

# The Relative False Negative Rate of Isosulfan Blue in Detecting Sentinel Lymph Nodes in Early Breast Cancer

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**Objective:** To determine the false negative rate of the isosulfan blue injection method of SLN detection in early breast cancer, relative to that of the combined blue dye and radiocolloid injection method.

**Material and Method:** Seventy women with early breast cancer underwent the combined method of SLN detection during the period between September 2007 and December 2008. Standard criteria for each method were used to identify SLNs. Each SLN was labeled as identified by the blue dye, the radiocolloid and as being positive or negative for cancer cells.

**Results:** Subjects were 50 years old with tumors of size 2.3 cm on the average. The average number of SLNs harvested was 2.5 nodes per subject. The detection rate for the isosulfan blue method was 91% (64/70). The relative false negative rate of the blue dye was zero (0/64).

**Conclusion:** Experienced surgeons who use the isosulfan blue method of SLN detection in early breast cancer can be reasonably confident that the false negative rate of the isosulfan blue method was similar to that of the combined method.

**Keywords:** Early breast cancer; Sentinel lymph node biopsy, Isosulfan blue, False negative rate

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Sentinel lymph node (SLN) biopsy is an established part of the management of early breast cancer. Many methods have been used to detect sentinel lymph nodes<sup>(1-3)</sup>. The most validated methods include the use of isosulfan blue ("blue dye") injection, radiocolloid injection or both. The most accurate and validated method at present is the combined blue dye and radiocolloid injection<sup>(1-5)</sup>. However, in many parts of the world, detection using radiocolloid injection is not feasible due to the lack of personnel, instruments, and other resources needed to establish a nuclear medicine service<sup>(2,5)</sup>. The most readily available and validated sentinel lymph node detection method is therefore the blue dye injection. Because it is unlikely that the blue dye method can be as sensitive as the combined injection method, the most important issue to address, if the blue dye were to be used alone, is the false negative rate of the blue dye method relative to that of the most accurate conventional method, *i.e.* the combined method. The aim of the present study

was to estimate the relative false negative rate of the blue dye injection method of SLN detection in early breast cancer.

## Material and Method

During the period between September 2007 and December 2008, consecutive female patients with early invasive breast cancer (AJCC TNM clinical stages I or II)<sup>(6)</sup> were approached for participation in the present prospective study, after approval by the hospital's research ethics committee. Demographic and clinical variables were collected for each subject. All subjects underwent the radiocolloid and blue dye (combined) injection methods of SLN detection. Standard methods and criteria, for each detection method, were used independently to detect the SLNs<sup>(3,7)</sup>. The radiocolloid method consisted of 0.4 mCi Technetium-99m labeled dextran injected intradermally at the periareolar or at the subareolar area, at least two hours prior to operation. A sentinel node was defined by this method as a node with an *ex vivo* 10-second radiation count higher than 10% of the count of the hottest node, as measured intraoperatively using a gamma probe system (Neo2000, Neoprobe Corp, Dublin, OH, USA). The blue dye method consisted of

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one to two milliliters of 1% isosulfan blue injected intradermally at the periareolar or at the subareolar area, followed by a two- to five-minute gentle massaging at the injection site. A sentinel lymph node was defined by this method as any node with blue dye stain, or any node with dye-stained afferent lymphatics, seen intraoperatively. Both the isosulfan blue and radiocolloid solutions were made (filtered and tagged) by local pharmacists and radiologists for use in Ramathibodi Hospital. All surgeons who participated in the present study had performed over 30 SLN biopsies prior to participation<sup>(2,5)</sup>. Standard sectioning methods and Hematoxylin and Eosin staining were used to define the cancer-positivity of the SLN. Each SLN was labeled as having been detected by radiocolloid injection, or by the blue dye injection, or both, and as being positive or negative for malignancy.

The detection (identification) rate, or sensitivity, in detecting the SLN for each method was defined as the ratio of the number subjects with detected SLNs by that method to the total number of subjects. The relative false negative rate of the blue dye method, in subjects whose SLNs were detected by that method, was defined as the ratio of the number of subjects with malignancy-negative SLNs as detected by the blue dye method. However, with malignancy-positive SLNs detected by the combined method, the total number of subjects with malignancy-positive SLNs were detected by the combined method and having at least one blue node. The relative false negative rate for the radiocolloid method was defined similarly.

Secondary outcomes included the relative detection rate (relative sensitivity) of a particular detection method for a particular subject, which was defined as the ratio of the number of detected SLNs by that method to the total number of SLNs detected by the combined method in that subject. Another secondary outcome was the relative missed cancer rate of a method for a particular subject given that at least one SLN was detected by that method, which was defined as the ratio of the number of malignancy-positive SLNs *not* detected by that method to the total number of malignancy-positive SLNs detected by the combined method in that subject.

Continuous variables and lymph node counts were summarized as mean (standard deviation, SD) or median (range) as appropriate. Categorical variables were summarized as counts and percentages. Estimates of the various lymph node-level rates were done using logistic regression analysis with random effects at the

patient-level<sup>(6)</sup>. All statistical analyses were performed using Stata version 9 (Stata Corp, College Station, TX, USA). Statistical significance was defined as a two-sided p-value of 0.05 or less.

## Results

Seventy patients with early breast cancer agreed to participate in the present study. The characteristics of the subjects in the present study are presented in Table 1. Outcomes of the present study are presented in Table 2. All subjects were women, with an average age of 50 years. All tumors were five centimeters or smaller and the average number of SLN removed was 2.5 nodes.

The combined and radiocolloid methods of SLN detection were able to identify SLNs in all 70 subjects (100% detection rate for the combined and radiocolloid methods). The findings of the radiocolloid method, in the present study, were identical to those of the combined method. The blue dye method was able to detect SLNs in 91% (64/70) of subjects (91% detection rate). In all, 29% of subjects (20/70) had cancer metastasis to the SLNs. The blue dye was able to identify a similar proportion of subjects (30%; 19/64) with lymph node metastasis. The false negative rate of the blue dye relative to the combined method (the relative false negative rate) was zero.

The total number of SLNs removed was 176 nodes in 70 subjects. At the lymph node level, for a given subject, the average relative detection rate of the blue dye was 75%. By the combined method, the average proportion of lymph nodes with metastasis was 20% per subject. This proportion was 23% for lymph nodes detected by the blue dye. The average relative missed cancer rate for the blue dye method was 11% of the positive lymph nodes per subject, and zero for the radiocolloid method. Based on a random effects logistic regression model, the corrected estimate

**Table 1.** Characteristics of the subjects

Characteristics	Summary (n = 70)
Age (years): mean (SD)	50.1 (12.4)
Side of lesion: right/left (%)	38/32 (54/46)
Size of primary cancer (cm):	
Mean (SD)	2.3 (1.0)
Median (range)	2.2 (0.5 to 5.0)
Number of SLNs harvested per patient	
Mean (SD)	2.5 (1.4)
Median (range)	2 (1 to 8)

**Table 2.** Detection rate, relative false negative rate and rates of lymph node metastasis for the blue dye, the radiocolloid and the combined method

Measures	Combined	Blue dye	Radiocolloid	p-value <sup>b</sup>
Detection rate (%; n = 70)	100 (70/70)	91 (64/70)	100 (70/70)	0.031 <sup>c</sup>
Number of pts with metastatic SLNs	20	19	20	
Proportion pts with metastatic SLNs (%)	29 (20/70)	30 (19/64)	29 (20/70)	
Number of pts with false neg SLNs <sup>a</sup>	NA	0	0	
Relative false negative rate <sup>a</sup>	NA	0 (0/19)	0 (0/20)	
Total number SLNs detected	176	122	176	
Total number metastatic SLNs	32	25	32	
Number detected SLNs, per pt (n = 70)				
Mean (SD)	2.5 (1.4)	1.7 (1.1)	2.5 (1.4)	
Median (range)	2 (1 to 8)	2 (0 to 6)	2 (1 to 8)	<0.001 <sup>d</sup>
Number detected met SLNs, per pt (n = 70)				
Mean (SD)	0.5 (0.9)	0.4 (0.7)	0.5 (0.9)	
Median (range)	0 (0 to 4)	0 (0 to 3)	0 (0 to 4)	0.025 <sup>d</sup>
Relative detection rate (%; n = 70)				
Mean (SD)	NA	75 (35)	100 <sup>e</sup>	
Median (range)	NA	100 (0 to 100)	100 <sup>e</sup>	<0.001 <sup>d</sup>
Proportion of metastatic SLNs, per pt (%)				
Mean (SD)	20 (36)	23 (39); n = 64	20 (36); n = 70	
Median (range)	0 (0 to 100)	0 (0 to 100)	(0 to 100)	
Relative missed cancer rate, per pt (%)				
Mean (SD)	NA	11 (23); n = 19	0 <sup>e</sup> ; n = 20	
Median (range)	NA	0 (0 to 75)	0 <sup>e</sup>	

SLN = sentinel lymph node; neg = negative; met = metastasis; pt = patient; NA = not applicable

<sup>a</sup>Relative false negative finding is as defined in the text, <sup>b</sup>Statistical contrasts between blue dye and radiocolloid methods, <sup>c</sup>p-value by exact symmetry test, <sup>d</sup>p-value by signed rank test, <sup>e</sup>all detected SLNs by combined method were also detected by radiocolloid method alone

of the relative missed cancer rate for the blue dye was 13% (95% CI: 3% to 41%).

The overall concordance between the blue dye and radiocolloid methods, defined as the detection of at least one node by both methods in a given subject, was 91% (64/70). The concordance of the blue dye given all subjects with SLNs identified by the radiocolloid method (“blue-hot” concordance)<sup>(9)</sup> was 91% (64/70). However, the concordance of the radiocolloid given all subjects with SLNs identified by the blue dye (“hot-blue” concordance)<sup>(9)</sup> was 100% (64/64).

## Discussion

In the present study, the sensitivity, or SLN detection rate, of the blue dye method in detecting SLNs in early breast cancer (91%) was shown to be less than that of the combined method (100%), as may be expected<sup>(1-5)</sup>. The radiocolloid method, which was able to detect all sentinel lymph nodes in the present study, was also more sensitive than the blue dye method

(Table 2). The blue dye’s relative detection rate of 75% at the lymph node level meant that for a given subject, on the average, the blue dye method could detect 75% of all detected nodes. This left 25% of the SLNs undetected by the blue dye method and raised the concern that if the blue dye were used alone for SLN detection the remaining undetected lymph nodes might contain metastasis, especially if the detected ones did not. However, according to the present study, this relative false negative rate of the blue dye method was zero (Table 2). That is, in all subjects with at least one blue dye-detectable SLN, the true or absolute false negative rate was the same for the blue dye as for the combined method. This also meant that none of the blue dye-undetected nodes contained metastasis if the blue dye-detected nodes did not, or, if the blue dye-undetected nodes contained metastasis, then at least one of the blue dye-detected nodes also contained metastasis.

The estimated relative false negative rate of zero was also reflected in the outcome called the

missed cancer rate. The motivation for this measure originated from the desire to quantify the difference between the blue dye and the combined methods in detail, that is, in terms of the ability to detect SLNs with cancer metastasis at the lymph node level, instead of simply counting the number of subjects with discordant findings. The lower the relative missed cancer rate (ideally zero) the better the given method is at detecting metastatic nodes compared with the most accurate method. The crude relative missed cancer rate of 11% (Table 2) for the blue dye meant that, on the average, for a given subject with detectable SLNs by the blue dye method, the blue dye method will not detect 11% of the metastatic-positive SLNs detected by the combined method. Under a more realistic statistical modeling framework, the corrected missed cancer rate was estimated to be 13%. That the range of the missed cancer rate (95% confidence interval) did not include 100% meant that the blue dye was able to detect at least one metastatic-positive SLN out of all metastatic-positive SLNs.

It was assumed in the present study that the accuracy of each detection method could be assessed by examining each component of the combined method. That is, it was assumed that the simultaneous or near simultaneous injection of the two tracer substances (blue dye and radiocolloid) does not interfere significantly with one another and hence does not limit the accuracy of either method. This assumption was probably justified by the results of previous studies<sup>(10,11)</sup>.

It might seem that a direct comparison between the false negative rate of the blue dye and the false negative rate of the combined method as reported in the literature can provide a valid estimate of the relative false negative rate of the blue dye. In fact, such a comparison is not valid. This is because the false negative findings of the combined method as reported in the literature included a component that must be excluded if a valid comparison were to be done. That component is the false negative finding in patients whose SLNs were detected solely by the radiocolloid method (*i.e.*, no blue nodes among the detected nodes). Detailed information such as the various components of the false negative findings is rarely provided in the literature (usually positive findings are given)<sup>(4,9)</sup>, so a valid comparison could not be carried out. In the present study, the calculation of the relative false negative rate estimate of the blue dye excluded the pure radiocolloid component. In addition, the most efficient comparison should be

between the same set of patients (same-group comparison), who must therefore undergo both the blue dye and radiocolloid methods of detection, with separate information at the lymph node level available for both methods. Such was done in the present study. A virtue of a same-group comparison is that the true or absolute false negative rate for each method need not be estimated since any overlapping true false negative findings will completely cancel out in the comparison.

The detection rate of the blue dye in the present study (91%) was within the range of those found in other studies<sup>(1,3-5)</sup>. The overall concordance between the blue dye and radiocolloid methods (91%) was also within the range seen in previous studies<sup>(9-11)</sup>. The detection rate for the radiocolloid method in the present study was equivalent to that of the combined method. This might have occurred by chance because of the relatively small sample size. The estimated relative false-negative rate of zero was probably a result of chance as well, although it was likely that the true relative false negative rate was low, if not zero.

In those areas of the world where sentinel lymph node detection can only be done using the dye injection method, it is important to know the method's limitations. The blue dye method's relatively low detection rate of 91% will mean that more patients will need a full axillary lymph node dissection, *i.e.* an additional 9% of those eligible for SLN biopsy. Although a low detection rate is of some concern<sup>(5)</sup>, a more serious limitation is the possibility of a higher rate of false negative SLN biopsy for the blue dye method, as compared with the most accurate conventional method. The finding in the present study that the false negative rate of the blue dye method was similar to that of the most accurate conventional method, *i.e.* the combined radiocolloid and blue dye method, should be reassuring to experienced surgeons who, by necessity, use solely the blue dye method of SLN detection.

### Conclusion

Although the SLN detection rate for the blue dye was 91%, its relative false negative rate of zero meant that the false negative rate of the blue dye method was similar to that of the combined blue dye and radiocolloid method. Experienced surgeons who, perhaps by necessity, used solely the blue dye method of SLN detection may be reasonably confident in foregoing a full axillary dissection following a cancer-negative, blue dye SLN biopsy.

### Potential conflicts of interest

None.

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### ผลลบเทียมสัมพันธ์ของสี isosulfan blue ในการตรวจต่อมน้ำเหลืองเซนติเนล ในมะเร็งเต้านมระยะต้น

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**วัตถุประสงค์:** เพื่อประมาณอัตราการเกิดผลลบเทียมของการตรวจต่อมน้ำเหลืองเซนติเนลด้วยวิธีฉีดสาร isosulfan blue อย่างเดียว เทียบกับวิธีตรวจผสมผสาน (combined method) ที่มีการฉีด isosulfan blue และสารคอลลอยด์กัมมันตรังสี (radiocolloid) ร่วมกัน ในผู้ป่วยมะเร็งเต้านมระยะต้น

**วัสดุและวิธีการ:** มีผู้ป่วยมะเร็งเต้านม 70 ราย เข้าร่วมการศึกษาและได้รับการตรวจต่อมน้ำเหลืองเซนติเนล ด้วยวิธีผสมผสานในช่วงเวลาระหว่างเดือนกันยายน พ.ศ. 2550 ถึงเดือนธันวาคม พ.ศ. 2551 โดยใช้เกณฑ์มาตรฐานเพื่อค้นหาต่อมน้ำเหลืองเซนติเนลแต่ละเม็ด และมีการระบุว่าต่อมน้ำเหลืองเซนติเนลแต่ละเม็ดนั้นถูกค้นพบด้วยวิธีตรวจวิธีใดบ้าง และเม็ดใดที่พบหรือไม่พบมะเร็งกระจายไปถึง

**ผลการศึกษา:** อายุเฉลี่ยของผู้ป่วยคือ 50 ปี และก่อนมะเร็งมีขนาดเฉลี่ย 2.3 เซนติเมตร จำนวนต่อมน้ำเหลืองที่พบโดยเฉลี่ย คือ 2.5 เม็ดต่อผู้ป่วย 1 ราย อัตราการตรวจพบต่อมน้ำเหลืองเซนติเนลด้วยวิธี isosulfan blue คือ ร้อยละ 91 (64/70) และอัตราเกิดผลลบเทียมสัมพันธ์เทียบกับวิธีผสมผสานคือ 0 (ศูนย์; 0/64)

**สรุป:** ศัลยแพทย์ที่มีประสบการณ์ในการใช้สี isosulfan blue เพื่อค้นหาต่อมน้ำเหลืองเซนติเนลในผู้ป่วยมะเร็งเต้านมระยะต้นอาจมั่นใจได้ว่าอัตราเกิดผลลบเทียมของวิธี isosulfan blue จะคล้ายคลึงกับวิธีผสมผสาน