INTRODUCTION

Surgical treatment of early breast cancer includes the removal of the primary tumor and the removal of axillary lymph nodes, which drain the lymph from the tumor site.

Apart from the removal of the whole breast and dissection of the lymph nodes, the early breast cancer treatment, according to Halsted and Meyer method, became more extensive and culminated with hyper-radical interventions of Urban in the middle of 20th century. Their aim was to eradicate the malignant tissue and in this way to eliminate the possibility of disease spreading into the body (1,2). In the meantime, the significance of lymph nodes involvement for disease staging and prognosis was recognized, influencing also the extent of further systemic treatment and radiotherapy.

Completely different surgical approach to the disease, in the early eighties, was introduced by Veronesi, with the idea of conserving the breast and removal only the quadrant of the breast where the tumor is situated. Since noninvasive diagnostic procedures could neither confirm nor deny the lymph nodes involvement, axillary dissection remained the "gold standard" of these operations, and they have always been performed together with conserving operations. The term "sentinel node" (SN) (guard node), indicating the first lymph node which drains the tumor region, was introduced by Donald Morton (3). Concept of the sentinel node biopsy (4) is dated in 1977, when Cabanas (4,5) performed the first operation of this type in the penile cancer. The largest number of studies in this field was conducted on malignant melanoma. Sentinel nodes were also studied on thyroid gland tumors, vulvar and uterine cervix cancer, and on gastrointestinal and colorectal tumors.

In 1994, Guliano introduced SN biopsy and lymphatic mapping (determination of lymph paths) in the breast cancer (4). Considering significant analogy with the malignant melanoma (predictability of the lymph drainage), the concept has become easily applicable and widely accepted. Numerous large...
projects are trying to prove the hypothesis that high quality and simple disease staging can be provided by histological analysis of SN biopsy.

The expected percentage of patients with the involved axillary nodes is about 30%-40%, which means that two thirds of patients undergo axillary dissection because of negative lymph nodes (2). On the basis of histopathological processing of the fast frozen preparation of SN, one could predict with statistical significance whether remaining lymph nodes contain metastases or not. When metastases are not present in SN, dissection of remaining axillary nodes and all potential complications related to axillary dissection could be avoided in precisely defined conditions. Consequently, the quality of life would be improved (6,7).

MATERIAL AND METHODS

Sentinel node biopsy has been performed since February 1999 at the Clinic for Surgical Oncology of the Institute of Oncology Sremska Kamenica. Until the preparation of this manuscript, 124 interventions were performed. Patients included in the analysis had tumors sized from T1 to T2a. Ultrasound and/or mammographic visualization of the tumor are assumed. Axillary nodes were not palpable in any of the patients. The patients with the earlier breast operation, multicentric tumors and presence of carcinomatous mastitis, and pregnant patients, were excluded from analysis. After sentinel node biopsy, radical breast surgery (conservative or mastectomy) and lymph node dissection of level I and II was performed in all cases.

Marking and identification of the sentinel nodes

Marking of sentinel nodes exclusively with stain was used in the first 50 patients, with intraoperative visual identification. In 73 patients combined SN marking technique was used (stain + radiotracer); intraoperative identification was done visually and with manual gamma counter (Gammed IV-Capintec).

We used original stain Patentblau V-Byk Gulden (2 ml). Radiotracer was Antimony sulfide (Sb2S3), marked with Tc 99 m, and of 0.3 mCy (9.6 MBq) activity. Application method of both contrasts was identical. Application depth depended on the primary breast tumor localization, and it was subdermal, subcutaneous, or peritumoral in deeply positioned tumors.

Radiotracer application time preoperatively was 16 hours, while the stain was applied immediately before operation (10-15 min). Preoperative scintigraphy was performed in 15 patients with stable gamma camera (Orbite 75-Siemens). X ray was done minimum 2.5 hours after the application of radiotracer.

Histopathological SN analysis in the first 100 patients was performed from definitive paraffin preparations with classical hematoxilin-eozin (HE) staining. In 22 patients, starting from January 2002, extempore SN analysis was introduced on fast frozen preparations with compulsory use of immunohistochemical analysis (EMA) in case of the absence of metastases on HE preparations. Other dissected axillary nodes were analyzed from definitive paraffin preparations. All treated patients were divided in three subgroups and separately analyzed.

Subgroup A: In 50 patients SN marking was performed only with stain and visual intraoperative identification, and the remaining lymph nodes were analyzed on paraffin preparations.

Subgroup B: In 50 patients combined SN marking was performed (with stain and radiotracer), while SN histopathological analysis and the other lymph nodes were analyzed on the paraffin preparations.

Subgroup C: In 22 patients SN marking was done as in subgroup B, but histopathological SN analysis was done on fast frozen preparations with immunohistochemical treatment in case of the absence of metastases on HE stained preparations. All other axillary nodes were treated with standard paraffin preparations.
RESULTS

Table 1 shows that SN marking exclusively with stain (Patenblau V-Byk Gulden) and with their exclusively visual intraoperative identification, false-negative result appeared in 3 patients (6%), i.e. in 3 cases histopathological SN analysis was negative (without metastases), while metastases were found in other axillary lymph nodes. This influenced the sensitivity to decrease to 80%, predictable negative finding was reduced to 81%, and the total accuracy to 89.29%.

False-negative finding was not noticed in subgroup B (SN marking performed with both contrasts, and identification was visual and with use of manual gamma counter) which reflected the value of other findings. In subgroup C (where extempore histopathological analysis of SN on fast frozen preparations was done), there were no false-negative findings, and the sensitivity, specificity, predictable positive finding, predictable negative finding, and the total accuracy were 100%.

DISCUSSION

Obtained SN biopsy results were mutually compared to determine the optimal method of their marking and optimal method of their histopathological treatment. SN marking and its precise identification, as well as histopathological analysis, are essential components, without which the complete procedure cannot be carried out. So, neither statistical data would be representative, nor they could be clinically used.

Our results showed that in SN marking combined techniques (staining used together with radiotracer) should be used, what a number of authors also confirmed (8-10). The application of preoperative scintigraphy with stable gamma camera is also important (10-12) because it enables preliminary insight into the lymphatic mapping and helps intraoperative identification of sentinel nodes with manual gamma camera.

Scintigraphy should be performed at least 2 to 2.5 hours after the application of radioisotope, since its adequate accumulation in SN cannot be expected earlier.

Preoperative time of stain contrast application is also an important factor. It may vary depending on position of the primary tumor in the breast. Practically, in case of all our patients the application time intervals for preoperative staining were between 10 and 20 minutes. Apart from making possible to stain the SN, the stain contrast can also stain lymph paths, which during preparation may serve as a direction to stained sentinel nodes.

Isolated stain marking technique has several objections. The most important one is the appearance of false-negative results, what was 6% on our sample. This value corresponds to the average value of false-negative results when isolated staining technique is applied. Also, it is possible not to stain axillary nodes. In the first place, drainage into internal mammary plexus disables SN biopsy, except when biopsy is routinely searched for in both localizations (in axilla and parasternally). Delay of operation with inadequate SN staining may cause difficulties in its identification.

Introduction of radioisotope Tc 99m antimony sulfide combined with stained contrast for SN marking brought, in case of our sample, to the improvement of method sensitivity from 80% to 100%, false-negative SN dropped from 6% to 0%, and predictable negative level increased from 81% to 100%. However, series is not big enough to allow speaking of absolute supremacy of combined identification method, but trend in this direction is very clear. Other authors also confirmed the above stated (9,13-15). Activity (9.6 MBq) of applied radiotracer is absolutely sufficient for apparatus used during intraoperative identification (GAMMED IV), giving the sufficient sensitivity (16). As many authors report, our findings also indicate that operation should be performed within 24 hours after the application of radioisotope. The use of the manual gamma camera-counter enables the precise SN localization in the axillary region and, with scintigraphic images made preoperatively, easily identifies SN in the internal mammary plexus or other localization (13). Biopsy of the mammary chain (IMC) was not performed. In the patients with the strongest activity in this region, this fact was only stated, and the node with the strongest activity was extirpated, regardless its staining, since the aim of the study was to detect the predictability of histopathological finding of axillary lymph node involvement. In future, it would be very interesting to do
the biopsy of IMC nodes as well, in order to determine the need for paraструктур irradiation (14), especially when primary tumor is localized in medial quadrant. Combination of drainage to both axillary nodes and IMC nodes was noticed in this study, what corresponds to data from literature.

Histopathological analysis is a third link required for the success of this method of SN biopsy. Precision of laboratory treatment, preparation cutting in search for metastatic deposits both in SN and other axillary nodes is essential for adequacy of this method. The percentage of micrometastases found in subgroup C (50%), which is substantially higher than literature data (17), alarmingly indicates that they can easily be overlooked and in that way compromise the complete procedure (18). Apart from a pathologist's experience in SN analysis on fast frozen preparation, the use of immunohistochemical methods of staining (18) is obligatory (in our case EMA-epithelial membrane antigen). Although some authors claim that dissection of other axillary nodes can be avoided in tumors under 1 cm in diameter and with SN metastases, our opinion is that the dissection of other lymph nodes is justified whenever micrometastases in SN are present (10). Histopathological treatment of preparations, complete with analysis on fast frozen preparations, cannot be performed under 40 minutes, what corresponds to literature data (19), but if it results in avoiding of axillary dissection, then it is absolutely justified.

Widespread use of SN biopsy in the breast cancer shows that it is easy for application. But since its introduction into the clinical practice, many authors are of the opinion that insufficient training leads to the occurrence of the false-negative results, especially in the first 30 operations (15). When surgeon is completely trained in this technique, the sensitivity of 98% and 100% can be expected, with the false-negative result maximum of 3% (20). Minimal predictive negative level should be 97% (21). It means that, on the basis of the absence of metastases in SN, we may claim that other axillary nodes do not contain metastases.

Combination of stained contrasts and radiotracers, and the obligatory use of intraoperative manual gamma camera-counter (14) should be used in marking and identification of SN. Preoperative scintigraphy with stable preparations, complete with analysis on fast frozen preparations, cannot be performed under 40 minutes, what corresponds to literature data (19), but if it results in avoiding of axillary dissection, then it is absolutely justified.

Experience of pathologists in extempore analysis of fast frozen SN preparations with the use of immunohistochemical staining is equally important as the experience of surgeon is.

CONCLUSION

This researching, and many others, indicates that in certain patients (especially T1a and T1b), under precise criteria, when SN metastases are not present, axillary dissection in the breast cancer (10, 22) and all its consequences (lymphoedeama, numbness, pain, limited movement in the shoulder joint) could be avoided.

We should remember that nowadays at least 50% of women undergo axillary nodes dissection within the breast cancer operative treatment because of histopathologically negative nodes.

REFERENCES