

Results of the Determination of Serum Markers in Patients with Malignant Melanoma

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ABSTRACT

Although there is no routine procedure for determination of serum markers in patients with malignant melanoma (MM), some markers are being studied as potentially useful prognostic tools. Serum lactate dehydrogenase (LDH), protein S-100B, melanoma-inhibiting activity (MIA) and tyrosinase may correlate with melanoma progression. In this study, the results of determination of S100 protein, LDH, MIA and tyrosinase in the serum of 50 patients with MM (stages I-IV) were determined. The increased values of MIA were found in 26% patients in stage I, while in 50% patients in stage IV. Increased S-100 protein was found in 13% patients in stage I while in 50% patients in stage IV. The increased values of LDH were found in 26% patients in stage I, while in 25% patients in stage IV. The positive serum tyrosinase was noticed in 17.3% patients in stage II, while in 25% patients in stage IV. The obtained results have revealed no significant differences between the groups in higher and lower stages of the disease, indicating that blood markers are not reliable prognostic factors for MM progression.

Key words: malignant melanoma, markers, lactate dehydrogenase (LDH), protein S-100, melanoma-inhibiting activity (MIA), tyrosinase

Introduction

The serum markers in patients with malignant tumours, including malignant melanoma (MM), may be valuable in clinical diagnosis, prognosis and monitoring of the disease and patients' response to therapy over time. The research data so far pointed out few blood markers as promising prognostic parameters in the detection of tumour progression. The most widely used serum markers are S-100, melanoma inhibitory activity (MIA), and lactate dehydrogenase (LDH). Different studies indicated close correlations between the serum concentrations of these and tumour load^{1,2}. Increased serum concentrations of these marker proteins being indicative of tumour growth^{3,4}, but they still cannot be used as an evidence of tumour progression. Until now there is no marker available to detect metastatic spread at an early stage in the progression of the disease. Laboratory tests and imaging procedures increase the probability of detecting occult metastases. The current treatment recommendations for the patients with MM, comprise various clinical and radiological examinations according to the stage of the disease (including chest x-rays, ultrasound of

lymph nodes and abdomen, CT-, MRI- and PET scans). However, there is no unique consensus in clinical practice⁵. Besides LDH, which is a leading blood parameter in patients with melanoma metastases, there are two serum proteins widely used in the follow-up of patients with MM; protein S-100, a member of the S-100 protein family, and melanoma-inhibiting activity (MIA), which is a soluble protein⁶. Increased levels of LDH in serum of melanoma patients were detected in 1954. Ever since the value of LDH as a tumour marker for MM has been discussed. LDH was reported to be an indicator for liver metastases, with high sensitivity and a specificity^{5,7}. Melanoma-inhibiting activity (MIA) is strongly expressed by malignant melanocytes while naevi show only moderate, low or no expression^{1,2}. The mature protein consists of 107 amino acids; its molecular weight is approximately 11 kd. MIA especially inhibits the attachment of melanoma cells to fibronectin and laminin, which leads to an increasing motility. Melanoma cells and mature chondrocytes express MIA whilst low levels of MIA-mRNA are found in various other cell types. In malignant tumours,

high MIA mRNA levels were detected in almost 100% of MM samples. S-100 protein is an acid calcium-binding protein with a molecular weight of 21kd found in the nervous system of vertebrates. Its name derives from its solubility in 100% saturated ammonium sulphate at neutral pH. It is dimerous protein, consisting of two isomeric subunits; (MW 10.4 kd) and (MW 10.5 kd), whereby each of the possible combinations occurs. S-100 has been found in melanoma cells and is used for the immunohistochemical diagnosis of amelanotic melanomas. In the recent literature, serum S-100 was suggested as a serum marker for staging and monitoring therapy of patients with MM^{1,2}. It has been indicated that levels of S-100 as well as of MIA in peripheral blood, correlate with melanoma progression, demonstrating a higher sensitivity, specificity, and diagnostic accuracy than other parameters in the diagnosis of newly occurring melanoma metastasis^{1,8}. Tyrosinase and tyrosinase-related protein-1 are enzymes involved in melanin synthesis and are specific to melanocytes and melanoma cells. Tyrosinase catalyses the oxidation of tyrosine to dopa and further to dopaquinone. Expression of the tyrosinase is confined to cells of the melanocytic lineage and Schwann cells. Determination of the serum concentration of tyrosinase may also be important marker in patients with MM. The aim of this study was to determine and compare levels of MIA, S-100 protein, LDH, and tyrosinase in the serum of patients with MM in different stages of disease, as well as to conclude whether these might be useful prognostic tools for MM progression.

Materials and Methods

In the present study, serum levels of MIA, S-100 protein, LDH, and tyrosinase were measured over a time period from 2002 to 2006. The markers in serum samples from 50 melanoma patients at different stages of disease were analyzed (Table 1).

Diagnosis of MM was based on the histopathological examination of the surgically removed lesions. Tumour thickness was determined according to the criteria of Breslow and tumour stage according to the TNM classification. Classification of the MM stages was based on UICC (according to Hermanek et al., 1987): T, N, M system: Stage I: T1-2, N0, M0; Stage II: T3-4, N0, M0; Stage III: T1-4, N1-2, M0; Stage IV: T1-4, N1-2, M1⁵. The following classification of the stages was used: I: (a) 0–0.75, (b) 0.76–1.5 mm; II: (a) 1.51–4.00 mm, (b) >4.00 mm; III: T1-4, N1, N2 (regional lymph nodes), M0; IV: T1-4, N1-2, M1 (distant metastases).

In our study 50 patients were included; 26 females and 24 males, aged from 22 to 83 (Figure 1). There were 15 patients in stage I; 23 in stage II; 8 in stage III, and 4 in stage IV of the disease. Serum concentrations of MIA were measured by enzyme-linked immunosorbent assays (ELISA), Photometric Enzyme Linked Immunosorbent Assay (Dia Sarin), (n.v.<10 ng/mL). Serum concentrations of S-100 were determined by Sangtec 100 ELISA (n.v.<0.150 g/L). Serum concentrations of LDH were

TABLE 1
PATIENTS WITH MM: CHARACTERISTICS

STAGE I n=15 (6 m : 9 f)
/age 17–71/ L: trunk 10, legs 3, arms 2
STAGE II n=23 (10 m : 13 f)
/age 22–71/ L: trunk 11, arms 6, legs 6
STAGE III n=8 (8 m : 3 f)
/age 45–74/ L: trunk 4, legs 3, arms 1
STAGE IV n=4 (3 m : 1 f)
/age 42–66/ L: trunk 4

L – localization, m – male, f – female

measured by UV assay (n.v.<241 U/L). Serum concentrations of tyrosinase were measured by RT – PCR (n.v.=negative).

Results

The results of the serum markers analysis are presented in the figures 1–5. The increased values of MIA were noticed in 26% of the patients in stage I, in 26% in stage II, in 0% in stage III and, in 50% in stage IV (Figure 2). The increased values of S-100 protein were found in 13% of the patients in stage I, in 9% in stage II, in 0% in stage III and, in 50% in stage IV (Figure 3). The increased values of LDH were noticed in 26% of the patients in stage I, in 4% stage II, 13% in stage III and, in 25% in stage IV (Figure 4). The positive serum tyrosinase was found in 0% of the patients in stage I, in 17.3% in stage II, in 12.5% in stage III and, in 25% in stage IV

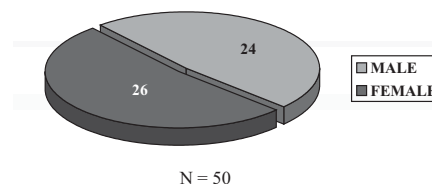


Fig. 1. The groups of patients with malignant melanoma according to the gender.

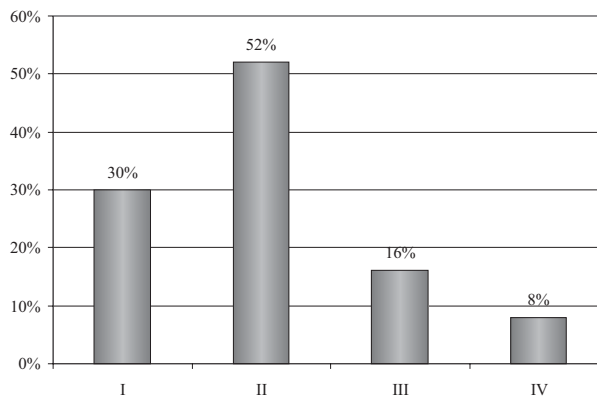


Fig. 2. The groups of patients with malignant melanoma according to MIA values and stages (I-IV).

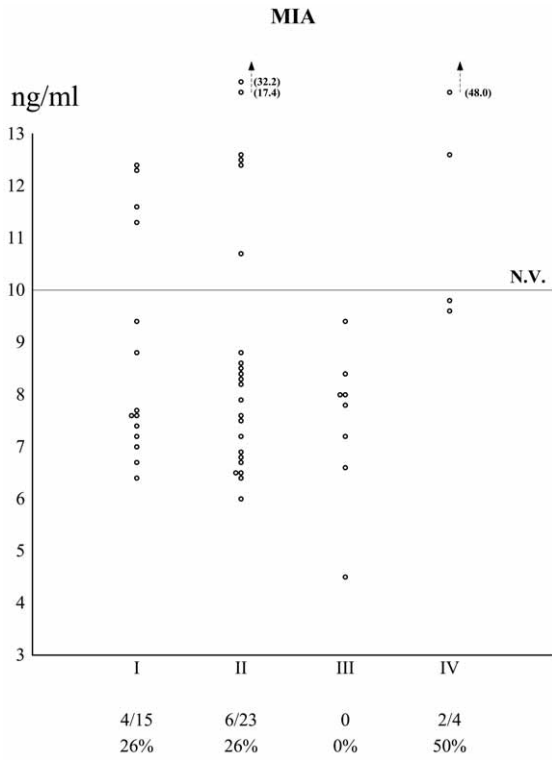


Fig. 3. The groups of patients with malignant melanoma according to MIA and stages.

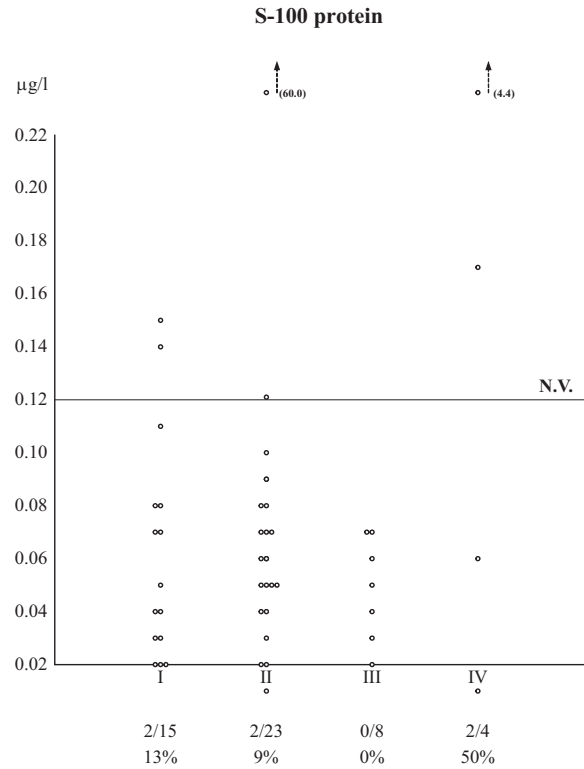


Fig. 4. The groups of patients with malignant melanoma according to S-100 protein and stages.

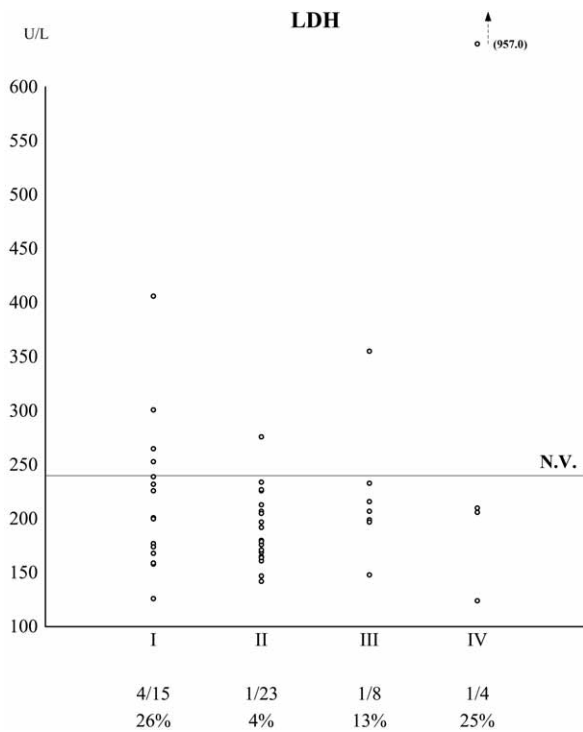


Fig. 5. The groups of patients with malignant melanoma according to LDH and stages.

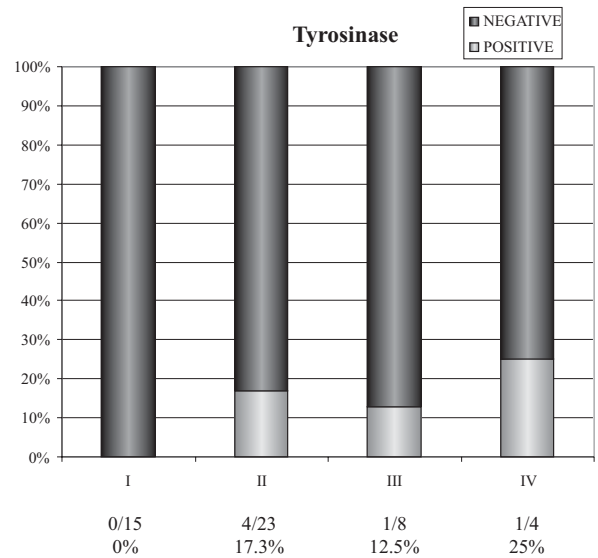


Fig. 6. The groups of patients with malignant melanoma according to tyrosinase and stages.

(Figure 5). The majority of MM patients had values of serum markers within the normal range. There was no significant difference in the number of elevated serum markers between the patients in the stage I, II, III or IV.

Discussion

Although there is no routine procedure for determination of serum tumour markers for the patients with MM, some markers may have a significant role in the follow-up, as possible prognostic factors in the early detection of disease progression or in the prediction of the therapy outcome⁴. Serum protein S-100 and MIA have been described as possible useful tumour markers for MM. For most of these markers, serum levels are more pronounced in more advanced stages of the disease. Therefore, these markers seem to have no place in the early detection of melanoma. On the other hand, sensitivity in the advanced stages of disease seems to be <100%, compromising their use as a new staging procedure. MIA was found to be more sensitive as a potential prognostic marker for patients with metastatic MM in comparison with S-100⁵. Regular determination of S-100 and MIA levels during follow-up can be used for early detection of the tumour relapse in melanoma patients, increased serum concentrations of these marker proteins being indicative of tumour growth⁹.

In our study, increased MIA level was found in 26% of the patients in stage I (localized disease), in none of the patients in stage III and, in only 50% of the patients with visceral metastases. Furthermore, increased S-100 level was found in only 50% of the patients with metastatic disease. These findings indicate that increased levels of both MIA and S-100 protein should not be considered confident markers of the tumour dissemination. It has been observed that patients with distant melanoma metastases with elevated serum S-100, MIA, or LDH had poorer overall survival than patients with normal serum concentrations^{3,8}. These three markers can also be used to monitor the course of the disease and therapy outcome in patients with distant metastases. Regular determination of S-100 and MIA levels during follow-up may be

used for early detection of a tumour relapse in melanoma patients^{10,11}. Thus increased serum concentrations of these marker proteins may be indicative of tumour growth. Auge et al. found the relation between S-100 and metastases site with higher sensitivity and mean concentrations in patients with brain metastases with the lowest in those with lung metastases¹. They found the correlation between MIA and the same metastases locations and considered that S100 and MIA are useful markers related to prognostic factors, being more effective when used in combination. Krahn et al. suggested that S-100 is a more reliable tumour marker in peripheral blood for patients with newly occurred melanoma metastases compared with MIA, albumin and LDH⁷. However, we found increased level of LDH similar in patients with both localized (26%) and disseminated disease (25%). Regarding to the given results, serum LDH in patients with MM should not be considered a strongly reliable indicator of the metastases occurrence. Since there are no marker proteins for melanoma that are not dependent on the tumour load, it is not currently possible to forecast the survival of patients who are tumour free after surgery¹². However, the serum markers currently available for melanoma have only limited clinical use.

Conclusion

Serum markers are not suitable for screening or the diagnostics of primary melanomas, but may be useful in detection of metastatic spread at an early stage of MM progression. The serum S-100 and MIA represent markers that may be valuable in metastases detection, tumour progression monitoring, or in the prediction of the therapy outcome. Thus, determination of serum markers in MM patients may be used in practice and indicate a widely disseminated incurable disease, but their use as tumour markers for melanoma is limited.

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REZULTATI MJERENJA SERUMSKIH MARKERA U BOLESNIKA S MALIGNIM MELANOMOM

SAŽETAK

Unatoč činjenici da danas još ne postoje točne smjernice za odabir serumskih markera u bolesnika s malignim melanomom (MM), određeni markeri mogli bi imati značajnu ulogu. Pokazalo se da razine laktat-dehidrogenaze (LDH), proteina S-100B, MIA (melanoma-inhibiting activity), kao i tirozinaze mogu korelirati s progresijom melanoma. U ovoj studiji navedeni pokazatelji mjereni su u 50 bolesnika s MM (stadij I-IV). Povišena razina MIA zabilježena je u 26% bolesnika sa stadijem I, te u 50% bolesnika sa stadijem IV. Povišena razina S-100 proteina zabilježena je u 13% bolesnika sa stadijem I, te u 25% bolesnika sa stadijem IV. Povišena razina LDH nađena je u 26% bolesnika sa stadijem I, te u 25% bolesnika sa stadijem IV. Pozitivna tirozinaza detektirana je u 17,3% bolesnika sa stadijem II, te u 25% bolesnika sa stadijem IV. Zabilježeni rezultati nisu otkrili statistički značajnu razliku između skupina bolesnika sa nižim, odnosno višim stadijem bolesti, ukazujući na to da serumski markeri nisu pouzdani pokazatelji progresije MM.