Evaluation of novel diagnostic kits for detecting metastatic lymph nodes in breast cancer patients treated with neoadjuvant chemotherapy using the semi-dry dot-blot method: A single-center prospective study

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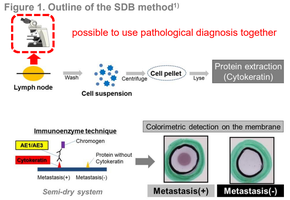
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Abstract

Background:
The semi-dry dot-blot (SDB) method is a diagnostic technique used to detect lymph node (LN) metastases (mets) by identifying cytokeratin (CK) proteins in the lavage fluid of sectioned LNs. This method is based on the principle that CK, an epithelial marker, is absent in normal lymphatic tissue. We prospectively evaluated novel SDB kits incorporating a newly developed anti-CK19 antibody and an automatic reader for diagnosing LN mets in breast cancer patients. In a previous analysis of 1734 sentinel LNs (SLNs) from patients not receiving **neoadjuvant chemotherapy (NAC)**, the kit demonstrated a sensitivity of 93.0%, specificity of 97.1%, and overall agreement of 96.7% for detecting macromets (>2.0 mm). However, the impact of NAC—especially in triple-negative and HER2-positive subtypes—on the diagnostic performance of the SDB method remains unclear, given that chemotherapy can cause cancer cell degeneration. This study aimed to evaluate whether the SDB kit could accurately detect macromets in patients undergoing NAC.
Methods:
A total of 127 LNs were collected from 74 breast cancer patients **who underwent NAC** followed by surgery between May 2022 and December 2024 at Nagasaki University Hospital. Both patients with and without axillary LN mets were eligible. Eligible LNs were defined as all SLNs in patients who underwent SLN biopsy and two axillary LNs in patients who underwent axillary LN dissection (ALND). Each LN was sliced at 2-mm intervals and rinsed with phosphate-buffered saline. The resulting lavage fluid was centrifuged, and the cells were lysed to extract protein, which was then analyzed using the SDB kit and automatic absorbance reader. Pathologists, blinded to the SDB results, independently diagnosed LN metastasis using hematoxylin and eosin (H&E) staining. SDB-based diagnoses were compared with permanent histopathological diagnoses.
Results:
Among the 127 LNs, 56 were obtained via SLN biopsy and 71 via ALND. Histological evaluation identified 16 macromets and 111 non-macromets (including 4 micromets and 1 case with isolated tumor cells). Using a CK19 absorbance cutoff of **11.0 milli-absorbance units (mAbs)** for detecting macromets, the SDB kit correctly identified 15 of the 16 macromets and all 111 non-macromets. **The resulting sensitivity, specificity, and overall agreement were 93.8%, 100%, and 99.2%, respectively.** The single false-negative case involved a sparsely distributed residual tumor measuring 2.1 mm. The diagnostic process required approximately 20 minutes and cost less than USD 30 per kit.
Conclusions:
The novel SDB kits combined with an automatic reader provided rapid, accurate, and cost-effective detection of LN macromets without tissue loss, even after NAC. These findings support the potential utility of this method in clinical decision-making. A prospective multi-center study is planned to further validate clinical performance.

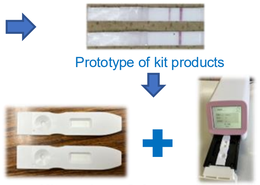
Background and Objectives

Figure 1. Outline of the SDB method[1)]



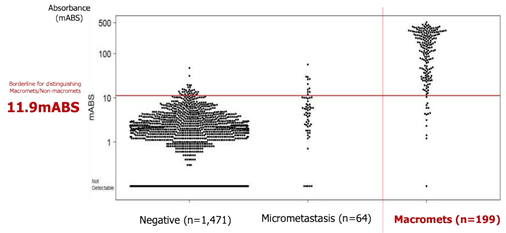
The original SDB method;

- **Accurate**
  sensitivity 93.3%, specificity 96.9%, accuracy 96.6%
- **Fast**
  about 40min for diagnosis
- **Cost-effective**
  only 10 USD per sample
- **Dual detection**
  possible to use pathological diagnosis together



(20min. for diagnosis, less than 30 USD for the kit and 3,000 USD for the automatic reader)

Figure 2. The prospective evaluation using SDB kits for breast cancer patient **without NAC (n=1,734)**[2)]



	SDB Macromets	SDB Non-macromets	Total
Macrometastases by permanent histology	185	14	199
Non-Macrometastases by permanent histology	39	1,496	1,535
Total	224	1,510	1,734*

*with immunohistochemical staining of CK19 and central pathological review

Sensitivity: 93.0% (95%CI: 79.0-95.2%)
Specificity: 97.5% (95%CI: 93.6-95.2%)
Overall agreement: 96.9% (95%CI: 92.3-95.2%)

This study aimed to evaluate whether the SDB kit could accurately detect macromets in breast cancer patients undergoing NAC.

Materials & Methods

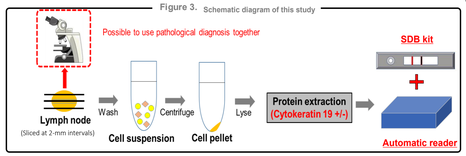
Materials: **127 LNs** from 74 breast cancer patients **undergoing NAC**

- **16 macromets**
- **4 micromets**
- **107 negative LNs**

Non-macromets

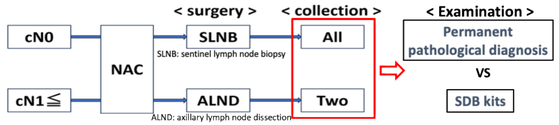
diagnosed by only permanent H&E stain

Figure 3. Schematic diagram of this study



[Definition of LN mets]
Macromets: >2.0mm
micromets: >0.2mm, ≦2.0mm
ITCs: ≦0.2mm
in maximum diameter of LN mets

Figure 4. Study design



Results

Table 1. Characteristics of patients

	Patients, N=74 (LNs, n=127)
Age (years), median [Q1-Q3]	**61** [52-69]
cT1/2/3/4, N(%)	**22**(29.7)/**39**(52.7)/**5**(6.8)/**8**(10.8)
cN0/1/2/3, N(%)	**46**(62.2)/**16**(21.6)/**5**(6.8)/**7**(9.5)
Regimen, N(%)	
dose dense	**24** (32.4)
anti-HER2	**40** (54.1)
immune checkpoint inhibitor	**5** (6.8)
Others	**5** (6.8)
ER, positive, N(%)	**31** (41.9)
PR, positive, N(%)	**22** (29.7)
HER2, positive, N(%)	**38** (51.4)
SLNB/ALND, N(%) n(%)	**37** (50.0) / **37** (50.0) **55** (43.3) / **72** (56.7)
Pathological LN status, n(%)	
Non-macrometastasis	**111** (87.4)
Negative	**106** (83.5)
ITCs	**1** (0.8)
micrometastasis	**4** (3.1)
Macrometastasis	**16*** (12.6)
Diameter of Macromets, median [Q1-Q3]	**5.5** [3.6-7.0]
pCR, N(%)	**35** (47.3)

*Fifteen of 16 macromets were obtained by ALND.

Figure 5. .Diagnoses made by permanent histology & Absorbance of kit reaction

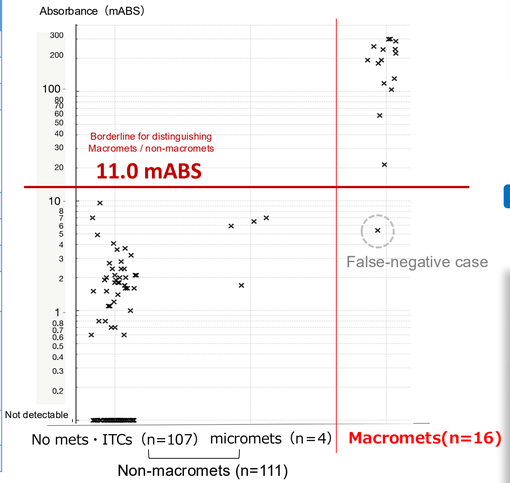


Table 2. Comparison between the SDB kit and the permanent histology for distinguishing macromets

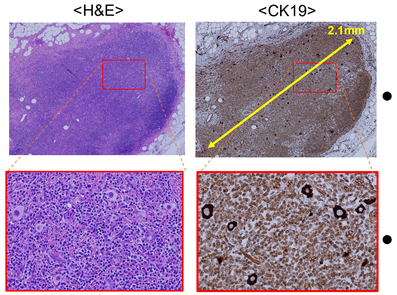
	SDB Macromets	SDB Non-macromets	Total
Macromets by permanent histology	**15**	**1***	**16**
Non-Macromets by permanent histology	**0**	**111****	**111**
Total	**15**	**112**	**127**

Sensitivity: 93.8% (95%CI: 80.8-93.8%)
Specificity: 100% (95%CI: 98.1-100%)
Overall agreement: 99.2% (95%CI:95.9 99.2-%)

* Macromets of 2.1mm in diameter
** Including 4 micromets

Discussion

Figure6. Hematoxylin & Eosin stain (H&E) and CK19 expression in **the false-negative case**.





- The permanent pathological diagnosis of this case was macromets, characterized by **sparsely remaining tumor cells** with a maximum diameter of **2.1 mm (morphological diagnosis).**

Discrepancy

- The SDB kit classified this case as non-macromets because of the **markedly reduced tumor burden (molecular diagnosis).**

- Micromets and ITCs after NAC have been suggested as poor prognostic factors[3)]. However, the optimal local management of the axilla in such situations following SLNB remains uncertain.
- The NEONOD2 trial, which is currently ongoing, is evaluating whether axillary surgery can be safely omitted in patients with micromets detected on SLNB after NAC[4)] .

Conclusions

- We plan to conduct a **multicenter prospective study** to evaluate the clinical utility of the SDB kit for diagnosing LN mets in breast cancer patients undergoing NAC.

The SDB kit with automatic reader;

- **Accurate** **sensitivity 93.8%, specificity 100%, and overall agreement 99.2%** for distinguishing macromets
- **Brief exam. time** about **20min.** for diagnosis
- **Cost-effective** **less than 30 USD** for the kit and **3,000 USD** for the automatic reader
- **Dual detection** possible to use pathological diagnosis together **for breast cancer patients undergoing NAC**

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