JBUON 2017; 22(5): 1296-1302
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Serum S100B levels correlate with stage, N status, mitotic rate and disease outcome in melanoma patients independent to LDH

Athanasios Karonidis[1], Marina Mantzourani[2], Helen Gogas[2], Dimosthenis Tsoutsos[1]

[1]Department of Plastic Surgery, Microsurgery and Burns Unit, General Hospital of Athens "G. Gennimatas", Athens; [2]First Department of Internal Medicine, National and Kapodistrian Universtity of Athens, General Hospital of Athens "Laiko", Athens, Greece

Summary

Purpose: *S100B protein is currently used as an immunohistochemistry marker to confirm melanoma diagnosis in biopsy specimens. Moreover, accumulating evidence supports its potential use as a tumor biomarker in blood. This study aimed to explore the potential uses of serum S100B protein as a biomarker in melanoma patients.*

Methods: *From 2012 to 2015, 107 sequential patients were diagnosed and treated for melanoma. All patients were tested for serum S100B and lactate dehydrogenase (LDH) at diagnosis and during their regular follow-up. Potential correlations between S100B serum levels and baseline characteristics and its impact on survival were assessed.*

Results: *S100B serum levels were within normal limits in patients with stages I and II, elevated in stage III, and very high in stage IV. In bivariate analysis, serum S100B levels >0.11μg/l and stage IV were the only independent prognostic factors associated with poor survival. Furthermore, S100B >0.5μg/l was associated with stage IV and poor survival. However, there was no significant association with LDH. S100B serum levels were positively correlated with mitotic rate (p=0.003), but only in stage IV patients (p=0.015). In stage III, a statistically significant difference in S100B serum levels were observed between N3, N2 and N1 stages, with higher levels for N2 (p=0.012) and N3 (p=0.009) compared to N1, and no difference between stages N2 and N3 (p=1.000). Also, no correlation was found between the number of primary melanoma lesions and S100B.*

Conclusions: *S100B serum levels reflect tumor load, correlate with response to treatment, might identify patients who are at increased risk of disease relapse, may predict prognosis independent to LDH, and could be used as early biomarkers of tumor recurrence.*

Key words: *LDH, melanoma, mitoses, S100B, tumor marker*

Introduction

S100B protein is a widely applied biomarker used to establish the diagnosis of melanoma in tumor specimens by immunohistochemistry [1,2]. S100B was first identified by Moore in 1965 who extracted a subcellular fraction from bovine collagen 'S100' because the constituents were soluble in 100% saturated ammonium sulphate at neutral pH [3]. In 1980, Gaynor et al. found that S100B protein was overexpressed in human malignant melanoma cells *in vitro* [4]. Since then, S100B has been established as the standard immunohistochemical marker for melanoma. However, the assessment of biomarkers in peripheral blood is simple, safe and cost-effective, and could provide the advantage of serial evaluation of tumor status in patients who are on antineoplastic treatment or follow-up.

S100B is a 21.5 kDa symmetric dimeric protein of neuroectodermal and mesodermal origin that consists of two subunits - isomers α (alpha) and β (beta) in all possible combinations (S100αα, S100ββ, S100αβ) [5-7]. α subunit is coded by 13 different genes located at the long arm of

Correspondence to: Athanasios Karonidis, MD, MBA, MRCS, FEBOPRAS. Department of Plastic Surgery, Microsurgery and Burns Unit, General Hospital of Athens "G. Gennimatas". Sifnou 33, Agia Paraskevi, 15343, Athens, Greece.
Tel : +30 6936798945, Fax: +30 2107709851, E-mail: thanasiskaronidis@hotmail.com
Received: 01/03/2017; Accepted: 18/03/2017

chromosome 1 (1q21), while β subunit is coded by one gene on the long arm of chromosome 21 (21q22.3) [5]. The αα homodimer is widely distributed in the skin (S100A7), keratinocytes, skeletal muscles (S100A13), heart (S100A13, S100A10), kidneys (S100A13), pancreas, ovary and lung (S100A10) [5]. The ββ homodimer is mainly produced by astrocytes, Schwann and Langerhans cells. The αβ heterodimer is found in melanocytes, chondrocytes and glial cells [5].

LDH is found in healthy tissues. Anormal LDH levels are mainly due to cell lysis that often occurs in malignant neoplasms, indicating high turnover of tumor cells, high tumor burden, and necrosis in fast-growing tumors [8]. Accumulating evidence supports the independent prognostic impact and superiority of S100B compared to LDH in melanoma [9,10].

The purpose of this study was to investigate the association of S100B serum levels in patients with melanoma stages I-IV with basic patient and tumor characteristics, treatment response and survival.

Methods

From 2012 to 2015, 107 consecutive melanoma patients (54 males and 53 females), 22 to 84 years old, were included in this study (Table 1). Patients with stages I-III underwent wide local excision and sentinel lymph node biopsy (SLNB) if indicated. Those who had positive SLNB underwent subsequently lymph node dissection. Patients with AJCC [13] stage III received adjuvant interferon while those with stage IV were treated with first-line systemic treatment according to local treatment guidelines. All patients underwent assessment of serum S100B protein and LDH preoperatively and during their regular follow-up. Considering that S100B serum levels might be elevated also in non-malignant conditions, we excluded patients with history of other cancers or any condition associated with brain injury, e.g. stroke, central nervous system trauma, neurodegenerative diseases. Patients were classified based on tumor stage, AJCC nodal status (N) [13], National Comprehensive Cancer Network (NCCN) [11] high risk features, defined as the presence of ulceration, high mitotic rate and lymphovascular invasion (LVI) in the primary tumor. Four patients had 2 primary melanomas and 1 had 3 melanomas (Table 1).

Table 1. Basic patient and tumor characteristics

Characteristics	*Mean*	*Range*
Age, years	59.22	22-84
Mitotic rate	6.16	0-42
Sex	*n*	%
Male	54	50.5
Female	53	49.5
Stage		
I	39	36.4
II	33	30.8
III	26	24.3
IV	9	8.4
N stage of primary melanomas		
1	102	95.3
2	4	3.7
3	1	0.9
Ulceration of primary lesion		
Yes	67	62.6
No	33	30.8
MD	7	6.5
Lymphovascular invasion		
Brisk	31	29.0
Intermediate	41	38.3
Small	20	18.7
No/Absent	5	4.7
MD	10	9.3
Total	107	100.0
N stage in stage III patients		
N1	11	42.3
N2	6	23.1
N3	9	34.6
Total	26	100

Serum S100B – LDH measurements

The mean S100B serum concentration of healthy individuals is 0.05 μg/L [14], levels are not influenced by age or sex [15], while 95% of normal controls have levels ≤ 0.105 μg/L [1,16]. S100B serum levels are slightly elevated in children compared to adults [17]. In this study, serum S100B levels were measured using the commercially available Cobas e411 S100B electrochemiluminescence assay (ECLIA) by Elecsys® S100 (Roche Diagnostics Mannheim, Germany) [1,18], according to the manufacturer's instructions and previously published protocols [8,19]. Levels up to 0.11 μg/L were defined as the upper limit of normal according to the Department of Biochemistry of the General Hospital of Athens "G. Gennimatas". LDH was assessed according to Abbott Diagnostics instructions.

Statistics

We followed the REMARK recommendations [12] to report our findings and used the SPSS 21.0 software to perform statistical analyses. Parametric and non-parametric tests were used to compare categorical and continuous variables. The Bonferroni adjustment test was applied in case of multiple comparisons. Median survival was calculated by Kaplan-Meier method and comparisons were performed by log-rank test. Cox proportional hazard models were used for multivariate analysis of prognostic factors. Overall survival (OS) was defined as the time from the date of histological diagnosis to the date of death from any cause. Alive patients were censored at the last date of follow-up or

contact. Analyses were censored at 4 years. All comparisons were two-tailed and a p-value <0.05 was defined as statistically significant.

Results

Table 2 summarizes the mean, the minimum, maximum and standard deviation of S100B serum levels according to tumor stage. The S100B mean level in stage I was 0.059μg/l, in stage II 0.073μg/l, in stage III 0.125μg/l and in stage IV 0.684μg/l. More patients with stage III/IV had high S100B serum levels (>0.11 μg/l) compared to those with stage I/II (Pearson's x^2 test, p<0.001).

Ulceration and S100B

S100B serum levels were not significantly different between patients with ulcerated and non-ulcerated primary melanomas (Mann-Whitney U test, p=0.121). The results were not different when analyzed for each stage (stage I, p=0.746; stage II, p=0.224; stage III, p=0.512; stage IV, p=0.857).

Mitoses and S100B

S100B serum values were positively correlated with the number of mitoses in primary melanomas (Pearson's correlation, p=0.003). However, this correlation was confirmed only for stage IV (stage I, p=0.767; stage II, p=0.851; stage III, p=0.425; stage IV, p=0.015) with a strong coefficient equal to 0.854 (Figure 1).

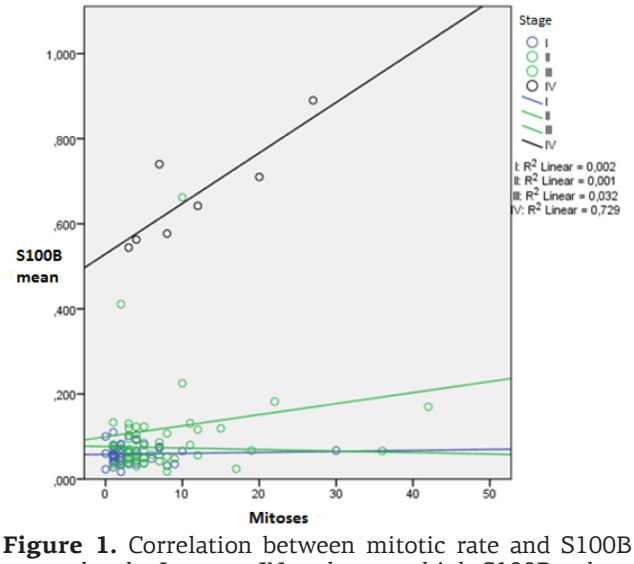


Figure 1. Correlation between mitotic rate and S100B serum levels. In stage IV melanoma, high S100B values correlated significantly with increased number of mitoses (p=0.015).

LVI

No statistically significant association was found between S100B levels and LVI (ANOVA test, p=0.368). This result was also confirmed for stages II (p=0.212) and III (p=0.657), whereas for stages I and IV, no statistical comparison was performed due to absolute value similarity (0 and 1 respectively).

S100B and N status in stage III

Among patients with stage III melanoma, those with N2/N3 disease had significantly higher S100B serum levels compared to those with N1 disease (Kruskal-Wallis test, p=0.002). Furthermore, multiple comparisons test (Figure 2) showed a statistical significant difference in S100B levels between stage N3 vs N1 (p=0.009) and stage N2 vs N1 (p=0.012), whereas there was no difference between stage N2 vs N3 (p=1.000).

S100B and multiple melanomas

No statistically significant difference in S100B serum levels was observed between patients with more than one concurrent primary melanoma and those with one primary melanoma (Mann-Whitney U test, p=0.456). As such there was no statistically significant correlation between the number of melanomas and S100B levels. In cases of multiple melanomas, S100B levels were not associated with the number of primary melanomas but with the stage of the more advanced melanoma.

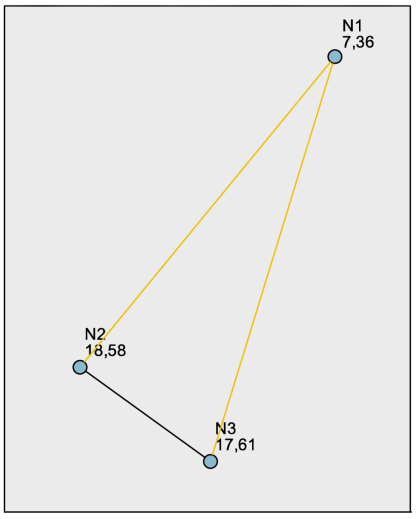
Prognostic significance of S100B serum levels compared to tumor stage

Tumor stage was a strong prognostic factor for OS (p<0.001). OS according to tumor stage is shown in Figure 3. Stage IV patients had by far the worst survival compared to earlier stages, while there was no significant difference in OS

Table 2. Distribution of S100B values according to different stages

Stage	*n*	*Mean*	*95% CI*	*Minimum*	*Maximum*
I	*39*	*0.059*	*0.052 - 0.066*	*0.017*	*0.110*
II	33	0.073	0.049 - 0.096	0.018	0.411
III	26	0.125	0.076 - 0.175	0.024	0.662
IV	9	0.684	0.449 - 0.918	0.177	1.310
Total	107	0.132	0.093 - 0.171	0.017	1.310

CI: confidence interval



Each node shows the sample average rank of Stage N (for Stage III).

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
N1-N3	-10,247	3,437	-2,982	,003	,009
N1-N2	-11,220	3,880	-2,891	,004	,012
N3-N2	,972	4,030	,241	,809	1,000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is ,05.

Figure 2. Pairwise comparisons of S100B serum levels between different N stages in patients with stage III disease.

between stages I,II, and III. Similarly, patients with high S100B serum levels (>0.11 μg/L) had significantly shorter OS compared to those with normal S100B serum levels (p<0.001; Figure 4). Furthermore, a bivariate Cox-proportional hazard model including tumor stage and S100B serum levels confirmed that both were independent predictors of OS. Moreover, S100B values >0.5μg/l were even more strongly correlated with both the stage of melanoma and survival (p<0.001). The great majority of patients with stage IV (88.89%) had S100B levels >0.5μg/l, while these high levels were much less common in earlier tumor stages (p<0.001). Furthermore, these patients died (death rate 88.89%), with survival range <0.5–2.5 years (mean 1.4 years) (p<0.001). Therefore the 'cutoff' level of 0.5μg/l, almost 5-fold above the upper limit of normal, was found to be associated with stage IV, low survival and increased death rate.

S100B – LDH

No statistically significant association was observed between S100B and LDH serum levels (p=0.323), and therefore both these variables are independent prognostic factors. However, the results were different when the analysis was stratified by stage. Although there were no associations

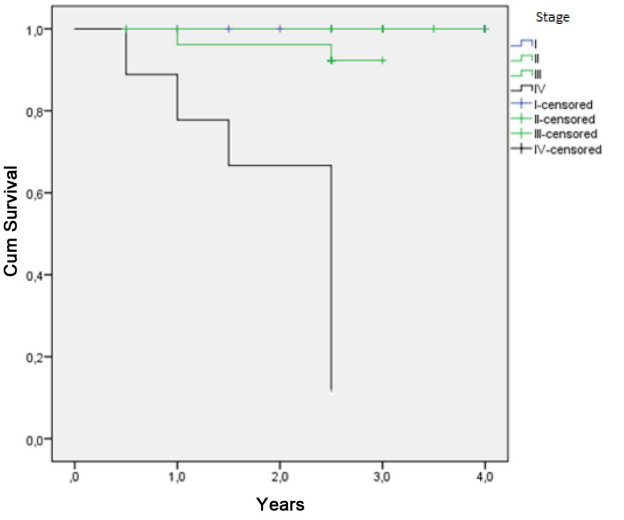


Figure 3. Kaplan-Meier overall survival according to melanoma stage. Stage IV patients had the worst survival compared to earlier stages (p<0.001), while there was no significant difference between stages I, II and III.

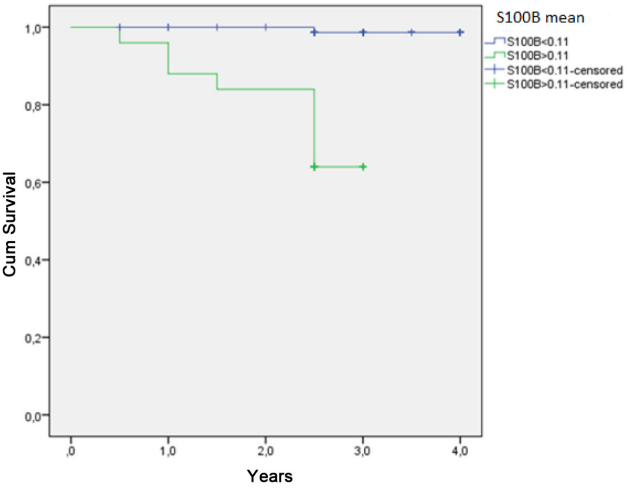


Figure 4. Kaplan-Meier overall survival according to S100B serum levels (below or above 0.11 μg/L) (p<0.001).

in stage I (p=0.935), stage II (p=0.602), and stage IV (p=0.093), a significant association was observed between S100B and LDH in stage III (correlation coefficient 0.496) . High LDH and S100B serum levels were recorded in 66.66% of stage IV patients. Importantly, all these patients had S100B levels >0.5μg/l and died within 2.5 years from diagnosis.

Discussion

S100B is an established tumor marker for the diagnosis of melanoma. Accumulating evidence supports its role as a useful serum biomarker for patients with melanoma [5]. S100B serum levels were found to be correlated with tumor load [20] and therefore could be useful to assess tumor status during follow-up [21]. Elevated S100B serum levels were associated with N1, N2 and N3

disease [22]. It was shown that S100B could be used to assess or even predict treatment response. Declining levels of S100B were associated with tumor response or successful treatment, whereas rising levels of S100B were correlated with disease progression [23-25]. Moreover, S100B levels could predict response to chemotherapy in stage IV patients [26]. S100B levels were also correlated with disease free survival (DFS) and OS. Wevers et al. [27] showed that preoperative levels of S100B were the strongest independent predictor of DFS. Nikolin et al. found statistically significant difference in DFS for stage IIB and IIC patients with S100 below and above 0.106 μg/l [28]. Hauschild et al. [29] demonstrated that patients with S100B levels <0.2μg/l had significantly longer OS (p<0.001) than those with S100B >0.2μg/l, regardless of stage. By contrast, other studies showed that neither S100B nor LDH could predict sentinel lymph node status as well as survival of patients who underwent melanoma dissection [30]. The results of the present study are consistent with the above findings showing that mean S100B serum levels are positively correlated with tumor stage. Although patients with stages I/II had mean serum levels within normal limits, these levels were slightly higher in stage II than in stage I. Thus, even in these very early stages, S100B serum levels might be a surrogate biomarker of tumor load.

For stage III, studies have shown that S100B is the strongest independent predictor of DFS [26] and a strong prognostic marker for OS [13,23-25]. In melanoma patients with palpable regional lymph nodes and AJCC stage III documented on PET/CT, the presence of raised preoperative S100B serum levels was associated with high regional tumor load [26]. Also, high S100B levels in postoperative melanoma patients without evidence of residual disease predicted a poorer DFS. A possible explanation could be that postoperative high S100B serum levels might reflect an ongoing process of a subclinical microscopic systemic disease that cannot be detected with imaging techniques [13,23-25]. Additionally, in patients with stage II-IB-IIIC, S100B serum levels demonstrated stronger prognostic significance when measured preoperatively than postoperatively on days 1 and 2 [27]. Bouwhuis et al. showed that raised S100B levels in patients with stage III melanoma were stronger prognostic factors than disease stage and the number of infiltrated lymph nodes [25]. Elevated S100B serum levels following lymph node dissection for stage III melanoma represented an independent prognostic factor [31]. Therefore, pre- and post-lymphadenectomy S100B serum levels might be useful to assess the completeness of tumor dissection [23]. In the present study, S100B serum levels in stage III patients reflected the loco-regional tumor load. Patients with elevated post-treatment levels were at increased risk of disease relapse and, therefore, underwent regular postoperative S100B assessments aiming at the early detection and treatment of a subclinical relapse. S100B serum levels were also associated with the N status of stage III patients. We found that S100B levels in patients with N2/N3 stages were significantly higher than in N1 stage. However, no statistically significant difference existed between N2 and N3 stages. Therefore, S100B serum levels might be important to monitor treatment response and disease status during follow-up, as well as to estimate the prognosis of stage III patients.

In contrast, stage IV melanoma patients with favorable performance status are treated with systemic treatment, with primary purpose to prolong life expectancy. Vrbic et al. showed that S100B is a reliable serum marker for the diagnosis of stage IV malignant melanoma [32]. Patients with generalized metastases usually have elevated S100B levels reflecting the extent of tumor load [23,31]. Rising S100B serum levels during treatment might be associated with disease progression, whereas effective treatment is often correlated with declining levels [26,33,34]. Rising S100B levels were able to detect tumor relapse 5-23 weeks before clinical examination or radiological methods could do [2,29]. In the present study, stage IV patients had S100B serum levels much higher than the upper limit of normal as well as the mean levels in earlier tumor stages. The majority (66.7%) of patients with stage IV disease was initially diagnosed as stage III with elevated S100B levels and their S100B serum levels became even higher when they relapsed with stage IV disease. Also, high S100B serum levels (>0.11μg/l) were associated with shorter survival in stage IV patients. Furthermore, S100B levels >0.5μg/l were associated with remarkably worse prognosis. Notably, 88.9% of patients with S100B >0.5μg/l died within 2.5 years with a mean survival of 1.12 years, and all of them had stage IV disease. These patients had high S100B levels even after adjuvant treatment. As a result, high levels of S100B more than 5-fold the upper limit of normal were associated with adverse disease outcome.

We evaluated S100B serum levels with regard to NCCN 'high risk features', i.e. ulceration, LVI, and mitoses and we did not observe any significant association between S100B and ulceration or LVI. However, there was statistically significant association between S100B and mitotic rate.

S100B was positively and strongly correlated with mitotic rate only in stage IV. To the best of our knowledge, this is the first study to demonstrate a connection between these two parameters. We also found that there was no statistically significant difference in S100B serum levels between patients with one primary melanoma and those with more than one concurrent primary lesion. In the case of a patient with multiple primary melanoma lesions, S100B serum levels were proportional to the lesion with the more advanced stage. To our knowledge, this finding is reported in the literature for the first time.

Multiple lines of evidence have demonstrated the independent to LDH prognostic impact of S100B serum levels [9,10]. For stages II and III, S100B has been superior to LDH in terms of sensitivity, specificity and diagnostic accuracy [35]. Also S100B is a stronger prognostic marker than LDH in patients with distant metastasis [9,18]. The results of the present study suggest that S100B offers superior and independent to LDH prognostic information, especially in advanced melanoma stages (III and IV). Patients in stage IV are more likely to have increased levels both of S100B and LDH and these are indicators of adverse prognosis.

In conclusion, S100B protein in peripheral blood represents a reliable, simple, safe and cost-effective biomarker, independently and strongly associated with poor disease outcome, and possibly a useful tool to monitor disease status in melanoma patients in combination with other diagnostic modalities.

Conflict of interests

The authors declare no confict of interests.

References

1. Peric B, Zagar I, Novakovic S, Zgajnar J, Hovekar M. Role of serum S100B and PET-CT in follow-up of patients with cutaneous melanoma. BMC Cancer 2001;11:328-34.
2. Harpio R, Einarsson R. S100 as cancers biomarkers with focus on S100B in malignant melanoma. Clin Biochem 2004;37:512-8.
3. Moore BW. A soluble protein characteristic of the nervous system. Biochem Biophys Res Commun 1965;19:739-44.
4. Gaynor R, Irie R, Morton D, Herschman HR. S100 protein is present in cultured human malignant melanomas. Nature 1980;286:400-1.
5. Sandru A, Voinea S, Bolovan M, Cinca S, Bordea C, Blidaru A. The significance of serum S100, MIA and LDH in cutaneous malignant melanoma. Rom J Biochem 2011;48:75-88.
6. Hartman KG, Mc Knight LE, Liriano MA, Weber DJ. The evolution of S100B inhibitors for the treatment of malignant melanoma. Futur Med Chem 2013;5:97-109.
7. Smit LH, Korse CM, Hart AA et al. Normal values of S-100B predict prolonged survival for stage IV melanoma patients. Eur J Cancer 2005;41:386-92.
8. Abusaif S, Jradi Z, Held L et al. S100B and lactate dehydrogenase as response and progression markers during treatment with vemurafenib in patients with advanced melanoma. Melanoma Res 2013;23:396-401.
9. Egberts F, Kotthoff EM, Gerdes S et al. Comparative study of YKL-40, S100B and LDH as monitoring tools for stage IV melanoma. Eur J Cancer 2012;48:695-702.
10. Weide B, Richter S, Büttner P et al. Serum S100B, Lactate Dehydrogenae and Brain Metastasis Are Prognostic Factors in Patients with Distant Melanoma Metastasis and Systemic Therapy. PLoS One 2013;8(11):e81624.
11. National Comprehensive Cancer Network (NCCN) Melanoma Version 1.2016. Available in internet at: http://www.nccn.org (assessed at 10.09.2016).
12. McShane LM, Altman DG, Sauerbrei W et al. Reporting recommendations for tumour MARKer prognostic studies (REMARK). Br J Cancer 2005;93:387-91.
13. Balch CM, Gershenwald JE, Soong S et al. Final Version of 2009 AJCC Melanoma Staging and Classification. J Clin Oncol 2009;27:6199-6206.
14. Astrand R, Undén J, Romner B. Clinical Use of the calcium-Binding S100B Protein. In: Heizmann CW (Ed): Calcium-Binding proteins and RAGE: From Structural Basics to Clinical Applications Methods in Molecular Biology 2013; vol 963, pp 373-384, Springer Science+Business Media, New York.
15. Wiesmann M, Missler U, Gottmann D, Gehring S. Plasma S-100b protein concentration in healthy adults is age- and sex- independent. Clin Chem 1998;44:1056-1058.
16. Muller K, Townend W, Biaska N et al. S100B serum levels predicts computer tomography findings after minor head injury. J Trauma 2007;62:1452-56.
17. Astrand R, Romner B, Lanke J, Unden J. Reference values for venous and capillary S100B in children. Clin Chim Acta 2011;412:2190-93.
18. Alber B, Hein R, Garbe C, Caroli U, Luppa PB. Multicenter evaluation of the analytical and clinical performance of the Elecsys® S100 immunoassay in patients with malignant melanoma. Clin Chem Lab Med 2005;43:557-63.
19. Reichenauer S. Luminometric measurement of protein

S100 in malignant melanoma. University of Tuebingen, Medical Faculty, 11 July 2006.

20. Martenson ED, Hansson LO, Nilsson B et al. Serum S-100b protein as a prognostic marker in malignant cutaneous melanoma J Clin Oncol 2001;19:824-31.
21. Garbe C, Hauschild A, Volkenandt M et al. Evidence and interdisciplinary consense-based German guidelines: diagnosis and surveillance of melanoma. Melanoma Res 2007;17:393-9.
22. Domingo-Domenech J, Castel T, Auge JM et al. Prognostic implications of protein S-100beta serum levels in the clinical outcome of high-risk melanoma patients. Tumour Biol 2007;28:264-72.
23. Kruijff S, Bastiaannet E, Muller-Kobold AC, van Ginkel RJ, Suumeijer AJ, Hoekstra HJ. S-100B concentrations predict disease-free survival in stage III melanoma patients. Ann Surg Oncol 2009;16:3455-62.
24. Tarhini AA, Stuckert J, Lee S et al. Prognostic significance of serum S-100B in surgically resected melanoma patients participating in intergroup trial ECOG 1694. J Clin Oncol 2008;27:38-44.
25. Bouhuis MG, Suciu S, Kruit W et al. Prognostic value of serial blood S100B determinations in stage IIB-III melanoma patients: a corollary study to EORTC trial 18952. Eur J Cancer 2011;47:361-8.
26. Hamberg AP, Korse CM, Bonfrer JM, de Gast GC. Serum S100B is suitable for prediction and monitoring of response to chemoimmunotherapy in metastatic malignant melanoma. Melanoma Res 2003;13:45-9.
27. Wevers KP, Kruijff S, Speijers MJ, Bastiaannet E, Muller Kobold AC, Hoekstra HJ. S100B: A Stronger Biomarker than LDH in Stage IIIB-C Melanoma. Ann Surg Oncol 2013;20:2772-9.
28. Nikolin B, Djan I, Trifunovic J et al. MIA, S100 and LDH as important predictors of overall survival of patients with stage IIb and IIc melanoma. JBUON 2016;21;691-7.
29. Hauschild A, Engel G, Brenner W et al. S100B protein detection in serum is a significant prognostic factor in metastatic melanoma. Oncology 1999;56:338-44.
30. Egberts F, Momkvist A, Egberts JH, Kaehler KC, Hauschild A. Serum S-100B and LDH are not useful in predicting the sentinel node status in melanoma patients. Anticancer Res 2010;30:1799-1805.
31. Martenson ED, Hansson LO, Nilsson B et al. Serum S-100b protein as a prognostic marker in malignant cutaneous melanoma J Clin Oncol 2001;19:824-31.
32. Vrbic S, Filipovic S, Pejic I, Vrbic M, Filipovic A. Sensitivity, specificity, positive and negative predictive value S100 beta protein in patients with malignant melanoma. JBUON 2005;8:139-41.
33. Kluger HM, Hoyt K, Bacchiocchi A et al. Plasma markers for identifying patients with metastatic melanoma. Clin Cancer Res 2011;15:2417-25.
34. Eisenhauer EA, Therasse P, Bogaerts J et al. New response evaluation criteria in solid tumors: revised RECIST guideline. Eur J Cancer 2009;45:228-47.
35. Diaz-Lagares A, Alegre E, Arroyo A et al. Evaluation of multiple serum markers in advanced melanoma. Tumor Biol 2011;32:1155-61.