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Preoperative axillary nodal staging of invasive lobular breast cancer with ultrasound guided fine needle aspiration in patients with suspicious ultrasound findings versus aspiration in all patients – a retrospective single institutional analysis.

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Abstract

Introduction – At present, surgical strategies for breast cancer patients with >2 lymph nodes (LN) involved differ from those with no or lower degree of nodal involvement. Preoperative assessment of the axilla is less sensitive in patients with lobular carcinoma (ILC) than patients with other histological tumour types.

Materials and Methods – A retrospective analysis of axillary staging by palpation, axillary ultrasound (AXUS) and AXUS-guided fine-needle aspiration cytology (FNAC) of 153 patients with ILC diagnosed and operated on between January 2013 and December 2020 was performed. Patients had either sentinel node biopsy or axillary lymph node dissection according to current practice. In period 1, patients had FNAC only when AXUS suggested nodal involvement (n=106), and in period 2, all ILC patients had axillary FNAC (n=47).

Results – Of the factors associated with >2LNs involvement, logistic regression suggested only AXUS/FNAC based staging as independent variable for all patients; and extracapsular extension of the metastasis plus lymphovascular invasion for clinically node negative patients. AXUS had similar sensitivities (68% overall, p=0.43), specificities (93% overall, p=0.61) and false-negative rates (32% overall, p=0.8) in the two periods. However, these were significantly different for AXUS-guided FNAC: sensitivity (90% vs 50%), specificity (60% vs 95%) and false-negative rate (10% vs 50%; all $p<0.001$).

Conclusions – AXUS-guided FNAC of all ILC patients did not result in improved preoperative identification of patients with >2 metastatic LNs but increased the false-negative rate of the assessment by producing false-negative results in patients who would not have undergone a biopsy due to negative AXUS findings.

Keywords: breast cancer, axillary metastasis, axillary ultrasound, fine-needle aspiration cytology, preoperative diagnosis

Abbreviations:

ACOSOG: American College of Surgeons Oncology Group

ALND: axillary lymph node dissection

AXUS: axillary ultrasound

CNB: core needle biopsy

ER: oestrogen receptor

FNAC: fine-needle aspiration cytology

FNR: false-negative rate

FRR: false-reassurance rate

HE: haematoxylin and eosin

HER2: human epidermal growth factor receptor-2

ILC: invasive lobular carcinoma

LN: lymph node

NPV: negative predictive value

NSP: no special type (invasive breast cancer)

PPV: positive predictive value

PR: progesterone receptor

SLNB: sentinel lymph node biopsy

US: Ultrasound

Invasive lobular carcinomas (ILC) of the breast differ from other special type and non-special type breast carcinomas in many aspects, including their propensity to cause architectural distortion or remain occult rather than forming masses on mammography, being composed of noncohesive cells due to their lack of functional E-cadherin, giving a different metastatic pattern… etc [1]. Sometimes massive axillary nodal involvement is found without prior clinical or imaging evidence of such involvement.

It is common practice to use ultrasonography (US) for the evaluation of the axilla during the preoperative evaluation of early breast cancers. Patients with no palpable lymphadenopathy and a negative axillary US (AXUS-) are considered clinically node negative (cN0), and are candidates for axillary sentinel lymph node biopsy (SLNB), whereas those who are judged to have suspicious or positive lymph nodes (LNs) (AXUS+) are subjected to a sampling of at least one LN. This may be a fine needle aspirate for cytological assessment (FNAC) or a core needle biopsy (CNB) for histology. Negative microscopic findings (i.e., the lack of evidence for metastatic involvement) also result in a cN0 preoperative staging and an indication for axillary SLNB as a surgical staging procedure. Patients with positive findings (FNAC+ or CNB+) are considered clinically node positive (cN+) and underwent ALND earlier, but are more and more commonly offered neoadjuvant systemic treatment. AXUS has been reported to be of lower sensitivity for ILC than invasive breast carcinoma of no special type (NST) [2].

Patients with a cN+ status diagnosed preoperatively are about three times more likely than cN0 patients to have substantial nodal involvement, at least pN2 disease with >3 LNs involved [3], as suggested by our previous findings and a meta-analysis [4, 5].

As concerns ILC, it seems that cytokeratin immunohistochemistry (IHC) of sentinel LNs (SLNs) may disclose nodal involvement in SLNs deemed negative on HE staining more often than in case of other histological types of breast carcinoma, the yield may be as high as 24% [6], with smaller macrometastases being also discovered with IHC only. Although the routine use of IHC for SLN assessment has declined, this method was and may still be more often used in cases of ILC [7], and some guidelines also support(ed) this approach [8, 9].

We retrospectively analysed a series of ILCs in order to look for the factors associated with nodal positivity in >2 LNs, and looked at how AXUS-guided FNAC of LNs in all ILC patients compares with FNAC restricted to patients with AXUS+.

Materials and methods

Consecutive primary ILCs or carcinomas with a lobular component diagnosed by core needle biopsy as such and surgically treated at the Bács-Kiskun County Teaching Hospital between January 2013 and June 2018 (period 1, P1) were retrospectively collected from the archives of the Pathology Department. Recurrent cases and cases with missing data on staging were excluded.

From July 2018 to December 2020 (P2), patients with a preoperative diagnosis of ILC and an AXUS- status were re-assessed by AXUS (Philips HD5, 3-12 MHz), and sampling at least one LN (generally the largest LN visualized) was attempted.

The preoperative assessment of the axilla in the patients reported followed the steps delineated in the introduction. The sampling procedure used for AXUS+ patients was FNAC. For P1, only standard staining (haematoxylin and eosin – HE and Giemsa) was used for evaluation, but from July 2018, cytokeratin immunohistochemistry was added in cases where the FNAC sample contained sufficient cells and was negative for metastasis by conventional staining. The smears were stained with AE1/AE3 (Biogenex, San Ramon, CA; 1:200 dilution, 20 minutes incubationat room temperature, citrate buffer). SLNB was generally performed with dual tracer administration with slight modification of the previously described method [10]. The radiocolloid (60–90 MBq 99mTc-labelled 40–80 nm particle size Nanoalbumon, Medi-Radiopharma Kft., Érd, Hungary; or similarly sized Nanocoll, Gipharma, Saluggia, Italy) was given under US-guidance into the breast parenchyma (intra- and/or peritumorally) for non-palpable or uncertainly palpable tumours to allow radioguided occult lesion localisation [11], whereas it was given superficially (periareolarly) for palpable lesions the day before surgery. Patent blue dye was given most commonly subareolarly 10-15 minutes before surgery. During a brief period in Spring 2020, the Nuclear Medicine department was shut down due to the COVID19 pandemic, and dual labelling was solved by indocyanine green (Verdye^{TM} (Diagnostic Green GmbH, Aschheim-Dornach, Germany) given instead of the radiocolloid. This was detected by means of a Visionsense VS Iridium system (EleVision^{TM} IR Platform, Medtronic PLC, New Haven, CT, USA) [12].

As part of changing practice, patients with positive SLNs generally underwent a level I+II ALND, but from 2016, the Hungarian National Guidelines allowed skipping ALND for patients operated on with breast conserving surgery and limited nodal involvement (up to 2

macrometastases) in conditions matching the American College of Surgeons Oncology Group trial (ACOSOG) Z-0011 [13-15].

The SLNs were assessed with gross slicing at about 2 mm intervals and HE staining of the initial cuts and two additional levels separated by 250 microns from each other (limited step-sectioning). When negative, cytokeratin (AE1/AE3) immunohistochemistry was also applied to one level for ILC cases, i.e., the reported cases. Two levels of cytokeratin staining were generally used for all SLNs till December 2014. Lymph nodes involved by isolated tumour cells were considered as negative for the purpose of the study [3].

The data collected included tumour size, histological type, grade, oestrogen receptor (ER) status, progesterone receptor (PR) status, HER2 (human epidermal growth factor receptor-2) status, focality, mammographic morphology; the number of LNs assessed and positive for metastasis, extracapsular extension of nodal metastases, data on the axillary status gained by palpation, AXUS and FNAC, and type of axillary surgery (SLNB vs ALND). Results of FNAC were classified as positive of metastasis vs negative, and this latter category also included inconclusive results.

For nodal positivity (as outcome value), the two categories of pN0 and limited nodal involvement (pN1 with 1-2 lymph nodes involved) versus more extensive nodal involvement (>2 LNs metastatic) were selected. This was done in order to follow the ACOSOG Z-0011 trial inclusion criteria and the practice of omitting further axillary surgery in cN0 patients with 1 to 2 metastatic SLNs.

Statistical analysis was performed with the SPSS Statistics software (IBM, SSPS 23.0, Armonk, NY USA) and Vassarstats [16] on the basis of Microsoft Excel stored data. For univariate analysis, the chi-square test or the Fisher exact test was used for categorical variables and the Mann-Whitney U test for continuous variables, whereas for the multivariable analysis of the factors influencing greater nodal involvement, a forward binary logistic regression was performed. Parameters of the diagnostic tests like sensitivity or specificity, including false reassurance rate (FRR; false-negatives / (false-negatives + true negatives), i.e., the proportion of cases being false negative among all testing negative) of P1 and P2 were compared with the binomial test with results of P1 used as standard. The significance level was $p<0.05$ for all statistical tests.

This retrospective analysis was approved by the Regional Ethical Committee of the University of Szeged as part of a larger clinicopathological analysis of ILCs.

Results

In the retrospective analysis involving P1 between January 2013 and June 2018, 106 ILC cases from 104 female patients were included. The median age of the patients was 65 years. Of the tumours, 59 were left sided, whereas 47 were right sided; at least 6 patients had bilateral tumours, including metachronous cases falling outside of the studied period. Axillary surgery was ALND in 37 cases (9 of these following SLNB) and SLNB in the remaining 69 (14 patients with limited nodal involvement and 55 with a pN0 status, including 7 pN0(i+) cases). Of the 106 ILCs analysed, 28 (26%) had more than 2 LNs involved by metastasis.

Main clinico-pathological features of patients and tumours and their relation to nodal status are summarized in Table 1. Most tumours were pure lobular carcinomas of histological grade II, and the analysis suggested that neither grade nor the presence of an NST component impacted on the pathological nodal status. On the other hand, age, pathological, mammographic and ultrasonographic tumour size, pT category, lymphovascular invasion, focality, ER status, radiomorphology, axillary palpation-based staging, AXUS/FNAC based staging, the presence of extracapsular extension of the metastasis, the use of neoadjuvant systemic therapy and the type of axillary surgery were all associated with the pathological nodal status on univariate analysis. The logistic regression suggested that of these factors, only AXUS/FNAC based staging, was a significant independent predictor of more than 2 involved LNs (p=0.019 odds ratio, OR=3.33 95%CI:1.29-9.13.). This is a factor that is available preoperatively, and can therefore be useful in the preoperative assessment. (In a logistic regression model including only factors available preoperatively (i.e., tumour size on US, on MG, palpation of the axilla, AXUS/FNAC based staging, mammographic appearance and ER status), AXUS/FNAC based staging was again the only independent predictor of more than 2 involved LNs, but with a higher hazard ratio: p<0.0001; OR 7.15, 95%CI: 2.47-20.79).

All cases that were deemed node-positive by palpation (n=8, 0.08), and all those which were thought suspicious on AXUS and had FNAC sampling, proved to be node-positive on final histology. Of the cases with positive palpation findings in the axilla, 7 were proven to be

positive by preoperative FNAC. All cases positive by palpation (8/8) and 20/25 (0.8; 95% confidence interval (CI): 0.61-0.91) positive / suspicious by AXUS and having an FNAC had >2 metastatic LNs. All cases with positive physical examination findings also had abnormal AXUS.

Table 1 Clinical and pathological factors and their association with nodal status

	pN0 or pN1 (1-2 LN+) (n=78)	pN1-3 (>2 LN+) (n=28)	p values or all cases
pN category			
pN0	55	0	
pN1	23	1	
pN2	0	11	
pN3	0	16	
Age	65.1	59.0	p=0.024
pT category			p<0.001
pT1	48	2	50
pT2	23	9	32
pT3	6	15	21
pT4	0	1	1
pTx, pT0*	1	1	2
Mean size (mm) (±S.D.)			
Pathological	21.0 (±14.0)	55.7 (±34.4)	p<0.001
Mammographic	19.8 (±18.9)	45.7 (±42.2)	p=0.027
Ultrasonographic	17.9 (±16.3)	38.2 (±38.5)	p=0.046
(Lympho)vascular invasion			p<0.001
Present	6	6	12
Absent	72	22	94
Distribution of the tumour			p=0.001
Unifocal	24	1	25
Multifocal	46	18	64
Diffuse	8	9	17
Histological grade (on CNB)			p=0.775
G1	3	1	4
G2	68	23	91
G3	7	4	11
Histological type			p=0.285
pure ILC	69	27	96
mixed ILC and NST	9	1	10
ER status			p=0.003
ER+	78	24	102
ER-	0	4	4
PR status			p=0.056
PR+	72	21	93
PR-	6	7	13

HER2 status			p=0.094
HER2+	1	1	2
HER2-	77	27	104
Palpation of the axilla			p<0.001
node-negative	77	20	97
node-positive	0	8	8
unknown	1	0	1
AXUS and FNAC			p<0.001
AXUS negative	73	8	81
FNAC negative or not diagnostic	3	2	5
FNAC positive	2	18	20
Extracapsular extension			p<0.001
Present	5	23	28
Absent	18	5	23
Not applicable	55	not applicable	55
Radiological changes			p=0.034
Mass	54	11	65
Architectural distortion or increased density	18	13	31
Other**	6	4	10
Neoadjuvant therapy given			p<0.001
Yes	3	8	11
No	75	20	95
SLNB only or ALND			p<0.001
SLNB only	68	0	68
ALND	10	28	38

* The pT0 refers to an occult carcinoma with pN3 nodal involvement; ** included in this category are cases not fitting into the other two categories with too few cases: 2 cases with microcalcification, 1 pN0, the other with a single node involved; 1 pN2 case unassessable with mammography due to mastitis; and 7 cases with mammographically occult lesions, 2 with pN0, 2 with 1-2 nodes involved and 3 with >2 nodes involved.

ALND: axillary lymph node dissection, AXUS: axillary ultrasound, CNB: core needle biopsy, ER: oestrogen receptor, FNAC: fine needle aspiration cytology, G: grade, HER2: human epidermal growth factor receptor-2, ILC: invasive lobular carcinoma, NST: no special type, PR: progesterone receptor, S.D.: standard deviation, SLNB: sentinel lymph node biopsy

Of the cases which were cN0 by palpation and AXUS or FNAC negative (n=81), 27 had axillary metastasis, and 8 had more than 2 LNs involved (Table 2). For these, the univariable analyses suggested that the factors being significantly associated with >2 LNs involved include age, the pT category, pathological tumour size, (lympho-)vascular invasion, the ER status, extracapsular extension of the metastasis, whether only SLN biopsy or ALND had been performed and whether or not primary systemic treatment was given or not. Of these

parameters, only the presence of extracapsular extension (p<0.001; OR=77.02 95%CI:7.49-791.9) and vascular invasion (p=0.045; OR=13.87 95%CI:1.01-189.1) remained significant independent predictors, neither of which is available preoperatively. Taking into account only factors available preoperatively, only age remained a significant predictor (p=0.038, OR=0.89 95% CI: 0.81-0.99), suggesting that with increasing age, the frequency of massive nodal involvement tends to decrease.

Table 2 Clinical and pathological factors and their association with nodal status in clinically completely negative cases (by palpation and AXUS)

	pN0 or pN1 (1-2 LN+) (n=73)	pN1-3 (>2 LN+) (n=8)	p values or all cases
pN category			
pN0	54	0	
pN1	19	0	
pN2	0	2	
pN3	0	6	
Mean age (years)	65.4	57.1	p=0.033
pT category			p=0.002
pT1	47	1	48
pT2	21	3	24
pT3	5	3	8
pT4	0	1	1
Mean size (mm) (±S.D.)			
Pathological	20.6 (±13.7)	65.6 (±52.8)	p=0.001
Mammographic	20 (±14.7)	19.1 (±18.8)	p=0.526
Ultrasonographic	18.0 (±16.8)	34.1 (±20.2)	p=0.084
(Lympho)vascular invasion			p=0.012
Present	6	3	9
Absent	67	5	72
Distribution of the tumour			p=0.123
Unifocal	23	0	23
Multifocal	42	6	48
Diffuse	8	2	10
Histological grade (on CNB)			p=0.932
No data	1	0	1
G1	13	2	15
G2	58	6	64
G3	1	0	1
Histological type			p=0.895
pure ILC	65	7	72
mixed ILC and NST	8	1	9
ER status			p=0.009
ER+	73	7	80

ER-	0	1	1
PR status			p=0.832
PR+	67	7	74
PR-	6	1	13
HER2 status			NA
HER2+	0	0	0
HER2-	73	8	81
Extracapsular extension			p<0.001
Present	4	6	10
Absent	69	2	71
Not applicable	54	not applicable	54
Radiological changes			p=0.499
Mass	49	3	52
Architectural distortion or increased density	18	3	21
Other*	6	1	7
Neoadjuvant therapy given			p<0.001
Yes	0	2	2
No	73	6	79
SNB only or ALND			p<0.0001
SNB only	66	0	66
ALND	7	8	15

* included in this category are cases not fitting into the other two categories with too few cases

ALND: axillary lymph node dissection, AXUS: axillary ultrasound, CNB: core needle biopsy, ER: oestrogen receptor, FNAC: fine needle aspiration cytology, G: grade, HER2: human epidermal growth factor receptor-2, ILC: invasive lobular carcinoma, NST: no special type, PR: progesterone receptor, S.D.: standard deviation, SLNB: sentinel lymph node biopsy

During the P2 period, 63 patients with ILC have been identified at our Unit. Ten of these had no FNAC of their ipsilateral axilla due to core needle biopsy misdiagnosis as non-lobular carcinoma (n=1), no AXUS identifiable LNs (n=2), initiation of primary endocrine therapy because of old age or presence of metastatic disease or COVID19 pandemic and a limited access to surgery (n=5) and no obvious reasons (n=2). The remaining 53 patients, all had AXUS-guided FNAC, and the results are summarized in Table 3. Six patients were not operated either because of death from unrelated cause (n=1), distant metastases, locally advanced tumour and/or primary systemic treatment (endocrine treatment in all cases) (n=5).

All cases with positive axillary palpation findings were also deemed positive by AXUS and proven to be metastatic by FNAC. Of the physically negative cases 10 were positive by

AXUS, and all but three cases (including one with non-diagnostic cytology sample, and no subsequent operation) had metastatic lymph nodes; the 5 FNAC-positive cases had >2 (range: 5-17) involved LNs, whereas only one of the 4 FNAC-negative cases fell into this category (with 10/10 involved LNs). Of the 39 AXUS-negative cases 11 had minimal nodal involvement and 4 patients with non-diagnostic FNAC results had a>2 metastatic LNs (with 3/12, 9/18, 5/10 and 15/16 involved LNs, respectively)(Table 3).

Statistical comparisons of accuracy, sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively), false negative rate (FNR) and FRR for each method of nodal status assessment (palpation, AXUS, AXUS guided FNAC, and the combination of the latter two: both positive vs either negative) are shown in Table 4. The highest FNR was seen with the least sensitive test, palpation. In principle, this is also true for the FRR, if we do not consider the FRR of AXUS guided FNAC for P1, when only 25 patients were investigated by this method, resulting in a very wide 95% CI.

Importantly, the parameters of P1 vs P2 were generally comparable for palpation, AXUS and the combination of AXUS and FNAC, but the FNR and sensitivity (and also FRR, specificity and NPV) of P2 vs P1 were highly significantly different (Table 4).

Table 3 Total number of cases with preoperative FNAC results in period P2

						Pathological nodal status			
Clinical nodal status by palpation		AXUS		FNAC	All	pNx (not operated)	pN0	pN1 (1-2 LNs)	pN+ (>2 LNs)
cN1	3	+	3	+	3	3	0	0	0
cNx	1	+	1	+	1	1	0	0	0
cN0	49	+	10	+	5	0	0	0	5 (ALND)
				-	4	0	2	1	1 (ALND)
				0	1	1	0	0	0
		-	39	+	2	0	0	2 (ALND)	0
				-	16	0	13	3	0
				0	21	1	11	5 (1 ALND)	4 (ALND)
All	53		53		53	6	26	11	10

ALND: axillary lymph node dissection; AXUS: axillary ultrasound; FNAC: fine-needle aspiration cytology; +: positive (metastatic); -: negative (no metastasis identified); 0 (as category of FNAC): not diagnostic (no lymphoid cells, no tumour cells); cN and pN: categories of the TNM system [UICC], with pN1 (1-2 LNs) denoting limited nodal involvement with 1 or 2 lymph nodes (LNs) involved and pN+ (>2 LNs) denoting more than 2 LNs involved.

Table 4. Statistical parameters of different methods for identifying lobular carcinomas with >2 metastatic lymph nodes

Method / Values	P1	P2	P1+P2	p
Axillary palpation				
n =	105	47	152	
Prevalence of >2 lymph nodes involved	0.267 (0.187-0.363)	0.213 (0.112-0.361)	0.25 (0.185-0.328)	0.261
Accuracy (95% CI)	0.810 (0.719-0.877)	0.787 (0.639.0.888)	0.803 (0.729-0.861)	0.69
Sensitivity (95% CI)	0.286 (0.140-0.489)	0 (0-0.345)	0.211 (0.101-0.378)	0.045
Specificity (95% CI)	1 (0.941-1)	1 (0.883-1)	1 (0.959-1)	n.a.
Positive predictive value (95% CI)	1 (0.598-1)	n.a.	1 (0.598-1)	n.a.
Negative predictive value (95% CI)	0.794 (0.697-0.867)	0.787 (0.639-0.888)	0.792 (0.714-0.853)	0.909
False negative rate (95% CI)	0.714 (0.511-0.861)	1 (0.656-1)	0.789 (0.622-0.899)	**0.045**
False reassurance rate (95% CI)	0.206 (0.133-0.303)	0.213 (0.112-0.361)	0.208 (0.147-0.286)	0.909
AXUS				
n =	106	47	153	
Prevalence of >2 lymph nodes involved	0.264 (0.185-0.360)	0.213 (0.112-0.361)	0.248 (0.184-0.326)	0.426
Accuracy (95% CI)	0.877 (0.796-0.931)	0.851 (0.711-0.933)	0.869 (0.803-0.916)	0.588
Sensitivity (95% CI)	0.714 (0.511-0.860)	0.6 (0.274-0.863)	0.684 (0.512-0.820)	0.425
Specificity (95% CI)	0.936 (0.850-0.976)	0.919 (0.770-0.979)	0.930 (0.863-0.967)	0.671
Positive predictive value (95% CI)	0.8 (0.587-0.924)	0.667 (0.309-0.910)	0.765 (0.584-0.886)	0.317
Negative predictive value (95% CI)	0.901 (0.810-0.953)	0.895 (0.743-0.966)	0.899 (0.827-0.944)	0.897
False negative rate (95% CI)	0.286 (0.140-0.489)	0.4 (0.137-0.726)	0.316 (0.180-0.488)	0.798
False reassurance rate (95% CI)	0.099 (0.047-0.190)	0.105 (0.34-0.257)	0.101 (0.056-0.173)	0.129
AXUS guided FNAC				
n =	25	47	72	
Prevalence of >2 lymph nodes involved	0.8 (0.587-0.924)	0.213 (0.112-0.361)	0.417 (0.304-0.539)	**0.0**
Accuracy (95% CI)	0.84 (0.631-0.948)	0.851 (0.711-0.933)	0.847 (0.739-0.918)	0.836
Sensitivity (95% CI)	0.9 (0.669-0.982)	0.5 (0.201-0.799)	0.767 (0.573-0.894)	**<0.001**
Specificity (95% CI)	0.6 (0.170-0.927)	0.946 (0.805-0.991)	0.905 (0.765-0.969)	**<0.001**
Positive predictive value (95% CI)	0.9 (0.669-0.982)	0.714 (0.303-0949)	0.852 (0.654-0.951)	0.101
Negative predictive value (95% CI)	0.6 (0.170-0.927)	0.875 (0.724-0.953)	0.844 (0.699-0.930)	**<0.001**

False negative rate (95% CI)	0.1 (0.018-0.331)	0.5 (0.201-0.799)	0.233 (0.106-0.427)	**<0.001**
False reassurance rate (95% CI)	0.4 (0.073-0.830)	0.125 (0.047-0.276)	0.156 (0.070-0.301)	**<0.001**
AXUS and FNAC (both positive vs either negative)				
n =	106	47	153	
Prevalence of >2 lymph nodes involved	0.264 (0.185-0.360)	0.213 (0.112-0.361)	0.752 (0.674-0.816)	0.264
Accuracy (95% CI)	0.887 (0.807-0.938)	0.894 (0.761-0.960)	0.889 (0.826-0.932)	0.887
Sensitivity (95% CI)	0.643 (0.441-0.807)	0.5 (0.201-0.799)	0.605 (0.435-0.755)	0.643
Specificity (95% CI)	0.974 (0.902-0.996)	1 (0.883-1)	0.983 (0.932-0.997)	0.974
Positive predictive value (95% CI)	0.9 (0.669-0.982)	1 (0.463-1)	0.92 (0.725-0.986)	0.9
Negative predictive value (95% CI)	0.884 (0.792-0.940)	0.881 (0.736-0.955)	0.117 (0.069-0.189)	0.884
False negative rate (95% CI)	0.357 (0.193-0.559)	0.5 (0.201-0.799)	0.395 (0.245-0.566)	0.357
False reassurance rate (95% CI)	0.116 (0.060-0.208)	0.119 (0.045-0.264)	0.117 (0.069-0.189)	0.116

AXUS: axillary ultrasound, CI: confidence interval, FNAC: fine needle aspiration cytology.

False reassurance rate is defined as false negatives/(false negatives + true negatives)

Discussion

Despite the improvement of diagnostic methods, ILCs of the breast may still lead to frustrating diagnostic experiences. Not only can they manifest as occult carcinomas [17], or carcinomas with more foci than expected [18], but they may also have massive nodal involvement without prior notice. Indeed, a clinically node negative status may hide multiple metastatic lymph nodes, as this happened in 14/153 (9%) of this overall cohort.

In keeping with current knowledge, axillary palpation had a low sensitivity to disclose significant axillary LN involvement. On the basis of systematic reviews, AXUS is said to identify every second case with metastasis to the axilla, but one of four cases with an AXUS-status harbours metastasis in the LNs [19, 20]. The Z-0011 trial completely changed the policy of preoperative nodal staging, and it is not sufficient to identify node-positive breast cancers, but involvement with higher nodal burden needs to be identified. On the basis of a report on 577 cases, it seems that a negative AXUS can predict for the lack of massive (pN2-pN3) nodal involvement in the majority of cases (NPV 95.5%), but an AXUS+ status cannot really distinguish between pN1 vs pN2-pN3 cases [3, 21]. In this respect, AXUS is not worse than standard or dedicated MRI assessment of the axilla [22, 23]. Most of the time, greater nodal burden is reflected by pN2 and pN3 categories, only a few studies have concentrated on a definition of >2LNs involved (i.e. inclusive of the upper pN1 category) matching the evidence of the Z0011 trial and the American Society of Clinical Oncology recommendations [24]. A meta-analysis of these studies (with results of 4271 patients reviewed) reported that 79% of AXUS- patients have low nodal burden (0-2 involved LNs) vs AXUS+ patients having only 43% with similar burden [25].

An AXUS-guided biopsy, when positive is much more likely to reflect greater degree of nodal involvement among node-positive cases than a negative needle biopsy (FNAC or CNB) [4, 5, 20], and the false-positive rate of FNAC or CNB is negligible, no false-positive cases occurred in this series.

The above data were all derived from series with a mixture of breast cancer types. As concerns the problem with ILC, several authors have highlighted that the imaging assessment of nodal status in these tumours is less reliable. The FNR for identifying a massive nodal metastatic load (pN2-pN3) is higher for ILC than for NST invasive breast carcinomas (17% vs 4%) by AXUS [26]. AXUS-guided FNAC is also significantly worse in detecting nodal involvement (with sensitivities of 55% for ILC vs 76% for NST) [2]. These data are reflected

by frustrating individual clinical experiences. Our series of 153 ILCs suggested that AXUS has an overall sensitivity of 68% (95%CI: 51-82%) for detecting >2LN involvement and the FNR is pretty high at 32% (95CI: 18-49%). These data reflect that lobular carcinomas are indeed different from NST breast cancers in the reliability of their nodal staging, and data derived from series without stratification by tumour type cannot be reliably be extrapolated to ILCs.

Since AXUS-guided biopsy has better sensitivity than AXUS alone, as a policy, we introduced AXUS-guided FNAC for all ILCs, to increase the detection rate of higher nodal burden. On retrospect, this policy failed. A higher proportion of patients were sampled by FNAC as compared to the previous policy in P1 (53/63, 87% vs 25/106, 24%). This has led to significant decrease in the sensitivity of the test along with an increase in the FNR (Table 4). These figures show that extending the sampling to all patients without abnormal AXUS finding does not improve the identification of patients with high nodal burden. Only half of the ten patients with a high nodal burden could be identified with this policy, whereas the remaining five had not only an AXUS- status, but also a negative (n=1) or inconclusive (n=4) FNAC result. The results have led to abandon this policy and limit AXUS-guided FNAC to patients with abnormal AXUS.

A possible refinement could be the extension of FNAC to patients with advanced T categories (T3, T4) as suggested by Morrow et al, on the basis of their multivariable analysis [2]. Indeed, these tumours were associated with a higher rate of >2LNs involved, but our multivariable analysis failed to reveal this variable as an independent one; of the factors available preoperatively, only traditional (P1-related) AXUS+FNAC (all patients) or age (only cN0 patients) remained significant in the multivariable analysis.

The limitations of the present work include the retrospective nature of the analysis. The periods (P1 and P2) compared had a different prevalence of patients with high nodal burden, and the case numbers are limited, despite the timescale of 7 years covered. Because of changing policy toward ALND, not all patients with positive SLNs had ALND, limiting the identification of greater nodal load. In fact, patients with cN0 status, including AXUS- and metastasis in 1 or 2 SLNs (n=14 and 8 in P1 and P2, respectively) without ALND might have harboured more involved lymph nodes, but could not be identified. This policy is becoming more and more general, and the data gained must be accepted as the best that could be reached in a non-prospective data collection outside clinical trial; no better data with higher rates of ALND can be expected in the future considering the conservatism in axillary surgery.

In summary, literature data suggest that preoperative nodal staging of ILC is less sensitive than that of NST carcinomas, a higher nodal burden (>2LNs involved) can more often remain hidden, but FNAC of the axilla of all AXUS- patients has not lead to better identification of cases with high nodal burden.

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