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Use of serum 5-S-CD and S-100B protein levels to monitor the clinical course of malignant melanoma

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Abstract

5-S-Cysteinyldopa (5-S-CD) is a precursor of pheomelanin. S-100B protein is a low molecular weight, acidic, calcium binding, cytoplasmic protein. In this study, the concentration changes of serum 5-S-CD and S-100B protein in melanomas of all stages were examined in parallel and patients were monitored during and after treatment. Serum samples were taken from 478 melanoma patients on 1924 occasions. Of these, 180 cases were regularly monitored. Concentrations of 5-S-CD were determined by high performance liquid chromatography (HPLC), S-100B protein by immunoluminometric assay. The mean/median concentrations of 5-S-CD and S-100B protein in Stage I, II and III patients and in the control group ranged around the normal level. In Stage IV patients, 58.4/50.6% sensitivity, 100% specificity and 100/86.6% positive predictive values were obtained concerning S-100B protein and 5-S-CD, respectively. Recurrence was observed in 57/180 of the regularly monitored patients in Stages I, II and III. In 10/57 (17.5%) of these patients suffering from any type of disease progression increases in both marker levels preceded the detection of metastasis by conventional methods. We can confirm that changes in both marker concentrations correlated with the stages of the patient. The markers are most sensitive in Stage IV patients and also have a high specificity in these patients. In Stage IV melanoma patients, 5-S-CD and S-100B protein levels are independent significant prognostic factors. In almost one fifth of patients both marker levels increased before the detection of metastatic disease with other appropriate, routinely scheduled investigations. This study suggests that serial serum marker measurements in the management and follow-up of melanoma patients should be examined further.

Keywords: Melanoma; Marker; 5-S-Cysteinyldopa; S-100B protein

1. Introduction

Malignant melanoma is the most dangerous skin cancer. Nevertheless, early detection of a primary results in a good survival. Patients with regional lymph node or organ involvement show an unfavourable prognosis. In metastatic cases, the early detection of progression, especially distant metastases, is important. However, current methods are not sensitive enough to detect organ metastases at such early stages. A simple, cheap, non-invasive serum marker would improve the management of high-risk patients.

The purpose of this study was to evaluate the use of serum 5-S-cysteinyldopa (5-S-CD) and S-100B protein as a prognostic marker during patient follow-up. The clinical significance of detecting a recurrence early was also analysed. Furthermore, we asked whether an elevation of 5-S-CD and S-100B protein levels precedes or follows other evidence of tumour spread. To our knowledge, this is the largest study to examine the clinical value of serum 5-S-CD and S-100B protein levels.

2. Patients and methods

Patients treated at the National Institute of Oncology in the Department of Dermatology from June 1996 to December 2000 were included. There were 478

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consecutive patients (260 males, 218 females). 21 were Stage I patients, 165 Stage II, 130 Stage III and 162 Stage IV patients. The age of patients ranged from 18 to 86 years (mean 56.7 years). Melanoma was histologically-proven [1], metastases were verified by histology or radiographic procedures (chest X-ray, abdominal ultrasound, magnetic resonance imaging (MRI), bone scan, computed tomography (CT). Mean/median follow-up time was 22/15 months. Patients were examined clinically at one to four months according to their stage of disease by means of physical and radiological examinations. At data analysis, they were divided into stages according to the American Joint Committee on Cancer (AJCC) classification [2]. Stage I included patients with primary tumour (Breslow thickness <1.49 mm), Stage II: primary tumour (Breslow value $\geqslant 1.50$ mm), Stage III: local recurrence, in transit and regional lymph node metastases; Stage IV: dissemination to distant organs. Sixty-three patients (with other skin diseases) were enrolled as the control group. In our institute, we performed chest X-ray and abdominal ultrasound (US) every 6 months after removal of the primary or the lymph node metastases. Thereafter, we carried out these examinations only occasionally. Other procedures were only implemented following a complaint from the patient.

Unfortunately, due to technical reasons, we received the marker results some weeks or months after the blood sampling. In some cases of elevated marker levels, when the results were obtained earlier, we implemented a thorough clinical examination followed by the repetition of the marker test. Following repeatedly elevated marker levels, we performed further investigations.

Stage I-II patients were treated usually with immunotherapy (interferon alpha [2]). Stage III patients, after lymph node dissection, were given monochemotherapy (dacarbazine) or chemo-immunotherapy. In Stage IV patients received combined chemotherapy (combination of bleomycin–viacristine–lomustine–dacarbazine or cisplatin–dacarbazine–BICNU–tamoxifen) was administered every 4 or 6 weeks. 180 patients (3 Stage I, 93 Stage II and 84 Stage III) were examined on 1210 occasions where serum S-100B and 5-S-CD protein levels were detected in parallel.

3. Method

The S-100B protein concentration was measured by the luminescence immunoassay in the tumour marker laboratory in our Institute. The LIA-mat Sangtec-100 is a monoclonal two-site immunoluminometric assay (sandwich principle). Antibody-coated polystyrene tubes serve as the solid phase. Sangtec-100 discriminates between the A1 and B subunit. Sangtec 100 measures the B subunit of the protein S-100 and was defined by three monoclonal antibodies SMST 12, SMSK 25 and SMSK 28. The coated antibody reacts with S-100 that is present in patient samples or standards during the first incubation. Unbound material is removed by washing. During the second incubation, the tracer antibody binds to the immobilised S-100. Non-reacted tracer is removed by a second washing step. The anti-S-100 tracer conjugates consist of an antibody and a covalently bound isoluminol derivative. The tracer-S-100 complex bound to the tube wall in the immunological reaction is detected by a light reaction. The light signal measured in relative light units is directly proportional to the amount of S-100 present in the standard and sample. The normal range in our study was between 0.010 and 0.120 μg/l. A cut-off level of 0.18 μg/l was used.

Serum 5-S-CD concentration was determined at the Biochemical Department of our Institute by Merck-Hitachi high performance liquid chromatography (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 s). Chromatography was performed on a Supelcosil LC-18 Column (25 cm×4.6 mm, 5 μ) using isocratic elution with a mobile phase (pH = 2.2) containing 10 g/l phosphoric acid, 0.1 mol/l Na_2 ethylene diamine tetra acetic acid (EDTA) and 7 g/l methanesulphonic acid. Analyses were performed at 35 °C, at a flow rate 0.7 ml/min. The method was calibrated with 5-S-CD as external and alpha-metil-DOPA as internal standards [3]. Data from the literature suggests that the normal range of 5-S-CD is between 1 and 10 nmol/l. Results were calculated using a 15 nmol/l cut-off value. All assays were carried out under the same conditions. Specimens were stored at −20 °C. Samples were analysed within 2 months after their collection, without knowledge of the clinical data.

3.1. Statistical analysis

Data were processed with an Excel program. *P* values of <0.05 were considered significant. For statistical significance, the Mann–Whitney test was used. The survival of patients with normal and elevated serum concentrations was also analysed by Kaplan–Meier survival curve. Differences between the curves were analysed using Cox-*f* test. We carried out a Cox univariate analysis taking into account the age and sex of the patients, the localisation of the melanoma, the histological type and Breslow values of tumours, serum S-100B protein and 5-S-CD in early melanoma cases. In advanced melanoma cases, we included in the analysis the serum 5-S-CD, S-100B protein and lactate dehydrogenase (LDH) concentrations, as well as the number (single or multiple organ involvement) and site (lung, liver, bone, brain, skin, lymph node node or multiple) of metastasis.

4. Results

Although it was a prospective study on a defined patient group, the analysis was retrospective. In the first part of the study, mean-median values, ranges, specificity, sensitivity and positive predictive values were calculated in all patients (Tables 1–2). Statistical analysis confirmed significant differences between serum 5-S-CD concentrations of Stage III and Stage IV patients. Significant differences were also found between the mean marker values of patients with no evidence of disease and those of patients with tumour burden.

Significant differences were found between mean S-100B protein concentrations of Stage II and III patients, as well as between Stage II and IV patients, and between Stage III and Stage IV patients. Similar significant differences were observed between patients with no evidence of disease and patients in Stage III and Stage IV ($P < 0.05$).

On the other hand, 10/115 (8.7%) and 27/115 (23.5%) false-positive results were found in the 5-S-CD and S-100B protein analyses, respectively. False-negative results were observed in 232/363 (63.9%) and 233/363 (64.2%) of all of the examined patients.

Significant differences were observed between the survival of patients whose S-100B protein concentrations were initially under 0.18 μg/l (97/335 cases were lost, their mean/median survival was 15/13.1 months, range 1–47.1 months) and of patients with a marker level above the cut-off value (100/143 patients died, mean/median survival was 6.7/4.4 months, range 1–41 months). Similarly, significant differences were observed between patients with normal and increased 5-S-CD values (117/321 lost cases with normal marker level, mean/median survival 13.5/10.9 months and 82/157 deceased patients above the cut-off level, with a months mean/median survival time of 7.4/5.3) (Fig. 1).

Initially, 157 (6 Stage I, 31 Stage II, 42 Stage III and 78 Stage IV) patients had increased 5-S-CD levels above the cut-off value, and in 143 cases (2 Stage I, 13 Stage II, 38 Stage III and 90 Stage IV), the S-100B protein concentration exceeded 0.18 ug/l. In 77 patients (2 Stage I, 1 Stage II, 17 Stage III and 57 Stage IV), both marker concentrations were elevated in parallel. The survival curves of these patients are shown in Fig. 2.

In patients with initially elevated S-100B protein and normal 5-S-CD levels (11 Stage II, 21 Stage III and 33 Stage IV) further progression was noticed in 48/65 (73.8%) cases. In contrast in patients with increased 5-S-CD and normal S-100B concentrations, only 35/80 (43.8%) cases progressed.

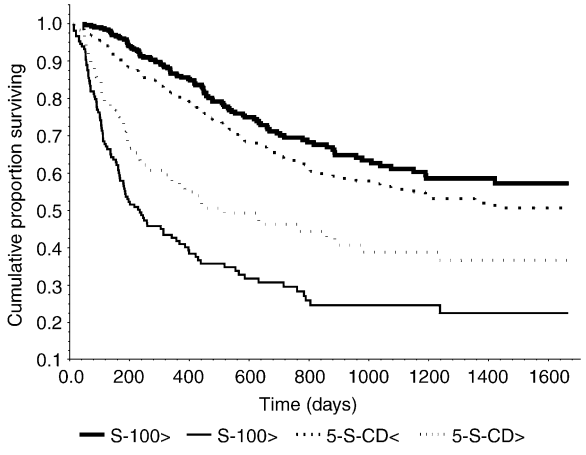

Fig. 1. The survival of patients with marker concentrations under or above the cut-off values (5-S-CD: 15 nmol/l, S-100B protein: 0.18 μg/l).

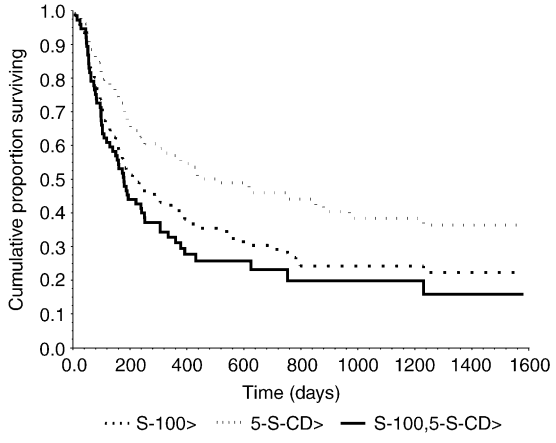

Fig. 2. Comparison of the survival of patients with initially elevated serum 5-S-CD and S 100B protein levels and with parallel increased marker levels. The survival was the shortest in the last group.

Table 1
Mean/median serum of concentrations 5-S-CD and S-100 protein in 478 patients univariate analysis

	S-100B protein				5-S-CD		
	Number	Mean	Median	Range	Mean	Median	Range
Stage I	21	0.05	0.03	0.01–0.19	11.34	9.77	1.50–28.22
Stage II	165	0.13	0.06	0.01–7.06	10.72	8.21	0.34–51.28
Stage III	130	0.48	0.09	0.01–23.05	13.05	9.55	0.63–67.13
Stage IV	162	2.07	0.24	0.01–103.59	77.90	13.92	0.14–998.6
Tumour-free	115	0.08	0.05	0.01–0.94	10.04	7.50	0.74–60.10
With tumour burden	363	1.41	0.11	0.01–103.59	41.55	10.63	0.14–998.6

Table 2
Specificity, sensitivity and positive predictive value of serum 5-S-CD and S-100B protein levels

	All (%)	Stage IV (%)
S-100B protein		
Sensitivity	36.8	58.4
Specificity	74.8	100.0
Positive predictive value	92.9	100.0
5-S-CD		
Sensitivity	35.80	50.60
Specificity	76.50	100.0
Positive predictive value	82.80	86.60

A Cox univariate analysis in early melanoma cases showed the Breslow thickness and the histological type of tumours to be the most relevant independent factors to predict survival. In advanced melanoma, serum 5-S-CD levels, S-100B protein levels, LDH levels, the number and site of metastasis proved to be independent significant prognostic factors (Table 3). In Stage IV patients, a strong correlation was found between concentrations of LDH, S-100B protein and 5-S-CD.

One-hundred and eighty patients (3 Stage I, 93 Stage II and 84 Stage III) were regularly monitored on 1210 occasions and serum S-100B protein and 5-S-CD were detected in parallel. Mean median values and ranges are shown in Table 4.

In 14 (24.6%) of the 57 patients suffering from any type of recurrence (3 regional lymph node involvements, 3 lung, 2 liver, 2 brain, 2 skin and 2 extraregional lymph node metastases), the elevated 5-S-CD level was the first sign of disease progression. In 13/57 (22.8%) of patients with progressive disease (4 lung, 2 liver, 1 skin, 1 brain, l regional and 3 extraregional lymph node metastases, 1 multiple organ involvement), high S-100B protein concentrations were measured before the detection of metastasis by conventional methods. In 10/57(17.5%) cases (4 lung, 1 skin, 1 brain, 1 liver metastases, 1 regional and 2 extraregional lymph node involvements), levels of both markers increased in parallel. False-negative results (ie positive in other investigations, but marker-negative) were found in 44 cases regarding S-100B protein and in 43 patients regarding 5-S-CD. The rising 5-S-CD and S-100B protein levels preceded by 1–3 months the detection of disease progression. (In the remaining cases, progression developed without any elevation of the markers.)

Table 3
Cox's proportional hazard analysis with respect to disease-specific survival

	β	Standard error	*t*-Value	Relative risk	Wald statistic	*P* value
Stages I–II						
Age	5.86E-05	4.36E-05	1.343291	1.000059	1.804431	0.179187
Sex	0.221524	0.416766	0.531531	1.247977	0.282525	0.595054
Localisation	0.258052	0.153346	1.682816	1.294407	2.831868	0.092421
Histological type	0.087494	0.028989	3.01816	1.091436	9.109292	0.002545
Breslow	0.284853	0.120893	2.356243	1.329567	5.551883	0.018467
5-S-CD	−0.006598	0.025612	−0.257605	0.993424	0.066361	0.796713
S-100B	−0.059109	0.481815	−0.12268	0.942604	0.01505	0.902361
Stage IV						
LDH	0.000185	5.03E-05	3.68449	1.000185	13.57547	0.00023
S-100B	0.026485	0.006652	3.981673	1.026839	15.85372	6.86E-05
5-S-CD	0.0023	0.00429	5.358766	1.002303	28.71637	8.44E-08
Metastases (number)	0.604538	0.12503	4.835129	1.830407	23.37847	1.34E-06
Metastatic (site)	0.080176	0.029131	2.752306	1.083478	7.575189	0.005921

Table 4
Mean/median values and ranges of serum 5-S-CD and S-100 protein concentrations of the 180 monitored patients

	Mean	Median	Range
5-S-CD			
Stage I–II	10.8	8.36	0.34–49.08
Stage III	16.49	13.43	0.67–67.13
S-100 protein			
Stage I–II	0.09	0.08	0.01–0.53
Stage III	0.15	0.08	0.01–23.04

5. Discussion

A common problem in treating melanoma patients is predicting their individual prognosis. The early detection of a recurrence might influence the results of treatment and could improve survival. Serum markers could potentially be used to follow disease-free patients and evaluate the response to therapy. The purpose of our work was to detect serum 5-S-CD and S-100B protein concentrations in patients with different stages of malignant melanoma, to examine the changes of both marker levels during patient follow-up and to evaluate the value of this diagnostic tool in the early detection of a recurrence.

Although many researchers have investigated the potential clinical significance of the S-100B protein as a circulating tumour marker, recent publications on serum markers of melanoma provide rather conflicting evidence with regard to its usefulness. In most studies, the assay was not considered practical due to its low detection limits [4,5]. On the other hand, elevated levels have been seen in patients with metastatic disease [6]. The marker is regarded to be a reliable prognostic marker in disseminated melanoma [7]. Serum concentrations obtained with lipid-bound sialic acid and S-100B protein have been compared previously [8]. An increasing S-100B concentration was found to correlate with recurrence and with survival and it was found to be superior versus lipid-bound sialic acid [9]. S-100B protein has proved superior compared with neurone-specific enolase and Mia [10–12]. Until now, Martenson has conducted assays involving the highest number of patients (1007 consecutive patients). Her observations seem to confirm the use of S-100 beta protein level as an independent factor in clinical stages II and III patients [13]. In another publication, it was strongly recommended that S-100B protein level be used as an adjunct to the conventional tools of clinical staging [14]. The calculated sensitivity and specificity ranged between 37 and 80% versus 50–80% in different studies and the values depended on the stage, on the investigated patient number and on the cut-off value used [14,15]. In our study, a 58.4% sensitivity and 100% specificity was calculated for Stage IV patients with regard to S-100B protein results.

5-S-CD, a precursor of reddish brown pheomelanin, is produced in melanocytes and melanoma cells, during biosynthesis of melanin's by a tyrosinase-dependent mechanism [16]. It is detectable in urine and sera. The value of urine cysteinyldopa measurements was analysed during follow-up of disseminated malignant melanoma patients and it was recommended as a reliable and valuable marker for the clinical follow-up of melanoma patients with advanced disease. In this study, 83% sensitivity was found in Stage IV patients, in our study this was only 50.6%. However, in the previously mentioned publication only 92 patients were examined [17].

Although the clinical significance of the urinary 5-S-CD level was more precisely analysed, than its serum concentrations, serum 5-S-CD has recently been recommended as a marker of disseminated malignant melanoma. Some publications have already reported it to be a useful marker for monitoring the clinical course of patients, and to be a prognostic factor with respect to survival time and death risk [18–21]. Among other investigated serum and urine markers for melanoma (circulating intercellular adhesion molecule-1, soluble interleukin-2 receptor level, 6 hydroxy-5-methoxy-indole), serum 5-S-CD has proved to be the most useful marker for disease progression with the exception of serum S-100B protein [22–24]. It has been observed that in disseminated patients serum and urinary 5-S-CD increased significantly earlier, and reflected melanoma progression better than physical examination and other laboratory tests. Our present results correlate with these data. In this study, statistical analysis confirmed significant differences between serum 5-S-CD concentrations of symptomatic and tumour-free patients. Significant differences were also found between Stage III and IV patients. A 50.6% sensitivity and 86.60% positive predictive value was calculated in Stage IV patients. Fifty-two per cent of patients having elevated marker levels at the time of enrolling died during the subsequent monitoring. In 14/57 (24.6%) patients with disease progression, the increasing marker level was the first sign of disease spread.

Seasonal variation in serum concentrations of 5-S-CD (higher values were measured in early summer and lower ones in early winter) is well known, but no individual concentrations exceeded the upper limit of the normal value [25]. That is why, in our work the summer and winter values were not analysed separately, either in the control or in the melanoma patients.

In some studies, the 5-S-CD level was elevated in patients whose metastases were amelanotic [18,23], although normal levels of 5-S-CD have also been observed in metastatic amelanotic melanoma patients, [19]. In our previous work, the marker concentration in patients with amelanotic primary or metastatic melanoma was analysed [21]. There was an improvement of 6% after the exclusion of the amelanotic cases. The possible role of pigment production in the calculation of significance was not analysed.

According to some authors, presurgical elevated values of 5-S-CD returned to normal after curative surgery in most patients, but did not predict the development of subsequent metastases [22]. In our study, we did not distinguish the data of patients before and after surgery.

In two previously published studies, the possible significance of an elevated marker level during the patient's

follow-up (214 and 466 consecutive patients, 496 and 666 samples) was analysed. In the first publication, the elevated S-100B protein level was the first sign of the recurrence in 19.5% of the metastatic patients. In the second work, S-100 protein was found to be a specific and sensitive tumour marker in melanoma patients, which preceded other evidence of melanoma recurrence [26,27].

In our study, the serum S-100B protein was measured in 180 melanoma patients with Stage I, II and III cancer. The S-100B level was determined on 1210 occasions. In 10/57 (17.5%) cases the levels of both markers increased in parallel, predicting haematogenous dissemination in nine patients. During the follow-up of patients physicians have difficulty in detecting early this type of recurrence.

Both markers had significant positive predictive value. In 22.8% and 24.6% of patients S-100 and 5-S-CD levels, respectively, could predict recurrence prior to the detection of a metastasis by conventional methods.

Summarising our results, we can confirm that in Stage IV patients serum 5-S-CD and S-100B protein levels are independent significant prognostic factors. In contrast, in Stage I-II patients, these markers will add no further information to the established prognostic factors. We can state that they are quite sensitive at detecting distant metastasis, and have an important predictive value. Alterations in the serum levels of these markers during follow-up, especially an observed elevation before recurrence, might require re-staging of the patient on changes in therapy. They are both reliable tools for monitoring the clinical course in progressing malignant melanoma, especially when used in parallel.

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