The association between lymphovascular invasion and other prognostic indicators in operable breast cancer: Experience at King Hussein Medical Center

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ABSTRACT

Objective: To evaluate the association between lymphovascular invasion (LVI) and different clinicopathologic features of invasive breast cancer.

Methods: Clinicopathologic and demographic data from a cohort of 298 patients, who were referred to surgery clinics at King Hussein Medical Center (KHMC) between 2007 and 2014, were retrieved and analyzed.

Results: The average age of the cohort under investigation was 51.2 years with most of the patients having invasive breast carcinoma. Lymphovascular invasion was detected in 53% of the patients. Patients with lymphovascular invasion were more likely to have larger tumors (4.2 vs. 3.3cm p=0.02). Additionally, they were more likely to have progesterone negative (p=0.032), and Her2 positive tumors (p=0.003). Patients with negative Lymphovascular were more likely to have negative perineural invasion (p=0.0001).

Conclusion: Lymphovascular invasion was associated with larger tumors, Progesterone negativity and Her-2 positivity. Lymphovascular invasion should be incorporated in tumor classification and treatment modality selection.

Key words: Hormone receptor and Her-2 status, invasive breast cancer, King Hussein Medical Center, lymphovascular invasion, prognostic marker.

JRMS Dec 2016; 23(4):11-16/DOI:10.12816/0032195

Introduction

Lymphovascular invasion (LVI) is an essential step for breast cancer progression and metastasis.[1] LVI has been suggested as a prognostic marker for long term survival in lymph node negative tumors.[1-4] Rakha, *et al*. studied the role of LVI as a prognostic marker for breast cancer,[3] concluding that LVI predicts worse outcome and higher metastatic potential.[3] This finding was consistent among different subgroup analyses based on established clinicopathologic features of breast cancer.[3] In a follow-up multi-institutional study, Rakha *et al*. reported that LVI is an important outcome predictor among metaplastic breast cancer patients.[5] However, Ejlertsen, *et al*. reported a low potential of LVI as a high-risk predictor.[6] In their study, 16172 patients were investigated, and LVI was found to be associated with overall survival only in the high-risk group .[6] Additionally, most of the data from the literature on the prognostic value of LVI were derived from lymph node negative patients.[1-4] Furthermore, most of the studies focused on the role of LVI in predicting disease survival and metastatic potential.[3] The association

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Manuscript received March 21,2016. Accepted July 14,2016.

between LVI and different clinicopathologic features has been rarely investigated.

The aim of this study is to evaluate the association between LVI and different clinicopathologic features of invasive breast cancer.

Methods

Two hundred and ninety eight patients with a diagnosis of invasive breast cancer, who had undergone surgery in King Hussein Medical Center (KHMC) between 2007 and 2014, were included in this study. The study was approved by the local ethics committee of the Royal Medical Services. All patients had full clinicopathologic evaluation at the pathology department of KHMC. Pathologic studies were conducted on paraffin embedded sections derived from tumor samples obtained during surgical management of the patients. The criteria for lymphovascular invasion were defined as the presence of tumor emboli in peritumoral lymphovascular spaces, identified microscopically with or without the aid of endothelial markers.[1,2] The status of estrogen receptor (ER) and progesterone receptor (PR) were determined by immunohistochemistry, positivity defined as the presence of staining of 1% or more of tumor cells with strong intensity. Only cases with score 3 on immunohistochemistry were included in our definition of Her2 positive cases and equivocal cases were excluded. A rabbit monoclonal primary antibody clones (Ventana) were used to assess hormonal and Her-2 status. Data were extracted from the patients' records and pathology reports, and slides were re-assessed by one of the authors (a senior pathologist). In some cases, further immunostaining were performed as needed to confirm the presence of LVI or hormonal/Her-2 status. The pathologic assessment and the ER, PR, and Her-2 status were interpreted according to the recommendations by the College of American Pathologists (CAP).[7,8]

Statistical analysis:

All statistical analyses were performed using statistical package for social sciences (SPSS) version 22.00 (SPSS Inc., Chicago, IL). Chi square test was used to assess significance between LVI and other clinicopathologic parameters evaluated on nominal and ordinal scale. Differences in age and size between groups with patients with present and absent lymphovascular invasion were compared using student t-test. A *p* value of less than 0.05 was considered significant.

Results

The average age of the cohort under investigation was 51.2 years (51.2±12.97). One hundred and eighty two patients (61.1%) had invasive ductal carcinoma, whereas 36 patients (12.1%) had invasive lobular carcinoma. The rest of the patients (26.8%) had other types. The average size of excised tumors was 3.86 cm (3.86±2.2). The majority of patients were estrogen and progesterone (PR) positive (72.1 and 68.1% respectively). In contrast, 78.2% of patients were Her2 negative. Clinicopathologic characteristics are outlined in Table I.

Patients with positive LVI had significantly larger tumor size (4.2 vs. 3.4 cm, p=0.001; Figure 1A), and they also had higher number of involved lymph nodes (5.6 vs. 2.7, p<0.000; Figure 1B). However, there was no significant change in the mean age of patients with present or absent lymphovascular invasion (50.9 vs. 49.4, p=0.771; Figure 1C).

About half of the cohort had intermediate grade tumors (137 patients) and 41.6% (116 patients) had high grade tumors. Of note, patients with higher grade tumors had higher likelihood of LVI (Figure 1D). Patients with low grade tumor were less likely to have LVI (30.4% vs. 69.6%). Patients with intermediate grade tumors had similar likelihood of having present or absent LVI (48.9% vs. 51.1% respectively). Patients with high grade were more likely to have LVI (63.8% vs. 36.2%; χ^2=11.5, p=0.009).

Hormone receptor status is an important determinant in the management of breast cancer patients. The presence of LVI was similar among patients with both estrogen receptor (ER) positive and negative tumors (Figure 2A; χ2=1.672, p=0.196). In contrast to ER status, patients with progesterone negative tumors were more likely to have LVI (Figure 2B; 62.1% vs. 37.9%; χ2=4.621, p=0.032). Interestingly, patients with

progesterone positive tumors has similar incidence of LVI (104 negative vs. 99 positive).

Patients with Her2 positive tumors were more likely to have LVI (Figure 2C; 69.2% vs. 30.8%; $\chi 2=8.77$, p=0.003). However, patients with Her2 negative tumors had similar incidence of LVI (120 negative vs. 113 positive).

Lymphovascular invasion and perineural invasion are important pathologic features to report in breast cancer. About 40% of the patients in our study had negative LVI and no perineural invasion. Patients with negative LVI were more likely to have negative perineural invasion (Figure 2D; 85.7% vs. 65.8%; $\chi 2=17.9$, p=0.0001).

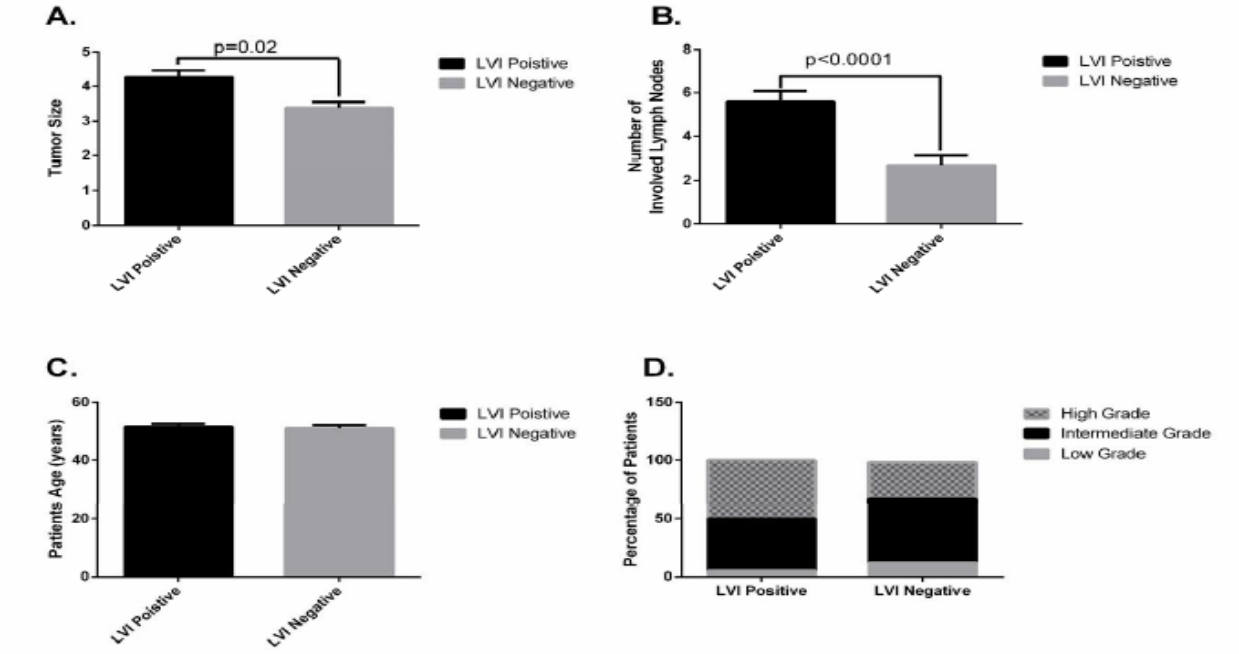

Fig. 1: Lymphovascular Invasion by tumor characteristics. (A) tumor size; (B) lymph nodes; (C) mean age; (D) tumor grade. Data presented as mean±SEM or percentage of involved patients.

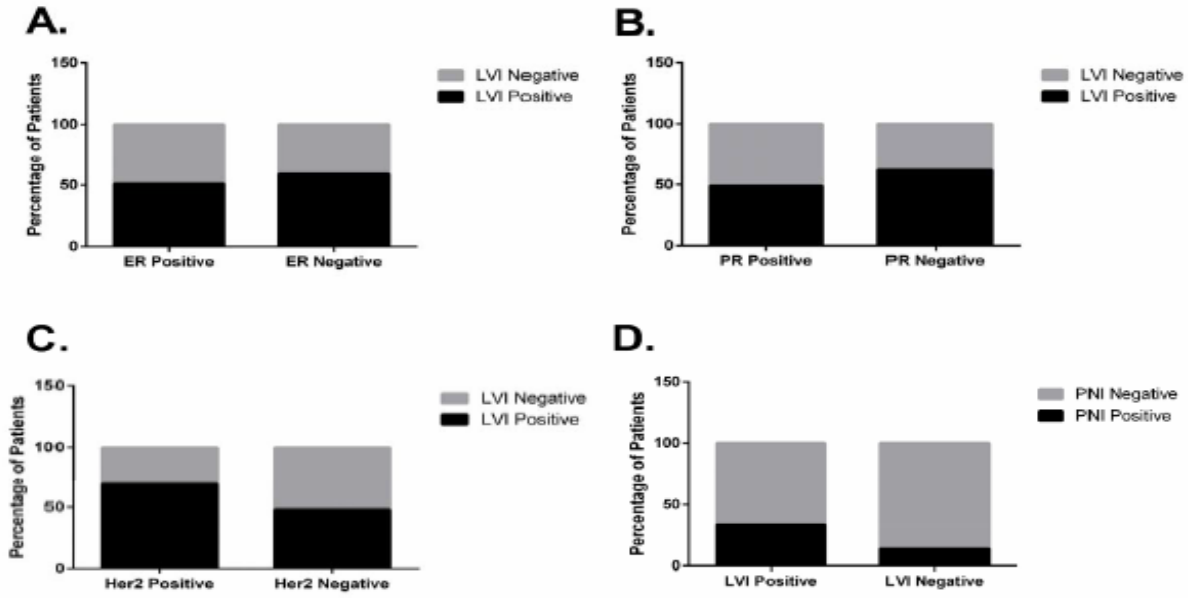

Fig. 2: Lymphovascular invasion by hormonal status of the tumor and perineural invasion. (A) ER status; (B) PR status; (C) Her2 status; (D) perineural invasion.

Table I: Population Characteristics

Characteristic		Number (%)
Age (years)	<45	99 (34.49)
	45-65	163 (47.39)
	>65	25 (18.12)
Histologic type	Ductal	182 (61.1)
	Lobular	36 (12.1)
	Others	80 (26.8)
Lymphovascular invasion	Yes	158 (53)
	No	140 (47)
Perineural invasion	Yes	73 (24.5)
	No	224 (75.5)
Histologic grade	High	116 (41.6)
	Intermediate	137 (49.1)
	Low	23 (8.2)
Number of involved lymph nodes	0	48 (28.2)
	1-3	107 (35.9)
	>4	143 (64.9)
Estrogen Receptor (ER)	Yes	215 (72.1)
	No	83 (27.9)
Progesterone Receptor (PR)	Yes	203 (68.1)
	No	95 (31.9)
Her2	Yes	65 (21.8)
	No	233 (78.2)

Discussion

Our data demonstrate a possible role of lymphovascular invasion (LVI) as a prognostic marker in locally invasive breast cancer patients. LVI was associated with larger tumors and more lymph node involvement. Additionally, it was associated with both progesterone negative and Her2 positive status. The incidence of LVI was not affected by ER status.

The role of LVI as a prognostic marker has been well established in patients with lymph node negative breast cancer. [2-4] Additionally, its importance across different subtypes of breast cancer has been well established.[3] Despite the well-established role as a prognostic factor, the association of LVI with different clinicopathological breast cancer features has been rarely addressed. In this study, an attempt to characterize the association between different breast cancer features and LVI was made. This characterization will provide insight on the interaction between LVI and other clinicopathological characteristics of breast cancer. It will also provide better understanding of the biology and molecular control of LVI in breast cancer.

Our data demonstrate an association between LVI and the extent of lymph node involvement. Patients with LVI were more likely to have lymph node involvement and higher number of involved lymph nodes. These findings are consistent with previous reports on the association between LVI and lymph node involvement.[2-4,9] Ugras *et al*,.reported low incidence of lymph node involvement in patients with negative LVI [10]. Additionally, Rakha, *et al.*, reported that about 80% of patients with lymph node negative breast cancer had negative LVI.[3] Similarly, our data on the association between tumor size and grade are in complete conformity with these reports.

Hormone receptor and Her2 status are well established prognostic factors in breast cancer patients , [1,7-11] and they have an essential role in selecting treatment modality.[11] The prognostic value of LVI in patients with different hormone receptor and Her2 status has been well established.[3] Interestingly, the association between LVI and these markers has not been investigated thoroughly. Rakha *et al.* reported that LVI is an independent prognostic factor in Her2 negative tumors. However, the occurrence of LVI was similar in patients with Her2 positive and negative

tumors. In contrast, our data indicates a higher occurrence of LVI in Her2 positive patients. Marinho *et al.* reported a negative association between ER, PR status and LVI.[12] Similarly in our study, patients with PR negative tumors were more likely to have LVI. On the other hand, LVI did not differ between patients with ER positive tumors and ER negative tumors. These findings indicated a critical role of PR in controlling LVI and supports previous reports on the negative association between ER and LVI. Additionally, they provide an insight on the possible mechanisms of LVI. However, other variables (such as the antibody clones and cold ischemia time) also need to be investigated.

Perineural invasion is considered a sign of aggressive behavior potential in most tumor systems.[13] However, its significance in invasive breast carcinoma is not yet well established and is currently only reported for completeness of pathologic reports.[14] Interestingly, a more recent publication concluded that perineural invasion were identified as parameters negatively associated with patient survival, based on both univariate and multivariate analyses. [15] In our study, patients with negative LVI were more likely to have negative perineural invasion (p=0.0001). Previous studies[14] showed that perineural invasion is seen more in patients with higher tumor stage, higher tumor grade and LVI.

Conclusions

Lymphovascular invasion varies by tumor size and number of lymph nodes involved but not by patient age. The incidence of LVI was related to tumor grade and is differentially affected by hormone receptor status: it appears to be more likely in patients with progesterone negative and in patients who are Her2 positive. Patients with negative LVI were more likely to have negative perineural invasion. A study of LVI in a multivariate analysis in relation to clinical outcome is needed to assess effect of LVI status on prognosis.

References

1. **Aleskandarany MA, Sonbul SN, Mukherjee A, Rakha EA.** Molecular Mechanisms Underlying Lymphovascular Invasion in Invasive Breast Cancer. *Pathobiology* 2015;82(3-4):113-23.
2. **Truong PT, Yong CM, Abnousi F, Lee J, Kader HA, Hayashi A, Olivotto IA.** ymphovascular invasion is associated with reduced locoregional control and survival in women with node-negative breast cancer treated with mastectomy and systemic therapy. *J Am Coll Surg* 2005;200(6):912-21.
3. **Rakha EA, Martin S, Lee AH, Morgan D, Pharoah PD, Hodi Z, *et al.*** The prognostic significance of lymphovascular invasion in invasive breast carcinoma. *Cancer* 2012;118(15):3670-80.
4. **de Oliveira Filho HR, Dória MT, Piato JR, Soares Junior JM, Filassi JR, Baracat EC, *et al.*** Criteria for prediction of metastatic axillary lymph nodes in early stage breast cancer. *Rev Bras Ginecol Obstet* 2015;37(7):308-13.
5. **Rakha EA, Tan PH, Varga Z, Tse GM, *et al.*** Prognostic factors in metaplastic carcinoma of the breast: a multi-institutional study. *Br J Cancer* 2015;112(2):283-9.
6. **Ejlertsen B, Jensen MB, Rank F, Rasmussen BB, Christiansen P, Kroman N, *et al.* Danish Breast Cancer Cooperative Group.** Population-based study of peritumoral lymphovascular invasion and outcome among patients with operable breast cancer. *J Natl Cancer Inst* 2009;101(10):729-35.
7. **Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, *et al.* American Society of Clinical Oncology; College of American Pathologists.** American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). *Arch Pathol Lab Med* 2010;134(7):e48-72.
8. **Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, *et al.* American Society of Clinical Oncology; College of American Pathologists.** Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. *Arch Pathol Lab Med* 2014 Feb;138(2):241-56.
9. **Choi SH, Barsky SH, Chang HR.** Clinicopathologic analysis of sentinel lymph node mapping in early breast cancer. *Breast J* 2003;9(3):153-62.
10. **Ugras S, Stempel M, Patil S, Morrow M.** Estrogen receptor, progesterone receptor,

and HER2 status predict lymphovascular invasion and lymph node involvement. *Ann Surg Oncol* 2014; 21(12):3780-6.

11. **Abdel-Fatah TM, Ball G, Lee AH, *et al.*** Nottingham Clinico-Pathological Response Index (NPRI) after neoadjuvant chemotherapy (Neo-ACT) accurately predicts clinical outcome in locally advanced breast cancer. *Clin Cancer Res* 2015;21(5):1052-62.
12. **Marinho VF, Metze K, Sanches FS, Rocha GF, Gobbi H.** Lymph vascular invasion in invasive mammary carcinomas identified by the endothelial lymphatic marker D2-40 is associated with other indicators of poor prognosis. *BMC Cancer* 2008;8:64.
13. **Liebig C, Ayala G, Wilks JA, Berger DH, Albo D.** Perineural invasion in cancer: a review of the literature. *Cancer* 2009;115(15):3379-91.
14. **Karak SG, Quatrano N, Buckley J, Ricci A Jr.** Prevalence and significance of perineural invasion in invasive breast carcinoma. *Conn Med* 2010; 74(1):17-21.
15. **Deger A, Ozyigit F, Arik O, Ekici F, Cinkaya A, Tayfur M, Deger H.** Association between well-known histopathological criteria and overall survival in invasive ductal carcinoma. *Int J Clin Exp Pathol* 2015;8(9):9772-81.