

HISTOPATHOLOGICAL CRITERIA THAT AFFECT AXILLARY LYMPH NODE INVOLVEMENT IN BREAST CANCER PATIENTS

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ABSTRACT

Objectives: The purpose was to assess the association between histopathologic findings and the increased risk of lymph node involvement in patients with invasive breast carcinoma among Jordanian population.

Methods: This retrospective study included 205 female patients, aged 30 - 84 years, diagnosed with invasive breast carcinoma (IBC) at Royal Medical Services (RMS)- King Hussein Medical Center and Queen Alia Military Hospital between 2015 and 2021. The patients had undergone either modified radical mastectomy or breast-conserving surgery alongside axillary dissection. In addition, the results of postoperative histopathologic findings were collected and analyzed.

Results: The mean age of the patients was 55 years (range 30–84). The mean number of dissected axillary lymph nodes was 15, and the range (10-38).

Of the 205 patients, 127 (62%) had positive axillary lymph node involvement. Of the 75 (37%) patients with a T1 tumor (≤ 2 cm), 64 (85%) had negative lymph node involvement. Of the 64 (31%) patients with a grade 1 tumor, 60 (93%) were negative for lymph node involvement. In tumors with lymphovascular invasion (LVI) and perineural invasion (PNI), 91 % had nodal metastasis. There was local nodal metastasis in 61%, 60%, and 72% of tumors positive for estrogen receptor, progesterone receptor, and HER2/neu, respectively.

Larger tumor size ($p < 0.001$), high histological grade ($p < 0.001$), HER-2/neu overexpression ($p = 0.040$), LVI ($p < 0.001$), and PNI ($p < 0.001$) were associated with higher risk for axillary lymph node involvement. However, there was no correlation between LN involvement and Hormonal receptor status ($p = 0.523$).

Conclusion: There is a strong correlation between axillary LN involvement and the size of the tumor, histological grade, HER2/neu amplification, LVI, and PNI. On the other hand, hormonal status does not have a significant correlation with axillary lymph node status.

Keywords: Breast cancer, histopathology, axillary lymph nodes

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INTRODUCTION

Breast cancer is a leading health concern: it is the most common malignancy among women worldwide. In 2020, there were around 2.26 million new breast cancer occurrences and 684,996 deaths⁽¹⁾. In Jordan, breast cancer is the most common cancer among females; it accounted for 38.4% of new cases in 2017 and is the leading cause of female cancer death, according to the Jordan Cancer Registry.⁽²⁾

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Lymph node (LN) involvement has a significant role in breast cancer tumors' staging, treatment, and prognosis. The staging of localized breast cancer depends on the tumor size, tumor extension, and LN involvement (number and station), according to the American Joint Committee on Cancer (AJCC) TNM staging. ⁽³⁾ Metastatic regional lymph nodes (LNs) are a prognostic factor and a strong predictor of distant metastasis, disease-free survival, and overall survival. ⁽⁴⁻⁶⁾ The characteristics of the primary breast cancer closely associated with axillary LN metastasis are tumor size, tumor grade, LVI, and hormonal status. ⁽⁷⁻⁹⁾

In this study, we have analyzed the correlation of histopathological factors (tumor size, grade, lymphovascular invasion [LVI], perineural invasion [PNI], hormonal receptors, and human epidermal growth factor 2 [HER2/neu] status) and LN involvement for patients with breast cancer at the Jordanian Royal Medical Services (RMS).

METHODS

We conducted a retrospective analysis in the King Hussein Medical Center and Queen Alia Military Hospital. The local ethics committee of the RMS directorate of the Jordanian army approved this study. We included 205 female patients with breast cancer who had been treated between 2015 and 2021. All patients had been diagnosed with invasive breast carcinoma and had undergone either modified radical mastectomy (removal of breast to the level of pectoralis minor muscle, ALND level I-II, and pectoralis major is spared) or breast-conserving surgery (lumpectomy) alongside axillary dissection (AD). The decision of surgical procedure depended on the clinical staging and patient preference. All histopathologic studies were performed on sections prepared from formalin-fixed, paraffin-embedded tissue. Histopathologic assessment by a histopathologist specialized in breast pathology included microscopic examination of hematoxylin and eosin-stained slides. The Elston modification of the Scharf–Bloom–Richardson classification was used for grading, with a resultant grade I–III classification. Immunohistochemical stains for ER, PR, and HER2 were also assessed. HER2/neu assessment by immunohistochemistry was appropriate for patients with tumors that scored 3+ (considered positive). Fluorescence *in situ* hybridization (FISH) was recommended for patients with 2+ immunohistochemistry scores (considered equivocal) to assess HER2/neu amplification more accurately and provide better prognostic information. The maximum tumor diameter was measured in millimeters to calculate the tumor size. We used the *AJCC Cancer Staging Manual* (eighth edition).

The exclusion criteria include pathology of *in situ* tumors without invasive components, recurrent tumors, previous malignancy, and patients who received neoadjuvant chemotherapy.

We collected the following data by reviewing the medical records: tumor size, tumor grade, number of dissected axillary LNs, estrogen receptor (ER), progesterone receptor (PR), HER2/neu, LVI, and PNI. Next, we divided patients into two groups: Group I had axillary LN metastasis, and Group II had no axillary LN metastasis. Finally, we analyzed the relationship between the histopathologic findings above and the axillary LN status.

Statistical analysis

We have presented the patient and the tumor characteristics as frequency, mean, and standard deviation or median, as appropriate. We determined the significance of correlations between patient and tumor characteristics and positive axillary LNs using the chi-square of independence test in SPSS Statistics Version 25 (IBM Corp., Armonk, NY, USA). Alpha set at <0.05 deemed statistically significant. We have reported the work in this article according to the STROCSS criteria (Strengthening the Reporting of Cohort Studies in Surgery).

RESULTS

The mean age of the patients was 55 years (range 30–84). The mean number of dissected axillary LNs was 15 and the range was 10-38 lymph nodes . Of the 205 patients, 127 (62%) were positive for axillary LN invasion. When examining hormonal receptor status and HER2/neu overexpression, 21 (10%) patients were triple negative.

There were 75 patients (37%) with a T1 tumor (≤ 2 cm) of whom 64 (85%) had negative nodes. This proportion fell sharply to 14% in those with T2 tumors (11 out of 70 patients) and to 8 % of those with a T3 tumors (3 of 38 patients), none of the 11 patients with a T4 tumors had negative lymph nodes (Table 1) .

Similar pattern was seen with histological grading (Table 2): 60 of 64 patients (94%) with grade I tumors had no LN involvement compared with 70% of those with grade II tumors and only 3% of those with grade III tumors.

Furthermore, there was nodal metastasis in 91.3% of tumors with LVI, indicating that the positive lymph node involvements were significantly associated with lymphovascular invasion Similarly, 91 % of tumors with PNI were significantly prevalent among patients with positive lymph nodes

Last but not least, 60 % of tumors positive for ER, PR were not significantly associated with nodal metastasis , and 43 (73 %) of those positive for HER2/neu, have nodal metastasis with statistically significant findings (Table 3) .

Table 1: Lymph node involvement according to T staging

Tumor size	Number of patients	Patients with positive lymph nodes, n (%)	Patients with negative lymph nodes, n (%)	X ²	p-value
T1	75	11 (14.7%)	64 (85.3%)	113.127	<0.001
T2	81	70 (86.4%)	11 (13.6%)		
T3	38	35 (92.1%)	3 (7.9%)		
T4	11	11 (100%)	0 (0%)		

T, tumor size and extension according to the *American Joint Committee on Cancer Staging Manual* (eighth edition)

Table 2: Lymph node involvement in grade I, II, and III tumors

Tumor grade	Number of patients	Patients with positive lymph nodes, n (%)	Patients with negative lymph nodes, n (%)	X ²	p-value
I	64	4 (6.3%)	60 (93.7%)	154.86	<0.001
II	20	6 (30%)	14 (70%)		
III	121	117 (96.7%)	4 (3.3%)		

Table 3: Lymph node involvement according to hormonal receptor status

Hormonal receptor status	Number of patients	Patients with positive lymph nodes, n (%)	Patients with negative lymph nodes, n (%)	X ²	p-value
ER positive	168	102 (60.7%)	66 (39.3%)	2.243	0.523
ER negative	37	25 (67.6%)	12 (32.4%)		
PR positive	167	100 (59.9%)	67 (40.1%)		
PR negative	38	27 (71.1%)	11 (28.9%)		
HER2/ neu positive	59	43 (72.8%)	16 (27.2%)	4.19	0.040
HER2/ neu negative	146	84 (57.5%)	62 (42.5%)		

ER, estrogen receptor; PR, progesterone receptor
human epidermal growth factor 2 (HER2/neu)

Discussion

Our findings on the close relationship between primary tumour size and axillary node involvement are broadly in line with other studies, but the differences are striking. Thus only 15% of our patients with T1 tumours had nodal metastases as opposed to 86% of those with T2 tumours. Similar results were reported in one study¹⁰, but the difference was less marked in another (31% versus 70%)¹¹.

Sopic et al.⁽¹²⁾ showed the relation of the increase in LN involvement with each 10 mm increase in tumor size. They categorized tumors into six size categories, and the prevalence of LN metastases increased stepwise as the tumor size increased from category 1 (1–10 mm; 10.8%) to category 6 (51–60 mm; 61.8%).

Another important histopathological factor is grading. The histological grade considers the cytological differentiation and the growth pattern of the carcinoma. The extent of tubule formation, nuclear pleomorphism, and the mitotic index are assessed the tumor grade is highly correlated with LN involvement in breast cancer. Kollias et al.⁽¹³⁾ retrospectively analyzed the medical records of 2684 patients with breast cancer. They found that 29% of the patients with grade III cancers had positive LN, while the proportion of positive LN with grade I and II was 11 and 18%, respectively. Bruno et al.⁽¹⁴⁾ revealed that the high pathological grade indicated high axillary nodal involvement even in small-size breast tumors. The risk of axillary LN involvement in grade III tumors doubled compared with grade I tumors (38% versus 18%).⁽¹⁴⁾ In our study, grade III tumors were associated with a very high incidence of LN involvement compared with grade I tumors (97% vs. 6%)

LVI is defined as tumor cells within a definite endothelium-lined space. LVI has been described as the strongest independent predictor of nodal involvement.⁽¹⁵⁾ Cornwell et al stated that LVI was noted to be present in 155 patients with positive LN (38.8%) and absent in 147 (36.8%), LVI was found with more positive nonsentinel nodes, and a higher lymph node ratio⁽¹⁶⁾. Our data are consistent with other studies showing a significant relationship between LVI and LN metastasis in patients with breast cancer.

PNI is another significant histopathological finding that correlates with the prognosis of a tumor.⁽¹⁷⁾ In 1985, Batsakis⁽¹⁸⁾ defined PNI for the first time: tumor cells' invasion, surrounding, or passing through of a nerve. Cetintaş et al, concluded in a univariate analysis that Perineural invasion affected both nodal positivity, and >4 nodal involvement.⁽¹⁹⁾ After analyzing our data, PNI-positive tumors had a more than double LN involvement compared with PNI-negative tumors (91% vs. 39%).

The presence or absence of ER and PR in the nucleus of tumor cells is critical in managing patients with breast cancer. Patients with receptor-positive primary tumors have a more favorable course than receptor-negative ones. There are conflicting data regarding the effect of hormonal status on LN involvement. Gann et al.⁽²⁰⁾ evaluated 18,025 breast carcinoma cases and suggested that tumors lacking ER have a significantly lower risk of LN metastasis than tumors with ER. On the other hand, Barry et al.⁽²¹⁾ showed that triple-negative patients (ER, PR, and HER2/neu negative) are more likely to have regional LN metastasis and distant metastasis. We found a non-significant increase in the LN involvement in ER- or PR-negative tumors compared with the ER- or PR-positive

tumors ($p=0.52$). However, we could not draw conclusions from the triple-negative patients due to the small sample (10.2%).

HER2/neu is a proto-oncogene that encodes a trans-membrane receptor with constitutive tyrosine kinase activity involved in cellular proliferation, differentiation, migration, and apoptosis. It is overexpressed in many tumors, including gastric, ovarian, and breast cancer. One study showed HER2/neu amplification in 15–30% of breast cancer cases. ⁽²²⁾Urgas et al. ⁽²³⁾ showed that HER2/neu overexpression significantly increases the risk of LN involvement compared with HER2/neu-negative tumors. Our data showed a significant increase in LN involvement in HER2/neu positive compared with HER2/neu negative breast cancer .

In conclusion, after evaluating the number of involved LNs relative to other histological and immunohistochemical findings, we found that the tumor size, grade, HER2/neu amplification, LVI, and PNI are associated with LN involvement. On the other hand, hormonal status is not significantly associated with axillary LN status

more in-depth evaluation is needed for these factors. As well as relation to other clinicopathological factors.

The main limitations of our investigation is that it was conducted at a single center , strength of the paper lies in the pathological detail.

Conflict of Interest Statement:

All Authors declare that they have no conflict of interest.

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