



## Intraoperative imprint cytology of sentinel lymph nodes in breast cancer patients: comparison with frozen section

Intraoperativni citološki otisak sentinelnih limfnih čvorova kod bolesnica sa karcinomom dojke: poređenje sa ledenim rezovima

<sup>1</sup>Tatjana Ivković-Kapicl\*<sup>†</sup>, <sup>1</sup>Ferenc Vicko<sup>†‡</sup>, Milana Panjković<sup>†§</sup>,  
Zoran Radovanović<sup>†‡</sup>, Tijana Vasiljević\*<sup>†</sup>, Dragana Radovanović<sup>†‡</sup>,  
Slavica Knežević-Ušaj\*<sup>†</sup>  
<sup>1</sup>Equal contribution

Oncology Institute of Vojvodina, \*Department for Pathology and Laboratory Medicine,

<sup>‡</sup>Clinic for Surgical Oncology, Sremska Kamenica, Serbia; University of Novi Sad,

<sup>†</sup>Faculty of Medicine, Novi Sad, Serbia; Clinical Center

of Vojvodina, <sup>§</sup>Department for Pathology, Novi Sad, Serbia

### Abstract

**Background/Aim.** Sentinel lymph node (SLN) biopsy has been established as the standard of care for axillary staging in patients with invasive breast carcinoma and clinically negative lymph nodes. Intraoperative assessment of sentinel lymph nodes might be done by frozen section (FS), touch imprint cytology (TIC) and one step nucleic acid amplification. The aim of this study was to review our institution's results with SLN biopsy using TIC and FS technique as intraoperative diagnostic tool for breast cancer patients. **Methods.** SLNs from 101 patients were examined intraoperatively by frozen hematoxylin-eosin (H&E) stain and by touch imprint cytology. Results of TIC were compared with FS and permanent histology sections. **Results.** The total number of dissected SLNs was 163 with a mean of 1.6 (1–4) per patient. The permanent H&E identified 19 (19%) patients with a sentinel lymph node metastasis and 82 (81%)

patients with tumor-free sentinel nodes. The sensitivity/specificity rates were 73.7%/99.3%, respectively for TIC and 84.2%/100%, respectively for FS. Relevant positive/negative predictive values were 93.3%/96.6%, respectively for TIC and 100%/97.9%, respectively for FS. **Conclusion.** Our experience with TIC and FS for the intraoperative evaluation of SLNs is similar to the findings from previously reported studies. We detected the high specificity for both methods, but TIC technique appeared to be less sensitive than FS in detecting SLN metastases in breast cancer patients. TIC could be recommended as reasonable alternative to frozen section due to its simplicity and low cost.

**Key words:**  
sentinel lymph node biopsy; breast neoplasms;  
intraoperative period; diagnostic techniques and procedures.

### Apstrakt

**Uvod/Cilj.** Biopsija limfnog čvora stražara (engl. *sentinel lymph node* – SLN) je standardna procedura za intraoperativnu procenu statusa aksilarnih limfnih čvorova kod bolesnica sa karcinomom dojke koje imaju klinički negativne limfne čvorove pazušne jame. Pregled SLN se intraoperativno izvodi tehnikama ledenih rezova (engl. *frozen section* – FS), citološke evaluacije otiska (engl. *touch imprint cytology* – TIC) i metodom amplifikacije nukleinskih kiselina u jednom koraku. Cilj studije bio je da se uporede i procene pouzdanost i tačnost tehnike FS i TIC kao metoda za pregled SLN kod bolesnica sa karcinomom dojke. **Metode.** SLN, dobijeni od 101 bolesnice, intraoperativno su pregledani na FS bojenim hematoksilin-eozin (HE) bojenjem i

citološkom analizom razmaza dobijenim otiskom limfnih čvorova. Rezultati citološke analize poređeni su sa FS nalazom i trajnim histološkim preparatima. **Rezultati.** Ukupan broj analiziranih SLN iznosio je 163, u proseku 1,6 (1–4) po bolesnici. Na definitivnim, parafinskim preparatima, metastaze u SLN ustanovljene su kod 19 (19%) bolesnica, dok kod 82 (81%) bolesnice u limfnim čvorovima nije bilo tumora. Senzitivnost i specifičnost za TIC iznosila je 73,7% i 99,3%, dok je za tehniku FS senzitivnost bila 84,2%, a specifičnost 100%. Pozitivna/negativna prediktivna vrednost za TIC je iznosila 93,3%/96,6%, a za metodu FS 100%/97,9%. **Zaključak.** Naše iskustvo sa metodama TIC i FS u intraoperativnoj proceni statusa SLN kod bolesnica sa karcinomom dojke slično je rezultatima ranije objavljenih studija. Utvrđena je visoka specifičnost

za obe metode, ali je senzitivnost tehnike TIC u detekciji metastaza u SLN nešto niža u odnosu na metodu FS. TIC metoda intraoperativnog pregleda SLN može predstavljati pouzdanu alternativu metodi FS zbog jednostavnosti i niske cene.

## Introduction

Axillary lymph node (ALN) status is an important prognostic factor and determinant of treatment for patients with breast carcinoma. Sentinel lymph node (SLN) biopsy has been established as the standard of care in assessing the axilla in patients with invasive breast carcinoma and clinically negative lymph nodes<sup>1</sup>. It is a minimally invasive procedure that accurately evaluates the status of the axilla and can obtain the same prognostic information derived from axillary lymph node dissection (ALND) with significantly less morbidity<sup>1,2</sup>. SLN is the first node receiving lymphatic drainage directly from the primary tumor. Thus, it is the node most likely to be the site of initial lymphatic metastasis. Currently, there is level 1 of evidence that documents that SLN biopsy is as accurate as ALND for breast cancer staging. If SLN is negative, it is predicted that the rest of the ALNs will also be negative<sup>3</sup>. Conversely, if SLN is positive the rest of the ALNs might also contain metastatic tumor deposits<sup>1,4,6</sup>.

SLN biopsies are performed in highly equipped institutions by lympho-scintigraphy scan or blue dye mapping of SLN or combined technique<sup>1,4</sup>.

Intraoperative evaluation of SLNs is enabled by various techniques such as frozen section (FS), touch imprint cytology (TIC) and one step nucleic acid amplification (OSNA)<sup>4</sup>. FS examination is the most common method for intraoperative diagnostics of SLN, but disadvantages are loss of valuable tissue for definitive histological examination, considerable time consumption, technical difficulty in preparation of fatty tissue, specific instrumentation and costs<sup>5-7</sup>. TIC is rapid, inexpensive, easy, widely available method with maximum tissue preservation that allows clear cytological details, but it requires well educated pathologist in terms of breast cytology. Immunohistochemical (IHC) staining for cytokeratins is not routinely used in intraoperative evaluation. ICH is very accurate technique, but is a time consuming and expensive method that requires a special equipment<sup>1,6-9</sup>.

All intraoperative diagnostic techniques are followed by routine examination of paraffin-embedded and hematoxylin-eosin (H&E) stained sections, which is a reference standard, after which the definite staging of axillary lymph nodes is performed<sup>1,3,8,10</sup>.

Both FS and intraoperative cytology imprints have a wide variety in sensitivity rates<sup>4,10,11</sup>.

The purpose of this study was to determine our institution's experience using both FS and TIC techniques for intraoperative detection of metastases in SLN biopsy and comparison with standard permanent section examination.

## Methods

Prospective study was performed at the Oncology Institute of Vojvodina, Sremska Kamenica, Serbia during 2014,

## Ključne reči:

limfni čvorovi, stražarski; biopsija; dojka, neoplazme; intraoperativni period; dijagnostičke tehnike i procedure.

2015 and 2016. Study included 101 patients with histologically confirmed breast cancer and clinically negative axillary lymph nodes treated operatively with SLN biopsy. Neoadjuvant chemotherapy was an excluding factor for the study. Detection of SLN was performed by combined method: preoperative application of 1 mL methylene blue dye and 1 mL (1mCi) of radioactive isotope (Tc99 nanocolloid).

SLN were identified successfully in all patients and were sent immediately for pathological examination. Fresh lymph nodes larger than 3 mm were bisected along long axis and each surface was touched on glass slide. The imprint samples were air-dried and fixed in 95% alcohol, than stained by May-Grünwald-Giemsa Quick-stain (Bio-Optica, Italy) and analyzed under microscope. Fresh cut lymph nodes were then frozen, cut at 5 µm, 3–5 sections *per* slide, stained in standard H&E stain and microscopically analyzed. Slides were analyzed by experienced pathologist and reported to surgeon intraoperatively.

The decision of performing ALND was made based on results of FS of SLN. The SLNs specimens were then placed in cassette, fixed in 10% buffered formalin for routine processing and standard pathological examination.

Breast cancer tumor staging was performed based on tumor size, status of axillary lymph nodes and metastases, determining pathological (p)TNM – tumor-nodus-metastasis status of breast cancer according to the American Joint Committee on Cancer (AJCC)<sup>12</sup>. Assessment of breast cancer differentiation was performed by modified Bloom-Richardson score<sup>13</sup>.

The results of FS and TIC were compared with definitive postoperative histopathology results of SLNs and analyzed using Statistical Package for Social Sciences (SPSS), version 18 (SPSS Inc. Chicago, USA).

## Results

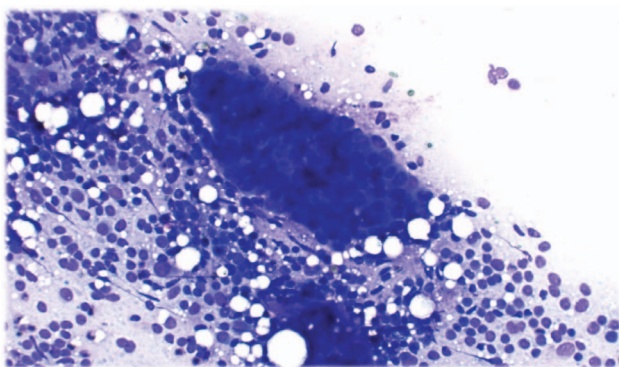
Breast surgery with SLN biopsy was made in 101 female patients with breast cancer and clinically negative axillary lymph nodes. Sentinel lymph node was successfully obtained in all patients (100%) and 163 lymph nodes were received for pathological analysis. The patients ranged in age from 29 to 82 (mean age 58.2). None of the 10 patients with non invasive extensive high-grade *in situ* carcinoma had SLN metastases. Invasive breast tumors were classified as pTis (n = 10; 9.9%), pT1 (n = 58; 58.4%) and pT2 (n = 33; 32.7%). Most frequent type of the tumor in analyzed group was ductal invasive carcinoma (n = 69; 68.3%). Primary tumor grading using Bloom-Richardson Grading System found that 28.7% patients had grade 1, 43.6% had grade 2 and 27.7% grade 3 of breast carcinoma. Tumor size varied from 3 mm up to 40 mm and 4 cases were multifocal carcinomas. Patients and tumor characteristics are summarized in Table 1.

**Table 1**  
**Patient and tumor characteristics**

Characteristics	Values
Age (years), mean (range)	58.19 (29–82)
Side of the tumor, n (%)	
left	44 (43.56)
right	57 (56.44)
Surgical procedure, n (%)	
quadrantectomy	97 (96.0)
mastectomy	4 (4.0)
Tumor stage, n (%)	
pTis	10 (9.90)
pT1	58 (57.42)
pT1a	4 (3.96)
pT1b	12 (11.88)
pT1c	42 (41.58)
pT2	33 (32.67)
Histologic tumor type, n (%)	
ductal invasive carcinoma	69 (68.32)
lobular invasive carcinoma	8 (7.92)
<i>in situ</i> carcinoma	10 (9.90)
other types	14 (13.86)
Histologic grade, n (%)	
1	29 (28.71%)
2	44 (43.56%)
3	28 (27.72%)

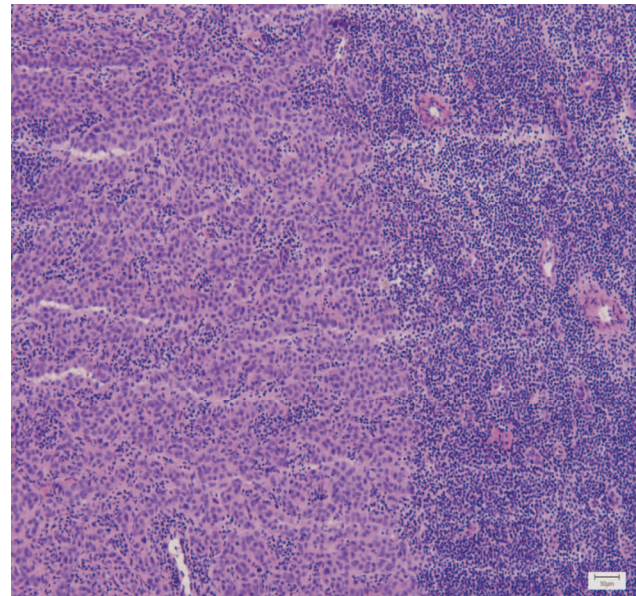
Average number of lymph nodes *per* patient was 1.6 (1 to 4). A total number of 163 SLN was examined by TIC, FS and permanent histopathological section methods. TIC was positive for SLN metastases in 15 cases (Figure 1) and negative for metastases in 148 cases. FS detected metastases in 16 SLN (Figure 2) and 147 were negative.

Metastatic deposits  $> 2$  mm were marked as micrometastases and  $\leq 2$  mm as macrometastases. Permanent histology sections, considered a gold standard in diagnostics of metastatic deposits, showed metastases in 19 SLN. Macrometastases were present in 17 SLN and micrometastases in 2 examined lymph nodes.



**Fig. 1 – Clusters of tumor cells in touch imprint cytology smears in lymph node with breast cancer metastasis (May-Grünwald-Giemsa,  $\times 400$ ).**

There was discordance between TIC and histopathology reports in 6 SLN. Five cases of negative TIC turned out positive for metastases in histopathology, and one of the positive TIC was found negative in histopathology. Two cases of false negative in TIC were micrometastases and other 3 were macrometastases. In total 163 SLN examined, 14 were positive for metastases on both TIC and permanent sections, and 143 were negative after analyzing with both methods.



**Fig. 2 – Metastasis of breast carcinoma in sentinel lymph node on frozen section (hematoxylin-eosin,  $\times 10$ ).**

Intraoperatively, FS technique found 16 positive cases and 147 negative cases for nodal metastases. Permanent sections and FS showed discordance in 3 false negative cases, two for micrometastases and one for macrometastases (Table 2).

Based on examination of 163 SLN acquired from 101 patients in our study, the sensitivity for metastases detected by TIC was (14/19) 73.9%, specificity was (143/144) 99.3%, positive predictive value was (14/15) 93.3% and negative predictive value was (143/148) 96.6%. Overall accuracy for TIC in detecting SLN metastases was (157/163) 96.3%.

The sensitivity of FS from our study was (16/19) 84.2%, specificity was (144/144) 100%, positive predictive value was (16/16) 100% and negative predictive value was (144/147) 97.9%. Overall accuracy of FS for detection of nodal metastases was (160/163) 98.1%.

## Discussion

Sentinel lymph node biopsy is a worldwide accepted concept for patients with breast carcinoma. Therefore, intraoperative detection of SLN is an imperative. However, because of the lack of equipment such as special infrastructure for preparation, storage and handling of radioactive technetium 99-label colloid or ineffective purchase of methylene blue dye, many facilities still use ALND<sup>6</sup>.

**Table 2**  
**Comparison of the results found in permanent histology sections, FS and TIC in 101 examined breast cancer patients**

Sentinel lymph node	Permanent histology sections	Frozen section	Touch imprint cytology smears		
	(n)	(n)	(n)		
Positive for metastases	19	16	15	True	14
				False	1
Negative for metastases	144	147	148	True	143
				False	5

FS – frozen section; TIC – touch imprint cytology.

Frozen section may provide information on the size of metastasis, but it causes loss of tissue for permanent sections, it is time consuming and expensive technique requiring a cryostat as well as skilled professionals. Touch imprint cytology requires less effort, it is faster, saves tissue for permanent sections, but pathologist needs to be trained for reporting cytology samples. Intraoperative cytology provides rapid results with minimal artifacts. However, number of examined cells in cytology samples is smaller<sup>4,11</sup>.

Numerous studies comparing FS and TIC in intraoperative evaluation of SNLs have demonstrated significant variation in sensitivity of 44–100% for FS and 34–95% for TIC<sup>4,10</sup>. However, the variations of the methodology involved in the intraoperative as well as permanent section histopathologic evaluation make it very difficult to reliably compare different studies.

Tew et al.<sup>10</sup> reviewed 31 studies comparing TIC and FS in the literature and overall sensitivity of TIC was 63%, with a pool sensitivity of 81% for macrometastases and 22% for micrometastases. A similar meta-analysis reporting on FS examination found an overall sensitivity of 78%, with 94% for macro- and 40% for micrometastases<sup>11</sup>.

In comparison of TIC and FS, although there was higher sensitivity of FS, no statistically significant difference between these two methods was found in the most of the studies<sup>7,11,13–16</sup>. The lower sensitivity of TIC is usually caused by inadequate sampling, and might be overcome when the number of slides during TIC is increased. This can improve sensitivity of the method without losing tissue for permanent histological examination<sup>7,15,17</sup>.

High specificity for both FS and intraoperative cytology approach, indicates that the false positive rates of these techniques are close to zero<sup>10,18</sup>. Higher false negative rates for both methods of intraoperative examination of SNLs are seen in low nuclear grade metastatic tumors and particularly lobular carcinomas, since these tumor cells are small and poorly cohesive<sup>11</sup>.

Our study showed 73.7% sensitivity and 99.3% specificity for TIC. The case of false positive imprint was due to misinterpretation of epithelioid histiocytes. Germinal center lymphocytes or activated endothelial cells could also rarely be mistaken for tumor cells<sup>15</sup>. The omission of micrometastases is the major cause of false negative intraoperative diagnoses. In accordance to literature data, our study showed two false negative imprints as the result of micrometastases. Reasons for false negative result is smaller number of examined

cells comparing to FS and unrecognized individual tumors cells in well differentiated carcinomas<sup>11,18</sup>.

The use of intraoperative immunohistochemistry with cytokeratins could minimize the intraoperative false negative rates. Such protocols are now available for using on either FS or cytology imprints. However, turnaround time for such protocols is 16–20 minutes, and it prolongs the time of the surgery and costs of the diagnostics and thus it is not a standardized procedure<sup>14,15,17</sup>. Recently, intraoperative ultrarapid IHC has been investigated for its feasibility, validity, and effectiveness in comparison with FS. Ultrarapid cytokeratin IHC significantly enhanced intraoperative detection of metastasis in SNLs without increased time for assessment. This technique is currently not widely available and requires specialized expertise<sup>7,9</sup>. Immunohistochemistry is a standard procedure if there is a suspicious presence of metastatic cells during permanent section examination.

The clinical prognostic significance of micrometastases in SLN remains controversial, and some authors consider micrometastases to behave similarly to macrometastases<sup>1,17,18</sup>. Several studies have questioned the clinical and pathologic significance of finding micrometastases in SLN, particularly in intraoperative consultations<sup>19,20</sup>.

Currently, the standard practice has been to offer completion axillary lymph node dissection in patients who are found to have positive SLN for metastatic carcinoma either during the primary surgical procedure or in permanent histopathology report<sup>20–22</sup>. However, the recently reported results from the American College of Surgeons Clinical Oncology Group (ACOSOG) Z0011 trial found that there was no statistically significant benefit from ALND for women who had clinically negative axilla but the SLN was positive<sup>3</sup>. Recommendations from recent studies advise that axillary lymph node dissection can be omitted in patients with one or two positive sentinel nodes when conventionally whole-breast radiation therapy is planned<sup>21,22</sup>. Therefore, the role of intraoperative assessment of SLN in breast cancer seems to be in evolution.

## Conclusion

Our experience with TIC and FS for the intraoperative evaluation of SNLs is similar to the findings from previously reported studies. We detected the high specificity for both methods, but TIC technique appeared to be less sensitive than FS in detecting SLN metastases.

TIC results can be obtained with reasonable accuracy within a short time frame, permitting intraoperative decisions regarding management of the axilla in the breast can-

cer patients. Therefore TIC could be recommended as an alternative to FS in view of its simplicity and low cost.

#### R E F E R E N C E S

- Hoda SA, Resekova E. Pathologic examination of breast and lymph node specimens, including sentinel lymph nodes. In: Brogi E, Hoda SA, Koerner FC, Rosen PP, editors. Rosen's breast pathology. 4th ed. Philadelphia: Wolters Kluwer Health; 2017. p. 1263–336. (English)
- Schrenk P, Rieger R, Shamiyeh A, Wayand W. Morbidity following sentinel lymph node biopsy versus axillary lymph node dissection for patients with breast carcinoma. *Cancer* 2000; 88(3): 608–14.
- Giuliano AE, Hunt KK, Ballman KV, Beitsch PD, Whitworth PW, Blumencranz PW, et al. Axillary dissection vs no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial. *JAMA* 2011; 305(6): 569–75.
- Omranipour R, Jaleefar A, Mirafsharieh A, Assasnik P. Intraoperative evaluation of sentinel lymph nodes by touch imprint cytology technique in breast cancer patients. *Annu Res Rev Biol* 2014; 4(24): 3751–7.
- Richards AD, Lakshani SR, James DT, Ung OA. Intraoperative imprint cytology for breast cancer sentinel lymph nodes: is it worth it? *ANZ J Surg* 2012; 83(7–8): 539–44.
- Safai A, Razeghi A, Monabati A, Azarpura N, Talei A. Comparing touch imprint cytology, frozen section analysis, and cytokeratin immunostaining for intraoperative evaluation of axillary sentinel lymph nodes in breast cancer. *Indian J Pathol Microbiol* 2012; 55(2): 183–6.
- Khanna R, Bhadani S, Khanna S, Padney M, Kumar M. Touch imprint cytology evaluation of sentinel lymph node in breast cancer. *World J Surg* 2011; 35: 1254–9.
- Chicken DW, Kojan G, Falzon M, Lee AC, Douek M, Sainsbury R, et al. Intraoperative touch imprint cytology for the diagnosis of sentinel lymph node metastases in breast cancer. *Brit J Surg* 2006; 93(5): 572–6.
- Franz M, Egervary K, Szollosi Z. Intraoperative evaluation of sentinel lymph nodes in breast cancer: comparison of frozen sections, imprint cytology and immunohistochemistry. *Cytopathology* 2011; 22(1): 36–42.
- Tew K, Irwig L, Matthews A, Crowe P, Macaskill P. Meta-analysis of sentinel node imprint cytology in breast cancer. *Br J Surg* 2005; 92(9): 1068–80.
- Craeger AJ, Geisinger KR, Perrier ND, Shen P, Shaw JA, Young PR, et al. Intraoperative imprint cytologic evaluation of sentinel lymph nodes for lobular carcinoma of the breast. *Ann Surg* 2004; 239(1): 61–6.
- Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. AJCC cancer staging manual. 7th ed. New York: Springer; 2009. p. 419–60.
- Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from large study with long-term follow-up. *Histopathology* 1991; 19(5): 403–10.
- Krishnamurthy S, Meric-Bernstam F, Lucci A, Hwang RF, Kuerer HM, Babiera G, et al. A prospective study comparing touch imprint cytology, frozen section analysis, and rapid cytokeratin immunostain for intraoperative evaluation of axillary sentinel lymph nodes in breast cancer. *Cancer* 2009; 115(7): 1555–62.
- Motomura K, Inaji H, Komoike Y, Kasugai T, Nagumo S, Noguchi S, et al. Intraoperative sentinel lymph node examination by imprint cytology and frozen sectioning during breast surgery. *Br J Surg* 2003; 87(5): 597–601.
- Perez-Sanchez VM, Vela-Chavez TA, Villarreal-Colin P, Bargallo-Rocha E, Ramirez-Ugalde MT, Munoz-Gonzales D, et al. Intraoperative touch imprint cytology of sentinel lymph nodes in breast cancer: experience at a tertiary care center in Mexico. *Med Oncol* 2010; 27(2): 233–6.
- Lumachi F, Marino F, Zanella G, Chiara GB, Basso SM. Touch imprint cytology and frozen-section analysis for intraoperative evaluation of sentinel nodes in early breast cancer. *Anticancer Res* 2012; 32(8): 3523–6.
- Elliot RM, Shenke RR, Thompson CL, Gilmore HL. Touch preparations for the intraoperative evaluation of sentinel lymph nodes after neoadjuvant therapy have high false-negative rates in patients with breast cancer. *Arch Pathol Lab Med* 2014; 138(6): 814–8.
- Gyorki DE, Henderson MA. Significance of sentinel lymph node micrometastases in patients with breast cancer. *J Clin Oncol* 2010; 28(9): e139; author reply e141–2.
- Maguire A, Brogi E. Sentinel lymph nodes for breast cancer. *Histopathology* 2016; 68(1): 152–67.
- Lyman GH, Somerfield MR, Bosserman LD, Perkins CL, Weaver DL, Giuliano AE. Sentinel Lymph Node Biopsy for Patients With Early-Stage Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. *J Clin Oncol* 2017; 35(5): 561–4.
- Maguire A, Brogi E. Sentinel lymph nodes for breast carcinoma: a paradigm shift. *Arch Pathol Lab Med* 2016; 140(8): 791–8.

Received on November 20, 2017.

Revised on March 14, 2018.

Accepted on April 4, 2018.

Online First April, 2018.