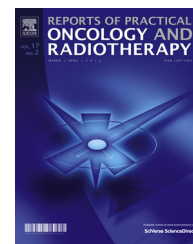


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## Preliminary communication

# Diagnostic value of intraoperative histopathological examination of the sentinel nodes in breast cancer and skin melanoma—Preliminary results of single centre retrospective study



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## ABSTRACT

**Objective:** Intraoperative histopathological examination of the sentinel nodes enables selection of patients who need dissection of the regional lymphatic system during the same operation. The aim of this study is to evaluate the diagnostic value of intraoperative histopathological examination of the sentinel nodes in breast cancer and skin melanoma. Intraoperative histopathology of the sentinel nodes as a diagnostic method is used in patients with melanoma and breast cancer. Recent studies have proved it to be an effective method for evaluating the nodes in the final histopathology. Intraoperative histopathological examination of the sentinel nodes is not performed routinely and there is no clear position on this issue. In this paper we try to prove that intraoperative test gives patients the simultaneous benefits of removal of regional lymph nodes metastases and earlier initiation of adjuvant therapy.

**Methods:** The study comprises 137 patients with breast cancer and 35 patients with malignant skin melanoma. Sentinel nodes were intraoperatively sectioned and examined by means of the imprint method and frozen section evaluation. The patients with positive sentinel nodes underwent immediate dissection of regional lymph nodes. Those with negative sentinel nodes diagnosed in the intraoperative examination, but positive in final pathologic results, underwent subsequent dissection of regional lymph nodes.

**Results:** 60 sentinel lymph nodes were found in 35 patients with skin melanoma. In 3 patients, 3 sentinel lymph nodes were false negative in the intraoperative histopathological examination. No false positive sentinel lymph nodes were found. 249 sentinel lymph nodes were found in the intraoperative histopathological examination in 137 patients with breast cancer. There were no false positive sentinel nodes, but there were 7 false negative sentinel nodes. In this study, only 5 (3.6%) patients with breast cancer and 3 (8.5%) patients with skin melanoma required another regional operation.

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**Conclusion:** The method of intraoperative histopathological evaluation of the sentinel nodes enables identification of metastases in these lymph nodes and gives a possibility to carry out a one-step regional lymphadenectomy and start the adjuvant therapy earlier.

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## 1. Background

The knowledge of the state of lymph nodes is necessary to evaluate the clinical progression of malignant tumours resulting in metastases through the lymphatic tract. Clinical examination is a basic evaluation method. Fine needle aspiration biopsy is applied in the case of enlarged lymph nodes and then specimens are sent for cytological examination. In the case of diagnostic doubt, open surgical biopsy should be carried out. If regional lymph nodes are clinically unchanged, the best method to assess their state is to carry out biopsy of the sentinel node with its cytological evaluation.<sup>1,2</sup>

By definition, the sentinel nodes (SNs) are the first lymph nodes on the way of the lymph flow from the area invaded by malignant cancer. The current state of knowledge requires an evaluation of sentinel nodes in solid neoplasms such as skin melanoma and breast cancer. When the sentinel nodes is free from metastases, the risk that another node in the lymph flow is invaded is almost none and there is no need to carry out total lymphadenectomy. The sentinel nodes may be examined intraoperatively. Intraoperative examination of the sentinel nodes gives a possibility to reduce the time before beginning the adjuvant therapy and to carry out a one-step regional lymphadenectomy if metastases have been diagnosed. This can be done by means of frozen section procedures and imprint techniques, immunohistochemical methods and tests on automatic platforms.<sup>2–9</sup> When it is impossible to make an intraoperative histopathological evaluation of the sentinel nodes and the results of the final examination prove a metastasis to the sentinel nodes, the patient needs to undergo another surgery and have regional lymph nodes resected. This exposes patients to additional stress related with the surgery and extends the time before beginning an adjuvant therapy. Lymph nodes involvement could be treated by surgical regional dissection of lymph nodes or radiotherapy, but distant metastases need to be treated by systemic chemotherapy.

There are new methods to examine the sentinel node. Indocarbocyanine green (ICG) visible in infrared light is one of them.<sup>10</sup>

The aim of this study is to assess preliminary results of the diagnostic value of intraoperative cytological examination of the sentinel nodes in breast cancer and skin melanoma.

## 2. Material and methodology

Between January 2009 and December 2009, 137 patients with breast cancer and between July 2009 and December 2009, 35 patients with malignant skin melanoma underwent the procedure of sentinel node biopsy with intraoperative histopathological examination. The patients were hospitalised at the

1st Department of Oncological and General Surgery, Greater Poland Cancer Centre, Poznań.

In the case of breast cancer primary tumour was diagnosed in the following number of patients: Tis – 33 patients, T1a – 10 patients, T1b – 26 patients, T1c – 62 patients, T2 – 6 patients. The criteria for exclusion from the study group were changes in clinical or radiological examinations of regional lymph nodes. Patients with T3 and T4 tumours in our group showed such changes.

The melanoma tumour was located on the back – 18 patients, neck – 1 patient, upper limb – 8 patients, lower limb – 4 patients, torso – 4 patients. The results of the histopathological examination of the tumour according to the Breslow scale were as follows: Breslow  $\leq 1$  mm – 4 patients, Breslow  $\leq 2$  mm – 11 patients, Breslow  $\leq 3$  mm – 7 patients, Breslow  $\leq 4$  mm – 7 patients, Breslow  $> 4$  mm – 6 patients.

The preoperative diagnostics of the state of the lymph nodes in the patients suffering from breast cancer and malignant skin melanoma comprised a clinical examination and ultrasonography. If no atypical lymph nodes were diagnosed, the patient underwent the procedure of sentinel node identification.

The sentinel nodes biopsy was carried out according to the following scheme. One day before the surgery, breast cancer patients received a sulphide colloid marked with a radioactive isotope  $^{99}\text{Tc}$  – 1 ml (1 mCi) to the site above the tumour. On the following day, immediately before the surgery, lymphoscintigraphy was carried out.

In the case of malignant melanoma, one day before the surgery the patients received a sulphide colloid marked with a radioactive isotope  $^{99}\text{Tc}$  – 2 ml (1.2 mCi) to the site surrounding the scar after the resected lesion through 4 punctures. After 2 h, lymphoscintigraphy was carried out. 10 min before the planned surgery the patients with malignant melanoma additionally received methylene blue 2.5% to the site surrounding the scar/tumour and they were massaged for 2 min (4 intradermal and subcutaneous injections – 2 ml).

The intraoperative evaluation consisted in sending the collected sentinel node to the histopathological laboratory, where the node was carefully dissected along the long axis, including the hilum and marginal sinuses. One half was used to make an imprint and it was stained with haematoxylin and eosin. The other half of the sentinel node was used to make frozen sections perpendicularly to the dissection plane and one of them was stained with H+E and the other one with thionine. The intraoperative evaluation result was sent to the operating surgeon with diagnosis of the presence or absence of metastases in the sentinel nodes. Then the specimens were sent for the final histopathological examination. The specimens were fixed in a 10% formalin solution for 24 h and embedded into paraffin blocks. Three sections were made and stained with H+E. In the case of the absence of metastases in the

sentinel nodes in traditional microscopic examinations, in the group of patients suffering from malignant melanoma, immunohistochemical examinations were additionally made with the use of antibodies against Melan A, HMB 45 and S 100.

### 3. Results

60 sentinel lymph nodes were found in 35 patients with skin melanoma, which makes the median of 1.7 sentinel nodes per patient. The detection concerned 100% of the patients. The lymph flows in the presented material were directed towards one lymphatic area in 92% of cases, whereas 8% were directed towards two surrounding areas. In the group of patients with malignant melanoma the intraoperative histopathological examination revealed 6 sentinel lymph nodes with metastases in 4 patients. In the final histopathological examination, the total of 9 lymph nodes with metastases were diagnosed in 7 patients. Thus, in 3 patients 3 sentinel lymph nodes were false negative in the intraoperative histopathological examination. No false positive sentinel lymph nodes were found. When analysing the causes of false negative results in the first case, the final examination revealed melanoma metastases in the lymph node capsule. In the second case, metastatic cells were identified after being compared with the neoplastic cells from the primary lesion, which was resected in a different centre. Intraoperatively, they were indistinguishable from melanocytes. Some patients are reoperated in our centre after primary excision of their tumours in other centres. In these centres surgeons could not perform sentinel node biopsy. In such cases, histopathologists compare specimens from our centre to those from centres of primary excision. In the third case, a metastasis to the lymph node was found in the final examination after making consecutive sections.

249 sentinel lymph nodes were found in the intraoperative histopathological examination in 137 patients with breast cancer, which makes the median of 1.8 sentinel nodes per patient. The detection concerned 100% of the patients. In the intraoperative histopathological examination 20 sentinel nodes with metastases were diagnosed. The final histopathological examination revealed 27 sentinel nodes with metastases. There were no false positive sentinel nodes, but there were 7 false negative sentinel nodes. The average number of lymph nodes found in intraoperative study was 1.82 in breast cancer, while the average number of lymph nodes found in the final histopathology was 1.98. For melanoma, an average of 1.69 lymph nodes were found in intraoperative study and 1.78 in final study. No serious complications were observed in either group. The difference between the number of sentinel lymph nodes found in the intraoperative study and final study is due to a more accurate detection of tissue taken during the final testing which enabled to find small nodes missed during intraoperative testing.

In the study, we compared the intraoperative histopathological examination with the final histopathological examination of the sentinel node. Assessment of regional nodal recurrence was not the subject of this investigation. Study assessing the reliability of sentinel lymph node was confirmed in a number of works.

In statistical analysis of this method on the group of patients with malignant melanoma, sensitivity was 66.7% and specificity was 100%. On the group of patients with breast cancer, sensitivity was 74.1% and specificity was 100%

### 4. Discussion

The assessment of progression of breast cancer by means of histopathological evaluation of the sentinel node has been evolving since the first report from Giuliano et al. in 1994 up to the general practice applied today.<sup>1,11,12</sup> The present recommendations of the Polish Union of Oncology to breast cancer patients suggest that in all justified cases elective axillary dissection should be replaced by the procedure of sentinel node biopsy, which involves a lower risk of post-operative complications. It is recommended when axillary lymph nodes are clinically free (N0 stage) and the surgeon has appropriate experience in carrying out sentinel node biopsies (at least 100 successful biopsies) because of the learning curve. The recommendations of the Polish Union of Oncology to patients suffering from skin melanoma suggest that a biopsy of the primary site of cancer should be followed by a sentinel node biopsy. This should be done if regional lymph nodes are clinically free (N0 stage).<sup>33</sup> Elective axillary dissection is an examination which enables evaluation of lymph nodes. The operation involves numerous side effects, such as wound infection, lymphoedema and nerve damage. A sentinel lymph node biopsy enables examination of clinically negative lymph nodes and clinical grading in patients with breast cancer and malignant melanoma. In most cases, it helps avoid the side effects of regional lymphadenectomy. Early diagnosis of metastases in the sentinel lymph node is possible due to the intraoperative histopathological examination.<sup>13-17</sup> In 2008, Alkhatib et al. examined intraoperatively a group of 133 patients with melanoma (271 nodes) by means of the frozen section method. They proved that the examination sensitivity was 91%, with the false negative coefficient of 0.4% (1 out of 271).<sup>30</sup> There are doubts concerning the application of intraoperative sentinel node examination in malignant melanoma. There are some apprehensions concerning the risk of overlooking micrometastases or individual metastatic cells of melanoma in intraoperative histopathological examinations. Furthermore, some authors claim that the examined tissue may be partly damaged during the frozen section procedure. Tanis et al. reported that the frozen section method is recommended in breast cancer, where the disease sensitivity reaches 74%, but they do not recommend the procedure in melanoma because of the sensitivity of 47%.<sup>18-21</sup>

Gipponi et al. evaluated 169 patients and in their examinations they proved false negative results of 5.3%.<sup>31</sup> Similarly, Ariyan et al. evaluated 263 patients and proved false negative results of 7% (2 out of 28).

The data from publications evaluates the sensitivity of the imprint biopsy in patients with malignant melanoma at 33-62% and the frozen section method at 73-90%.<sup>22-26,29,32,35</sup>

In our study on the group of patients with malignant melanoma, sensitivity was 66.7% No false positive results were observed.

In breast cancer, according to data from the literature, the sensitivity of the method of intraoperative histopathological sentinel node examination with imprint procedure fluctuates between 72% and 86% and the specificity, between 90.8% and 100%.<sup>27,28</sup> Low sensitivity, which is quoted in some publications, may be accounted for by inaccurate imprint of the specimen, the presence of metastatic cells under the node capsule or high adhesive capacity of lymphatic cells, which is higher than that of epithelial cells. According to the literature, the sensitivity of the frozen section method ranges between 75% and 90%.<sup>34</sup> Low sensitivity, which is reported by some authors, may be related to making an individual frozen section. Making a larger number of frozen sections increases the chance to identify metastases in the intraoperative histopathological examination. According to some authors, the frozen section procedure involves the risk of losing 25–50% of the specimens and producing a larger number of artefacts.

The results of our own study on breast cancer proved to be similar to the data from publications, with the sensitivity of 74.1% and absence of false positive results.

To sum up, the method of intraoperative histopathological evaluation of the sentinel nodes enables identification of metastases and gives a possibility to carry out a one-step regional lymphadenectomy and start an adjuvant therapy earlier. When observing the data quoted in the literature and our own data, this thesis is justified in the case of patients with breast cancer. However, it requires further research in the case of other neoplasms, including patients with malignant melanoma.

### Conflict of interest

None declared.

### Financial disclosure

None declared.

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