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ARTICLE

Comparison of prognostic significance of serum 5-S-Cysteinyldopa, LDH and S-100B protein in Stage III-IV malignant melanoma

Teodóra BÁNFALVI,[1] Mariann BOLDIZSÁR,[2] Maria GERGYE,[3] Katalin GILDE,[1] Tibor KREMMER,[2] Szabolcs OTTÓ[3]

[1]Department of Dermatology, [2]Biochemistry and [3]Central Clinical Laboratory, National Institute of Oncology, Budapest, Hungary

5-S-cysteinyldopa is a precursor of pheomelanin. S-100B protein is a low molecular weight, acidic, calcium binding, cytoplasmatic protein. LDH was defined as the most important serum parameter in disseminated melanoma. The aim of the present study was to compare the prognostic values of serum 5-S-Cysteinyldopa, S-100B and LDH concentrations in Stage III-IV melanoma patients. Serum samples were taken from 179 Stage III-IV melanoma patients at diagnosis. Serum 5-S-CD concentrations were determined by HPLC, S-100B protein by immunoluminometric assay while LDH by UV kinetic method. The mean/median concentrations of LDH, S-100B protein and 5-S-CD in Stage III patients ranged around the normal level. In Stage IV, the markers ranked as S100B = 5-S-CD > LDH for sensitivity, S-100B > LDH > 5-S-CD for specificity and LDH = S100B = 5-S-CD for positive predictive value, respectively. Furthermore, mean marker concentrations of patients with progressive disease differed significantly from nonprogressive cases (when staging categories have been disregarded). Survival analysis indicated, that the initially elevated LDH and S-100B level in Stage IV disease predicts comparably short survival. Results of our study suggest that these serum marker values correlate well with Stages and disease progression. In Stage IV melanoma, the markers had appropriate sensitivity, high specificity as well as important positive predictive value. Among the studied serum markers S-100B protein and LDH proved to be similarly reliable in respect to the clinical outcome. (Pathology Oncology Research Vol 8, No 3, 183–187)

Keywords: melanoma, progression, 5-S-Cysteinyldopa, LDH, S-100B

Introduction

Recently, many efforts had been made to analyse the potential clinical significance and the possible relationship of disease progression and circulating markers in malignant melanoma. Serum LDH, S-100B protein and 5-S-Cysteinyldopa concentrations were elevated in the majority of metastatic melanoma patients, but their sensitivity is still questionable. Patients with distant metastasis have an unfavourable prognosis and will die. Exact staging would be important for the management and prognosis, as well. Elevated marker level usually correlates with survival and predicts the outcome of advanced disease. A simple, cheap, sensitive and specific, noninvasive serum marker would improve the possibilities of the monitoring of high risk patients. Recently, among other investigated serum markers (5-S-CD, MIA, tyrosinase, NSE and cytokines) S-100B protein and LDH are in the highlight of interest.[2,5,11,13,16,22,25,27,29] S-100B protein, member of a family of 19 proteins, was first isolated from bovine brain in 1965. Lactate dehydrogenase has been described as leading blood parameter in patients with melanoma metastases and now it is involved into the new UICC staging system. 5-S-cysteinyldopa, a precursor of pheomelanin, is produced in melanocytas and melanoma cells, and is detectable in urine and sera of patients. Several studies reported on promising results concerning these markers, but according to our knowledge this is the first study in comparing the clinical significance of 5-S-CD, LDH and serum S-100B protein in Stage III-IV melanoma patients.

The purpose of this study was to evaluate and compare the possible prognostic role of serum LDH, S-100B pro-

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Correspondence: Teodóra BÁNFALVI, Department of Dermatology, National Institute of Oncology, Ráth György u.7-9. Budapest, H-1122 Hungary. Tel: 36 1 224 8600, fax: 361 224 8620, E-mail: banfalvit@freemail.hu

tein and 5-S-Cysteinyldopa (5-S-CD) levels and to test their ability to discriminate between progressive and non-progressive disease in a large number of patients suffering from metastatic melanoma.

Materials and Methods

Patients

This study includes data of patients from June 1996 to January 2001 treated at the National Institute of Oncology, on the Department of Dermatology. A total of 179 patients (85 males, 94 females) were involved (37 in Stage III, 142 in Stage IV). The age of patients ranged from 22 to 88 years (mean 59.8). Median follow up time was 15 months. Melanoma was proven by histology,[8] while metastases have been verified by histology or other imaging procedures (chest X-ray, abdominal ultrasound, MRI, bone scan, CT). Patients were divided into AJCC Stages.[7] Stage III included patients with local recurrence, in transit and regional lymph node metastases; Stage IV cases with dissemination to distant organs. Sixty three patients (with other skin diseases) were enrolled as control group.

Patients in Stage III after lymph node dissection were treated usually with monochemotherapy (dacarbazine) or chemo-immunotherapy (dacarbazine and interferon alfa). In Stage IV disease combined chemotherapy (combination of bleomycin-oncovine-lomustine-dacarbazin or platidiam-dacarbazine-BCNU-tamoxifen) were administered in every four or six week.

Method

The S-100B protein concentration was measured by luminescence immunoassay following the kit instructions (LIA-mat Sangtec-100, Byk Lab,Budapest, Hungary).[2] The normal range in our study was between 0.010 - 0.120 µg/l and 0.12 µg/l and used as cut-off.

Serum 5-S-CD concentration was determined by a 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).[14] Normal range of 5-S-CD was between 1-10 nmol/l, in accordance with the literature. Results were calculated with 10 nmol/l cut off value.

LDH was determined by optimized UV kinetic method (Reanal Finechemical Co). As cut off value 460 U/l was used.

Assays were made under same conditions every time. Specimens were stored at -20C. Samples were analysed within two months after their collection, without knowledge of clinical data.

Statistical analysis

Data were processed with MS-Excel statistical programme using Sudent t-test or Mann-Whitney method. values < 0.05 were considered to be significant. The survival of patients with normal and elevated serum marker concentrations was illustrated by Kaplan Meyer survival curve and analyzed by using Mantel Cox test (Statsoft Inc.).

Results

Mean-median serum marker values and ranges at the diagnosis were calculated for the Stage III and IV melanoma patients and were demonstrated on ***Table 1.*** Statistical analysis confirmed significant differences of mean serum concentrations of LDH, 5-S-CD, S-100B protein between Stage III and Stage IV disease.

Specificity, sensitivity and positive predictive values were also calculated on the basis of the number of disease free patients and of patients with symptoms. In Stage III 5-S-CD was superior with 60 % sensitivity, 91.6 % specificity and 93.8 % positive predictive value in comparison to any other markers. In Stage IV for sensitivity S 100B and 5-S-CD were equally superior, for specificity S 100B was the best while for positive predictive value all the three markers were equally good (***Table 2***).

Initially, 118 (21/37 in Stage III and 97/142 in Stage IV) patients had increased 5-S-CD levels, 71 cases (3/37 in Stage III and 68/142 in Stage IV) had LDH levels above the cut off value, and in 107 patients (11/37 in Stage III and 96/142 in Stage IV) the S-100B protein concentration exceeded the upper limit of normal value (0.12 µg/l). In 46/179 patients (1/37 in Stage III and 45/142 in Stage IV)

Table 1. **Comparison of serum marker concentrations of melanoma patients in Stage III and IV**

	St-III (n=37)			*St-IV (n=142)*		
	mean	*median*	*range*	*mean*	*median*	*range*
LDH (U/l)	359	349	220–655	612*	447	104–4585
S100B (µg/l)	0.35	0.07	0.01–5.00	1.41*	0.27	0.01–24.49
5-S-CD (nm/l)	13.91	1.04	0.65–34.67	71.42*	13	0.14–825

*P<0.05 (Student t-test)

Table 2. **Characteristics of serum markers in Stage IV melanoma patients**

	Sensitivity (%)	*Specificity (%)*	*Positive predictive value (%)*
LDH	48.5	83.3	98.5
S 100B	70.5	100	100
5-S-CD	69.1	50	96.9

all the three marker concentrations elevated parallel. Twenty eight patients from 179 did not have any of the markers increased (11 in Stage III, and 17 in Stage IV).

During the follow up period significant difference was observed between the survival of patients whose S-100B protein concentrations were initially under or above the cut off value (25/72 and 84/107, respectively: $p<0.05$). Similarly significant difference was found between patients with normal and increased 5-S-CD values (32/61 and 77/118 respectively, $p<0.05$). Forty eight from 128 patients having initially normal LDH level died at the end of the study while only 10/71 patients were alive from cases with elevated LDH level. The calculated median survival data proved to be 4.6 month. Kaplan-Meyer analysis of the survival of patients with elevated versus normal marker levels indicated significant differences in case of all the three markers, with the shortest survival of patients with elevated S 100B or LDH levels ($p<0.05$, ***Figure 1***)

Furthermore, significant difference was found between survival of patients with all the three elevated LDH, 5-S-CD and S-100B protein levels and of patients with normal marker concentrations, either. LDH was found to be the most relevant parameter with the shortest survival time. From the 46/179 patients with three elevated marker only three out of 46 patients were alive at the end of the follow up time and their median survival was only 3.4 month.

Last, we have established two patient groups based on the outcome of the disease irrespective of the initial Stage. The first group consisted 127 patients with progressive disease (local recurrence, metastasis or death during the follow up) while the second of 52 cases with nonprogressive disease (patients were disease free at the end of the follow up). We found that the serum concentrations of all the three markers was found to be significantly elevated in patients with later progression compared to the non-progressing cases (***Figure 2***).

Discussion

Serum 5-S-Cysteinyldopa, S-100B protein and LDH used to be elevated in the majority of patients with metastatic malignant melanoma and correlate well with the disease progression. However, their significance in clinical practice is not clear enough. The increasing LDH concentration does not necessarily indicate liver involvement in progression of melanoma. LDH was reported to have prognostic value for several tumors and reflected tumor cell turnover and tumor burden[1]

In most studies for screening and early diagnosis, the S-100B protein assay was not considered practical for its low detection limit.[6,13] On the other hand, the marker was regarded to be a reliable prognostic marker in disseminated melanoma and was strongly recommended as an adjunct to the conventional tools of clinical staging.[27] S-100 B protein was proved to be superiority compared to NSE and MIA.[16,26,29] Until now, Martenson has conducted assays in the highest number (1007 patients). Her observations seem to confirm the use of S-100B protein level as an independent factor in the clinical stage II and III.[22] The calculated sensitivity and specificity ranged between 37-80% versus 50-80% in different studies and data depended strongly on the stage, the number of the investigated patients and the cut off value.[1,6]

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.[20] Some publications have already reported on the usefulness of this marker for monitoring the clinical course of patients, and found to be a good prognostic factor concerning the survival time and death risk. It was previously published that in the serum and urine of progressing patients, 5-S-CD concentrations increased significantly earlier and reflected melanoma progression better than the physical

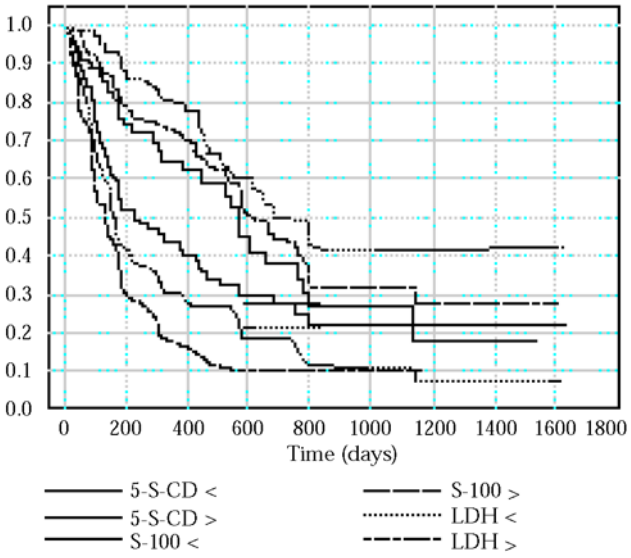

Figure 1. *Kaplan-Meyer survival curves of Stage III-IV melanoma patients according to the level of serum markers (normal< or elevated>). Significant differences ($p<0.05$) were found between survival of patients with normal and elevated levels in case of all the three markers (Mantel-Cox test).*

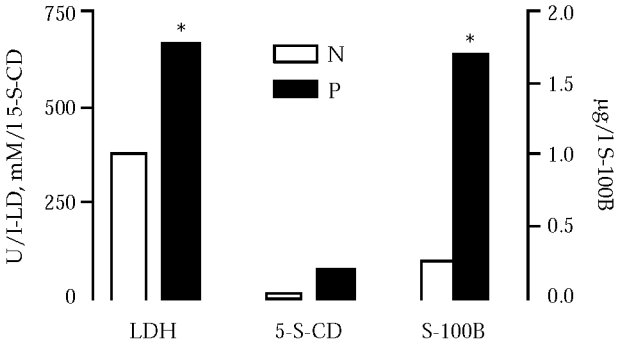

Figure 2. *Serum concentrations of S-100B, 5-S-CD and LDH in the serum of patients with nonprogressive and progressive disease. S 100B = µg/l, LDH = u/l, 5-S-CD = nM/l*
** p<0.05, Student t-test.*

examination or other laboratory tests.[3,4,18,24,30] Among other investigated serum and urine melanoma 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 disease progression except serum S-100B protein.[15,23,28] The overall survival rate was most strongly associated with the serum levels of S-100B protein but there was also a significant correlation to urinary levels of 5-S-CD.[19] Our present results correlated well with these publications concerning S-100B protein and 5-S-CD. In this study statistical analysis confirmed significant difference between serum 5-S-CD concentrations of symptomatic (progressing) and tumor-free patients as well as between Stages III and IV.

Publications are available in the literature comparing the value of LDH to S-100B protein, MIA and sVCAM-1, but there is no data concerning 5-S-CD. In the first study, involving 71 Stage IV melanoma patients the highest sensitivity was found for S-100B protein (91%) and the highest specificity for LDH (92%). Elevated serum levels of S-100B, MIA and LDH indicated disease progression in AJCC Stage IV melanoma. By multiple logistic regression, LDH was identified to be the only statistically significant marker for progressive disease and was stated to be the most relevant parameter.[9] In the second study including 89 Stage III/IV patients (UICC Stages) sensitivity for newly occurred metastases was highest for S-100B protein (86%), followed by MIA (80%) and LDH (48%). Specificity for LDH (98%) was higher than for S-100B protein (91%) and MIA (62%). On the basis of their results serum S-100B protein appeared to be the most appropriate tumor marker for newly developed progression.[21] In the third study (on 67 consecutive Stage IV melanoma patients) authors declared that both sensitivity and specificity as well as the decreases of serum S-100B and MIA concentrations were associated with the response to the treatment, while increasing levels indicated progressive disease. S-100B beta and MIA were not superior to the conventional LDH and CRP.[10] In the fourth study (489 serum samples from 64 patients with advanced melanoma), the sensitivity of S-100B protein was compared to the conventional blood parameters. S-100B represented the only relevant independent prognostic marker in a multivariate analysis.[17] In another publication on 97 consecutive patients with metastatic cutaneous melanoma elevated pre-treatment serum levels of sVCAM-1 and of LDH associated with unfavourable outcome.[12]

The above mentioned literature is rather conflicting in the evaluation of prognostic significance of LDH and S-100B protein. LDH seemed to be more specific, S-100 protein more sensitive tumor marker in metastatic melanoma patients. In our study in Stage IV melanoma S-100B protein proved to be the most sensitive and specific marker with 70.5% sensitivity, 100% specificity and 96.9% positive predictive value, respectively. The elevated marker levels correlated strongly with disease progression and survival with the shortest survival of patients with elevated LDH or S 100B markers.

In conclusion, we have confirmed that LDH, S-100B protein and 5-SCD correlated well with the prognosis of stage IV melanoma patients. On the basis of our results S-100B could be regarded the most sensitive circulating tumor marker of the investigated ones.

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