

Long-Term Follow-up Results of Fluorescence and Blue Dye Guided Sentinel Lymph Node Biopsy in Early Breast Cancer

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Abstract

Purpose This study aimed to assess the value of the combination of indocyanine green (ICG) and methylene blue (MB) dye in early breast cancer patients undergoing sentinel lymph node biopsy (SLNB).

Methods Between January 2011 and December 2015, 1061 early breast cancer patients underwent SLNB were included. SLNB was performed for enrolled patients by injection of both ICG and MB. Detection rate, positivity, and number of sentinel lymph nodes (SLNs) by ICG and MB were analysed. Axillary recurrence and arm lymphedema at 5.6 years follow-up were reported.

Results The combination of ICG and MB resulted in a very high detection rate of 99.6%, the median number of sentinel lymph nodes was 3. A total of 374 histologically confirmed positive SLNs were detected in 237 patients, 96.6% of the positive patients and 94.1% of the positive nodes could be identified by the combination of ICG and MB. After a median follow-up of 5.6 (2 to 9.3) years, 0.64% of patients with negative SLNs had ipsilateral axillary recurrence, and the incidence of arm lymphedema was 2.1%.

Conclusions Although the missing isotope control weakens the interpretation of the findings, the dual tracing modality of ICG and MB, without involvement of radioactive isotopes, was an effective method for SLNB in patients with early breast cancer.

Introduction

Sentinel lymph node biopsy (SLNB) was first applied in breast cancer care in the 1990s by Krag et al [1], and now has become the standard procedure for patients with clinically negative axilla [2]. SLNB can accurately stage axillary lymph node metastasis and avoid unnecessary axillary lymph node dissection (ALND) [3], and the risk of lymphedema, paresthesia, and pain syndromes is reduced subsequently [4].

The existing tracer-based techniques for SLNB have involved blue dye, technetium-99m (^{99m}Tc)-labeled nanocolloid, or both [5]. Isosulfan blue and patent blue have been the most commonly used blue dyes in developed countries and methylene blue has been widely used in developing countries like China [6, 7]. A dual tracer of blue dye and radioisotope (RI) can improve detection of SLNs and reduce the false negative rate (FNR) [8] and thus has been recommended as the standard technique in guidelines [9]. However, the use of RI creates some logistical challenges for hospitals, such as the handling and disposal of isotopes, training of staff, legislative requirements, and the reluctance of patients to be exposed to radiation [9]. The application of RI is limited to breast centers with RI facilities and nuclear medicine [10]. And the time-window for SLN identification is limited due to the short half-life (6 hours) of ^{99m}Tc [9]. Although SLNB was performed routinely in 76% of hospitals in China, only 14.6% of hospitals used dual-tracer method including RI [11]. It may be due to the inconvenience of RI application. Constraints of radioisotopes have led to the development of non-radioactive alternatives. Innovative tracers such as indocyanine green (ICG), superparamagnetic iron oxide (SPIO), and microbubbles have been explored [12]. The main disadvantages of SPIO include the need to remove metal retractor from the wound during

surgery to obtain magnetometer readings, and SPIO may interfere with contrast-enhanced MRI [13]. It was reported that the standard dual technique of RI and blue dye was significantly better than microbubbles in terms of SLNs identification rate [9]. ICG was one of the most promising technique because of the high SLNs identification rate (93%-100%) and was the most commonly used non-radioactive tracer in clinical trials of SLNB [12]. ICG is a FDA-approved organic molecule with near infrared (NIR) fluorescence [14]. SLNB with NIR fluorescence imaging using ICG was first reported by Kitai et al, and showed high detection rate for SLN with the rate of 94% [15]. As ICG can be transcutaneously detected similar to radioactive agents, the combination of ICG and methylene blue (MB) shows great potential as an alternative to standard dual mapping methods involving RI. Previously, we reported that dual tracer of MB and ICG improved the mapping performance in 198 patients with early breast cancer [16]. Now we report the final results for 1061 patients from this prospective cohort study, not only for SLN detection, but also for long-term axillary recurrence.

Methods

Study Design and Patients

The study was registered retrospectively as Australia and New Zealand Clinical Trials Registry No.12612000109808 in January 2012. The first participant was enrolled in January 2011. From January 2011 to May 2012, we conducted a randomized controlled study comparing MB and MB combined with ICG in SLNB for early breast cancer patients according to the registered content. The preliminary results have showed that MB combined with ICG is better than MB alone in identification of SLNs, which has been published in a local journal [17]. Therefore, dual tracers were used for all patients since May 2012. A total of 1061 consecutive early breast cancer patients underwent SLNB with dual tracing by MB and ICG were enrolled in the present study between January 2011 and December 2015. Primary breast cancer was confirmed in eligible patients by core needle biopsy or surgical biopsy, and eligible patients had negative axillary lymph nodes by clinical examination and ultrasound. Patients were not admitted to the study if any of following criteria were present: distant metastasis, inflammatory breast cancer, clinically or radiologically suspicious lymph nodes, previous axillary surgery, and hypersensitivity to iodine or ICG. Patients with previous breast surgery were not excluded. Written informed consents were obtained from all included patients. The study was approved by the Ethics Committee of Peking University People's Hospital, Beijing.

Procedure

MB (Jizhou Pharmaceutical, Suzhou, China) was diluted in saline to a final concentration of 1%. ICG (Dandong Pharmaceutical, Jilin, China) was dissolved and diluted in distilled water to a final concentration of 0.5 mg/mL before use, and 1 mL MB and 1 mL ICG were injected intradermally in the periareolar region 5 minutes before surgery. ICG fluorescence was stimulated and detected by the fluorescence detector (MingDe Medicine, China) and lymphatic drainage could be navigated in real time. Blue-stained nodes and fluorescent nodes were removed and deemed SLNs. Nodes that were hard and

highly suspicious for metastatic tumor were also removed and defined as SLNs irrespective of fluorescence or MB staining. Lymph nodes labeled by MB and/or ICG are shown in Fig. 1. SLNs were classified into four groups by different tracers: ICG(+)/MB(+), ICG(+)/MB(-), ICG(-)/MB(+), and ICG(-)/MB(-). Patients with tumor-positive SLNs had ALND unless they met entry criteria for the ACOSOG Z0011 study. All patients received standard adjuvant therapy including radiotherapy, chemotherapy, and endocrine therapy, according to the National Comprehensive Cancer Network (NCCN) guideline.

Outcomes and statistical analysis

The total numbers of SLNs and the numbers of positive SLNs detected by MB alone and by the combination of MB and ICG were recorded. Patients were followed up every six months. Follow-up was ended on April 15, 2020.

Relapse free survival (RFS) was defined as the time from diagnosis to the recurrence of tumor, including locoregional disease or distant metastasis, or death from any cause. Breast cancer specific survival (BCSS) was defined as the time from diagnosis to death from breast cancer.

Lymphedema was recorded as self-reported lymphedema [18]. Patients were asked to indicate the presence of swelling during follow-up by answering following yes or no questions: 1) Have you experienced swelling of the upper-limb on the breast cancer treated side in the past year?; 2) Is the arm on the treated side thicker than the other arm?; 3) Have you seen a doctor for arm swelling?

The associations between different tracers and identification of SLNs were evaluated using χ^2 tests. Mann-Whitney U tests were used to compare the median of SLNs in different tracers. The Kaplan-Meier method was used to draw survival curves. A 2-sided p value < 0.05 was considered significant. Statistical analyses were performed with statistical software (SPSS, version 20.0; RStudio, version 1.3.1056).

Results

Patient characteristics

The present study enrolled 1061 patients with early breast cancer (Fig. 2). Their characteristics are presented in Table 1.

Table 1
Patient and tumor characteristics

Characteristics	N	%
Total	1061	
Age(years)		
53 (20–89)*		
≥50	648	61.1%
<50	413	38.9%
BMI		
≥ 24	541	51%
< 24	520	49%
Histological type		
In situ	87	8.2%
Invasive ductal	803	75.7%
Specified carcinoma	171	16.1%
HR		
Positive	810	76.3%
Negative	239	22.5%
Unknown	12	1.1%
HER2		
Positive	181	17%
Negative	822	77.5%
Unknown	58	5.5%
Molecular subtype (immunohistochemical)		
Luminal A	379	35.7%
Luminal B	429	40.4%
BMI, Body mass index; HR, Hormone receptor; HER2, Human epidermal growth factor receptor 2; p, Pathologic.		
* Age is the median age, including the maximum and minimum.		
# Eleven patients received excisional biopsy in other hospital, and pT of them were unknown. One patient with pN2 was classified as stage pIII, and stage of the remaining 10 patients was unknown.		

Characteristics	N	%
HER2 enriched	90	8.5%
Triple negative	142	13.4%
Unknown	21	2.0%
pT		
pTis	87	8.2%
pT1	766	72.2%
pT2	187	17.6%
pT3	10	0.9%
pTX	11	1.0%
pN		
pN0	803	75.7%
pN1	214	20.2%
pN2	34	3.2%
pN3	10	0.9%
pStage		
p0	87	8.2%
pI	594	56.0%
pII	324	30.5%
pIII	46	4.3%
Unknown [#]	10	0.9%
Excisional biopsy		
Yes	796	75%
No	265	25%
Surgery		

BMI, Body mass index; HR, Hormone receptor; HER2, Human epidermal growth factor receptor 2; p, Pathologic.

* Age is the median age, including the maximum and minimum.

[#] Eleven patients received excisional biopsy in other hospital, and pT of them were unknown. One patient with pN2 was classified as stage pIII, and stage of the remaining 10 patients was unknown.

Characteristics	N	%
Mastectomy	600	56.6%
Breast conserving	461	43.4%
Adjuvant chemotherapy		
Yes	665	62.7%
No	396	37.3%
Adjuvant radiotherapy		
Yes	617	58.2%
No	444	41.8%
BMI, Body mass index; HR, Hormone receptor; HER2, Human epidermal growth factor receptor 2; p, Pathologic.		
* Age is the median age, including the maximum and minimum.		
# Eleven patients received excisional biopsy in other hospital, and pT of them were unknown. One patient with pN2 was classified as stage pIII, and stage of the remaining 10 patients was unknown.		

Sentinel lymph node detection

All of 1061 patients received SLNB traced by combination of ICG and MB. The staining for each lymph node of 92 patients was not recorded clearly by different tracers as ICG(+)/MB(+), ICG(+)/MB(-), ICG(-)/MB(+), or ICG(-)/MB(-). Therefore, the remaining 969 patients were included in the analysis for sentinel lymph node detection.

SLNs were identified in 910 (93.9%) patients by MB alone, and in 965 (99.6%) patients by the combination of ICG and MB ($p < 0.001$) (Table 2). The dual tracer had a significantly higher sentinel node detection rate than MB alone, and the absolute difference was 5.7%. Older age, higher BMI, upper outer quadrant tumor, prior excisional biopsy, and larger tumor size were associated with a reduced detection rate with MB alone but not with the combination method (Online Resource 1). The combination method also resulted in the identification of more lymph nodes in each patient (Table 2). The median number of SLNs detected by MB alone was 2, while the median number of SLNs detected by the dual tracer was 3 ($p < 0.001$).

Table 2
Comparison of identification by different tracers

	MB	ICG + MB	p
Identification rate	910/969 (93.9%)	965/969 (99.6%)	< 0.01
Positive SLNs identified	259/374 (69.3%)	352/374 (94.1%)	< 0.01
Patients with N (+) identified	201/237 (84.8%)	229/237 (96.6%)	< 0.01
Patients with more than 2 N (+)	9 (0.9%)	28 (2.9%)	< 0.01
SLNs, Sentinel lymph nodes; MB, Methylene blue; ICG, Indocyanine green.			

A total of 374 histologically confirmed positive SLNs were detected in 237 patients, of which 84.8% (201/237) could be identified by MB, and 96.6% (229/237) by dual tracers, resulting in an additional 28 (11.8%) node positive patients identified by the dual tracer ($p < 0.001$) (Table 2). Among the 374 positive nodes excised, 69.3% (259/374) could be identified by MB alone and 94.1% (352/374) by the dual tracer ($p < 0.001$). Among 28 patients who had more than 2 positive SLNs detected by dual tracer, 19 (67.9%) would have been identified as ≤ 2 if using MB alone.

Survival

Among 1061 patients, 32 were lost to follow-up; therefore, 1029 patients were included in the survival analysis. After a median follow-up of 5.6 years (2 to 9.3 years), there were 73 RFS events including 38 recurrences without death, 19 deaths with recurrence, and 16 deaths without recurrence. The 6-year RFS was 93.5% in node negative patients and 91.3% in node positive patients ($p = 0.21$) and the 6-year BCSS was 97.8% and 97.7% ($p = 0.47$) (Fig. 3).

Five of 777 patients with negative SLNs were diagnosed with ipsilateral axillary recurrences as the first events after a median follow-up of 5.6 years. The ipsilateral axillary recurrence rate was 0.64% (Online Resource 2).

Lymphedema

Details on lymphedema were collected from 973 of 1061 patients. Among 766 patients who had SLNB, 16 reported ipsilateral arm lymphedema and among 207 patients with ALND, 29 reported lymphedema. The incidence of upper extremity lymphedema following SLNB was 2.1% and was significantly lower versus ALND (14%) ($p < 0.001$).

Discussion

The present prospective study reports a radioisotope-free method of SLNB using a combination of ICG and MB that had a 99.6% detection rate. After a median follow-up of 5.6 years, the ipsilateral axillary recurrence rate in patients with negative SLNs was 0.64% and the incidence of upper extremity arm

lymphedema was 2.1%. The dual tracing modality of ICG and MB, without radioactive isotopes, was an effective method for SLNB in patients with early breast cancer.

Although blue dye has no radiation exposure and is not expensive, its injections may cause skin necrosis and anaphylaxis [19], which was absent when ICG was used. The approach of blue dye was cheap but achieved lower identification rate. Both RI and ICG could increase the detection rate but need additional apparatus which means increased cost [8, 9]. Previous studies have compared the cost of RI and ICG, which showed \$331 – \$420 per patient for RI and \$5 - \$111 per patient for ICG [10], and it cost \$18 per patient in China similarly. It seemed a little bit cheaper for ICG than RI, however, we could not conclude ICG is more cost-effective than RI. Based on our practical experience, the use of ICG made the surgical operation more visualized, it's easier and quicker for us to find the SLN. But we did not have accurate records of the surgery time. Other study have reported that the average time between skin incision and SLN identification was 7 ± 6 min when using ICG [20]. And it has been showed in a randomized controlled trial that the time to detect the first SLN was similar between multimodal method (combination of RI, ICG and blue dye) and RI alone (6.5 ± 5.16 vs. 8.0 ± 4.35 min; $p = 0.13$) [21].

The fluorescent ICG tracer helps to visualize lymphatic drainage from breast to axilla for incision localization and SLN identification, and, as in our study, previous trials have shown detection rates as high as 99 percent [22, 23]. Although age, BMI, tumor location, and excisional biopsy have all been reported to associate with decreased SLN detection rates [24–27], we achieved very high sensitivity in our study independent of negative factors when ICG and MB were used together.

FNR is a crucial indicator for assessing the accuracy of SLNB. However, FNR was not analyzed in our study. Since MB was already considered a reliable tracer method in China [7], and no additional ALND was performed in patients with negative SLNs, calculating the FNR of ICG and MB would have been difficult.

Instead, the efficacy of SLNB traced by combination of ICG and MB was evaluated in terms of long term ipsilateral axillary recurrence. Although previous studies have examined the rate of ipsilateral axillary recurrence after SLNB with ICG tracer, they have enrolled relatively few patients and the time of follow-up was short [28, 29]. In our study, after a median follow-up of 5.6 years, the ipsilateral axillary recurrence rate of was 0.64%, which was comparable to the result reported in the NSABP B32 trial and previous studies using different tracers (Table 3) [28–32].

Table 3
Ipsilateral axillary recurrence rates

Author	Tracers	Patients with SLNB alone	Follow-up time	Axillary recurrence rate
Krag et al ^[30]	RI + blue dye	2011	8y	0.4%
Inoue et al ^[28]	ICG + blue dye	464	3.2y	0.4%
Boniface et al ^[31]	RI + blue dye	2216	10.5y	1.6%
Galimberti et al ^[32]	RI	5262	7.0y	1.7%
Toh et al ^[29]	ICG	153	2.5y	0
Our study	ICG + blue dye	777	5.6y	0.67%
SLNB, Sentinel lymph node biopsy; RI, Radioisotope; ICG, Indocyanine green.				

The number of SLNs that should be excised for accurate lymph node staging is controversial. Is detection of relatively more SLNs helpful in accurate axillary staging? And how many SLNs are enough? Several studies suggested that SLNB should not remove only one or two lymph node(s) when other labeled nodes are present, and in cases where 4 or more SLNs were removed accuracy reached 100% and the FNR dropped to zero [33, 34]. In patients undergoing SLNB after neoadjuvant chemotherapy, a dual tracer reached an acceptable FNR when more than 2 SLNs were detected [35]. In our study, the median number of SLNs detected by the combined method was 3, a little bit higher than traditional methods. It was reported that more SLNs identified by ICG was probably due to the low molecular weight and high degree of diffusion of ICG allowing it to spread beyond the SLN to secondary draining lymph node [36]. ICG could bind rapidly and completely to proteins. After intradermal or subcutaneous injection, ICG could theoretically bind to proteins and consequently became larger molecular tracer [37]. And it has been shown in a double-blind randomized trial that the average number of detected SLNs by ICG or ICG:HSA (adsorption of ICG to human serum albumin) were not significantly different [37]. We think the more reasonable explanation for a little bit more SLNs was due to the higher sensitivity of ICG method. It does not rely on visual search for dye but on light of fluorescence. Fluorescence can penetrate through thin layers of tissue and reveal nodes otherwise hidden when using MB [14]. We believe that the dual tracer with ICG and MB would be useful in SLNB, even after neoadjuvant chemotherapy. Further studies will be needed.

Our study showed that the detection rate of positive lymph nodes was 69.3% by MB alone. More positive lymph nodes could be detected by dual tracers. However, it was comparable with the results of NSABP B32 study. The detection rate of positive lymph nodes was 77.5% and 93% by blue dye and combined method, respectively [24]. The number of positive lymph nodes at SLNB is very important for subsequent

decision making. Patients undergoing breast-conserving surgery with only 1 or 2 positive SLNs who meet the entry criteria for the ACOSOG Z0011 trial can be exempted from ALND [38]. In our study, among 28 patients with more than 2 positive SLNs detected by the dual tracer, 67.9% had only 2 or less positive SLNs detected by MB alone. Therefore, the application of ICG could be helpful in accurate decision making with a higher number of positive SLNs.

Some may argue that excision of more SLNs detected by ICG will increase the incidence of lymphedema. The present study is the first one to report the incidence of lymphedema after SLNB with ICG tracer in a large sample cohort. The incidence of breast cancer treatment-related lymphedema following SLNB was 2.1%, whereas that following ALND was 14%. Goldberg reported on a series of 600 patients received SLNB by combined dye-isotope mapping technique, the incidence of patient-reported lymphedema was 3% [39]. And another report showed that the incidence of subjective lymphedema after SLNB was 4.1% [40]. Therefore, from the results of our study, it seems that SLNB with a dual tracer of combined ICG and MB is not related to an increased risk of lymphedema.

Our study has some shortcomings. RI has not been available in the majority of the hospitals in China, and it is regrettable we did not compare the dual ICG and MB tracer with the standard method of RI and blue dye recommended by the guidelines. We have also noticed that several studies compared the combination of ICG and RI to RI and blue dye [10]. with the purpose of replacing blue dye with ICG in the dual tracer method. Clearly, this was not our goal. Since previous studies have shown that ICG performed similarly to RI with regard to the number of SLNs identified, rate of failed mapping, and identification of pathologically positive SLNs [41, 42], the hypothesis of replacing RI by ICG in a dual tracer method was reasonable.

Conclusion

Although the missing isotope control weakens the interpretation of the findings, the dual tracing modality of ICG and MB, without involvement of radioactive isotopes, was an effective method in SLNB for patients with early breast cancer.

Declarations

Authors' contributions: Chaobin Wang participated in the study design and coordination and participated in the data analysis. Shu Wang conceived the study and participated in its design and coordination. Fuzhong Tong, Yingming Cao, Peng Liu, Bo Zhou, Hongjun Liu, Lin Cheng, Miao Liu, Jiajia Guo, Fei Xie, Houpu Yang, Siyuan Wang, Yuan Peng participated in the clinical study implementation and data collection. All authors approved the final manuscript.

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Conflict of interest: The authors declare that they have no conflict of interest.

Ethics approval: This study was approved by the Ethical Committee of Peking University People's Hospital, Beijing.

Consent to participate: Written informed consent was obtained from all patients.

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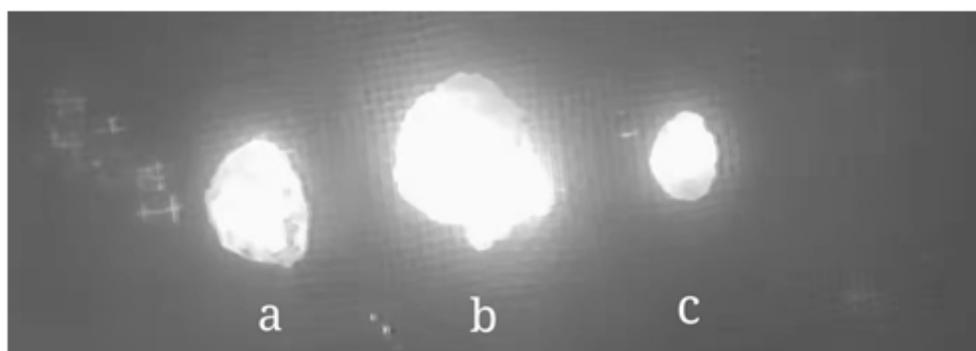
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Figures



(1) MB



(2) ICG

Figure 1

Lymph node labeled by MB and/or ICG . Three sentinel lymph nodes were identified. Lymph node a, b were labeled as MB(+)/ICG(+), lymph node c was labeled as MB(-)/ICG(+).

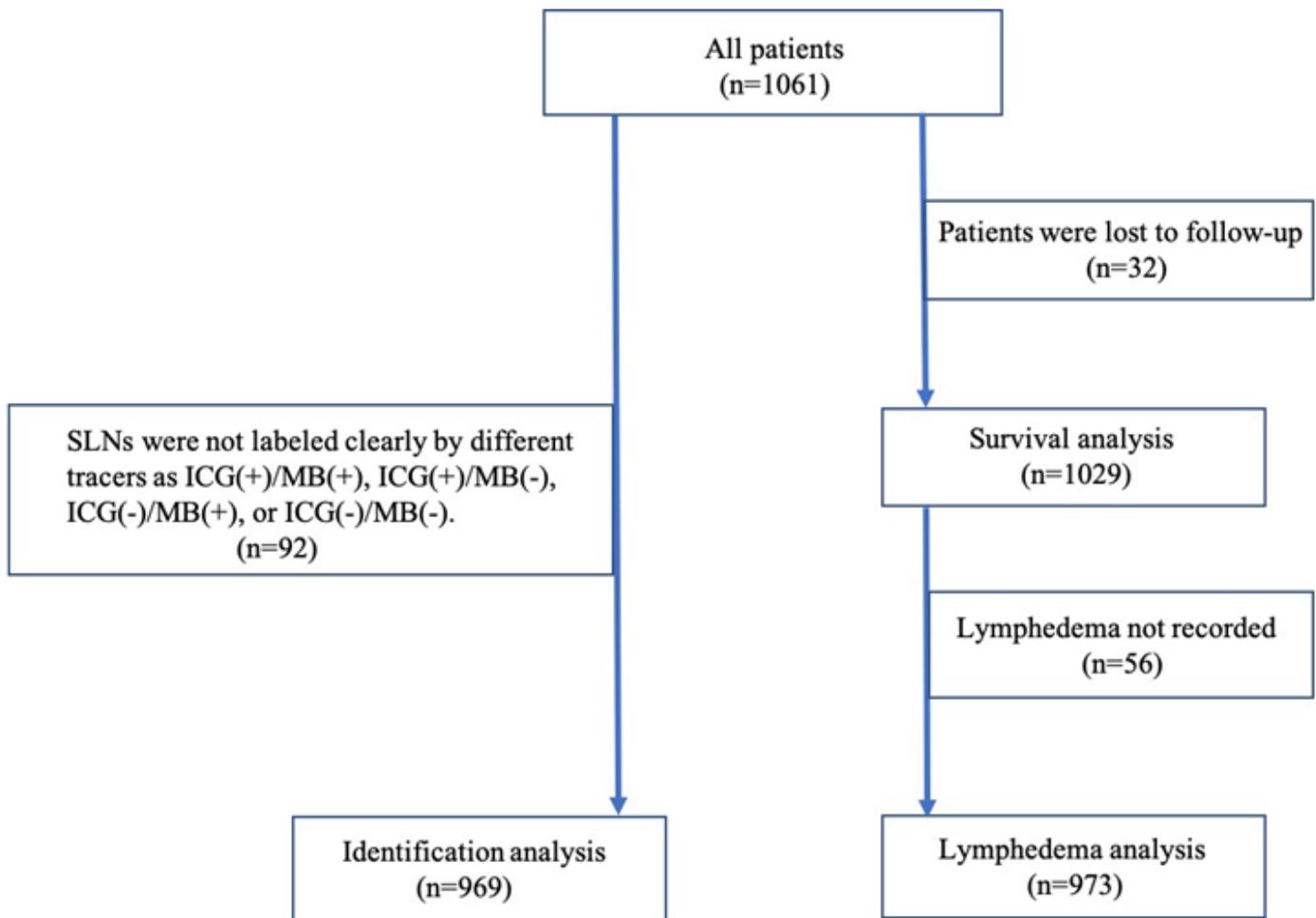
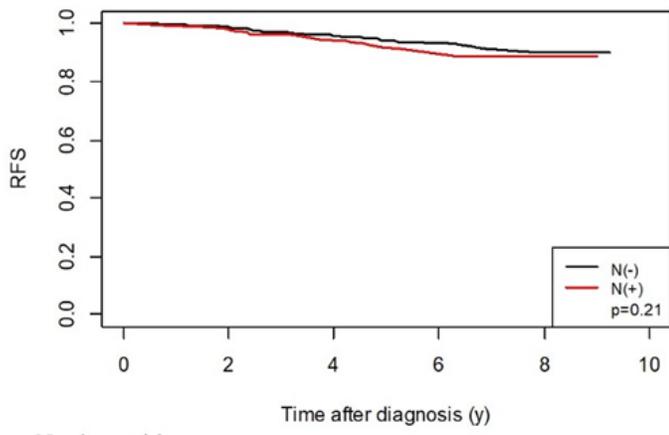


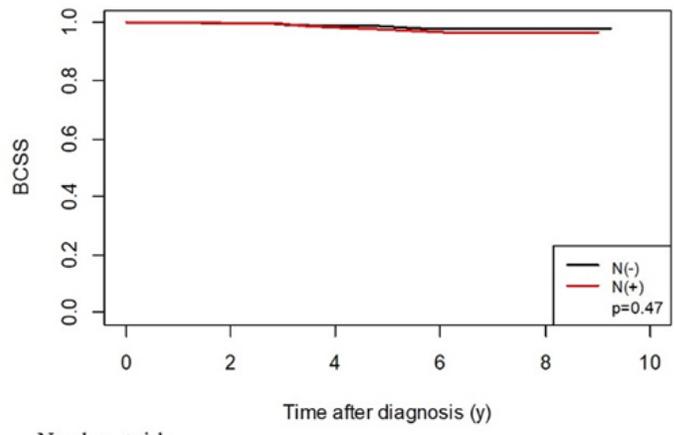
Figure 2

Study enrollment flow chart A total of 1061 patients were included, sentinel lymph nodes (SLNs) of 92 patients were not labeled clearly, but follow-up were performed. 32 patients were lost to follow-up, but SLNs of them were labeled clearly. Therefore, different analyses were performed in different populations.



Number at risk		Time after diagnosis (y)					
		0	2	4	6	8	10
N(-)	777	769	734	266	52	0	
N(+)	252	248	231	85	11	0	

a Relapse Free Survival



Number at risk		Time after diagnosis (y)					
		0	2	4	6	8	10
N(-)	777	777	753	274	55	0	
N(+)	252	252	239	89	12	0	

b Breast Cancer Specific Survival

Figure 3

Kaplan–Meier Estimates of Relapse Free Survival and Breast Cancer Specific Survival Panels a and b show relapse free survival (RFS) and breast cancer specific survival (BCSS), respectively. N(-) denotes lymph nodes negative and N(+) denotes lymph nodes positive.