Anatomical site and size of sentinel lymph node metastasis predicted additional axillary tumor burden and breast cancer survival

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Introduction: Intraoperative sentinel lymph node (SLN) biopsy is the current standard of care in the assessment of axillary lymph node (ALN) status in breast cancer patients with early stage (T1-2) disease and clinically nonpalpable lymph node. Patients with negative SLN (SLN-) can be exempted from axillary lymph node dissection (ALND), a procedure that is associated with significant complications such as wound infection, seroma, lymphedema, and restricted shoulder mobility. To better predict the benefits of ALND, different pathologic determinants associated with non-SLN tumor burden were investigated. Results from early studies showed that the presence of even ITC and MiM in SLN with no ALND conferred a worse survival, but with adjuvant therapy, this can be improved. In addition, presence of lymphovascular invasion (LVI), perineural invasion, multifocality of the primary tumor, HER2 status and Ki-67 index, extranodal extension, and a larger metastatic focus were found to be predictive but results have been contradicting across different reports. In this study, we attempted to identify parameters that are predictive of non-SLN status and patient survival, as well as to determine the prognostic significance of SLN features, in particular the size of tumor invasion and their anatomical location in SLN.

Materials and methods:

Patient data: All cases with a diagnosis of invasive breast cancer and a positive SLN biopsy on either intra-operative frozen section (FS) or in the re-processed formalin fixed paraffin embedded (FFPE) tissue sections were retrieved from one of the involved institutions from 2006 to 2019. The same number of cases with a diagnosis of invasive breast cancer but a negative SLN biopsy result, matched by year and grade, were also retrieved. Demographics, prognostic data and biomarker results were obtained from medical records.

Protocol for SLN handling: All submitted lymph nodes were embedded in total. Those that were >2 mm in greatest dimension were sliced at 2 mm intervals along the short axis and were embedded on alternating cut surfaces. Two levels, separated by approximately 40 µm, were cut and examined per FS block. One H&E section would subsequently be cut from the reprocessed FFPE tissue. In cases where the FS diagnosis was reported as negative, immunostaining for pan-cytokeratin would be performed on each FFPE tissue section.

Assessment of SLN: For all positive cases, all the intra-operative FS and the re-processed FFPE sections were reviewed. The total number of SLN, number of positive SLN, size of the largest metastatic focus, the deepest anatomical location of the tumor in SLN and the presence and size of ENE were recorded.

Assessment of ALND: All H&E sections on cases with ALND performed were reviewed. The total number of lymph nodes retrieved, and the number of positive lymph nodes were assessed.

Statistical analysis: Statistical analysis was performed with the statistical software SPSS for Windows version 26. Pearson's chi-square test or Fisher's exact test was used to examining the association of categorical variables. Mann Whitney U test was used for the analysis of continuous variables. Survival data were evaluated using Kaplan-Meier analysis. Multivariate Cox regression analysis using the backward Wald method was used to determine the independent prognostic values. $P < 0.05$ was considered to indicate statistical significance.

Results:

Association of pathologic determinants with ALND status: Consistently, ALN+ cases had a higher number of positive nodes in SLN (p=.001). There was also significantly more MaM in SLN+ALN+ than SLN+ALN- cases (p=.014). In addition, more frequent ENE was found in ALN+ cases (p = .035). It is interesting to note that the presence of ENE was associated with a higher final pN stage (p<.001). However, there was no significant difference in the number of small and large ENE between ALN- and ALN+ cases. Comparing the pathologic features in primary tumors between the ALN+ and ALN- groups, ALN+ cases showed a larger primary tumor size (p = .002), and a higher pT stage (p = .004). The tumor grade, tumor histotype, ER, PR, HER2, Ki-67 index, and subtype showed no correlation with ALN+. Only the type of SLN metastasis and tumor size were independently associated with the ALN+ group in multivariate analysis. Of note, the number of SLN retrieved and SLN positive node were associated with a high ALN tumor burden (ALN+ node>2).

Association of pathologic determinants with SLN tumor invasion level

The SLN+ cases were also examined according to the deepest anatomical location of the tumor in SLN. The invasion level was closely associated with the size of tumor invasion. Significant associations of invasion level were found with a higher number of positive SLN, MaM, larger size of MaM foci and the presence of ENE (p<.001 for all). In line, a deeper level of tumor invasion, with a higher proportion of medulla involvement, was also found in ALN+ cases (p=.008) and a higher final pN stage (p<.001).

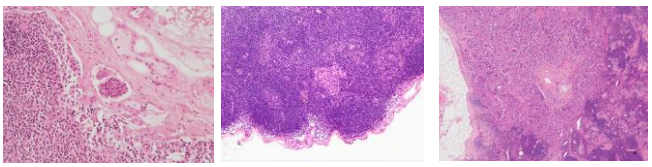
In addition, the deeper level of invasion was found associated with larger tumor size, pT stage and the presence of LVI (p≤.001). Of note, invasion level, particularly to the subcapsular/lymphatic region only, was associated with ER negativity (p=.030).

Survival data

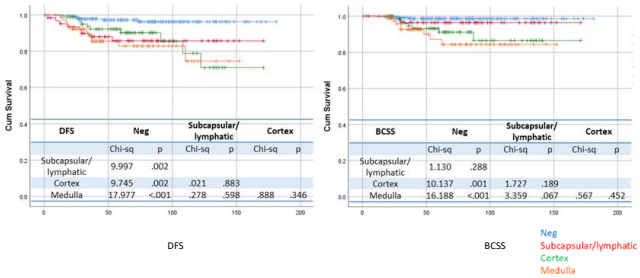
Kaplan Meier analysis showed that the categories of SLN metastasis showed a significant difference in DFS, BCSS and OS. Particularly, when compared to SLN- cases, SLN+ cases with ITC and MaM demonstrated worse outcomes (χ2 in DFS and BCSS for ITC: 14.553 and 4.314 respectively, p≤.038; χ2 in DFS, BCSS and OS respectively for MaM=1: 17.351, 13.815 and 6.591, p≤.010; for MaM>1: 14.036, 13.791 and 9.413, p≤.004). Such survival difference was not observed for the MiM group. Interestingly, there are no significant differences between the ITC and MaM cases in terms of survival.

For the SLN tumor invasion level, when compared to SLN- cases, cases with tumor invasion to the cortex and medulla of the SLN were found to have worse outcome (χ2 in DFS, BCSS and OS respectively for cortex: 9.745, 10.137 and 7.473, p≤.006; for medulla: 17.977, 16.188 and 10.443, p≤.001). By contrast, cases with tumor invasion to the subcapsular/lymphatic region demonstrated a worse DFS than SLN- cases (χ2=9.997, p=.002) but not BCSS and OS.

In multivariate analysis which also included age, tumor size, grade, biomarker statuses, CT, RT and HT, the presence of MaM in SLN was an independent prognostic parameter for OS (MaM>1: HR=6.824, p=.005) and marginally for DFS (MaM>1: HR=4.947, p=.056) while ITC was an independent feature for DFS (HR=8.688, p=.002). Tumor invasion to cortex/medulla in SLN was an independent prognostic feature for BCSS (HR=9.376, p=.001).



Left: subcapsular sinus invasion; Middle: cortical invasion; Right: medulla invasion



Conclusions:

Our study identified features in SLN, namely the level of tumor invasion at SLN and tumor size in SLN, as predictors for both ALN metastasis and breast cancer outcome. These features, together with primary tumor size, the number of positive SLN and presence of ENE were associated with nodal metastases in ALN. We also showed that the presence of ITC, particularly those with a deeper invasion in SLN portended a worse prognosis. SLN ITC+ cases tend to receive less treatment than other cases with nodal involvement, but with worse outcomes. They should be taken into account for the adjuvant treatment decision.