Breast cancer patients with micrometastases in sentinel lymph nodes: differences considering additional metastatic lymph nodes

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Summary

Aims: Characterization of breast cancer patients with micrometastases in sentinel lymph node (SLN) and establish differences between micrometastatic breast cancers with additional metastatic lymph nodes (LNS) versus no other lymph node invasion. *Methods:* Analysis of 30 breast cancers, N1mi or pN0(i+), diagnosed and treated in our department from July 2000 to July 2008. *Results:* Micrometastases in SLNs were found in 30 patients. Complete axillary dissection revealed other metastatic LNs in 24%. Concerning breast cancers with additional LN invasion versus no other LN invasion, tumors located in the superior-external quadrant were more frequent in the former group. Other characteristics as clinical presentation, histological subtype, focality, cytonuclear grade, hormone receptors and Her2 expression were not significantly different in either group. Regarding SLN invasion, the presence of at least two micrometastatic foci were significantly more relevant in patients with other metastatic LN invasion ($p < 0.01$). Micrometastases diagnosed only after immunohistochemistry (IHC) were exclusively found in patients without other LN invasion, reaching statistical significance ($p < 0.05$). *Conclusions:* Complete axillary dissection revealed additional LN invasion in 24% of patients with micrometastases in the SLN. Tumors with additional LN invasion were more frequently found in the superior external quadrant and SLNs harbored at least two micrometastatic foci. Micrometastases diagnosed exclusively by IHC techniques were more relevant in cases without additional lymph node invasion.

Key words: Micrometastases; Sentinel lymph node.

Introduction

Lymph node (LN) metastases are considered the most important prognostic factor in breast cancer patients [1, 2]. Axillary LN status is determined after histological examination of at least ten LNs are surgically removed [3]. Axillary dissection was first considered a therapeutic intervention to eliminate disease from regional LNs [4]. It later became the only surgical staging procedure available [5]. With the advancement of sentinel lymph node (SLN) biopsy in early breast cancers (T0-T2, N0), this same information can be obtained by removal of only a few nodes, avoiding much of the morbidity associated with axillary dissection [6]. Histopathologic examination of SLNs is more detailed than traditional examination of axillary nodes [1, 2]. Further axillary surgery depends on SLN status. Serial sectioning of SLNs and use of immunohistochemistry (IHC) with cytokeratin have led to increased detection of minimal lymph node involvement, classified as micrometastases (0.2-2 mm; pN1 (mi)) and isolated tumor cells (ITCs) (< 0.2 mm; pN0(i+)) [1, 7].

After detection of micrometastases that the SLN has incorporated into its staging system, many breast tumors become upstaged [8]. The significance of micrometastases remains controversial. Value of complete axillary dissection after finding micrometastases is still not uniformly defined. It would be of great value to identify a guide for estimating risk of non SLN involvement.

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The aims of this study were to identify parameters more associated with other LN involvement in breast cancer patients with micrometastases in SLN. We also intended to characterize breast cancers with micrometastases in SLN and prevalence of additional metastatic invasion after axillary dissection.

Methods

Analysis of 30 breast cancer patients with micrometastases in SLNs diagnosed in our department from July 2000 to July 2008 was carried out.

In breast cancers (T0-T2) with clinical and imaging negative axillary nodes, SLN biopsy is performed using a blue dye technique.

Twenty-five patients with micrometastatic SLNs were submitted to axillary dissection. Clinical and histological parameters were compared in cases with other metastatic LNs versus no other metastatic LNs besides micrometastatic SLN.

The analytic data included patient characteristics (age, hormonal status) and clinical characteristics (presentation, location). Histopathological data studied breast tumor histological type, size, cytonuclear (Bloom Richardson) grade, focality and hormone (estrogen and progesterone) receptors. SLN was histologically analyzed after serial section (100 μm thickness) using classical staining – hematoxylin and eosin (H&E) and IHC (MNF116) when classic staining was negative or inconclusive. Lymph nodes obtained from later axillary dissection were studied to identify additional invasion.

Descriptive statistics are reported as frequencies, percentages, means and standard deviations. Distribution of categorical

variables were compared using the chi-square test. Parametric variables were tested using Student's t-test. Statistical analysis was performed using SPSS version 15.0.

Results

Micrometastases in SLNs were found in 30 patients. Mean age was 55.2 ± 12.0 (40-81) and 50% post-menopausal with a mean menopausal age of 50.7 ± 3.1 (45-56). Tumors presented clinically as palpable lesions in 22 cases (74%). The remaining patients showed image (mammography or mammary ultrasound) alterations (n = 8). Lesions were described in the following quadrants: superior-external in 60% (n = 18), superior-internal in 20% (n = 6), inferior-internal in 10% (n = 3), inferior external in 3% (n = 1) and retroareolar in 7% (n = 2). Histology revealed mainly ductal invasive carcinoma (n = 26) but also two invasive lobular carcinomas and another two mucinous invasive carcinomas were reported. Multifocal lesions were found in 21%. Tumors were pT1 in 25 cases (83%) and pT2 in five cases (17%). Concerning cytonuclear grade, G2 tumors were found in 73% (n = 22) and G1 (n = 4) and G3 (n = 4) tumors in 13% each. Lymphovascular invasion was present in four tumors (13%). Estrogen receptors (ERs) were positive in 97% (n = 29). Progesterone receptors (PRs) were positive in 68% (n = 17) and negative in 32% (n = 8). Cerb2 was positive in 21% (n = 6) and negative in 89% (n = 22).

Micrometastases in SLN measured between 0.2-2 mm in 27 patients. In the remaining three cases ITCs were found (≤ 0.2 mm). Considering the focality of micrometastases in SLNs, 25 were related only to one focus and in the other five at least two foci were noted. Diagnosis of micrometastases was performed using H&E in 23 cases and IHC in the seven cases with negative standard staining.

Completion of axillary dissection was performed in 25 patients after SLN analysis. On average 13.9 LNs were removed. Six patients (24%) had additional metastatic invasion revealed by axillary dissection, including two (8%) macrometastases (≥ 2 mm) and four (16%) micrometastases (≤ 2 mm). Additional micrometastatic LNs harbored one focus in two cases (8%) and two foci in another two cases (8%). Table 1 summarizes the comparison between tumors with SLN micrometastases with additional metastases versus without other metastatic LNs reported in axillary dissection. Significant differences were found considering the presence of tumors in the superior-external quadrant. Table 2 correlates the number of micrometastatic foci and additional LN invasion. The presence of at least two micrometastatic foci in SLN was more frequent in patients with other metastatic LNs. Table 3 compares the method of detecting micrometastases and implications considering other LN invasion. Diagnosis of micrometastases by IHC (negative H&E) was more associated in patients without other metastatic LNs, reaching statistical significance ($p < 0.05$).

Table 1. — *Comparison between micrometastatic tumors without additional LN invasion vs with additional LN invasion. (LN: lymph node; SLN: sentinel lymph node; n.s.: non-significant; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; IMC: invasive mucinous carcinoma; DCIS: ductal carcinoma in-situ; ER: estrogens receptor; PR: progesterone receptor).*

	Micrometastases in SLN		
	Other LN invasion n = 6 24%	Without LN invasion n = 19 16%	*p* value
Premenopausal/post-menopausal	3/3 50%/50%	8/11 42%/58%	n.s.
Clinical presentation palpable lump/imaging alteration	5/1 83%/17%	16/3 84%/16%	n.s.
Location (quadrant): superior external/other	6 100%	8/11 42%/58%	< 0.005
IDC/ILC/IMC	5/1 83%/17%	16/1/2 84%/11%	n.s.
Multifocal	0	4 21%	n.s.
DCIS associated	5 83%	14 74%	n.s.
G1	2 (33%)	2 (11%)	n.s.
G2	4 (67%)	14 (74%)	n.s.
G3	0	3 (15%)	n.s.
pT1/pT2	5/1 83%/17%	16/3 85%/15%	n.s.
Lymphovascular invasion	2 33%	2 11%	n.s.
ER+/-	6 100%	18/1 95%/5%	n.s.
PR+/-	5/1 83%/17%	10/4 71%/29%	n.s.
Cerb2+/-	0/6 0/100%	5/12 30%/70%	n.s.

Table 2. — *Number of micrometastatic foci and correlation with LN invasion. (LN: lymph node; SLN: sentinel lymph node).*

	Micrometastases in SLN		
	Other LN invasion n = 6 24%	Without LN invasion n = 19 16%	*p* value
Micrometastatic focus in SLN	2 33%	18 95%	
1	4 67%	1 5%	< 0.01
≥ 2			

Table 3. — *Method of detecting micrometastases in SLNs and correlation with presence of additional LN invasion. (SLN: sentinel lymph node; LN: lymph node; H&E: Hematoxylin and Eosin; IHC: immunohistochemistry).*

Histological analysis SLN	Micrometastases in SLN		
	Other LN invasion n = 6 24%	Without LN invasion n = 19 16%	*p* value
H&E+	6 100%	14 74%	
1			< 0.05
H&E-	0	5	
IHQ (MNF16)+	0%	26%	

Discussion

SLN biopsy aims to allow a correct staging procedure, avoiding the need of complete axillary dissection in cases without SLN invasion. A meticulous pathologic study is performed in SLNs, detecting the presence of metastatic disease as small as 0.2 mm.

In this series, in face of micrometastases in SLNs, we found an incidence of 24% additional LN invasion. Several studies suggested that presence of micrometastases in SLNs correlates with absence of metastases in non-sentinel LNs [8, 9]. Langer *et al.* reported in a prospective trial that SLN micrometastases do not harbor risk of axillary recurrence or distant disease [11]. This emphasizes the idea of avoiding further axillary dissections. In contrast, other investigators suggested a relevant percentage of non-SLN involvement in micrometastatic SLNs [12, 13], reaching 26% [14]. A recent meta-analysis reported the risk of non-SLN metastases associated with micrometastatic disease in SLNs of around 10-15%, depending on the method of detecting SLNs [15].

The greatest aim with a micrometastatic SLN is to identify patients at risk of additional LN invasion. Considering patients submitted to complete axillary dissection (n = 25), we compared clinical and histological parameters between tumors with other LN invasion (n = 6) and those without other LN invasion (n = 19). We evaluated, tumor characteristics, hormonal stage, clinical presentation, location (quadrant), histological subtype, focality, DCIS associated, cytonuclear grade, stage, hormonal receptors (ER, PR) and Her2 expression. Significant differences between breast cancers with other metastatic invasion besides the SLN were related to primary tumor location, with the superior-external quadrant being significantly more associated with additional invasion. Other parameters were not significantly different (Table 1).

Some previous studies have emphasized the role of some tumor aspects, as tumor size and lymphovascular invasion, as predictive factors for the presence of metastatic LNs [8, 16]. In a multicentric study predictive factors were pT stage, menopausal status, grade, lymphovascular invasion and histological type [17]. One study reported micrometastases from invasive lobular carcinoma was related with other LN involvement; on the contrary all invasive ductal carcinomas had negative axillary dissection [18].

Our results emphasize the location of the primary tumor as the only predictive factor, considering primary tumor characteristics for additional LN invasion. Lymph flows unidirectionally from the superficial to deep plexus and from the de subareolar plexus through the lymphatic vessels of the lactiferous ducts to the perilobular and deep subcutaneous plexus [19]. Intramammary lymphatic vessels move centrifugally toward axillary and internal mammary lymph nodes [19]. With this drainage system in mind, it seems logical that tumors located in the superior external quadrant originate more frequently from small metastatic foci in the axillary lymph nodes. In consonance with the centrifugal movement in lymphatic vessels, metastases from tumors with this topographic location flow a shorter way toward the axilla.

Focusing on SLN analysis, the presence of at least two micrometastatic foci was significantly more associated with additional axillary invasion. It has been reported that risk of non-SLN metastasis is related to the size of disease in SLNs [1, 20], being the greatest for macrometastases, intermediate for micrometastases, and the least for ITCs (14.8%), more frequently detected by IHC [21]. Besides classical H&E, the advent of IHC directed to cytoqueratin can identify up to 34% initially negative SLNs [22, 23]. With IHC we could identify more (7/30) micrometastatic SLNs. In our study, micrometastases detected by IHC were not associated with additional metastatic invasion besides the SLN, reaching statistical significance. These results emphasize the idea that SLN metastases diagnosed by routine H&E have a higher risk of non-SLN metastases than metastases detected by IHC, reflecting a larger metastatic size in the former group [24]. Studies confirm the increase incidence in the detection of micrometastases by IHC [1, 17, 21]. The prospective use of IHC by Rydén *et al.* only showed stage migration in 3/132 cases [1]. All these reports supports the idea that patients with micrometastases detected by IHC have a low risk of additional LN invasion.

Controversy exists surrounding the best management of patients with SLN micrometastases. In approximately 80% of patients with SLN micrometastases, the SLN is the only LN involved [20]. These particular patients would not benefit from further axillary surgery. Recent studies report that selected patients with micrometastases without further axillary dissection will not suffer from a higher incidence of regional recurrence [25, 26].

Various studies suggest that the prognosis of breast cancer patients with micrometastases should not be considered the same as that of truly node-negative patients (pN0), with a poorer survival for micrometastatic patients [27, 28]. This means that minimal LN invasion cannot be safely overlooked, particularly when tumors are located in the superior-external quadrant and SLN harbors at least two micrometastatic foci, as we proved in our results. This is the only possible way to solve this problem - the identification of predictive factors associated with LN invasion besides SLN micrometastases. The real clinical impact of micrometastases will only be assured after results of on-going randomized controlled trials are known [29, 30].

Conclusions

In this study the incidence of additional LN invasion in face of micrometastatic SLNs was 24%. Primary tumor characteristics predictive of other metastatic involvement besides SLNs was the superior-external quadrant location, reaching statistical significance. Other parameters like hormone stage, clinical presentation, histological type, focality, cytonuclear grade, hormone receptors and Her2 expression were not significantly different. Consid-

ering analyzes of SLN, the presence of at least two metastatic SLN foci was significantly more frequent in patients with additional LN invasion. Finally, micrometastases detected by IHC with negative H&E were exclusively reported in patients without other LN invasion, also statistically significant.

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