Morphological characteristics of the primary tumor and micrometastases in sentinel lymph nodes as a predictor of melanoma progression

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Summary

Presence or absence of micrometastases in sentinel lymph nodes (SLN) is a reliable prognostic factor. SLN involvement in the neoplastic process may vary from single melanoma cell to complete replacement of lymph node. Thereby several classification systems were proposed for more accurate prognosis and determination criteria for selecting treatment strategies. We studied morphological characteristics of primary tumor and micrometastases affecting on the disease course, which could serve as a criteria for selecting further treatment strategy on the example of 195 melanoma patients with who were treated at the National Cancer Institute. The most significant unfavorable factors for the further progression of disease are thickness of primary tumor byBreslow more than 2.0 mm, ulceration, extensive (more than 5 mm in diameter) and extracapsular spread of tumor.

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

Introduction

Presence of metastases in sentinel lymph nodes (SLN) is an important prognostic factor, that was confirmed by the study MSLT-I (Multicenter Selective Lymphadenectomy Trial): 5-year survival of patients with SLN metastases is 72.3$\pm$4.6 % and SLN without metastases is 90.2$\pm$1.3 % ($p<0.001$) [1]. In this regard since 2002 the classification of melanoma American Joint Committee on Cancer (AJCC) introduced a definition of regional micrometastases (indicated by letter a) and macrometastases (indicated by letter b). According to the definition of AJCC, macrometastases are clinically and/or radiologically defined metastases in

regional lymph nodes, confirmed histologically after therapeutic lymphadenectomy and micrometastases are diagnosed after SLN biopsy or elective regional lymph node dissection. The presence of single micrometastases is defined as N1a, 2 or 3 nodes as N2a [2]. It was noted in subsequent versions of AJCC classification in 2009 that the presence of micrometastases in lymph nodes can be checked not only histologicaly but by immunohistochemistry [3].

Despite the fact that as a result of MSLT-I the importance of SLN biopsy for staging and prognosis of melanoma was proved, the question about its independent medical significance and performing regional lymphadenectomy after detection of micrometastases is open. Threby in 2004 MSLT- II study started, during which melanoma patients with micrometastases identified by histology, immunohistochemistry or PCR are randomized into 2 groups. The first group of patients undergoes immediate lymphadenectomy. The second group is observed using US of regional lymph nodes and performing of delayed regional lymph node dissection in case of developing clinical metastases. In this study will be included 4,200 patients by December 2017 [4].

Nowdays in the most countries the current standard of care is the complete removal of lymph nodes in the regional collector , in which the tumor micrometastases were detected, despite the fact that only 10-15% of patients with positive SLN after complete lymph node dissection revealed metastatic lesion. However, SLN involvement in the neoplastic process may be different from the presence of single melanoma cell to the full replacement of the lymph node. In this regard, several classification systems were proposed for more accurate prognosis and determination of criteria for selecting treatment strategies.

In 2004 D. Dewar published results of study that included 146 patients with melanoma micrometastasis in SLN. Histological examination of both SLN and nonSLN obtained after the subsequent regional lymphadenectomy was made. Melanoma metastases in each case were assessed in accordance with their microanatomical localization within lymph node: subcapsular, combined (subcapsular and parenchymal), parenchymal, multifocal multiple, extensive

involvement of lymph nodes larger than 5 mm or extracapsular spread of tumor. The author found a correlation between microanatomical localization of metastases in the SLN and the frequency of lesions in nonSLN. In particular, 38 (26%) patients had subcapsular micrometastases in SLN, while in any cases weren't detected metastases nonSLN. For other microanatomical localizations defeat of nonSLN observed in average 22.2 % of the cases: the combined localization in 6 (11.1%) patients, the parenchymal in 3 (18.8 %) patients, multifocal multiple localisation in 7 (36.8 %), the extensive lymph node involvement or extracapsular extension of tumor in 8 (42.1%). In author's opinion, it possibly to refrain from doing regional lymphadenectomy in patients with subcapsular micrometastasis [5].

H. Starz proposed so-called micromorfometric S- classification, which based on the depth of invasion of tumor cells in SLN that measured using an ocular micrometer and defined as the distance between the inner layer of lymph node's capsule and melanoma cells in node's parenchyma. Author identified 4 stages: S0 defined as histological absence of metastases, SI with depth of invasion <0.3 mm; SII with depth of invasion - 0.3-1 mm; SIII with depth of invasion > 1.0 mm. At stages SI and SII defeat nonSLN was detected in 15 % of patients. 5 year survival in these stages does not differ from the survival of patients with negative SLN. In stage SIII metastases in nonSLN are found in 50% of patients, and almost all of them are progressing over 5 years follow-up [6] .

A. van Akkoi, after morphological examination of 388 melanoma patients with SLN micrometastasis proposed to use the maximum size of the largest SLN micrometastases as a prognostic factor (Rotterdam classification). Dividing patients depending on the size of micrometastasis in 3 groups (less than 1.0 mm, from 0.1 mm to 1.0 mm and more than 1.0 mm), it is studied their 5-year overall survival rate, which was 91 % , 61 %, and 51 % respectively ($p < 0.001$). Author proposed to asses SLN micrometastases less than 0.1 mm as submicrometastasis or "biologically false positive» metastases. Patients with submicrometastasis may be equated to SLN-negativepatients and do not require implementation of regional lymph node dissection [7].

A. Meier in the so-called Hannover scoring system used one main parameter (the maximum size of the largest micrometastases (<0.1 mm, > 0.1 mm)) and 2 extra (depth of invasion of tumor cells in the lymph node parenchyma (<2 mm, > 2 mm) and involvement of lymph node capsule (presence or absence)). The maximum size of micrometastases <0.1 mm and the absence of additional parameters assessed as favorable prognosis, similar to negative SLN. The maximum size of micrometastases > 0.1 mm and the presence of two additional parameters assessed as unfavorable prognosis [8].

In 2009 I.Van der Ploeg published the comparative assessment of the three histological subclassification of SLN. After studying 116 patients with SLN metastases has been proven that the defeat of nonSLN the best correlates with the depth of tumor invasion SLN and the maximum size of micrometastases 0.4 mm. Overall 5 -year survival rate depends on the depth of invasion of tumor cells in SLE : the SI it is 92 %, with SII - 83 %, with SIII - 68%. According to the authorA, with depth of invasion of tumor cells less than 0.4 mm should refrain from carrying out lymph node dissection [9].

Materials and Methods

SLN biopsy has used in the research department of tumors of the skin and soft tissuesf the National Cancer Institute since 2009 and was performed 195 melanoma patients.

Radionuclide method is used for the detection of SLN. Before treatment excision biopsy of skin tumor is performed. After verification of the diagnosis patient examined with X-rays of the chest, US of the abdomen and lymph nodes. Lymphoscintigraphy is performed before surgery is to identify approximate location of SLN. We use colloids «Nanocis» or «Nanoalbumon», radiolabeled 75-100 MBq 99mTc activity that are introduced around scar intradermally. Lymphoscintigraphy is performed immediately after administration of the cooloid and after 2 hours on a gamma camera or SPECT. SLN location is marked on the patient's skin with a marker. The next day during surgery we perform firstly wide excision of scar and then remove SLN.

Removed SLN are studied after fixation in paraffin blocks, as the use of fresh-frozen slices accompanied by a large number of false-negative results and the part of the material may be lost during the procedure of freezing. The slices were removed SLE performed with a slice thickness of 2 mm, are investigated after staining by hematoxylin-eosin.

Positive SLN are evaluated according to microanatomical localization by D. Dewar, ie devided subcapsular, combined (subcapsular and parenchymal), parenchymal, multifocal localization of micrometastases, as well as extensive involvement of lymph node more than 5 mm or extracapsular spread of tumor.

In addition, we studied the degree of SLN damage accoding to our own method under which metastases allocated as single tumor cells, clusters of tumor cells, the extensive involvement of lymph nodes larger than 5 mm and extracapsular spread of the tumor.

Statistical processing of the data used methods for quantitative descriptive statistics (mean, median, standard deviation) and qualitative (n, %) signs. For comparative analysis of the distributions characteristics were used criterion $\chi 2$ and $\chi 2$ adjusted Yats when number of observations in groups less than 5. Differences were evaluated as statistically significant when $p < 0.05$.

Results

SLN biopsy was performed to 195 melanoma patients (mean age 50.2$\pm$13.7 years, 115 women, 80 men, the average thickness of the primary tumor is 2.6$\pm$1.2 mm, 67 (34.3%) primary tumors have ulceration). In 34 patients (17.4%) stage IA was set.

SLN metastases were identified in 35 patients, accounting for 17.9% (mean age 50.0$\pm$13.4 years, 20 women, 15 men, the average thickness of the primary tumor is3.8$\pm$1.7 mm, 14 (40.0 %) primary tumors have superficial ulceration). Among patients with positive SLN subcapsular localization of metastasis was detected in 3 (8.6%) patients, parenchymal in 6 (17.1%), combined (subcapsular and

parenchymal) in 3 (8.6%), multifocal multiple in 14 (40.0 %), extensive involvement of lymph node or extracapsular spread of tumor in 9 (25.7%) patients.

Metastases as single tumor cells were detected in 9 (25.7 %) patients, as clusters of tumor cells in 17 (48.6%), extensive involvement of lymph in 5 (14.3%) and extracapsular spread in 4 (11.4%) patients.

During the follow-up period (median 38 months) in 10 (28.6 %) patients with SLN metastases was identified further progression of the disease: in 2 (5.7 %) patients were found metastases in regional lymph nodes, in 2 patients (5,7 %) in nonregional lymph nodes, in 6 (17.2%) in internal organs.

There were not any cases of disease progression when thickness of primary tumor was less than 2.0 mm. Equal numbers of patients had tumor thickness 2,01-4,0 mm and more than 4,0 mm.

Among the 10 patients who revealed progression of the disease superficial ulceration of the primary tumor was found in 7 cases (70%); of 25 patients without progression this figure was also 7,however it accounting 28 % ($\chi 2=5,25$; $p=0.022$, hazard ratio (HR)-2.5 (95% CI 1,2-5,3)) .

There were not any cases of disease progression among patients with combined and subcapsular localization of metastases. When parenchymal localization was noticed disease progression was found during follow-up period in 1 of 6 patients (16.7 %), when it was multifocal multiple in 3 of 14 (21.4%), when it was extensive involvement of lymph node or extracapsular extension of the tumor in 6 of 9 (66.7 %) patients (Figure 1).

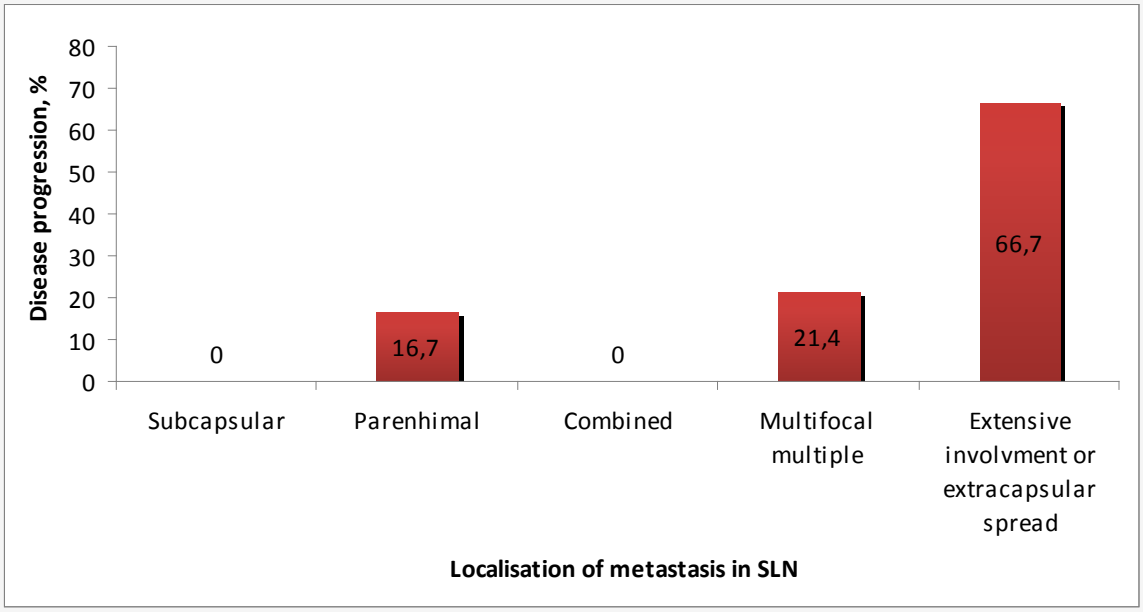

Fig. 1. Frequency of disease progression depend on localisation of metastasis in SLN

So, the maximum frequency of progression is correlated with extensive lymph node involvement or extracapsular extension of the tumor (6/9 (66.7 %)), which significantly exceeds the rate of progression for all other localisations of metastases (4/16 (15.4%)) ($\chi 2$=8,62; p=0.0033; OR 4.3 (95% CI 1,6-11,9)).

Disease progression was observed for metastases as a single tumor cells in 1 of 9 patients (11.1%), for metastases as clusters of tumor cells in 3 of 17 (17.6%), for extensive involvement of lymph nodes in 3of 5 (60.0 %) and extracapsular extension of tumor in 3 of 4 patients (75.0 %) (Fig. 2).

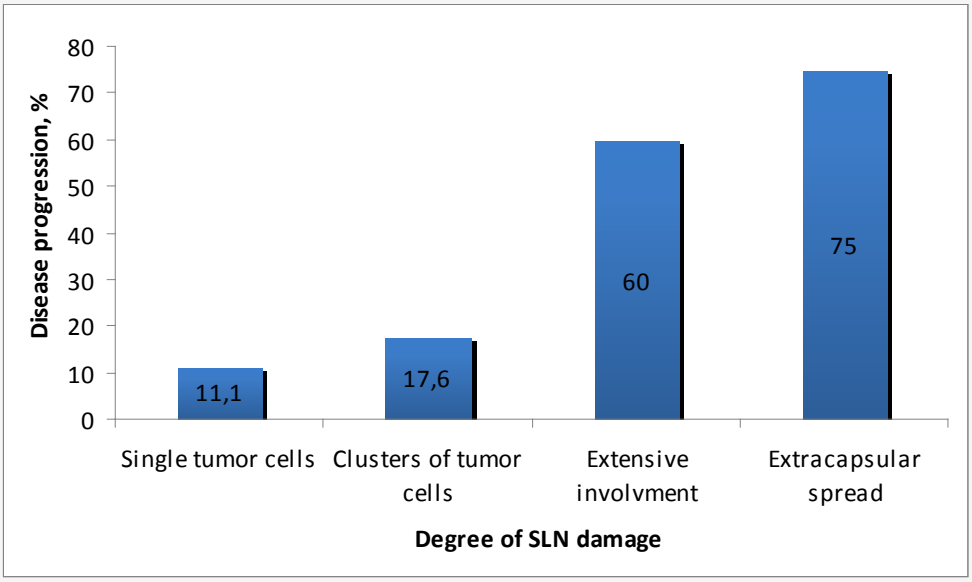

Fig. 2. Frequency of disease progression depend on degree of SLN damage

Disease progression often found when was extensive lymph node involvement ($\chi 2$ =2.6%; p=0.10) and frequency of progressionis increase significantly when extracapsular extension of tumor was found ($\chi 2$=3.96; p=0.043).

We studied the frequency of disease progression depending on the thickness of melanoma by Breslow and microanatomical localization of metastasis and the damage of lymph node (Tables1, 2).

Table 1. Disease progression depend on thickness of primary tumor by Breslow and localisation of metestases in SLN

Thickness of primary tumor by Breslow	Localisation of metestases in SLN					All
	Subcapsular	Parenchymal	Combined (subcapsular and parenchymal)	Multifocal multiple	Extensive involvement of lymph node or extracapsular spread	
< 1,0 mm	-	-	-	-	-	-
1,01-2,0 mm	-	-	-	-	-	-
2,01-4,0 mm	-	1/10	-	1/10	3/10	5/10 (50.0 %)
> 4,01 mm	-	-	-	2/10	3/10	5/10 (50.0 %)
All	-	1/10	-	3/10	6/10	10/10

		(10.0 %)		(30.0 %)	(60 %)	(100.0 %)

Table 2. Disease progression depend on thickness of primary tumor by Breslow and degree of SLN damage

Thickness of primary tumor by Breslow	Degree of SLN damage				All
	Single tumor cells	Clusters of tumor cells	Extensive invoivment	Extracapsular spread	
< 1.0 mm	-	-	-	-	-
1.01-2.0 mmm	-	-	-		-
2,01-4.0 mm	1/10	1/10	1/10	2/10	5/10 (50,0 %)
> 4.01 mm	-	2/10	2/10	1/10	5/10 (50,0 %)
All	1/10 (10.0 %)	3/10 (30.0 %)	3/10 (30.0 %)	3/10 (30.0 %)	10/10 (100.0 %)

The most frequently (in 60% of cases) metastases were detected for extensive involvement in SLN or extracapsular extension of the tumor and the tumor thickness more 2.0 mm.Slightly less (30%) disease with manifested withmultifocal multiple metastases in SLN and similar thickness of skin tumor. Frequency of progression does not increase with increasing of tumor thickness depends on damage of SLN.

Conclusions

Thus, the most significant unfavorable factors for the further disease progression are tumor thickness more than 2.0 mm by Breslow, its surface ulceration, extensive metastases (more than 5 mm) of SLN and extracapsular spread of tumor. Morphological and quantitative characteristics of subclinical SLN in melanoma patients with may determine the future course of disease and serve as a criterion for selecting further treatment strategy.

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