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THE ROLE OF SENTINEL LYMPH NODE BIOPSY IN BREAST CANCER DIAGNOSIS

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Summary. *Sentinel lymph node biopsy (SLNB) is a relatively new method of evaluation of breast cancer spread. The procedure is a specific test able to demonstrate whether the breast cancer has spread and invaded axillary lymph glands. Axillary dissection is still being performed in over 50% of breast cancer patients with histologically negative disease, in spite of the significant associated postoperative morbidity (prolonged axillary pain, lymphedema, arm numbness, wound seroma formation). Axillary lymph node status is also an important prognostic factor in breast cancer patients and a primary factor for systemic adjuvant therapy institution. Aim of this work is to stress the importance of this surgical method in diagnosis and treatment of breast cancer and to demonstrate technical performance details (technique of SLN marking, technique of SLN dissection, pathologic evaluation etc.). The method has been utilized in many European surgical centres for more than a decade, and the acceptable standard of 95% of successful SLN dissections further affirmates the method, so it is expected that the method should become a routine procedure in surgical breast cancer management.*

Key words: *Breast cancer, sentinel lymph node biopsy, dissection of axillary lymph nodes, staging*

Introduction

Sentinel lymph node biopsy (SLNB) is a method applied for the first time in patients with carcinoma of the penis in 1970 (1). Later, the method has also been used to detect invaded lymph nodes in malignant melanoma (2), breast cancer (3,4,5,6,7), thyroid carcinoma and other cancer sites. Sentinel lymph node (SLN) is the first node receiving lymphatic drainage, hypothetically also the metastases from malignant tumours. Theoretically negative SLN in malignant tumours demonstrate the negative status of other lymph nodes as well. Axillary lymph node dissection is performed only for patients with SLN metastases. Patients are thus spared of serious postoperative complications occurring after axillary dissection (prolonged axillary pain, lymphedema, arm numbness, wound seroma creation etc.).

Morton et al. (1992) have stated that the first lymph node on the metastasizing tumour cell route, the SLN, reflects the status of other nodes in the regional system of lymphatic drainage. These authors were the first to perform histopathologic analyses of the SLN-detected regional lymph nodes in patients with malignant melanoma. The methods of lymph node labeling were later applied for breast and thyroid cancer as well.

Krag et al. (1993) published their pilot study on successful SLN identification in 18 out of 22 evaluated breast cancer patients, using non-filtrated technetium-sulphur coloid and hand-gamma probe.

Giuliano et al. (1994), applying 1%-isosulphane blue stain, precisely identified SLN in 114 (66%) assayed patients. The identified SLNs reflected precise axillary status in 109 (96%) patients. In the studies to follow, Giuliano et al. (1995) used haematoxylin-eosine (H&E) staining anticytokeratine IHC check to achieve higher level of precision. Based on these investigations, the authors conclude that SLN dissection is not only a precise index of axillary lymph node status, but it also improves axillary "staging", which is advantageous compared to standard axillary lymph node dissection.

Albertini et al. (1996) used vital blue solution and filtrated technetium-sulphur coloid to mark SLN. Out of 62 evaluated patients, SLN was indentified in 57 (92%). Metastases were found in 18 SLNs, which were all marked with blue stain and radioactive coloid. There were no false-negative SLNs in this study. The authors recommend utilization of the staining and radionuclide combination to mark SLN.

Veronesi et al. (1997) used human albumine labeled with technetium to label SLN, and radioisotope was applied superficially into the tumour. Identification was performed with gamma-counter. These authors identified SLN in 160 out of 163 evaluated cases, and identified SLN reflected axillary status in 97%.

Imoto et al. (1998) used indocarmine and successfully identified SLN in 78% of the patients, with the sensitivity of the method of 86%. Axillary lymphonodal status was precisely determined in 94% of the cases. Regardless of the precisely defined nodal status, these authors prefer the combination of blue stain and radioisotopic coloids in SLN identification.

Motomura et al. (1999) successfully identified SLN in

127 (73.8%) out of 172 patients with indocyanine green staining (Diagnogreen 0.5%). Association between SLN and axillary lymph node status was demonstrated in 96.1% of the cases, with false negativity rate of 11.1%. Tumour size, surgical procedure and tumour histology did not affect SLN identification. However, the level of success was markedly higher in patients without (79.0%) than in those with involved axillary lymph nodes (65.7%). Success was also dependent on the presence or absence of lymphatic or vascular invasion and patient age. SLN detection level was higher in patients without (78.9%) than in those with lymphatic or vascular invasion (63.8%), as well as in those aged below 50 years (83.3%) compared with the patients over 50 (64.8%).

Haigh et al. (1999) have ten years long experience in labeling and dissection of SLN in breast cancer patients. Based on their investigations these authors point out that the successfulness of the technique depends primarily on the proper patient selection, injection technique, dissection technique and histopathologic evaluation.

Method technique

Isosulphan-blue stain (commercially LIMPHAZURIN) or indocyanin-green (commercially DIAGNOGREEN) is nowadays used for intraoperative mapping of SLN. Methylen-blue was abandoned as an insufficiently reliable method and indocarmine, also commonly used, weakly permeates lymphatic vessels compared to isosulphane-blue. With LYMPHAZURIN, the percentage of successful SLN identification is above 90% (5).

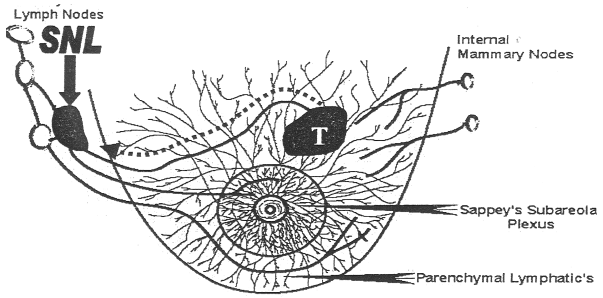

Fig. 1. Axillary lymphatic drainage

The procedure of lymphatic mapping begins with 5 ml injecton of isosulphan-blue 5 minutes before lymph node biopsy. Injection site is determined by palpation, echosonography or wire-lead and mammography. Usually utilized are peri-tumoral, intra-tumoral or subdermal injections. After the injection, hand massage of the injection site is recommended (lasting 1-2 minutes).

In order to increase the method sensitivity, in addition to stains, redionuclides are utilized as well at the present. Radionuclide of choice is the technetium-sulphate colloid at the dose of 0.5-1 ml. Radioactive albumines are less sensitive than technetium-sulphate, and on account of smaller diameter of the particles they can pass SLN and reach second order lymph glands, which is the indication for axillary dissection. Dilemma on the successfuness of SLN identification with only stains, only radionuclides or their combination is now solved in many studies (4,5,6). According to the Bassa et al. (1999) report SLN is identified in 75% of the cases with only staining, in 90% with radioactive isotopes, and in 95% with their combination.

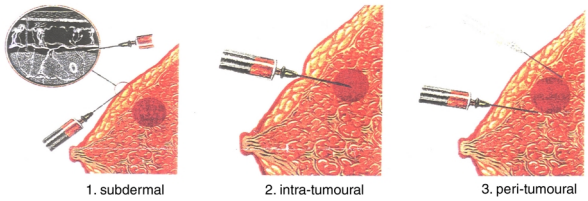

Fig. 2. Staining application mode

The technique of radioactive contrast injection connotes intraparenchymal or intradermal application. Intradermal and subareolar mode is preferred at the present – the injection procedure is simpler, the interval to SLN dissection is shorter and dissection technique less complex (11).

Attitudes on preoperative lymposcintigraphy are different. Lymphoscintigraphy aims at identification of the lymphatic confluence towards the SLN. It is not of any help for intraoperative SLN detection, nor it reduces the rate of false-negative biopsies (5). The method is mainly utilized by less experienced surgeons. Routine application of preoperative lymphoscintigraphy is furher impeded by its high price of around 550 $US.

Surgical intervention in general anaesthesia is recommended. Before isosulphane-blue injection percutaneous gamma probe is performed to detect the radioactivity zone. Aim of the procedure is differentiation of the primary application site radioreflection from radioactivity of the suspect SLN (5). Simultaneously, incision preparations are performed. Incision should be made in the region of highest radioactivity within the axilla, so called "hot spot" zone. If this zone cannot be identified, the incision should be made 1 cm below the line of axillary crease. Blue-green afferent lymphatic flow is followed over the clavico-pectoral fascia leading to SLN. The interval between the stain application and axillary incision should be 3-10 minutes. This interval should be acted upon; if not, this could hinder the identification of afferent lymphatics and SLN localization. After identification and removal of one node, the total number of involved nodes is determined with gamma probe. It is also used for identification of so called "hot nodes" within the axilla, if the blue-green lymphatic is not identified visually, which enables identificaton and removal of SLN.

After the identification of the first SLN, secondary SLN should be determined as well. All "hot nodes" within axilla should be also removed and sent for histopathologic evaluation. Among the patients with metastatic changes within lymph nodes in whom more than one SLN are removed, in 88.5% metastatic changes are present in the "hot nodes" (5). If a conclusive SLN is not found, standard three-level axillary dissection of

lymph nodes should be done.

Pathologic evaluation of SLN is performed intraoperatively on frozen sections. The waiting period for the histologic finding is used to complete the definitive surgical procedure, or for segmental or total mastectomy. If metastases are identified on frozen sections, standard axillary dissecton should be done. If axillary metastases are not identified, we should stop at the SLN lymphadenectomy.

Negative result on the frozen sections is then permanently H&E stained. If metastases are not identified, cytokeratin IHC staining is applied (4). This staining detects "hidden" micrometastases. Some lymph nodes are also used for multiple marker analysis with RT-PCR method (reaction of chained multiplication with reverse transcriptase). The method is able to detect hidden lymphatic metastases which remained unidentified with IHC method.

Discussion

Limited breast cancer which does not penetrate the basal membrane is usually called *carcinoma in situ*, in contrast to the cancer which penetrates the basal membrane and spreads beyond milk channels (invasive cancer). With cancer spread along the milk channels cancer cells may invade blood and lymphatic vessels. Via blood or lymph cancer process is disseminated thoughout the body. Lymphatic vessels of the breast drain into the axillary lymph nodes, but also along the sternum and clavicula. Dissection of axillary lymph nodes is recommended for invasive breast cancers. However, this procedure may induce seroma formation within the operative wound and edema of the upper extremities, requires drainage and markedly prolongs hospitalization.

"Sentinel" lymph node biopsy is a relatively new method for assessment of cancer spread in breast cancer but also for other cancer sites (head and neck cancers, colorectal cancer, carcinoma of the penis, malignant melanoma etc.). The technique represents a test aiming to establish whether the cancer has spread lymphatically and invaded axillary lymph nodes. Axillary lymph nodes usually drain into one lymph node and then in other axillary nodes. This node, so called "sentinel" lymph node, may serve as an index of lymphatic cancer spread. Application of radioactive marker or stain may detect "sentinel" lymph node, which is the first to receive lymphatic drainage and, hypothetically, malignant tumour metastases.

The method has various advantages compared to standard dissection of axillary lymphatics. It enables more precise determination of lymphatic dissemination of a malignant process. With standard axillary dissection a pathologist get on the average 10-15 lymph nodes and in search for cancer he has to make sections of all these nodes. With one lymph node obtained during operation, a pathologist make a number of sections in order to find the elements of a malignancy. Negative findings in SLN suggest the absence of lymphatic dissemination, sparing the patient of axillary dissection and all the complications associated with the procedure (pain, seroma, arm edema, etc.).

The procedure is not appropriate for all patients. Generally, SLN biopsy cannot be performed in all patients with invasive breast cancer. In those with previous breast surgery or radiotherapy the procedure should not be applied too, since inadequate results may be obtained. In patients with large palpable axillary lymph nodes, axillary dissection should be performed. Classic axillary dissection should also be done in patients with tumour larger than 5 cm since they have high incidence of axillary lymph node involvement. SLN biopsy is not the appropriate procedure for occult forms of cancer, multifocal tumours and for patients with difficulties for the procedure to be performed.

The general opinion is that SLNB should be done in patients with stages I and II of breast cancer, and that stage III is not appropriate due to high percentage of lymphatic (I-III level) involvement.

For SLN labeling only staining may be used, only radioactive marker or their combination. The dilemma is nowadays resolved: most of the centres worldwide utilize the combination of staining and radiopharmaceuticals due to the high identification rate of 95% (10). Indocyanin-green (DIAGNOGREEN 0.5%) and isosulphan-blue (LYMPHAZURIN) are most frequently used. Indigocarmine and methylen-blue are now aborted due to their insufficient reliability. Tecnetium sulphur colloid (0.5-1 ml), filtrated or not, is the most commonly used radioactive marker. Application of human serum albumin labeled with technetium is disputable due to its lesser sensitivity and smaller diameter of the particles which may pass through SLN I and reach II level lymph nodes.

Marker application may be intratumoral, peritumoral, subdermal and subareolar. Subdermal and subareolar administration are nowadays preferred due to the simplicity of the procedure, shorter interval to SLN dissection, and simpler dissection technique.

The interval between staining and axillary incision is recommended to be 3-10 minutes. Exceeding of this interval may cause transfer of the stain into the non-SLNs, which afterwards impede SLN identification. The inteval primarily depends on the tumour site. Shorter transition-time is required for tumours situated high towards the axilla, compared to those in the lower inner quadrant which require prolonged transition-time.

Histopathologic check-up proceedings are now uniform for all centres dealing with SLN identification. If there is a negative result on frozen sections, haematoxyllin-eosin (H&E) staining is performed first, and then anticytokeratin IHC check-up. To detect hidden micrometastases RT-PCR method may be used to analyse multiple markers (reaction of chained multiplication with reverse transcriptase).

False negative results may be the consequence of:

- lack of some SLN (3);
- extraaxillary localization of positive SLN (3);
- embolism of lymphatic channels with tumour cells;

- presence of tumour within lymph nodes may prevent tumours to take up stain (12);
- patient age (in older individuals lymph nodes may be replaced with fat tissue, which induces lower capacity for radiopharmaceuticals uptake;

Experience of the surgeons is very important for successful SLN detection. Giuliano et al. (1997) even think that there is a »learning curve«, ie. that the increase of SLN detection percentage correlates with the experience of the surgeon.

Conclusion

Labeling and SLN dissection is a new surgical procedure in breast cancer diagnosis, gaining significant acceptance among the surgeons. Conservative breast surgery and selective axillary dissection represent central trends in breast cancer surgery. These surgical procedures significantly reduce the rate of postoperative complications. Conservative surgical approaches resolve the problem of whole breast removal and reconstructive breast interventions, and with selective axillary dissection we may avoid otherwise unresolvable arm lymphedema, arm stiffness, prolonged postoperative axillary pain and wound seroma formation. Axillary dissection is indicated only in case of metastases within SLN.

Labeling and dissection technique of SLN is very delicate, requiring a well trained team consisting of a surgeon, radiologist, pathologist and oncolog. Many European surgical centres have large experience with labeling and SLN dissection. Acceptable standard of 95% of successful dissections in these centres further affirms the method, so it is to be expected that SLN biopsy become a routine procedure in surgical breast cancer management.

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ULOGA BIOPSIJE »STRAŽAR« LIMFNOG NODUSA U DIJAGNOZI KARCINOMA DOJKE

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Kratak sadržaj. *Biopsija »stražar« limfnog nodusa (SLNB) je relativno novija metoda za procenu rasprostranjenosti kancerskog procesa kod karcinoma dojke. Ovaj postupak predstavlja svojevrstan test kojim može da se utvrdi da li se karcinom dojke proširio limfnim putem i zahvatio limfne žlezde aksile. Danas se disekcija aksilarnih limfnih nodusa još uvek izvodi u više od polovine pacijenkinja sa karcinomom dojke koje imaju histološki negativne noduse, uprkos značajnom postoperativnom morbiditetu (prolongirani aksilarni bol, limfedem, ukočenost ruke, stvaranje seroma u rani). Status aksilarnih limfnih nodusa je takođe važan prognostički faktor kod bolesnica sa karcinomom dojke i primarni faktor za korišćenje sistemske adjuvantne terapije. Cilj ovog rada je da se ukaže na značaj ove hirurške metode u dijagnostici i terapiji karcinoma dojke i da se prikažu tehnički detalji izvođenja metode (tehnika obeležavanja SLN, tehnika disekcije SLN, patološka procena i drugo). Metoda se u mnogim evropskim hirurškim centrima primenjuje više od jedne decenije, a prihvatljiv standard od 95% uspešnih disekcija SLN afirmiše metodu, te treba očekivati da ona postane rutinska procedura u hirurškom lečenju karcinoma dojke.*

Ključne reči: *Karcinom dojke, biopsija »stražar« limfnog nodusa, disekcija aksilarnih limfnih nodusa*