Lateral versus Classical Blue Dye Injection in SLNB for Breast Cancer Patients

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ABSTRACT

Background*: Breast cancer is the most common type of cancer and the second leading cause of cancer deaths among women. The evolution and the now widespread use of the SLNB as the gold standard for the management of axillary disease in early breast cancer, as compared to the classic Axillary Lymph Node Dissection has reduced morbidities related to the ALND. The classical sites for injection of the blue dye are sub-areolar or peri-areolar and peri-tumoral. However, there is variable incidence of failure of visualization of the dyed SLN in as many as 47% of attempted cases in some centers.* ***Objective****: Evaluation of the lateral injection technique of blue dye as compared to the classical techniques in the identification of the SLN in early breast cancer patients (Tis-T2).* ***Methods****: forty patients eligible for SLNB were included in the study, twenty were subjected to the lateral injection technique (intra- parenchymal injection of 5 ml of 1% patent blue dye in the UOQ) and twenty were subjected to the classical injection techniques (peri-tumoral or retro areolar).* ***Results****: The mean age of the study population was 49.28 ± years. SLNB was done with 1% patent blue dye with an identification rate of 100% for both arms of the study. The median number of colored lymph nodes that were retrieved through the lateral injection technique were 4 as compared to a median of 2.5 lymph nodes retrieved through the classical injection, this difference was statistically significant (p value=0.001). The number of palpable non colored lymph nodes encountered in the control group (classical injection) was greater than those in the study group (p value=0.06). The operative time with the lateral injection technique was shorter than the classical injection techniques, this was statistically significant (p value=0.002).* ***Conclusion****: The use of 1% patent blue dye for SLNB is a safe, easy and effective as compared to other tracers including radio-colloids. The lateral injection technique is superior to the classical injection techniques as regarding the colored lymph node yield and shorter operative time. There was no loco-regional recurrence after 6 months follow up (clinical and ultrasound follow up). Further studies are needed to correlate between effect of the breast cup size, site of injection and number of retrieved Colored lymph nodes.*

Keywords*: Lateral injection, SLNB, Retro-areolar injection, Peri-tumoral injection.*

BACKGROUND

The Sentinel Lymph Node (SLN) is one or a small group of lymph nodes that first drain the entire breast [(1)]. Breast tumors are therefore likely to produce lymph node metastasis in this SLN. The evolution and the now widespread use of the SLN biopsy (SLNB) as the gold standard for the management of axillary disease in early breast cancer, as compared to the classic Axillary Lymph Node Dissection (ALND) has reduced morbidities related to the ALND [(2)].

Internationally accepted SLNB methods currently use a combination technique of radioactive tracer and different variations of vital stains. Due to its lymphatic tropism, radioactive tracer can increase the chances for SLN identification [(3)]. However, in those centers where the radio-active colloid or the gamma cameras are not easily available the use of blue dye alone has shone comparable results to the combination technique [(4)].

The classical sites for injection of the blue dye are sub-areolar or peri-areolar and peri-tumoral. However there is variable incidence of failure of visualization of the dyed SLN in as many as 47% of attempted cases in some centers, factors affecting the failure of visualization include inexperienced surgeons, and obstruction of the peri-tumoral lymph vessels by tumor emboli, and also large sized breasts that can lead to formal ALND with greater morbidity, also smearing of the breast surgical field with the blue dye can be inconvenient if the injection was peri-tumoral [(5)].

The aim of the study is to compare the effectiveness of the lateral blue dye injection technique at the upper outer quadrant of the ipsi-lateral breast, to the classic retro-areolar or peri-tumoral injection of blue dye in identification of the SLN in terms of identification rate, operative time and the number of colored LNs retrieved.

METHODS

Participants

This prospective randomized controlled trial (RCT) study was conducted at Kasr Al-Ainy teaching Hospitals, Faculty of Medicine, Cairo University between 1st July 2018 and 1st March 2019. The study protocol was reviewed and permitted by the institutional research and ethics committee. Forty patients with clinically node negative early breast cancer who are candidates for Sentinel Lymph Node Biopsy were studied. Patients proved to have clinically positive axillary lymph nodes were excluded as well as patients with metastatic disease and pregnant women as it is contra-indicated to expose them to blue dye.

Study interventions

Patients presenting to the breast clinic with suspicious breast lumps were subjected to triple assessment in the form of clinical examination, sono- mammography (MRI breast in selected cases) and tru-cut biopsy, when it was proved to be a case of a malignant breast mass, metastatic work-up was performed (chest X-Ray and Abdominal Ultrasound in early cases) and Immunohistochemistry on the biopsy was done (ER and PR receptors, HER2NEU, and Ki67).

The patient „s data was there after presented at the weekly multidisciplinary team (MDT), where Surgical Oncology, Medical Oncology, Radiotherapy, Radiology and Pathology Consultants who are experts in breast cancer management discuss the results of the investigations; patients who were categorized as node negative early breast cancer (Tis-2 N0) were eligible to partake in the study and undergo a SLNB.

The forty patients were randomly allocated equally to the study group or the control group via a computer generated sequence. The patients" data was collected preoperatively, including the patients" age and breast size (cup size) by examination.

Surgical procedure

The procedure is done under general anesthesia with the patient supine and the ipsi-lateral upper limb abducted at 90 degrees. Patent blue dye is used in SLNB. Axillary incision followed by opening of axillary fascia, retrieval of all colored LNs and all palpable (non-colored) LNs, closure with no drain (in case of a negative frozen section).

A. Technique of SLNB for the control group

Peri-tumoral or sub-areolar injection of 5 ccs 1% Patent Blue dye as shown in (Figure 1), followed by breast massage for 5 minutes to facilitate migration of the blue dye.

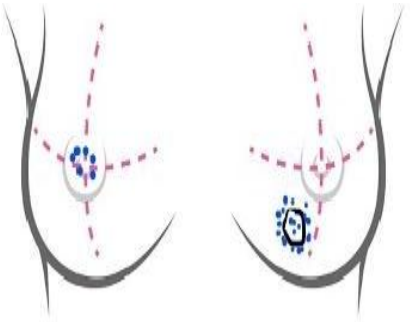

Fig. 1: Illustration of classical injection sites

B. Technique of SLNB for the study group

Patent blue dye is used for the SLNB for the study group as well with the same concentration and amount as that used for the control group with the exception that the dye was injected intra-parenchymal in the upper outer quadrant of the breast midway between areola and anterior axillary line) the needle directed to the axilla as shown in (Figure 2) taking care not to inject too close to the axilla in the case of small sized breasts to avoid smearing of the surgical field.

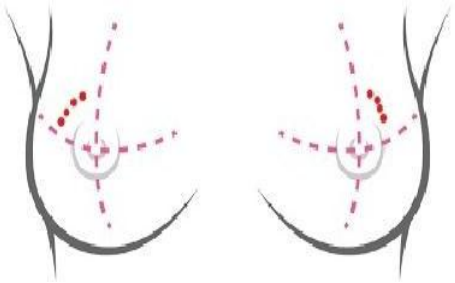

Fig. 2: Illustration of the lateral injection site

Breast massage was not employed in this technique and the axilla was opened and explored for the sentinel lymph node almost immediately as the initial pilot cases showed that the blue streaks and the lymph nodes colored quite quickly.

Data collected intra-operatively included the time of completion of the biopsy and retrieval of all colored LNs and all palpable LNs from the skin incision till the conclusion of the biopsy (closure was postponed till the frozen section result was conveyed to the surgeon) As well as the number of colored lymph nodes retrieved and also the number of palpable (non-colored lymph nodes that were encountered and subsequently excised).

Data collected post operatively included the size and the pathological origin of the tumor (invasive ductal carcinoma or invasive lobular carcinoma) as well as the presence of lympho-vascular emboli; this was collected from the final paraffin block report. The patients were subjected to follow-up (clinical and ultrasound examination) for detection of loco- regional recurrence for an average of 6 months.

Statistical analysis of data

Data was entered on the computer using "Microsoft Office Excel Software" program (2010) for windows, then coded and transferred to the Statistical Package of Social Science Software program, version 25 (SPSS) to be statistically analyzed.

A. Descriptive statistics

Quantitative data were statistically described in terms of mean ± standard deviation (± SD), median and range while qualitative data were statistically described in terms of frequencies (number of cases) and percentages when appropriate.

B. Analytical statistics

- Mann Whitney U test: was used for independent samples for Comparison between two groups with quantitative data and not normally distributed (6).
- Chi-Square test: was used for Comparison between groups with qualitative data. Exact test was used instead when the expected frequency is less than 5 (7).

The confidence interval was set to 95% and the margin of error was set to 5%. The p value was considered significant as follows:

p value <0.05: Significant.

p value < 0.01: Highly significant.

RESULTS

Patient Demographics

The total number of patients enrolled in this study were 40 patients, with a mean age of 49.28 with a SD of 14.03 and an inter quartile range of 40 – 59 years.

Breast cup size

17 patients had a reported breast cup size C (42.5%), 16 had a cup size B (40%), 4 had a cup size of A (10%) and 3 of cup size D (7.5%) as shown in (Figure 3)

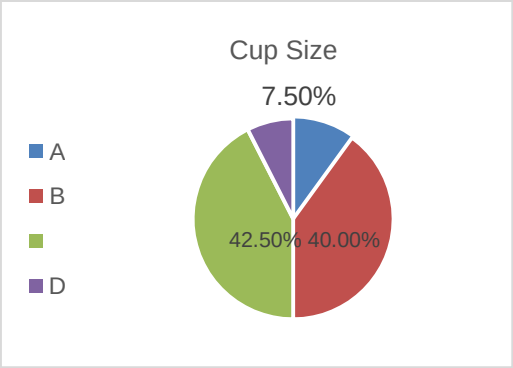

Fig. 3: Percentage of different cup sizes

Tumor Characteristics

36 patients (90%) had a tumor with an invasive ductal origin while four patients enrolled in the study had an invasive lobular carcinoma (10%). As for tumor location, the commonest was the Upper outer quadrant (UOQ) with 21 patients (52.5%) followed by the retro-areolar location (RA) with 8 patients (20%), 4 patients had a tumor located in the upper inner quadrant (UIQ) (10%), 2 patients had tumors that were located upper central above the nipple areola complex (5%) and the lower inner quadrant (LIQ) and the lower outer quadrant (LOQ) were each represented by 3 and 2 patients respectively.

The mean tumor size for all the patients enrolled in the study was 27.1 mm with a SD of 11.11 mm and a median value of 26 with an interquartile range from 20 – 31 mm With the mode of a T2 according to the TNM classification. Only one case had reported lympho-vascular emboli as evidenced by the post- operative paraffin section pathology report.

Operative technique

The sentinel LN identification rate was 100% for both groups. The number of colored lymph nodes for the study group had a median of 4 and a mean of 4 +/- 1.56 as compared to the control group which had a median of 2.5 and a mean of 2.4 +/- 0.94. This difference was statistically significant with a p value of 0.001. The colored number of lymph nodes that were collected in both groups are shown in (Table 1) and (Figure 4).

The number of palpable non colored LNs that were retrieved through both techniques is illustrated in (Figure 5), and the difference between the occurrences of a palpable non colored LN through both techniques had a p value of 0.060 which was statistically non-significant.

Table 1: Shows the numbers of colored lymph nodes in both groups

		Study group		Control group	
		Count	**%**	**Count**	**%**
Coloured LN	**1**	0	0.0%	4	20.0%
	2	4	20.0%	6	30.0%
	3	4	20.0%	8	40.0%
	4	5	25.0%	2	10.0%
	5	4	20.0%	0	0.0%
	6	1	5.0%	0	0.0%
	7	2	10.0%	0	0.0%

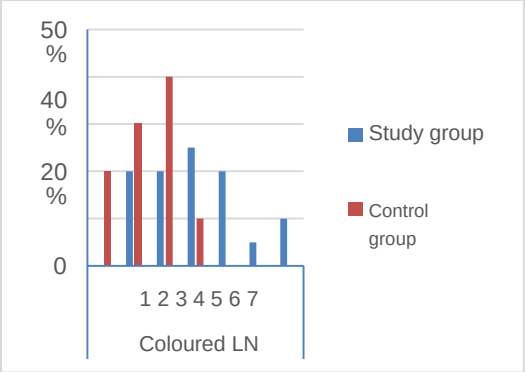

Fig. 4: chart shows the number of colored lymph nodes retrieved in both groups

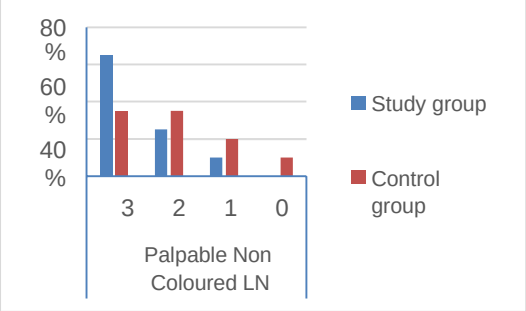

Fig. 5: Frequency of palpable non colored lymph nodes

The operative time for the study group had a mean of 11.5 +/- 1.32 minutes as compared to the control group that had a mean operative time of 13.05 ±1.39 minutes. This difference had a p value of 0.002 and was statistically significant as shown in (figure 6)

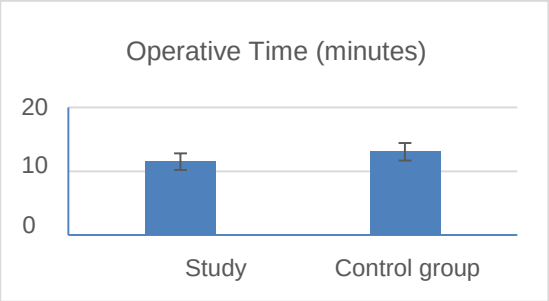

Fig. 6: chart shows comparison between both groups regarding operative time

Effect of Breast (cup) size

The effect of the breast size on the operative time is shown in (Table 2); this difference however for the operative times for different cup sizes was statistically insignificant with a p value of 0.214.

Table 2: Cup size and operative time

		Cup Size				P value
		A	B	C	D	
Operative Time (minutes)	**Mean**	10.33	12.00	11.25	12.50	
	SD	1.53	1.41	1.04	0.71	
	Median	10.00	12.00	11.00	12.50	
	1st quartile	9.00	11.00	10.50	12.00	
	3rd quartile	12.00	13.00	12.00	13.00	0.214

The effect of the cup size however on the number of colored LN retrieved through both SLNB injection techniques is shown in (Table 3) and this difference has a p value of 0.043 which is statistically significant

Table 3: Cup size and number of colored LNs

		Cup Size				P value
		A	B	C	D	
Coloured LN	**Mean**	6.00	2.7	3.8	2.50	
	SD	1.40	0.8	1.4	0.7	
	Median	6.00	2.50	3.50	2.50	
	1st quartile	5.00	2.00	3.00	2.00	0.043
	3rd quartile	7.00	3.00	4.00	3.00	

DISCUSSION

Over the last decade, SLNB has been adopted worldwide as an alternative to axillary lymph node dissection (ALND) as a standard method of staging patients with node negative early stage breast cancer[8]. Our study aimed at the assessment of the injection of the blue dye laterally in the upper outer quadrant as compared to the classical peri-tumoral or sub-areolar injection of the blue dye for the identification of the sentinel LN. This being based on the hypothesis that the shorter distance, between the lymphatics of the upper outer quadrant of the breast and the axilla, and any other site in the breast will lead to better delivery of the blue dye to the SLN leading to better visualization, identification and subsequent retrieval of the SLN and the secondary LN. This would hypothetically be of an even greater value in patients with large breast sizes.

In our study, SLNB was done using 1% Patent Blue dye with lymph node identification in all surgical specimens (identification rate 100%). It was found that identification rates (IRs) for SLNB has increased over time from 88 % in 1992-2000 to 97% in 2007- 2012, the increase in IR during the last 18 years is attributed to the likely increase in gained experience by the surgeons performing SLNB [9].

The effect of the breast size (cup size) on the operative time for retrieving the sentinel lymph node and its effect on the number of colored lymph nodes retrieved has not been reported in literature this was after a thorough search through the Medline database using keywords including "breast size" , "cup size" and "sentinel lymph node" returned no relevant papers. Our study results show that there is no statistically significant difference found in the operative times for sentinel lymph node retrieval for the different breast sizes for both techniques. However, the effect of the breast size on the number of colored lymph nodes retrieved through both techniques was statistically significant with an inverse relationship between the cup size and the number of colored lymph nodes retrieved with a p value of 0.043.

The median number of colored lymph nodes retrieved in the study group was 4 as compared to 2.5 for the control group; this difference was statistically significant with a p value of 0.001. The difference between the incidence of palpable non colored lymph nodes between both groups was statistically non- significant. Therefore by comparing the results between the study and control group we found that the lateral injection of the blue dye in the upper outer quadrant is superior to the classical peri-tumoral or retro-areolar injection in terms of the number of colored lymph nodes retrieved.

The mean operative time was 11.5 minutes in the study group while 13.05 minutes in the control group which is significant with p value 0.002. While there was no statistically significant difference on the number of positive lymph nodes found by paraffin section.

In our study, injection of 1% patent blue was safe; no allergic or anaphylactic reactions were observed during the SLNB procedure, in accordance with most published studies, except Teknos et al. reporting a pulmonary edema during a SLN procedure using blue dye [10].

In our clinical trial, no necrotic skin lesions were observed, in contrast to published data. Stradling et al. reported 5 necrotic skin lesions (21%) after the injection of 3 to 5 mL of blue dye in a study of 24 patients. Blue dye was injected in the aforementioned study in both the parenchyma and the skin; none of the patients however had required surgical debridement [11]. A severe skin and fat necrosis was reported by Salhab et al with a peri-tumoural blue dye injection, but the concentration of the blue dye used was not mentioned in the case report [12].

These skin complications seemed to be mainly due to intradermal injection of the blue dye more than related to dye concentration, intradermal injection leads to vasoconstriction which may end in skin sloughing due to inhibition of NO synthesis and prostacyclin release from the vascular endothelium [12]. In our study no intradermal injection was performed, only retro-areolar, peri-tumoral or intra- parenchymal, this explains the absence of these complications in our cases.

Upon searching the Medline database only one study was found which employed a technique similar to ours, this study was performed in 2000 by Borgstein et al. That study aimed to validate the intradermal injection of 2.5% Patent Blue dye in combination with peri-tumoral radio-colloid injection for SLNB followed by routine ALND. The study design was different to our study where the intradermal blue dye was injected either in the

skin above the tumor (group 1) or in the skin at the lateral areolar border (group 2), the study showed an overall identification rate with this combination technique exceeding 96% with blue nodes being retrieved in 91% and a concordance rate or more than 95% for both hot and blue nodes. The study also reported a mean number of colored LNS to be 1.2 ±0.5. Our study showed an IR of 100% for both techniques and a mean number of colored LNS of 4 ±1.56 for the lateral injection technique[(13)].

Study limitations

The false negative rate for SLNB could not be calculated in our study as we did not routinely perform ALND after each SLNB so as not to increase the morbidity on the patients as per the study design and the recommendations of the ethical committee. However, the addition of routine ALND after the SLNB could give as an insight of the FNR of this injection technique.

The use of a combination technique with a peri- tumoral radio-colloid injection could also allow us to validate this new injection technique by means of comparing the concordance rate of the hot and blue dyes with the other injection techniques.

CONCLUSION

The use of 1% Patent Blue dye for identification of SLNB by any injection technique is safe and effective with an identification rate of 100%. The lateral injection technique is superior to the classical technique in terms of the number of colored LNs retrieved and reduction in the number of palpable non colored LNs. The breast cup size is an important factor in determining the number of colored LN that can be retrieved through SLNB. . There was no loco- regional recurrence after 6 months follow up (clinical and ultrasound follow up). Further studies are needed to correlate between effect of the breast cup size, site of injection and number of retrieved Colored lymph nodes.

Compliance with ethical standards

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

Ethical approval: approval was given by the Ethical Committee for Researches of General Surgery Department, Cairo University Hospital and the Research committee of Faculty of medicine, Cairo University.

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