Original Article

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ประสิทธิภาพของการตรวจหากรดนิวคลีอิคแบบหนึ่งขั้นตอนในการประเมินการแพร่กระจายของมะเร็งเต้านมสู่ต่อมน้ำเหลืองเซนติเนลในโรงพยาบาลสงขลานครินทร์

Efficacy of One-Step Nucleic Acid Amplification Assay for Evaluation of Sentinel Lymph Node Metastasis of Breast Cancer in Songklanagarind Hospital

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บทคัดย่อ:

วัตถุประสงค์: เพื่อศึกษาความไว ความจำเพาะและความถูกต้องของการตรวจต่อมน้ำเหลืองเซนติเนลด้วยวิธี one-step nucleic acid amplification assay (OSNA assay) เทียบกับวิธี frozen section และผล permanent

วัสดุและวิธีการ: รวบรวมข้อมูลผู้ป่วยมะเร็งเต้านมระยะเริ่มต้น 120 ราย โดยได้รับการผ่าตัดต่อมน้ำเหลืองเซนติเนล ร่วมกับการเจาะต่อมน้ำเหลืองออกในระดับที่ 1 และ 2 ในโรงพยาบาลสงขลานครินทร์ ตั้งแต่วันที่ 1 มีนาคม พ.ศ. 2554 – 30 พฤศจิกายน พ.ศ. 2557

ผลการศึกษา: วิเคราะห์ต่อมน้ำเหลืองเซนติเนล 213 ต่อม (เฉลี่ย 1.84 ต่อม) ผลของการตรวจด้วยวิธี OSNA assay เทียบกับผล permanent มีความไวร้อยละ 100 (25/25) ความจำเพาะร้อยละ 95.2 (179/188) ความถูกต้องร้อยละ 95.7 ค่าพยากรณ์ผลบวกร้อยละ 73.5 (25/34) และค่าพยากรณ์ผลลบร้อยละ 100 (179/179) ระยะเวลาการรายงานผลโดยเฉลี่ย 39.6 นาที เมื่อเทียบกับผลการตรวจด้วยวิธี frozen section ซึ่งมีความไวร้อยละ 92 (23/25) ความจำเพาะร้อยละ 100 (188/188) ความถูกต้องร้อยละ 99.1 ค่าพยากรณ์ผลบวกร้อยละ 100 (23/23) และค่าพยากรณ์ผลลบร้อยละ 98.9 (188/190) โดยหลังจากที่ทำ discordance analysis เมื่อเปรียบเทียบ

ได้รับเงินทุนวิจัยจากคณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์

รับต้นฉบับวันที่ 11 กรกฎาคม 2559 รับลงตีพิมพ์วันที่ 20 ตุลาคม 2559

ในการแพร่กระจายแบบ macrometastasis มีค่าพยากรณ์ผลบวกร้อยละ 100 (25/25) และการแพร่กระจายแบบ micrometastasis มีค่าพยากรณ์ผลบวกร้อยละ 75 (3/4) และมีค่าความถูกต้องร้อยละ 99.5

สรุป: การตรวจต่อมน้ำเหลืองเซนติเนลด้วยวิธี OSNA assay มีความไว ความจำเพาะ ความถูกต้อง ให้ผลการตรวจที่รวดเร็ว สามารถใช้แทนการตรวจต่อมน้ำเหลืองด้วยวิธี frozen section ได้

คำสำคัญ: การตรวจหานิวคลีอิคแบบหนึ่งขั้นตอน, ความจำเพาะ, ความถูกต้อง, ความไว, ออสนา

Abstract:

Objective: To compare the sensitivity, specificity and accuracy of the one-step nucleic acid amplification (OSNA) assay and frozen sections.

Material and Method: One hundred and twenty patients with early breast cancer and no clinical nodal involvement underwent intraoperative sentinel lymph nodes (SLNs) evaluation and undertaken axillary lymph nodes dissection (ALND) at the Department of Surgery, Songklanagarind Hospital from March 1st, 2011-November 30th, 2014.

Results: A total of 213 SLNs were evaluated (mean 1.84 nodes). The accuracy of the OSNA assay compared to histological evaluation was 95.7%. The OSNA assay performance was: sensitivity 100% (25/25); specificity 95.2% (179/188); positive predictive value (PPV) 73.5% (25/34) and negative predictive value (NPV) 100% (179/179). Turnaround time was an average 39.6 minutes. The accuracy of frozen sections compared to histological evaluation was 99.1%. The frozen sections performance was: sensitivity 92% (23/25); specificity 100% (188/188); PPV 100% (23/23) and NPV 98.9% (188/190). After discordant analysis, the PPV of the OSNA assay on nodes with macrometastases (++) was 100% (25/25), and the result on nodes with micrometastases (+) was 75% (3/4). The accuracy of OSNA after review was thus 99.5%.

Conclusion: The OSNA assay is accurate and rapid for SLN evaluation and it can replace the frozen section in general practice.

Keywords: accuracy, one-step nucleic acid amplification, OSNA, sensitivity, specificity

Introduction

Sentinel lymph node (SLN) biopsy is the standard procedure for patients with early breast cancer and no clinical nodal involvement. If the SLNs are negative, axillary lymph node dissection (ALND) can be omitted to reduce the morbidity. If the SLNs is positive, an ALND should be done to evaluate the other lymph nodes.[1-4]

SLN evaluation may include frozen section or touch imprint cytology or a combination of both methods with high specificity but the sensitivity varies widely, ranging from 44-75%.[5,6]

Reverse transcription–polymerase chain reaction was the first molecular approach used for the detection of SLN metastases through analysis of tumor-specific mRNA. The one-step nucleic acid amplification (OSNA) assay (Sysmex, Kobe, Japan) is a molecular technique that combines node tissue homo-genization and sub-sequent reverse-transcription loop-mediated isothermal

amplification of cytokeratin (CK)-19 mRNA in a single quick step. The assay can accurately detect nodal metastases, comparable to conventional pathological examination, and yields quantitative results. The initial purpose of OSNA was to provide a means to objectively assess the occurrence of SLN metastasis during an operation for breast cancer that would reduce the workload of pathologists and technicians. Numerous studies have demonstrated that the ability of OSNA to detect SLN involvement is comparable to that of pathological examination in breast cancer surgery.[7-10] To date, the OSNA system is used in approximately 290 hospitals worldwide.

To validate the OSNA assay in our hospital, we performed this study to evaluate the sensitivity, specificity and accuracy of the OSNA assay and compared these results with frozen sections.

Material and Method

When we began the study, the OSNA system had already been used with 120 patients with breast cancer T1-3, clinically N0, who had had intraoperative SLN evaluations, and had also undergone axillary lymph node dissection at the Department of Surgery, Songklanagarind Hospital during March 2011-November 2014.

The test was performed by one technician who was contacted on the morning of the operation to come and prepare the device and the reagent. When the patient entered the operating theater, dye was injected into the subdermal circumareolar area and the SLN biopsy performed. After the fatty tissue was removed, the SLN was weighed and each node was cut into 3 pieces, with 1 piece sent for a frozen section and the other 2 pieces sent to the molecular lab for OSNA assay as shown in Figure 1. The SLNs were homogenized using LYNORHAG lysis buffer (Sysmex Corp., Hyogo, Japan). The CK19 mRNA in each lysate was amplified using a LYNOAMP BC gene amplification reagent (Sysmex Corp.), then a 2-µl sample of each lysate was subjected to an RT-LAMP reaction. The CK19 mRNA copy number was detected by measuring the rise time based on a standard curve using an RD-100i (Sysmex Corp.).[11-13]

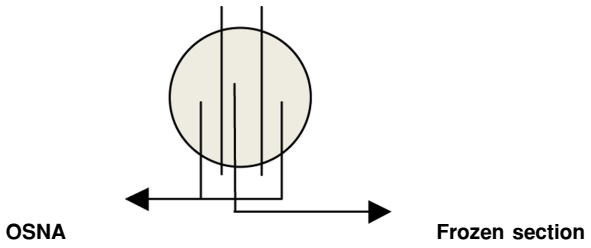

Figure 1 The SLN was cut into 3 pieces, with 1 piece sent for a frozen section and 2 pieces sent to the molecular lab for OSNA assay.

The results were expressed as the number of CK19 mRNA copies per microliters (copies/µL), and the metastatic load was assessed. The (+) symbol corresponded to 250 to 5000 CK19 mRNA copies/µL and was defined as "micrometastases," and the (++) symbol corresponded to >5000 CK19 mRNA copies/µL and was defined as "macrometastases." A negative result (–) had less than 250 CK19 mRNA copies/µL and reflected the absence of cancer cells or the presence of isolated tumor cells.[14-16]

Statistical analysis

Two analytic values were determined by comparing the results of the OSNA assay and frozen section with the histologic results (defrosted tissue) using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, United States). Continuous data are presented as median (range) unless otherwise stated. Sensitivity, specificity, positive

predictive value, negative predictive value, and accuracy were calculated.

Results

There were 120 patients enrolled in this study, of whom 4 were excluded due to incomplete information. The median age of the patients was 54.7 (range 32–79). All patients had a tumor size less than 5 cm. (<T3 lesion) except one patient had a tumor size of 6 cm. Of the 116 patients, 82 percent were categorized as early breast cancer. The patient characteristics are shown in Table 1.

Table 1 Patient characteristics

Patient characteristic	Number (%)
Enrolled	120
Excluded	4
Analyzed	116 (100.0)
Age (years)	
Median (range)	54.7 (32–79)
<45	24 (20.7)
≥45	92 (79.3)
T classification	
Tis	4 (3.4)
T1a	4 (3.4)
T1b	14 (12.1)
T1c	48 (41.4)
T2	45 (38.8)
T3	1 (0.9)
Histological type	
Invasive ductal carcinoma	104 (89.7)
Invasive lobular carcinoma	4 (3.4)
Ductal carcinoma in situ	4 (3.4)
Mucinous carcinoma	3 (2.6)
Papillary carcinoma	1 (0.9)
Nuclear grade	
1	19 (16.4)
2	49 (42.2)
3	37 (31.9)
Unknown	11 (9.5)
Estrogen receptor	
Positive	87 (75.0)
Negative	29 (25.0)
Progesterone receptor	
Positive	73 (62.9)
Negative	43 (37.1)
HER2 status	
3+	12 (10.3)
2+	25 (21.6)
Negative	75 (64.7)
Unknown	4 (3.4)
Stage	
1	51 (44.0)
2a	45 (38.8)
2b	8 (6.9)
3a	5 (4.3)
3b	0 (0.0)
3c	3 (2.6)
DCIS	4 (3.4)

HER2=human epidermal growth factor receptor 2, DCIS=ductal carcinoma in situ

A total of 213 SLNs were removed (mean 1.84 nodes). The SLNs were identified by isosulfan blue dye alone (47%) and combined with 99mTc sulfur colloid (53%). The mean turnaround time of the assay (the time from receiving the nodes to acquiring the results) was 39.6 minutes.

As shown in Table 2, the accuracy of the OSNA assay compared to histological evaluation was 95.7%.

The OSNA assay performance was: sensitivity 100% (25/25); specificity 95.2% (179/188); positive predictive value (PPV) 73.5% (25/34); and negative predictive value (NPV) 100% (179/179).

As shown in Table 3, the accuracy of frozen section compared to histological evaluation was 99.1%. The frozen section performance was: sensitivity 92% (23/25); specificity 100% (188/188); PPV 100% (23/23); and NPV 98.9% (188/190).

Table 2 OSNA results compared with histologic results

OSNA assay results	Histologic results		Total
	H&E +	H&E −	
Positive	25	9	34
Negative	0	179	179
Total	**25**	**188**	**213**

Table 3 Frozen sections compared with histologic results

Frozen sections results	Histologic results		Total
	H&E +	H&E −	
Positive	23	0	23
Negative	2	188	190
Total	**25**	**188**	**213**

Discordant case analysis: there were 9 nodes that were OSNA assay positive and histology negative. All of these nodes had negative frozen section results. After the OSNA assay positive nodes were carefully reviewed, 5 revealed less than 250 CK19 mRNA copies/μL that meant a negative result, 3 revealed 250–5,000 CK19 mRNA copies/μL and histological results showed micrometastasis, and 2 nodes that were positive frozen section of these 2 nodes were initially negative and the histologic results were changed to positive. The frozen section results were changed to positive in both after the slides were reviewed.

The PPV value of the assay result (++) on nodal macrometastases was 100% (25/25), and the result (+) on micrometastases was 75% (3/4), as shown in Table 4. Thus the accuracy of OSNA after the second review was 99.5%.

Table 4 OSNA assay results after discordant case analysis

OSNA assay results	Histologic results		Total
	H&E +	H&E −	
Positive ++ (>5,000 copies/μL)	25	0	25
Positive + (250–5,000 copies/μL)	3	1	4
Negative	0	184	184
Total	**28**	**185**	**213**

Discussion

The OSNA test can be used to detect the occurrence of SLN metastasis of breast cancer expressing CK19. The existence of CK19 negative breast cancer was estimated to occur in 1–2% of breast cancer cases evaluated using immunohistochemistry. However, Pegolo, et al.[17] showed that cases with negative CK19 immunostaining had significant CK19 expression at the mRNA level.

To date, the OSNA assay is used in close to 300 hospitals worldwide. Recent studies from around the world have confirmed that the OSNA assay can be used intraoperatively to detect SLN involvement in early breast cancer patients.[18–21] Our results were similar to several previous published studies that found high sensitivity

(range 83.1–95%) and specificity (range 92.9–97.1%) for the OSNA test.[14,24–26] The high NPV provides strong evidence that the ALND can be safely omitted in patients with SLNs assessed as negative for the presence of cancer cells. Our result found that the NPV value of the OSNA assay was 100%, similar to the result of Smolaz, et al.[27]

The OSNA assay detects a greater number of metastases in SLNs compared to frozen section analysis because of the OSNA assay's ability to detect more low-volume nodal metastases. In our study, 3 micrometastases and 5 isolated tumor cells were found by the OSNA assay but not found by frozen section analysis, which was similar to the results of studies by Osako, et al.[19] and Santaballa, et al.[28] However, the NSABP-B32 study[29] and IBCSG 23–01 trial[30] demonstrated that occult metastases were not clinically significant in early breast cancer patients.

In our study, a laboratory technician performed the OSNA test within 40 minutes, similar to the time reported in many other studies. After receiving the results the surgeons make the decision to either finish and close the incision if the result was negative or continue with the ALND with a breast surgery if the result was positive.

The advantage of the OSNA assay over conventional pathology is that it allows semiquantitative result of the total tumor load in the SLNs when the whole SLNs are examined, although 100% concordance between the OSNA assay and the pathological examination cannot be achieved because of tumor allocation bias. The total tumor load evaluated by the OSNA assay is also used as a predictor for non-SLN metastasis in an attempt to select those SLN metastasis patients.[31,32] Moreover, the OSNA assay can be used to detect the occurrence of metastasis of other cancers expressing CK19 and is expected to provide a more accurate assessment of lymph node metastasis and staging. Kumagai, et al.[33] performed a multicenter prospective study for gastric cancer and reported that the sensitivity, specificity, and concordant rate of OSNA were 83.3, 95.9 and 94.2%, respectively. Yamamoto, et al.[34] also reported results from a multicenter prospective study for colorectal cancer, where the sensitivity, specificity, and concordant rate were 86.2, 96.5 and 96.5%, respectively. It is interesting to note that the sensitivity, specificity, concordance, PPV and NPV in each study for the various cancers were found to be similar to that of breast cancer. However, it is not a standard treatment for intraoperative lymph node evaluation in gastric and colorectal cancer.

This study had some important limitations. First, there were no data about CK19 expression in primary tumors to avoid false-negative cases. Despite this limitation, however, the sensitivity, specificity and accuracy of OSNA were excellent. Second, there was low numbers of cases. Further studies with larger numbers of cases are necessary.

Conclusion

The OSNA assay can be used to detect the occurrence of SLN metastasis of cancers expressing CK19. Using the OSNA assay provides a more reliable indicator of the need for an ALND in the same surgical procedure. The accuracy of the OSNA assay in this study was excellent and the sensitivity and specificity were high. It provided satisfactory results in a short time. The OSNA assay can thus be used as an alternative method for determinating metastasis in SLNs, which can reduce the workload of pathologists or be used in hospitals which don't have a pathologist.

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References

1. Rubio IT, Espinosa-Bravo M, Rodrigo M, Amparo Viguri Diaz M, Hardisson D, Sagasta A, et al. Nomogram including the total tumoral load in the sentinel nodes assessed by one-step nucleic acid amplification as a new factor for predicting non sentinel lymph node metastasis in breast cancer patients. Breast Cancer Res Treat 2014; 147: 371 – 80.
2. Tiernan JP, Varghese ET, Nair A, Pathak S, Kim B, White J, et al. Systematic review and meta-analysis of CK-19 based one-step nucleic acid amplification versus histopathology for sentinel lymph node assessment in breast cancer. Br J Surg 2014; 101: 298 – 306.
3. Lyman GH, Giuliano AE, Somerfield MR, Benson AB, Bodurka DC, Burstein HJ et al. American Society of Clinical Oncology guideline recommendations for sentinel lymph node biopsy in early-stage breast cancer. J Clin Oncol 2005; 20: 23 – 30.
4. Jakub JW, Bryant K, Huebner M, Hoskin T, Boughey JC, Reynolds C, et al. The number of axillary lymph nodes involved with metastatic breast cancer does not affect outcome as long as all disease is confined to the sentinel lymph nodes. Ann Surg Oncol 2010; 18: 86 – 93.
5. 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: 569 – 75.
6. Van la Parra RF, Peer PG, Ernst MF, Bosscha K. Meta-analysis of predictive factors for non-sentinel lymph node metastases in breast cancer patients with a positive SLN. Eur J Surg Oncol 2011; 37: 290 – 9.
7. Csermin G, Amendoeira I, Apostolikas N, Bellocq JP, Bianchi S, Bussolati G, et al. Pathological work-up of sentinel lymph nodes in breast cancer. Review of current data to be considered for the formulation of guidelines. Eur J Cancer 2003; 39: 1654 – 67.
8. Giuliano AE, Dale PS, Turner RR, Morton DL, Evans SW, Krasne DL. Improved axillary staging of breast cancer with sentinel lymphadenectomy. Ann Surg 1995; 222: 394 – 9.
9. Tsujimoto M, Nakabayashi K, Yoshidome K, Kaneko T, Iwase T, Akiyama F, et al. One-step nucleic acid amplification for intraoperative detection of lymph node metastasis in breast cancer patients. Clin Cancer Res 2007; 13: 4807 – 16.
10. Visser M, Jiwa M, Horstman A, Brink AA, Pol RP, van Diest P, et al. Intra-operative rapid diagnostic method based on CK19 mRNA expression for the detection of lymph node metastases in breast cancer. Int J Cancer 2008; 122: 2562 – 7.
11. Schem C, Maass N, Bauerschlag DO, Carstensen MH, Loning T, Roder C, et al. One-step nucleic acid ampifications-a molecular method for the detection of lymph node metastases in breast cancer patients; results of the German study group. Virchows Arch 2009; 454: 203 – 10.
12. Tamaki Y, Akiyama F, Iwase T, Kaneko T, Tsuda H, Sato K, et al. Molecular detection of lymph node metastases in breast cancer patients; results of a multicenter trial using the one-step nucleic acid amplification assay. Clin Cancer Res 2009; 15: 2879 – 84.
13. Feldman S, Krishnamurthy S, Gillanders W, Gittleman M, Beitsch PD, Young PR, et al. A novel automated assay for the rapid identification of metastatic breast carcinoma in sentinel lymph nodes. Cancer 2011; 117: 2599 – 607.
14. Snook KL, Layer GT, Jackson PA, de Vries CS, Shousha S, Sinnett HD, et al. Multicentre evaluation of intraoperative molecular analysis of sentinel lymph nodes in breast cancer carcicoma. Br J Surg 2011; 98: 527 – 35.
15. Veronesi U, Viale G, Paganelli G, Zurrida S, Luini A, Galimberti V, et al. Sentinel-lymph-node biopsy in breast cancer: ten years results of a randomized controlled study. Ann Surg 2010; 251: 595 – 600.
16. Veronesi U, Viale G, Paganelli G, Luini A, Zurrida S, Galimberti V, et al. Sentinel-lymph-node biopsy as a staging procedure in breast cancer: update of a randomized controlled study. Lancet Oncol 2006; 7: 983 – 90.
17. Pegolo E, Puppin C, Gerometta A, Damante G, Puglisi F, Di Loreto C. One-step nucleic acid amplification (OSNA) for intraoperative evaluation of sentinel lymph node status in breast cancer: a comparative study between CK19 protein expression and CK19 mRNA level in primary tumors and lymph node metastasis. Virchows Arch 2013; 463: 7 – 15.
18. Cserni G. Intraoperative analysis of sentinel lymph nodes in breast cancer by one-step nucleic acid amplification. J Clin Pathol 2011; 3: 193 – 9.
19. Osako T, Iwase T, Kimura K, Yamashita K, Horii R, Yanagisawa A, et al. Intraoperative molecular assay for SLN metastases in early stage breast cancer. A comparative analysis between

one-step nucleic acid amplification whole node assay and routine frozen section. Cancer 2001; 117: 4365 – 74.

20. Osako T, Iwase T, Kimura K, Yamashita K, Horii R, Akiyama F. Accurate staging of axillary lymph nodes from breast cancer patients using a novel molecular method. Br J Cancer 2011; 105: 1197-202.
21. Castellano I, Macri I, Deambrogio C, Balmativola D, Bussone R, Ala A, et al. Reliability of whole sentinel lymph node analysis by one-step nucleic acid amplification for intraoperative diagnosis of breast cancer metastases. Ann Surg 2011; 4: 11 – 20.
22. Laia BV, Marcos MB, Refael CM, Francisco SC, Jose T, Blai BS, et al. Molecular diagnosis of sentinel lymph nodes for breast cancer: one step ahead of standardization. Diagn Mol Pathol 2011; 20: 18 – 21.
23. Alix-Panabieres C, Vendrell JP, Slijper M, Pelle O, Barbotte E, Mercier G, et al. Full length cytokeratin-19 is released by human tumor cells: a potential role in metastatic progression of breast cancer. Breast Cancer Res 2009; 11: R39.
24. Wang YS, Ou-yang T, Wu J, Liu YH, Cao XC, Sun X, et al. Comparative study of one-step nucleic acid amplification assay, frozen section, and touch imprint cytology for intra-operative assessment of breast sentinel lymph node in Chinese patients. Cancer Sci 2012; 103: 1989 – 93.
25. Tamaki Y, Akiyama F, Iwase T, Kaneko T, Tsuda H, Sato K, et al. Molecular detection of lymph node metastases in breast cancer patients: results of a multicenter trial using the one-step nucleic acid amplification assay. Clin Cancer Res 2009; 15: 2879 – 84.
26. Sato N, Honma K, Noguchi S, amaki Y, Tsuda H, Kinoshita T, et al. Multi-institutional evaluation of sentinel lymph node (SLN) examination by one-step nucleic acid amplification OSNA) assay in breast cancer: performance of metastases detection and prediction of additional non-sentinel lymph node (non-SLN) involvement. J Clin Oncol 2011; 29: 1 – 6.
27. Smolarz B, Krawczky T, Westfal B, Maciejczyk R, Zadrozny M, Samulak D, et al. Comparison of one-step nucleic acid amplification (OSNA) method and routine histological investigation for intraoperative detection of lymph node metastasis in Polish women with breast cancer. Pol J Pathol 2013; 64: 104 – 8.
28. Santaballa A, De La Cueva H, Salvador C, Garcia-Martinez AM, Guarin MJ, Lorente D, et al. Advantages of one step nucleic acid amplification (OSNA) whole node assay in sentinel lymph node (SLN) analysis in breast cancer. Springerplus 2013; 2: 542 – 4.
29. Krag DN, Anderson SJ, Julian TB, Brown AM, Harlow SP, Costantino JP, et al. Sentinel-lymph-node resection compared with conventional axillary-lymph-node dissection in clinically node-negative patients with breast cancer: overall survival findings from the NSABP B-32 randomised phase 3 trial. Lancet Oncol 2010; 11: 927 – 33.
30. Galimberti V, Cole BF, Zurrida S, Viale G, Luini A, Veronesi P, et al; The International Breast Cancer Study Group trial 23-01 investigators. Axillary dissection versus no axillary dissection in patients with sentinel-node micrometastasis (IBCSG 23-01): a phase 3 randomised controlled trial. Lancet Oncol 2013; 14: 297 – 305.
31. Espinosa-Bravo M, Sansano I, Perez-Hoyos S, Ramos M, Sancho M, Xercavins J, et al. Prediction of non-sentinel lymph node metastasis in early breast cancer by assessing total tumoral load in the sentinel lymph node by molecular assay. Eur J Surg Oncol 2013; 39: 766 – 73.
32. Peg V, Espinosa-Bravo M, Vieites B, Vilardell F, Antunez JR, de Salas MS, et al. Intraoperative molecular analysis of total tumor load in sentinel lymph node: a new predictor of axillary status in early breast cancer patients. Breast Cancer Res Treat 2013; 139: 87 – 93.
33. Kumagai K, Yamamoto N, Miyashiro I, Tomita Y, Katai H, Kushima R, et al. Multicenter study evaluating the clinical performance of the OSNA assay for the molecular detection of lymph node metastases in gastric cancer patients. Gastric Cancer 2014; 17: 273 – 80.
34. Yamamoto H, Sekimoto M, Oya M, Yamamoto N, Konishi F, Sasaki J, et al. OSNA-based novel molecular testing for lymph node metastases in colorectal cancer patients: results from a multicenter clinical performance study in Japan. Ann Surg Oncol 2011; 18: 1891 – 8.