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Original Article

Predictors of False-Negative Axillary FNA Among Breast Cancer Patients: A Cross-Sectional Study

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Abstract

Introduction

Fine-needle aspiration (FNA) is commonly used to investigate lymphadenopathy of suspected metastatic origin. The current study aims to find the association between nodal characteristics and cancer-related factors with the rate of false-negative preoperative FNA.

Methods

This retrospective, single-center, cross-sectional study included breast cancer patients with negative preoperative axillary FNA results who underwent postoperative histopathological evaluation. Data were collected from electronic medical records, including clinical, imaging, cytological, and pathological findings. Patients with incomplete records, non-axillary or inconclusive FNAs, positive preoperative FNAs, or unsampled axillae postoperatively were excluded. Key variables analyzed included lymph node size, cortical thickness, tumor grade, histological type, immunohistochemical subtype, and metastatic patterns.

Results

A total of 209 negative axillary FNA samples were analyzed, with a mean patient age of 46.13 years. Invasive ductal carcinoma was the most common diagnosis, and ER-positive tumors were the predominant subtype. Ultrasonography identified suspicious axillary nodes in 20.57% of cases. Histopathology revealed a 27.75% false-negative rate, with a negative predictive value of 78.3%. Larger lymph node size and cortical thickness exhibited lower false-negative rates, while histologic type and ER status showed significant associations with false-negative outcomes ($P < 0.05$).

Conclusion

The 27.75% false-negative rate of preoperative FNA remains concerning and may not be sufficiently low to justify foregoing definitive axillary staging. The current study found significant associations between false-negative FNA rates and histological subtype and ER status, the latter of which is not explicitly mentioned in the literature.

1. Introduction

Breast cancer represents the most commonly diagnosed malignancy among women globally. In 2020, it surpassed lung cancer to become the leading cancer diagnosis in women worldwide, with an estimated 2.3 million new cases reported [1,2]. Precise clinical staging plays a pivotal role in assessing prognosis, informing treatment strategies, and anticipating clinical outcomes in breast cancer. A critical component of

staging is the evaluation of axillary lymph node involvement, as the presence or absence of metastasis in these nodes substantially influences therapeutic approaches and overall survival rates [3].

Conventional techniques, including sentinel lymph node biopsy (SLNB) and axillary lymph node dissection (ALND), have long been the standard approaches for axillary staging in newly diagnosed breast cancer patients. Despite their diagnostic utility, these invasive procedures are associated with inherent risks and potential postoperative complications, notably lymphedema [1].

Axillary lymph node fine-needle aspiration biopsy (FNAB) has gained recognition as an effective method for staging breast cancer. When performed under ultrasound guidance (US-FNA), this technique allows for targeted sampling of suspicious axillary lymph nodes through a minimally invasive approach, providing a less invasive and potentially less morbid alternative to traditional methods such as SLNB and ALND [4]. This technique exhibits high diagnostic accuracy in detecting metastatic disease. Although the overall diagnostic accuracy of ultrasound-guided FNAB is well documented, the understanding of lymph node characteristics that influence the technique's sensitivity for detecting metastatic disease is limited [5]. Ewing et al. demonstrated that true-positive FNAB results were more likely in larger lymph nodes compared to false-negative results. Furthermore, true positive FNABs were associated with a higher percentage of nodal replacement by carcinoma, in contrast to false negative FNABs [6]. Establishing a precise correlation between the diagnostic sensitivity of FNAB for detecting metastatic malignancies and specific nodal characteristics remains challenging. The current study aims to establish the relationship between nodal characteristics and cancer-related factors with the false-negative FNAB rate through a retrospective analysis of data from 209 patients.

2. Methods

2.1. Study design

This was a retrospective, single-center, cross-sectional study. Data were collected over two months, from November to December 2024. All participants provided informed consent, including agreement to the use of their anonymized data for publication purposes. This study was approved by the Ethics Committee of the Kscien organization (Approval No. 2025-34).

2.2. Data source

Data were obtained through a review of electronic medical records, which included demographic information, medical history, presenting complaints, preoperative imaging findings (ultrasound, magnetic resonance imaging, and computed tomography), FNA results, diagnosis, treatment approaches, and postoperative histopathological outcomes.

2.3. Eligibility criteria

The study included patients diagnosed with breast cancer who had negative axillary FNA results before surgery and underwent histopathological assessment postoperatively. Exclusion criteria included patients with incomplete medical records, FNAs not sourced from the axillary region, positive preoperative axillary FNA results, FNAs conducted outside the study center, inconclusive FNA findings, cases where the axilla was not sampled after surgery, and hemorrhagic FNA samples. The following characteristics were analyzed: lymph node size, histological cancer type, immunohistochemical subtype, cortical thickness of axillary lymph nodes, tumor grade, and metastatic patterns.

2.4. Procedure

Ultrasound-guided FNA was systematically performed, adhering to established clinical protocols. Verbal informed consent was obtained before the procedure, with the target axillary lymph nodes initially identified through palpation and subsequently confirmed using high-frequency linear ultrasound imaging (7–15 MHz transducer). Patients were positioned in supine orientation with ipsilateral arm abduction to optimize anatomical access. Following alcohol antisepsis of the procedural field, a sterile 23-gauge Chiba-type needle connected to a 10 mL syringe via a pistol-grip aspiration device was percutaneously introduced into the lymph node cortex under continuous real-time sonographic visualization. Dynamic negative pressure (5-10 mL suction) was maintained during 3-5 controlled, multidirectional needle passes within the target lesion, with vacuum release executed before needle withdrawal to minimize peripheral blood admixture. Aspirates were promptly processed by smearing on glass slides and fixing in 95% alcohol for hematoxylin and eosin staining. Post-procedural hemostasis was achieved through sustained manual compression for 3 minutes, followed by clinical monitoring for immediate complications.

2.5. Statistical analysis

The data were initially collected and recorded in a Microsoft Excel (2024) spreadsheet and subsequently imported into version 25 of the Statistical Package for Social Sciences (SPSS). Categorical data were analyzed using the chi-squared test and Fisher's exact test. The results were presented as frequencies, ranges, percentages, means with standard deviations, and medians. A significance level of $P < 0.05$ was adopted.

3. Results

A total of 209 negative samples were included in the study. Ages ranged from 25 to 84, with a mean age of 46.13 years. A family history of cancer was reported in 70 patients (33.49%). The right breast was affected in 106 cases (50.72%). Invasive ductal carcinoma (IDC) was the most common diagnosis (156, 74.64%). Grade II was the most abundant tumor grade (105, 50.24%). Upon immunohistochemical (IHC) examination, it was revealed that ER+ was the most common subtype of cancer (126, 60.29%) (Table 1).

Ultrasonography of the axilla showed 43 (20.57%) suspicious nodes. The mean size of the lymph nodes was 11.7mm. Among the included samples, 58 (27.75%) were false negatives upon histopathological examination, while the true negatives were

Table 1. Demographic and clinical data of the patients.

Variable	Frequency (%)
Age groups	Total (209)
25-34	29 (13.9%)
35-44	60 (28.7%)
45-54	77 (36.8%)
55+	43 (20.6%)
Mean age ± SD	46.13 ± 11.79
Median age	49 (IQR=15)
Family history of cancer	70 (33.49%)
Affected side	Total (209)
Right side	106 (50.72%)
Left side	103 (49.28%)
Diagnosis	Total (209)
IDC	156 (74.64%)
DCIS	20 (9.57%)
ILS	20 (9.57%)
Others	13 (6.22%)
Tumor grade	Total (209)
Grade I	17 (8.13%)
Grade II	105 (50.24%)
Grade III	52 (24.88%)
N/A	35 (16.75%)
Cancer subtype (IHC)*	
ER+	126 (60.29%)
PR+	98 (46.89%)
HER2+	23 (11%)

IDC: Invasive ductal carcinoma, DCIS: Ductal carcinoma in situ, ILS: Invasive lobular carcinoma, N/A: Not applicable, IHC: Immunohistochemistry

**More than one receptor can be positive in a single patient, hence why the percentages don't add up to 100%.*

151 (72.25%). Negative Predictive Value (NPV) was 78.3%. The rate of false negative readings decreased as the size of the lymph nodes increased above the mean size (11.7 mm), and a similar trend was also seen in cortical thickness. However, a statistically significant association wasn't established (Table 2).

Among the analyzed characteristics, histologic cancer type and ER status were associated with false-negative readings (P-value < 0.05) (Table 3).

4. Discussion

Recent studies have provided insights into the factors contributing to false-negative FNA results in axillary lymph node evaluation for breast cancer. Earlier research by Ewing et al. identified smaller lymph node size (<1.2 cm) as a significant factor associated with false-negative FNA findings [6]. However, in the present study, lymph node size did not show a statistically significant association with false-negative results. This may be attributed to the fact that lymph node measurements were reported in only 20% of the cases, limiting the power of the analysis. Notably, consistent with previous literature, there was a trend of decreased rate of false-negative results as lymph node size increased beyond the mean threshold of 1.17 cm. It is plausible that a significant association might have emerged had a larger proportion of cases included lymph node size data.

Few recent studies have directly compared false-negative FNA rates among IDC, ILC, and other histological subtypes, representing a notable gap in the literature. Prior work, such as Chung et al., suggested that ILC's diffuse, discohesive growth and smaller metastatic foci may contribute to higher false-negative rates [7]. Supporting this, Sauer and Kåresen reported false-negative rates of 16% for ILC versus 6% for IDC, indicating that histological subtype may impact FNA sensitivity [8]. In the present study, a statistically significant association was found between histological subtype and false-negative FNA rates: IDC accounted for 75.86% of false negatives, ILC for 18.97%, and other types for 5.17%. It is worth mentioning that this association may be attributed to the higher prevalence of IDC compared to other cancer subtypes. Further research comparing subtype-specific FNA accuracy, particularly between IDC and ILC, is warranted to optimize preoperative axillary staging.

Regarding receptor and HER2 status, current evidence does not support a consistent association with false-negative rates of axillary FNA in breast cancer. Some studies suggest that axillary FNA sensitivity may not significantly differ by breast cancer subtype (including ER/PR/HER2 status), though negative predictive values can vary [9]. The current study found a significant association between ER-positive tumors and false-negative FNA readings. However, PR and HER2 status had no significant association with false FNA readings.

Cortical thickness is a key factor influencing the accuracy of axillary FNA. Thinner cortices (<3.5 mm) are linked to a higher risk of false-negative results, likely due to lower tumor burden and sampling difficulties [6]. Although a statistically significant association was not demonstrated, likely because only ~17% of cases reported cortical thickness, the data suggest an inverse relationship between cortical thickness and false-negative rates, consistent with existing literature. Raising the cortical thickness threshold when selecting nodes for FNA may reduce false negatives and enhance diagnostic accuracy, though this must be balanced against the need for sensitivity in specific clinical contexts.

Tumor grade, which reflects the degree of cellular differentiation and is often associated with tumor aggressiveness and metastatic potential, was not found to be significantly associated with false-negative FNA rates. This aligns with existing literature, where tumor grade has not been identified as a major predictive factor for FNA accuracy. Notably, a higher rate of false negatives was observed among lower-grade tumors. This finding is consistent with the biological behavior of such tumors, which are typically less aggressive and may present with smaller or fewer nodal metastases, making detection more challenging.

Although this study did not demonstrate a statistically significant association between false-negative FNA rates and the

Table 2. Nodal characteristics and FNA accuracy statistics.

Variable	Frequency (%) *
Suspicious LNs on US	43 (20.57%)
Mean LN size (cm)	1.17
Mean cortical thickness (mm)	3.39
Malignancy upon FNA	Total (209)
Positive	0
Negative	209 (100%)
Malignancy upon HPE	Total (209)
Positive	58 (27.75%)
Negative	151 (72.25%)
Negative Predictive Value	78.3%
FNA accuracy relative to mean LN size	
<1.17	Total (18)
False negative	5 (27.78%)
True negative	13 (72.22%)
>1.17	Total (24)
False negative	7 (29.17%)
True negative	17 (70.83%)
FNA accuracy relative to the histological subtype of cancer	
IDC	Total (156)
False negative	44 (28.21%)
True negative	112 (71.79%)
ILC	Total (20)
False negative	11 (55%)
True negative	9 (45%)
DCIS	Total (20)
False negative	2 (10%)
True negative	18 (90%)
Others	Total (13)
False negative	1 (7.69%)
True negative	12 (92.31%)
FNA accuracy relative to receptor status	
ER+	Total (126)
False negative	41 (32.54%)
True negative	85 (67.46%)
PR+	Total (98)
False negative	31 (31.63%)
True negative	67 (68.37%)
HER2+	Total (35)
False negative	11 (31.43%)
True negative	24 (68.57%)
FNA accuracy relative to mean cortical thickness (3.39mm)	
<3.39	Total (80)
False negative	24 (30%)
True negative	56 (70%)
>3.39	Total (69)
False negative	20 (28.99%)
True negative	49 (71.01%)
FNA accuracy relative to tumor grade	
Grade I	Total (17)
False negative	6 (35.29%)
True negative	11(64.71%)
Grade II	Total (105)
False negative	35(33.33%)
True negative	70(66.67%)
Grade III	Total (52)
False negative	10 (19.23%)
True negative	42 (80.77%)

LN: Lymph nodes, FNA: Fine needle aspiration, US: Ultrasound, HPE: Histopathological examination, IDC: Invasive ductal carcinoma, ILC: Invasive lobular carcinoma, ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor 2.

**The number in parentheses represents the count of patients for whom data were available.*

pattern of metastasis (micrometastasis vs. macrometastasis), prior literature has identified a notable link. Iwamoto et al. reported that 25% of patients with negative FNA results were subsequently found to have micrometastases on sentinel lymph node biopsy, contributing to a false-negative rate of approximately 31.5 [10]. Similarly, Fung et al. found micrometastases in 5 out of 16 patients with false-negative FNAs, underscoring the cytological challenge in detecting smaller metastatic foci [11].

In addition to nodal and patient-related factors, human error remains a significant contributor to false-negative FNA results and may compromise the validity of data analysis. These errors include sampling issues, operator dependency, interpretive variability, and subjective clinical decision-making. Alkuwari and Auger reported that nearly all false-negative cases in their study were due to sampling errors rather than misinterpretation [12]. Although less frequent, diagnostic challenges in cytology, particularly with scant cellularity or atypical morphology, can lead to interpretive errors. Moreover, the decision to perform FNA is often based on the clinician's subjective assessment of nodal suspiciousness, which may result in missed metastases if abnormal nodes are not sampled [13].

A key limitation of this study was the incompleteness of the collected data, with several variables lacking sufficient information. Although the results were mostly in line with the

Table 3. Extent of significance between analyzed characteristics and the rate of false negatives

Variable*	False negative	True negative	P-value
Lymph node size (Mean ± SD)	1.26 ± 0.5	1.29 ± 0.6	0.969
Cancer type (N, %)			
IDC	44 (28.21%)	112 (71.79%)	0.013
ILC	11 (55%)	9 (45%)	
DCIS	2 (10%)	18 (90%)	
Others	1 (7.69%)	12 (92.31%)	
Receptor status			
ER+	41 (32.54%)	85 (67.46%)	0.038
PR+	31 (31.63%)	67 (68.37%)	0.411
HER2+	11 (31.43%)	24 (68.57%)	0.594
Cortical thickness (Mean ± SD)	3.54 ± 0.74	3.56 ± 0.92	0.206
Tumor grade			
Grade I	6 (35.29%)	11 (64.71%)	0.235
Grade II	35 (33.33%)	70 (66.67%)	
Grade III	10 (19.23%)	42 (80.77%)	

**The number in parentheses represents the count of patients for whom data were available.*
IDC: Invasive ductal carcinoma, ILC: Invasive lobular carcinoma, DCIS: Ductal carcinoma in situ, ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor 2.

literature, with some unusual findings, it likely affected the data analysis and impacted the overall findings.

5. Conclusion

The 27.75% false-negative rate of preoperative FNA remains concerning and may not be sufficiently low to justify foregoing definitive axillary staging. The current study found significant associations between false-negative FNA rates and histological subtype and ER status, the latter of which is not explicitly mentioned in the literature.

Declarations

Conflicts of interest: The authors have no conflicts of interest to disclose.

Ethical approval: Not applicable.

Patient consent (participation and publication): Patients provided consent to participate in the study and to authorize the publication of any related data.

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Role of Funder: The funder remained independent, refraining from involvement in data collection, analysis, or result formulation, ensuring unbiased research free from external influence.

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Use of AI: ChatGPT-3.5 was used to assist in language editing and improving the clarity of the manuscript. All content was reviewed and verified by the authors. Authors are fully responsible for the entire content of their manuscript.

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