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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

HİSTOPATOLOJİK PARAMETRELER VE İNTRATÜMÖRAL LENFOSİTLER: ÜÇLÜ NEGATİF MEME KANSERİNDE SAĞKALIM SONUÇLARI İLE KORELASYON - KAPSAMLI RETROSPEKTİF BİR ANALİZ

HISTOPATHOLOGICAL PARAMETERS AND INTRATUMORAL LYMPHOCYTES: CORRELATION WITH SURVIVAL OUTCOMES IN TRIPLE NEGATIVE BREAST CANCER - A COMPREHENSIVE RETROSPECTIVE ANALYSIS

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ÖZET

AMAÇ: Üçlü-negatif meme kanseri (TNBC) agresif tümör davranışı ve sınırlı tedavi seçenekleriyle karakterize bir alt tiptir. Bu çalışmanın amacı TNBC'de yaş, patolojik evre, proliferatif indeks, tümör infiltre eden lenfositlerin (TIL) varlığı ve sağkalım sonuçları arasındaki ilişkiyi araştırmaktır.

GEREÇ VE YÖNTEM: Üçlü negatif meme kanserli 31 hastanın tümör lamları ve blokları patoloji arşivinden alındı ve retrospektif olarak yeniden değerlendirildi. Hasta yaşı, tümörün histopatolojik alt tipi, tümör derecesi, lenf nodu derecesi, Ki-67 proliferasyon indeksi ve sağkalım arasındaki ilişki değerlendirildi. TIL'ler hafif, orta ve şiddetli olarak skorlandı ve sağkalım ile ilişkisi değerlendirildi.

BULGULAR: Yaş ve tümör evresi ile ilgili olarak, anlamlı bir korelasyon bulunmamıştır (sırasıyla p=0,81 ve p=0,89). Bununla birlikte, N evresi analiz edildiğinde, 65 yaş ve üzeri hastaların daha yüksek bir oranının ileri N3 evresi meme kanseri göstermesiyle açık bir ilişki gözlenmiştir (p=0,000013). TIL'ler ile Ki-67 proliferatif indeks arasında anlamlı bir ilişki bulunmuş, yüksek TIL sergileyen vakalar aynı zamanda yüksek proliferatif indeks de göstermiştir (p=0,003). Ayrıca, artmış TIL konsantrasyonu TNBC hastalarında tedaviye olumlu yanıt ve genel sağkalımda iyileşme ile ilişkilendirilmiştir (p=0.001).

SONUÇ: Bu bulgular, TNBC prognozunda yaş, patolojik evre, proliferatif indeks ve TIL varlığının dikkate alınmasının önemini vurgulamaktadır. TIL'lerin rutin histopatolojik incelemede değerlendirilmesi ve özellikle postmenopozal hastalarda patoloji raporlarına dahil edilmesi, gelecekteki çalışmalar için değerli bilgiler sağlayabilir ve tedavi kararlarını yönlendirebilir. TIL'leri hedefleyen immün m odüle edici tedaviler üzerine ek araştırmalar, TNBC hastalarında sonuçları iyileştirmek için umut vaat edebilir.

ANAHTAR KELİMELER: Üçlü negatif meme kanseri, Tümör infiltre eden lenfositler, Evre, Sağkalım.

ABSTRACT

OBJECTIVE: Triple-negative breast cancer (TNBC) is a subtype characterized by aggressive tumor behavior and limited treatment options. This study aimed to investigate the relationship among age, pathological stage, proliferative index, presence of tumor infiltrating lymphocytes (TILs), and survival outcomes in TNBC.

MATERIAL AND METHODS: Tumoral slides and blocks of 31 patients with triple negative breast cancer were retrieved from the pathology archive and retrospectively re-evaluated. The relationship among patient age, histopathological subtype of the tumor, tumor grade, lymph node grade, Ki-67 proliferation index and survival was evaluated. TILs were scored as mild, moderate and severe and the relationship with survival was evaluated.

RESULTS: Regarding age and tumor stage, there was no significant correlation found (p=0,81 and p=0,89 respectively). However, when analyzing the N stage, a clear association was observed, with a higher proportion of patients aged 65 years or older displaying advanced N3 stage breast cancer (p=0.000013). A significant relationship was found between TILs and the Ki-67 proliferative index, with cases exhibiting high TILs also demonstrating a high proliferative index (p=0.003). Furthermore, increased TIL concentration was associated with a positive response to therapy and improved overall survival in TNBC patients (p=0.001).

CONCLUSIONS: These findings emphasize the importance of considering age, pathological stage, proliferative index, and the presence of TILs in TNBC prognosis. Evaluation of TILs in routine histopathologic examination and inclusion in pathology reports, particularly in postmenopausal patients, could provide valuable information for future studies and guide treatment decisions. Additional research on immune-modulating therapies targeting TILs may hold promise for improving outcomes in TNBC patients.

KEYWORDS: Triple negative breast cancer, Tumor infiltrating lymphocytes, Grade, Survival.

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INTRODUCTION

Breast cancer remains one of the most prevalent forms of cancer affecting women, and is ranked as the second primary cause of cancer-related fatalities (1). One particular form of breast cancer, known as triple negative breast cancer (TNBC), is characterized by the absence or low expression of Estrogen receptor (ER), Progesterone receptor (PR), and c-erbB-2 (HER2/neu) (2). TNBC is notorious for its aggressive tendencies, accounting for 15-20% of all cases of invasive breast cancer and is frequently diagnosed in younger patients (3). Unfortunately, traditional endocrine therapy and trastuzumab appear to offer little therapeutic benefit to TNBC patients, thus requiring chemotherapy as the primary approach to systemic medical intervention. Nevertheless, TNBC sufferers often have poor long-term prognosis due to the high rates of both relapse and disease recurrence (4). Recent studies have highlighted the crucial role of tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment and their impact on cancer progression and growth. Prior investigations on breast cancer have revealed that substantial infiltration of TILs may confer a favorable prognosis, although some studies have reported a negative or no correlation with prognosis (5 - 9).

The inclusion of TILs within TNBC has demonstrated a positive correlation with enhanced prognosis and heightened chemotherapeutic responsiveness. Specifically, elevated levels of TILs have been linked to heightened rates of pathologic complete response (pCR), denoting the eradication of all observable cancer manifestations subsequent to treatment (10). Conversely, certain investigations have posited a negative association between a heightened TILs rate and overall survival (8).

Tumor infiltrating lymphocytes possess predictive value concerning the response to neoadjuvant chemotherapy in TNBC. In this study, we conducted an assessment of the interrelationship between TILs and histopathologic parameters, disease stage, and survival among patients diagnosed with TNBC.

MATERIAL AND METHODS

The study encompassed metastatic primary TNBC patients as its subjects. Among the participants, 31 individuals underwent surgical resection, whereby paraffin blocks containing tumor samples, accompanying hematoxylin and eosin (HE) stained slides and immunohistochemistry (IHC) stained slides were retrieved from the archival records in pathology department. It was confirmed that all cases exhibited negative immunohistochemical results for Estrogen receptor, Progesterone receptor, and C-erbB-2.

Tumor size classification, following the guidelines stipulated by T grading, was implemented as T1 for tumors measuring less than 2 cm, T2 for tumors ranging from 2 to 5 cm, and T3 for tumors equal to or exceeding 5 cm (11). Lymph node staging was conducted to classify patients as either N2 or N3 based on the extent of lymph node involvement (11). The investigation encompassed an evaluation of the association between patient age and both tumor stage and nodal stage.

Considering the Ki67 proliferation index immunohistochemically exhibited a minimum value of 15% across all cases, the range of 15-45% was designated as intermediate, while a threshold of ≥46% was considered indicative of high proliferation, drawing upon prior studies (12).

The investigation entailed an assessment of the interplay between histopathologic diagnosis and proliferation rates. The assessment of stromal TILs involved the application of standardized methodologies, as per the guidelines outlined by the international TILs working group, to determine the quantity of stromal TILs. TILs were categorized into three predefined groups: mild (0-10% immune cells within the stromal tissue within the tumor), moderate (11-59%), and severe TIL (≥60%) (10).Subsequently, the collected data was subjected to statistical analysis, utilizing Kaplan-Meier analysis, to explore the association between TIL quantity and overall survival among all patients. The study also aimed to investigate the correlations between TILs and other pertinent histopathologic parameters, including pathologic stage and proliferation index.

Ethical Committee

Compliance with ethical standards Clinical Research Ethics Committee of Canakkale Onsekiz Mart University approved the study (date: 01.02.2023/14).

Statistical Analysis

SPSS program was used for statistical analysis and chi-square test and Kaplan-Meier survival analysis were applied. A significance value (P-value) and 95% Confidence Interval (CI) of the difference was reported.

RESULTS

The study population comprised patients who underwent surgical intervention for breast masses within the timeframe spanning from 2016 to 2022. Specifically, individuals diagnosed with triple negative breast cancer at the Department of Pathology of our institution were selected as subjects for this investigation. From the archival repository of pathological specimens, a total of 31 cases with various histopathological manifestations were identified, including invasive ductal carcinoma, mucinous carcinoma, medullary carcinoma, invasive papillary carcinoma, invasive micropapillary carcinoma, apocrine carcinoma, metaplastic carcinoma, neuroendocrine carcinoma, with corresponding case counts of 11, 6, 4, 3, 3, 2, 1 and 1 respectively. Clinicopathological features of breast cancer patients have been shown in **Table 1**.

Table 1: Clinicopathological features of breast cancer patients

Features	N	%
Age		
<65 years	14	45.1
≥ 65 years	17	54.8
Histological types		
invasive ductal carcinoma	11	35.4
mucinous carcinoma	6	19.3
medullary carcinoma	4	12.9
invasive papillary carcinoma	3	9.6
invasive micropapillary carcinoma	3	9.6
apocrine carcinoma	2	6.4
metaplastic carcinoma	1	3.2
neuroendocrine carcinoma	1	3.2
Tumor stage		
T1	5	16.1
T2	10	32.2
T3	16	51.6
Lymph node stage		
N1	1	3.2
N2	12	38.7
N3	18	58
Tumor infiltrating lymphocytes		
mild	3	9.6
moderate	15	48.3
severe	13	41.9
Ki-67 proliferative index		
minimum	0	0
intermediate	13	41.9
high	18	58.0

The age range of the 31 patients included in our study was 35-88 years with a mean age of 66.4 years and a median age of 66 years (Std 66.4839 ±4.185). Upon comprehensive analysis of all groups, it was observed that 16.1% of patients exhibited stage T1 breast cancer, while 32.2% and 51.6% of patients were classified with stage T2 and T3 breast cancer, respectively. No cases of stage T4 breast cancer were detected within the study cohort.

In the context of investigating the relationship between T2 and T3 stage breast cancer groups, it was noted that patients diagnosed with T2 stage breast cancer displayed a comparatively longer overall survival. However, statistical analysis did not yield a significant difference (p=0.89).

Regarding the examination of the association between age and T staging, T3 stage tumors were observed in 57.1% of patients aged 65 years or older and in 61.5% of patients below the age of 65 years. Nevertheless, no statistically significant correlation was identified between age and T staging (p=0.81).

The analysis based on the N stage revealed that 58% of the entire patient cohort exhibited N3 stage, whereas 38.7% displayed N2 stage and 3.2% displayed N1 stage breast cancer. None of the patients were categorized N0. Further investigation showed that among patients aged 65 years or older, 94.1% were classified as N3 stage, whereas 84.6% of patients below the age of 65 years were identified with N2 stage breast cancer. These findings were highly associated between age and N staging, and the association was found to be statistically significant (p=0.000013).

The Ki-67 proliferative index of the examined cases exhibited a wide range from 15% to 80%, with a mean value of 51 and a median value of 55. However, upon statistical analysis, no significant correlation was found between the Ki-67 proliferative index and clinicopathological parameters (p=0.34).

The assessment of TILs involved a scoring system comprising three levels: mild, moderate, and severe **(Figure 1)**. The observed TILs percentages in the cases ranged from 9% to 90%, with a mean TILs value of 49.6% across all cases. Specifically, 3 cases exhibited low TILs, 15

cases displayed moderate TILs, and 13 cases exhibited severe TILs. A statistically significant correlation was observed between tumor stage and the number of TILs, indicating that T3 stage tumors had a higher rate of TILs compared to other tumor stages (p=0.002). Utilizing the chi-square test, a statistically significant relationship was identified between TILs and the Ki67 proliferative index (p=0.003). Additionally, it was observed that cases with severe TILs also demonstrated a high Ki67 proliferative index.

Throughout the 5-years follow-up period, when assessing the relationship between TILs and overall survival (OS), it was observed that an increase in TILs was significantly associated with a longer OS in patients with TNBC (p=0.001). In the context of investigating the relationship between T2 and T3 stage breast cancer groups, it was noted that patients diagnosed with T2 stage breast cancer displayed a comparatively longer overall survival. However, statistical analysis did not yield a significant difference (p=0.89) **(Figure 2)**.

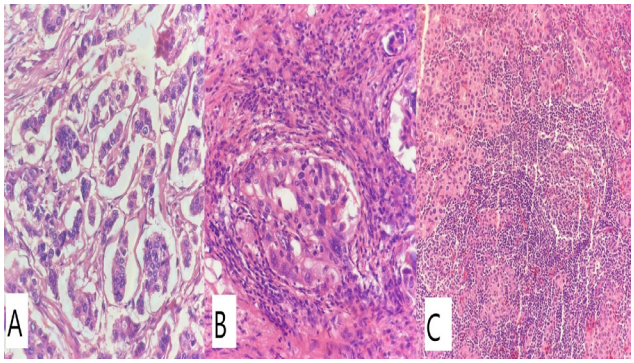

Figure 1: Hematoxylin and eosin stained images of cases with mild (A), moderate (B) and severe (C) intratumoural lymphocyte counts in microscopic examinations of tumour specimens (A, B, C: HEx400).

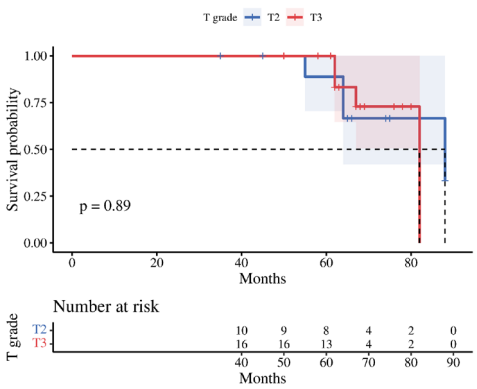

Figure 2: Association between tumor grade and overall survival. In the context of investigating the relationship between T2 and T3 stage breast cancer groups, it was noted that patients diagnosed with T2 stage breast cancer displayed a comparatively longer overall survival.

DISCUSSION

This study is characterized as a retrospective cohort investigation conducted utilizing a database comprising 31 women who were diagnosed with TNBC at Canakkale Onsekiz Mart University Hospital within the period spanning from January 2016 to December 2022. Prior studies have reported that TNBCs predominantly affect young patients during the premenopausal period. However, contrary to these findings, our study observed a mean age of 66 years, indicating that the patients included in our cohort were in the postmenopausal period (13).

Previous studies have indicated that T3 stage breast cancer is more commonly diagnosed in postmenopausal patients aged 65 years or older when compared to younger and premenopausal patients (13). However, in our study, we found that the mean age of patients with both T3 and T2 stage breast cancer was 65 years, and no significant difference in terms of age relation was observed between these two stages.

Upon analysis of lymph node involvement, our findings revealed that patients diagnosed with N3 stage breast cancer had the highest rate (58%) at the time of diagnosis, and this rate exhibited a gradual increase beyond the age of 65 years. In line with our study, Botteri et al. also reported a similar trend, where lymph node stage increased among individuals aged 65 years and above (14). Moreover, another study conducted by Wildiers et al. observed a high lymph node stage at the time of diagnosis in patients aged 70 years and older who were diagnosed with breast cancer (15).

In a comprehensive investigation conducted by Liu et al., the role of TILs was examined in a large cohort consisting of breast cancers representing all four major intrinsic biological subtypes. The study revealed significant correlations between the presence of TILs and various factors, including estrogen receptor negativity and the core basal intrinsic subtype. Moreover, the presence of TILs was found to be significantly associated with high tumor grade, as demonstrated by the findings of the study (16).

Triple-negative breast cancer is characterized by the presence of large anaplastic cells and is associated with a poor prognosis. Several stu-

dies have underscored the prognostic significance of TILs in high-grade breast cancers (17, 18). For instance, Kurozumi et al. conducted an investigation involving 294 cases, wherein they explored the relationship between TILs and prognosis. Their findings revealed that severe stromal TIL expression served as a favorable prognostic marker specifically in Estrogen receptor-cancers (19). Similarly, Ibrahim et al., through a meta-analysis encompassing data from 2,987 patients, demonstrated a significant association between TILs and a favorable outcome in Estrogen receptor -negative breast tumors (20).

In accordance with our study, Jamiyan et al. also discovered a comparable relationship between survival outcomes, thus supporting our findings (9). Collectively, our results indicate that the prognosis of TNBC is, to some extent, influenced by the presence of immune cell types closely associated with the tumor.

Our findings demonstrate that higher concentrations of TILs predicted a positive response to neoadjuvant chemotherapy across all evaluated molecular subtypes. Moreover, in the context of TNBC, increased TILs concentration was associated with a survival benefit. These results provide support for the hypothesis that breast cancer exhibits immunogenic characteristics, thereby highlighting the potential of immune modulating therapies as viable treatment approaches for this disease.

In conclusion; in breast cancer, various factors including tumor size, proliferative score, histological type, molecular subtype and lymph node metastasis have been established as prognostic indicators for patient survival. Additionally, recent evidence highlights the significance of TILs and their scoring in directly influencing survival outcomes specifically in TNBC. Our study revealed an association between increasing tumor and lymph node stages with advancing age. Furthermore, higher TIL rates within tumors were correlated with prolonged OS. Given these findings, it is recommended that routine histopathologic examinations and pathology reports include the evaluation and documentation of TIL scores, particularly in postmenopausal patients diagnosed with TNBC. This approach would contribute valuable insights for future research and aid in the identification of potential treatment options.

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