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Review

Breast cancer micrometastasis and axillary sentinel lymph nodes frozen section. Our experience and review of literature

Alessandro Sanguinetti [a,*], Andrea Polistena [a], Roberta Lucchini [a], Massimo Monacelli [a], Roberta Triola [a], Stefano Avenia [a], Ivan Barillaro [a], Micol Sole Di Patrizi [a], Andrea Boccolini [a], Claudia Conti [a], Giovanni Bistoni [b], Nicola Avenia [a]

[a] Struttura Complessa di Chirurgia Generale e Specialità Chirurgiche, Università degli Studi di Perugia, Azienda Ospedaliera "S.Maria", Via Tristano di Joannuccio, 1, 05100 Terni, Italy
[b] Struttura Complessa di Chirurgia Plastica e Ricostruttiva, Università degli Studi "La Sapienza", Dipartimento di Chirurgia Generale "Pietro Valdoni", Viale del Policlinico, 186, 00186 Roma, Italy

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ABSTRACT

Background: Sentinel lymph node (SLN) biopsy plays a major role in the surgical management of primary breast cancer. The aim of this study was to assess the diagnostic accuracy of the assessment of axillary frozen sections of SLNs for micrometastasis diagnosis.
Patients and methods: This study focused on 250 SLNs from 137 patients. Each lymph node was fully analyzed by frozen section. After fixation, serial sections were cut and stained by hematoxylin and eosin (HE) and for pan-cytokeratins by immunohistochemistry (IHC).
Results: Tumor cells were detected in 57 SLNs, 37 on frozen sections and 20 on controls. Of these 57 positive SLNs, 38 contained metastases, 9 contained micrometastases and 10 contained isolated tumor cells. The specificity and positive predictive value of SLN frozen sections for micrometastasis was 100%. The sensitivity was 83.3% for metastasis, 40% for micrometastasis; the false-negative rate was 16.7% for metastasis and 60% for micrometastasis.
Conclusion: Analysis of frozen section of SLNs is an accurate method for metastasis detection, allowing concurrent axillary dissection when positive. The protocol for SLN analyses described herein shows good sensitivity for micrometastasis detection.

1. Introduction

Axillary lymph node status remains one of the most important prognostic factors in primary breast carcinoma, despite the development of a range of other prognostic markers [1]. The sentinel lymph node (SLN) biopsy technique was introduced to permit an accurate staging of the axillary lymph node status for T1-2, N0 breast carcinoma [2,3]. Examination of the SLN by frozen section is known to be less accurate than analysis of permanent sections, but allows re-intervention for axillary lymph node dissection to be avoided if metastases are detected. Some controversies remain in the literature concerning the utility of routine SLN frozen section in breast cancer. Some centers prefer touch imprinting or delayed histopathologic of analysis. Recently, studies have focused on the implication of SLN micrometastasis [3–6], Viale et al. found that axillary lymph node metastases were present in 21.6% of cases, and they discussed whether axillary lymph node dissection should under taken in cases of sentinel lymph node micrometastasis [1]. In our institution, SLN biopsy frozen section was introduced in the late 2010s, in a multidisciplinary setting, in order to avoid unnecessary axillary lymph node dissection. At the end of 2011, we modified our SLN protocol following the recommendation of Turner et al. [3]. The aim of this study was to estimate the sensitivity, specificity, negative predictive value and false-negative rate of frozen section in the detection of SLN micrometastasis.

2. Patients and methods

2.1. Patients

For this retrospective study, we collected 250 SLNs from 137 patients with breast carcinoma who underwent SLN mapping

* Corresponding author. Strada di Santa Filomena 3, 05100 Terni, Italy.
E-mail address: a.sanguinetti@aospterni.it (A. Sanguinetti).

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during the two years following the introduction of an SLN protocol for frozen section. The median number of SLNs examined per patient was 2.45 (range 1–7). There were 9 cases of mastectomy and 128 cases of lumpectomy. In 10 cases, no invasive carcinomas were retrieved, but ductal carcinoma *in situ* was present in 7 cases and no residual tumor was found in 3 cases of neo-adjuvant therapy. A total of 250 SLNs were thus included in this study.

2.2. Histopathological analysis of the tumor

The relevant clinicopathological characteristics were tumor type, Bloom- Richardson grade, lympho vascular invasion, perineural invasion, proliferation index (MIB-1), estrogen and progesterone receptor status as analyzed by immunohistochemistry and scored with Allred score [4], HER2/neu amplification assessed by fluorescence *in situ* hybridization (FISH) analysis and gene amplification determined by a ratio of HER2/CEP17 > 2.

2.3. Histhopathological analysis of the lymph nodes

Axillary SLNs were analyzed by frozen section intra-operatively, using a protocol modified from Turner et al. [3]. Lymph nodes less than 4 mm in diameter were included in totality for frozen section; lymph nodes between 5 and 7 mm in diameter were bisected and each part was included for frozen section; lymph nodes equal or greater than 8 mm in diameter were cut into 4 mm slices and totally included for frozen section. Control sections were performed after formalin fixation and paraffin embedding; every paraffin block was totally cut into 4 μm-thick serial sections at 250 μm intervals. These sections were stained with hematoxylin and eosin (HE). If no metastasis was visible on HE staining, immunochemistry was performed on 3 sections separated by 500 μm as follows. A pool of monoclonal mouse anti-human cytokeratin antibodies (Monoclonal Mouse Anti-Human Cytokeratin 7 Clone OV-TL) was used from Dako, Baar, Switzerland (AE1-AE3, M3515; dilution 1:100) and from BioGenex, San Ramon, USA (anti-cytokeratin 8 and 18 (5D3), AM131; dilution 1:25), with microwave antigen retrieval and 3,3-diaminobenzidine as chromogen. Metastasis was defined as a tumor infiltrate larger than 2 mm and micrometastasis defined as tumor involvement $\geq$0.2 mm but <2 mm. The presence of isolated tumor cells (ITCs) was defined as existence of single tumor cells or small cell clusters not greater than 0.2 mm according to the AJCC TNM Classification of Malignant Tumors, 6th Edition [5].

3. Results

3.1. SLN metastases

Over a two year period, we performed the same SLN protocol as described in the Materials and Methods section. Of a total of 250 SLNs retained for this study, 56 were positive (22.7%): 36 were diagnosed using the intraoperative frozen section (65%) and 20 positive SLNs were found on control sections (35%). Metastases greater than 2 mm were present in 37 SLNs: 31 diagnosed on frozen section and 6 on control sections. SLN metastasis that were not diagnosed on frozen section were considered as false-negative SLN, so 6 out of 37. Of the six false-negative SLN frozen sections, in 3 cases the slides were sub-optimal, rendering a correct diagnosis impossible; in 2 cases, the metastases were undiagnosed on frozen section and in 1 case the metastasis appeared only at the levels performed for the control sections. In two cases of lobular carcinoma the lymph node metastasis were not seen but were revealed with cytokeratin IHC. Micrometastases were diagnosed in 9 SLNs: 4 on frozen section and 5 on control sections. There was 5 false-negative SLN on frozen section. The size of the micrometastases ranged from 0.25 mm to 1.9 mm. Of the 5 cases diagnosed on control sections, 2 were found at successive levels and 4 only by IHC. For the 2 cases diagnosed at successive levels, micrometastases were first found in Section 1 of 5, Section 2 of 4 and Section 7 of 7; this one illustrates the usefulness of serial sections for micrometastasis detection. ITCs were found in 10 cases, two on frozen sections and 8 cases on controls sections, only by IHC. There was 7 false-negative SLN on frozen section.

3.2. Sensitivity and specificity

The specificity of frozen section analysis was 100% for metastasis, micrometastasis and ITC. The sensitivity of frozen section in metastasis was 83.3% and decreased to 40% for micrometastasis and 18.2% for ITC. The negative predictive value was 91.9% for metastasis, 80.6% for micrometastasis and 70% for ITCs. The false-negative rate was 16.7% for metastasis, 60% for micrometastasis and 81.8% for ITCs. There was no false positive case.

3.3. Axillary lymph node dissection

An axillary lymph node dissection was performed in 22 out of the 25 patients (89.3%) with SLN metastasis: 20 during the same surgery and 2 during a second operation. In 8 cases, there were positive axillary lymph nodes. Three out of the seven patients with SLN micrometastasis underwent axillary dissection: 1 during the same operation and 2 in a second intervention. All axillary lymph nodes were negative. Only 1 out of the 9 patients with SLN ITCs underwent axillary lymph node dissection during the same operating time, which one was positive.

3.4. Extra capsular SLN infiltration

Interestingly, of the 24 axillary dissections performed [25], 9 patients had axillary lymph node metastasis. Of them, in 6 cases (63.6%) the SLN had metastatic extra capsular growth and adipose tissue infiltration. Furthermore, in the 15 cases of negative axillary dissection, only 3 cases (22.2%) had SLN metastatic extra capsular growth and adipose tissue infiltration.

4. Discussion

While the detection of rate of SLN and the accuracy of SLN histology as a predictor of axillary lymph node status have been extensively studied [6,8–10], information about the utility of intraoperative frozen section for SLN micrometastasis is sparse. In this study of 278 SLN frozen sections, 63 SLNs were infiltrated by tumoral cells including 66.7% SLN metastasis, 15.9% of SLN micrometastasis and 17.4% of ITCs. We demonstrated a sensitivity of 83.3% for this method in detecting metastasis, but a lower sensitivity, 40%, for micrometastasis. Previous studies published on the sensitivity of frozen section for micrometastasis confirmed the variable rate, ranging from 10% to 61%. This large variation depends on the different histopathological techniques used for frozen section analysis, including the cutting intervals, the number of sections and the use of IHC on control, as well as if perioperative IHC was used [6]. In our opinion, IHC during intraoperative SLN examination is time consuming and not practicable for most small institutions. Recently, reverse-transcription polymerase chain reaction (RT-PCR) assay for cytokeratin-19 and mammaglobin are beginning to be evaluated during intraoperative SLN exam [7–9]. At the present time, no clear international directive exists regarding the use of frozen section. In 2004, the European Working Group for Breast Screening Pathology (EWGBP) reported a high degree of variability among European pathology institutes for SLN examination [10].