Clinical significance of 5-S-cysteinyldopa monitoring in patients with malignant melanoma

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5-S-cysteinyldopa is a precursor of melanin. Its serum and urinary level can reflect melanoma progression. In this study the concentration changes of 5-S-CD in melanosas of all stages were examined, and patients were monitored during and after treatment. Serum samples were taken from 479 melanoma patients with different Stages on 1924 occasions, from June 1996 to December 2000. Levels of 5-S-CD were determined by HPLC.

The mean/median value of 5-S-CD in the Stage I-II-III patients and in the control group ranged around the normal level. Significant difference was found between Stage III and Stage IV as well as between patients with no evidence of disease and patients with tumor burden.

In Stage IV 69.7% sensitivity, 61.5% specificity and 79.3% positive predictive value were evaluated. The survival of patients with normal 5-S-CD level (n=235) differed significantly from cases having elevated marker concentration (n=244). One hundred eighty cases were regularly monitored on 1210 occasions. Recurrence development was noticed in 57 patients. In 24.6% of these patients suffering from any type of disease progression the increasing marker level preceded the detection of metastasis by conventional methods.

Serum 5-S-CD in Stage IV is sensitive enough to detect distant metastasis, and its predictive value has a great importance. It is a reliable marker for monitoring the clinical course in malignant melanoma.

Key words: Melanoma, monitoring, 5-S-CD.

In the treatment of melanoma patients a common problem is predicting their individual prognosis and detecting recurrence early. Nowadays there are no proper tools (except from some well-known histological parameters) to help in the solution. A cheap, easy detectable and well repeatable serum marker with high sensitivity and specificity could help in the management of melanoma patients. Unfortunately, publications on different serum markers (S 100 protein, NSE, lipid-bound sialic acid, RT-PCR, MIA) provide rather conflicting evidence for their usefulness [3, 5, 7, 9, 19, 22].

In our present work changes of serum 5-S-CD level were examined measuring it serially in different stages of melanoma. The aim of the study was to analyse the value of marker concentration during the patient follow up, the clinical significance of early detection of a recurrence and to establish whether elevation of 5-S-CD precedes or follow other evidence of tumor spread. To our knowledge this is the largest number of patients in evaluating the value of serum 5-S-CD and the first study about patient monitoring with serum 5-S-CD involving great patient number.

Material and methods

This study includes 479 patients suffering from malignant melanoma treated at the Department of Dermatology, National Institute of Oncology, Budapest, from June 1996 to December 2000. Serum samples were obtained on 1924 occasions. The diagnosis of malignant melanoma and lymph node metastasis was verified by histology [8]. For histopathological investigations paraffin embedded haematoxylin eosin staining method and immunohistochemistry ($100, HMB-45, MART-1) were generally used. The presence of metastasis was proved by various imaging techniques, like
chest X-ray, abdominal US, MRI, CT and bone scintigraphy. The age of patients ranged from 18 to 86 years (mean 56.7), including 260 males, and 219 females. Patients were clinically evaluated from one to four months related to their stages, by means of physical and radiological examinations.

At data analysis they were divided into stages according to AJCC Stages [6]. Stage I included patients with primary tumour (Breslow thickness < 1.49 mm); Stage II primary tumor (Breslow value > 1.50 mm); Stage III local recurrence, in transit and regional lymph node metastases; Stage IV dissemination to distant organs. Fifty-nine patients (with other skin diseases) were enrolled as control group. The values of different stages were analysed in details. Patients in Stage I-II were treated usually with immunotherapy (interferon alfa 2), in stage III after lymph node dissection with monochemoterapy (dacarbazine) or chemo-immunotherapy, in Stage IV with combined chemoterapy (combination of bleomycin-oncovine-lomustine-dacarbazin or platidiam-dacarbazine-bleomycin-tamoxifen) administering them in every four or six week.

Serum 5-S-CD concentration was determined at the Biochemical Department of our Institute by Merck-Hitachi HPLC consisting of an L-6200A Intelligent Pump, D-2500 Chromato Integrator, AS 2000A Autosampler and equipped with a LaChrom L-3500A amperometric detector (settings: +0.75 V, filter: 2 sec) [8]. Chromatography was performed on a Supelco LP-C18 Column (25 cm x 4.6 mm, 5 µ) using isocratic elution with a mobile phase (pH=2.2) containing 10 g/l phosphoric acid, 0.1 mmol/l Na₂EDTA and 7 g/l methanesulfonic acid. Analyses were performed at 35 °C, at flow rate 0.7 ml/min. The method was calibrated with 5-S-CD as external and alpha-metil-DOPA as internal standards. The specimens were stored at -20 °C. Samples were analysed within two months after their collection, without knowledge of clinical data. Normal range of 5-S-CD was between 1—10 nmol/l, on the basis of the literature.

Data were processed with an Excel programme. At statistical analysis p values <0.05 were considered to be significant. For statistical significance Mann Whitney test was used. The survival of patients with normal and elevated serum 5-S-CD concentrations was also analysed by Kaplan Meyer survival curve. Differences between curves were analysed using Cox-f test.

**Results**

During the 5-year study period 1924 serum samples from 479 melanoma patients were examined. In the first part of our work results were analysed according to AJCC Stages. The mean/median serum 5-S-CD concentrations of patients were compared with one another.

The patients included 21 initially in Stage I, 165 initially

| Table 1. Number of patients according to AJCC Stages, and according to their clinical symptoms. Mean-median 5-S-CD values of patients enrolled to the study |
|---|---|---|---|---|
| Number | Mean | Median | Range |
| Stage I | 21 | 11.34 | 9.77 | 1.50 | 28.20 |
| Stage II | 165 | 10.73 | 8.21 | 0.34 | 51.28 |
| Stage III | 130 | 13.05 | 9.55 | 0.62 | 67.13 |
| Stage IV | 163 | 77.89 | 13.92 | 0.14 | 998.58 |
| Control | 59 | 10.04 | 8.81 | 2.09 | 29.15 |
| Tumor free | 116 | 11.39 | 7.50 | 0.74 | 60.10 |
| With tumor burden | 363 | 41.55 | 10.63 | 0.14 | 998.58 |

**Patients**

Figure 1. Distribution of 5-S-CD concentrations of patients with no evidence of disease. 116/479 (24.2%) patients were tumor free at the beginning of the study. Mean/median concentration: 11.38/7.5 nmol/l. 47/116 (40.5%) disease free patients had elevated marker value.

Figure 2. Distribution of 5-S-CD concentrations in malignant melanoma patients with symptoms of melanoma. 363/479 (75.8%) patients had initially tumor burden, from which 198/363 (54.5%) cases exceeded the normal level. Mean/median value: 41.55/10.63 nmol/l.

in Stage II, 130 in Stage III and 163 in Stage IV. At the time of patients enrolling 116 patients were tumor free and 363 patients suffered from any sign of melanoma, independent
of Stages. The mean/median values, ranges are shown in Table 1.

Significant differences were disclosed between Stage III and Stage IV as well as between patients with no evidence of disease and symptomatic patients, furthermore between control group and Stage IV, just as between control group and symptomatic patients (p < 0.05).

It was surprising that 47/116 (40.5%) disease free patients had elevated marker value and only 198/363 (54.5%) symptomatic patients exceeded the normal level. Distributions of serum 5-S-CD of patients with NED (no evidence of disease) concentrations are shown on Figure 2. In eleven (9.5%) cases from 116 initially NED patients with elevated marker level were noticed further progression, from whom later 7/116 (6%) patients died. In 78/198 patients with tumor burden further progression developed and 75/198 (37.9%) were lost.

The survival of patients with normal 5-S-CD level (n=235, mean/median: 23.1/19.2 months, range: 1–55 months) differed significantly from 244 cases having elevated marker concentration (mean/median: 16.3/12.6 months, range: 1–55.2 months) (Fig. 3). Metastatic disease was noticed in 104/235 (44.3%) patients with normal 5-S-CD level at the first visit, and during follow up 84/235 (35.7%) patients were lost. In 140/244 (57.4%) cases with elevated marker concentrations at the first measure further progression occurred, from which 116/244 (47.5%) patients later died.

In patients with solitary and multiplex metastasis, elevated marker concentration was found in 32/60 (53.3%) patients with one metastatic site and 73/113 (64.6%) with multiple dissemination. Data of remaining cases could not be evaluated.

Sensitivity (significance of 5-S-CD in detecting metastasis), specificity (evaluation of the absence of metastasis) and positive predictive value (probability of progression in cases with elevated marker level) were calculated in all patients, in stage III and in stage IV. Data are shown in Table 2.

The second part of our study was the patients monitoring. One hundred and eighty patients (3 in Stage I, 93 in Stage II, and 84 in Stage III) were examined on 1210 occasions. In 14 (24.6%) of the 57 patients suffering from various type of recurrence (8 lung, 4 liver, 4 regional lymph node involvement, 2 brain, 2 extraregional lymph node and 2 skin metastases) the elevated serum 5-S-CD level was the first sign of the disease progression. The increasing 5-S-CD level preceded 1–3 months the detection of progression by conventional methods.

**Table 2. Sensitivity, specificity and positive predictive value calculated in all patients, in Stage III and Stage IV**

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Stage III</th>
<th>Stage IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>198/363 (54.5%)</td>
<td>46/91 (50.6%)</td>
<td>108/155 (69.7%)</td>
</tr>
<tr>
<td>Specificity</td>
<td>69/116 (59.5%)</td>
<td>24/39 (61.5%)</td>
<td>58/86 (62.3%)</td>
</tr>
<tr>
<td>Pos. predict. value</td>
<td>155/245 (63.3%)</td>
<td>32/61 (52.5%)</td>
<td>88/111 (79.3%)</td>
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</table>

**Discussion**

The incidence of melanoma is increasing worldwide. Early detection of distant metastasis and a reliable tool in therapy monitoring and during patient follow up would be able to improve the prognosis of the disease. The purpose of our study was to examine the serum 5-S-CD concentration changes in different stages of malignant melanoma, to analyse the value of this marker concerning the extent of metastasis, and to evaluate the clinical significance of patient’s monitoring in every stage.

5-S-cysteinyldopa, a precursor of reddish brown pheomelanin is produced in melanocytes and melanoma cells during biosynthesis of melamins by a tyrosinase dependent mechanism [4]. It is detectable in urine and sera. Increased urinary excretion of 5-S-cysteinyldopa was reported in 1979 in patients with metastasis, suggesting that the determination of 5-S-CD might have a prognostic value in the presence of metastasis [1]. In another study no significant increase was found in patients free of metastasis and in patients with regional lymph node involvement and in ame-
lanotic melanoma. The increase of urinary 5-S-CD indicated the presence of metastasis and also provided prognostic information [21]. The value of urine cysteinyldopa was also analysed in the follow up of disseminated malignant melanoma and it was recommended as a reliable and valuable marker to clinical follow up of melanoma patients with advanced disease. In this study 83% sensitivity of urine 5-S-CD was found in Stage IV, while our result with serum 5-S-CD was only 69.7%. In the previously mentioned publication fifty percent of patients with one metastatic site had increased serum level, but in this study 92 patients were only examined [15]. In our present work 53.3% elevated marker concentrations were found in patients with solitary distant metastasis, and 64.6% in patients with multiple organ involvement. The rising marker level correlated well with the tumor burden [13, 14]. With determination of a proper cut off value it would be perhaps possible to differentiate between solitary and multiplex distant metastases.

Seasonal variation in serum concentration of 5-S-CD (higher values were measured in early summer and lower ones in early winter) are well known, but the individual marker concentration never exceeded the upper limit of normal value [23]. That is why in our study the summer and winter values were not separated and analysed from this point of view, either in control, or in melanoma patients. Serum concentrations of 5-S-CD have also been examined in some publications in healthy subjects, in patients with nevus pigmentosus and malignant melanoma [20, 23].

Although, the clinical significance of urinary 5-S-CD level was more precisely analysed than its serum concentration, serum 5-S-CD has been recently recommended as a marker of the disseminated malignant melanoma. Some publications have already reported the usefulness of this marker for monitoring the clinical course of patients, and as a prognostic factor concerning survival time and death risk [10, 11, 16, 17]. Among other investigated melanoma serum and urine markers (circulating intercellular adhesion molecule-1, soluble interleukin-2 receptor level, 6 hydroxy-5-metoxyindol) serum 5-S-CD proved to be the most useful marker for estimation of disease progression, except from serum S 100 protein [10, 11, 12, 18]. In disseminated patients serum and urinary 5-S-CD were found to increase significantly earlier, and to reflect melanoma progression better, than the physical examination and other laboratory tests. Our present results correlated well with these publications. In our study 69.7% significance, 79.3% positive predictive value was found in Stage IV. In 14/57 (24.6%) patients with progressing disease the increasing marker level was the first sign of the disease spreading.

In some studies 5-S-CD level was elevated in patients whose metastases were amelanotic [11, 21, 24]. In our previous work the serum concentration of 5-S-CD in patients with amelanotic primary or metastatic melanoma were analysed [2]. From the examined 132 patients with clinical symptoms of melanoma in 48 patients (36%) the tumor marker level was under the normal upper limit. In 6% of cases, presence of an amelanotic primary tumor could be verified by histology, which improved the calculated significance (70%). In the present study the possible role of pigment production in calculation of significance was not analysed.

Presurgical elevated values returned to normal level after curative surgery in most of the patients, but it did not predict the development of subsequent metastases [10]. In the present work we did not distinguish data of patients before and after surgery.

In this study statistical analysis confirmed significant difference between marker concentrations of symptomatic and tumor free patients. In the group of disease free patients the deviation from the normal level was lower. In the group of patients with tumor burden, serum 5-S-CD concentrations were much higher than the average. The mean/median marker levels were also grouped according to AJCC Stages. Significant difference was found between Stages III and IV. Fifty seven percent of patients having elevated marker level at the time of enrolling died during monitoring. In 24.6% percent of cases suffering from metastasis development, the elevating marker level was the first sign of progression.

Summarising our results we can confirm that serum concentrations of 5-S-CD correlates with Stages. The marker has the greatest significance in Stage IV and has important positive predictive value. This study of 479 patients with all stages of melanoma might help to establish the place of serial 5-S-CD measurements in the management and follow up of melanoma.

References

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