Methylene Blue as Inexpensive and Reliable Sole Sentinel Lymph Node Mapping Agent for Patients with Invasive Ductal Carcinoma

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ABSTRACT

Background: Invasive ductal cancer (IDC) represents about 75% of all breast malignancies. There are many breast cancer prognostic factors, but the ones that have the most impact on the survival rates in advanced breast cancer are tumor size and regional lymph node involvement. Axillary lymph node dissection (ALND) has particularly important and undoubtful role in current surgical options for breast cancer treatment. With the introduction of sentinel lymph node biopsy (SLNB) for breast cancer patients it was possible to identify those to whom regional spread of the disease did not occur at the time of surgery, and thus spare them an unnecessary ALND procedure. Objective: To determine the rate of sentinel lymph node (SLN) detection using only methylene blue dye as a mapping agent, as well as to correlate the number of positive SLNs with the number of positive non-sentinel lymph nodes (non-SLNs). Methods: The study represents a prospective study that included 50 female patients with histologically confirmed invasive ductal carcinoma (IDC) who underwent SLNB using only methylene blue dye as the mapping agent, while the detection and harvest of SLN was done by visual control only. All patients also underwent an obligatory complete ALND, which was as that time the institutional oncological protocol for surgical treatment of histologically confirmed IDC. The final data such as tumor size, SLN and non-SLN status were obtained by further analysis of pathohistological reports from tumor biopsy and other surgical specimens. Results: The accuracy rate of SLN detection was 98%. The number of detected SLN was in the range of 1 to 6, with an average of 2 for each patient. The number of positive SLN was in significant correlation with the number of tumor-affected non-SLN (p<0,001). Further analysis showed that for each increase in the number of positive SLN by 1, the risk of positive non-SLN increased 6-fold, OR=6.22 (p<0,001).

Conclusion: Use of methylene blue dye as a sole mapping agent when performing SLNB in patients with IDC is a reliable and effective method that can be safely implemented in medical institutions that lack availability of nuclear medicine services or significant monetary funds.

Keywords: Breast cancer, sentinel lymph nodes, methylene blue, lymph nodes mapping, SLNB.

1. BACKGROUND

Invasive ductal cancer (IDC) represents about 75% of all breast malignancies. Breast cancer survival rates vary from country to country. In North America and in countries such as Sweden and Japan they reach 80% and over, while for the developing countries the survival rates are at about 60%, and under 40% in underdeveloped countries.

There are many breast cancer prognostic factors, but the ones that have the most impact on the survival rates in advanced breast cancer are tumor size and regional lymph node involvement. It is a well-known fact that breast cancer patients with higher number of positive regional lymph nodes (higher N status according to TNM classification) have a poorer prognosis (1). Axillary lymph node dissection (ALND) has particularly important and undoubtful role in...
current surgical options for breast cancer treatment. It provides us with invaluable information that guide our decision for or against an adjuvant chemotherapy, gives us an excellent locoregional disease control and, to lesser extent, it leans favorably to overall survival rates. However, ALND does carry a certain number of unwanted postoperative complications that usually progress to chronic state and significantly affect the quality of life in such patients. The most notable ones are arm oedema, dysesthesia, paresthesia and permanent shoulder pain with reduced shoulder mobility (2). In the study conducted by Toonkel et al. in 1986, it was found that over 50% of women who had undergone ALND for breast cancer had negative lymph nodes after histopathology evaluation. Looking back this means that over 50% of women with diagnosed breast cancer could have been spared such procedure. With the introduction of sentinel lymph node biopsy (SLNB) for breast cancer patients it was possible to identify those to whom regional spread of the disease did not occur at the time of surgery, and thus spare them an unnecessary ALND procedure (3).

Sentinel lymph node (SLN) represents the regional „guardian” lymph node that will most likely be the one to contain metastatic cancer cells (4). The procedure of SLNB in breast cancer patients is usually performed by using two mapping agents: radiocolloid and blue dye. These are then preoperatively injected in subareolar plane of affected breast, and consequently identified within ipsilateral axillary SLNs using gamma detection probe and visual control. This way the rate of detection of SLNs is usually over 96% (5).

There were many studies done where different mapping agents were used and detection rate of SLNs was evaluated. While some studies where methylene blue or isosulfan blue dye were used as only mapping agents have shown very high SLN detection rates (97% and 99%), others have not obtained the same results (85,7% and 71,4%) (6, 7, 8).

As methylene blue was readily available at our institution, it was our intention to determine weather this inexpensive mapping agent could yield high SLNs detection rates and thus be used in future as a sole mapping agent in patients with breast cancer, without need for expensive and time consuming nuclear medicine services. In order to avoid many different variables, it was decided to conduct the study with female patients that had the most common breast malignancy – invasive ductal carcinoma.

It is important to mention that up until this study was performed in our institution (University Clinical Centre Tuzla), the surgical treatment protocol of breast cancer in any stage was a rather simple one: radical mastectomy with obligatory ALND. It was therefore extremely rewarding to conduct this prospective study which consequently served as a milestone in our future modality of breast cancer treatment.

2. OBJECTIVE

The main aim of this study was to determine the rate of sentinel lymph node (SLN) detection using only methylene blue dye as a mapping agent, as well as to correlate the number of positive SLNs with the number of positive non-sentinel lymph nodes (non-SLNs).

3. PATIENTS AND METHODS

Fifty female patients with no age restriction who had invasive ductal breast carcinoma (IDC) diagnosed preoperatively by biopsy and histopathology were enrolled in the study after approval and informed consent. The study was approved by the Research Ethics Committees of our institution and reviewed in accordance with the precepts established by the Helsinki Declaration.

None of the patients was pregnant, had undergone chemotherapy or radiotherapy. Patients with clinically palpable lymph nodes (LN) were not enrolled in the study. Patients with specific pathological conditions such as glucose-6-phosphate dehydrogenase (G6PD) deficiency, thalassaemia or drepanocytosis were also not included since the use of methylene blue (MB) may aggravate methemoglobinemia or lead to haemolytic anaemia.

The procedure of SLNB was initiated as soon as the patient was under general anesthesia with the injection of 2ml of MB (Metilitionino®, Bergamo, Italy) was placed in subareolar plane of tumor involved breast. Circular motion hand-massage of the injected breast was then immediately started using sterile Vaseline (Vaselinum Album®, Gradac, Bosnia and Herzegovina) as a lubricant. The massage was conducted starting at medial upper quadrant of the breast, going to medial lower, then lateral lower and up towards lateral upper breast quadrant with final push towards the breast tail and the axilla. This massage was performed intensively during next 5 minutes. The next step was to perform SLNs harvest. An incision 4 cm in length was made in the region of ipsilateral anterior axillary line. Using visual control, every blue colored or tinged LN was then harvested and sent for histopathology frozen section evaluation. Regardless of the SLNs histopathology result, radical mastectomy with ALND was performed in every patient and removed tissue was sent for definitive histopathology examination.

All harvested SLNs were sent for frozen section procedure. Every SLN was measured, macroscopically examined and described, then bisected along their major axes and the two slice sections were further bisected along their major axis. The two halves with the largest diameter were then frozen inside of cryostat at -20°C. The frozen samples were then cut to slices of 5 µm thickness (2 slices from each half) and stained using the standardized Hematoxylin and Eosin (H&E) staining method. The prepared tissue samples were then microscopically examined using Olympus BX41 microscope.

All the tumor-free SLNs detected by frozen section were examined by immunohistochemistry (IHC) with cytokertatin AE1/AE3 (diluted at 1/200; Dako, Carpinteria, CA, USA) on consecutive sections, 5 µm thick, cut at 250 µm intervals, until exhaustion of the SLNs. According to the guidelines on the interpretation of TNM categories issued by the European Working Group for Breast Screening Pathology (EW-GBSP), micrometastases were defined as clusters of cancer cells ≥2 mm, micrometastases as clusters of cancer cells ≥0.2 mm and ≥2 mm and isolated tumour cells as clusters of cancer cells <0.2 mm. The other LNs (non-SLNs) were analyzed using a standard protocol without IHC but with serial sections (3 slices 5 µm thick at 500 µm intervals).
4. RESULTS

Out of 50 patients randomly included in the study based mainly on their histopathology finding of IDC, 32 women (70%) were between 45 and 65 years old (χ² test 49.02; df=2; p<0.0001). There were 12 (24%) patients over 66 years old and only 3 (6%) patients who were younger than 45 years old, which shows significant difference in these two age groups (Fisher's exact test; p<0.05).

It was found that most patients were presented for surgery with IDC tumor size over 20mm (X²=51.4; df=4; p<0.001) which indirectly also points out the lack of proper screening program in our country resulting in patients’ first specialist consultation when the breast mass is already significant and palpable (Figure 1).

ALDN was performed in all of 50 patients and histopathology was done for each surgical specimen, it was found that 40% of treated patients with IDC had no regional spread of the disease. Most of the patients with positive regional nodal disease had 1 to 3 metastatic LNs, SLNs and non-SLNs included (X²=46.96; df=7; p<0.001) (Figure 2). We were able to visually detect SLN in every patient and number of harvested SLNs was between 1 and 6, with 6 SLNs harvested in only one patient. Median number of SLNs harvested was 2 (IQ range: 2-3).

Median number of non-SLNs found in ALND specimen was 12 (IQ range: 10-15), with minimum number of 7 and maximum number of 23 examined non-SLNs per ALND specimen. Only in one patient with gigantomastia where a single SLN was detected and came back as negative on histopathology, it was found upon further examination of ALND specimen that one of the non-SLNs was positive. This shows that SLN detection rate accuracy using MB first specialist consultation when the breast mass is already significant and palpable (Figure 1).

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| Number of SLN | Number of patients | Percentage |
|---------------|--------------------|------------|
| 1             | 12                 | 24.0       |
| 2             | 17                 | 34.0       |
| 3             | 16                 | 32.0       |
| 4             | 4                  | 8.0        |
| 6             | 1                  | 2.0        |

Table 1. Number of detected SLNs per patient with IDC

| SLNs status | non-SLNs status | Total |
|-------------|-----------------|-------|
|             | Negative        | Positive | |
| N           | 20              | 1     | 21 |
| %           | 62.5%           | 5.6%   | 42.0% |
| Positive    | N               | 12    | 17 | 29 |
| %           | 37.5%           | 94.4%  | 58.0% |
| Total       | N               | 32    | 18 | 50 |
| %           | 100.0%          | 100.0% | 100.0% |

Table 2. SNLs status vs. non-SLNs status. SNLs detection rate accuracy = 98%.

Statistical analyses were performed using SPSS software ver. 20.0. The Kolmogorov-Smirnov test of normality was used to investigate the assumption of normality. Furthermore, the basic parameters of descriptive statistics, i.e. measures of central tendency (mean and median) and dispersion (standard deviation and interquartile range) were also analyzed. To examine statistical evidence that the associated population means are significantly different Student’s t-test with, where necessary, correction for unequal variances was used. When the assumptions of the independent-samples t-test were violated, the Mann-Whitney U test, as the alternative non-parametric test, was used. The chi-squared test was chosen to compare the distribution of a categorical variable in a sample, with Yates’ continuity correction in the case of 2x2 contingency tables or Fisher’s exact test in the case when more than 20% of cells have expected frequencies < 5. Association between variables was tested by using parametric Pearson or non-parametric Spearman correlation. Cramér’s phi was used as a measure of association between two categorical variables. In order to get a better understanding of the impact of selected independent variables on dependent variable logistic regression model was chosen as a primary methodological approach. All tests were performed considering 95% confidence interval (p<0.05).
as a sole mapping agent for SLNB in this study was 98% (Table 2).

As expected, there was clear significant correlation between tumor size (T) and positive SLNs, which shows also indirectly to codependence of regional LN status, in other words with the increase in tumor size there was notable increase in number of positive SLNs of the total number of harvested SLNs, as well as overall increase in number of tumor involved regional LN (N) (Pearson’s correlation coefficient r=0,3323; 95%CI= 0,05944 to 0,5590; p<0,05).

The most of positive SLNs had tumor metastatic deposits that were larger than 10mm in diameter (Figure 3), while only two patients had tumor deposits less then 2mm in diameter (X2=23,55; df=3; p<0,001).

After univariant logistic regression analysis was applied, it was shown that a positive SLN was a significant predictor of LN positivity in dissected regional LN basin (OR=28,33; %95CI=5,33-240,82; p=0,002). This practically translates that with a positive SLN the risk of other positive LN in regional LN basin increases 23-fold. As far as diagnostic accuracy was concerned in terms of SLN being a predictor of regional LN basin status, sensitivity was calculated to be 94,4%, with negative predictive value of 95,2%. Number of positive SLN was also strong predictor of non-SLNs positivity with OR=6,22 (%95 CI=2,23-17,33; p<0,001), which means that with every additional positive SLN the risk for non-SLN positivity increased 6-fold. This is nonetheless exponential relation which means that with finding of 2 positive SLNs the risk of non-SLNs positivity in regional nodal basin increases 6,22 x 6,22, thus over 36-fold.

5. DISCUSSION

According to latest collected data the incidence of IDC in Bosnia and Herzegovina is 58,9 newly diagnosed IDC patients per 100.000 women which correlates well with the incidence values of around 60 for neighboring countries (Serbia, Monte Negro, North Macedonia). When compared to the incidence value of 80–90 in western European countries (Germany, Netherlands, France and others) one could deduce that the women in our region are less likely to develop IDC than the women of western European countries (9). However, there is big difference in prevalence of well-organized breast cancer screening programs that the countries in west Balkans such as ours clearly lack, which can be further showed through the incidence of ductal carcinoma in situ (DCIS) that is in constant increase in the developed countries and it has values as high as 32 in USA and 16 in UK (10). While in Bosnia and Herzegovina there is no exact data on the incidence of DCIS, there is however statistical value of 0,26% in situ breast cancer prevalence in Tuzla County that account for 1/6th of Bosnia and Herzegovina population. The data was collected over a period of 10 years (from 2001. to 2011.) and it has shown that only 2,6% of breast cancer patients were diagnosed with in situ determinant (27 out of 1032 women diagnosed with breast cancer) (11). If fair distribution is to be assumed for all regions of our small country the prevalence of in situ breast cancer for Bosnia and Herzegovina would theoretically be 1,5%. This clearly shows the lack of efficient and substantial breast cancer screening programs in our country. This fact can be further extrapolated from the same study (11), where it has been shown that most of the women in Tuzla County are diagnosed with breast cancer >2cm in diameter – T2 and T3 (61%), while only 21% of women were diagnosed with breast cancer <2cm in diameter - T1. In comparison, the percentage of newly diagnosed women with early-stage breast cancer and tumor size <2cm in diameter for western European countries is 53% in Croatia, 52,3% in France, 46% in Netherlands, 44% in Italy, 42% in England etc. (12). Evaluating the above data, it may be inferred that the true incidence of IDC in Bosnia and Herzegovina is probably much closer to those values of western European countries, therefore it is safe to assume that a great number of women in our country are not timely diagnosed and are consequently treated at later stages of the disease, making the treatment outcome less favorable. It is thus expected that with better organization and implementation of breast cancer screening programs there will be a proportional increase in the incidence of breast cancer in our country (13).

The most common agents used in SLN mapping are artificial dyes and radiocolloid substances. In Western European countries and in North America it is advised that SLNB should be performed using combination of isosulfan blue dye and radiocolloid since it is considered that such combined method of SLN mapping is the most effective for SLNs detection (14, 15) although there are some studies that have not confirmed such an advantage in comparison when a single SLN mapping agent was used (16, 17). Performing a SLNB using a radiocolloid as a mapping agent is somewhat complex and expensive procedure (especially for the underdeveloped and countries in transition). Such procedure is also very dependent of nuclear medicine services that should be readily available. There are number of studies that show different rates of improved detection rates when radiocolloid is used in combination with blue dye vs. using blue dye only varying from 0% to 18% (15, 16, 17). Since about 60% of clinically negative node axilla in breast cancer patients is found to be also negative on histopathology (18) combining radiocolloid to methylene blue in SLN mapping potential benefit for patients who would be spared of ALND ranges from 0% to 11%. This represents unreasonably high cost for such a small benefit furthermore since SLNB does not represent therapeutical procedure and it does not affect the rate of overall survival for breast cancer patients (19). Use of methylene blue as a sole SLN mapping agent allows surgeons to, even in countries of poor economic status, offer their patients SLNB as proper invasive diagnostic procedure in treatment of breast cancer without compromising its efficacy.

The results from our study clearly confirm that size of primary tumor (T) presents strong independent predictive factor od SLN positivity in breast cancer patients, which correlates with the results of other similar studies (20). Some authors investigated the size of metastatic deposits within SLNs and found that both the number of positive SLNs as well as the size of metastatic deposits within SLNs independently serve as predictive factors of other non-SLNs involvement (21). There are also other studies that further analyze the predictive value of SLN. By composing different formulas for calculating the number of positive SLNs
and non-SLNs and thus predicting the overall regional LN status, surgeons are guided to further perform even less radical ALND, such as removal of only first level axillary LN, when certain parameters regrading positive SLNs and primary tumor size are applied into such equation (22, 23).

The main advantage of SLNB in breast cancer patients is appreciated in cases where patients are able to be spared from unnecessary surgical procedure such as ALND. With the wide-spread use of SLNB procedure many authors have published interesting data that about 50% of breast cancer patients with positive SLN do not have associated positive non-SNLs when ALND is completed (24, 25). Our study showed that 38% of patients with positive SLNs had negative non-SLNs when ALND was performed (Table 2). Because of this there were many studies conducted trying to determine proper predictors of non-SLN status with positive SLNs. One of the most interesting ones is certainly the size of metastatic deposits within SLNs that are analyzed as two distinct groups, those with micrometastasis (< 2mm) and those with macrometastasis (> 2mm). The results of those studies clearly point that SLNs with macrometastasis are related to higher number of positive non-SLN in comparison to those where SLNs had micrometastatic deposits (44,4%:24,4% respectively) (24, 25). Positive and border-significant correlation between the size of metastatic deposits of SLNs with the number of positive non-SLNs was also noted in our study (p=0.357; p=0.05) which would probably yield a stronger significance if the number of involved patients was greater. Other studies were conducted where only group was to try to determine the prognostic significance of micrometastasis involved SLNs, and therefore its guidance in opting for ALND and/or adjuvant oncological therapy in breast cancer patients. There are more and more studies that are giving support towards new guidelines where positive SLNs with micrometastasis obviates the need for further ALND (26).

6. CONCLUSION

This prospective study has a great value for our institution as well as for our region since it was with this study that SLNB became a standard surgical diagnostic procedure in patients diagnosed with breast cancer. It was especially rewarding to show that even inexpensive and readily available mapping agent such as methylene blue yielded high accuracy SLN detection rate in our hands as well (98%).

SLNB is an indesepensible diagnostic tool in breast cancer management that has a great prognostic significance and gives a precise insight into regional LN status. It is procedure that is usually performed using combined SLN mapping method that includes use of radiocolloid as well as an artificial dye agent in order to both make SLN detection easier and more accurate. Our study has shown and further attested that use of MB as a sole SLN mapping agent yields high SLN detection rate and may be used as such without fear of compromising the effectiveness of SLNB procedure. MB is inexpensive and readily available mapping agent that obviates the need for nuclear medicine services in SLNB procedure thus making the procedure available in most any hospital setting.

• Patients Consent Form: All participants were informed about subject of the study.
• Author’s contribution: E.H gave substantial contributions to the conception and design of the work, as well as in in analysis, interpretation of data for the work and drafting and revising the article. E.I. gave substantial contribution in acquisition, drafting and revising the article. Z.M., E.B. and G.S. gave substantial contribution in acquisition, analysis and revising the final version. All coauthors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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REFERENCES

1. Carter CL, Allen C, Henson DE. Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases. Cancer. 1989; 65: 181–187.
2. Schenk P, Rieger R, Shamiyeh A, Wayand W. Morbidity following sentinel lymph node biopsy versus axillary lymph node dissection for patients with breast carcinoma. Cancer. 2000; 88: 608–614.
3. Giuliano AE. Mapping a pathway for axillary staging: a personal perspective on the current status of sentinel lymph node dissection for breast cancer. Archives of Surgery. 1999; 134(2): 195-159.
4. Turner RR, Ollila DW, Krasne DL, et al.: Histopathologic validation of the sentinel lymph node hypothesis for breast carcinoma. Annals of Surgery. 1997; 226(3): 271–276.
5. McMasters KM, Wong SL, Martin RC 2d, et al. Dermal injection of radioactive colloid is superior to peritumoral injection for breast cancer sentinel lymph node biopsy: results of a multi-institutional study. Annals of Surgery. 2001; 233: 676–687.
6. Mathelin C, Croce S, Brasse D, Gairard B, Gharbi M, et al. Methylene Blue Dye, an Accurate Dye for Sentinel Lymph Node Identification in Early Breast Cancer. Anticancer Res. 2009; 29(10): 4119–4125.
7. Vohra LM, Memon AA, Khaliq T, Lehri AA. Sentinel lymph node biopsy in breast cancer by isosulfan blue. Pak J Med Sci. 2009; 25(5): 786–790.
8. Ollila DW, Brennan MB, Giuliano AE. The role of intraoperative lymphatic mapping and sentinel lymphadenectomy in the management of patients with breast cancer. Adv Surg. 1999; 32: 349–364.
9. Ferlay J, et al. Cancer Incidence, Mortality, and Prevalence Worldwide. Version 1.0. IARC CancerBase No. 5. Lyon, GLOBOCAN 2001: IARC Press.
10. Virinig B, Tuttle T, Shamiyan T and Kane R. Ductal Carcinoma In Situ of the Breast: A Systematic Review of Incidence, Treatment, and Outcomes. J Natl Cancer Inst. 2010; 102 (3): 170–178.
11. Pašić A. Clinical and morphological characteristics of recurring breast cancer – master’s theses. University of Tuzla. Tuzla, Bosnia and Herzegovina. 2013.
12. Sant M, Allemani C, Berrino F et al. Breast cancer survival in Europe and the United States. A Population-Based Study. Cancer. 2004; 100(4): 715–722.
13. Miller AB. Screening in developing countries: problems and opportunities. Cancer Treatment and Research. 1996; 86: 185–189.
14. Derossis AM, Fey J, Yeung H, Yeh SD, Heerdt AS et al. A trend analysis of the relative value of blue dye and isotope localization in 2,000 consecutive cases of sentinel node biopsy for breast cancer. J Am Coll Surg. 2001; 193: 473-478.

15. McMasters KM, Tuttle TM, Carlson DJ, Brown CM, Noyes RD, Glaser RL et al. Sentinel lymph node biopsy for breast cancer: a suitable alternative to routine axillary dissection in multi-institutional practice when optimal technique is used. J Clin Oncol. 2000; 18: 2560-2566.

16. Varghese P, Mostafa A, Abdel-Rahman AT, Akberali S, Gattuso J, Canizales A. et al. Methylene blue dye versus combined dye-radioactive tracer technique for sentinel lymph node localisation in early breast cancer. Eur J Surg Oncol. 2007; 33: 147-152.

17. Morrow M, Rademaker AW, Bethke KP, Talamonti MS, Dawes LG, Clauson J, at al. Learning sentinel node biopsy: results of a prospective randomized trial of two techniques. Surgery. 1999; 126: 714-720.

18. Fisher B, Redmond C, Fisher ER, et al. Ten year results of randomized clinical trial comparing radical mastectomy and total mastectomy with or without radiation. New England Journal of Medicine. 1985; 312(11): 674-682.

19. Mansel RE, Fallowfield L, Kissin M, Goyal A, Newcombe RG, Dixon JM et al. Randomized multicenter trial of sentinel node biopsy versus standard axillary treatment in operable breast cancer: the ALMANAC Trial. J Natl Cancer Inst. 2006; 98: 599-609.

20. Hansen NM, Ye X, Grube BJ, Giuliano AE. Manipulation of the primary breast tumor and the incidence of sentinel node metastases from invasive breast cancer. Arch Surg. 2004; 139(6): 634-639.

21. Durak MG, Akansu B, Akin MM, Sevınç AI, Koçdor MA, Saydam S. at al. Factors predicting non-sentinel lymph node involvement in sentinel node positive breast carcinoma. Turk Patoloji Derg. 2011; 27(5): 189-195.

22. Unal B, Gur AS, Beriwal S, Tang G, Johnson R, Ahrendt G, et al. Predicting likelihood of having four or more positive nodes in patient with sentinel lymph node-positive breast cancer: a nomogram validation study. Int J Radiat Oncol Biol Phys. 2009; Nov 15; 75(4): 1055-1040.

23. Kohrt HE, Oshen RA, Bermas HR, Goodson WH, Wood DJ, Henry S. et al. Bay Area SLN Study: New models and online calculator for predicting non-sentinel lymph node status in sentinel lymph node positive breast cancer patients. BMC Cancer. 2008; Mar 4; 8:66.

24. Turner RR, Chu KU, Qi K, et al. Pathologic features associated with nonsentinel lymph node metastases in patients with metastatic breast carcinoma in a sentinel lymph node. Cancer. 2000; 89: 574-581.

25. Reynolds C, Mick R, Donohue JH, et al. Sentinel lymph node biopsy with metastasis: can axillary dissection be avoided in some patients with breast cancer? J Clin Oncol. 1999; 17: 1720-1726.

26. Langer I, Guller U, Viehl CT, Moch H, Wight E, Harder F et al. Axillary lymph node dissection for sentinel lymph node micrometastases may be safely omitted in early-stage breast cancer patients: long-term outcomes of a prospective study. Ann Surg Oncol. 2009; Dec;16(12): 3366-3374.