THE SIGNIFICANCE OF SERUM S100, MIA AND LDH IN CUTANEOUS MALIGNANT MELANOMA

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Malignant melanoma is a disease with a continuously increasing incidence and an unfavorable outcome in stage IV. It is a tumor with intense metabolic activity that produces and releases in the bloodstream enzymes, cytokines and growth factors. Although several proteins were suggested as serum markers in malignant melanoma, until now none has proven itself sensitive enough to be currently used in treatment monitoring and patient follow-up.

The target of this paper is to synthesize the information from the literature referring to three of the most commonly used serum markers in malignant melanoma. With the exception of LDH, whose role is well established, MIA and S100B are still the subject of various studies.

Keywords: malignant melanoma, tumors markers, LDH, MIA, S100B

Malignant melanoma is a surprising disease, with a clinical evolution that cannot be patterned. It is hard to imagine that, although correctly excised, a tumor with a thickness of less than 1 mm cannot be cured. Nevertheless, 5–15% of these patients develop metastases (1, 2) and eventually die. On the other hand, a large number of thick, ulcerated melanomas never relapse (3).

Because the evolution of malignant melanomas it is hard to predict, mostly in stages I and II, efforts are being made in order to identify markers that can correlate with disease progression and its response to treatment.

Tumor markers are molecules produced either by the tumor cells or by the normal ones, as response to cancerous invasion (4). Many of the tumor markers are intracellular or extracellular proteins released into the body’s fluids. The ideal tumor marker should be: specific to a precise cancer type, undetectable in healthy subjects or in subjects with benign pathology and sensitive enough to detect small, incipient tumors.

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Unfortunately, none of the presently known markers has either a specificity or sensibility of 100% (3). These inconveniences come from the fact that, on the one hand, the majority of the proteins are also produced in small quantities in many normal cells, and on the other hand, some are released in the circulation during the course of some benign diseases. If in a given population, we investigate 13 tumor markers, we find that 49% of the participants develop at least one positive test (5). Despite these restrictions, tumor markers represent one of the main tools that can be used for:

- screening and early diagnosis (PSA, AFP);
- guiding the diagnosis: in the case of a patient with disseminating disease, it is helpful in determining the starting point (calcitonin in medullary thyroid cancer, vanillylmandelic acid for pheochromocytoma);
- suggesting the disease prognosis (LDH in melanoma);
- monitoring the treatment response;
- early identification of relapse.

Serum tumor markers are not routinely used in malignant melanoma (7), excepting LDH which is used in AJCC staging system 2009. In this neoplasia, the prognosis is established taking into account the histology-Breslow score and ulceration, and the clinical setting – the number of the regional involved lymph nodes and the presence of metastases. But none of the conventional clinico-pathological parameters was able to reliably identify those patients who are likely to relapse. Therefore, new biomarkers that predict the clinical outcome are urgently needed.

Similar to other forms of cancer, melanoma is accompanied by some alteration of normal protein activity or concentration. From the long list of biologic serum markers proposed for MM (S100B, MIA – Melanoma inhibitory activity, LDH – Lactic dehydrogenase, TA90 – tumor associated antigen 90, 5SCD – 5Scysteinyldopa, NSE – Neuron specific enolase, CRP – C Reactive Protein, etc.), only few have been rigorously investigated in retrospective or prospective studies. This paper reviews the most significant and best characterized serum markers in malignant melanoma – S100, MIA and LDH – as well as their indications and limits.

**S100B**

S100B was the first molecular marker to be used in the immunohistochemical diagnosis of pigmented skin lesions and is still the most sensitive one. S100B is part of the S100 protein family, having at least 21 different members (11).

S100B protein was isolated by Moore, in 1965, from the bovine brain (9) and got its name due to its solubility in 100% saturated ammonium sulphate solution at neutral pH. The S100 proteins are dimers made of two subunits, α and β, in all possible combinations (αα, αβ, ββ). α subunit is coded by 13 different genes located on the long arm of chromosome 1 (1q21), while β subunit is coded by one gene on the long arm of chromosome 21 (21q22.3) (5, 12).
Protein S100B is secreted by a large number of cells of neuroectodermal and mesodermal origin, each dimer being specific to certain types of tissues:

- the ββ dimer is mainly made by the astrocytes but also by Schwann and Langerhans cells (13);
- the αα dimer has a wide distribution in: skin (S100A7), keratinocytes, skeletal muscles (S100A13), heart (S100A13, S100A10), kidneys (S100A13), pancreas, ovary, lung (S100A10);
- the αβ dimer is found in melanocytes, glial cells, chondrocytes.

The S100B protein family represents the most numerous group from the calcium binding proteins. This property is given by their structure, which contains the EF-hand, made out of the helix-loop-helix sequence, which binds the calcium selectively and with great affinity (14). Each protein contains two of these sequences, at the N and C ends, and it can bind four calcium ions. It also has four binding sites for copper and six for zinc, which also influence the ability to bind calcium (15, 16).

Within the cell, S100B is found both in the nucleus and cytoplasm, exhibiting intra- and extracellular functions (12). The action mechanism is not fully elucidated, but it is accepted that they are involved in several processes through the activation of some enzymes and the modulation of the interaction between several proteins:

- inhibits the assembly of microtubules through sequestration of tubulin, thus maintaining the integrity of the cytoskeleton (13);
- regulates the progression of the cell cycle and cell differentiation;
- inhibits p53 protein phosphorylation by the C protein kinase, thus compromising the tumor suppressor activity of the P53 gene (17,19);
- in nanomolar concentration, in vitro, stimulates neurite outgrowth in cerebral cortex neurons and increases neuronal life (13);
- in micromolar concentrations, in vitro, stimulates apoptosis and determines the raise of β-amyloid precursor protein in neuronal cultures (18);
- stimulates the inflammatory response.

The over expression of S100B was initially described in neurodegenerative diseases: Alzheimer’s disease, epilepsy, lateral amyotrophic sclerosis, the Down syndrome. Consequently, it was demonstrated that increased blood levels appear in traumatic brain lesions, hemorrhagic or ischemic, the values of S100B being proportional with the lesion dimension and patient evolution (13).

In tumor samples, S100B is identified by immunohistochemistry with the help of monoclonal antibodies. It has a high sensibility for melanic lesions, but, has not a good specificity. In skin, the protein may be seen in melanocytes, Langerhans cells, Schwann cells, the acini and ducts of sweat glands, sensorial corpuscles and, of course in the tumors derived from these structures: melanomas, schwannomas, liposarcomas, histiocytic tumors, clear cell sarcomas (20). S100B is also expressed in large quantities in gliomas, neurofibromas, malignant tumors of the nerve sheath, chondrosarcomas.
In blood, S100B may be determined using two assays, immunoradiometric or immunoluminometric, both having as a starting point the same method: the use of two different monoclonal antibodies directed against two distinctive epitopes of the β subunit of S100B. Because the β subunit is the target, this reaction allows to identify both the αβ dimers released from melanocytes and the ββ dimers released from the CNS (19). Given these conditions, it is possible for a patient with malignant melanoma to exhibit a raised S100B value as result of a stroke.

The first data on the significance of serum values of S100B protein in malignant melanoma were published in 1995 (21). From then on, multiple prospective studies emphasized the importance of this tumor marker in MM. It can be used to:

(i) **Appreciate the extent of the disease, knowing that serum values increase in parallel with the clinical stage** (22) – It is commonly accepted that S100B values are proportional with the tumor load of the body. In a study on 126 patients, at a cut off of 0.15 µg/l, Guo et al. (21) noticed that S100B sensibility changed with the progression of the disease; in stages I and II, the overvalues were observed in only 1.3% of cases, in stage III in 8.7%, and in stage IV, 73.9% of patients presented high S100 levels. In another study, that established a 0.09 µg/l cut off, a sensibility of 75% was reached in identifying cases with metastasis (23). S100B values depend on the location of the metastases, with maximum values in cerebral and hepatic metastases (24).

(ii) **Evaluate the treatment response** – Evaluating treatment response can be done through a wide range of paraclinical investigations (echo, CT scan, MRI, PET-CT). It seems that similar results may be obtained through the monitoring of pre- and post-therapy S100B serum values (25), which would represent an important benefit, given the lower costs and the possibility to repeat them any time. Treatment response is indicated by the decrease of serum values, while a raise in concentration suggests tumor progression. Smit et al. even claim that a quick normalization of the S100B during polichemotherapy or immunotherapy is accompanied by a longer survival (28). On the contrary, an increase in S100B during systemic treatment attests failure and strongly suggests that the treatment plan should be changed (25).

(iii) **Predict the disease free survival and the overall survival** – The ability of S100B to suggest evolution in patients with malignant melanoma was the subject of many papers. In a study on 412 patients with malignant melanoma, Hauschild et al. demonstrated that the estimated overall survival was significantly higher (p<0.01) in subjects with S100B<0.2 µg/l, comparing with those having S100B>0.2 µg/l, regardless of the clinical stage (26). The same conclusion was reached in a study where S100B’s threshold was 0.15 µg/l (27). Patients with metastatic MM, with normal values of S100B, live longer than those who have a S100B higher than the upper normal limit (28): the median survival was 14 months for S100B<0.16 µg/l and 6.6 months for S100B>0.16 µg/l (28). A large prospective
study on 1007 patients with MM in stages I–III showed that S100B was a more sensitive survival predictor, stronger than the clinical stage (22): 30% of patients with S100B<0.10 µg/l were alive after five years, comparing to only 6% of those with S100B>0.10 µg/l (22). However, for stage I it is considered that S100B has a lower prognostic significance, here being undermined by the thickness of the tumor and the presence of ulceration.

(iv) Post-therapy follow-up period – Although commonly debated on, no protocol on post therapy follow-up was established in malignant melanoma. In Germany, it is recommended that patients with thin melanomas should be evaluated every six months, and those with thick and intermedia melanoma, every three months. At each check up, except for the clinical exam, one has to determine S100B and also to do an echo of the regional lymph nodes (29).

There are opinions stating that S100B sensibility in identifying metastases is higher than that of other conventional methods (30, 31) The serum protein concentration exceeded the threshold limit of 0.2 µg/l established in this study, with 5–23 weeks before the appearance of other clinical and paraclinical signs of disease (30). Martensen et al. consider that for a stage III melanoma, increased values of S100B after lymph node dissection represent an independent prognostic factor (22). It has been suggested even that S100B monitoring pre- and post-lymphadenectomy could be used as a quality control marker for dissection (31).

It has to be noticed that it is difficult to compare the results obtained by different authors because there are many variables: methods to determine S100, reference values (threshold limits), melanoma stage system, features of different patient groups.

In a meta-analysis of 116 articles concerning S100B, published until January 2008, Mocellin et al. concluded that S100B could have an important role in establishing the therapy plan and patient follow-up for stages I-III malignant melanoma (32). Then, why has it not been implemented in routine practice so far? Because studies stating the exact opposite opinion also exists:

- in a prospective analysis of 266 patients in stages I-III malignant melanoma, Curry et al. noticed that S100B has a minimum value in identifying patients who would develop relapse (33);
- Egberts et al. measured the S100B level before and after sentinel node biopsy in 259 patients with stages I-II malignant melanoma, reporting a reference limit of 0.12 µg/l. They found no correlation between serum S100B concentration, on the one hand, and histopathology of the sentinel node, overall survival and disease free survival, on the other hand (34);
- increased values of S100B may also be found in other circumstances: healthy patients 4%, septic patients 20% (35);
- no general normal threshold was accepted, although a kit manufacturer recommended 0.12 µg/l (LIA – mat Sangtec –100).
Taking into account the diverse opinions, we can conclude that S100B has a higher significance in evolved forms of melanoma, but for stages I-II we have to be cautious, and new data should be needed.

**MIA**

Melanoma inhibitory activity (MIA) was purified in 1989 (36, 37) and cloned in 1994 (38, 39). Its discovery is linked to the search for autocrine growth factors in malignant melanoma.

Initially, it was considered that MIA has a tumor suppressor activity, hence the name (38, 39). In vitro, MIA alters the morphology of melanoma cells and inhibits thymidine incorporation in the DNA (41, 42). Adding MIA to melanoma cell lines determines cells rounding with the loss of intercellular links and growth inhibition. Consequent studies showed that in vivo, MIA’s function is opposite to the above mentioned one, its active secretion by the melanoma cells promoting invasion and metastasis (40).

MIA is a small protein coded by a gene on the long arm of chromosome 19 (19q13.27–13.33), made out of four exons (42). Through its translation, a precursor of 131 amino acids is formed and later through its cleavage, the mature protein is generated. Mature MIA consists of 107 amino acids and has a molecular weight of 11 kDa (36, 37). The first 24 aminoacids (signal peptide) from the precursor control MIA’s transport in the extracellular compartment (42, 39). At this level, MIA interacts with fibronectin, a glycoprotein from the extracellular matrix with a major role in intercellular adhesion, differentiation, raise and cell migration (46). Fibronectin contains multiple binding sites for integrins, membrane receptors that mediate cell attachment to surrounding tissues, but also to other structures, such as collagen, fibrin, heparan sulfate, and proteoglycans (47).

Data from the literature suggest MIA’s mechanism of action: four MIA molecules bind to specific sites of a fibronectin molecule, thus impeding integrin coupling with fibronectin (48). MIA sterically interferes with the fibronectin binding to α4β1 and α5β1 integrins, with subsequent inactivation of their function (49). All these MIA properties in vivo may explain its inhibitory activity in vitro: the protein impedes the attachment of melanoma cells to the culture dishes.

In vivo, MIA is synthesized both by normal tissue and malignant tumors, and it can also be detected in some other pathological states. In healthy tissue, MIA expression is limited to chondrocytes (40, 43). Its synthesis is initiated at the beginning of chondrogenesis, intrauterine, and continues during cartilage development and also during fractures’ healing (40). Some authors suggest that at this level MIA functions as a chemotactic factor for mesenchymal stem cells (40), thus favoring the chondrogenic phenotype and inhibiting the osteogenic one.

In vitro, in chondrocyte cell cultures, MIA expression is inhibited by retinoic acid adding, which explains why it was also called CD-RAP (cartilage derived retinoic acid-sensitive protein) (44).
During chondrogenesis, MIA serum values increase significantly. In a study published in 2004, Bosserhoff et al. stated that pregnant women after week 38, and children and teenagers under 17 have MIA serum values above median (45).

MIA may be identified in several tumor types through immunohistochemistry, Western-blot or RT-PCR, but with higher specificity for malignant melanoma and chondrosarcomas. It is also expressed, in variable quantities, in certain advanced stage adenocarcinomas: breast, gastric, pancreatic, colic, ovarian cancer (35, 50–54). MIA is not expressed in either normal skin or benign melanocytes (38, 39). It is revealed in small to moderate levels in the majority of benign nevi and in very large quantities in malignant melanomas, in the primary tumor and in secondary deposits as well (54). In malignant melanoma, the induction of MIA synthesis seems to be an early event in carcinogenesis, taking into account that all in situ tumors express MIA (40).

The active secretion of MIA by malignant melanocytes allows them to interrupt the connections with the extracellular matrix. In malignant melanoma, the number of contacts between tumor cells and fibronectin, laminin and tenascin drops with 30–50% comparing to normal cells (55). The detachment of malignant melanocytes from the extracellular matrix and the basal membrane increases their motility, thus favoring local invasion and metastases.

Another possible MIA’s mechanism of action is the inhibition of the cellular mediated immune antitumor response. As the immune system cells express α4β1 integrins, it was stated that MIA blocks the receptors on the leukocyte surface. In vitro experiments showed that MIA inhibits peripheral blood mononuclear proliferation and decreases the cytotoxic activity of lymphokine-activated killer cell (LAK) in a dose dependent process (39, 40). These data suggest that MIA may contribute to the immunosuppression, which is frequently seen in melanomas. MIA might also favor melanoma progression by inducing the expression of other tumor associated genes (40).

Taking into account that MIA is a protein made by malignant melanocytes in amounts correlated to tumor progression, it was investigated whether MIA serum values may represent a useful marker in the follow-up of patients with malignant melanoma.

In one of the first studies published by Bosserhoff et al. in 1997, serum MIA was detected using an ELISA test. The upper normal limit was established at 6.5 ng/ml by measuring MIA in the blood of 72 healthy donors. The determined values overcame the limit established in 13% of patients with stage I malignant melanoma, 23% in stage II, 81% in stage III and 97% in stage IV (35, 56).

These promising results encourage the initiation of other trials. Further studies raised the cut-off value in order to increase specificity and sensibility. Thus, considering pathological MIA serum values greater than 8.5 ng/ml, Farries et al. obtained a specificity of 100% in identifying relapse in stage III malignant melanoma, with a sensibility of only 46% (57). Relapse occurred in all patients with positive MIA in the next two years (57). Most authors believe that values higher than 14 ng/ml are pathologic. With this threshold, the specificity of
metastases detection varies between 96.8% (24) and 98.9% (58), with a sensibility between 50% (58) and 70% (24).

The serum concentration of MIA is directly proportional to the tumor mass, and this is why it may be used as a marker of the treatment response (56, 59). The surgical removal of metastasis or the answer to chemotherapy is accompanied by normalization of MIA values (58, 59). The persistence of high values, despite of the systemic treatment, suggests an unfavorable prognosis (62).

Some enthusiastic researchers affirm that MIA measurement may replace other staging methods (59) and could represent the ideal test for screening of the sentinel node (60). Post-surgery follow-up of patients in stages I and II can be made by determining MIA, which has a positive predictive value big enough to suggest occult metastases, clinically undetectable (66).

Before fully accepting this new marker, one must take into account several elements:

- MIA is positive in 17% of patients with malignant epithelial tumors;
- rheumatic diseases accompanied by joint destruction present raised values of MIA (even above 100 ng/ml): rheumatoid arthritis, psoriatic arthritis (61). MIA is probably being actively freed from chondrocytes during cartilage destruction;
- during chondrogenesis, MIA serum concentration is increased. This is the reason why it is not indicated in the follow-up of patients with less than 17 years old and of pregnant women having more than 38 weeks of pregnancy (45);
- in stages I and II of disease, MIA specificity is less than 67% (63);
- there are few studies which deny MIA usefulness in the follow-up of patients with malignant melanoma (64, 65), but they are performed on a small number of subjects.

LACTIC DEHYDROGENASE

LDH is an enzyme found in all body cells in different forms and proportions. There are four different classes, two of which are dependent on cytochrome c, and the other two are NAD dependent (nicotinamid adenin dinucleotid). In this review, we refer only to the LDH NAD(P) dependent form, which exists as five isozymes. Each isozyme is a tetramer composed of a combination of two different subunits: M and H. Their physical, catalytic and immunological properties differ, depending on the monomer type they consist of (67).

LDH2 (3HM) is produced by the reticuloendothelial system and it is usually the predominant form in serum. Elevated levels of LDH accompany any disease characterized by cell destruction (11): hemolysis, myocardial infarction-LDH1(4H), acute pancreatitis, hepatic cytolysis-LDH5(4M), meningitis, encephalitis. It is often used as a marker of tissue breakdown. Serum concentrations above the upper
admitted threshold were also documented in different cancer forms: Hodgkin lymphoma and non-Hodgkin lymphoma, multiple myeloma, lung cancer, prostate cancer, ovarian and testicular dysgerminomas. Elevated serum LDH levels reflect tumour cell turnover and tumour burden (19).

LDH is not useful in screening and diagnosis of malignant melanoma, as it is not specific for it. Nevertheless, its efficacy was shown in monitoring patients in advanced stages. TNM staging of malignant melanoma from 2002 introduced a new M category – M1c, which corresponds to any distant metastatic lesion accompanied by the raise of serum LDH. This initiative was motivated by the fact that in all published studies, after a multivariate analysis, the increase of serum LDH in stage IV patients was associated with an unfavourable prognosis (68).

The M1c subcategory was maintained in 2009 staging system (69). By analyzing patients with distant metastasis from the AJCC melanoma staging database in 2008, it was noticed that their evolution was strongly linked to serum LDH. Thus, stage IV patients with normal LDH had an overall survival rate of 65% at 1 year and 40% at 2 years, respectively. On the other hand, patients with LDH above the upper normal limit at the time of staging had 1 year overall survival of only 32% and a 2 year overall survival of 18% (69).

Various studies compare the characteristics of the three markers discussed in this paper and try to identify their role in each stage of the disease. The first one was published in 1999 by Deichmann et al. and it followed 71 stage IV malignant melanoma patients (70), aiming to determine the markers ability to discriminate between progressive and stable disease.

The study showed that all three markers were able to predict disease evolution, but MIA and S100B brought just as much information as LDH in metastatic disease (70).

The situation is different for localized melanoma. Garbe et al. surveyed 296 clinically disease free patients with stages II and III malignant melanoma for 19 months. During the follow-up, 41 of them developed metastatic disease (71). Table 2 shows the ability of the three markers to detect metastatic disease – both MIA and S100B have a higher accuracy than LDH in the diagnosis of newly occurring metastasis; therefore, they may be useful in the follow-up of disease-free stage II and III melanoma patients (71).
Table 2

Correlations between S100B, MIA, LDH in stage II-III MM

<table>
<thead>
<tr>
<th></th>
<th>Cut off</th>
<th>Sensibility (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
</tr>
</thead>
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<tr>
<td>S100B</td>
<td>0.12 µg/l</td>
<td>29</td>
<td>93</td>
<td>84</td>
</tr>
<tr>
<td>MIA</td>
<td>10.49 ng/ml</td>
<td>22</td>
<td>97</td>
<td>86</td>
</tr>
<tr>
<td>LDH</td>
<td>240 U/l</td>
<td>2</td>
<td>90</td>
<td>77</td>
</tr>
</tbody>
</table>

Adapted after (71)

CONCLUSIONS

There is no international agreement regarding the number and type of serum markers required in the follow-up of patients with malignant melanoma. From the multitude of proposed markers, LDH, S100B and MIA seem to be the most trustworthy.

S100B and MIA may be used in the screening of patients with stage II and III in order to identify occult metastases (71). Elevation of S100B in stages II and III melanoma patients might detect a haematogenic or lymphatic dissemination with several months before the onset of overt disease (72). For this reason, Bouwhuis et al. recommend serial determinations of S100B serum levels (73). Both S100B and MIA correlate with DFS and OS of melanoma patients, but simultaneous use of the two markers increases sensibility and maintains specificity (24).

The sensibility of S100B and MIA is high enough to predict the treatment response, which is not valid for LDH.

LDH is not recommended in routine monitoring of patients with stages I-III malignant melanoma, because it does not have the ability to detect occult metastases. On the other hand, it is compulsory to measure LDH in patients with metastatic melanoma in order to have a correct staging (stage IV M1c). In stage IV, LDH increase represents the most powerful prognostic determinant of diminished survival (74).

We believe that the evaluation of these three serum markers in combination with the established prognostic parameters (Breslow thickness, lymphnode status) might provide a better stratification of patients in new adjuvant therapeutic trials. For the future, the development of new predictive markers that can monitor response to therapy in patients who are clinically disease-free would substantially improve treatment and disease management.

REFERENCES


