Blue dye versus combined blue dye—radioactive tracer technique in detection of sentinel lymph node in breast cancer

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Sentinel lymph node biopsy; Breast cancer; Staging; Blue dye; Gamma probe

Abstract  
Background. Sentinel lymph node biopsy in breast cancer can be used to select patients in which axillary lymph node dissection could be avoided. In this study we compared the value of two methods for identification of sentinel node (SN) using either only blue dye or combination of blue dye and radioactive tracer.

Material and methods. All patients were women with clinically T\textsubscript{1,2}N\textsubscript{0}M\textsubscript{0} breast cancer. They were randomized into two groups. In Group A (50 patients) SN marking was performed only with blue dye and in Group B (100 patients) combined SN marking with blue dye and radiotracer was done. We used 2 ml of blue dye Patentblau V\textsuperscript{®} (Byk Gulden). Radiotracer was Antimony sulfide marked with Tc 99m and of 0.3 mCy (11.1 MBq) activity. Application method of both contrasts was peritumoral. After SN biopsy all patients underwent mastectomy or conservative surgery with axillary lymph node dissection of levels I and II.

Results. In Group A mean of 1.7 SNs were identified (median 1, range 1-4). False-negative rate in this group was 3/17 (17.6%) with negative-predictive value 20/23 (86.9%), sensitivity 14/17 (82%), specificity 20/33 (60%) and accuracy 34/50 (68%). In Group B mean number of SNs excised per case was 1.6 (median 1, range 1-5). False-negative rate was 2/44 (4.5%), negative-predictive value 41/43 (95.3%), sensitivity 42/44 (95%), specificity 41/56 (73%) and accuracy 83/100 (83%). The combination technique was significantly superior to blue-dye alone technique for negative-predictive value ($p=0.033$) and overall accuracy ($p=0.048$).
Conclusions. The prediction of axillary lymph node status in breast cancer patients using combined technique has significantly higher accuracy than marking of SN with blue dye alone and therefore should be preferred.
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Introduction

One of the most important prognostic indicator in patients with breast cancer is axillary lymph node involvement. It can be determined with three surgical modalities: axillary lymph node dissection (ALND), sentinel lymph node biopsy (SLNB) and axillary node sampling (ANS).

SLNB in breast cancer represents the best diagnostic modality to accurately stage axilla and to select patients in which ALND could be avoided. It is minimally invasive, fast, safe and accurate procedure with less morbidity than ANS and ALND. Introduced in breast surgery by Giuliano and Krag this procedure is one of the most important surgical innovations in treatment of breast cancer patients. Using this procedure many patients with early-stage breast cancer can be spare of unnecessary ALND and its possible short and long-term morbidity such as seromas, pain, infection, motor and sensory nerve injury, lymphedema, restricted arm mobility, etc.

Various factors can influence on results of SLNB and one of them is type of contrast used to mark sentinel nodes. In this study we compared the value of two methods for identification of sentinel node (SN) using either only blue dye or combination of blue dye and radioactive tracer.

Materials and methods

This prospective study has been performed between February 1999 and August 2003 at the Department of Surgical Oncology, Institute of Oncology Sremska Kamenica, Novi Sad, Serbia. All patients were women with clinically T1N0M0 breast cancer. Preoperative diagnosis was obtained with the aid of physical examination, mammography, ultrasonography and fine needle aspiration cytology or core biopsy. The exclusion criteria were pregnancy, palpable suspicious axillary lymph nodes, prior breast or axillary surgery, multicentric tumours, neoadjuvant chemotherapy or radiotherapy and refusal for procedure by the patient.

Patients were randomized into two groups. Group A: In 50 patients SN marking was performed only with blue dye with visual intraoperative identification of SNs. Group B: In 100 patients combined SN marking was performed (blue dye and radiotracer) with intraoperative visual and gamma probe identification of SNs.

The day before surgery, Antimony sulfide (Sb2S3), marked with Technetium (Tc 99m) was injected peritumorally. Mean injected radioactivity was 0.3 mCi (11.1 MBq). Lymphoscintigraphy was performed only in first 15 patients. 10-15 min before incision 2 ml of blue dye Patentblau Vr (Byk Gulden) was injected peritumorally. The breast was massaged for a few minutes to facilitate identification of blue lymphatic vessels and nodes more easily. After general anaesthetic induction, the axilla was searched for radioactive areas using the gamma probe (Gammed IVr-Capintec) and for blue-stained lymph nodes and afferent lymphatics.

SN was defined as any node that was blue, hot and blue, hot alone or node whose afferent blue lymphatic vessel was coming directly from tumour.

After SN biopsy all patients underwent mastectomy or conservative surgery with ALND of levels I and II. All procedures were performed by two surgeons.

Immediately after harvesting, sentinel lymph nodes were sent to pathology department for intraoperative frozen section evaluation. SNs larger than 5 mm were bisected along the longitudinal axis, while smaller nodes remained uncut. Ten sections were obtained from each block of tissue and stained with hematoxylin and eosin (H&E) or immunostained for epithelial membrane antigen (EMA; DAKO, Denmark). Remaining SNs tissue was fixed in formalin and embedded in paraffin for routine pathologic examination. Sections of paraffin embedded tissue blocks were stained with H&E or EMA immunohistochemistry and these results were compared with frozen section analysis.

Formalin fixed paraffin embedded tissue sections of non-sentinel lymph nodes were stained with H&E. In the first 50 cases frozen sections were not performed.

All data were analyzed with SPSS statistical package for Windows version 12.0 (SPSS, Chicago, IL, USA). Fisher’s exact test, chi-square test and t test were used to compare data between the two groups. Values of $P \leq 0.05$ were considered to be statistically significant.
Results

Patients characteristics in groups A and B such as age, histological tumour size, type of cancer and type of surgery are presented in Table 1. In groups A and B number of harvested SNs was 1.7 (median 1, range 1-4) and 1.6 (median 1, range 1-5), respectively.

In the first set of patients (blue-dye group) SNs were positive in 27 cases and in 13/27 (48%) SNs were the only positive nodes (false-positive cases). Three cases out of 23 (13%) were false-negative. False-negative rate in this group was 3/17 (17.6%) with negative-predictive value 20/23 (86.9%), sensitivity 14/17 (82%), specificity 20/33 (60%) and accuracy 34/50 (68%). Seventeen patients (34%) in this group had positive axillary nodes. Mean number of removed axillary nodes was 12.5 (range 4-18) and mean number of positive axillary lymph nodes (pN+) was 3.2 (55/17, range 1-14). Micrometastases in SNs were detected in two cases and axilla was negative in these patients.

In group B (blue-dye + radiotracer) the SN was the only positive node in 15/57 (26%) of the cases while number of false-negative cases was 2/43 (4.6%). Positive axillary lymph nodes with negative SNs were found in 2/44 patients with metastatic axillary involvement (false-negative rate 4.5%). Negative-predictive value was 41/43 (95.3%), sensitivity 42/44 (95%), specificity 41/56 (73%) and accuracy 83/100 (83%). Mean number of removed axillary nodes was 13.4 (range 7-23). Forty-four (44%) patients in this group had positive axillary nodes and mean number of pN+ nodes was 4.6 (201/44, range 1-22). In three cases SNs contained only micrometastases while most of removed axillary nodes were metastatic in these patients. On the other hand, two patients with micrometastases in axillary nodes had negative SNs at final histology.

No statistically significant differences were seen between two groups regarding to number of false-negative (p=0.333) and false-positive (p=0.081) cases, false-negative rate (p=0.127), sensitivity (p=0.129) and specificity (p=0.244). However, differences in negative-predictive value and overall accuracy reached statistical significance (p=0.033 and p=0.048, respectively) (Table 2).

Discussion

A sentinel lymph node is defined as the first lymph node in a regional basin that receives lymphatic drainage from the site of a primary tumour. For more than a decade, SLNB in breast cancer patients is used to predict axillary lymph node status and avoid ALND if possible. Sentinel nodes are marked with either blue-dye, radioactive tracer or their combination. Because it is cheap and safe many institutions use blue dye as contrast of first choice. Furthermore, there is no need for nuclear medicine department and gamma probes, so procedure can be performed even in small hospitals or hospitals with limited financial support. Sometimes fear of radiation exposure is reason for avoiding use of radioactive tracer although it has been proven that radiation dose levels are very low.10–13

With only blue dye marking (injected peritumoral) in 50 patients we achieved false-negative rate of 17.6%, sensitivity of 82%, specificity of 60% and accuracy of 68%. In literature, false-negative rates for SLNB using only blue dye are usually higher than the 5% guidelines, even as high as 55%.14–17 However, results in number of studies represents the first experience with SLNB and therefore do not represent the real capability of procedure. The addition of radioactive tracer to the blue-dye (Group B, 100 patients) increased sensitivity, specificity and accuracy to 95, 73 and 83%, respectively. False-negative rate in this group was 4.5%. Motomura et al. also compared these two techniques with results that combined SLNB was significantly superior to the blue-dye alone method for sensitivity and accuracy.18 Similar study by Canavese et al. showed that addition of radiotracer to blue-dye increased sensitivity and overall accuracy from 77 to 87% and from 91.5 to 95.5%, respectively.19 Looking at our own results, with combined technique we achieved recommended false-negative rate of less than 5% and sensitivity higher than 95%. Statistically significant differences in negative-predictive value and overall accuracy confirmed that combined method of SLNB is highly recommended.

Number of removed SNs and axillary nodes was similar in both groups with no statistical significance. In three cases in group B SNs contained only micrometastases although large number of axillary nodes removed in these patients had macrometastases. This emphasizes the role of thorough pathohistological examination of SNs and search for micrometastases. There is an ongoing debate in literature should every SN positive patient underwent routine ALND especially in case of micrometastases.20–22 Our data suggests necessity of ALND in all SN positive patients.

High percentage of mastectomies, 60% in group A and 45% in group B, is mainly due to limited radiotherapy facilities and often malfunction of LINAC (unfortunately this situation can be seen in
many developing countries in Eastern Europe). Preoperative lymphoscintigraphy was performed only in first 15 patients in group B; it was not performed in other patients due to organizational reasons. Results of lymphoscintigraphy from our study as well as others show that, irrespective of the tumour site, most cancers drain to the axilla, obviating the need of a preoperative lymphoscintigram. We find lymphoscintigraphy very helpful in detection of SNs especially those outside of the axilla but this procedure is not necessary for axillary SLNB. Concerning injection site, peritumoral injection was simply our matter of choice without any ideas that this injection site is better than others. Many studies compared subareolar, intradermal, subcutaneous, peritumoral and intratumoral injection. Some of these studies showed that there is no statistical difference in results of SLNB due to place of injection. On the other side, some authors claim that subareolar, intradermal and subcutaneous injections are better than other sites. Team from NKI/AvL Amsterdam prefers intratumoral injection for years with results comparable to other institutions experienced in this procedure. From our point of view every site has its potential advantages and disadvantages and experienced surgeon will achieve good results with axillary SLNB no matter of injection site.

Conclusion

This study confirms that prediction of axillary lymph node status in breast cancer patients using combined technique has significantly higher accuracy than marking of SN with blue dye alone and therefore should be preferred.

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