Prognostic factors of appearing micrometastases in sentinel lymph nodes in skin melanoma

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Summary

For many years, the search of demographic, clinical and histological factors that have predictive value for the appearence melanoma micrometastases in sentinel lymph nodes (SLN) is conducted. The most significant of them are the tumor thickness by Breslow more than 1.0 mm, the level of invasion by Clark IV-V and ulceration of the primary tumor. In addition, some studies have shown that the mitotic rate > 0, the absence of tumor-infiltrating lymphocytes, male sex, the regression of primary tumor also increase the risk of SLN metastasis. Prognostic factors influencing on the incidence of micrometastases in SLN are studied on the example of 156 patients with primary melanoma who were treated in National Cancer Institute. Such factors as the localization of the primary tumor on the body and tumor thickness by Breslow more than 4 mm are significant. The optimal threshold value of tumor thickness by Breslow for prediction SLN metastasis is 2.5 mm. This threshold is characterized of maximum sensitivity (86.2%) and specificity (68.5%). Younger age of patients is also associated with increased incidence of micrometastases, but these data are not statistically significant.

Keywords: skin melanoma, sentinel lymph nodes, micrometastases, prognostic factors.

Introduction

Nowadays sentinel lymph node biopsy (SLN) is the standard diagnostic procedure for skin melanoma. A presence or an absence of micrometastases in SLN is a reliable predictor: according to different authors 5-year overall survival in

patients with micrometastases is 64-67% and in patients without metastatic disease - 86-92% [1].

The indications for SLN biopsy in the most clinics in the world are primary skin melanoma with tumor thickness by Breslow 1.0 mm or more, and/or level of invasion by Clark IV-V, and/or ulceration of the primary tumor without clinical signs of metastases in regional lymph nodes. In this case micrometastases in SLN are defined in 13-30% of patients [5,12].

For many years, the search of demographic, clinical and histological factors that have predictive value for the appearence melanoma micrometastases in SLN is conducted. It will more exactly predict the outcome of disease and in future may individualize treatment for each patient.

Almost in all studies that searched prognostic factors for skin melanoma it has been shown that with the tumor thickness by Breslow increase the likelihood of metastase in SLN (Table 1). It should be noted that according to the literature in "thin" melanomas frequency of clinically defined metastases in regional lymph nodes in the delayed period is higher than micrometastases after SLN biopsy. So when tumor thickness less than 0.75 mm these figures are 2.3% and 0.94% respectively, and when it when tumor from 0.75 mm to 1.00 mm these figures are 8.6% and 5.5% [6].

Table 1. Detection rate of micrometastases in SLN depending on the thickness of primary tumor

Tumor thikness by Breslow, mm	Detection rate of micrometastases in SLN, %
$\leq$1.0	0.9-5.5
1.01 – 2.0	12-19.7
2.01 – 4.0	28-33.2
$\geq$ 4.01	28-44

The second most important prognostic factor is ulceration of the primary tumor. In particular, patients with ulceration of the primary tumor micrometastases in SLN are detected in 30-35% [4,9].

The level of invation by Clark for many years was considered as an independent prognostic factor. Number of studies show that among patients with the level of invasion by Clark IV-V SLN lesion occurs in 20-25% of cases.

It's interesting that increase of patients' age is associated with reduction of metastases in regional lymph nodes: among patients older than 50 years the frequency of metastases in regional lymph nodes, including SLN, significantly reduced compared to younger patients. This increases the risk of distant metastasis and reduced overall survival [3,11].

In addition, in some publications, was shown that the mitotic rate > 0, the absence of tumor-infiltrating lymphocytes, male sex, the regression of the primary tumor also increase the risk of SLN metastasies [7, 13,14,15].

However, these studies are often contradictory. For example, S.C.Paek examined data 910 patients who underwent SLN biopsy believes that the prognostic significance have the tumor thickness by Breslow, young age, angiolymphatic invasion, mitotic rate, tumor location in the trunk and lower extremities [10]. At the same time L.L.Kruper, based on data from 628 patients, says that the prognostic significance have tumor thickness by Breslow, tumor-infiltrating lymphocytes and mitotic rate [8].

Numerous studies in this area have not led to a change in the indications for SLN biopsy. According to A.Cadili and K.Dabbs it connect with their conflicting results. It's caused by lack of standardized histological examination of SLN, which leads to the variability of measurements and reporting in various clinics. In particular, due to the tumor thickness by Breslow is the most objective and accurate procedure than others this figure only referred to the prognostic factors in virtually all studies. In this regard, the authors consider that it is necessary to study the relative prognostic factors in the clinic where SLN biopsy is a routine procedure [2].

The purpose of the study

Explore the prognostic factors influencing on the incidence of micrometastases in SLN on the example of 156 patients who were underwent SLN biopsy in the Department of skin and soft tissues tumors in the National Cancer Institute.

Materials and Methods

The study included 156 patients with clinical signs of melanoma or after prior biopsy of the tumor localized on the trunk and extremities, without clinical and radiological data of the regional metastases and advanced disease. The patients were divided into 2 groups: one of them had micrometastases in SLN (SLN +) and other hadn't (SLN-). We have studied such characteristics as sex, age, location of primary tumor and SLN, tumor thickness by Breslow and presence of ulceration.

Technology SLN biopsy

For SLN identifying was used the method of radionuclide detection. On the eve of the operation (24 hours before surgery) lymphoscintigraphy was performed for identification of lymph node region and approximate location of SLN in it. As lymphotropic radiopharmaceutical used colloids «Nanocis» or «Nanocoll» radiolabeled with 75-100 MBq of ^{99m}Tc, which was injected around the primary tumor or scar poslebiopsiynogo intradermally. Lymphoscintigraphy was performed immediately after administration of the isotope and 2 hours later in the gamma camera or single photon emission computed tomography gamma (OFET). Dynamic lymphoscintigraphy helped identify lymph nodes in which provide a direct lymphatic drainage from the primary tumor and determine cases where lymph takes several regions. SLN location was marked on the patient's skin with a marker. It should be noted that, due to the relaxation and the changing position of the patient during surgery markings do not always coincide with the location of SLN. Therefore, before the operation once more refined localization of SLN using a handheld gamma counter.

The next day during the operation firstly performed wide excision of the primary tumor or biopsy scar and then removed SLN. During the surgery searching of SLN was performed using a handheld gamma scanner EuroProbe. After skin incision lymph nodes with high accumulation of radionuclide were determined using portable gamma counter and removed. When we identified SLN we were focused on a intensity of the accumulation of radiopharmaceuticals, which in SLN should be greater than those of the neighboring non-sentinel lymph nodes in vivo in 3 times, and ex vivo in 10 times. After removal of the SLN wound again was studied gamma-counter.

The sections were removed SLN made in steps of 2 mm, were investigated after staining with hematoxylin-eosin, immunohistochemistry was not conducted.

Statistical analysis

Results

Lymphoscintigraphy was successful in 156 of 162 patients (96.3%). In 6 patients SLN weren't detected for the following reasons. 2 patients had significant inflammation around the primary tumor, in 3 people melanoma was located close to the area of regional limph node region (axillary and inguinal region), in 1 patient lymphoscintigraphy was unhelpful for unknown reasons.

After lymphoscintigraphy 95/156 patients had 1 SLN (60.9%), 57/156 patients had 2 SLN (36.5%) and 4/156 patients had 3 lymph nodes (2.6%). On average one patient had 1.4 SLN.

Micrometastases in SLN were detected in 29 patients (18.6%). Among 156 patients with verified melanoma in 24 patients (15.4%) set the stage IA disease. The distribution of patients by stage is shown in Table 2.

Table 2. Distribution of patients by stage

Stage	Number of patients	%	Number of patients in groups
IA (T1aN0M0)	24	15.4	

IB (T1b-2aN0M0)	38	24.4	127 (SLN-)
IIA (T2b-3aN0M0)	28	17.9	
IIB (T3b-4aN0M0)	29	18.6	
IIC (T4bN0M0)	8	5.1	
IIIA (T1a-4aN1a-2aM0)	16	10.3	29 (SLN+)
IIIB (T1b-4bN1a-2aM0)	13	8.3	
All patients	156	100	156

By the studying influence of the sex on the incidence of metastases in SLN was noted about the same proportion of men and women in both groups, with a slight predominance of women. The average age of patients with metastases in the SLN was slightly below the average age in the group with negative nodes, but these differences were not statistically significant (Table 3).

Table 3. The influence of gender and age on the incidence of metastases in SLN

Characteristic	**SLN- (127)**	**SLN+ (29)**	**All patients (156)**	**p**
Gender				
Men	52 (40.9 %)	12 (41.4 %)	64 (41.0 %)	$\chi^{2=}0.002$ p=0.965
Women	75 (59.1 %)	17 (58.6 %)	92 (59.0 %)	
Mean age				
Men (x±σ)	53.9 ±13.0	52.6 ±14.3	53.7±13.2	0.74
Women (x±σ)	48.5 ± 4.1	43.2 ±15.7	47.5±5.8	0.18

With the localization of melanoma on the trunk SLN lesions statistically significant increased; when melanom localized in the extremities these differences were not significant. Also, we have not observed the influence of SLN localization. In both groups of 2/3 of the SLN were localized in the axillary region. Number of identified SLN didn't influence on their lesions (Table 4).

Table 4. The influence of localization of primary tumor and number of SLN on the incidence of metastases in SLN

Characteristic	**SLN- (127)**	**SLN+ (29)**	**All patients (156)**	**p**
Localization of primary tumor				
Upper extremities	25 (19.7 %)	3 (10.3 %)	28 (18.0 %)	0.16
Lower extremities	34 (26.8 %)	5 (17.3 %)	39 (25.0 %)	0.23
Trunk	69 (53.5 %)	21 (72.4 %)	89 (57.0 %)	0.046
Localization of SLN				
Supraclavicular region	3 (2.4 %)	0 (0.0 %)	3(1.9 %)	0.08
Axillary region	77 (60.6 %)	19 (65.5%)	96 (61.5 %)	0.62
Ilio-inguinal region	47 (37.0 %)	10 (34.5 %)	57 (36.6 %)	0.79
Number of identified SLN				

1	76 (59.8 %)	19 (65.5 %)	95 (60.9 %)	0.56
2	47 (37.0 %)	10 (34.5 %)	57 (36.5 %)	0.79
3	4 (3.2 %)	0 (0.0 %)	4 (2.6 %)	0.043

An effect of the tumor thickness on the frequency of SLN lesions is statistically significant. The median of thickness by Breslow in the group with negative nodes was 1.9 mm (interquartile range 1.1-3.0 mm), and in the group with positive nodes - 3.8 mm (interquartile range 3.0-6.0 mm) (Figure 1).

We have split each of the groups into 4 subgroups according to the thickness of primary tumor and revealed a statistically significant reduction in the frequency of SLN lesions with a thickness less than 1 mm and from 1 to 2 mm. With a thickness from 2.0 to 4.0 mm a trend toward increased frequency of metastases was showed, and with a thickness more than 4 mm, these differences become significant.

There was not statistically significant effect of ulcerations in the subgroups in both groups (Table 5).

Table 5. The influence of tumor thickness by Breslow and ulceration on the incidence of metastases in SLN

Characteristic	**SLN-** **(127)**	**SLN+** **(29)**	**All patients** **(156)**	**p**
Tumor thikness by Breslow, mm				
≤ 1	30 (23.6 %)	1 (3.4 %)	31 (19.9 %)	0.0001
1.01 - 2.0	40 (31.5 %)	2 (6.9 %)	42 (26.9 %)	0.0001
2.01 - 4.0	42 (33.1 %)	14 (48.3 %)	56 (35.9 %)	0.14
≥ 4.01	15 (11.8 %)	12 (41.4 %)	27 (17.3 %)	0.002

Mean thikness	2.28 ± 1.1 мм	4.1 ± 1.6 мм	2.6±1.2	< 0.0001
The frequency of ulceration depends on the thickness of the primary tumor by Breslow, mm				
< 1	4/30 (13.3 %)	0/1 (0.0 %)	4/31 (12.9 %)	0.69
1.01 - 2.0	11/40 (27.5 %)	0/2 (0.0 %)	11/42 (24.4 %)	0.39
2.01 - 4.0	22/42 (52.4 %)	7/14 (50.0 %)	29/56 (51.8%)	0.87
> 4.01	8/15 (53.3 %)	5/12 (41.7 %)	13/2 (48.1 %)	0.55
All	45/127 (35.4 %)	12/29 (53.3 %)	57/156 (36.5 %)	0.54

Conclusions

Thus, our study revealed that on the incidence of metastases in SLN influence such factors as the localization of primary tumor in the body and tumor thickness by Breslow more than 4 mm.

The younger age of patients and tumor thickness from 2 to 4 mm is also associated with increasing incidence of metastases in SLN but these data are not statistically significant.

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Figure 1. The thickness of primary tumor.

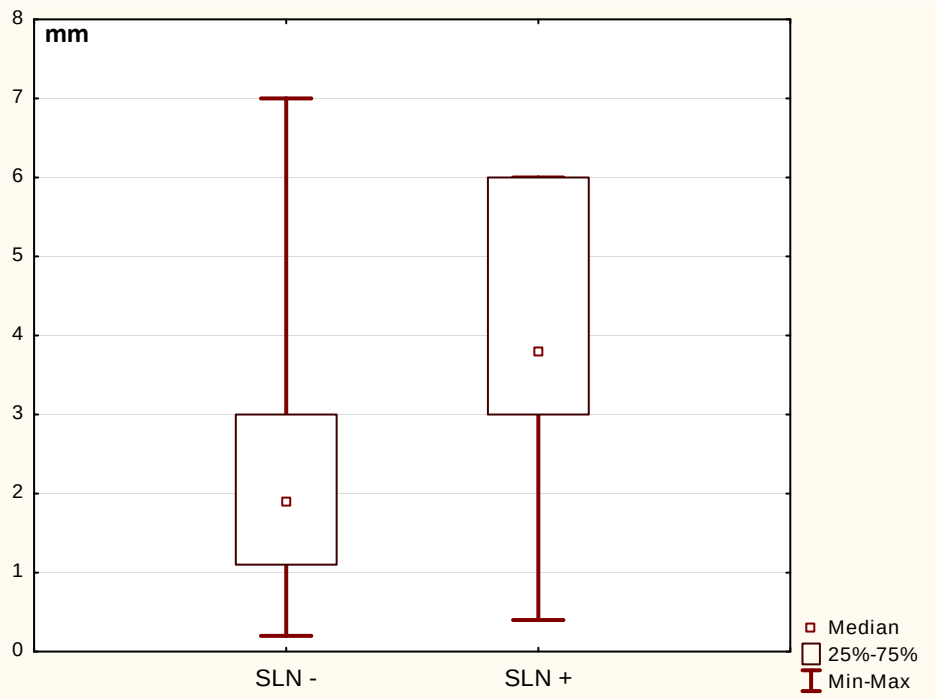

Figure 2. Dependence of the frequency of SLN lesions on tumor thickness by Breslow