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การทำนายการแพร่กระจายของต่อมน้ำเหลืองที่ไม่ใช่เซนติเนล โดยการวิเคราะห์ปริมาณเนื้องอกจากต่อมน้ำเหลืองเซนติเนลในผู้ป่วยมะเร็งเต้านม

Prediction of Non-Sentinel Lymph Node Metastasis by Molecular Assay in Whole Sentinel Lymph Node Analysis in Breast Cancer Patients

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บทคัดย่อ:

วัตถุประสงค์: เพื่อค้นหาว่าปริมาณเนื้องอกจากการตรวจหากรดนิวคลีอิคแบบหนึ่งขั้นตอนสามารถเป็นปัจจัยที่ทำนายการแพร่กระจายของมะเร็งไปยังต่อมน้ำเหลืองที่ไม่ใช่เซนติเนลในผู้ป่วยมะเร็งเต้านมระยะต้น

วัสดุและวิธีการ: รวบรวมข้อมูลผู้ป่วยมะเร็งเต้านมระยะเริ่มต้น 102 ราย โดยได้รับการผ่าตัดต่อมน้ำเหลืองเซนติเนลและส่งตรวจด้วยวิธีออสนาในโรงพยาบาลสงขลานครินทร์ ระหว่างวันที่ 1 มกราคม พ.ศ. 2558-30 พฤษภาคม พ.ศ. 2559

ผลการศึกษา: ในการวิเคราะห์ตัวแปรเดียวพบว่าปริมาณเนื้องอกจากต่อมน้ำเหลืองเซนติเนล ขนาดก้อนมะเร็ง การแพร่กระจายในหลอดเลือดและทางเดินน้ำเหลือง การแพร่กระจายแบบมาโครเป็นปัจจัยที่มีผลต่อการแพร่กระจายของต่อมน้ำเหลืองที่ไม่ใช่เซนติเนลอย่างมีนัยสำคัญทางสถิติ ในขณะที่การวิเคราะห์หลายตัวแปรพบว่ามีเพียงปริมาณเนื้องอกจากต่อมน้ำเหลืองเซนติเนลเท่านั้นที่มีผลต่อการแพร่กระจายของต่อมน้ำเหลืองที่ไม่ใช่เซนติเนลอย่างมีนัยสำคัญทางสถิติ อัตราส่วนออด 1.1 ค่าความเชื่อมั่นที่ร้อยละ 95=1.0, 1.2 โดยมีพื้นที่ใต้โค้งเท่ากับ 0.9 ค่าความเชื่อมั่นที่ร้อยละ 95=0.8, 0.9

รับต้นฉบับวันที่ 27 ตุลาคม 2559 รับลงตีพิมพ์วันที่ 16 กุมภาพันธ์ 2560

สรุป: ปริมาณเนื้องอกเป็นปัจจัยสำคัญที่สามารถทำนายการแพร่กระจายของต่อมน้ำเหลืองที่ไม่ใช่เซนติเนล

คำสำคัญ: ต่อมน้ำเหลืองเซนติเนล, ปริมาณเนื้องอก, มะเร็งเต้านม, ออสนา

Abstract:

Objective: To determine whether the total tumor load (TTL) as indicated through the one-step nucleic acid amplification (OSNA) assay can be a predictive factor of non-sentinel lymph node (SLN) metastasis in early breast cancer patients.
Material and Method: The records of 102 patients with cT1-3N0 breast cancer who had an intraoperative SLN evaluation performed through an OSNA assay at Songklanagarind Hospital between 1 January 2015 and 30 May 2016 were examined.
Results: Univariate analysis found TTL, tumor size, presence of lymphovascular invasion, and macrometastasis were significant predictive factors of non-SLN metastasis. In the multivariate analysis, TTL was the only predictive factor with statistical significance (OR 1.1, 95% CI=1.0, 1.2). The area under the receiver operating characteristics (ROC) curve of TTL was 0.9 (95% CI=0.8, 0.9).
Conclusion: TTL is a significant predictive factor of non-SLN metastasis in early breast cancer patients.

Keywords: breast cancer, OSNA, total tumor load, whole sentinel lymph node

Introduction

The axillary lymph node is the most important prognostic factor in breast cancer patients. The sentinel lymph node (SLN) biopsy is the standard method that can represent overall axillary lymph node status in patients who are clinically negative for lymph nodes.[1-4] The incidences of non-SLN metastasis in SLN micrometastasis and macro-metastasis are 5.0-25.0% and 40.0-60.0%, respectively.[5,6]

The ACOSOG Z0011 trial found that there are some patients with a SLN metastasis in which axillary lymph node dissection (ALND) can be safely omitted as it does not show differences in the locoregional recurrence rates nor in the survival rate.[7] There is still a debate on which patients with a positive SLN can still benefit from ALND.

Numerous studies have been designed to identify variables that are predictive of non-SLN metastasis to avoid the complete ALND. Such studies have shown that different pathological characteristics of the primary tumor and the SLN metastasis are linked with higher chances to find additional positive non-SLN.[8,9] The traditional intraoperative histological exams are not standardized and have limited ability to detect metastasis. This is due to the partial evaluation of the node, with the risk of leaving a non-identified metastasis, thus underestimating the stage of the lymph node disease.[10,11]

The current method of lymph node metastasis is the One-Step Nucleic Acid Amplification (OSNA) assay (Sysmex Corporation, Kobe, Japan), which does the analysis through the detection and amplification of cytokeratin 19 (*CK19*) mRNA. This test shows a semi-quantitative result for the detection of nodal metastasis, differentiating micro-metastasis from macrometastasis.

The objective of this study was to determine the total tumor load (TTL), which is one of the variables determined with the OSNA assay, and is predictive factor of non-SLN metastasis in breast cancer patients.

Material and Method

The records of 238 SLNs from 102 patients with cT1-T3N0 breast cancer who had SLN analysis by OSNA assay at Songklanagarind Hospital between 1 January 2015 and 30 May 2016 were collected. The records of patients who had undergone systemic neoadjuvant chemotherapy, or who had breast cancer with metastasis at the time of diagnosis, were excluded.

The data collected from the medical records included age, type of surgery, tumor size, tumor site, histologic subtype, grade, estrogen receptor and progesterone receptor status, human epidermal growth factor receptor 2 (HER2) status, presence of lymphovascular invasion, SLN identification technique, turnaround time, total number of SLNs, number of positive and negative SLNs, and number of positive and negative non-SLNs. TTL was calculated as number of *CK19* mRNA copies/μLin the positive SLNs.

SLN evaluation by OSNA assay

The SLNs identification technique using periareolar injection of 1.0% isosulfan blue dye, 99mTc sulfur colloid, or both, depended on the surgeon. SLNs were denoted as blue node, hot node, blue+hot node, and/or palpable node. All whole SLNs were sent for an OSNA assay (Sysmex, Kobe, Japan). The results were assessed by the cut off level of calculated *CK19* mRNA copies per μL: macrometastasis was defined as >5,000 copies/μL of *CK19* mRNA, micrometastasis as 250-5,000 copies/μL, and non-metastasis as <250 copies/μL.[12]

Several studies have found that the OSNA assay could accurately detect SLN metastasis at rates comparable with the conventional pathological examination.[12-15] Axillary lymph node dissection at levels I and II was done in cases of SLN metastasis.

Statistical analysis

Statistical analysis was done with Statistical Package for the Social Science (SPSS) for Windows version 17.0 (SPSS Inc., Chicago, IL, United States). Categorical data were analyzed by Pearson's chi-square test. Continuous variables were analyzed by Mann-Whitney U-test. In SLN positive patients, univariate and multivariate logistic regression models were used to evaluate the risk factors that were different between the non-SLN positive and negative groups. The power of the TTL was quantified with a receiver operating characteristics (ROC) curve as measured by the areas under receiver operating characteristic curves (AUC). A p-value of <0.050 indicates statistical significance. All tests were two-tailed with a 95% confidence interval.

Results

A total of 238 SLNs from 102 patients were examined. Almost 80.0% of the patients were more than 45 years old, and all had a tumor size less than 5 cm (<T3 lesion) except for one patient with a 6 cm tumor. Thirty-nine patients (38.0%) had SLN metastasis. The average number of SLNs removed in the groups with negative and positive SLNs were 2.4 and 2.2 respectively. Patients and disease characteristics divided by negative and positive SLNs are reported in Table 1.

In patients with ALND after positive SLN, 25 patients (64.0%) had no additional axillary lymph node metastasis. Fourteen patients had additional non-SLN metastasis. In the SLN metastasis without additional non-SLN metastasis group, 17 patients (68.0%) had a micrometastasis (OSNA+) and 8 patients (32.0%) had a macrometastasis (OSNA++) in the SLN. Nineteen patients had only one positive SLN in the SLN metastasis without additional non-SLN metastasis group. Only 2 patients had 4 positive SLNs in the intraoperative evaluation. The characteristics of the patients who had an ALND are described in Table 2.

Table 1 Patient and tumors characteristics divided by negative and positive SLNs obtained by OSNA assay

Characteristic	SLN		P-value
	Negative (n=63) Number (%)	Positive (n=39) Number (%)	
Age (years)			
Mean	54	52	0.312[a]
<45	13 (20.6)	8 (20.5)	0.981[b]
≥45	50 (79.4)	31 (79.5)	
Tumor site			
Left	36 (57.1)	19 (48.7)	0.405[b]
Right	27 (42.9)	20 (51.3)	
Breast surgery			
Conservative	27 (42.9)	18 (46.2)	0.741[b]
Simple mastectomy	36 (57.1)	21 (53.8)	
SLN identification			
Dye alone	12 (19.0)	11 (28.2)	0.287[b]
Combined	51 (81.0)	28 (71.8)	
Average SLNs (node)	2.41	2.21	0.323[a]
OSNA turnaround time (min)	42.24	42.18	0.604[a]
Mean tumor size (mm)	22	24.6	0.302[a]
Pathological T stage			
pT1a	2 (3.2)	2 (5.1)	0.531[b]
pT1b	5 (7.9)	1 (2.6)	
pT1c	26 (41.3)	15 (38.5)	
pT2	30 (47.6)	20 (51.3)	
pT3	0 (0.0)	1 (2.6)	
Histologic type			
Invasive ductal carcinoma	54 (85.7)	39 (100.0)	0.108[b]
Invasive lobular carcinoma	2 (3.2)	0 (0.0)	
Mucinous carcinoma	6 (9.5)	0 (0.0)	
Medullary carcinoma	1 (1.6)	0 (0.0)	
Histologic grade			
Grade I	21 (33.3)	7 (17.9)	0.072[b]
Grade II	15 (23.8)	17 (43.6)	
Grade III	27 (42.9)	15 (38.5)	
Estrogen receptor			
Positive	47 (74.6)	30 (76.9)	0.794[b]
Negative	16 (25.4)	9 (23.1)	
Progesterone receptor			
Positive	40 (63.5)	25 (64.1)	0.953[b]
Negative	23 (36.5)	14 (35.9)	

Table 1 (continued)

Characteristic	SLN		P-value
	Negative (n=63) Number (%)	Positive (n=39) Number (%)	
HER2 status			
Positive	11 (17.5)	10 (25.6)	0.327[b]
Negative	52 (82.5)	29 (74.4)	
Lymphovascular invasion			
Present	11 (17.5)	19 (48.7)	0.003[b]
Absent	48 (76.2)	19 (48.7)	
Unknown	4 (6.3)	1 (2.6)	

[a]p-value by Mann-Whitney U-test, [b]p-value by chi-square test

SLN=sentinel lymph node, OSNA=One-Step Nucleic Acid Amplification, HER2=human epidermal growth factor receptor 2

Table 2 Characteristics of patients with ALND after positive SLN

Characteristic	Non-SLN in ALND		P-value
	Negative (n=25) Number (%)	Positive (n=14) Number (%)	
Age (years)			
Mean	54.5	48.6	
<45	3 (12.0)	5 (35.7)	0.084[b]
≥45	22 (88.0)	9 (64.3)	
Tumor site			
Left	13 (52.0)	6 (42.9)	0.587[b]
Right	12 (48.0)	8 (57.1)	
Breast surgery			
Conservative	12 (48.0)	6 (42.9)	0.760[b]
Simple mastectomy	13 (52.0)	8 (57.1)	
SLN identification			
Dye alone	8 (32.0)	3 (21.4)	0.484[b]
Combine	17 (68.0)	11 (78.6)	
Average SLNs (node)	2.4	1.86	0.067[a]
OSNA turnaround time (min)	43.4	39.9	0.044[a]
Mean tumor size (mm)	20.4	32.2	0.004[a]
Pathological T stage			
pT1a	2 (8.0)	0 (0.0)	0.323[b]
pT1b	1 (4.0)	0 (0.0)	
pT1c	11 (44.0)	4 (28.6)	
pT2	11 (44.0)	9 (64.3)	
pT3	0 (0.0)	1 (7.1)	

Table 2 (continued)

Characteristic	Non-SLN in ALND		P-value
	Negative (n=25) Number (%)	Positive (n=14) Number (%)	
Histologic grade			
Grade I	5 (20.0)	2 (14.3)	0.548[b]
Grade II	12 (48.0)	5 (35.7)	
Grade III	8 (32.0)	7 (50.0)	
Estrogen receptor			
Positive	21 (84.0)	9 (64.3)	0.167[b]
Negative	4 (16.0)	5 (35.7)	
Progesterone receptor			
Positive	18 (72.0)	7 (50.0)	0.171[b]
Negative	7 (28.0)	7 (50.0)	
HER2 status			
Positive	6 (24.0)	4 (28.6)	0.759[b]
Negative	19 (76.0)	10 (71.4)	
Lymphovascular invasion			
Present	9 (36.0)	10 (71.4)	0.103[b]
Absent	15 (60.0)	4 (28.6)	
Unknown	1 (4.0)	0 (0.0)	
Type of SLN metastasis			
Micrometastasis	17 (68.0)	2 (14.3)	0.001[b]
Macrometastasis	8 (32.0)	12 (85.7)	
Number of positive SLN			
1	19 (76.0)	8 (57.1)	0.476[b]
2	5 (20.0)	5 (35.7)	
4	1 (4.0)	1 (7.2)	
SLN TTL (copies/μL)			
Mean	50,484	113,919	0.001[a]
(range)	(250, 440,000)	(470, 380,000)	

[a]p-value by Mann-Whitney U-test, [b]p-value by chi-square test

SLN=sentinel lymph node, ALND=axillary lymph node dissection, OSNA=One-Step Nucleic Acid Amplification, HER2=human epidermal growth factor receptor 2, TTL=total tumor load

Univariate and multivariate analysis of non-SLN metastasis

The univariate analysis included age, tumor site, type of breast surgery, SLN identification technique, average number of SLNs, turnaround time, tumor size, histologic type, estrogen receptor (ER), progesterone receptor (PR), HER2 status, lymphovascular invasion (LVI) status, type of SLN metastasis, number of positive SLNs, and the number of TTL in the SLN. The TTL (p-value=0.040, OR=1.1, 95% CI=1.0, 1.2), tumor size (p-value=0.021, OR=3.4, 95% CI=1.2, 9.6), presence of lymphovascular invasion (p-value=0.043, OR=4.3, 95% CI=1.1, 17.4), and macro-metastasis in the SLN (p-value=0.004, OR=12.7, 95% CI=2.2, 70.9) were statistically significant predictive factors of non-SLN metastasis.

The TTL was the only predictive factor of non-SLN metastasis in the multivariate analysis (OR=1.1, 95% CI=1.0, 1.2), p-value=0.042 (Table 3).

The power of the TTL, quantified with the ROC curve as measured by the AUC, was 0.9 (95% CI=0.8, 0.9) (Figure 1).

Table 3 Univariate and multivariate analysis of prognostic factors with positive non-SLNs

	Univariate		Multivariate	
	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
Age ≥45 years	0.2 (0.1, 1.2)	0.091		
Tumor site, right	1.4 (0.3, 5.3)	0.588		
Conservative surgery	0.8 (0.2, 3.0)	0.783		
SLN: dye alone	0.5 (0.1, 2.6)	0.487		
Average SLNs	0.6 (0.3, 1.1)	0.151		
Turnaround time	0.9 (0.9, 1.0)	0.388		
Tumor size	3.4 (1.2, 9.6)	0.021	8.0 (0.9, 67.7)	0.059
Histologic grade III vs I, II	2.1 (0.3, 15.0)	0.559		
ER positive	0.3 (0.1, 1.5)	0.172		
PR positive	0.3 (0.1, 1.5)	0.172		
HER2 status positive	1.2 (0.2, 5.5)	0.757		
Presence of LVI	4.3 (1.1, 17.4)	0.043	0.1 (0.0, 2.8)	0.234
SLN macrometastasis	12.7 (2.2, 70.9)	0.004	0.0 (0.0, 3.2)	0.112
Number of positive SLN	1.5 (0.6, 3.7)	0.337		
SLN TTL (copies/μL)	1.1 (1.0, 1.2)	0.040	1.1 (1.0, 1.2)	0.042

CI=confidence interval, SLN=sentinel lymph node, ER=estrogen receptor, PR=progesterone receptor, HER2=human epidermal growth factor receptor 2, LVI=lymphovascular invasion, TTL=total tumor load

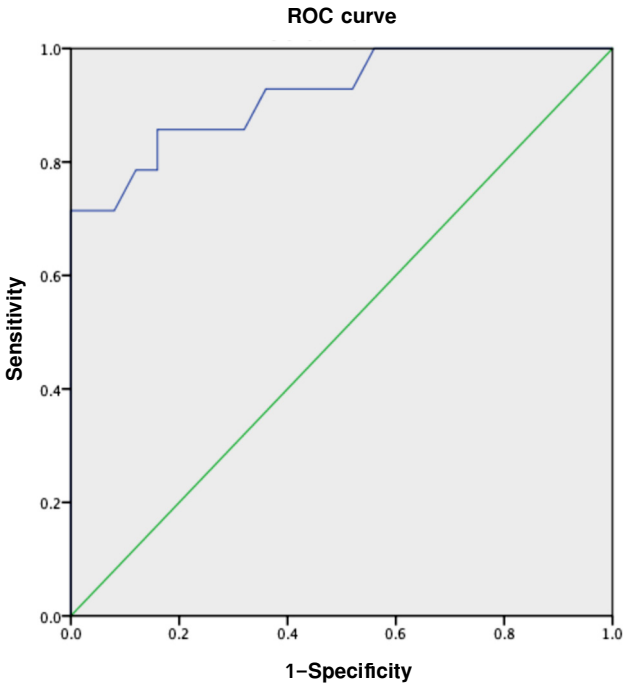

Figure 1 The power of the total tumor load analysed against the occurrence of tumor metastasis, in non-sentinel lymph nodes quantified with the receiver operating characteristics curve as measured by the area under curve, was 0.9

Discussion

Intraoperative assessment of SLN by OSNA assay is a simple and effective technique which is more sensitive than frozen section and touch imprint cytology.[16-18] When compared with the conventional histology in Songklanagarind Hospital, the OSNA assay had a sensitivity of 100.0%, specificity 95.2%, positive predictive value 73.5%, and negative predictive value 100.0%.

To avoid sampling errors, the concept of whole SLN analysis was reported as standard technique.[19] It also shows a quantitative result of the TTL.[22-25]

Several studies have reported on significant predictive factors of additional non-SLN metastasis in patients with positive SLN who met the ACOSOG Z0011 trial but may not have completely met all the criteria of the research study. A study by Mittendorf et al.[26] tested 6 variables: number of SLNs identified, number of positive SLNs, SLN metastasis size, extranodal extension, tumor size, and histology in an MD Anderson nomogram for predicting the likelihood of having additional axillary metastasis. The main problem is the results of these variables revealed after surgery in postoperative period.

The TTL model, which was used to intraoperatively evaluate the likelihood of metastasis in positive SLN, was generated as a new predictor. Espinosa-Bravo et al.[27] reported that TTL and hormonal status were statistically significant predictive factors for non-SLN metastasis. Peg et al.[28] found that TTL, tumor size, number of affected SLNs, HER2 status and LVI were predictors of axillary status. Our study found that only TTL was a significant predictive factor for additional non-SLN metastasis, while the size of SLN metastasis, hormonal status, tumor size, number of affected SLNs, HER2 status and LVI did not have statistical significance. The TTL was quantified by the AUC, with a value of 0.9 (95% CI=0.8, 0.9).

Our study had an important limitation, which was the low number of positive SLN cases. Further studies with l arger number of cases are necessary to find a reliable TTL cut-off point for determining non-SLN metastasis.

Conclusion

The TTL as assessed intraoperatively by whole SLN analysis is a diagnostic technique for predicting additional non-SLN metastasis that can help surgeons to make timely decisions concerning ALND for their patients. The limitation of this study was the low number of positive SLN cases.

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