

# Evaluation of Intraoperative Frozen Section Analysis with Final Histopathology Results for Sentinel Lymph Node Biopsy: 2011 Criteria Eligible Versus Ineligible Breast Cancer Patients

## Bedeutung von intraoperativer Gefrierschnittanalyse und histopathologischen Ergebnissen von Wächterlymphknotenbiopsien: Vergleich von 2011-Studie-geeigneten mit ungeeigneten Brustkrebspatientinnen



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### Key words

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### Schlüsselwörter

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### ABSTRACT

**Background** Intraoperative frozen section analysis (FSA) of sentinel lymph nodes (SLNs) declined in the post American College of Surgeons Oncology Group Z0011 (ACOSOG Z0011) trial era. However, for those patients who do not meet the ACOSOG Z0011 criteria, FSA continues to be a valuable tool in intraoperative decision-making for axillary lymph node dissection (ALND). The aim of this study was therefore to retrospectively evaluate the benefit and accuracy of FSA of Z0011 criteria eligible versus ineligible patients and identify possible predictive factors for false negative results.

**Methods** Intraoperative FSA was performed on SLNs of 522 cT1–T3 breast cancer patients between 2008 and 2013. Clinicopathologic characteristics were retrospectively assessed by chart review.

**Results** Overall FSA sensitivity and specificity was 67.8% and 100%. Sensitivity was generally higher for macrometastasis than for micrometastasis. The Z0011 eligible group showed a sensitivity and specificity of 72.7% and 100% versus 62.1% and 100% in the Z0011 ineligible group. Importantly, subgroup analysis of  $\leq 2$  versus  $> 2$  positive SLNs of the Z0011 eligible group demonstrated both a 100% specificity and sensitivity. Several clinicopathologic factors were associated with a higher rate of false negative results in the Z0011 ineligible patient group. FSA was beneficial for 22.2% of Z0011 ineligible patients and for only 0.6% of Z0011 eligible patients regarding intraoperative decision-making for ALND.

**Conclusions** FSA continues to be especially beneficial in the intraoperative assessment of SLNs in the Z0011 ineligible group to prevent second stage ALND. Despite an overall lower FSA sensitivity in the Z0011 eligible patient group, FSA offers in both groups a comparable high sensitivity and diagnostic accuracy for macrometastasis.

## ZUSAMMENFASSUNG

**Hintergrund** Nach der Veröffentlichung der American College of Surgeons Oncology Group Z0011-Studie (ACOSOG Z0011) ist die Analyse der intraoperativen Gefrierschnitte von Wächterlymphknoten zurückgegangen. Aber für Patientinnen, welche die ACOSOG Z0011-Kriterien nicht erfüllen, bleibt die Gefrierschnittanalyse ein wichtiges Instrument für die intraoperative Entscheidungsfindung im Hinblick auf eine Axilladisektion. Ziel dieser Studie war es, eine retrospektive Evaluierung der Vorteile und der diagnostischen Genauigkeit von Gefrierschnittanalysen bei Brustkrebspatientinnen durchzuführen, welche die Kriterien der Z0011-Studie erfüllten oder nicht erfüllten, und dabei mögliche prädiktive Faktoren für falsch negative Ergebnisse festzustellen.

**Methoden** Zwischen 2008 und 2013 wurde eine intraoperative Gefrierschnittanalyse der Wächterlymphknoten bei 522 cT1–T3-Brustkrebspatientinnen durchgeführt. Die klinisch-pathologischen Merkmale wurden retrospektiv mithilfe der Krankenakten evaluiert.

**Ergebnisse** Insgesamt betrug die Sensitivität und Spezifität für alle Gefrierschnittanalysen 67,8% bzw. 100%. Generell war die Sensitivität für Makrometastasen höher als für Mikrometastasen. Bei der Gruppe, welche die Kriterien der Z0011-Studie erfüllte, betrug die Sensitivität und Spezifität 72,7% bzw. 100%, verglichen mit 62,1% bzw. 100% für die Gruppe, welche die Z0011-Kriterien nicht erfüllte. In der Gruppe, welche die Z0011-Kriterien erfüllte, wurde eine Untergruppenanalyse durchgeführt, und die Ergebnisse für  $\leq 2$  positiven Wächterlymphknoten wurden mit den Ergebnissen für  $> 2$  verglichen. Bei beiden Untergruppen betrug Spezifität und Sensitivität jeweils 100%. In der Patientinnengruppe, welche die Z0011-Kriterien nicht erfüllte, waren mehrere klinisch-pathologische Faktoren mit einer höheren Rate an falsch positiven Ergebnissen assoziiert. Im Hinblick auf die intraoperative Entscheidungsfindung für eine Axilladisektion brachte die Durchführung einer intraoperativen Gefrierschnittanalyse Vorteile für 22,2% der Patientinnen, welche die Z0011-Kriterien nicht erfüllten, aber nur für 0,6% der Patientinnen, welche die Z0011-Kriterien erfüllten.

**Schlussfolgerungen** Die Gefrierschnittanalyse ist besonders für die intraoperative Evaluierung von Wächterlymphknoten bei Patientinnen, welche die Z0011-Kriterien nicht erfüllen, vorteilhaft, da dadurch eine Zweitoperation zur Axilladisektion vermieden werden kann. Obwohl die Sensitivität der Gefrierschnittanalyse in der Gruppe, welche die Z0011-Kriterien erfüllte, insgesamt niedriger war, hat die Gefrierschnittanalyse in beiden Gruppen eine vergleichbar hohe Sensitivität und diagnostische Genauigkeit für Makrometastasen.

## Introduction

Evaluation of the axillary lymph nodes is one of the most important prognostic factors of patients with breast cancer. Historically, axillary lymph node dissection (ALND) had been the gold standard to determine the axillary stage in these patients. In the past decades, surgical management of the axilla in breast cancer patients has become progressively less extensive. Sentinel lymph node biopsy (SLNB) has largely replaced ALND for axillary staging in clinically-node negative (cN0) patients with similar oncological results and lower morbidity [1–6]. Moreover, indications to carry out an ALND in case of a positive SLN have been further adapted according to the American College of Surgeons Oncology Group Z0011 trial (ACOSOG Z0011) and the International Breast Cancer Study Group 23-01 trial (IBCSG 23-01). The prospective randomized ACOSOG Z0011 and IBCSG 23-01 trials demonstrated that ALND could be avoided in patients with T1 to T2 primary breast cancer and 1–2 positive SLNs [7, 8]. The ACOSOG Z0011 trial revealed no difference in local, regional recurrence and survival between early breast cancer patients (cN0, tumor size  $< 2$  cm and 1–2 positive

SLNs) who were randomized into a SLNB only or SLNB and ALND group [8]. Results of the IBCSG 23-01 trial showed that ALND can be safely omitted in T1 to T2 breast cancer patients with SLN micrometastasis ( $\leq 2$  mm). Since then, clinical management of the axilla was modified for T1 to T2 breast cancer patients with 1–2 positive SLNs in many guidelines and the routine clinical use of frozen section analysis (FSA) for intraoperative SLNs assessment was called into question [9, 10].

Intraoperative assessment of SLNs by FSA has the advantage of allowing patients with positive SLNs to avoid a second surgery by directly proceeding to ALND. FSA is currently the most widely used method in the intraoperative assessment of SLNs. Studies have shown a high specificity (98–100%) and a variable sensitivity from 44 to 100% that reduce the risk of a deferred ALND in a non-negligible percentage [11]. The use of FSA for SLN assessment has declined since the published ACOSOG Z0011 and IBCSG 23-01 trials. Main factors are the risk of false negative or false positive results, which may mislead the surgeon's decision for/against ALND, as well as the costs [12]. However, for those patients who do not meet the ACOSOG Z0011 criteria, FSA continues to be a valuable

tool in intraoperative decision-making for immediate ALND. Respectively, FSA accuracy and factors associated with false negative or false positive FSA results are now especially essential in this subgroup. Therefore, the aim of this study was to retrospectively evaluate the benefit of FSA for immediate ALND as well as the accuracy of patients eligible for ACOSOG Z0011 criteria versus ineligible patients and to identify possible predictive factors for false negative results.

## Materials and Methods

### Patients

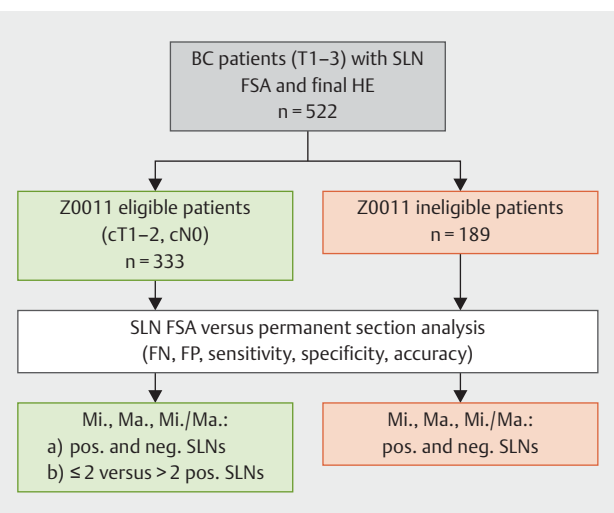
All primary breast cancer patients with stage T1–3 who underwent intraoperative FSA and permanent section analysis from our institution were retrospectively selected over a period of 5 years (2008–2013) (► **Fig. 1**). Breast cancer patients with neoadjuvant therapy and patients with T4 staged breast cancer were excluded. In this regard, selective patients with Her2-positive or triple negative breast cancer treated with an adjuvant therapy in the era under study (2008–2013) would nowadays rather be treated by neoadjuvant therapy. Data were collected from the patient records, which included clinicopathologic characteristics, results of FSA and permanent section analysis of SLNs. The study was carried out in accordance with Good Clinical Practice guidelines and was approved by the local Ethics Committee (4409).

### SLN mapping

SLN mapping was performed by using lymphoscintigraphy. Lymphoscintigraphy was performed by peritumoral or periareolar injection of Tc-99m-nanocolloid on the day of/before surgery. Scans of the involved breast and axilla were acquired 90–120 minutes after injection. SLNs were localized before skin incision and for guidance during surgery by using a gamma probe. Radioactive nodes with counts greater than 10-fold, in relative to the background, were regarded as SLNs and sent for FSA to the Department of Pathology. Two pathologists evaluated the frozen sections independently. Each SLN was initially longitudinally bisected, macroscopically assessed and afterwards cut into 100–250 µm slices dependent on SLN size by using a freezing microtome, as recommended by the College of American Pathologists (CAP) and the American Society of Clinical Oncology (ASCO) [13]. Intraoperative examination was carried out on at least three frozen sections. The remaining nodal tissue was embedded in paraffin and serial sections were made of the SLN for permanent hematoxylin & eosin staining. In selected cases with suspicious histologic findings additional cytokeratin staining was performed. The size of SLN metastasis was assessed and categorized into isolated tumor cells ( $\leq 0.2$  mm or  $< 200$  cells), micrometastasis ( $> 0.2$  mm and  $\leq 2$  mm, or  $\geq 200$  cells) and macrometastasis ( $> 2$  mm). Isolated tumor cells were considered as non-metastases.

### Statistical analysis

Data of FSA and permanent section analysis was extracted from the medical records and processed with SPSS software version 22 and Graph pad prism version 5. In cross tabulation, false negative results were defined as SLNs in which



► **Fig. 1** Study design. A total of 522 patients with primary breast cancer were subdivided into Z0011 eligible patients (n = 333) and Z0011 ineligible patients (n = 189). Performance of FSA (FN, FP, sensitivity, specificity, accuracy) of SLNs was evaluated in regard to permanent section analysis results. Analysis was done for micrometastasis, macrometastasis and for both. In the Z0011 eligible patient group, FSA performance was additionally analyzed for  $\leq 2$  versus  $> 2$  positive SLNs. BC: breast cancer, SLN: sentinel lymph node, FSA: frozen section analysis, Mi.: micrometastasis, Ma.: macrometastasis, FP: false positive, FN: false negative.

- no micrometastasis were found in the FSA, but which showed micrometastasis in permanent section analysis;
- no macrometastasis were found in the FSA, but which showed macrometastasis in permanent section analysis;
- no micro- and/or macrometastasis were found in the FSA, but which showed metastasis in permanent section analysis.

For univariate analysis of false negative results, the chi-squared, the Cochran–Armitage test for trend or the Mann–Whitney U-test were used for categorical or numerical variables, respectively. Sensitivity, specificity, negative predictive value, positive predictive value and accuracy were calculated accordingly. A p-value less than 0.05 was considered statistically significant.

## Results

### Patient characteristics

FSA of the SLN was performed on 522 patients (► **Table 1**). The mean age was 57.5 years (standard deviation  $\pm 12.39$  years). On histopathology, 59.7%, 37.7% and 2.6% of patients had T1, T2 and T3 primary tumors. Most of the patients were diagnosed with a HR-positive/Her2-negative subtype (84%), followed by a HR-positive/Her2-positive subtype (7%) and a triple negative subtype (6%). Patients were grouped into Z0011 eligible (cT1–T2, cN0; n = 333) and Z0011 ineligible (n = 189) patients, respectively.

► **Table 1** Patient and tumor characteristics.

Characteristic	Total (n = 522)	Z0011 eligible group (n = 333)	Z0011 ineligible group (n = 189)	p-value*
Age	57.5 ± 12.39	58.30 ± 11.74	57.69 ± 13.46	0.36 <sup>§</sup>
pT stage				
▪ T1	312 (59.7%)	229 (68.7%)	82 (43.3%)	0.0012 <sup>†</sup>
▪ T2	197 (37.7%)	104 (31.2%)	93 (49.2%)	
▪ T3	14 (2.6%)	0 (0%)	14 (7.4%)	
Histology				
▪ Ductal	410 (78.5%)	271 (81.3%)	139 (73.5%)	0.11 <sup>‡</sup>
▪ Lobular	79 (15.1%)	39 (11.7%)	40 (21.1%)	
▪ Other type	33 (6.3%)	23 (6.9%)	10 (5.2%)	
Histological grade				
▪ G1	73 (13.9%)	59 (17.7%)	14 (7.4%)	0.0036 <sup>†</sup>
▪ G2	337 (64.5%)	209 (62.7%)	128 (67.7%)	
▪ G3	112 (21.4%)	65 (19.5%)	47 (24.8%)	
ER				
▪ ER positive	468 (89.6%)	301 (90.3%)	167 (88.3%)	0.33 <sup>‡</sup>
▪ ER negative	54 (10.3%)	32 (9.6%)	22 (11.6%)	
PR				
▪ PR positive	425 (81.4%)	277 (83.1%)	152 (80.4%)	0.11 <sup>‡</sup>
▪ PR negative	93 (17.8%)	56 (16.8%)	37 (19.5%)	
HER2				
▪ HER2 positive	81 (15.5%)	45 (13.5%)	36 (19.0%)	0.49 <sup>‡</sup>
▪ HER2 negative	438 (83.9%)	286 (85.8%)	152 (80.4%)	
▪ N.A.	3 (0.5%)	2 (0.6%)	1 (0.5%)	
FSA of SLNs				
▪ Macrometastasis	85 (16.2%)	42 (13.5%)	43 (22.7%)	0.40 <sup>‡</sup>
▪ Micrometastasis	9 (1.7%)	6 (1.8%)	3 (1.5%)	
▪ ITC	0 (0%)	0 (0%)	0 (0%)	
Permanent section analysis of SLNs				
▪ Macrometastasis	108 (20.6%)	52 (15.6%)	56 (29.6%)	0.36 <sup>†</sup>
▪ Micrometastasis	37 (7.0%)	16 (4.8%)	21 (11.1%)	
▪ ITC	19 (3.6%)	12 (3.6%)	7 (3.7%)	

\* All tests without unknowns, <sup>§</sup> Two-sided Mann–Whitney U-test, <sup>†</sup> Two-sided Cochran–Armitage test for trend, <sup>‡</sup> Two-sided chi-square test. Values are presented as mean ± standard deviation or number (%).

ER: estrogen receptor, PR: progesterone receptor, HER2: human epidermal growth factor receptor 2, N.A: not available, FSA: frozen section analysis, ITC: isolated tumor cells.

## Intraoperative FSA and permanent section analysis results

Intraoperative FSA and permanent section analysis results of identified SLNs are shown in ► **Table 2**. Taking permanent section analysis as the gold standard, there were 18.1% true positive (n = 95) and 73.1% true negative cases (n = 382) for FSA. 8.6% of patients had false negative results (n = 45) and none of the patients had a false positive result. Sensitivity, specificity, positive and negative predictive values were 67.8%, 100%, 100% and 89.4%. Accuracy of FSA was 91.3%. The division into cases with micrometastasis and macrometastasis revealed a significantly higher sensitivity of FSA in the macrometastasis group (76.8%) in comparison to the micrometastasis group (23.0%). Moreover, two

patients were reported with macrometastasis during FSA that were changed to micrometastasis in permanent section analysis.

Subgroup analysis was performed of patients eligible for Z0011 criteria (► **Table 3 a**), including comparison of ≤ 2 versus > 2 positive SLNs (► **Table 3 b**), and patients not fulfilling the Z0011 criteria (► **Table 3 c**). In patients eligible for Z0011 criteria, 14.4% SLNs were true positive (n = 48) and 80.1% were true negative (n = 267) with permanent section analysis being the gold standard (► **Table 3 a**). There were 5.4% false negative results (n = 18) and none false positive report. Sensitivity, specificity, positive and negative predictive values and accuracy were 72.7%, 100%, 100%, 93.6% and 94.5%. A comparison of the micrometastasis and the macrometastasis patient group showed a signifi-

► **Table 2** Cross tabulation between FSA and permanent section analysis (n = 522).

	TP, n (%)	FP, n (%)	FN, n (%)	TN, n (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Mi.	9* (1.7)	0 (0)	30 (5.7)	483 (92.5)	23.0	100.0	100.0	94.1	94.2
Ma.	83 (15.9)	2# (0.3)	25 (4.7)	412 (78.9)	76.8	99.5	97.6	94.2	94.8
Mi./Ma.	95 (18.1)	0 (0)	45 (8.6)	382 (73.1)	67.8	100.0	100.0	89.4	91.3

\* Two patients were reported with micrometastasis during FSA that were changed to macrometastasis in permanent section analysis.

# Two patients were reported with macrometastasis during FSA that were changed to micrometastasis in permanent section analysis.

Mi.: micrometastasis, Ma.: macrometastasis, TP: true positive, FP: false positive, FN: false negative, TN: true negative, PPV: positive predicted value, NPV: negative predicted value.

► **Table 3** Subgroup analysis.

	TP, n (%)	FP, n (%)	FN, n (%)	TN, n (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
<b>a</b> Cross tabulation between FSA and permanent section analysis of patients eligible for Z0011 criteria (n = 333).									
Mi.	6* (1.8)	0 (0)	12 (3.6)	315 (94.5)	33.3	100.0	100.0	96.3	96.3
Ma.	41 (12.3)	1# (0.3)	11 (3.3)	280 (84.0)	78.8	99.6	97.6	96.2	96.3
Mi./Ma.	48 (14.4)	0 (0)	18 (5.4)	267 (80.1)	72.7	100.0	100.0	93.6	94.5
<b>b</b> Cross tabulation between FSA and permanent section analysis of patients fulfilling the Z0011 criteria in regard to ≤ 2 versus > 2 positive SLNs (n = 333).									
Mi.	0 (0)	0 (0)	0 (0)	333 (100)	100.0	100.0	100.0	100.0	100.0
Ma.	2 (0.6)	0 (0)	0 (0)	331 (99.3)	100.0	100.0	100.0	100.0	100.0
Mi./Ma.	2 (0.6)	0 (0)	0 (0)	331 (99.3)	100.0	100.0	100.0	100.0	100.0
<b>c</b> Cross tabulation between FSA and permanent section analysis of patients not fulfilling the Z0011 criteria (n = 189).									
Mi.	3 (1.5)	0 (0)	18 (9.5)	168 (88.8)	14.2	100.0	100.0	90.3	90.4
Ma.	42 (22.2)	1# (0.5)	14 (7.4)	132 (69.8)	75.0	99.4	97.6	93.3	92.0
Mi./Ma.	46 (24.3)	0 (0)	28 (14.8)	115 (60.8)	62.1	100.0	100.0	82.5	85.1

\* Two patients were reported with micrometastasis during FSA that were changed to macrometastasis in permanent section analysis.

# One patient was reported with macrometastasis during FSA that was changed to micrometastasis in permanent section analysis.

Mi.: micrometastasis, Ma.: macrometastasis, TP: true positive, FP: false positive, FN: false negative, TN: true negative, PPV: positive predicted value, NPV: negative predicted value.

cantly higher sensitivity of the macrometastasis group (78.8% versus 33.3%). One patient was reported with macrometastasis during FSA that was changed into micrometastasis in permanent section analysis.

A cross tabulation between FSA and permanent section analysis of patients fulfilling the Z0011 criteria regarding to ≤ 2 versus > 2 positive SLNs was done (► **Table 3b**). Cross tabulation analysis revealed 99.3% true negative cases (n = 331) and 0.6% true positive cases (n = 2). There were no false positive or false negative cases leading into sensitivity, specificity, positive and negative predictive values of 100%.

In patients not fulfilling the Z0011 criteria, there were 24.3% true positive cases (n = 46) and 60.8% true negative cases (n = 115) (► **Table 3c**). 14.8% of SLNs were false negative (n = 28) without any false positive case. Sensitivity, specificity, positive and negative predictive values and accuracy were 62.1%, 100%, 100%, 82.5% and 85.1% in this group. The division into cases with micrometastasis and macrometastasis revealed a significantly higher sensitivity in the macrometastasis group (75.0%) in comparison

to the micrometastasis group (14.2%). Moreover, one patient was reported with macrometastasis during FSA that was changed into micrometastasis in permanent section analysis.

### Clinicopathological characteristics associated with false negative results for Z0011 ineligible patients

For Z0011 ineligible patients, clinicopathological characteristics associated with false negative results of FSA are demonstrated in ► **Table 4**. Univariate analysis revealed that lymphatic invasion (p = 0.004), number of positive SLNs (p = 0.005) and type of metastasis (p = 0.002) were significantly associated with false negative diagnosis compared to the control group. A trend was observed regarding the pT stage (p = 0.09).

### Relevance for intraoperative ALND decision

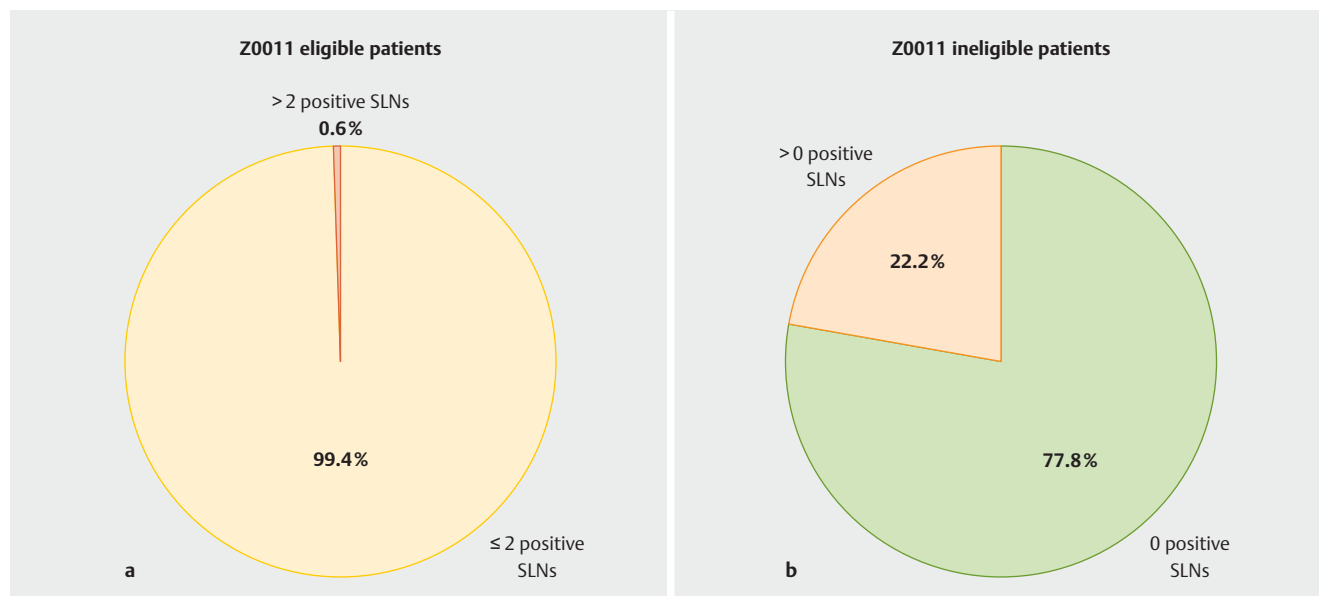
Case numbers of positive SLNs detected by FSA relevant for ALND decision are depicted in ► **Fig. 2a** and **b**. In the Z0011 eligible group, FSA detected ≤ 2 positive SLNs with macrometastasis in 99.4% of patients. Only 0.6% of patients showed > 2 positive SLNs

► **Table 4** Subgroup analysis: Clinicopathological characteristics associated with false negative versus accurate FSA results of patients not fulfilling the Z0011 criteria (n = 189).

Variable	Accurate FSA group (n = 161)	False negative FSA group (n = 28)	p-value*
Age	57.3 ± 13.2	57.6 ± 14.3	0.9 <sup>§</sup>
pT stage			
▪ T1	76 (47.2%)	6 (21.4%)	0.09 <sup>†</sup>
▪ T2	72 (44.7%)	21 (75%)	
▪ T3	13 (8.0%)	1 (3.5%)	
Histology			
▪ Ductal	120 (74.5%)	19 (67.8%)	0.8 <sup>‡</sup>
▪ Lobular	35 (21.7%)	6 (21.4%)	
▪ Other type	7 (4.3%)	3 (10.7%)	
Multifocality			
▪ Yes	41 (25.4%)	9 (32.1%)	0.6 <sup>‡</sup>
▪ No	120 (74.5%)	19 (67.8%)	
Histological grade			
▪ G1	13 (8.0%)	1 (3.5%)	0.1 <sup>†</sup>
▪ G2	111 (68.9%)	17 (60.7%)	
▪ G3	37 (22.9%)	10 (35.7%)	
ER			
▪ ER positive	144 (89.4%)	23 (82.1%)	0.6 <sup>‡</sup>
▪ ER negative	17 (10.5%)	5 (17.8%)	
PR			
▪ PR positive	128 (79.5%)	24 (85.7%)	0.6 <sup>‡</sup>
▪ PR negative	33 (20.4%)	4 (14.2%)	
HER2			
▪ HER2 positive	29 (18.0%)	7 (25%)	0.5 <sup>‡</sup>
▪ HER2 negative	131 (81.3%)	21 (75%)	
▪ N.A.	1 (0.6%)	0 (0%)	
L0	126 (78.2%)	14 (50%)	0.004 <sup>‡</sup>
L1	29 (18.0%)	12 (42.8%)	
N.A.	6 (3.7%)	2 (7.1%)	
V0	152 (94.4%)	27 (96.4%)	0.9 <sup>‡</sup>
V1	6 (3.7%)	1 (3.5%)	
N.A.	3 (1.8%)	0 (0%)	
SLNs from permanent section analysis			
▪ 1 positive SLN	22 (13.6%)	23 (82.1%)	0.005 <sup>†</sup>
▪ 2 positive SLNs	14 (8.6%)	5 (17.8%)	
▪ > 2 positive SLNs	6 (3.7%)	0 (0%)	
▪ Macrometastasis	42 (26.0%)	14 (50%)	0.0002 <sup>‡</sup>
▪ Micrometastasis	4 (2.4%)	14 (50%)	

\* All tests without unknowns, <sup>§</sup> Two-sided Mann–Whitney U-test, <sup>†</sup> Two-sided Cochran–Armitage test for trend, <sup>‡</sup> Two-sided chi-square test. Values are presented as mean ± standard deviation or number (%).

ER: estrogen receptor, PR: progesterone receptor, HER2: human epidermal growth factor receptor 2, L: lymphatic invasion, V: vascular invasion, SLN: sentinel lymph node, N.A: not available.



► **Fig. 2** FSA results relevant for intraoperative ALND decision of Z0011 eligible and ineligible patients. **a** In the Z0011 eligible patient group, FSA detected in 99.4% of patients  $\leq 2$  positive SLNs with macrometastasis and in 0.6% of patients  $> 2$  positive SLNs with macrometastasis. **b** In the Z0011 ineligible patient group, FSA detected in 77.8% of patients SLNs without macrometastasis and in 22.2% of patients SLNs with at least one macrometastasis.

with macrometastasis. In the Z0011 eligible group, FSA identified 77.8% of patients with no positive SLNs and 22.2% with at least one positive SLN for macrometastasis.

## Discussion

Intraoperative assessment of SLNs by FSA confers the benefit of allowing breast cancer patients with positive SLNs to avoid a second surgery by immediately proceeding to ALND. Whereas ALND was traditionally advised for all breast cancer patients with positive SLNs, the ACOSOG Z0011 and IBCSG 23-01 trials have demonstrated safety of omitting ALND in a substantial number of patients. Since then, the routine clinical intraoperative assessment of SLNs by FSA was called into question. This study evaluated the benefits and accuracy of FSA for SLNs for patients with eligible ACOSOG Z0011 criteria versus ineligible patients and identified factors associated with false negative results.

Several methods are used for the intraoperative assessment of axillary lymph nodes, including FSA, intraoperative cytology (IC) or one-step nucleic acid amplification (OSNA). In this regard, FSA is currently the most used method of intraoperative assessment of SLNs. Advantages of FSA are a greater sensitivity than IC [14] and the possibility to provide information on the size of metastases in regard to OSNA. Limitations of FSA include that it is an expensive and time-consuming procedure, the requirement of an experienced pathologist, the risk of destroying the diagnostic tissue for permanent section analysis and that FSA has not been standardized [15].

Performance of FSA of SLNs was investigated in various studies. According to the meta-analysis of Liu et al. (47 studies, for a total of 13 062 patients), the mean sensitivity for FSA was 73% (range

44–100%) with a mean specificity of 100% (range 98–100%) [11]. Another meta-analysis reported sensitivity rates ranging from 57–74% [16]. In both meta-analysis, intraoperative FSA was more reliable for detecting macrometastasis than for detecting micrometastasis. This is consistent with our study with an overall sensitivity and specificity of 67.8% and 100%. Moreover, the micrometastatic subgroup also demonstrated a great loss of sensitivity in regard to the macrometastatic subgroup (23.0% versus 76.8%). This might be mainly dependent on technical reasons such as the step size and the number of slices as well as sampling errors [17]. The IBCSG 23-01 trial showed no benefit of performing ALND compared to omitting ALND in patients with SLNs containing micrometastasis. Thus, the necessity for intraoperative SLN micrometastasis detection can be questioned and the low sensitivity for micrometastasis identification might not be clinically relevant.

With regard to FSA performance in patients meeting Z0011 eligible criteria versus ineligible patients, subgroup analyses are missing so far. The division of patients into Z0011 eligible versus ineligible groups, as performed in our study, has become important since the routine performance of FSA for intraoperative SLN assessment was queried by the ACOSOG Z0011 and the IBCSG 23-01 trials [7, 8]. The ACOSOG Z0011 trial revealed no difference in local or regional recurrence between patients with 1–2 positive SLNs who were randomized into a SLNB only or SLNB and ALND group [8]. Moreover, they also showed that the use of SLNB alone compared with ALND did not result in minor survival in a limited patient population (cN0, tumor size  $< 2$  cm and 1–2 positive SLNs). The findings were supported by the IBCSG 23-01 trial, which also demonstrated that ALND could be avoided in patients with early breast cancer and limited SLN involvement [7]. These

trials changed axillary management in many guidelines and lead to a progressive decline in the use of FSA for SLNs. Cipolla et al. recently demonstrated that FSA was only useful in 7.7% of the patients who met the criteria of the IBCSG 23-01 and ACOSOG Z0011 trials [18]. In our study, intraoperative FSA prevented a delayed ALND in 0.6% of Z0011 eligible patients only and 22.2% of Z0011 ineligible patients. The overall sensitivity of SLNs-FSA from patients not fulfilling the Z0011 criteria was reduced by 10% compared to the Z0011 eligible group. This was due to the lower sensitivity of detecting micrometastasis in the Z0011 ineligible group. The current clinically decision-relevant macrometastatic sensitivity rates did not significantly differ between both groups. In this regard, the additional cut off of 2 positive SLNs plays an important role in the Z0011 eligible patient group for ALND decision. To date, published studies only compared positive versus negative SLNs in regard to FSA performance without considering the precise number of affected SLNs. For the first time, a subgroup analysis was therefore performed in this study containing the cut off of 2 positive SLNs. Importantly, subgroup analysis of  $\leq 2$  versus  $> 2$  positive SLNs increased both specificity and sensitivity to 100% in the Z0011 eligible patient group.

The greatest drawback of FSA results is the frequency of FN cases because of which these patients are still subject to recall for ALND. Reports revealed FN rates of FSA up to 33% with a higher frequency of cases with micrometastasis [19]. In our study, the overall FN cases were 8.6% with lower FN cases in the Z0011 eligible patient group compared to the Z0011 ineligible patient group. Notably, there were no FN cases in the Z0011 patient group when the cut off of 2 was included. A few studies have tried to determine predictive factors for FN cases of FSA by univariate and multivariate analysis. Analysis of different patient subgroups, e.g. Z0011 eligible or Z0011 ineligible patients' cohorts, have not been done so far. An invasive lobular histology and lymphovascular invasion were found to be independent predictors for FN cases [20]. Takei et al. identified a positive PR and a low nuclear grade as patients' favorable prognostic factors, while an unfavorable prognostic factor was positive lymphovascular invasion [21]. In another study, positive ER and PR status were favorable prognostic factors whereas young age was an unfavorable prognostic factor [22]. In a recent study, multifocality, lymphovascular invasion, tumor size and biological subtype were independent factors [23]. In our study, FN rates were significantly dependent on size of SLN metastasis, lymphatic invasion, number of positive SLNs and a trend was observed regarding tumor size. In contrast to recent studies, analysis of predictive factors for FN cases was only performed for the Z0011 ineligible subgroup in our study, due to none FN case of patients fulfilling the Z0011 criteria in regard to  $\leq 2$  versus  $> 2$  positive SLNs, that should be taken into account for already described predictive factors. Further general factors regarding FN rates are technical limitations, institutional experience, sampling error or tissue loss [24, 25]. However, a metastatic lesion so small that it would be exhausted at the time of FSA indicates a very small LN tumor burden that would be questionable in terms of clinical significance.

## Conclusion

Management of the axilla in breast cancer is still evolving and FSA analysis can guide de-escalation of axillary management. In our study, FSA was highly sensitive for macrometastasis in both the ACOSOG Z0011 eligible and ineligible patient group whereas both patient groups showed a low sensitivity of FSA for micrometastasis. Several clinicopathologic factors were associated with a higher rate of false negative results in the ACOSOG Z0011 ineligible patient group. Importantly for Z0011 eligible patients, subgroup analysis of  $\leq 2$  versus  $> 2$  positive SLNs demonstrated a 100% specificity and sensitivity preventing second stage ALND. Moreover, our study showed that for selective breast cancer patients (e.g. ACOSOG Z0011 eligible patients without any high-risk features for a higher propensity for axillary lymph node metastasis), intraoperative assessment of SLNs by FSA might be unnecessary whereas FSA continues to be especially beneficial in the ACOSOG Z0011 ineligible group to avoid second stage ALND.

## Declarations

**Ethics approval and consent to participate:** The study was carried out in accordance with Good Clinical Practice guidelines and was approved by the Ethics Committee of the Medical Faculty of the Heinrich-Heine-University Duesseldorf (Ref-No: 4409). Written informed consent was obtained from the patients.

**Consent for publication:** Not applicable.

**Availability of data and materials:** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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**Author's contributions:** F.R. and S.M. were responsible for conceptual design of the study. A.F. collected the data. F.R. conducted the primary data analyses and provided the primary data interpretation. F.R. drafted the manuscript and prepared the figures and tables. A.F., F.B., P.K., I.E., K.M.-G., M.D., V.F., N.K., T.K., J.H., E.R., T.F., K.R., S.M. provided critique to refine the manuscript. All authors read and approved the final version of this manuscript.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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