

Ultrasound Assessment of Regional Lymph Nodes in Melanoma Staging

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ABSTRACT

Background: Melanoma can early metastasize to regional lymph nodes. The sentinel lymph node (SLN) is the first lymph node draining directly from the site of primary melanoma, and the pathohistological status of the SLN is the most significant prognostic factor for overall survival prevalence and prognosis in patients with melanoma. Ultrasound is a very useful for the imaging of regional lymph node metastases, combined with Doppler and cytopuncture.

Objective: The aim of this study was to investigate the role of ultrasound assessment of regional lymph nodes in melanoma staging.

Patients and methods: The study included all patients with primary melanoma detected in the period between 2003 and 2012, in whom diagnostic processing has not proven distant metastases or physical examination did not find enlarged lymph nodes. In total, 202 surgically treated patients were included in the study, of which 101 patients underwent ultrasound examination of regional lymph nodes using a linear probe of at least 12 MHz, while ultrasound of regional lymph nodes was not performed for 101 patients.

Results: The results of this study emphasize the importance of ultrasound in the diagnostics and treatment of patients with melanoma. Based on the observation of the occasional positive ultrasound and fine needle aspiration cytology (FNAC) in regional lymph nodes, our results indicate that a proportion of patients can avoid sentinel lymph node biopsy (SLNB). In case of a positive ultrasound findings (complemented with FNAC of suspicious nodes), direct dissection of regional lymph nodes is recommended. However, negative ultrasound findings do not exclude the presence of micrometastases due to poor sensitivity of this method and is not a contraindication for SLNB.

Conclusion : Therefore, there is a need for further studies on metastatic melanoma, especially those in the sentinel lymph nodes and in its early stage.

KEY WORDS: melanoma, ultrasound, lymph nodes, sentinel lymph node biopsy, elective lymph node dissection

INTRODUCTION

Melanoma is a type of skin cancer that is derived from melanocytes, skin cells that produce pigment (1). In the last twenty years, melanoma has become

one of the most frequent cancers in the western world, with one of the fastest growing incidence rates among all cancers (2). Although melanoma

accounts for only 4% of all skin tumors, it is responsible for 80% of skin cancer deaths (3). Melanoma has an unpredictable course, and no prognostic factors have been found that would fully predict the biological behavior of the tumor and its metastatic potential (4,5). Today, patients with primary melanoma are relatively successfully treated in the early stages of the disease by radical surgical removal of the primary tumor (6). Improved survival has been recorded in recent years, but despite advances in diagnostics and current knowledge about the mechanism of its occurrence, melanoma poses an increasingly significant public health problem worldwide due to the growing incidence of this cancer (7).

Long-term impact studies on patient survival shed new light and defined cases in which elective and therapeutic lymph dissection (sentinel node biopsy (SLNB)) is justified (8). It has been scientifically proven that melanoma, depending on the thickness of the primary tumor, can metastasize to regional lymph nodes early on (8-10). The sentinel lymph node (SLN) is the first lymph node draining directly from the site of primary melanoma (11). Examination of such a node (or nodes) is an important step of surgical procedure among patients with melanoma and offers new diagnostic and prognostic insights based on the role of lymph in metastatic malignancy (10). The histological status of SLN is the most significant prognostic factor for overall survival prevalence and prognosis in patients with melanoma (11-15). Today, SLNB is considered an acceptable method of nodal staging and melanoma treatment for the majority of patients with newly diagnosed melanoma (11). The presence of metastasis in SLN is the most important prognostic indicator in patient survival: the five-year survival of patients with lymph node metastasis is 72.3% and is 90.2% without metastasis (16).

Ultrasound is an easily accessible diagnostic technique, both harmless and with the possibility of frequent reapplication, combined with Doppler and cytopuncture. This technique is very useful for the imaging of regional lymph node metastases – size, structure, blood supply, number of enlarged lymph nodes and the nature of the enlarged node (inflammatory or metastatic). Use of a high frequency linear transducer plays an important diagnostic role in the recognition of small, clinically occult micrometastases, the gradation of local/regional spread, and the follow-up of patients with melanoma (17). There is a lack of evidence on the accuracy of ultrasound as an imaging technique for the recognition of metastases in SLN due to commonly misdiagnosed micrometastases (18). Ultrasound combined with fine needle aspiration cytology reduces the need for SLNB to

some extent (19,20). Ultrasound is also used to diagnose distant metastases in the liver, spleen, kidneys, pancreas, and adrenal gland. The main diagnostic challenges are bleeding, extensive colliquation, and low specificity of this imaging technique. As a non-invasive method, ultrasound cannot replace SLNB in determining the staging of melanoma because it is impossible to show minimal amounts of metastatic deposit without immunohistochemistry (19,21-23). However, SLNB is an expensive and complex surgical procedure. When preliminary sonography shows a finding of suspected lymph node metastasis, SLNB is no longer necessary and the patient may undergo ultrasound-guided fine needle aspiration cytology or may be subjected directly to elective lymph node dissection (ELND).

The aim of this study was to determine the clinical value of routine ultrasound of regional lymph nodes in patients with primary melanoma, without clinically notable regional lymph nodes and with no evidence of distant metastases, in order to detect pathologically altered lymph nodes. Confirmation of the benefits of routine ultrasound of regional lymph nodes would bring a shortened and simplified diagnostic procedure for some patients with melanoma. Implementation of ultrasound imaging technique might lower healthcare costs and reduce duration of the treatment, while also reducing the patient's exposure to stress by avoiding unnecessary surgical procedures.

PATIENTS AND METHODS

Clinical features of patients

Patients with histopathologic confirmation of melanoma were hospitalized for further treatment at the Department of Dermatology and Venereology (which includes the Referral Center for Melanoma of the Croatian Ministry of Health). The stage of the disease was determined by diagnostic processing prior to continuation of the treatment.

Patients in whom ultrasonography did not find distant metastases, and no enlarged lymph nodes were found on clinical examination, were subjected to the SLNB procedure. Given the complexity of the SLNB procedure, an ultrasound was performed during the treatment for one group of treated patients. In case of possible detection of a pathologically altered node evaluated with ultrasound, fine needle aspiration cytology was performed under ultrasound control. In case of positive cytology results, SLNB was not performed, but ELND was immediately indicated.

The study included all patients with primary melanoma treated at the Department of Dermatology and Venereology, Sestre milosrdnice University Hospital



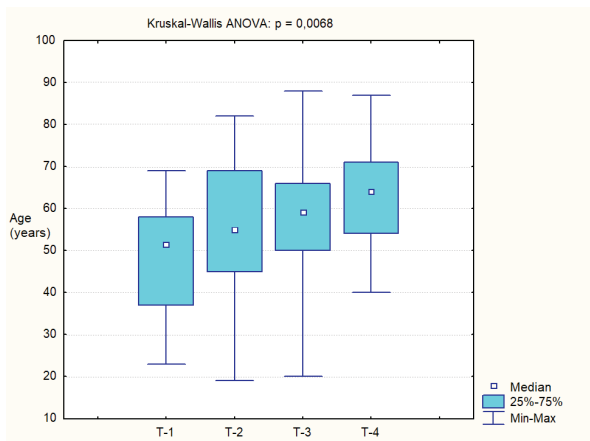


Figure 1. T-classification according to patient age.

Center within the Referral Center for Melanoma of the Ministry of Health between 2002 and 2012, in whom diagnostic processing had not proven distant metastases or physical examination did not find enlarged lymph nodes.

Patients were divided in 2 groups according to additional criteria: ultrasound examination of regional lymph nodes using a linear probe of at least 12 MHz.

Input parameters in this study were age, sex, histopathologic analysis of primary melanoma (tumor thickness, presence of ulceration), clinical status of regional lymph nodes, ultrasound findings of regional lymph nodes, and findings of fine needle aspiration cytology under ultrasound control. The output parameters were performed SLNB and ELND.

In total, 101 patients underwent ultrasound analysis of regional lymph nodes, while ultrasound of regional lymph nodes was not performed for 101 patients.

Diagnostic and surgical procedures

The lymphoscintigraphy procedure was performed with a gamma camera (with prior lymphatic labeling with radioactive technetium) on the day of surgery. A mean volume of 0.18 mL (0.1 to 0.3 mL) of ^{99m}Tc -human serum albumin (Nanocoll Amersham Sorin, Milano Italy) was injected intradermally at two sites along the lesion, with a total dose of 104.7 MBq (99-150 MBq). Early and late images were taken after 20 minutes and 2 hours. The location of the biggest accumulation was marked on the skin. On the day of surgery, 20 minutes before skin incision, blue dye (lymphotropic dye) was injected, with a mean volume of 1.1 mL (0.6 to 3.3 mL) and an average of 8 stitches around the scar (3-16 stitches). The median interval between injection and surgery was 22-25 minutes (6-41 minutes).

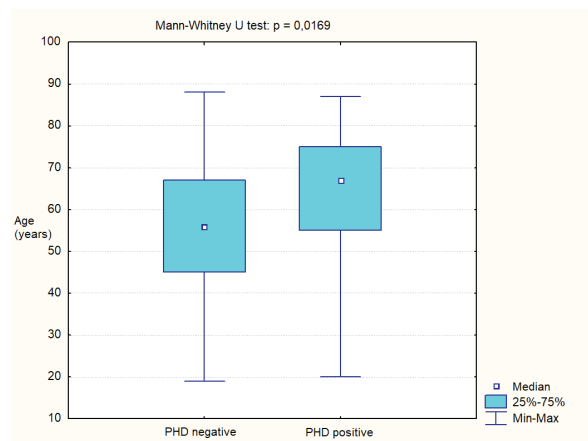


Figure 2. Correlation of the histopathologic report and age of patients.

A manual gamma camera probe was used for intraoperative SLN detection. The probe was placed vertically, in contact with the skin surface, and drawn transversely at a speed of 1-5 cm/s to the marked place. Upon detection of the point of highest radioactivity, the surgeon made an incision of the skin and subcutaneous tissue and searched for SLN using a gamma probe. Visualization of blue-colored lymphatics and lymph nodes was also helpful. After surgical incision (intraoperatively), SLN was identified as a hot spot and/or blue nodule(s) of the highest radioactivity. This node(s) was removed and sent for further histopathologic analysis. Background emissions and SLNs were recorded preoperatively, intraoperatively, and extraoperatively. Extraoperative evaluation was performed outside the operative field, after SLN excision.

SLNB was performed under general or regional anesthesia (with prior lymphatic labeling and lymphotropic staining). Intraoperatively, SLN preparation was referred for urgent cytodiagnostics. The method of imprinting a tumor, metastasis, or sentinel lymph node is used for urgent intraoperative analysis because it detects occult micrometastases of melanoma. The removed SLN was referred to the Department of Pathology in 10% formalin for paraffin sections where the sample was stained with a hematoxylin-eosin stain and subjected to immunohistochemical analysis (S-100 HMB45 and MART-1).

SLNs were cut at the point of entry of the hilus and then every 1-2 mm into thick fragment cuts including the entire node. All fragments were embedded in paraffin, and 10 sections were prepared from each inclusion for histological and immunohistochemical analysis (S-100 protein and HMB 45).

Ultrasound of regional lymph nodes was performed with a PHILIPS device, model HDI 5000 SONO

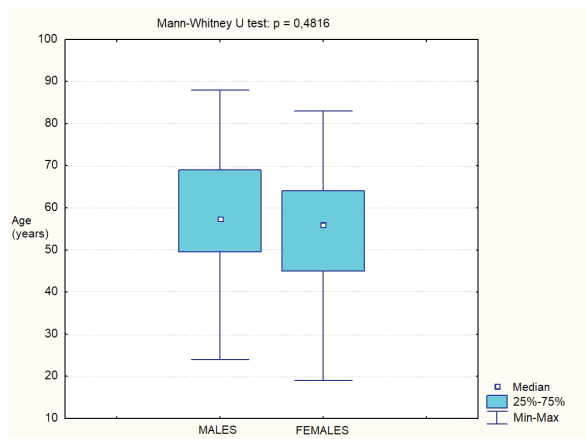


Figure 3. Age range according to sex in group 1 (patients who underwent ultrasound).

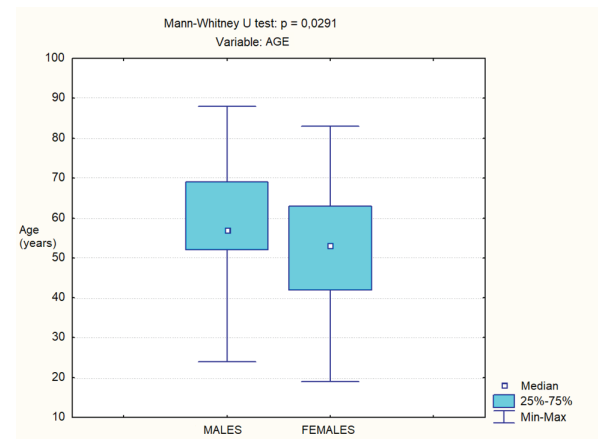


Figure 4. Age range according to sex in group 2 (ultrasound was not performed).

CT, using a linear probe L 12 MHz. At the same time, normal ultrasound finding of regional lymph nodes did not exclude the existence of micrometastases. In the case of suspicious altered node during ultrasound analysis, i.e. before the indication of SLNB, targeted fine needle aspiration cytology under the control of ultrasound was performed. If the fine needle aspiration cytology finding was positive (proven lymph node metastasis), a no complicated SLNB procedure was required, but ELND was indicated. All cytological samples were stained in the same way: May-Grunwald-Giemsa stain and Papanicolaou.

Statistical processing

For the analysis, 202 patients were selected for whom no enlarged lymph nodes were found by clinical examination. Among this group of patients, 101 patients had undergone ultrasound analysis of the regional lymph nodes while no ultrasound was performed for 101 patients. The samples were matched by sex and age – equal numbers of men and women, within the same age range. Comparison by sex was performed using the chi-square test, and by age by the Mann-Whitney U test (depending on sample size and value distribution). Differences between qualitative variables were tested by the chi-square test. Differences in quantitative variables between the two groups were tested by the nonparametric Mann-Whitney U test, while differences between three or more groups were analyzed by the nonparametric analysis of Kruskal-Wallis variance. Univariate logistic regression was performed, and specificity and sensitivity were calculated. The limit of statistical significance was considered to be $P=0.05$. The effect of prior use of ultrasound on subsequently performed or uncompleted biopsy was examined by logistic

regression (OR with 95% CI). The influence of age and sex on SLNB was also examined by logistic regression. Statistical analysis of the data was performed in the statistical package Statistica for Windows, USA (on a personal computer in the program Statistica 6).

RESULTS

The study included a total of 202 patients with skin melanoma detected in the period between 2003 and 2012 and surgically treated at the Department of Dermatology and Venereology. The mean patient age was 56 years (range 19-88 years). Out of all the patients included in the study, 96 were men (47.5%) and 106 were women (52.5%). There was no statistically significant difference in the sex of the patients. The primary melanoma was most commonly localized on the trunk (100 patients, 49.5%), followed by the lower (62 patients, 30.7%), and upper extremities (31 patient, 15.3%) and finally by the head and neck (9 patients, 4.5%). However, there was no statistically significant difference in the localization of primary melanoma.

In the total sample with respect to the thickness of the primary melanoma (according to T-classification), patients with T-1 thickness were slightly younger, while patients with T-4 thickness of primary melanoma were slightly older (~71 years old). The difference was statistically significant ($P=0.0068$) (Figure 1).

Out of the total number of patients, 165 (81.7%) showed 1 SLN, 34 of them had 2 SLNs (16.8%), while only 3 patients had 3 SLNs (1.5%). The numbers of SLNs was associated with the localization of primary tumor localization; patients with 2 or 3 SLNs most commonly had primary melanoma on the trunk. With regard to age, patients with 2 or 3 SLNs were slightly

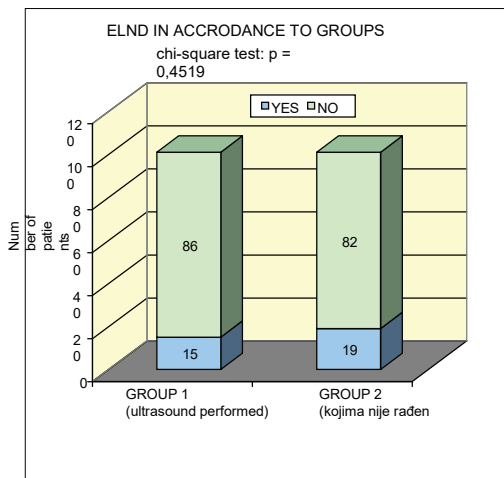


Figure 5. Distribution of ELND by groups (ultrasound was or was not performed).

younger, but without a statistically significant difference (Mann-Whitney U test, $P=0.5062$).

A total of 34 patients (17%) underwent ELND, where 18 histopathologic reports of removed lymph nodes were positive (52.9% of all ELND, or 8.9% of the total number of patients). ELNDs were performed both among patients with positive SLN and among some patients in whom SLN was not positive (in case of high risk of lymph nodes metastases, ELND was performed at the same time as SLNB). Given the T-classification of primary melanoma, ELND was most commonly performed in T-4 category patients (18 of 33 patients, 54.5%). The association between T-classification and ELND was statistically significant ($P=0.0001$). The results did not show statistical significance in the correlation of primary melanoma localization and the final histopathologic report ($P=0.9542$), nor any influence of sex on the outcome of the final histopathologic report ($P=0.8256$). However, a statistically significant difference was observed depending on patient age with regard to histopathologic analysis ($P=0.0169$) (Figure 2).

In group 1 (patients who underwent ultrasound) there was 101 patients (50%), the same number as in group 2 (patients who did not undergo ultrasound). The mean follow-up length was 3 years (range 1-12 years). The mean age among patients who underwent ultrasound was 57 years (range 19-88 years), while the mean age of patients who did not undergo ultrasound was 56 years (range 19-88 years). There was no statistically significant difference between the groups with respect to the age ($P=0.2747$).

In group 1 (patients who underwent ultrasound), there was no statistically significant difference in the age with respect to sex ($P=0.4816$), while in group 2 (patients who did not undergo ultrasound) there was

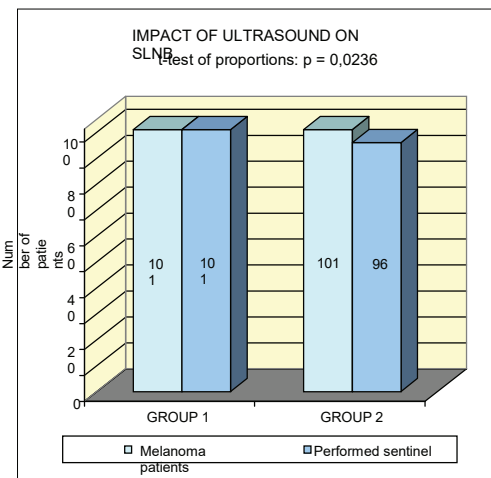


Figure 6. Influence of lymph nodes ultrasound on SLNB.

a statistically significant difference in age with respect to sex ($P=0.0291$) (Figure 3, Figure 4).

Among patients who underwent ultrasound, the number of positive histopathologic reports was 5 (5.0%), while the number of positive histopathologic findings was 13 (12.9%) among patients with no ultrasound performed. When comparing these groups, we found a statistically significant difference ($P=0.0482$). Ultrasound findings of lymph nodes were compared with a final histopathologic report of SLNB and ELND. The ultrasound finding of the lymph nodes was a true positive in 5 patients, a false positive in 3 patients, true negative in 91 patients, and false negative in 2 patients.

In group 1 (where ultrasound was performed), ELND was performed in 15 patients (14.6%), while in group 2 (in which no ultrasound was performed) ELND was performed in 19 patients (18.8%). There was no statistically significant difference between the groups ($P=0.4519$) (Figure 5).

Given the presence of true positive ultrasound findings, it was interesting to analyse the correlation between ultrasound examination and the need to perform SLNB. Although all patients underwent SLNB, we can hypothetically exclude 5 patients out of patients from group 1 (ultrasound was performed) who had true positive ultrasound findings of lymph nodes in whom it was not really necessary to perform SLNB (95.0%), while in group 2 (in whom no ultrasound was performed) we had 101 patients with SLNB. Statistical significance was noted when comparing these 2 groups of patients ($P=0.0236$). Therefore, we can say that the number of patients who underwent SLNB was lower by 5% in group 1 than in group 2 due to the performance of ultrasound examination (Figure 6).

However, since this was a marginal percentage (less than 10%), it was necessary to perform Poisson's test, which showed no statistical significance.

DISCUSSION

Melanoma has been the subject of intensive study by the global professional and scientific medical community for the past few decades. Given that the incidence of malignant melanoma has grown in recent years (24,25), it has become one of the main public health problems in many countries (25). Lymph node metastases represent the first site of melanoma spread from the primary tumor (9,10). The histological status of SLN is the most important prognostic factor for overall survival of patients with melanoma, independent of primary tumor thickness (26). The significance of this prognostic factor was additionally acknowledged by the inclusion of SLN in the current classification of melanoma (27,28). Today, SLNB with eventual radical lymph node dissection of regional lymph nodes (ELND) is the standard procedure in the treatment of melanoma in the world. Sentinel-node biopsy is a minimally invasive and low-morbidity procedure performed with the use of blue dye and radio-labeled colloids (16,29).

In the total sample of this study, the average age of men was higher than the average age of women, and the difference was statistically significant, which is congruent with the known literature data claiming melanoma is diagnosed earlier in women. The distribution according to decade of life according to sex indicates women with primary melanoma were more prevalent in the younger age groups up to the age of 50, while there were more men with primary melanoma among participants older than 50. Primary melanoma was most often localized on the trunk, followed by the leg, arm, and most rarely on the head and neck, which is consistent with known epidemiological data (3). There was no statistically significant difference in terms of sex in the overall sample, and there were no statistically significant differences in terms of sex and age between the groups, except in group 2 (where no ultrasound of lymph nodes was performed) where there was a statistically significant difference in favor of men. Most studies state that the incidence of melanoma in men is higher than in women (2,30,31).

The thickness of primary melanoma shown through the T classification indicates that the highest prevalence was in the T2 category, followed by T3, then T4, and the lowest in T1. If the sex distribution of melanoma thickness is examined, it can be seen that the T2 category is more common in women and

the T3 category is more common in men, which corresponds to the fact that melanoma is generally detected earlier in women, but the sex difference was not statistically significant. If the thickness of primary melanoma is compared by age, it can be seen that categories T1 and T2 are more common in younger patients than categories T3 and T4, which also corresponds to the known data that melanoma in younger patients is diagnosed earlier, but no statistically significant difference was confirmed. Thickness is one of the features of the tumor itself with prognostic significance on disease outcome (8,16), and it is also a dominant prognostic indicator of melanoma metastasis in SLN (8,16). It has been proven that malignant melanoma, depending on the thickness of the primary tumor, can metastasize to regional lymph nodes early on (8,9). Guidelines in the use of lymphatic labeling and SLNB in staging of patients with newly diagnosed melanoma demonstrate that SLNB is an acceptable method for staging a large number of patients with newly diagnosed melanoma (10,29). Long-term studies proved the validation of SLNB usage in patients with intermediate-thickness or thick primary melanomas. This method is of key importance in collecting valuable information and ultimately improving melanoma-specific survival (8).

Considering the total sample, the share of definitely positive PHD findings in this sample was less than 10%: a total of 34 (16.8%) underwent ELND due to clinical suspicion of metastases, of which in 18 (52%, or 8.9% of the total number) patients the final PHD findings confirmed the existence of lymph node metastases. The localization of the primary melanoma and the sex of the patient did not affect the frequency of positive PHD findings or whether ELND was performed. A statistically significant difference was observed with respect to the definitive PHD finding in favor of elderly patients, which can be attributed to the fact that melanoma is diagnosed later in the elderly than in younger patients. Of all the analyzed variables, only age affected the PHD finding, in that with each year of age the risk of a positive PHD finding increased by 4%. Given the thickness of primary melanoma, there was a statistically significant difference with regard to the definitive PHD finding. Thus, the most positive patients were in the T4 category, followed by T3, while there were no positive PHD findings in the T1 and T2 categories. There was a statistically significant difference between the groups with respect to the definitive PHD finding ($P=0.0482$). In group 2, in which no ultrasound of lymph nodes was performed, there were several positive PHD findings. This finding can be explained by the fact that group 1, in which ultrasound of lymph nodes was

performed, was formed mainly from patients from a later period in the study, because routine ultrasound of lymph nodes in that period was more frequent and performed due to better accessibility than in the previous period, coupled with the fact that there have been more younger patients with melanoma at an earlier stage in recent years. There was no statistically significant difference between the groups with respect to the number of ELNDs performed ($P=0.4519$). This finding was expected, regardless of the routine use of ultrasound in group 1.

Ultrasound in combination with fine needle aspiration cytology (FNAC) has proven to be useful in identifying regional metastases earlier than by physical examination. It is also known to have a high sensitivity and specificity during the follow-up of patients with melanoma (32). Voit *et al.* (2006) showed the great value of ultrasound detection of SNs before surgery (33). Ultrasound examination possesses the ability to detect SNs via their morphology very accurately, with 79% sensitivity (33). Additionally, pre-operative ultrasound in combination FNAC can accurately detect SN metastases before surgery (19,20). Although SLNB was performed in all patients in this study, according to our results, the SLNB procedure could have been avoided in a smaller number of patients, since in group 1 the ultrasound finding (confirmed by cytopuncture) was truly positive in 5% of cases. Namely, the SLNB procedure in these patients did not have to be performed in a separate procedure, with the ELND procedure performed later in general anesthesia (after obtaining a definitive pathohistological finding) because the ultrasound confirmed by cytopuncture was actually an indication for ELND. Taking into account that there were 5 patients with a positive ultrasound finding, we demonstrated a statistically significant difference ($P=0.0236$), i.e., we can say that the number of patients with SLNB was lower by 5% in group 1 than in group 2 thanks to the use of ultrasound. However, since this is a marginal percentage (less than 10%), it was necessary to perform Poisson's test, not the proportionality test used. Using the Poisson test, the statistical significance was lost.

Nevertheless, although ultrasound can never replace SLNB in melanoma staging, this method can be very useful in providing more detailed findings and planning patient treatment. Moreover, routine ultrasound in combination FNAC of the SLN can replace surgical SLNB or lymph node dissection, preventing the need for a second surgical procedure (20).

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