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Clinical features and their effect on outcomes of patients with triple negative breast cancer with or without lymph node involvement

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Abstract

Objective: Clinical and pathological characteristics of triple negative breast cancer (TNBC) treatment are required for escalation or de-escalation of treatment because of a lack of druggable targets. This study aimed to identify the factors affecting the risk of disease recurrence and disease-related death in patients with TNBC.

Methods: Patients with TNBC who were treated at the University Medical Centre Maribor between January 2010 and December 2017 were studied. Clinical and pathological data were analyzed using multivariate analysis and non-parametric tests. Subgroup analysis was performed to examine additional factors that affect 5-year overall survival (OS) and recurrence-free survival.

Results: Multivariate analysis showed that tumor size and the lymph node ratio (LNR) were significant risks in our population. Better discrimination of patients at risk of a shorter recurrence-free survival and OS was achieved by using the LNR. Only lymphovascular invasion was significant for predicting 5-year OS.

Conclusion: For risk-based decision-making systems, the LNR is useful for discriminating between high- and low-risk patients with TNBC.

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Introduction

Breast cancer (BC) is the most common malignancy in women worldwide. BC accounts for one of the main causes of cancer-related deaths.[1] Multifactorial involvement in the development of BC is also a cause of variability in BC subtypes. Because of this variability, BC is a heterogeneous disease with many different subtypes.[2] BC is classically grouped into subtypes on the basis of the presence of hormonal receptors, such as estrogen receptor and progesterone receptor, as well as the presence of human epidermal growth factor receptor 2 (HER2).[3] One of the subtypes of BC is triple-negative breast cancer (TNBC), which is defined by a lack of ER, PR, and HER2 overexpression and amplification.[4] TNBC represents approximately 12%–20%[4,5] of all new cases of BC every year and usually has a poorer prognosis compared with other BC subtypes. TNBC is an aggressive type of cancer that affects younger patients and is frequently diagnosed in an advanced stage.[4] Patients with TNBC have larger tumors and a higher tumor grade, usually grade three, compared with patients without TNBC.[6] Overall survival (OS) and recurrence-free survival (RFS) are worse in patients with TNBC than in those with non-TNBC disease.[4,7] The 5-year OS rate of patients with TNBC is 72%–86%.[8–11] To some extent, survival is also affected by a lack of hormonal receptors and HER2. Therefore, there is no specific target treatment in this BC subtype.[12]

With regard to management and prognosis of BC, there are several important characteristics, such as tumor size,[13] tumor grade,[3] presence of different receptors,[13] amount of proliferation factors, presence of metastases,[13] and lymph node (LN) status.[14] Positive LNs are correlated with a higher recurrence rate of BC and poorer clinical outcomes.[14,15] Recent studies[8,13,16–18] have indicated that the number of positive LNs compared with the total number of LNs removed has an important effect. This rate is defined as the lymph node ratio (LNR).[14] Even though the number of positive LNs is important for establishing a treatment plan,[13] the LNR might be a more accurate prognostic factor in LN-positive TNBC, especially being better for predicting mortality (OS), than the number of LNs involved.[8,13,16–18]

Understanding the relation between clinical prognostic factors in TNBC is important, especially because of the heterogeneous nature of this disease. This study aimed to understand the relation of traditional risk factors to novel risk factors, such as the LNR, to use them for risk stratification and predicting the outcome of patients.

Patients and methods

We retrospectively identified patients with TNBC who were treated at the University Medical Centre Maribor (UMC Maribor) Centre for Breast Disease from January 2010 until December 2017. This study was approved by the Institutional Review Board (Reg. No. UKC-MB-KME-9/19).

All patients signed a written informed consent form to allow the use of their medical records retrospectively for research purposes.

Detailed data on histopathological features and clinical outcomes were recorded. The clinical data included demographic information on age, treatment procedures (neoadjuvant chemotherapy regimens, surgical treatment, adjuvant chemotherapy regimens), the number of positive LNs, and the LNR. The LNR was calculated by dividing the number of positive LNs by the number of all dissected LNs. Patients had to have at least five LNs removed to be included in the LNR calculation. Patients who showed a low-risk LNR (<21%) or no LN involvement were compared with a group of patients with their LNR status classified as middle- and high-risk levels (>21%). Further analysis was then performed by comparing LNR status outcomes with LN status outcomes.

Recorded histopathological features were histological subtype, lymphovascular invasion (LVI), the proliferation marker Ki-67, and tumor differentiation. Tumor differentiation was categorized as good (G1), moderate (G2), or poor (G3). Data were collected on BC-specific death, as well as disease recurrence or metastases. Disease stage was determined according to the American Joint Committee on Cancer, 8th Edition Cancer Staging Manual.

The patients' data were analyzed using descriptive statistics, the Mann–Whitney U test, and multivariate analysis of variance. Analysis of OS and RFS was performed in a subgroup of patients from January 1 2010 until December 31 2013. Statistical analysis was performed using SPSS software for Mac version 23.0 (IBM Corp., Armonk, NY, USA). A p value of <0.05 was considered statically significant.

Results

Patients' characteristics and disease management

A total of 136 consecutive patients with TNBC were identified in our analysis. Patients were then appropriately evaluated for their disease status. We excluded patients with primary metastatic disease, tumors arising after previous BC therapy and those that presented with a different histological character, patients lost to follow-up (e.g., continued treatment at another institution), and patients with no medical data available. Therefore, the final number of patients included in this analysis was 88. Patients were diagnosed from 32–87 years old. The biological characteristics of the patients are shown in Table 1.

Patients were treated according to the Interdisciplinary Tumor Board decision. Neoadjuvant chemotherapy was administered in seven (22%) LN-positive patients and in 15 (27%) LN-negative patients. A total of 18 (81.8%) patients received neoadjuvant chemotherapy of epirubicin and cyclophosphamide (EC). Three (13.6%) patients received combined therapy of EC following treatment with taxanes and cyclophosphamide (TC); data for one of these patients were missing. All patients then underwent surgical treatment. In the LN-positive group, 22 (69%) were treated with breast-conserving surgery and 10 (31%) underwent a mastectomy. In the LN-negative group, 46 (82%) patients were treated with breast-conserving surgery and 10 (18%) were treated with mastectomy. A total of 72 (82%) patients then underwent radiotherapy. Adjuvant chemotherapy was administered to 22 (69%) LN-positive women and to 38 (68%) LN-negative women. Among the patients who were treated with adjuvant chemotherapy, 33 (55%) underwent treatment with EC

Table 1. Patients' characteristics.

Characteristics	LN-positive TNBC (n = 32)	LN-negative TNBC (n = 56)
Age at time of diagnosis (years)	57.4 (SD: 13.1)	59.1 (SD: 13.5)
Tumor size (mm)	29.8 (SD: 19.7)	26.8 (SD: 17.5)
Grade (n)		
2	8	9
3	24	46
Lymphovascular invasion (n)	11	6
Number of involved LNs	11.39 (SD: 6.9)	/
Disease stage (n)		
IA	None	24
IB	6	None
IIA	3	19
IIB	12	8
IIIA	9	2
IIIB	2	3
Neoadjuvant chemotherapy (n)	7	15
Surgery (n)	32	56
Adjuvant chemotherapy (n)	22	38
Ki-67	58.7 (SD: 28.5)	67.7 (SD: 26.4)

Data are mean ± SD or number. LN: lymph node; TNBC: triple negative breast cancer; SD: standard deviation.

alone. Twenty-one (35%) patients were treated with a combination of TC and EC regimens. Five (8.3%) patients, among whom one had already been treated with neoadjuvant chemotherapy, received different forms of chemotherapy. Data for two (3.3%) patients were missing. Disease recurrence or metastasis was present in 10 (11.4%) patients. Disease-related death was recorded in 12 (13.6%) patients.

Multifactorial analysis of clinical prognostic factors

By using multifactorial analysis of variance, we analyzed the effect of specific potential prognostic factors for disease outcomes adjusted for LN status. We found that histological tumor grade ($p < 0.536$, partial $\eta^2 = 0.005$) and any clinical prognostic effect of the proliferation marker Ki-67 ($p < 0.581$, partial $\eta^2 = 0.004$) were not significant for disease-specific death. The correlation between disease-specific death and clinical prognosis was significantly affected by tumor size ($p < 0.008$, partial $\eta^2 = 0.082$) and the LNR ($p < 0.010$, partial $\eta^2 = 0.079$). Further analysis showed that prognostic factors of disease-specific death were a larger tumor size ($U = 264.5$, $p < 0.022$) and a higher LNR ($U = 211.5$, $p < 0.001$). None of the clinical pathological factors, such as Ki-67 ($p < 0.963$), tumor size ($p < 0.149$), histological tumor grade ($p < 0.913$), and LNR ($p < 0.069$), were significant for disease recurrence.

Prognostic factors in 5-year RFS and OS

In a subgroup of patients, who were treated between January 1 2010 and December 31 2013, 5-year analysis of the patients' (n = 50) outcomes was performed. There were no significant differences in the duration of RFS and OS regarding surgical management of the tumor (RFS: $p < 0.552$; OS: $p < 0.903$) and management

of the axilla (RFS: $p < 0.908$; OS: $p < 0.830$).

Using Kaplan–Meier survival curves, we compared the predictive value of LN positivity compared with the LNR ratio on OS and RFS (Figure 1). For RFS, the LN status did not show a significant difference on RFS between LN-negative and LN-positive patients ($p = 0.108$). Categorization of patients into a low-risk group of the LNR < 21% and a high-risk group of the LNR > 21% showed that the LNR was a significant ($p = 0.014$) factor for distinguishing between these groups in patients with RFS. LNs ($p = 0.007$) and the LNR ($p = 0.002$) were significant for predicting OS.

Using Cox multifactorial analysis of clinical characteristics, we analyzed the correlations of 5-year RFS with Ki-67 ($p < 0.567$), tumor size ($p < 0.267$), histological tumor grade ($p < 0.407$), LVI ($p < 0.065$), and LNR ($p < 0.013$). When we adjusted for a low-risk LNR, multifactorial analysis showed that the LNR ratio was the only significant factor for RFS ($p < 0.037$). The LNR was not a significant factor for OS ($p < 0.069$). When we evaluated predictive factors for OS, LVI predicted a worse outcome of patients ($p < 0.013$), but not the overall LNR ($p < 0.069$). There was no significant effect of tumor size ($p < 0.826$), histological tumor grade ($p < 0.326$), or Ki-67 ($p < 0.300$) on OS.

Discussion

This study supports the importance of evaluating the LNR in women with operable

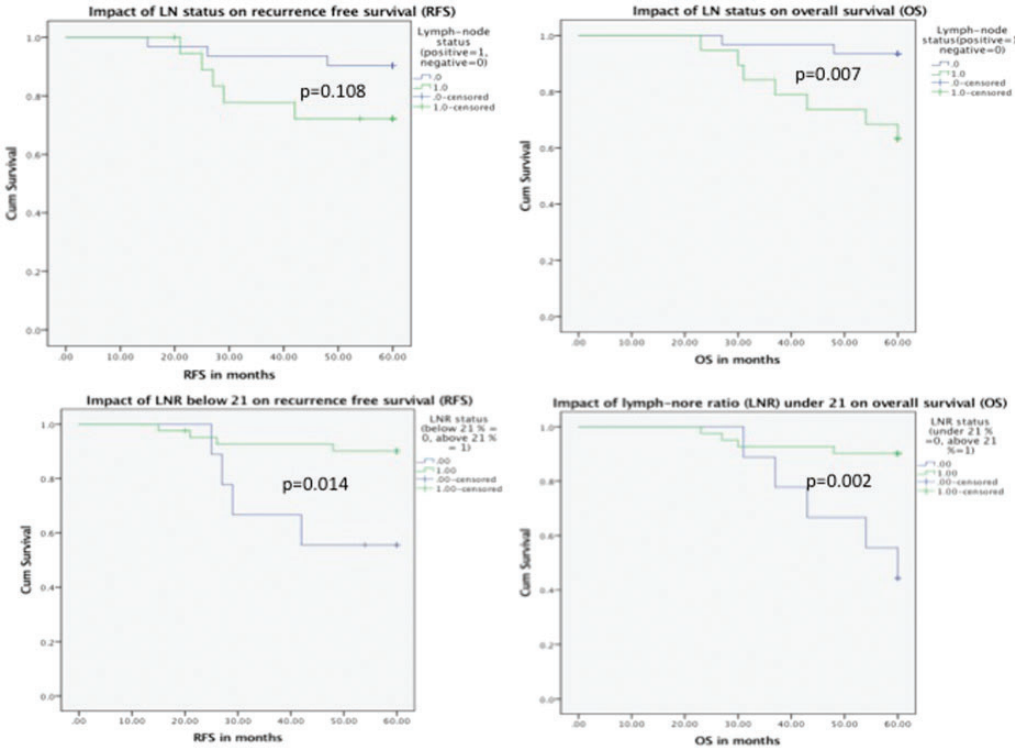

Figure 1. Comparison of overall survival and recurrence-free survival using stratification by lymph nodes or the lymph node ratio status.

TNBC as an early prognostic factor of worse outcome for disease recurrence and disease-specific death. The importance of the LNR status was validated in our survival analysis. Interestingly, the risk factor of tumor size was not significant in survival analysis when adjusted for other clinical characteristics that are proposed to be relevant, such as the LNR, tumor grade, and the proliferation factor Ki-67. In a larger analysis of the Surveillance, Epidemiology and End Results database, Wang et al.[19] also showed that tumor size did not affect survival in patients with TNBC.

There is growing evidence that, especially in TNBC, tumor size cannot be correlated with nodal involvement, which is consistent with our study. A study on the effect of tumors <1 cm showed that, regardless of the small size, TNBC was a risk factor for lower overall survival.[20] Braunstein and colleagues[21] investigated factors that affect local recurrence in early BC treated with breast-conserving therapy. These authors found that a younger age and increasing LN involvement were the main risk factors for local recurrence in non-luminal A subtypes of BC. They also showed that every additional positive LN increased the risk of local recurrence. Furthermore, attempts at understanding the effect of clinical characteristics in a heterogeneous group of patients with different molecular subtypes of BC showed that there was merit in using the LNR in predicting OS and disease-free survival (DFS).[22] This previous study showed that the LNR and LN status were independent prognostic parameters for the entire cohort, but that discrimination in 10-year DFS was better in hormone-positive patients than in patients with TNBC.

Our data are in accordance with the understanding that the association between traditional staging systems, prediction of survival, and therapeutic outcome might not be as accurate in TNBC as in hormone receptor-positive BC.[19] A study that compared the LNR in women with pN1 status after operative treatment of BC showed that only the LNR status was able to discriminate between high- and low-risk women and enable prediction of DFS in multivariate analysis.[23] Therefore, discovery of different clinical characteristics is warranted to better optimize patient prediction models in the future.[19]

Currently, to the best of our knowledge, there is only one nomogram available specifically for TNBC.[24] This nomogram incorporates age at diagnosis, race, tumor size, number of positive LNs, grade, and histological subtype in its analysis.[24] To improve the currently available models, pathological factors must also be considered.

Our study suggested the importance of LVI in OS because it was the only significant factor for predicting OS after multivariate analysis. A few studies have shown that LVI is an independent predictor of a poor outcome.[25–27] LVI is defined as invasion of lymphatic spaces, blood vessels, and/or the peritumoral area.[28] Studies that analyzed the predictive value of LVI in early BC showed that LVI did not affect locoregional recurrence and survival.[29] However, studies that analyzed the effect of LVI on patients with operable BC and positive LNs showed that LVI was an independent prognostic factor.[28] A large cohort study in Italy showed that, when taking into account multiple clinicopathological characteristics in TNBC, LVI might not be a relevant prognostic marker of mortality.[8] Therefore, while LVI was significant in our study, the relevance of the association of LVI with the LNR status should be further examined in larger cohorts. With regard to other factors, the level of Ki-67 was not significantly related to survival in our study. Most women in our study had Ki-67 levels >30% in LN-negative and LN-positive women, regardless of LN status. Therefore, we could not discriminate between high and low risk

based on Ki-67 status. This was the case in some previous studies, which showed that there was a correlation between high Ki-67 expression (>10%, >16%, or even >35%) and poor TNBC outcomes.[30–32] There are relatively conflicting opinions regarding the importance of Ki-67 as a marker in TNBC because the results are inconsistent.[8,30–34] In contrast to the above-mentioned research, other studies also indicated that, as in our study, there was no association between Ki-67 expression and survival outcomes in TNBC.[33,34] This discrepancy between studies could be mainly due to a lack of standardization of this marker between institutions.[35] Conflicting data on Ki-67 in TNBC could also be attributed to different cut-off points defining the positivity of Ki-67.[30–32] However, this possibility did not affect our study because we tested mean values of different levels of Ki-67 against survival data.

Study limitations

This study has several limitations. One of the main limitations is the retrospective nature of the study. Therefore, some of the patients' records had to be excluded from the study because of a lack of clinical information, thus narrowing the patient pool. As a single-institution study, the narrow patient pool provided us with a limited analysis of cases. Regardless of these limitations, our model enables a good starting point for development and exploration of clinical decision-making models for TNBC and better prediction of therapy requirements.

Conclusion

Developing future risk stratification models for TNBC should be based on the knowledge of the LNR in predicting RFS and OS. Furthermore, investigation of robust pathological markers with high standardization should be performed. Our study supports the idea that use of the LNR in a heterogeneous group of patients with TNBC ensures discrimination between high and low risks for early, 5-year RFS. Moreover, the correlation of LVI and the LNR should be further examined in larger cohorts.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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