The characteristics of the sentinel lymph node metastasis in predicting the axillary lymph node status in patients with breast carcinoma

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Introduction: Lymph node metastases are the most significant prognostic factors in patients with breast carcinoma. A positive sentinel lymph node (SLN) biopsy is followed by an axillary lymph node (ALN) dissection. In sentinel lymph node negative cases the risk of positive non-sentinel ALN is very low though not absent.
The aim of this study was to determine predictive factors for non-sentinel lymph node metastases on the basis of sentinel lymph node metastasis characteristics as well as features of the primary tumour.
Material and methods: 128 patients who had a positive SLN biopsy for breast carcinoma in 2005-2007 were identified. The breast carcinoma metastases in each SLN were assessed according to their location within the node (subcapsular, mixed subcapsular and parenchymal, parenchymal, multifocal or extensive) and metastatic infiltration of perinodal tissue was also reported. These data were correlated with the ALN involvement and characteristics of the primary tumour.
Results: The strong predictors of the ALN metastasis included the SLN metastasis diameter (7.6 vs. 4.4 mm) and size classified according to WHO classification (ITC 0 vs. 100%, micrometastasis 23.5 vs. 76.5%, macrometastasis 51.9 vs. 48.1%). The SLN metastases with a diameter of above 3 mm were associated with approximately twice more frequent ALN metastases. In an extensive location of SLN metastasis the highest percentage of ALN metastases was found (65 vs. 35%). The weak predictors of ALN metastases were: primary tumor diameter (> 2 cm), immunohistochemical HER2 positive status, infiltration of sentinel perinodal tissue by metastasis, histological primary tumour grade.
Conclusions: Some additional details, which can be easily evaluated in a routine SLN examination in breast carcinoma, have a predictive value of the ALN metastatic status and should be included in the histopathological report.

Key words: breast carcinoma, sentinel lymph node, axillary lymph node metastases, microanatomic location of metastasis.

Introduction

An invasive breast carcinoma is the most common carcinoma of women. It accounts for 22% of female carcinomas, 26% in affluent countries, which is more than twice the occurrence of carcinoma in women at any other site [1, 2]. In Poland, statistically one of eight women will be confronted with breast carcinoma during her life. It is assessed that the incidence of breast carcinoma is 84.2 per 100,000 with a mortality of 26.5 per 100,000 [3]. Nevertheless, due to screening and new therapeutic

possibilities (adjuvant systemic and hormone treatment) the overall survival is gradually improving, over the past few years approximately by 7-11% [2, 3].

A sentinel lymph node (SLN) biopsy for breast carcinoma is a standard surgical procedure. The proper assessment of the SLN metastasis is essential for making decisions about the avoidance of unnecessary axillary lymph node (ALN) dissection and the qualification of the patients for an adequate adjuvant therapy. It will decrease morbidity and adverse effects after ALN dissection (arm lymphedema in 10-30% and pain in 10-20% of patients) and moreover improve the quality of life of patients [4, 5]. The predictive negative value of the SLN biopsy in staging patients with clinically node-negative breast carcinoma allows nearly 65-75% of patients to be spared ALN dissection and it is suggested to have a strong association with morbidity [6]. On the other hand, ALN involvement is not identified in a half of SLN positive cases; approximately 50% of patients will have additional non-sentinel axillary node metastases [7, 8]. The results of the American College of Surgeons: Oncology Group (ACOSOG) after 5-year observation of patients with SLN metastases who were randomly appointed to no additional surgery or to complete ALN dissection to assess differences in axillary recurrences and survival were not sufficiently satisfactory. The number of axillary recurrences in a group without ALN dissection was lower than expected [9].

Several features of the primary tumour were investigated for their possible value in predicting the risk for further axillary involvement, i.e. histological type, size, receptors' status [10-14]. Because of the lack of standardization of the investigational procedures the conclusions from different studies lead to incoherent results [15]. Independently, the SLN characteristics including the size of metastasis, the percentage of positive SLNs and extracapsular extension of metastasis [16-18] influence the ALN status. Though, none of these just by themselves can be a hallmark to identify a group of patients for whom ALND is unnecessary. In the latest edition of TNM classification of breast carcinoma [19] the patients with isolated tumour cells (ITC) in the regional lymph nodes are classified separately as pN0 (i+) category. The definition of ITC is single tumour cells of small clusters of cells, not more than 0.2 mm at the greatest dimension, which do not show evidence of any metastatic activity of penetration of vascular or lymphatic sinus walls [20]. This category was established for prevention of overstaging and hence, overtreatment of the patients. The ITC and micrometastases pose a clinical dilemma with regard to adjuvant treatment decisions because their prognostic meaning is currently unclear.

Moreover, the methods of the pathologic examination of SLNs and detection of metastasis still remain controversial [21]. There are differences between procedures starting from the preparation techniques of material, cutting and metastasis evaluation.

The studies under microanatomic location of metastasis in SLN were initiated in melanoma. The results were promising. Most of the authors have confirmed a predictive value of SLN metastasis location in evaluation of the non-sentinel lymph node status [22-24].

The aim of this study was to evaluate the predictive value of SLN metastasis features, i.e. metastasis location as well as clinicopathological data on the ALN status.

Material and methods

The material consisting of both SLN and ALN were obtained from patients who underwent surgery for breast carcinoma (breast-conserving surgery or mastectomy) between January 2005 and February 2007 at the Breast Cancer Department of the Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology. The criteria of the patients' exclusion were: more than 6 SLN, bilateral breast carcinoma, neoadjuvant chemotherapy, histopathological consultation only, significant lack of data.

SLNs were identified using a standard technique of preoperative dynamic lymphoscintigraphy using radiolabeled colloid, followed by an intraoperative injection of vital blue dye and use of a hand-held gamma probe. The SLNs were removed and sent to the Department of Pathology. After fixation in 10%

Table I. The definitions of microanatomic locations

Location	Definition	Scheme
Subcapsular	Subcapsular sinus only	
Mixed	Subcapsular and parenchymal	
Parenchymal	Paracortical area of parenchyma without contact with capsule smaller than half of the node	
Multifocal	Multiple discrete deposits	
Extensive	Deposit larger than half of the node	
Extracapsular spread		

buffered formalin they were serially sectioned at 2-mm intervals, perpendicular to the long axis of the node and processed according to standard procedures. Routine haematoxylin and eosin (H&E) staining was performed. The SLNs were evaluated separately by two pathologists. SNL characteristics included metastatic, size both as a maximal diameter of the largest metastases and its classification according to the 6th edition of WHO staging system of metastasis, its location and perinodal tissue infiltration by metastasis [19]. The definitions of microanatomic locations are summarized in Table I.

Table II. The clinical and histopathological data of the primary tumour

Feature	N	%
Mean age (SD/range)	53.9 years (10.41/21-81)	
Side of the primary tumour:		
Left	72	56.3
Right	56	43.7
Invasive carcinoma subtype:		
Ductal	83	64.8
Lobular	24	18.8
Other	21	16.4
Grading		
1	29	22.6
2	65	50.8
3	34	26.6
Mean tumour size (SD/range)	1.9 cm (0.87/0.2-5)	
pT		
1	69	53.9
1a	2	1.5
1b	12	9.4
1c	55	43.0
2	44	34.4
3	1	0.8
4	4	3.1
X	10	7.8
Immunohistochemical status of the steroid receptor and HER2		
ER(–)PGR(–)HER2(–)	7	5.5
ER(–)PGR(–)HER2(1+, 2+)	2	1.5
ER(–)PGR(–)HER2(3+)	7	5.5
ER(+)PGR(+)HER2(–)	48	37.5
ER(+)PGR(+)HER2(1+, 2+)	42	32.8
ER(+)PGR(+)HER2(3+)	22	17.2

ALNs were bivalved along the long axis. The routine pathological analysis was performed with formalin-fixed paraffin-embedded sections which were stained with H&E, then they were evaluated microscopically and reported as positive or negative for carcinoma metastasis.

From the institutional breast carcinoma registry, retrospectively some clinical data and features of the primary tumour were obtained for analysis.

The statistical analysis was performed using Statistica 7.0 (StatSoft Inc, USA) and Excel 2003 (Microsoft Corp, USA). The Kruskal-Wallis ANOVA, Mann-Whitney U, χ^2 test and Spearman's rank correlation coefficient were used where appropriate. The cut-off point for statistical significance was $p < 0.005$ or $p < 0.001$ for a strong statistical significance.

Results

Between January 2005 and February 2007, 128 patients with a positive SLN biopsy were reported and all of them had ALN dissection performed. The summary of clinical and histopathological data of the primary tumour is presented in Table II. Table III summarises the characteristics of SLN metastases.

All data were analyzed between two groups: with and without ALN metastases. The strongly

Table III. The summary of SLN metastasis features

Feature	N	%
Mean number of SLN	1.7	
Mean number of SLN with metastases		
Number of SLN with metastases	145	
1	121	83.4
2	20	13.8
3	4	2.8
Mean diameter of SLN metastases (range)	5.8 mm (0.09-26 mm)	
WHO classification of the SLN metastasis size		
ITC	3	2.1
Micrometastasis	34	23.4
Macrometastasis	108	74.5
SLN metastasis location		
Subcapsular	33	22.8
Mixed	25	17.2
Parenchymal	8	5.5
Multifocal	19	13.1
Extensive	60	41.4
Extracapsular extension	52	35.9

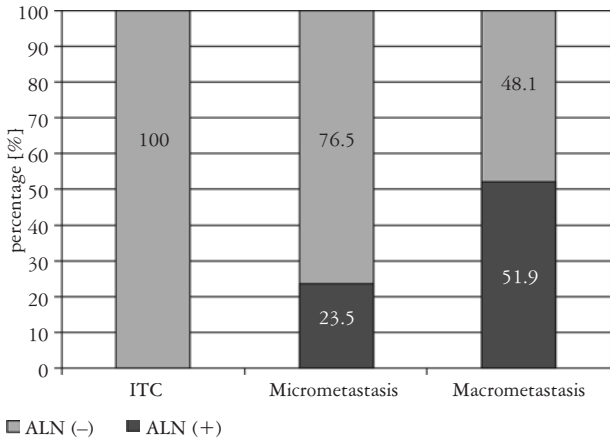

Fig. 1. The percentage of ALN metastases in subgroups assigned according to the WHO classification of SLN metastasis size ($p < 0.001$)

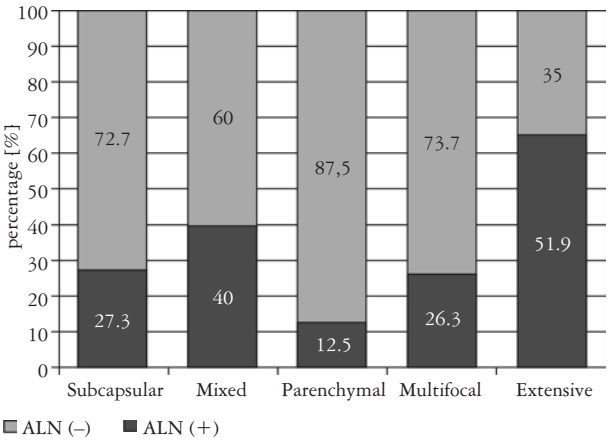

Fig. 2. The percentage of ALN metastases in particular locations of SLN metastasis ($p < 0.001$)

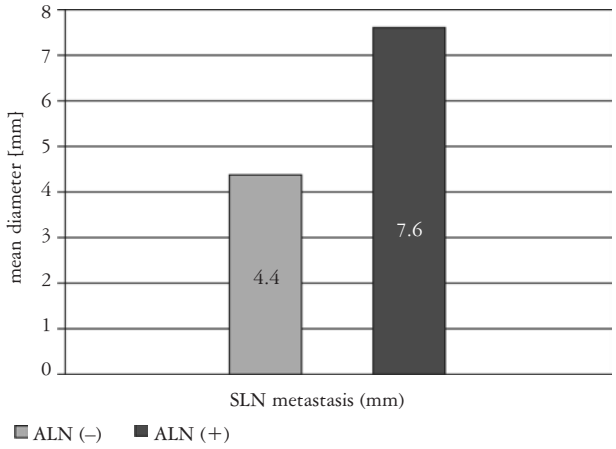

Fig. 3. The mean diameter of SLN metastasis in ALN with and without metastasis ($p < 0.001$)

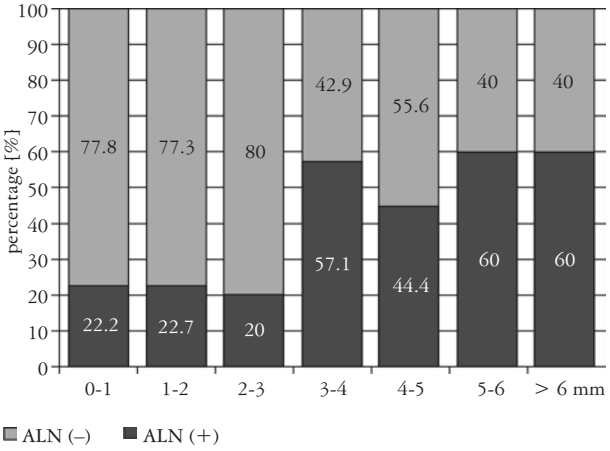

Fig. 4. The percentage of ALN metastasis in connection with the size (mm) of SLN metastasis ($p < 0.001$)

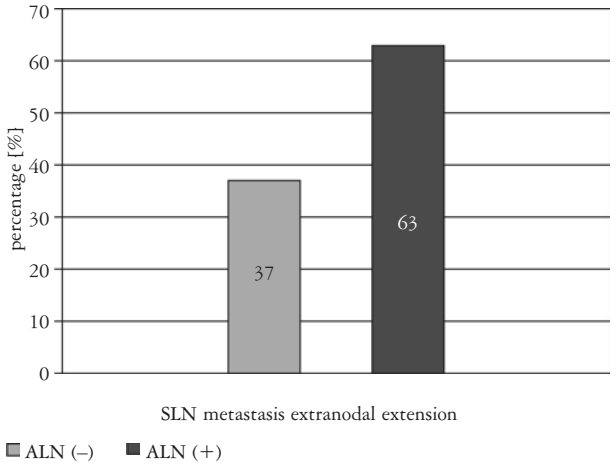

Fig. 5. The extranodal extension of SLN metastasis in comparison with an ALN involvement

significant statistical differences between groups were presented in Fig. 1-5.

The slight significant differences ($p < 0.05$) were found between subgroups with and without ALN metastasis and the primary tumour diameter (> 2 cm), immunohistochemical HER2 positive status, histological grading of the primary tumour and infiltration of perinodal tissue.

Discussion

Sentinel lymph node biopsy is a rapidly emerging treatment option for the patient with an early stage invasive breast carcinoma and clinically negative axillary lymph nodes. With experience, using radioisotope, blue dye or both, SLNs are successfully localized in more than 90% of cases [25, 26]. There are convincing arguments that the lymph node

metastases in SLN may be a source of further metastases to subsequent lymph nodes. Most of the latest investigations are related to predicting the risk for non-SLN metastases by evaluation of SLN characteristics. The SLNs are evidently the primary targets of lymphatic metastases and the sources of further dissemination to secondary lymph nodes. In solid malignancies, neoplasmatic cells initially flow in the subcapsular sinuses through an afferent capsular lymph node vessel. Subsequently, the malignant cells spread in marginal sinuses, cortical parenchyma and eventually invade lymph node parenchyma [27, 28]. On the basis of the metastatic cell migration route in the lymph node, briefly presented above, the studies concerning the microanatomic location of metastatic deposits and their predictive value in non-SLN involvement, have started.

The first reports which embrace both, the SLN metastasis size and its microanatomic location were referred to melanoma. Starz *et al.* [22] proposed S-staging concept – the classification based on two parameters: the number of 1-mm-thin SLN slices and the maximum distance of tumour cells to the inferior margin of the lymph node capsule. Regardless of the correlation between the recommended classification and non-SLN involvement, consecutive studies on larger groups of patients did not confirm its predictive value. In studies of Reeves *et al*. [23] from many characteristics of SLN melanoma metastases only two were significantly associated with the positive non-SLN status: the size of metastasis and ulceration of primary melanoma. Dewar *et al*. [24] suggested the microanatomic classification of melanoma metastasis to SLN which predicted more accurately a non-SLN involvement than the size and depth of metastases alone. The patients with only subcapsular deposits did not present any non-SLN metastases. Therefore, the authors concluded that the completion lymphadenectomy among patients with subcapsular SLNs metastases might be safely avoided. On the contrary, in Frankel *et al.* study [29], the location of metastases showed no correlation with positive non-SLN involvement. The independent predictive value in that report has Breslow thickness over 4 mm, the presence of angiolymphatic invasion, satellitosis, extranodal extension, three or more positive SLN and tumour burden within the SLN over 1%.

The predictive value of selective characteristics of SLN metastases was evaluated in breast carcinoma as well. Viale *et al.* [18] affirmed, in a multivariate analysis, that further axillary involvement was significantly associated with the type and size of SLN metastases, the number of affected SLNs and the occurrence of peritumoral vascular invasion in the primary tumour. In our study the mean maximal diameter was statistically nearly twice greater if ALNs were positive (4.6 mm vs. 7.6 mm). The percentage of ALN metastases in subgroups assigned according to the WHO classification of the SLN metastasis size (1) was similar to results obtained by Viale *et al.* and van Deurzen *et al.* [18, 30]. The analysis of SLN metastasis in 1 mm size interval has revealed that SLN metastasis above 3 mm was correlated with an over double increase of the ALN metastasis incidence (20-22.7% vs. 44.4-60% of positive ALN). Our results seem to be convergent with the recent studies on the prognostic value of micrometastases in SLN of breast carcinoma patients. Gobardhan *et al.* [31] concluded that although the risk of distant metastases was higher in patients with micrometastases than in the pN_0 or pN_{ITC} no statistically significant differences in overall or disease-free survival between these three groups were observed. At present micrometastatic lymph node involvement in itself is not an indication for adjuvant chemotherapy in breast carcinoma. Recently, a study related to the microanatomic location of SLN metastases in breast carcinoma was presented by van Deurzen *et al.* [30]. Patients with subcapsular, combined subcapsular and parenchymal, parenchymal and extensive tumour deposits showed an ALN involvement in 25%, 42%, 27% and 54% of cases, respectively. Our results are similar and amount to 27.3%, 40%, 12.5% and 65% adequately; in addition, the multifocal location was assessed and the percentage of positive ALNs in that group was 26.5%. The extranodal extension of SLN metastases was significantly higher in a group with positive ALNs (63% vs. 37%).

After a review of up-to-date literature concerning SLN in melanoma and breast carcinoma persistent questions about the appreciable differences of sectioning protocols and their influence on results, are not yet answered entirely. The latest report presented by Riber-Hansen R *et al.* focused on "the protocol trap" [32]. The authors compared the differences between results of the maximal metastasis diameter, maximum centripetal tumour depth, microanatomic location of metastases and complete metastasis volume in positive SLN from melanoma patients when complete step-sectioning or less extensive protocols of sectioning were applied. The results showed that adding extra steps to pathology protocols when assessing semiquantitative parameters led to unidirectional stage migration, i.e. the number of SLN-positive patients was increased by up to 41% using complete step-sectioning compared with less extensive protocols as well as the maximum metastasis diameter and maximum centripetal tumour depth were up-staged.

In conclusion, some additional details (maximal metastasis diameter, its location and perinodal tissue invasion) which can be easily reported in a routine SLN evaluation in breast carcinoma have a predictive

value of the ALN metastatic status. Nevertheless, uniform and distinct consensus under the SLN sectioning protocols is required.

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