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Does the subtype of breast cancer affect the diagnostic performance of axillary ultrasound for nodal staging in breast cancer patients?

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ABSTRACT

Introduction: Imaging findings can be affected by histopathological characteristics, such as breast cancer subtypes. The aim was to determine whether the diagnostic performance, in particular negative predictive value (NPV), of axillary US differs per subtype of breast cancer.
Methods: All patients diagnosed between 2008 and 2016 in our hospital with primary invasive breast cancer and an axillary US prior to axillary surgery were included. Histopathology of axillary surgery specimens served as gold standard. The NPV, sensitivity, specificity, positive predictive value (PPV) and accuracy of the axillary US were determined for the overall population and for each subtype (ER+/PR+HER2-,HER2+, triple negative tumors). The Chi-square test was used to determine the difference in diagnostic performance parameters between the subtypes.
Results: A total of 1094 breast cancer patients were included. Of these, 35 were diagnosed with bilateral breast cancer, resulting in 1129 cancer cases. Most common subtype was ER+/PR+HER2- in 858 cases (76.0%), followed by 150 cases of HER2+ tumors (13.3%) and 121 cases of triple negative tumors (10.7%). Sensitivity, specificity and accuracy of axillary US did not significantly differ between the subtypes. There was a significant difference for NPV between triple negative tumors and HER2+ tumors (90.3% vs. 80.2%, $p = 0.05$) and between HER2+ and ER/PR+HER2- tumors (80.2% vs. 87.2%, $p = 0.04$).
Conclusion: There was no significant difference in the diagnostic performance of axillary US between the subtypes, except for NPV. This was highest in triple negative subtype and lowest in HER2+ tumors. This can be explained by the difference in prevalence of axillary lymph node metastases in our cohort.

Introduction

According to current European guidelines, physical examination followed by axillary ultrasound (US) is routinely performed to assess the preoperative axillary lymph node status in newly diagnosed breast cancer patients [1]. In case of suspicious axillary lymph node(s), axillary US is combined with ultrasound-guided tissue sampling. Preoperative axillary US combined with ultrasound-guided tissue sampling has a pooled sensitivity of 50.0% (95%CI 43.0–57.0), specificity of 98.3% (95%CI 97.2–99.0), positive predictive value (PPV) of 100.0% (95%CI 100.0–100.0) and negative predictive value (NPV) of 67.4% (95%CI 60.0–76.2) [2,3]. These results imply that prior to surgery, only half of the patients with axillary lymph node metastases can be identified with axillary US. Otherwise, one in four patients with a negative axillary US appear to have axillary lymph node metastases after axillary surgery at pathology.

Imaging findings can be affected by histopathological characteristics, such as (invasive carcinoma of no special type (NST) versus

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invasive lobular carcinoma), tumor size, and size of axillary lymph node metastases [4,5]. For instance, preoperative breast magnetic resonance imaging (MRI) is more likely to detect axillary lymph node metastases in luminal B (35.6%) and HER2+ (34.6%) tumors compared to luminal A (17.3%) and basal like tumors (24.7%) ($p = 0.014$) [6]. The influence of breast cancer subtypes on the diagnostic performance of axillary US is unknown. Therefore, the aim of this study was to determine whether the diagnostic performance (sensitivity, specificity, PPV, accuracy), and in particular the NPV of axillary US differs per subtype of breast cancer.

Materials and methods

Data collection and study population

All patients diagnosed between 2008 and 2016 in our hospital with primary invasive breast cancer and an axillary US prior to surgery were included. Exclusion criteria were: ductal carcinoma *in situ*, recurrent breast cancer, patients without any surgical nodal staging (i.e. sentinel lymph node biopsy (SLNB) or axillary lymph node dissection (ALND)), and patients who were primarily treated with neoadjuvant systemic therapy. Data on patient and tumor characteristics, diagnostic work-up, surgical procedures and data on the histopathological outcome of the axillary lymph nodes were retrospectively collected.

Due to the retrospective design of this study, the necessity to acquire informed consent from the study subjects was waived by the local medical ethics committee. We wish to disclose that a subset of this cohort ($n = 577$) has been included in earlier publications on the accuracy of axillary ultrasound to predict final pN status in terms of the total number of metastatic lymph nodes [7,8]. However, these studies did not separate the different subtypes of breast cancer.

Clinical and nodal status

Pre-operative nodal staging consisted of physical examination combined with an axillary US. The axillary US was performed by dedicated breast radiologists using an iU-22-xMATRIX ultrasound system in combination with a linear 2–17 MHz array transducer.

Prior to 2011, an ATL-HDI5000 system was used (both Philips Healthcare, Best, the Netherlands). The following criteria were used to identify suspicious axillary lymph nodes: diffuse cortical thickening, focal cortical mass and/or effacement or replacement of the fatty hilum [9]. Tissue sampling was performed in case of suspicious axillary lymph node(s) using 16–18 gauge core needle biopsy. Fine needle aspiration cytology was performed when core needle biopsy was deemed technically challenging. In case multiple axillary lymph nodes were suspicious, only one of these lymph nodes was sampled. In patients with bilateral breast cancer, axillary lymph nodes in both cases were assessed. Clinical nodal status was defined as cN0 in case of no evidence for axillary lymph node metastases and cN+ in case of ≥1 axillary lymph node metastases.

Sentinel lymph node procedure

In clinically node negative patients an SLNB was performed. The SLNB procedure was performed using the following triple technique: lymphoscintigraphy (using 80 MBq Technetium-99 m nanocolloid injected peri-areolar), blue dye to detect lymphatic vessels (Bleu Patente®; Guerbet Aaulnaysous-Bois, France) and intraoperative use of a gamma probe to detect radioactivity. In case of one or more SLN metastases, a completion ALND or radiotherapy of the axilla was performed. In clinically node positive patients, an ALND was performed directly.

Pathological assessment sentinel lymph node

Sentinel lymph node(s) were mostly lamellated and paraffin embedded for histological evaluation. Each sentinel lymph node was examined at three histological levels at 500-μm intervals and were stained with haematoxylin and eosin (H&E). If H&E staining was negative, cytokeratin immunohistochemical (IHC) staining was done. All ALND lymph nodes were paraffin embedded after at least 16 h of fixation for histological evaluation.

Isolated tumor cells (clusters <0.2 mm and/or less than 200 cells) and micrometastasis (≥0.2 and/or more than 200 cells, but ≤2.0 mm) were considered as negative, and macrometastasis (>2.0 mm) as positive. Pathological nodal staging was based on the number of malignant axillary lymph nodes: pN0 is none or micrometatasis, pN1 is 1–3 (at least one larger than 2,0 mm), pN2-3 ≥4 (at least one larger than 2,0 mm) axillary lymph node metastases. In this study, non-axillary lymph node metastases are not taken into account.

Receptor status

Biomarker status was determined by immunohistochemical (IHC) staining and was considered positive if ≥ 10% of the cells stain positive, according to Dutch guidelines [10]. HER2neu status was determined by IHC or FISH according to ASCO-CAP guidelines [11]. Three subtypes of breast cancer were distinguished, namely ER/PR+HER2-, HER2+ and triple negative (ER/PR-HER2-).

Statistical analysis

Data were summarized as proportion with 95% confidence intervals or means with standard deviations (SD). For both the overall population and subtypes of breast cancer the sensitivity, specificity, PPV, NPV, and accuracy was determined. Accuracy was calculated as the sum of true positives and true negatives divided by total number of cases. The Chi square test was used to determine the difference in sensitivity, specificity, NPV, and accuracy between the breast cancer subtypes. Statistical analyses were performed using the statistical Package for the Social Sciences (version 22, IBM, Armonk, New York, USA). P-value ≤ 0.05 was considered statistically significant.

Results

Between 2008 and 2016, 1094 patients were diagnosed with primary invasive breast cancer patients and underwent an axillary US. Of these, 35 were diagnosed with bilateral breast cancer, resulting in 1129 examined axillae (Fig. 1). Most common subtype was ER/PR+HER2- in 858 tumors (76.0%), followed by HER2+ in 150 tumors (13.3%) and triple negative tumors in 121 examined axillae (10.7%). Grade III was most common in triple negative tumors (80.2%) compared to HER2+ tumors (60.7%) and ER/PR+HER2- tumors (18.9%). Patient and tumor characteristics are summarized in Table 1.

Diagnostic performance axillary US – overall population

The prevalence of axillary lymph node metastasis, based on histopathological examination, was 21.2% (239 of 1129 cases) in the overall population. Using pre-operative axillary US, 1027 of 1,129, (91.0%) cases were clinically node negative (cN0) (Fig. 2). Of the 1027 cases preoperatively staged as cN0, 890 (86.7%) were true negative and 137 cases (13.3%) were false negative; 113 with pN1 (11.0%), and 24 with pN2-3 (2.3%). Of the 24 false negative patients with pN2-3, ER/PR+Her2- was the most common subtype with

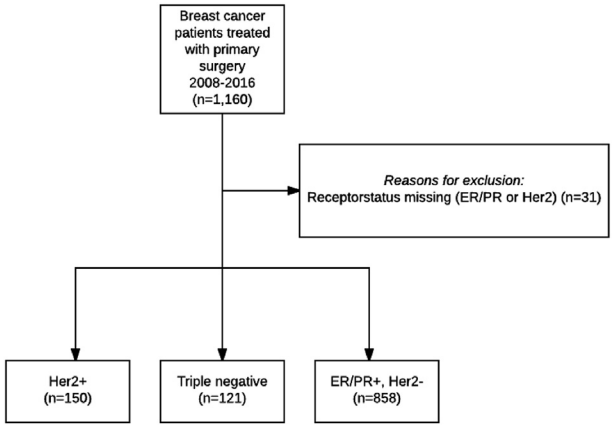

Fig. 1. Flowchart patient inclusion.

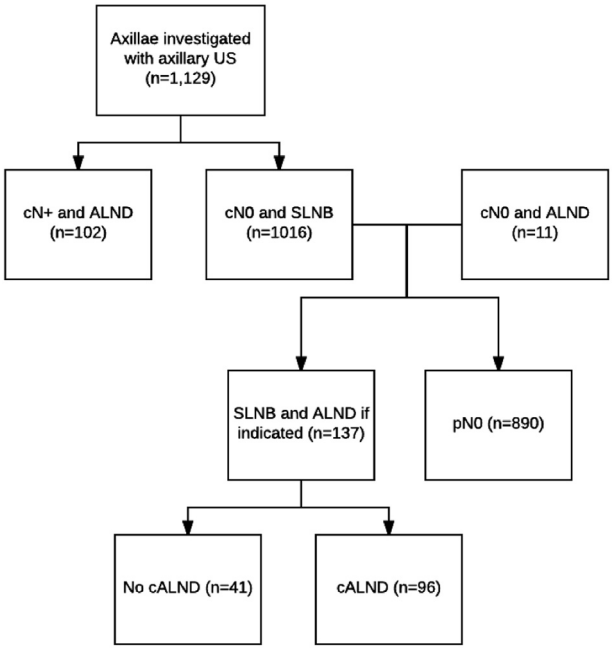

Fig. 2. Flowchart of axillary nodal staging.

62.5% (15/24), followed by Her2+ with 25.0% (6/24) and triple negative tumors with 12.5% (3/24). Based on pre-operative axillary US, 102 of 1129 (9.0%) cases were clinically node positive (cN+). The sensitivity, specificity, PPV, NPV, and accuracy in the overall population were 42.7% (102/239), 100% (8890/890), 100% (102/102), 86.7% (890/1027), and 87.9% (992/1129), respectively (Table 2.)

Diagnostic performance axillary US – subtypes

The prevalence of axillary lymph node metastases differed between breast cancer subtypes. The prevalence was highest in HER2+ tumors with 32.7% (49/150), followed by 19.9% (171/858) in ER/PR+HER2- tumors and 15.8% (19/121) in triple negative tumors.

Triple negative tumors were most often (93.4%) preoperatively staged as cN0 (113 of 121 cases). Of these, 102 (90.3%) were true negative and 11 (9.7%) false negative. In ER/PR+HER2- tumors, 788 of 858 cases (91.8%) were staged as cN0, whereof 687 (87.1%) were true negative and 102 (12.9%) false negative. In HER2+ tumors 126

Table 1
Patient and tumor characteristics.

Characteristic	Total	ER/PR+HER2-	HER2+	Triple negative
Number of cases evaluated, n	**1129**	**858**	**150**	**121**
Mean age (y) (range)	61 (21–90)	62 (31–90)	59 (21–84)	60 (26–86)
Mean cT-size, mm (range)	22 (1–100)	21 (2–100)	28 (3–100)	24 (0–78)
Uni-/Multifocal tumors, n (%)				
Unifocal	936 (82.9)	707 (82.4)	123 (82.0)	106 (87.6)
Multifocal	193 (17.1)	151 (17.6)	27 (18.0)	15 (12.4)
Tumor type, n (%)				
Invasive carcinoma NST	911 (80.7)	679 (79.1)	133 (88.7)	99 (81.8)
Lobular	144 (12.8)	127 (14.8)	12 (8.0)	5 (4.1)
Other	62 (5.5)	42 (4.9)	5 (3.3)	15 (12.4)
Missing	12 (1.0)	10 (1.2)	0 (0)	2 (1.7)
Tumor grade, n (%)				
1	241 (21.3)	228 (26.7)	7 (4.6)	6 (5.0)
2	537 (47.6)	468 (54.4)	52 (34.7)	17 (14.0)
3	350 (31.0)	162 (18.9)	91 (60.7)	97 (80.2)
Missing	1 (0.1)	0 (0)	0 (0)	1 (0.8)
cN stage, n (%)				
cN0	1027 (91.0)	788 (91.8)	126 (84.0)	113 (93.4)
cN+	102 (9.0)	70 (8.2)	24 (16.0)	8 (6.6)
pN stage, n (%)				
pN0	890 (78.8)	687 (80.1)	101 (67.3)	102 (84.3)
pN+	239 (21.2)	171 (19.9)	49 (32.7)	19 (15.7)
pT stage, n (%)				
T1	765 (67.8)	612 (71.3)	84 (56.0)	69 (57.0)
T2	319 (28.3)	209 (24.4)	61 (40.7)	49 (40.5)
T3	39 (3.4)	31 (3.6)	5 (3.3)	3 (2.5)
T4	6 (0.5)	6 (0.7)	0 (0)	0 (0)
Axillary surgery, n (%)				
SLNB	890 (78.9)	694 (80.9)	97 (64.7)	99 (81.9)
SLNB with completion ALND	127 (11.2)	88 (10.3)	26 (17.3)	13 (10.7)
ALND	112 (9.9)	76 (8.8)	27 (18.0)	9 (7.4)

N number of cases, cT clinical tumor stage, cN clinical nodal stage, pN pathological nodal stage, pT pathological tumor stadium, SLNB sentinel lymph node biopsy, ALND axillary lymph node dissection.

Table 2
Diagnostic performance axillary ultrasound per subtypes.

	All cases % [95%CI]	HER2+ % [95%CI]	Triple negative % [95%CI]	**ER+/PR+HER2-% [95%CI]**
Sensitivity	42.7 [36.6–49.0] (102/239)	49.0 [35.6–62.5] (24/49)	42.1 [23.1–63.8] (8/19)	40.9 [33.8–48.4] (70/171)
Specificity	100.0 [100.0–100.0] (890/890)	100.0 [100.0–100.0] (101/101)	100.0 [100.0–100.0] (102/102)	100.0 [100.0–100.0] (687/687)
PPV	100.0 [100.0–100.0] (102/102)	100.0 [100.0–100.0] (24/24)	100.0 [100.0–100.0] (8/8)	100.0 [100.0–100.0] (70/70)
NPV	86.7 [84.4–88.6] (890/1027)	80.2 [72.3–86.2] (101/126)	90.3 [83.3–94.6] (102/113)	87.2 [84.7–89.3] (687/788)
Accuracy	87.9 [85.8–89.7] (992/1129)	83.3 [76.5–88.5] (125/150)	90.9 [84.3–95.0] (110/121)	88.2 [85.9–90.2] (757/858)

of 150 (84.0%) were staged as cN0, whereof 101 (80.2%) were true negative and 25 (19.8%) false negative.

NPV was highest in triple negative tumors with 90.2%, followed by 87.2% in ER/PR+HER2- tumors and 80.6% in HER2+ tumors. The difference in NPV was statistically significant for triple negative and HER2+ subtype ($p = 0.05$), as well as for HER2+ and ER/PR+HER2- subtype ($p = 0.04$). There was no significant difference between triple negative and ER/PR+HER2- tumors ($p = 0.36$). Sensitivity was highest for HER2+ subtype (49.0%), followed by triple negative tumors (42.1%) and ER/PR+HER2- tumors (40.9%), but these differences were not statistically significant ($p = 0.60$, $p = 0.90$ and $p = 0.30$, respectively). The accuracy was highest for triple negative tumors (90.9%), followed by ER/PR+HER2- tumors (88.2%) and lowest for HER2+ tumors (88.3%), but were not statistically significant ($p = 0.40$, $p = 0.10$ and $p = 0.07$, respectively). There was no difference between the breast cancer subtypes for the specificity and PPV (Table 2).

Discussion

In the current era, breast cancer subtypes have become more important for patient tailored treatment, since they have different patterns of disease presentation [12], metastatic spread [13] and response to treatment [14–16]. The panel of the eighth edition of the TNM classification of the American Joint Commission of Cancer (AJCC) for breast recognized the need to incorporate breast cancer subtypes [17]. It is therefore arguable whether breast cancer subtypes should be kept in mind while interpreting results in the diagnostic pre-operative work-up. No prior study investigated the diagnostic performance of axillary US for different subtypes among breast cancer patients. Current randomized controlled trials (e.g. BOOG 2013-08, SOUND, INSEMA and NCT01821768 trial) investigate whether SLNB can be safely omitted in clinically node negative patients treated with BCT [18–21]. Most important for these trials, is to accurately select true negative patients since patients are randomized for omission of the SLNB.

The NPV in the overall population was 86.7% compared to the pooled NPV of 67.4% in the meta-analysis of Houssami et al. and Diepstraten et al. [2,3]. This present study showed that there is a significant difference for NPV between triple negative tumors and HER2+ tumors (90.3% vs. 80.2%, $p < 0.05$) and between HER2+ and ER/PR+HER2- tumors (80.2% vs. 87.2%, $p = 0.04$). This can be explained by the difference in prevalence of axillary lymph node metastases between breast cancer subtypes. Highest prevalence of axillary lymph node metastases and thus lowest NPV was seen in HER2+ tumors and highest NPV and thus highest NPV for triple negative tumors. Triple negative tumors often have the lowest prevalence of axillary lymph node metastases compared to other breast cancer subtypes [22–24].

Other diagnostic performance parameters, sensitivity, specificity and accuracy of axillary US did not significantly differ between the breast cancer subtypes.

Besides breast cancer subtypes, other histopathological characteristics could affect the diagnostic performance of axillary US as well (e.g. tumor size, tumor type, and size of lymph node metastases). Stachs et al. showed that the diagnostic performance of axillary US depends on the size of axillary lymph node metastases. Only 9.8% (4 of 41) axillary lymph node metastases were identified with a preoperative axillary US in metastases $\leq$5 mm versus 72.4% (55 of 76) in metastases >10 mm [4]. Schipper et al. previously demonstrated with a subset of this cohort that none of the patients with isolated tumor cells or micrometastases were identified by axillary US [7]. In this present study, isolated tumor cells and micrometastasis were considered as node negative. Results on the influence of tumor type, (invasive carcinoma NST versus invasive lobular carcinoma) on the diagnostic performance of axillary US are inconsistent. Choi et al. and Schipper et al. showed no significantly difference between invasive carcinoma NST and lobular carcinoma [25]. In contrast, Neal et al. and Johnson et al. reported a significantly higher NPV invasive carcinoma NST (96%) compared to invasive lobular carcinoma (83%, $p < 0.01$) [9,26].

Despite the difference in NPV between breast cancer subtypes, the clinical consequence remains questionable. The axillary US has a NPV of 96% to exclude advanced nodal disease (i.e. pN2-3) in the overall breast cancer population [7]. Potential metastases that are preoperatively not diagnosed with axillary US are therefore limited (1–3 metastases). Additionally, in these clinically node negative patients with limited axillary lymph node metastases in the SLNB; completion axillary treatment can be safely omitted [27,28].

Triple negative tumors have generally the worst prognosis at baseline compared to other breast cancer subtypes. For current randomized controlled trials it can be reassuring that the risk of metastases in patients with negative axillary US is lower than 10% triple negative tumors, because NPV is 90.3% due t rather relatively low prevalence of axillary lymph node metastases. Prevalence is higher and NPV lower in HER2+ tumors compared to the other breast cancer tumors. However, potential axillary lymph node metastases that are left *in situ* in HER2+ tumors can be diminished by (neo)adjuvant treatment with chemotherapy and trastuzumab (with or without pertuzumab). The review of Moja et al. showed that adjuvant treatment with trastuzumab led to a significant improvement in disease-free and overall survival in HER2+ breast cancer patients (HR 0.66, 95%CI 0.57–0.77, $p < 0.0001$; and HR 0.60, 95%CI 0.50–0.71, $p < 0.00001$) [29].

To our knowledge, this is the first study assessing the effect of breast cancer subtypes on the diagnostic performance of the axillary US. Another strength is that these results are based on a large single center study and an extensive study period. This study has several limitations. First, reporting diagnostic performance of axillary US highly depends on prevalence of nodal tumor burden [2]. The median prevalence of histology proven axillary lymph node metastases in this present study is low compared to the meta-analyses of Houssami et al. and Diepstraten et al. (21.0% versus 47.2%). Diepstraten et al. also demonstrated that the sensitivity of axillary US increased with an increasing prevalence of histology proven axillary lymph node metastases, 0.62 (95%CI 0.55–0.68) in the group with a high prevalence compared to 0.38 (95%CI 0.3–0.46) in the group with low prevalence [2]. High prevalence

was defined as ≥40% and low prevalence as <40% of the patients with axillary lymph node metastases. The lower sensitivity in this present study can be explained by the low prevalence of axillary lymph node metastases in this cohort. Interpreting results of the present study should therefore incorporate the prevalence of axillary lymph node metastases in this cohort. Most of the clinically node positive breast cancers patients within our institution were treated with neoadjuvant systemic therapy and therefore excluded in the present study. This results in a lower prevalence of patients with axillary lymph node metastases. Final limitation of the present study is that information on size of the axillary lymph node metastases from axillary US was not available. Therefore it is unknown whether the size of the axillary lymph node metastases could also have affected the diagnostic performance of axillary US.

In conclusion, there was no significant difference in sensitivity of axillary US between the subtypes of breast cancer. However, the NPV was highest in triple negative subtype and lowest for HER2+ tumors. This difference can be explained by the different prevalence of axillary lymph node metastases among the breast cancer subtypes.

The clinical consequence of the difference in NPV between breast cancer subtypes remains questionable. Recruitment of patients of all different breast cancer subtypes remains important for the current randomized controlled trials that investigate whether the SLNB can be safely omitted in all breast cancer subtypes.

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