CLINICOPATHOLOGICAL FACTORS ASSOCIATED WITH POSITIVE PREOPERATIVE AXILLARY ULTRASOUND SCANNING IN BREAST CANCER PATIENTS

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ABSTRACT

Background: Axillary lymph node status is the most important breast cancer prognostic factor. Preoperative axillary ultrasound examination (PAUS) is used to triage patients for sentinel lymph node biopsy (SLNB) or axillary lymph node dissection (ALND). We assessed the detection rate of lymph node metastases by PAUS in a screening unit and evaluated associations between clinicopathological factors and PAUS positivity. **Patients and Methods:** This was a single-centre retrospective analysis of data extracted from a hospital breast cancer database and clinical records. Clinical, radiological, and pathological and prognostic indices were compared between PAUS-positive and PAUS-negative patients subsequently found to have lymph node metastases on histopathological analysis. Results: Two hundred and two patients were eligible for analysis. 50.5% of lymph node-positive patients were correctly identified as PAUS positive. Patients with PAUS-positive lymph nodes had less favourable disease characteristics, namely clinically palpable lymph nodes, higher Nottingham prognostic index (NPI), high lymph node burden according to the European Society of Medical Oncology (ESMO) group classification, and larger, grade 3 tumours with lymphovascular invasion and extranodal spread. Moreover, PAUS-positive patients had more macrometastases and lymph node involvement than PAUS-negative patients. **Conclusion:** PAUS-positive patients and PAUS-negative (SLNB-positive) patients have different clinicopathological characteristics. The presence of LVI, extranodal spread, grade 3 histology, or large tumours with poor prognostic indexes in PAUS-negative patients should be regarded with caution and perhaps prompt second-look ultrasound examination.

KEYWORDS: Preoperative axillary ultrasound scan; sentinel lymph node biopsy; breast cancer

Introduction

Axillary lymph node status is the single most important prognostic factor in breast cancer patients [1]. There is a strong association between axillary tumour burden and the risk of recurrence [1,2]. Axillary staging allows local and regional control and provides relevant information to direct adjuvant systemic therapy.

Current nodal staging involves sentinel lymph node biopsy (SLNB) in all patients diagnosed with early stage breast cancer unless lymph node metastases are identified preoperatively, in which case axillary lymph node dissection (ALND) is instead

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offered as part of the definitive surgery. Accurate preoperative imaging and axillary testing can, therefore, triage patients appropriately, assist surgical treatment planning, and provide the opportunity for a single-stage operation [2].

Some imaging modalities are used to stage the axilla. The sensitivity and/or economic impact of high-resolution computed tomography (CT), positron emission tomography (PET), magnetic resonance imaging (MRI), and, more recently, shear wave ultrasound examination have all been evaluated [3,4]. Current United Kingdom guidelines [5] recommend preoperative axillary ultrasound scanning (PAUS) in combination with fine needle aspiration (FNA) or core biopsy as gold standard axillary staging. It is in contrast to many other countries, notably the United States, where ultrasound examination and subsequent core biopsy are only performed in the context of a clinically palpable lymph node, despite a 30-50% clinical examination false negative rate [6,7]. Furthermore, the accuracy of PAUS is dependent on the experience and skill of the operator, with the accuracy of PAUS estimated to range between 45 and 68% [6].

The Nottingham Prognostic Index (NPI) is used to determine prognosis following breast cancer surgery. It combines nodal status, tumour size and histological grade to categorise patients as excellent, good, moderate, or poor prognosis [8]. The European Society of Medical Oncology (ESMO) group stratifies patients into low, moderate, and high risk based on calculated risk of recurrence based on patients' age, tumour size, grade, vascular invasion, ER, HER2 status, and lymph node involvement [9]. Both are calculated postoperatively following a complete examination of the surgical specimen.

Here we report the detection rate of positive axillary lymph nodes using PAUS at our unit. We compared the clinicopathological features of PAUS positive and PAUS negative (SLNB+) groups and examined the association(s) between clinicopathological factors and PAUS positivity (PAUS+) group. Finally, we assessed associations between NPI and the high-risk ESMO group and PAUS positivity.

Patients and Methods

It was a retrospective analysis of patients treated over a two-year period for early-stage primary breast cancer and found to have histologically confirmed lymph node metastases. Our unit is a regional breast cancer centre that follows national guidelines on triple assessment and PAUS. No ethical approval was required since this was a retrospective review of service provision.

A consultant radiologist or specialist consultant radiographer performed PAUS examinations using a Hitachi ultrasound machine with a linear 13-18 MHz small plate probe. Specialist consultant breast pathologists performed all histopathological assessments.

Clinicopathological data including patient demographics, clinical assessment findings, radiological findings at initial evaluation, and histopathological findings including hormone receptor and HER2 status were retrieved from the hospital cancer database, case notes, and pathology records. Patients who had pre-surgical diagnostic procedures completed elsewhere or had received neoadjuvant chemotherapy were excluded from the analysis. HER2 immunohistochemistry (IHC) was scored from 0 to 3+ according to the assay and clinical guidelines for HercepTest (Dako, Glostrup, Denmark) [10].

Briefly, breast cancer tissue was considered positive for HER2 when strong (3+) membranous staining was observed, and cancer cells scoring 0 or 1+ were regarded as negative. Equivocal 2+ cases were further tested and scored negative or positive based on gene amplification verified by fluorescence in situ hybridization (FISH) using the PathVysion HER2 DNA Probe Kit (Abbott, Chicago, IL).

The NPI score for each patient was extracted from the breast cancer database. In multifocal cancer cases, the highest score was recorded as the final overall score. The ESMO high-risk group was calculated based on available data using the ER and HER2 status and the number of lymph nodes involved.

Axillary lymph nodes were considered radiologically abnormal if they showed any of the following features: evidence of diffuse or focal cortical enlargement of more than 2.3 mm, loss of the lymph node fatty hilum, Solbiati index (longitudinal-transverse diameter ratio) less than 2, disrupted lymph node capsule, or increased intranodal vascularisation. The largest or most abnormal lymph node was biopsied in cases with multiple morphologically abnormal axillary lymph nodes. The nodal status assessed by PAUS was compared with final histological findings.

Patients diagnosed preoperatively with lymph node metastases were offered axillary lymph node clearance (ANC). All other patients were offered sentinel lymph node biopsy (SLNB).

Statistical methods

All data analyses were performed using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA). Pearson's chi-squared test, Fisher's exact test, and the Mann-Whitney U test were used where appropriate to assess for associations between PAUS positivity and clinicopathological characteristics. A p-value ≤ 0.05 was considered statistically significant.

Results

Two hundred and five patients were diagnosed with histopathologically positive metastatic lymph nodes between 2012 and 2014. Three patients underwent other imaging modalities before PAUS and were excluded from the study. Therefore, a total of 202 cases were available for study.

The clinicopathological characteristics of the study cohort are shown in Table 1 (a,b) . The mean and median age of patients was 61 and 60 years, respectively (range 31-90 years). One hundred and ninety-eight patients were female (98.02%) and four patients (1.98%) were male. One hundred and thirty-eight patients (68.3%) were symptomatic and 64 (31.7%) patients were screen-detected. Lymph nodes were palpable in 38 (18.8%) patients, 33 (86.8%) of which were also positive on PAUS.

One hundred and forty-eight patients (73.3%) had invasive ductal carcinoma (IDC) of no special type with or without DCIS, 24 (11.9%) had invasive lobular carcinoma (ILC) with or without DCIS, and 30 (14.8%) patients had special-type (ST) carcinomas. The mean and median tumour diameters were 48.5 mm and 41.5 mm, respectively (range 7-90 mm). HER2 was negative in 121 cases, unequivocally positive in 33 cases, and equivocal (2+) in forty-eight patients. The latter were further tested with FISH, of which seven were positive resulting in a total of forty positive (score 3+) cases (19.8%) and 162 negative cases (80.2%).

PAUS correctly identified 102/202 (50.5%) patients with axillary lymph node metastases and were offered (and received) ANC. The 100 PAUS-negative cases subsequently received SLNB that revealed metastases on histopathological assessment. These patients received further axillary surgery, radiotherapy, or no further intervention as deemed appropriate by the multidisciplinary breast team.

Table 1a Clinicopathological characteristics of the study population and their differences between PAUS-positive and PAUS-negative patients.

		PAUS-positive	PAUS-negative	p-value
Total no. patients		102	100	
Age	<50	16	23	**0.38**
	51 - 69	62	53	
	>70	24	24	
Gender	Female	99	99	**0.62**
	Male	3	1	
Year of diagnosis	2012	27	32	**0.02**
	2013	47	27	
	2014	28	41	
Palpable lump	Yes	33	5	**<0.0001**
	No	69	95	
Symptomatic	Yes	72	66	**0.48**
	No	30	43	
Tumour size	<20 mm	14	43	**<0.0001**
	20 - 30 mm	23	24	
	>30 mm	65	33	
Histopathological subtype	Invasive ductalcarcinoma	76	72	**0.156**
	Invasive lobular carcinoma	15	9	
	Special type	11	19	
Tumour grade	1	4	10	**0.027**
	2	45	55	
	3	53	35	

Table 1b Clinicopathological characteristics of the study population and their differences between PAUS-positive and PAUS-negative patients.

		PAUS-positive	PAUS-negative	p-value
Total no. patients		102	100	
ER status	Positive	88	94	**0.098**
	Negative	14	6	
HER2 status	Positive	24	16	**0.179**
	Negative	78	84	
Multifocal	Yes	22	12	**0.069**
	No	80	88	
Lymph nodes removed	Total, median (range)	15 (4-39)	7 (2-34)	**<0.0001**
	1-2 nodes	53	84	
	3 or more	49	16	
Size of metastases	Macrometastases	94	76	**0.0008**
	Micrometastases	7	24	
	Isolated tumour cells	1	0	
Lymphovascular invasion	Yes	70	55	**0.04**
	No	31	45	
Extranodal extension	Yes	54	28	**0.0003**
	No	48	78	
NPI	Total	102	100	**0.0007**
	$\leq$ 2.4	0	1	
	2.41-3.4	3	9	
	2.41-3.4	45	64	
	2.41-5.4	54	26	
ESMO high-risk group	Total	47	22	**<0.0001**
	LN 1-3, ER-	5	3	
	LN 1-3, HER2+	7	14	
	LN $\geq$ 4	35	5	

Associations between clinicopathological characteristics and PAUS positivity

Associations between clinicopathological characteristics and PAUS-positivity are shown in Table 1. Tumour size $\geq$ 5 cm (χ^2 = 7.743, p = 0.0054), clinically palpable lymph nodes (χ^2 = 24.74, p < 0.0001), grade 3 tumours (χ^2 = 4.072, p = 0.04), and lymphovascular invasion (LVI; χ^2 = 4.374, p = 0.04) were significantly associated with PAUS positivity. More cases were diagnosed as PAUS-positive in 2013 (p=0.02).

A greater number of lymph nodes were removed from the axilla in PAUS-positive patients, a larger number of which had macrometastasis than PAUS-negative patients (p=0.0008). Extranodal extension and LVI was more common in PAUS-positive patients than PAUS-negative (SLNB+) patients. The age, gender, symptomatic lump, breast cancer subtype, ER and HER2 status, and multifocality were not associated with a positive PAUS.

Associations between NPI subgroups, the "high-risk" ESMO group, and PAUS positivity

Associations between NPI subgroups, the high-risk ESMO group, and PAUS-positivity are shown in Table 1 (a,b). The majority of our patients were in the moderate or poor prognostic group, with 109 patients (53.96%) in the moderate (NPI 3.41-5.4) NPI prognostic group and 80 (39.6%) in the poor ($\geq$ 5.41) NPI prognostic group. There was a significant association between a high NPI score and PAUS positivity, with more than twice as many cases correctly identified as PAUS+ in the NPI $\geq$ 5.41 group. Within this group, 38 (70.4%) were symptomatic at presentation and 43 (79.6%) had two or more lymph nodes with macrometastasis on pathological examination (χ^2=17.09, p=0.0007). Within the poor NPI group, 26 cases (32.5%) were PAUS negative, of which only four (15.4%) were subsequently found to have heavy axillary tumour burden with four or more lymph nodes involved, five (19.2%) had micrometastasis only, and seventeen (65.4%) had one lymph node with macrometastasis.

There were 69 cases (34.2%) in the ESMO high-risk group, the only ESMO group that could be assessed since the low- and medium-risk groups are node negative. Forty (58%) had four or more lymph nodes involved on final pathological examination, of which 35 (87.5%) were correctly identified preoperatively. There was a significant association between PAUS positivity and high axillary tumour burden with four or more lymph nodes involved (χ^2=18.73, p<0.0001).

Discussion

Here we show that patients that have positive lymph nodes identified by PAUS have different clinicopathological characteristics to those who subsequently have lymph node metastases identified by SLNB. PAUS-positive patients have less favourable disease characteristics with higher NPI, greater axillary tumour burden based on ESMO classification, clinically palpable lymph nodes, and larger, higher-grade tumours with LVI and extranodal spread. Moreover, PAUS-positive patients have more macrometastases and more lymph nodes involved than those who have metastases identified by SLNB alone. These results are consistent with those of Wely et al. [11] and others [12,13], who also showed that patients with ultrasound-positive lymph nodes often have more extensive lymph node involvement and unfavourable disease characteristics. In particular, in their consideration of the impact of the Z0011 trial, Verheuvel et al. found that the PAUS-positive group also had unfavourable disease characteristics and a worse prognosis than SLNB positive patients. They concluded that omission of ALND is as yet only applicable to patients with SNLB-positive axillae [13]. The significant association between the poor prognostic NPI group and PAUS positivity is partly due to the presence of significant macrometastasis within this group of patients.

Within the high-risk ESMO group, those with four or more lymph nodes involved on final pathological examination were mostly identified correctly preoperatively as PAUS positive. It is likely to have been due to the lymph node burden resulting in marked morphological changes in the involved nodes that were readily detected by the radiologist at the time of presentation. These findings are consistent with those of Wely et al. [11] and Ertan et al. [12], who also showed that patients with ultrasound-positive lymph nodes often have more extensive lymph node involvement. The overall PAUS positive detection rate in our unit was 50.5%. Previous studies have shown that the overall sensitivity, specificity, and accuracy of ultrasound-guided lymph node biopsy is 50-70%, 100%, and 75%, respectively [14-16]. The detection rate improved in our centre in 2013 due to the introduction of specialist radiologists. However, the 2014 data only covered the first half of the year and, therefore, did not fully reflect that year's PAUS detection rate. In a recent review [17] and meta-analysis [18] of 21 studies (n = 4313 patients) on the clinical utility of ultrasound-guided needle biopsy for pre-operative staging of the axilla in breast cancer, the median sensitivity (correctly identified axillary metastases) and specificity (correctly identifying those without axillary metastases) of ultrasound alone was 61.4% and 82%, respectively. The inclusion of core biopsy and histopathological diagnosis increased the sensitivity and specificity to 79.4% and 100%, respectively.

In another pooled meta-analysis [15], the false negative rate of axillary ultrasound examination with or without biopsy was 25%, i.e., one in four patients will have axillary nodal metastases at SLNB despite negative PAUS. Also, a positive ultrasound examination cannot differentiate between high or low burden (1-2 lymph nodes) disease [19]. Despite this, PAUS remains a cost-effective staging strategy that can identify those with axillary metastasis and reduce the need for unnecessary SLNB. It has been calculated to result in cost savings of approximately $4000 per patient [19,20]. To improve pre-operative diagnostic accuracy, Swinson et al. [21] recommended sampling multiple lymph nodes with abnormal characteristics by fine needle aspiration. Other investigators have suggested using 3-dimensional (3D) ultrasonography to predict breast cancer prognosis [22], since the radiological convergence sign is correlated with lymph node metastases even when tumours are small (diameter <2 cm) [22]. Amonkar et al. examined the features considered abnormal as detected on PAUS and developed a structured ultrasound scoring algorithm of lymph node features to triage patients with the low metastatic burden to SLNB and those with the higher nodal burden to core biopsy and subsequent ALND if biopsy positive [23]. A 3mm cortical thickness threshold (in contrast to the 2.3 mm used in our centre) for nodal biopsy was most effective for identifying abnormal lymph nodes (sensitivity 68%, specificity 64%), emphasising the need for balance between detection of nodal metastases but minimising unnecessary biopsy.

In our PAUS-negative group, 57 out of 76 (76%) patients were subsequently shown to have macrometastases; PAUS failed to detect these preoperatively. It is significant since poor preoperative nodal metastasis detection increases the need for subsequent surgery and increases patient anxiety. Current standard UK PAUS practice, which is protocol-driven, is highly operator

dependent. Also, further inaccuracy can be introduced since there is no definitive way of knowing whether the observed lymph node is the sentinel lymph node. The sentinel node can be missed on PAUS, since the radiologist may target lymph nodes at or near the axillary vein, thereby missing the crucial lower axillary nodes low in the lymphatic chain, which by definition form the majority of sentinel nodes.

To overcome this limitation, recent attempts have been made to improve identification of the SLNB preoperatively. Britton [24] described systematic axillary scanning, in which the lowest one or two nodes are identified. Contrast-enhanced ultrasound with microbubbles has also been described; using this technique, the SLNB was successfully identified in 89-93% of breast cancer patients. Next-generation microbubble agents and vacuum-assisted core biopsy of sentinel node preoperatively may help reduce sampling error and improve the accuracy of axillary imaging, thereby reducing the false negative rate [25,26]. To date, we are unaware of any published studies where vacuum-assisted core biopsy combined with microbubble technology have been used to reduce the sampling error of SLNB, although the results of one such study in the UK is eagerly awaited.

Conclusion

We have shown that there are substantial clinicopathological differences between PAUS-positive and PAUS-negative (SLNB-positive) patients. LVI, extranodal spread, grade 3 histology, or large tumours with poor prognostic indices in patients with negative PAUS should be treated with caution and perhaps prompt a second-look ultrasound examination. Although our data are limited by being retrospective, prospective data collection of more patients will increase the statistical power and permit multivariate analysis; prospective data collection is currently underway. However, despite their limitations, these retrospectively collected data are representative of a typical case mix in a busy screening unit in a district general hospital. Future analyses with longer follow-up will permit analysis of overall and disease-free survival between the positive PAUS and positive SLNB group.

Authors' Statements

Competing Interests

The authors declare no conflict of interest.

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