Retrospective analysis of sentinel lymph node biopsy using methylene blue dye for early breast cancer

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Abstract

Background: Methylene blue is the most commonly used tracer for sentinel lymph node (SLN) biopsy (SLNB) in China. This study aimed to investigate the feasibility of clinical application of SLNB using methylene blue dye (MBD) for early breast cancer and the prognosis of patients with different SLN and non-SLN statuses.

Methods: We retrospectively analyzed the clinicopathological data of patients with early breast cancer treated at the Peking University First Hospital between 2013 and 2018. We calculated the SLN identification rate (IR) in SLNB with MBD and the false-negative rate (FNR), and analyzed the prognosis of patients with different SLN and non-SLN statuses using Kaplan-Meier curves.

Results: Between January 2013 and December 2018, 1603 patients with early breast cancer underwent SLNB with MBD. The SLN IR was 95.8% (1536/1603). Two SLNs (median) were detected per patient. There were significant differences in FNR between patients with SLN micrometastasis and macrometastasis (19.0% vs. 4.5%, \( \chi^2 = 12.771, P < 0.001 \)). Chi-square test showed that there were significant differences in SLN successful detection rates among patients with different vascular tumor embolism status (96.3% vs. 90.8%, \( \chi^2 = 9.013, P = 0.003 \)) and tumor (T) stages (96.6% vs. 94.1%, \( \chi^2 = 5.189, P = 0.023 \)). Multivariate analysis showed that vascular tumor embolism was the only independent factor for SLN successful detection (odds ratio: 0.440, 95% confidence interval: 0.224 - 0.862, \( P = 0.017 \)). Survival analysis showed a significant difference in disease-free survival (DFS) between patients with non-SLN metastasis and patients without non-SLN metastasis (\( P = 0.006 \)).

Conclusion: Our single-center data show that, as a commonly used tracer in SLNB in China, MBD has an acceptable SLN IR and a low FNR in frozen sections. This finding is consistent with reports of dual tracer-guided SLNB. Positive SLNs with non-SLN metastasis are associated with DFS.

Keywords: Breast cancer; Identification rate; Methylene blue dye; Prognosis; Sentinel lymph node biopsy

Introduction

The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-32 study laid the foundation for the clinical application of SLNB.[1-2] Based on high-level evidence, the 2020 National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines on Breast Cancer recommended sentinel lymph node biopsy (SLNB) for the evaluation of axillary lymph nodes in patients with clinical node-negative early breast cancer.[3] In 2020, the Chinese Society of Breast Surgeons of the Chinese Medical Association conducted a 37-hospital retrospective analysis and found that methylene blue dye (MBD) was used as the only tracer in more than 70% of SLNBs in breast cancer patients in China.[4] To investigate the clinical SLN identification rate (IR) using MBD and to calculate the false-negative rate (FNR) in frozen sections, we retrospectively analyzed the clinicopathological data of patients with early breast cancer who underwent SLNB with MBD at the Peking University First Hospital between January 2013 and December 2018.

Methods

Ethical approval

This study was approved by the Institutional Ethics Committee of Human Research of Peking University First Hospital (No. 2018-46). Given the study’s retrospective nature and because data analysis was performed anonymously, it was exempt from obtaining informed consent from patients.

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Participants

Eligible patients with early breast cancer treated at the Peking University First Hospital between January 2013 and December 2018 were included in this retrospective study. Inclusion criteria: (1) female; (2) clinical carcinoma in situ (cTis)-T3N0, histopathologically confirmed invasive breast cancer or carcinoma in situ; (3) axillary lymph nodes clinically negative on physical examination and ultrasound; (4) axillary lymph node dissection (ALND) and systemic treatment in patients with positive SLN after SLNB; and (5) complete clinical and pathologic data. Exclusion criteria: (1) stage IV breast cancer at the time of initial diagnosis; (2) inflammatory breast cancer; (3) positive axillary lymph nodes on clinical evaluation; (4) patients who were contraindicated for SLNB or declined SLNB; (5) patients who did not undergo breast surgery; or (6) missing clinical or pathology data.

SLNB surgical procedure

A total of 0.1 to 0.3 mL of 1% MBD (20 mg/2 mL; Jumpcan Pharmaceutical Group Co., Ltd., China) was intra-dermally injected into each of the injection sites (1–3 sites) in the affected areola area using a 1-mL syringe. The surgeon performed a 3 to 5 minute breast massage after MBD injection. Three to 5 min later, an incision was made along the lower border of the axillary hair, the outer edge of the pectoralis major, and the anterior edge of the lattissimus dorsi. Next, blue-stained lymphatic vessels were traced to locate blue-stained SLNs deep in the axillary fascia. SLN was considered successful if one to five blue-stained lymph nodes were identified during the procedure. SLNB failure was considered if the number of blue-stained lymph nodes was zero or ≥6. The SLNB surgeries in this study were performed by eight surgeons. Two of them performed more than 50 surgeries per year, which was defined as surgeon A group, while the others were defined as surgeon B group.

SLN sampling and pathological evaluation

The SLN sampling procedures were standardized according to the College of American Pathologists (CAP) recommended techniques. According to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual (8th Edition), the total number of SLNs detected should be <6. A positive SLN was defined as a macrometastatic (maximum diameter of metastatic lesion >2.0 mm) or micrometastatic SLN (maximum diameter of metastatic lesion >0.2 and ≤2.0 mm and/or >200 tumor cells in a section). A negative SLN was defined as one lacking tumor cells or with isolated tumor cells (maximum diameter of metastatic lesion ≤0.2 mm and ≤200 tumor cells in a section). The SLN FNR in frozen sections = number of MBD-negative frozen sections with a positive paraffin result/total number of positive-SLN paraffin sections.

Immunohistochemistry (IHC) and histological grade

IHC results were interpreted according to the 2010 American Society of Clinical Oncology (ASCO)/CAP guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer and the 2013 recommendations for human epidermal growth factor receptor 2 (HER2) testing in breast cancer. The criteria for Nottingham Combined Histologic Grade for invasive breast cancer include duct formation, nucleus size and shape, chromatin atypia, and mitotic phase.

Tumor stage and molecular subtyping

Anatomic and prognostic staging was performed according to the AJCC Cancer Staging Manual (8th Edition). Breast cancer was categorized as luminal A, luminal B, HER2 positive, or triple-negative according to the 2011 St. Gallen International Expert Consensus.

Follow-up

The clinicopathological data of the enrolled patients in this study were collected, sorted and logged by the fixed staff of Peking University First Hospital, after which the data were re-verified by the authors. All follow-up data were obtained from outpatient medical records as well as from telephone and mail inquiries and were updated until January 31, 2020. The follow-up data included disease-free survival (DFS) and overall survival (OS).

Statistical analysis

Categorical variables were presented as numbers (percentages) and analyzed using Chi-squared test or Chi-squared test with Yates’ continuity correction. Continuous variables with normal distribution were expressed as mean ± standard deviation while those with skewed distribution were shown as median (Q1, Q3). Multivariate logistic regression analysis was adopted to identify influencing factors of SLN detection. Kaplan-Meier survival curves were applied for survival analyses. The differences between groups in DFS and OS were performed using the log-rank test and a Cox proportional hazards model. SPSS software version 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. A two-sided P < 0.05 was considered statistically significant.

Results

Baseline clinicopathological data

A total of 2486 breast cancer patients were admitted to the Peking University First Hospital between January 2013 and December 2018, 1731 of whom (69.6%) underwent SLNB with MBD. Patients who did not undergo breast surgery and those with missing clinical or pathological data were excluded. Finally, 1603 patients were included in this study [Figure 1]. Of them, 1393 (86.9%) had invasive breast cancer, and 210 (13.1%) had carcinoma in situ. The patients were aged 21 to 91 (55.5 ± 12.6) years [Table 1].

SLN detection and frozen sections

Among the 1603 patients, SLNs were successfully detected in 1536 patients (95.8%) and the median SLNs (Q1, Q3) were 2 (1, 3) [Table 2]. A total of 1 to 2 SLN(s) were...
detected in 72.5% (1113/1536) of patients. SLNB failed in 67 patients, including zero blue-stained nodes in 11 patients and ≥6 nodes in 56 patients.

Among the 1536 patients who underwent SLNB, SLN metastasis was identified in 310 patients (20.2%) in paraffin sections [Table 2], including micrometastasis in 42 patients (13.5%) and macrometastasis in 268 patients (86.5%). All 310 patients underwent ALND, and 117 of them (37.7%) had non-SLN metastasis. Among the 268 patients with macrometastasis, 112 (41.8%) had non-SLN metastasis. Among the 42 patients with micrometastasis, five had non-SLN metastasis (11.9%).

All 310 SLN-positive patients had invasive breast cancer, 20 of whom had a negative result with frozen sections but a positive result with paraffin sections. Therefore, the FNR was 6.5%. Specifically, the FNR was 19.0% (8/42) for SLN micrometastasis and 4.5% (12/268) for SLN macrometastasis. In frozen sections, there were significant differences in FNR between patients with SLN micrometastasis and macrometastasis in frozen sections ($\chi^2 = 12.771, P < 0.001$).

**Factors for SLN detection**

Analysis of the 1603 SLNB cases showed that there were significant differences in SLN successful detection rate among patients with different vascular tumor embolism status (96.3% vs. 90.8%, $\chi^2 = 9.013, P = 0.003$) and tumor (T) stages (96.6% vs. 94.1%, $\chi^2 = 5.189, P = 0.023$) [Table 3]. Multivariate analysis with non-conditional logistic regression input showed that vascular tumor embolism (odds ratio: 0.440, 95% confidence interval: 0.224–0.862, $P = 0.017$) was the only independent factor for SLN successful detection [Table 4].

**Prognostic analysis**

A total of 1603 patients with early breast cancer were included in this study. Of them, 113 patients were lost to follow-up, and 1490 (93.0%) completed the follow-up.
The patients were followed up for 9.8 to 72.5 months (median: 35.7 months). A total of 1429 patients successfully underwent SLNB and completed the follow-up. Of them, 46 patients had relapse or metastasis, including simple locoregional recurrence (LRR) \((n = 10, 0.7\%)\), simple distant metastasis \((n = 30, 2.0\%)\), and LRR with distant metastasis \((n = 6, 0.4\%)\). Nineteen patients died of breast cancer.

A prognostic analysis based on 1429 patients showed that the 5-year DFS rate was 94.8% and that the 5-year OS rate was 96.0% [Figure 2]. No significant difference was observed in DFS \((P = 0.152)\), OS \((P = 0.538)\), or LRR \((P = 0.926)\) between SLN-positive and SLN-negative patients. Among the 292 SLN-positive patients with complete follow-up data, no significant difference was observed in DFS \((P = 0.460)\) or OS \((P = 0.855)\) between patients with macrometastasis and patients with micro-metastasis; no significant difference was observed in OS \((P = 0.928)\), but a significant difference was observed in DFS between patients with non-SLN metastasis and patients without non-SLN metastasis \((P = 0.006)\) [Figure 3]. Although the Cox regression analysis showed that non-SLN metastasis had no significant impact on OS \((\text{hazard ratio [HR]}: 1.069, 95\% \text{ confidence interval [CI]}: 0.255–4.478, P = 0.928)\), a higher probability of recurrence and metastasis was observed in the non-SLN metastasis group \((\text{HR}: 4.367, 95\% \text{ CI}: 1.369–13.924, P = 0.013)\).

### Discussion

Many studies, such as the NSABP B-32, have confirmed that SLNs can predict the pathologic status of axillary lymph nodes in patients with clinically axillary node-negative early breast cancer in clinical practice.\(^{[12-14]}\) In 2005, ASCO first recommended SLNB as a surgical method for evaluating the status of axillary lymph nodes.\(^{[15]}\) Subsequently, NCCN, and guidelines implemented by the China Anti-Cancer Association have generally accepted SLNB as the standard procedure for evaluating axillary lymph nodes.\(^{[16]}\)

Due to the qualification requirements for the use of radionuclides and the limited availability of isosulfan blue, MBD is the most commonly used tracer in SLNB for breast cancer in China. In 2020 the Chinese Society of Breast Surgeons of the Chinese Medical Association conducted a 37-hospital multicenter analysis and found that MBD was used as the only tracer in more than 70% of SLNBs of breast cancer patients. In this study, we retrospectively analyzed the clinicopathological data of patients with early breast cancer treated at the Peking University First Hospital between January 2013 and December 2018. Patients with SLNB indication were selected according to the SLNB criteria recommended by NCCN clinical practice guidelines for breast cancer, and the SLN IR with MBD

| Parameters                  | n   | %   | Positive SLN(s) | n   | %   |
|----------------------------|-----|-----|-----------------|-----|-----|
| Age (years)                |     |     |                 |     |     |
| <35                        | 70  | 4.4 |                 |     |     |
| ≥35                        | 1533| 95.6|                 |     |     |
| BMI (kg/m²)                |     |     |                 |     |     |
| <24.0                      | 823 | 51.3|                 |     |     |
| ≥24.0                      | 780 | 48.7|                 |     |     |
| Menopause                  |     |     |                 |     |     |
| No                         | 708 | 44.2|                 |     |     |
| Yes                        | 895 | 55.8|                 |     |     |
| Pathological type          |     |     |                 |     |     |
| Carcinoma in situ          | 210 | 13.1|                 |     |     |
| Invasive breast cancer     | 1393| 86.9|                 |     |     |
| Invasive ductal carcinoma  | 1137| 70.9|                 |     |     |
| Invasive lobular carcinoma | 65  | 4.1 |                 |     |     |
| Other                      | 191 | 11.9|                 |     |     |
| Histological grade         |     |     |                 |     |     |
| 1                          | 335 | 24.0|                 |     |     |
| 2                          | 652 | 46.8|                 |     |     |
| 3                          | 406 | 29.1|                 |     |     |
| Molecular typing           |     |     |                 |     |     |
| Luminal A                  | 266 | 19.1|                 |     |     |
| Luminal B                  | 814 | 58.4|                 |     |     |
| HER2 negative              | 646 | 46.3|                 |     |     |
| HER2 positive              | 168 | 12.1|                 |     |     |
| HER2-enriched              | 111 | 8.0 |                 |     |     |
| TNBC                       | 202 | 14.5|                 |     |     |
| Vascular tumor embolism    |     |     |                 |     |     |
| No                         | 1473| 91.9|                 |     |     |
| Yes                        | 130 | 8.1 |                 |     |     |
| T stage                    |     |     |                 |     |     |
| T0                         | 28  | 1.7 |                 |     |     |
| Tis                        | 210 | 13.1|                 |     |     |
| T1                         | 894 | 55.8|                 |     |     |
| T2                         | 443 | 27.6|                 |     |     |
| T3                         | 28  | 1.7 |                 |     |     |
| Anatomic staging           |     |     |                 |     |     |
| 0                          | 228 | 14.2|                 |     |     |
| I                          | 725 | 45.2|                 |     |     |
| II                         | 567 | 35.4|                 |     |     |
| III                        | 83  | 5.2 |                 |     |     |
| Prognostic stage           |     |     |                 |     |     |
| 0                          | 228 | 14.2|                 |     |     |
| I                          | 912 | 56.9|                 |     |     |
| II                         | 313 | 19.5|                 |     |     |
| III                        | 150 | 9.4 |                 |     |     |

**BMI:** Body mass index; **HER2:** Human epithelial growth factor receptor 2; **TNBC:** Triple negative breast cancer.
and the FNR of frozen sections were calculated. In all, 2486 breast cancer patients were admitted to our hospital over 6 years, 1731 of whom (69.6%) underwent SLNB. After excluding patients with stage IV breast cancer or positive axillary nodes at the time of diagnosis, nearly all patients with SLNB indications underwent SLNB, suggesting that SLNB is widely accepted in clinical practice. It effectively reduces ALND rate and improves the quality of life. Studies in other countries show that the SLN IR is 81.7% to 98.0% with dual tracer (radionuclides combined with isosulfan blue)-guided SLNB. In this study, 1603 patients underwent SLNB, and SLNB was successful in 1536 (95.8%) patients; the IR was consistent with that of published studies on dual tracer-guided SLNB.

Table 3: Analysis of clinicopathological factors for SLN detection in early breast cancer (n=1603).

| Parameters                        | SlN successful detection (n=1536) | χ²   | P value |
|-----------------------------------|----------------------------------|------|---------|
| Age (years)                      |                                  |      |         |
| <35                               | 70                               | 64 (91.4) | 2.472 | 0.116 |
| ≥35                               | 1533                             | 1472 (96.0) |      |       |
| BMI (kg/m²)                      |                                  |      |         |
| <24.0                             | 823                              | 788 (95.7) | 0.023 | 0.881 |
| ≥24.0                             | 780                              | 748 (95.9) |      |       |
| Menopause                        |                                  |      |         |
| No                                | 708                              | 677 (95.6) | 0.125 | 0.723 |
| Yes                               | 895                              | 859 (96.0) |      |       |
| Pathological type                |                                  |      |         |
| Carcinoma in situ                | 210                              | 201 (95.7) | 0.007 | 0.934 |
| Invasive breast cancer           | 1393                             | 1335 (95.8) |      |       |
| Histological grade               |                                  |      |         |
| 1                                 | 335                              | 324 (96.7) | 2.488 | 0.288 |
| 2                                 | 652                              | 619 (94.9) |      |       |
| 3                                 | 406                              | 392 (96.6) |      |       |
| HR                                |                                  |      |         |
| Negative                         | 311                              | 301 (96.8) | 0.940 | 0.332 |
| Positive                         | 1075                             | 1027 (95.5) |      |       |
| HER2                             |                                  |      |         |
| Negative                         | 1111                             | 1067 (96.0) | 0.703 | 0.402 |
| Positive                         | 275                              | 261 (94.9) |      |       |
| Ki67                              |                                  |      |         |
| ≤14                               | 414                              | 398 (96.1) | 0.184 | 0.668 |
| >14                               | 1170                             | 1119 (95.6) |      |       |
| Vascular tumor embolism          |                                  |      |         |
| No                                | 1473                             | 1418 (96.3) | 9.013 | 0.003 |
| Yes                               | 130                              | 118 (90.8) |      |       |
| T stage                           |                                  |      |         |
| 1                                 | 1132                             | 1093 (96.6) | 5.189 | 0.023 |
| ≥2                                | 471                              | 443 (94.1) |      |       |
| Tumor location                   |                                  |      |         |
| Upper outer quadrant              | 896                              | 852 (95.1) | 4.273 | 0.370 |
| Lower outer quadrant              | 204                              | 195 (95.6) |      |       |
| Upper inner quadrant              | 338                              | 327 (96.7) |      |       |
| Lower inner quadrant              | 98                               | 96 (98.0) |      |       |
| Central                           | 67                               | 66 (98.5) |      |       |
| Surgeon group                     |                                  |      |         |
| A                                 | 827                              | 797 (96.4) | 1.300 | 0.254 |
| B                                 | 776                              | 739 (95.2) |      |       |

Data are presented as n (%). Continuity correction. BMI: Body mass index; HER2: Human epithelial growth factor receptor 2; HR: Hormone receptor; SLN: Sentinel lymph node.

Table 4: Multivariate logistic regression analysis of clinicopathological factors for SLN detection in early breast cancer.

| Parameters                        | β       | SE      | Wald | P value | OR (95% CI)   |
|-----------------------------------|---------|---------|------|---------|---------------|
| Vascular tumor embolism           | -0.821  | 0.343   | 5.719| 0.017   | 0.440 (0.224–0.862) |
| T stage                           | -0.450  | 0.262   | 2.946| 0.086   | 0.637 (0.381–1.066) |

CI: Confidence interval; OR: Odds ratio; SE: Standard error; SLN: Sentinel lymph node.
data showed that the incidence of LRR was as low as 0.7%, which suggests that MBD is a safe and viable tracer for SLNB.

The AJCC Cancer Staging Manual (8th Ed) clearly specifies that the total number of SLNs detected should be fewer than six, which has been incorporated into the technical specifications for SLNB in clinical practice. Of the 1603 patients who underwent SLNB, the SLNB failed in 67 patients, including no blue-stained nodes in 11 patients (0.7%) and ≥6 SLNs detected in 56 patients (3.5%). In the remaining patients, the number of SLNs detected was $1.99 \pm 1.12$ nodes per patient, which is consistent with AJCC guidelines. We believe that the same basic principles should be followed regardless of the specific tracer(s) used in SLNB. Dual tracer-guided SLNB differs from single tracer-guided SLNB, which explains the difference in the number of SLNs detected. When using MBD as a tracer in SLNB, only stained nodes should be resected for pathological evaluation of SLNs, and it is not advisable to dissect unstained nodes to increase the number of SLNs detected.

The NCCN clinical practice guidelines for breast cancer recommend that patients with newly diagnosed breast cancer undergo ultrasound to evaluate axillary lymph nodes and that clinically node-negative early breast cancer is an indication for SLNB. However, the SLN-positive pathological rate is still as high as 25% in patients with such clinical indications, suggesting that histopathological evaluation of SLNs provides more objective node (N) staging, and imaging evaluation cannot replace pathological evaluation of the SLN status. In this study, the SLN-positive rate was 20.2%, which was lower than reported earlier. This discrepancy may be caused by the more comprehensive pre-operative ultrasound evaluation at our hospital, which includes detailed information about

![Figure 2: Kaplan-Meier analyses of disease-free survival (A) and overall survival (B) in 1429 patients. SLNB: Sentinel lymph node biopsy.](image)

![Figure 3: Kaplan-Meier analyses of disease-free survival (A) and overall survival (B) in the non-SLN, non-metastasis group, and non-SLN metastasis group. SLN: Sentinel lymph node.](image)
the number of axillary lymph nodes, maximum diameter, length-to-width ratio, cortical and medulla structure, hilum of lymph nodes, and blood flow to lymph nodes.[20] Patients with suspected axillary node metastasis on ultrasound undergo ultrasound-guided lymph node biopsy, and SLNB is avoided if metastasis is confirmed by cytological examination. Therefore, careful ultrasound evaluation before operation may have contributed to the lower SLN-positive rate in this study than that reported in other studies.

Intraoperative frozen sections examination is a main method for evaluating the status of SLNs. The 1997 AJCC Cancer Staging Manual (5th Edition) clearly specified that a maximum diameter of a metastatic lesion >2 mm meets the criterion for macrometastasis.[21] In 2012, the CAP recommended that SLN sampling must be adequate to detect all macrometastases. Thus, each grossly negative node should be thinly sliced along the long axis of the node (2 mm slices), and all slices should be macroscopically examined. At least one representative hematoxylin and eosin-stained section must be examined. These standards are still in use. We searched English-language papers published in 2015 to 2019 with keywords including “breast cancer,” “SLN,” and “frozen section” and retrieved more than 70 papers, suggesting that the SLN frozen section pathology is still a popular topic. We retrospectively analyzed the data of previous studies with more than 2000 cases and found that the FNR of macrometastasis in frozen sections is 3.1% to 8.9%.[22,23] Given that the diagnostic criteria for lymph node micrometastasis include >200 tumor cells in a section, even deep dissection may not prevent false negatives. In this study, using frozen sections to evaluate the SLN status intra-operatively, the FNR of macrometastasis was 4.5%, which was relatively low compared with that in previous studies. This finding might have been observed because our pathology department is in strict compliance with the CAP technical standards of pathological sampling and evaluation of SLN.

The American College of Surgeons Oncology Group Z0011 study shows that eligible early breast cancer patients with macrometastases in ≤2 SLNs may avoid ALND.[24] The IBCSG 23-01 study shows that eligible early breast cancer patients with SLN micrometastasis may not require ALND.[25] The current study showed that among patients with SLN metastasis, 37.7% had non-SLN metastasis. The proportions were 41.8% and 11.9% among patients with macrometastases in tumor (T) stage ≥2, the omission of ALND should be carefully chosen in clinical settings.

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**Conflicts of interest**

None.

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