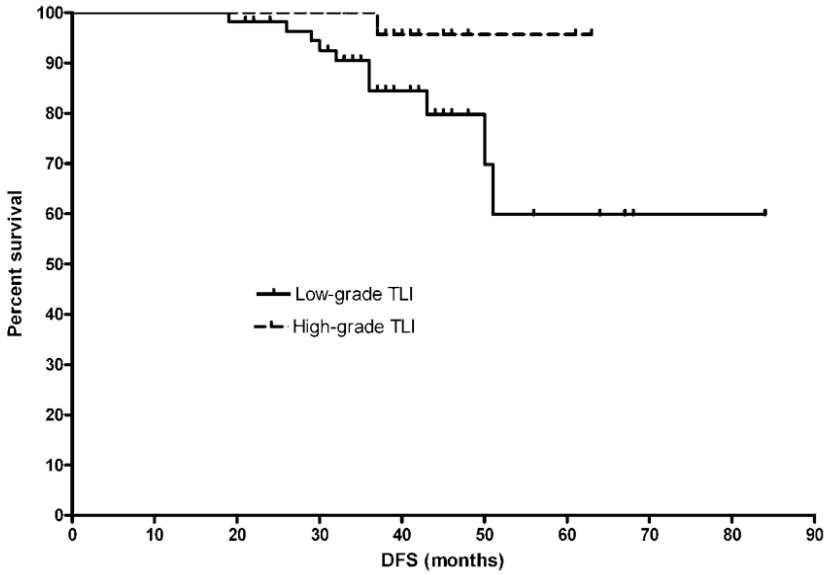

Figure 1. Kaplan-Meier plots for disease-free survival in breast cancer patients undergoing NACT according to the presence of low-grade vs high-grade TLI ($p<0.05$).

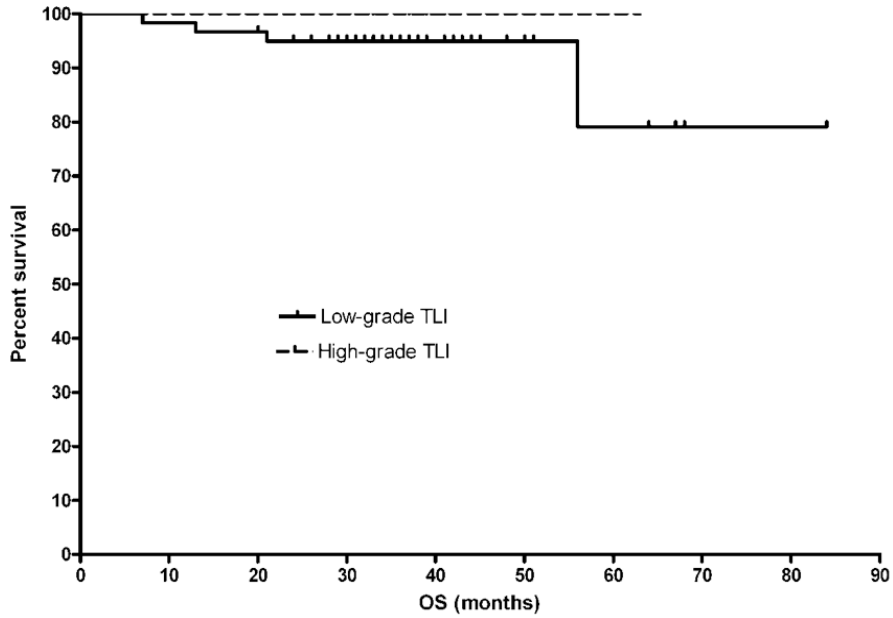

Figure 2. Kaplan-Meier plots for overall survival in breast cancer patients undergoing NACT according to the presence of low-grade vs high-grade TLI ($p<0.04$).

patients undergoing NACT. Because the clinical outcomes of this patient group continue to be heterogenous [5-7], there is an urgent need of biomarker tools that may improve their prognostic stratification. This study revealed three principal findings. First, significant associations of TLI with preoperative primary tumor stage, clinical TNM stage, and postoperative tumor size were found. Second, patients with high-grade TLI after NACT showed a better prognosis both in terms of DFS and OS. Third, TLI was an independent prognostic factor in our patients undergoing NACT. Taken together, these results indicate that the assessment of TLI using the Klintrup criteria should be

routinely included in the pathological reporting of breast cancer specimens obtained after NACT. Notably, the Klintrup method used in the present study allowed a structured assessment of all white cell types at the primary tumor and can be applied to routine hematoxylin and eosin specimens [13].

The association between TLI and prognosis in patients with breast cancer is still a matter of debate. Although TLI has been related to better clinical outcomes in some studies, other series have identified a significant association between inflammatory inflitrates and reduced survival [15]. The apparent controversy clearly highlights the need for further research on this topic. Our study supports the notion that the presence of TLI after NACT is a favorable prognostic factor in breast cancer patients. This association can be explained by the fact that tumor cells can be cleared by host innate and adaptive immuno-inflammatory cells, a process known as tumor immunosurveillance [18]. During tumor-specific adaptive immune responses, some inflammatory cells infiltrating the tumor (e.g., cytotoxic T lymphocytes) can induce the production of tumor-associated antigens and the cytokine interferon-gamma (IFN-γ) [19]. Notably, IFN-γ can modulate prognosis in patients with malignancies by influencing cell cycle arrest, apoptosis, differentiation, angiogenesis, and macrophage activity [20]. These observations may explain the favorable prognostic impact of TLI as observed in our report. In particular, we postulate that an increased inflammatory response may have stimulated the clearance of rapidly dividing tumor cells. All of the patients enrolled in this study underwent NACT. In a previous study, Denkert et al. [16] reported a strong association between lymphocytic infiltrate and chemotherapy response in a large set of more than 1,000 samples enrolled from two prospective, randomized clinical breast cancer trials. The authors speculated that chemotherapy could trigger an immune response directed against the tumor cells, which can be particularly strong in the subset of patients in whom a sensitization of the immune system against some tumor antigens is present before the onset of chemotherapy [16]. Further studies are needed to shed more light on this possibility.

Our findings should be interpreted within the context of some limitations. First, this investigation was conducted in Turkish individuals, so results cannot be simply extrapolated to populations with different ethnic backgrounds. Second, our study should be considered as an exploratory analysis and independent replication is needed to extend and confirm our results. Moreover, breast cancer patients were treated on an individual basis according to each patient's disease characteristics based on clinical trial data and influenced by the personal experience of the surgeon [7]. Finally, due to financial constraints, IFN-γ and molecular markers of white blood cell recruitment and infiltration were not measured in this study.

Notwithstanding the study caveats, our results suggest that the assessment of TLI provides independent prognostic information in breast cancer patients undergoing NACT and should therefore be considered for future inclusion in routine pathological reporting of this patient population.

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