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ARTICLE

Serum levels of S-100 protein and 5-S-cysteinyldopa as markers of melanoma progression

Teodóra BÁNFALVI, Katalin GILDE, Mariann BOLDIZSÁR,[1] Tibor KREMMER,[1] Szabolcs OTTÓ[2]

Departments of Dermatology, [1]Biochemistry and [2]Central Clinical Laboratory, National Institute of Oncology, Budapest, Hungary

Serum S-100 protein is widely used as a marker of melanoma and since 5-S-cysteinyldopa (5-S-CD) is a precursor of melanin its serum and urinary levels can reflect melanoma progression. In this study we examined the concentration changes of serum S-100 protein and 5-S-CD in 252 melanoma patients of different clinical stages. Serum samples were taken from 252 melanoma patients at 860 times, from June 1996 to July 1998. The serum S-100 protein was measured by the immunoluminometric assay, levels of 5-S-CD was determined by HPLC. The value of S-100 protein in patients with primary melanoma (0.11 μg/l) and in patients without symptoms (0.15 μg/l) ranged around the normal level (0.01–0.12 μg/l). There was a significant difference between the values of patients with or without symptoms. There was a similarly significant difference between the S-100 values of clinical Stage I (0.11 μg/l) and Stage III (2.91 μg/l) as well as between those of clinical Stage II (0.47 μg/l) and Stage III (2.91 μg/l), respectively. Analyzing the values of patients with symptoms we observed significant difference between the S-100 protein values of patients with primary tumor and those with solitary or multiple distant metastases. In case of 5-S-CD significant difference was found between clinical Stage I and III as well as clinical Stage II and III. Furthermore, there was a significant difference between the mean marker values of patients with primary tumor, lymph node, lung metastasis and clinical stage III. (Pathology Oncology Research Vol 5, No 3, 218–222, 1999)

Keywords: melanoma, S-100 protein, 5-S-cysteinyldopa

Introduction

The incidence of melanoma is increasing worldwide. The early detection of distant metastasis with tumor markers would be effective in improving the prognosis of the disease. S-100 protein is an acidic, calcium binding, low molecular weight, nervous system specific, cytoplasmatic protein which was isolated from bovine brain. Its function is unknown up to the present. Gaynor succeeded in demonstrating the presence of S-100 protein in human melanoma cell lines and its discovery was explained by the neuroectodermal origin of the melanocytes.[7] Early detection of S-100 was based on the complement fixation test.[8] Detection of the S-100 has become a widely used immunohistochemical method in the routine and differential diagnosis of malignant melanoma.[19] It was claimed that the expression of monoclonal anti S-100 beta activity indicates the vertical progression of the melanoma and the beginning of invasiveness.[5] Later, S-100 was detected in serum of patients with malignant melanoma, too.[6] Recently, serum S-100 measurement has been used as a marker of the disease.[2,9]

5-S-cysteinyldopa (5-S-CD) is a precursor of pheomelanin. It is detectable in urine and sera. In the literature, level of 5-S-CD has been analysed in healthy subjects, in patients with naevus pigmentosus and malignant melanoma. Serum 5-CD was found in a higher concentration in early summer than in early winter. Nowadays, serum level of 5-S-CD has been shown to be useful marker of melanoma progression.[1,21]

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Correspondence: Teodóra BÁNFALVI, MD, Department of Dermatology, National Institute of Oncology, Ráth György u. 7-9. H-1122, Budapest, Hungary; Tel: 36-1-224-8600, fax: 36-1-224-8620, E-mail: banfalvi@oncol.hu

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The aim of our study was to examine serum S-100 and 5-S-CD concentrations in different stages of malignant melanoma and to determine the values of these markers during and after treatment in patients with different clinical symptoms of malignant melanoma.

Materials and Methods

This study includes patients with malignant melanoma treated on the Department of Dermatology, National Institute of Oncology, Budapest, from June 1996 to July 1998. Serum samples were taken from 252 patients on 860 occasions. The clinical diagnosis of malignant melanoma and lymph node metastasis was proven by histology and the presence of metastasis was verified by various imaging techniques, like chest X-ray, abdominal US, MRI, CT and bone scintigraphy. Patients were divided into special groups according to their clinical symptoms, and the classical clinical stages. In the three stage system, Stage I included patients with primary tumor and local recurrence; Stage II – regional lymph node and in transit metastases; Stage III – dissemination to distant organs.[16] In clinical Stage I and Stage II blood sampling was made preoperatively. Several patients were selected for serial serum assays therefore, it may happen that due to the progression of the disease the same patient is included in more than one studied groups.

The S-100 concentration was measured by luminescence immunoassay in the Byk Laboratory of our Institute. The LIA-mat Sangtec-100 is a monoclonal two-site immunoluminometric assay (sandwich principle). Antibody-coated polystyrene tubes serve as solid phase. Sangtec-100 discriminates between the α1 and β-subunit. It measures the β-subunit of S-100 as defined by the three monoclonal antibodies SMST 12, SMSK 25 and SMSK 28. The coated antibody reacts with the S-100 protein present in patients samples, during the first incubation. During the second incubation the tracer antibody binds to the immobilised S-100. The tracer-S-100 complex bound to the tube wall in the immunological reaction is detected by a light reaction.[3,20] In our study the normal range of serum S-100 concentration was found between 0.010–0.120 µg/l.

5-S-CD was analysed by HPLC with electrochemical detection. The mobile phase contained 10 g/l phosphoric acid, 0,1 mmol/l Na_2EDTA and 7g/l methanesulfonic acid. Analyses were performed at 35 °C, at flow rate 0,7 ml/min.[15] The normal range of serum 5-S-CD ranged between 1–10 nmol/l.

The data were processed with MS-Excel program and analyzed with Tukey's post hoc test.

Results

During the 2-year study period 860 serum samples (860 S-100 protein, 765 5-S-CD assays) from 252 patients were examined. The results were analyzed according to the individual patient groups specified above.

S-100 protein

We have examined 124 symptom-free patients in average of 3 tests/patient. Symptom free means, that at the time of blood sampling they had no evidence of disease. However, these patients previously had primary tumor, regional lymph node metastasis, or other type of metastasis (in transit, skin metastasis, etc.). The metastasis was surgically removed or treated with chemotherapy. In this group the mean concentration of S-100 protein was 0.15 µg/l. Furthermore, we have analyzed 194 patients with clinical signs of melanoma (primary tumor, regional metastasis, solitary metastasis, multiple metastasis, 2–3 tests/patients). The mean serum S-100 protein concentration in this group was significantly higher compared to the symptom-free group($p<0.05$). (*Figure 1a*) The mean S-100 values of patients classified into classical clinical stages were also compared. In stage I (82 patients) the mean value of S-100 was measured at 0.11 µg/l. In stage II (80 patients) the mean marker concentration was proved to be 0.47 µg/l. while in stage III (131 patients) the mean S-100 level was 2.91 µg/l. Each stage differed from the other significantly ($p<0.05$)

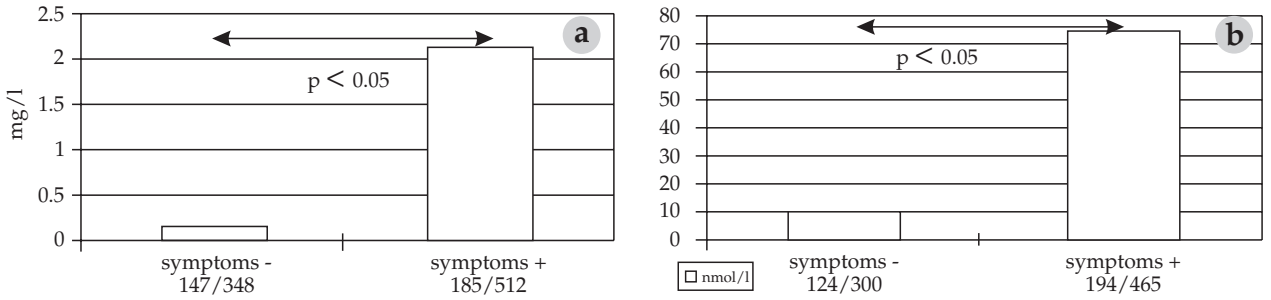

Figure 1 (a) *– S-100 protein levels in malignant melanoma patients with or without clinical symptoms. Symptom-free patients: 124 patients, 348 assays. Symptomatic patients: 194 patients, 512 assays. (**b**) 5-S-CD levels in malignant melanoma patients with or without clinical symptoms. Symptom-free patients: 124 patients, 300 assays. Symptomatic patients: 194 patients, 465 assays.*

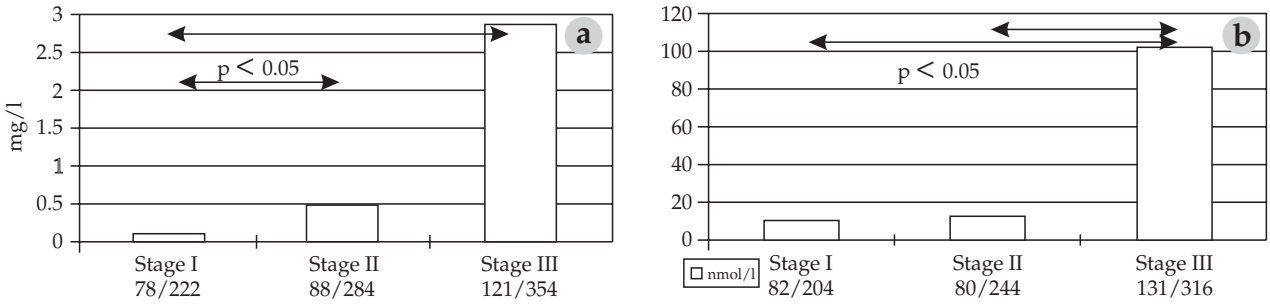

***Figure 2 (a)**. S-100 protein levels in melanoma patients at various clinical stages. Stage I: 82 patients, 222 assays. Stage II: 80 patients, 284 assays. Stage III: 131 patients, 354 assays. **(b)** 5-S-CD levels in melanoma patients at various clinical stages. Stage I: 82 patients, 205 assays. Stage II: 80 patients, 244 assays. Stage III: 131 patients, 316 assays.*

(*Figure 2a*). Finally, the S-100 protein values were also analyzed according to the stage of progression; primary tumor (38 patients), regional lymph node metastasis (43 patients, pulmonary metastasis (26 patients), hepatic metastasis (9 patients); multiple distant dissemination (64 patients) (*Figure 3a)*. The highest S-100 level was found in the multiple dissemination group followed by hepatic metastases and regional lymphatic ones. Interestingly, patients with lung metastases have a relatively low S-100 level.

5-S-cysteinyldopa measurements

124 patients without clinical symptoms of melanoma and 194 patients with clinical symptoms were analyzed for serum-5-CD levels. The mean 5-S-CD concentration proved to be significanly higher in the symptom+ group (74,6 nmol/l versus 10,21 nmol/l, respectively; p<0.05 (*Figure 1b*.)

In stage I (82 patients) the mean 5-CD value was 10,16 nmol/l which was higher in stages stage II (80 patients, 12,52 nmol/l), but increased extremely in stage III (131 patients, 103,2 nmol/l.) (*Figure 2*). Finally, the 5-S-CD values were also correlated to the progression of the disease. Compared to the primary stage, patients with regional lymph node involvement or lung metastasis exhibited a significantly higher 5-CD level (*Figure 3b*), however in the progression groups of liver metastasis or multiorgan involvement the 5-CD levels were 5–7 times higher (*Figure 3b*).

Discussion

S-100 is a calcium-binding protein with a molecular weight of 21.000 Dalton. It is composed of two subunits (alpha and beta) of homo- and heterodimeric forms. Physiologically, it is present in glia cells (beta-beta), in Schwann cells of the peripheral nerves (beta-beta), in the satellite cells of the ganglia of sympathetic plexuses, and in striated muscle and macrophages (alpha-alpha). It appears to play an important role in various cellular processes such as cell division and differentation. It has been reported to regulate the cytoskeletal system, such as microtubules and microfilaments in the presence of calcium. S-100 protein is a sensitive indicator of damage to the central nervous system.[6] Elevated serum S-100 concentration in disseminated melanoma was first described by Fagnart in 1988.[6] It was also a novelty that Guo, in 1995, qualified the S-100

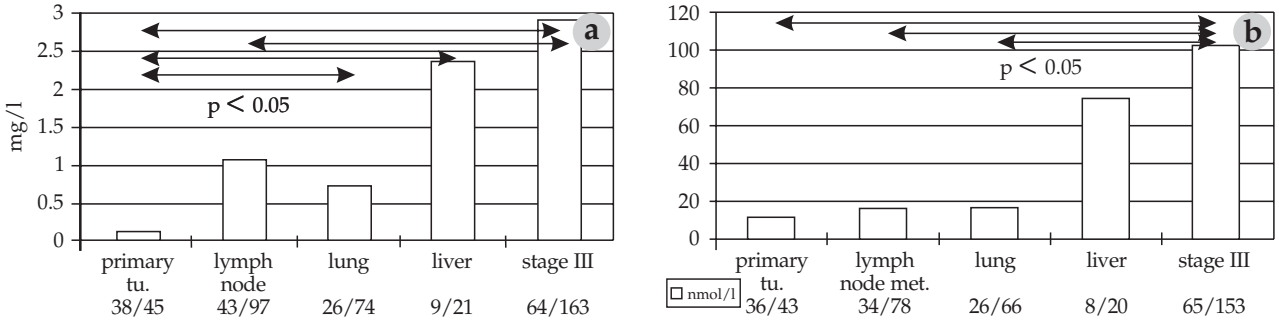

***Figure 3 (a)** S-100 protein levels in various stages of disease progression. Primary melanoma: 36 patients, 45 assays. Regional lymph node metastasis: 43 patients, 97 assays. Pulmonary metastasis: 26 patients, 74 assays. Hepatic metastasis: 9 patients, 21 assays. Multiple distant dissemination: 64 patients, 163 assays. **(b)** 5-S-CD levels in various stages of disease progression. Primary melanoma: 36 patients, 43 assays. Regional lymph node metastasis: 34 patients, 78 assays. Pulmonary metastasis: 26 patients, 66 assays. Hepatic metastasis: 8 patients, 20 assays. Multiple distant dissemination: 65 patients, 153 assays.*

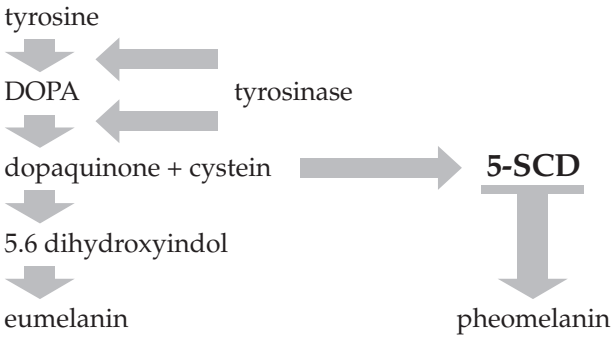

***Figure 4**. The mechanism of melanin biosynthesis.*

as a potentially useful adjunct in the clinical monitoring of patients with metastatic malignant melanoma during therapy. For screening and early diagnosis, however, the assay was not considered practical due to its low detection limits.[9] The potential clinical significance of S-100 as a circulating tumor marker has been investigated repeatedly. Due to its high specificity but low sensitivity Bonfrer et al suggest that the assay should be the subject of further studies.[3] On the other hand, Buer et al regard the S-100 protein to be a reliable prognostic marker in disseminated melanoma.[4] Hanson et al find the assay suitable for the screening of high risk patients and for the monitoring of therapy.[10] As to the latter, Henze et al also recommend it as a proper tool for the monitoring of therapy of patients with disseminated melanoma.[12] Results obtained with lipid-bound sialic acid and S-100 protein were compared and found that the increasing S-100 concentration correlated with the appearance of the recurrence and with survival, unlike the lipid-bound sialic acid.[18] Assays in the highest number were conducted by Schoultz et al who confirmed the use of S-100 beta levels as a prognostic factor independently of the clinical stage, either, provided that the cut-off values are proper.[22] It was strongly recommended to add S-100 as an adjunct to the conventional markers of clinical staging.[23]

5-S-cysteinyldopa is formed during biosynthesis of melanins by a tyrosinase dependent mechanism.[2] (*Figure 4*.) In patients with dysplastic naevus syndrome serum 5-S-CD levels did not differ from those in controls moreover, plasma 5-S-CD levels correlated with the spread of disease and were useful in distinguishing primary melanoma and Stage III and IV melanoma.[21] Its measurement has been suggested as diagnostic tool, indicating growth or malignant transformation in giant melanocytic naevi during childhood.[17] The seasonal variation in serum concentration of 5-S-CD was found to be higher in early summer and lower in early winter. The difference in the average levels was approximately twofold, but among the 240 samples studied no individual values exceeded the upper limit of normal value.[25] However, 5-S-CD levels were not elevated in patients, whose metastases were amelanotic.[13] It was reported that the urinary excretion of 5-S-cysteinyldopa was increased in patients with metastasis and that determination of 5-S-CD may have prognostic value in the presence of metastasis.[1] It was revealed that the increase of urinary 5-S-CD indicated the presence of metastasis and also provided prognostic information. It was also shown that in patients' serum and urine, 5-S-CD was elevated significantly earlier, and reflected melanoma progression better, than the physical examination and laboratory tests.[14] On the contrary, Hirai et al found elevated 5-S-CD level only in patients Stage IV.[13]

In this study we analyzed the S-100 and 5-S-CD levels in patients with malignant melanoma of different clinical stages and different symptoms. We observed significant differences in the level of both markers in case of symptomatic and asymptomatic patients. In the group of symptom-free patients deviation from the normal level was rather sporadic while in the group of symptomatic patients S-100 and 5-S-CD concentrations were significantly higher than the average normal values. Furthermore, significant differences could only be found between Stages I and III as well as between Stages II and III patients in case of 5-S-CD.

Most patients with primary tumor had a poor prognosis based on the general parameters of staging (Breslow thickness), although the S-100 and 5-S-CD values were around the normal range. However, significant differences were observed between the mean S-100 concentrations of patients with primary tumor and pulmonary metastases as well as between patients having primary tumors and hepatic metastases, respectively. Since ocular melanoma predominantly results liver metastasis, highly elevated S-100 level in a symptom free patient may suggest the presence of a metastasis. In case of 5-S-CD we found significant difference between the mean values for primary tumor, lymph node, lung metastasis and stage III, with a proper cut off value, it would be possible to differentiate between solitary and multiplex distant metastasis.

In summary, we can state that the mean value of S-100 and 5-S-CD assays show significant differences between clinical Stages I and III, and Stages II and III melanomas, respectively. In the symptomatic patients, disease progression was accompanied by increasing marker concentrations. A novel observation is the significant difference between the mean serum S-100 values of patients with primary tumor and pulmonary metastasis and those with primary tumor and hepatic metastasis. In case of 5-S-CD significant differences were found between the mean values of primary tumor, lymph node, lung metastasis and stage III. According to the high marker level in lung and liver metastasis, this marker might be useful to monitor patients with disease free ocular melanomas, to detect liver metastasis and high risk patients after removing the primary tumor to reveal lung metastasis.

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