

## Original contributions

### Sentinel node biopsy in patients with cutaneous melanoma and clinically non-palpable lymph nodes (stage N0) – the importance for diagnosis and prognosis

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*The aim of the study was to assess the percentage of metastases in regional lymph nodes detected by means of sentinel lymph node (SLN) biopsy technique and to analyse the survival of patients with cutaneous melanoma and clinically non-palpable lymph nodes (N0) in correlation to the number of involved lymph nodes.*

*Materials and methods.* The data of 400 melanoma patients (437 biopsies) treated during the period between April 1995 and September 1999 in the Department of Soft Tissue/Bone Sarcomas of the Maria Skłodowska-Curie Memorial Cancer Centre and Institute of Oncology, Warsaw was enrolled for the analysis. In most of the cases SLN biopsy was performed by vital blue dye in combination with intraoperative lymphoscintigraphy.

*Results.* Micrometastases in SLNs were identified in 25% of patients (100/400). Estimated 3-year disease free survival (DFS) and overall survival (OS) rate in the group of patients without metastases to SLNs were 79,3% and 85% respectively, as compared to 41% DFS and 57% OS in the group of patients with metastases to SLNs [ $p < 0.0001$ ]. Significant differences in DFS and OS were also observed between groups of patients with metastases to SLN only, and patients with 2 or more lymph nodes involved (DFS: 52% vs. 28% [ $p < 0.02$ ]; 3-year OS: 67% vs. 33% [ $p = 0.006$ ]).

*Conclusions.* SLN biopsy is an effective and reliable diagnostic method in clinical stage I and II cutaneous melanoma patients (N0). It seems that this technique allows to distinguish the group of high-risk melanoma patients.

#### **Biopsja węzła wartowniczego u chorych na czerniaka skóry z klinicznie niezmiennymi węzłami chłonnymi (N0) – ważna metoda w diagnostyce i rokowaniu**

*Wstęp.* Celem pracy było określenie odsetka przerzutów do węzłów wartowniczych oraz analiza przeżyć chorych na czerniaka skóry, z klinicznie niezmiennymi węzłami chłonnymi w zależności od stwierdzanych zmian przerzutowych do tych węzłów. *Materiał i metoda.* Analizie poddano dane dotyczące 400 chorych (437 biopsji węzłów wartowniczych), leczonych w Klinice Nowotworów Tkanek Miękkich i Kości Centrum Onkologii-Instytutu im. M. Skłodowskiej-Curie w Warszawie w okresie od kwietnia 1995 do września 1999. W większości przypadków biopsje węzła wartowniczego były wykonywane techniką wybarwienia węzłów wartowniczych barwnikiem PatentBlau V, w połączeniu ze śródoperacyjną limfoscyntyografią przy pomocy ręcznej przenośnej gamma-kamery.

*Wyniki.* Mikroprzerzuty w węzłach wartowniczych stwierdzono u 25% operowanych (100/400). Oszacowane 3-letnie przeżycia całkowite (OS) oraz wolne od nawrotu choroby (DFS) wyniosły odpowiednio: 85% i 79,3% u chorych z niezmiennymi węzłami wartowniczymi i były istotnie lepsze ( $p < 0.0001$ ) od 57% OS i 41% DFS w grupie chorych z mikroprzerzutami czerniaka do węzłów wartowniczych. Podobnie oszacowane 3-letnie przeżycia całkowite i bezobjawowe chorych z 1 węzłem wartowniczym, zmienionym przerzutowo, były istotnie lepsze od przeżyć chorych z dwoma lub więcej przerzutami czerniaka do regionalnych węzłów chłonnych ( $p < 0,02$ ) (DFS: 52% vs. 28% [ $p < 0,02$ ] i OS: 67% vs. 33% [ $p = 0,006$ ]).

*Podsumowanie.* Biopsja węzłów wartowniczych skóry jest ważną i wiarygodną metodą diagnostyczną u chorych na czerniaka w I, II klinicznym stopniu zaawansowania (N0). Dzięki jej zastosowaniu możemy dokładniej i pewniej określić grupę chorych o dobrym rokowaniu oraz ustalić grupę chorych obarczonych wysokim ryzykiem rozsiewu ogólnoustrojowego.

**Key words:** cutaneous melanoma, sentinel lymph node biopsy

**Słowa kluczowe:** czerniak skóry, biopsja węzła wartowniczego

## Introduction

In Poland the frequency of malignant melanoma is increasing more rapidly than that of other solid tumors [1]. Sentinel lymph node (SLN) biopsy has reached a great popularity as a standard management in melanoma stage I,II patients (with clinically N0 lymph nodes) throughout the world [2]. The hypothesis of SLN was presented by *Morton et al.* in the early 1990s, when their first experimental (1991) and clinical (1992) papers describing the idea of SLN and the technique of its staining and intraoperative localization were published [3, 4]. Briefly, this concept is based on two assumptions: 1<sup>st</sup> that particular parts of the dermis are characterized by lymphatic drainage to a single lymph node of a regional basin and 2<sup>nd</sup> that distant organ melanoma metastases are mostly preceded by the spread to regional nodes. It means that SLN is, in a high percentage of patients, the first site of metastatic disease.

Introduction of intraoperative lymphoscintigraphy to clinical practice in the mid-nineties has improved the operative technique and allowed to achieve accurate SLNs identification in more than 95% of investigated lymphatic basins [5, 6].

The aim of the study performed in the Department of Soft Tissue/Bone Sarcomas of the Maria Skłodowska-Curie Memorial Cancer Centre – Institute of Oncology was to evaluate the percentage of metastases in regional lymph nodes detected with SLN biopsy method and to analyse the survival of patients with cutaneous melanoma in correlation to the number of involved lymph nodes.

## Material and methods

A prospective study of sentinel lymph node biopsy in melanoma patients was started in April 1995 in the Department of Soft Tissue/Bone Sarcomas of the Maria Skłodowska-Curie Memorial Cancer Centre – Institute of Oncology (MSCMCCIO). All patients enrolled into the study met the following criteria:

- primary sites of cutaneous melanomas were: the trunk or the extremities,
- primary focus was present or patients underwent previous excisional biopsy,
- patients were feasible for general anaesthesia,
- N0 regional lymph nodes (nonpalpable).

Criteria of exclusion from SLN biopsy were as follows:

- presence of mucosal melanomas;
- melanomas which were previously radically, widely excised or a skin graft was applied or skin flap was used during the previous operation;
- patients were not feasible for general anaesthesia;
- pregnant women.

Each patient provided informed consent in accordance with institutional regulatory requirements. Between April 1995 and July 2001 the sentinel lymph node biopsies were performed in 704 patients (801 lymphatic basins). 412 patients treated in the period between April 1995 – September 1999 were included in the analysis. In these 412 patients 449 sentinel lymph node biopsies in regional lymphatic basins were performed. In a majority (91%, 376/412) of patients only one regional lymphatic basin was identified, in 35 patients two lymphatic basins were identified and in 1 patients three lymphatic basins were identified. The patients' characteristics are summarized in Table I.

Table I. Patient characteristics

No of patients	412
male/female	130/282
Age (median)	
male/female	50 yrs/48 yrs
Melanoma thickness:	
Breslow (mm)	
average/median	4.86/4.00 mm
Clark (median)	III°
SLN biopsy technique:	
vital blue staining only	169 patients
blue dye + intraoperative lymphoscintigraphy	243 patients
Operated regional lymph node basins:	in total
one	449 basins
two	376 patients
three	35 patients
	1 patient
No of patients:	
without micrometastases	300
with micrometastases to SLNs	100
lack of clinical data	12

During the 53 months of performing the biopsy the details of the SLN biopsy technique were modified due to surgical and organisational experience. The initial 169 patients underwent vital blue-dye lymphatic mapping only [Patent Blau V®]. In the subsequent 243 patients intraoperative lymphatic mapping was carried out with a combination of dying and intraoperative lymphoscintigraphy with hand-held gamma-detecting probe [Neoprobe 1000®, Neoprobe Corp., Dublin, OH, USA or Navigator®, RMD Watertown, MA, USA].

In all cases preoperative lymphoscintigraphy was performed one day before SLN biopsy in the Department of Nuclear Medicine, MSCMCCIO [Head: Prof. I. Kozłowicz-Gudzińska]. For lymphoscintigraphy sulphur colloid was used (particle size less than 220nm, radioisotope labelling – <sup>99m</sup>Tc, activity <44.4 Bq = 1.2 mCi). On the day of the biopsy, after the induction of anaesthesia, the place with the highest residual radioactivity was localized on the skin of the regional lymphatic basin with the use of the gamma probe and marked. Then an intradermal injection of 1-2 mL of 2.5% Patent Blau V using tuberculin syringes was administered around the primary lesion or on both sides of the scar after excisional biopsy. After 10-15 minutes an incision over the marked lymph node basin was made, and after careful exploration of the tissue with the help of a hand-held gamma-probe the detection of the stained lymphatic vessels and blue stained SLN or SLNs was performed. SLNs were considered as those which demonstrated distinctive staining or 15 to 20 fold more radioactivity than background tissue or both features. After excision of the SLN the biopsy was finished by routine careful hemostasis and primary wound closure without the drainage.

SLN specimens were evaluated histologically in the Department of Pathology [Head: Prof. W. Olszewski]. In our centre the intraoperative examination of the frozen sections was not performed. During routine pathologic examination SLNs were cut in half along the major axis and stained with Hematoxylin and Eosin. Paraffin embedded specimens were examined in light microscopy (x40; x200).

Further surgical therapy was dictated by the results of the histopathological analysis. In case of positive SLN regional lymphatic basin lymphadenectomy was performed two weeks later.

The hospital system "Oncosys" and data base "Sarcoma" were applied for data collection.

Statistical analysis was performed using Statistica software [Statsoft®]. For survival analysis the Kaplan-Meier method in

combination with the log-rank test was used for univariate analysis. Differences were considered statistically significant if  $p$ -values were  $<0.05$ .

Follow-up time for survivors was 1-73 months, median: 21 months.

## Results

Among the 412 operated patients, 12 patients (2.9%) were excluded from statistical computations due to the lack of fully recorded data or failure in the detection of the SLN in the regional lymph node basin. Therefore 400 patients were included in the analysis. Metastases were identified in 100 patients (25%), and in 300 patients (75%) the sentinel lymph nodes were tumour cell-free.

The estimated 3-year disease free survival (DFS) rate in the group of patients without metastases to SLNs was 79.3%, as compared to 41% DFS in the group of patients with SLNs metastases [ $p<0.0001$ ] [Figure 1A]. Similarly there were significant differences in overall survival (OS) between the group of patients without metastases to SLNs and the group of patients with nodal involvement: 85% vs. 57% respectively [ $p<0.0001$ ] [Figure 1B].

An analysis of the sites of the first recurrences in 300 patients without SLN metastases demonstrated that in 14 cases symptoms of isolated regional lymph node metastases were found within a previously mapped lymph node basin (false-negative results = 4.6%; Table II). All these patients underwent lymphadenectomy, 10 of them are still alive without evidence of the disease. In 10 consecutive patients the first symptoms of recurrence were local recurrence and/or unresectable "in-transit" metastases on the extremities. Remaining 38 patients (62%) were affected by multifocal, hematogenous metastases to distant organs during follow-up.

Detailed analysis of the group of 100 patients with regional lymph node metastases identified by means of SLN biopsy demonstrated that in 61% of them SLNs were the only focus of metastases. The final pathological examination of specimens after lymphadenectomy revealed that the remaining lymph nodes were metastases-free. In 39% of cases the patients showed additional involved lymph nodes besides SLNs. Estimated 3-year DFS in the group of patients with metastasis to 1 lymph node was significantly better than in the group of patients with

**Table II. Analysis of the first site of relapse in patients without metastases in SLN (300 patients)**

First relapse	No of patients (percentage)
Isolated regional lymph node recurrences (after negative SLN biopsy)	14 (4.6%)
Local recurrences and/or "in-transit" metastases and/or extraregional lymph node metastases	10 (3.3%)
Distant organ metastases (hematogenous)	38 (12.7%)
in total:	62 (20.6%)

metastases to 2 or more lymph nodes ( $p<0.02$ ): 52% vs. 28%, respectively [Figure 2A]. Patients with 1 positive lymph node demonstrated 67% 3-year OS, as compared to 33% 3-year OS in patients with metastases to 2 or more lymph nodes ( $p=0.006$ ) [Figure 2B].

## Discussion

Sentinel lymph node biopsy is a new technique which allows to identify occult regional node metastases in patients with clinical N0 (stage I and II) melanoma. Using intraoperative radiolymphoscintigraphy (radioguided surgery) and vital dye lymphatic mapping the identification of the SLN is possible in over 95% of the sampled nodal basins [7, 8, 9, 10]. Preoperative lymphoscintigraphy is necessary to identify the lymphatic basis/basins draining and helps to mark the position of the SLN. It was estimated that as many as approximately 25% of patients have more than one SLN [7, 10]. According to our experience (consisting with the opinions of other authors) SLN biopsy is a very safe procedure of low aggression without concomitant morbidity.

It seems that the status of regional lymph nodes as evaluated by means of SLN biopsy is the most important predictive factor for patients with cutaneous melanoma. The prognosis of patients with tumor-free SLNs is very good with 85% 3-year OS and about 80% 3-year DFS. Most of the recurrences observed in this group of patients (38/62–62%) are hematogenous and multifocal. The introduction of SLN biopsy to oncological arma-

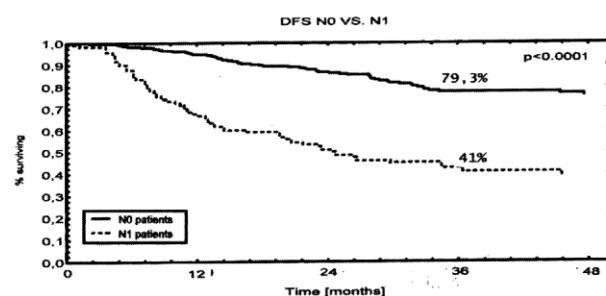


Figure 1a

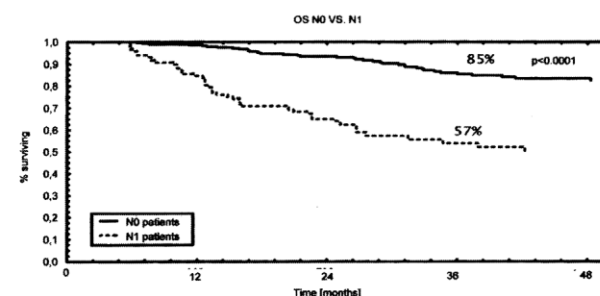


Figure 1b

Figure 1. 3-year disease free survival (A) and 3-year overall survival (B) in group of patients with metastases to SLNs (N1 patients) and without metastases (N0 patients)

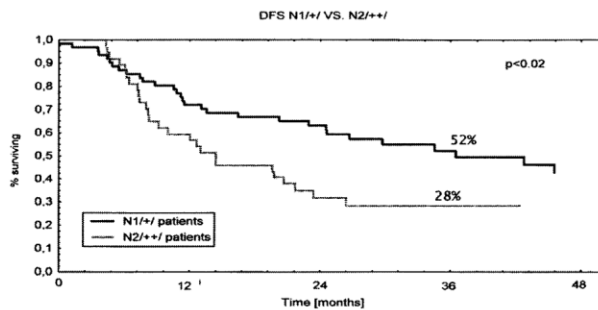


Figure 2a

Figure 2. 3-year disease free survival (a) and 3-year overall survival (b) in group of patients with metastases to 1 regional lymph node (N1/+ patients) and with metastases to 2 or more regional lymph nodes (N2/+ patients)

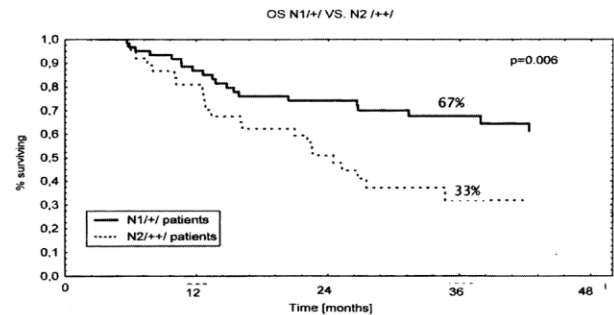


Figure 2b

mentarium can help to assess the group of patients with good prognosis, who unmeaningfully benefit from any adjuvant therapy independently from the primary tumor thickness. Speculations as to whether it is worth to perform elective lymphadenectomy on the basis of indications arising from by primary tumour parameters are aimless.

The results of the present study suggest that the introduction of SLN biopsy into clinical practice must bring on changes within the actual TNM system AJCC-UICC [12]. First proposals concerning a new staging system have been already published [13, 14]. 80% 3-year DFS rate in patients without metastases to SLNs impairs prognostic significance of primary tumor thickness according to Breslow or Clark. The differences in survival for patients with stage I or II melanoma including the 4 levels of primary tumour invasion (pT1, pT2, pT3, pT4) are comprised within the limits of 20%. Moreover, patients with clinically occult micrometastases to SLNs (stage I or II) "migrate" to clinical stage III. On the other hand the dichotomization of the clinical stage III into two groups basing upon the diameter of involved lymph nodes (<3 cm and >3 cm) is not useful, if it is possible to identify the micrometastases in clinically uninvolved regional lymph nodes by means of SLN biopsy.

We have demonstrated that the presence of tumour cell spread to regional lymph nodes, even microscopically observed, significantly impairs prognosis (3-year DFS: 41%). The most important feature is the number of involved nodes and not their size. 3-year OS in patients with metastases to 1 lymph node, as compared to 3-year OS in patients with metastases to 2 or more lymph nodes, is significantly better: 67% vs. 33% ( $p=0.006$ ). The results of survival in this last subgroup suggest that patients with more than 1 metastasis to regional lymph nodes have a high risk of systemic metastases, even in subclinical cases. The detection of early lymph node involvement may improve the benefit from adjuvant therapy in stage III melanoma patients. Current trials will further estimate the survival advantages of SLN biopsy in patients with early-stage melanoma.

In the analysed group of 300 patients with uninvolved SLNs we observed 4.6% of false negative results (14/300). It is necessary to stress that in our institution we

only used standard pathological examination in light microscopy of two sections after hematoxylin and eosin staining. Special immunohistochemical analysis [HMB-45, S-100] and serial sectioning in negative results may improve nodal micrometastases detection [2, 11]. However, it does not seem useful to apply molecular methods [RT-PCR] for the detection of occult metastases, due to the distinctive number of false-positive results [15].

It appears that in the nearest future SLN biopsy will become a standard procedure in the management of clinical stage I and II cutaneous melanoma (clinically uninvolved lymph nodes). There are some principal conditions which this procedure has to fulfill in order to be considered successful and reliable. SLN biopsy should be performed only in those oncological centres, in which interdisciplinary cooperation between surgeons, nuclear medicine specialists and pathologists may be possible. SLN biopsy results may be the basis for further clinical decisions only if the SLN detection rate is over 85% for axillary basins and more than 95% for inguinal basins. Short training in well experienced centres is very helpful. It should be considered good practice that the first 20 SLN biopsies are followed by a synchronic elective lymphadenectomy. This will help to confirm whether the SLN technique is performed properly. Last but not least, SLN biopsy must be performed by surgical teams which are capable of performing subsequent lymphadenectomy, because the lymph node involvement is found in, approximately, 25% of patients, being an indication for radical excision of regional lymph nodes.

## Conclusions

Sentinel lymph node biopsy is a valuable and effective diagnostic method in cutaneous melanoma patients. However it must be performed by experienced multidisciplinary teams. This technique allows to assess the patients with good prognosis and to evaluate which patients have a high risk of organ metastases.

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