JBUON 2016; 21(3): 691-697
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

MIA, S100 and LDH as important predictors of overall survival of patients with stage IIb and IIc melanoma

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Summary

Purpose: *Melanoma represents the most severe form of skin cancer. Detection of specific tumor markers is an important step in disease diagnosis and treatment, contributing to personalized therapy. The purpose of this study was to evaluate the potential of MIA, S-100 and LDH as biomarkers for the estimation of overall survival and disease-free survival rate in patients with stage IIa, IIb vs stage IIc melanoma.*

Methods: *Selected biomarkers MIA, S-100 and LDH were prospectively evaluated in 80 patients with melanoma. Patients were divided in two groups according to tumor thickness. The first group (group A) consisted of patients with primary tumor thickness between 2.0 – 4.0 mm (N=40), i.e. IIa and IIb stage of disease (16 males; 40%, and 24 females; 60%). The second group (group B) consisted of 40 patients with primary tumor thickness over 4.0 mm, i.e. IIc stage, which is considered as high risk group (26 males; 65%, and 14 females 35%). Statistical analyses were performed to estimate overall survival and disease-free survival in both patient groups.*

Results: *In group A a significant difference in overall survival was found among MIA1, MIA2 and MIA3 scores, while the other 2 markers didn't show significant differences. In group B statistically significant differences in overall survival were found regarding all three biomarkers. Statistically significant differences in disease-free survival were found for MIA1 score compared to MIA2 and MIA3 scores. Also, very significant difference was detected in patients with S-100 below 0.106 and above 0.106. The same was confirmed for normal and increased LDH level in group B for disease-free survival.*

Conclusion: *MIA score, S100 protein and LDH in the IIC group B patients might be useful in the prediction of overall survival and disease free survival.*

Key words: *biomarkers, melanoma, MIA, LDH, S100*

Introduction

Melanoma represents the most severe form of skin cancer. Only in USA during 2012, 68130 new cases were registered and 8700 had a fatal outcome [1]. Melanoma incidence is constantly growing, therefore better prevention measures and early detection of the disease are required. Registered patients are mostly young and in late phases of disease. Melanoma usually evolves through 3 phases: *in situ* proliferation, radial growth and the phase of vertical growth that represents the key moment in cell migration into deeper dermis layers, into lymph vessels and blood stream [2]. Detection of specific tumor markers is an important step in disease diagnosis and treatment, contributing to personalized therapy and they may become a diagnostic tool in modern medicine. Even though in melanoma it may be that the most important prognostic factor regarding adjuvant therapy is the sentinel lymph node status, biomarkers can be valuable indicators for systemic therapy.

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Received: 09/06/2015; Accepted: 26/07/2015

Prognostic markers are necessary for more precise determination of the risks for disease progression and estimation of response [3].

Precise prognosis of melanoma represents a challenge. Different serum tumor markers have been tested as prognostic factors in melanoma. Among them are S100, melanoma-inhibiting activity (MIA) and lactate dehydrogenase (LDH) [4-6]. MIA protein has been isolated for the first time from melanoma cell culture. High values of MIA were detected in 96-100% of IV stage melanoma [6]. S100 protein is also highly specific. Increased levels of S100 protein are registered in 74-100% of patients in IV stage melanoma. LDH has been proved to have prognostic value in several tumor types, such as NSC (non-small-cell) and SC (small-cell) lung carcinomas, Hodgkin and non-Hodgkin lymphomas and prostate cancers. Increased serum values of LDH are not obligatorily related to tumor-related liver damages, as it was earlier believed. Since LDH is metabolized at very constant level, it is believed that increased levels of LDH are a consequence of metabolic activity of cancer cells and presence of cancer. In the Waldmann et al. study [7] 43% of the patients had increased level of LDH, and only 21% had liver metastasis detected by CT scan. Even though no 100% accurate and melanoma-specific marker has been found so far, S-100 protein, MIA protein and LDH may be considered as highly specific biomarkers for melanomas [7-9].

The aim of this study was to evaluate the potential of MIA, S-100 and LDH as biomarkers for the estimation of overall survival and disease-free survival rate in patients with stage IIa, IIb vs stage IIc melanoma.

Methods

MIA, S-100 and LDH biomarkers were prospectively evaluated in 80 patients with melanoma. All patients had undergone surgical treatment. Regarding the thickness of the primary tumor, patients were divided in 2 groups. The first group (group A) consisted of patients with primary tumor thickness between 2.0 – 4.0 mm (N=40), i.e. IIa and IIb stage of disease (16 males; 40%, and 24 females; 60%). At present, these patients are treated as patients with moderate risk. The second group (group B) consisted of 40 patients with primary tumor thickness over 4.0 mm, i.e. IIc stage, which is considered as high risk group (26 males; 65%, and 14 females;35%). MIA samples were stored at –70oC until assayed and MIA score was determined by semi-quantitative method. Semi-quantitative assessment of MIA protein expression was performed by immunohistochemistry staining, based on cytoplasmic staining intensity and the percentage of immunoreactive tumor cells. The total possible maximum score for the evaluation of the MIA expression, which is obtained by adding up points for the percentage of immunoreactive cells and the intensity of immunoreactive staining, was 8. Analytically, the estimatd scores were as follows: score 0 for patients with 0 and 1 point; score 1 for patients with 2 and 3 points; score 2 in patients with 4 or 5 points; and score 3 for patients with 6, 7 and 8 points (Table 1).

S-100 serum samples were obtained by venous puncture, centrifuged and immediately analyzed. S-100 protein was estimated using a commercial immunoluminometric assay (Liaison ®Diasorin®, Italy). 0.105 mg/L was taken for the upper limit of the expected value (cut-off) of serum S-100 protein because these were the measured values in 95% of healthy subjects.

LDH levels were measured by multislice dry biochemical method using clinical-biochemistry kit on Ectachem 250 analyzer (Johnson & Johnson Clinical Diagnostic, N.Jersey, USA).

Statistics

Statistical analyses were performed using the software Statistica 10.0 [10]. Parameters of basic descriptive statistics were calculated for all biomarkers (average value, minimum-maximum and standard deviation/SD). Chi-square test was used for pairwise comparison between groups. Significance was set at $p<0.05$. Kaplan–Meier method was performed to estimate overall survival and disease-free survival in both patient groups and comparison of the curves was carried out using log-rank test.

Results

Values of MIA scores were significantly different in group A and B (x^2=14.90588, p=0.00190). In group A all the values of MIA scores were found and were equally distributed. In group B, no MIA1 value has been observed. The most common MIA score in group B was MIA3 (27.5%) (Figure 1).

The average value of S-100 protein of all patients was 0.48±0.53 (range 0.005-1.565). Statistically significant differences were noted between the mean values of S-100 protein in patients

Table 1. MIA score analysis

Points for immunoreactive cells	*Intensity of immunostaining*
0 = No stained cells	0 = No cytoplasmic staining
1 = < 1% stained cells	1 = Low staining intensity
2 = 1-10% stained cells	2 = Moderate staining intensity
3 = 11-33% stained cells	3 = Very intense staining
4 = 34-66% stained cells	
5 = 67-100% stained cells	

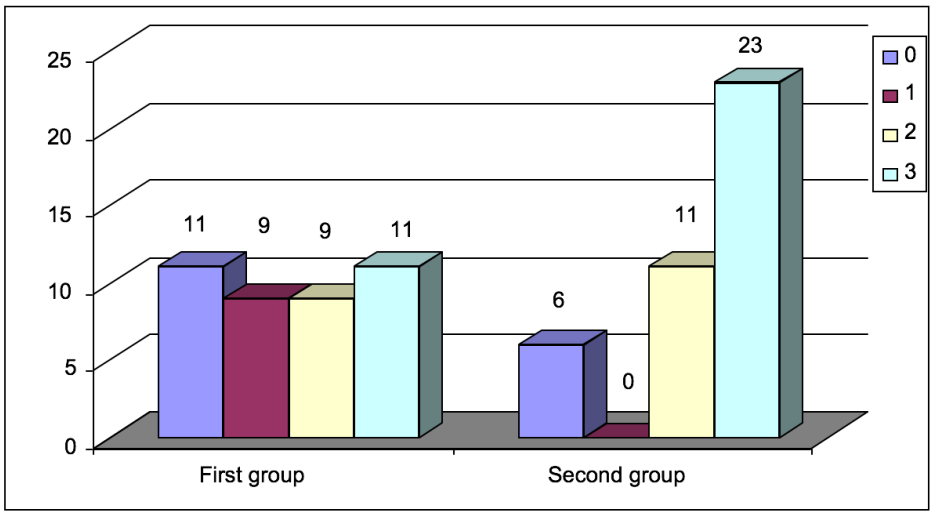

Figure 1. Distribution of MIA scores in both patient groups.

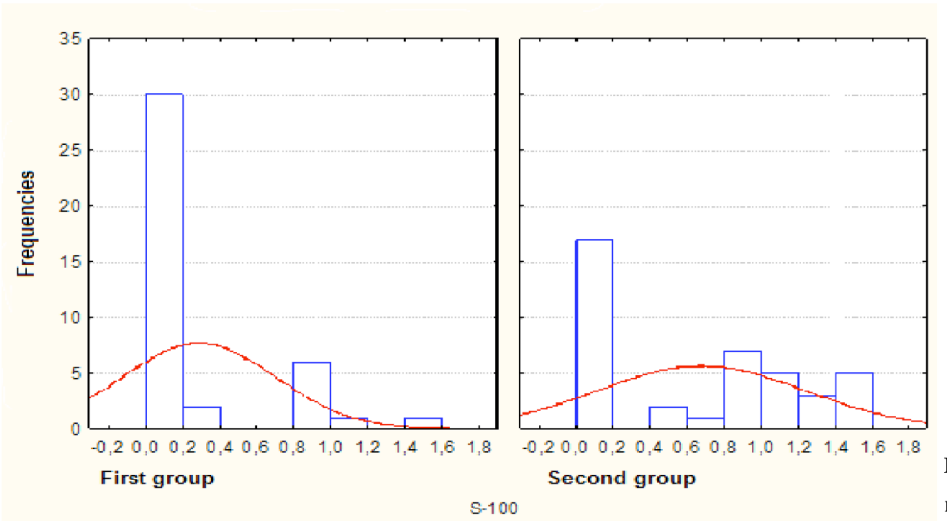

Figure 2. S-100 levels in the first and second group of patients.

from group A and B (p=0.003). The general trend showed that S-100 values were lower in group A than in group B (Figure 2).

LDH values in group A where at the level of reference value in the majority of the patients (67.5%), while LDH values in group B were higher than the reference value in 55% of the cases (Figure 3).

Regarding the pairwise tumor marker and overall survival and disease-free survival in patients from group A, statistically significant difference in overall survival was found among MIA score 1 and 2 comparing to MIA score 3. No statistically significant difference was registered in overall survival between patients with S100 protein level below 0.106 and above 0.106, nor comparing patients with a value over 1. Similarly, no differences among patients from group A were detected for normal and increased level of LDH (Figure 4).

In group B of patients statistically significant differences in overall survival were found regarding all three biomarkers. Significantly lower survival in patients with MIA3 score compared to MIA2 score was detected, and also in patients with increased LDH compared to those with normal LDH levels. Also, significantly shorter survival rate was found in patients with S100 protein level above 0.106 (Figure 4).

Disease-free survival in group A patients was

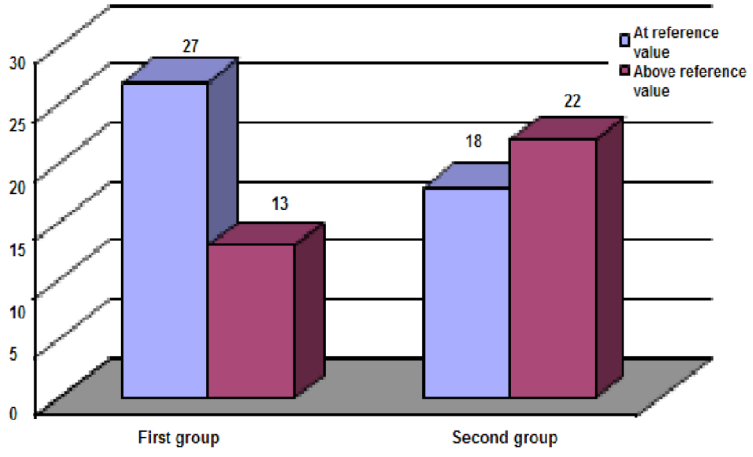

Figure 3. LDH values in the first and second group of patients.

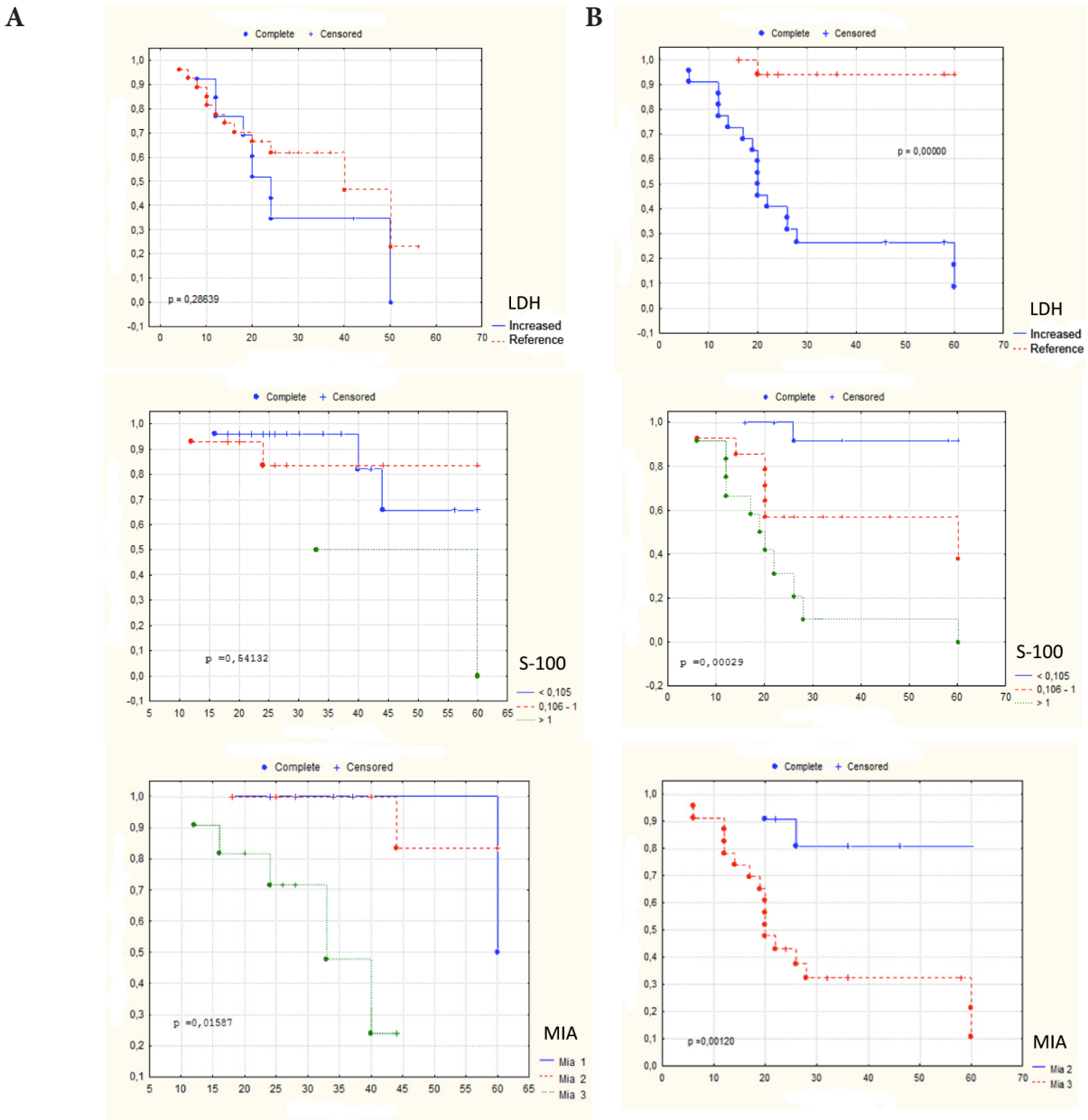

Figure 4. Kaplan–Meier curves with log-rank test showing overall survival in both patient groups (4A: group A and 4B: group B) in relation to LDH, S-100 and MIA scores.

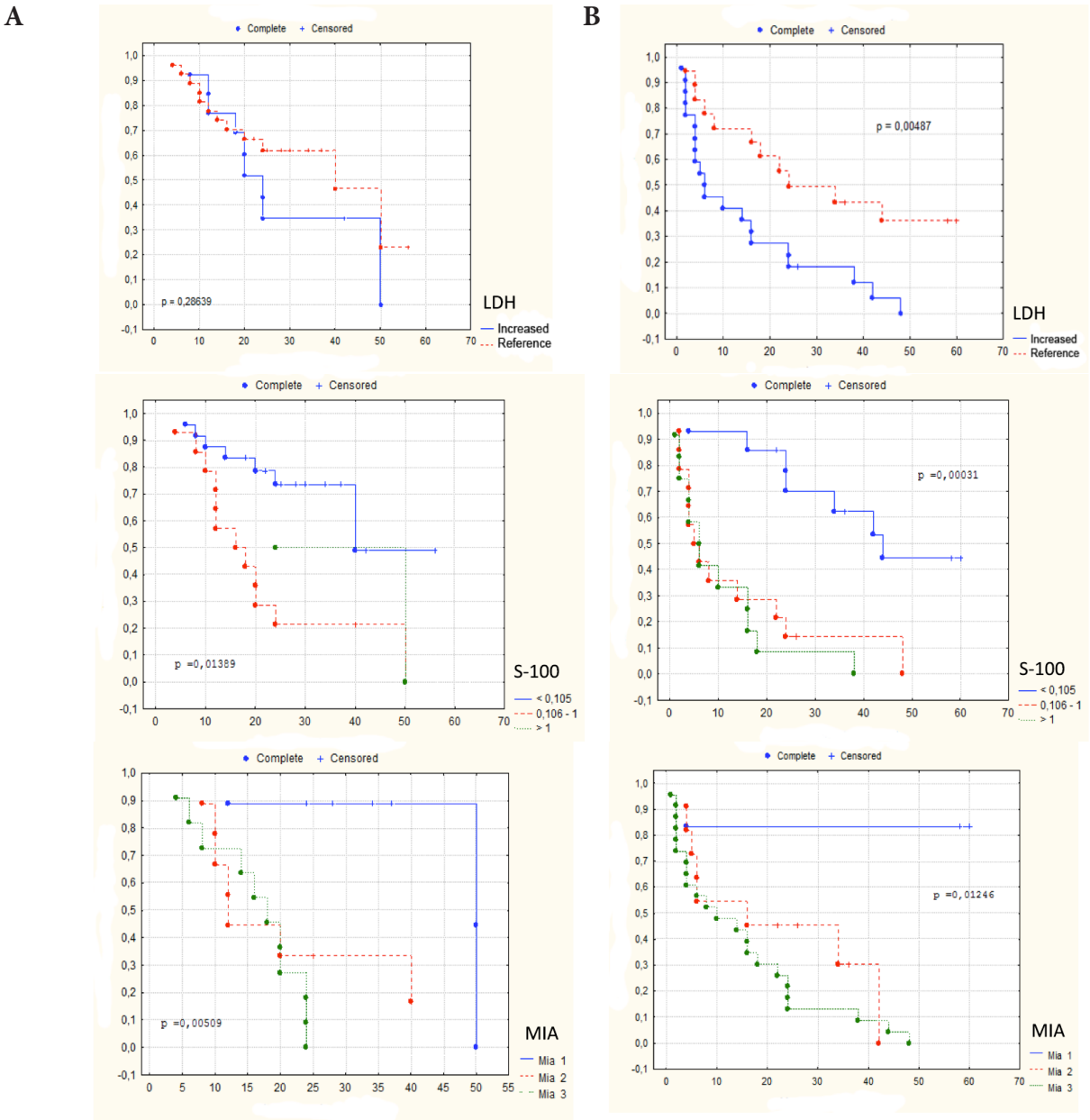

Figure 5. Kaplan–Meier curves with log rank test showing disease-free survival in both patient groups (5A: group A and 5B: group B) in relation to LDH, S-100 and MIA scores..

significantly different among MIA1, MIA2 and MIA3 scores. Comparing patients with S-100 below and above 0.106 in group A, significant difference was noted, as well as for values above 1. No statistically significant difference was found for normal and increased level of LDH (Figure 5).

In group B patients statistically significant differences in disease-free survival were found for MIA1 score compared to MIA2 and MIA3 scores. Also, very significant difference was detected in patients with S-100 below 0.106 and above 0.106 for disease-free survival. The same was confirmed for normal and increased LDH level in group B (Figure 5).

Discussion

MIA score has proved to be a reliable diagnostic biomarker in both groups of patients for both parameters measured: overall survival and disease-free survival. Higher values of MIA led to shorter overall survival and disease free survival in both groups. Stahlecker et al. [5] and Goggas et al. [11] showed that there is no development of metastasis in patients with normal MIA values. Several studies concluded that higher MIA values are indicative for poor prognosis [12-14]. Garbe et al. [15,16] also found positive correlation between higher MIA score and shorter disease-free survival. MIA score was also helpful in detecting the

progression from localized to metastatic disease and in monitoring the treatment of melanoma [17].

S-100 protein was proved to be strong prognostic marker for disease-free survival in both groups of patients, while its potential as a biomarker for overall survival was proved only in the second group (IIc stage). In the first group of patients increased S100 protein was connected with decrease in overall survival, but without statistical significance. Increased level of S-100 protein over 0.106 led to decrease in overall survival and disease-free survival in the second group. Several recent studies also confirmed positive correlation between advanced stage of disease and desreased disease-free survival [18]. Schultz et al. [19] also proved that increase in S100 protein level was related to decrease in overall survival [20]. Stages of malignant melanoma and the relative hazard of death increased 5-fold when circulating S100B exceeded 0.6 µg/l. These serum S100B survival data were confirmed for prediction of survival [21,22]. Also, it should be stressed that decrease in S100 protein level increases disease-free survival for 26 months. Patients with lower mean values survive up to 29.6 months [23]. The results of our study show that S100 level is also significant for patients in II disease stage. The level of the S100 protein was previously shown to be related to the stage of melanoma [24], the likelihood of metastasis and the disease-free and overall survival. It has also been shown that S100 is promising in monitoring of therapeutic interventions in relapsed disease, predicting also the overall survival estimation [25].

The increased LDH level in group B patients led to decrease in disease-free survival. Previous studies showed that increased LDH was related to metastasis and the most frequent location was the liver. Patients with abnormal LDH levels had significantly decreased survival. LDH might serve as a prognostic factor in late-stage malignant melanoma [8,11]. Several authors [3,8,11] believe that the increase in LDH is one of the most important prognostic factors in patients with advanced melanoma. This has been discussed in a study where LDH was evaluated in combination with other tumor markers such as S100B and MIA. Patients in advanced stage with normal LDH had an overall survival rate of 65% at 1 year and 40% at 2 years. On the contrary, patients with LDH above the upper limit of normal at the time of staging had 1 year overall survival of only 32% and a 2 year overall survival of 18% [21].

Conclusion

MIA proved to be an important prognostic parameter in both groups of patients. Higher values of the MIA score were connected with decrease in overall survival and disease free survival.

Increase in S100 proteins was related to decrease in overall survival in both groups of patients. In group B (stage IIC) statistically significant decrease in overall survival was observed. MIA score, S100 protein and LDH were significant predictive markers for B group patients compared to group A. It can be concluded that using MIA score and S100 protein and LDH levels in stage IIC patients might be useful in the prediction of overall survival and disease free survival.

Conflict of interests

The authors declare no confict of interests.

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