Melanoma Inhibiting Activity Protein (MIA), Beta-2 Microglobulin and Lactate Dehydrogenase (LDH) in Metastatic Melanoma

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Abstract. *Background: Serum levels of melanoma markers may have a role in monitoring disease evolution in metastatic melanoma. Patients and Methods: Serial measurements of melanoma inhibiting activity protein (MIA), lactate dehydrogenase (LDH), S-100 and β2-microglubulin were obtained from 42 metastatic melanoma patients during their biochemotherapy treatment. Results: High pre-treatment serum levels of S-100, LDH, MIA and β2-microglobulin were detected in 50%, 57%, 50% and 24% of the patients, respectively. Only S-100 had prognostic significance for both disease-free (p=0.011) and overall survival (p=0.021). In patients who responded to treatment, S-100 levels decreased significantly from pre-treatment to the time of response (p=0.050). When patients progressed, levels of MIA and β2-microglobulin increased significantly (p=0.028 and p=0.030, respectively). Conclusion: Correlation with disease evolution was found for S-100, MIA and β2-microglobulin levels. Despite the small sample size of the study, S-100 was a significant prognostic marker for overall survival and disease-free survival.*

Accurate prognosis of metastatic melanoma remains challenging. Various tumor markers in serum have been investigated as prognostic factors in metastatic melanoma, including S-100, melanoma inhibiting activity protein (MIA) and lactate dehydrogenase (LDH) (1). Prognostic serum markers could be helpful in staging melanoma and monitoring the disease evolution.

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S-100 is a 21-kDa acidic calcium-binding protein whose presence is used to confirm an immunohistochemical diagnosis of melanoma (2). Elevated S-100 serum levels have been reported in 74-100% of patients with stage IV AJCC (American Joint Committee on Cancer) melanoma (3-5). Some studies have suggested that serum S-100 level has a prognostic significance for survival (6) and could serve as a useful marker for monitoring patients during chemotherapy and predicting response to treatment (7-11).

MIA is an 11-kDa protein with growth inhibitory properties that was first isolated from tissue culture of malignant melanoma cells. High serum levels of MIA have been reported in 96-100% of stage IV melanoma patients (12-13). Its value as a prognostic factor or as a marker for monitoring disease evolution in melanoma has not been clearly assessed.

The presence of high serum LDH levels is a well-known adverse prognostic factor in metastatic melanoma and is now included as such in the AJCC staging system (14). High serum levels of β2-microglobulin are found in lymphoma and myeloma patients (15). Preliminary studies have shown high serum levels of β2-microglobulin also in melanoma patients, but this finding has not been confirmed by others (16, 17). This study was undertaken to evaluate the prognostic value of these tumor markers and their correlation with disease progression in patients with metastatic melanoma receiving treatment with chemoimmunotherapy.

Patients and Methods

Study design. The correlation between disease evolution and serum levels of LDH, S-100, MIA and β2-microglobulin in individuals participating in either of two phase II trials assessing treatment of metastatic melanoma with a combination of cisplatin, vinblastine, dacarbacine or temozolomide and subcutaneous IL-2 plus IFN-alpha (18-19) has been analysed. These trials were performed at

Table I. *Patient characteristics.*

Characteristics	No.
No. of patients	42
Gender male/female	23/19
Age median (range)	49 (26-73)
PS median (range)	1 (0-2)
No. of disease sites	4 (1-8)
Sites of metastases	
Lung	26 (62%)
Lymph nodes	23 (55%)
Cutaneous/soft	19 (45%)
Liver	8 (19%)
Bone	9 (21%)
CNS	5 (12%)
Others	6 (14%)

CNS: Central nervous system; PS: ECOG performance status.

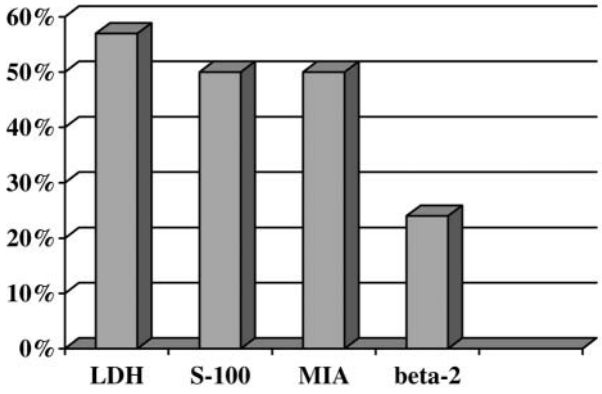

Figure 1. *Patients (%) with high serum levels of LDH, S-100, MIA, β2-microglobulin prior to treatment.*

the Hospital Clinic, Barcelona, Spain, between December 1996 and December 2002. The ethics committee of the Hospital Clinic approved the protocols and all patients signed informed consent documents prior to their inclusion in the studies.

The inclusion criteria were as follows: 18 years of age or older; presence of histologically confirmed metastatic melanoma; South-West Oncology Group (SWOG) performance status of 0 to 2; estimated life expectancy of at least eight weeks, and normal hematological, renal and hepatic function. Women of child-bearing potential were required to use an effective form of contraception. The exclusion criteria were a history of other malignancies and concomitant corticosteroid treatment. Women who were pregnant or breastfeeding were ineligible for inclusion in the studies.

Patients underwent a study-specific baseline staging evaluation consisting of a computed tomography (CT) of the chest and abdomen, a bone scan and cerebral magnetic resonance imaging (MRI). Treatment cycles were repeated every three or four weeks. Before each treatment cycle, clinical anamnesis and physical examination were performed, including routine hematological, renal and hepatic tests. Serum samples for analysis of tumor markers were collected before the initiation of each treatment cycle. Samples were analyzed during the trials but the results did not affect clinical decisions. Clinical response to treatment was evaluated every three cycles by complete body CT, bone scan and cerebral MRI.

Tumor measurement. Tumor size measurements were performed following WHO criteria (20). Clinical response was assessed as complete response, partial response, disease progression, or stable disease. A complete response (CR) was defined as the disappearance of all evidence of cancer for at least 4 weeks. A partial response (PR) was defined as a 50% reduction in the sum of the products of the perpendicular diameters of all lesions for at least 4 weeks, without any evidence of the development of new lesions or of the progression of any lesions. Stable disease (SD) was defined as a <50% reduction or <25% increase in the sum of the products of the perpendicular diameters of all lesions, without any evidence of new lesions. Progressive disease (PD) was defined as a >25% increase in one or more lesions or the appearance of new lesions. Clinical response was evaluated every three cycles.

Serum measurements. Serum samples were obtained before the start of chemotherapy treatment and at each subsequent response evaluation.

S-100. S-100 serum levels were measured by a two-site immunoluminometric assay according to the manufacturer's instructions (Liaison Diasorin, Italy). The upper limit of the normal range for S-100 for a healthy individual was established in our laboratory as 0.20 μg/l (21).

MIA. MIA levels were measured using a manual commercial ELISA (Roche Diagnostics, Mannheim, Germany). The cut-off value established in our laboratory was 14 ng/l (21).

β2-microglobulin. β2-microglobulin was measured by automatic nephelometry (BN II) using a comercial kit (Dade Behrig, Germany) The cut-off value established in our laboratory was <2.5 mg/l.

LDH. Serum LDH levels were measured using an automatic analyzer ADVIA 1650 (Bayer, Tarrytown, NY, USA) and a commercial kit (Roche Diagnostics, Manheim, Germany). The cut-off value established in our laboratory was 450 U/l.

Data analysis. The pretreatment serum levels of each marker and their evolution during treatment were compared with the clinical response and the survival time. Univariate survival analysis was performed by the Kaplan-Meier method. The significance of the changes in serum levels of the selected markers was tested using the Wilcoxon signed rank test. A value of $p<0.05$ was considered statistically significant.

Results

Serum samples from 42 stage IV melanoma patients undergoing chemotherapy were evaluated. Patient characteristics are presented in Table I. Pre-treatment serum levels of S-100, LDH, MIA and β2-microglobulin were elevated in 50%, 57%, 50% and 24% of patients in comparison to controls, respectively (Figure 1).

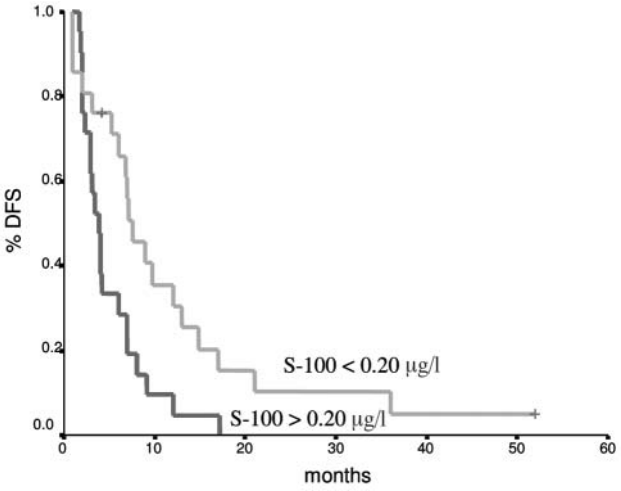

Figure 2. *Disease-free survival in relation to S-100 serum levels. Patients were subdivided into two groups: S-100>0.20 µg/l and S-100< or equal to 0.20 µg/l; p=0.011.*

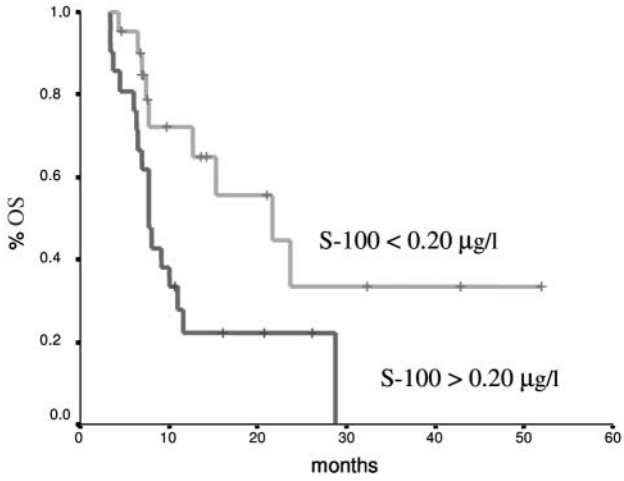

Figure 3. *Overall survival in relation to S-100 serum levels. Patients were subdivided into two groups: S-100>0.20 µg/l and S-100< or equal to 0.20 µg/l; p=0.021.*

Table II. *Descriptive values of serum levels of S100, MIA, LDH and β2-microglobulin pre-treatment (Pre S-100, Pre MIA, Pre LDH, Pre Beta-2) and at the time of response (Post S-100, Post MIA, Post LDH, Post Beta-2).*

	Pre S-100 (µg/l)	Post S-100 (µg/l)	Pre MIA (ng/ml)	Post MIA (ng/ml)	Pre LDH (U/l)	Post LDH (U/l)	Pre Beta-2 (mg/l)	Post Beta-2 (mg/l)
Mean	2.8	1.5	25.0	16.0	489	382	1.8	2.46
Median	0.5	1.0	23.0	12.0	354	320	1.7	2.00
Min	0.0	0.01	5.0	4.4	235	249	1.3	3.10
Max	14.0	6.0	48.0	37.8	1097	802	2.6	2.00

Table III. *Descriptive values of serum levels of S100, MIA, LDH and β2-microglobulin pre-treatment (Pre S-100, Pre MIA, Pre LDH, Pre Beta-2) and at the time of progressive disease (Post S-100, Post MIA, Post LDH, Post Beta-2).*

	Pre S-100 (µg /l)	Post S-100 (µg /l)	Pre MIA (ng/ml)	Post MIA (ng/ml)	Pre LDH (U/l)	Post LDH (U/l)	Pre Beta-2 (mg/l)	Post Beta-2 (mg/l)
Mean	1.40	2.00	18.34	46.62	601.48	563.84	1.81	2.17
Median	0.20	0.87	12.15	25.65	351.50	435.00	1.60	1.90
Min	0.00	0.01	5.00	5.00	235.00	248.00	0.90	1.00
Max	14.00	16.20	48.00	244.00	7467.0	2184.0	3.40	4.00

High pre-treatment levels of S-100 (upper limit 0.20 mg/l) had prognostic significance for both disease-free survival (DFS) ($p=0.011$) and overall survival (OS) ($p=0.021$) (Figures 2 and 3). High pre-treatment levels of the other three serum markers had no prognostic significance for either disease-free or overall survival.

Of the patients who responded to treatment, three (7.1%) patients with a complete response and eleven with a partial response (26.2%), showed a decrease in S-100 levels at the time of response evaluation relative to pretreatment S-100 levels ($p=0.074$). In these patients, no significant changes were found between pre- and post-treatment levels of LDH ($p=0.19$), MIA ($p=0.10$) or β2-microglobulin ($p=0.23$) (Table II).

When patients finally progressed, a significant increase in serum levels of MIA ($p=0.028$) and β2-microglobulin ($p=0.030$) was detected, but not in LDH serum levels ($p=0.68$) or S-100 ($p=0.61$) (Table III).

Discussion

Two different aspects of our study results merit special comment: first, the prognostic significance of S-100 in melanoma patients, and second, the correlation of tumor markers with response and progression after treatment.

A role for S-100 as a prognostic marker in patients with metastatic melanoma treated with chemotherapy is supported

by our results. Patients whose pre-treatment serum S-100 levels were higher than 0.20 μg/l had a shorter DFS and OS compared to those whose pre-treatment levels were within the normal range. Although these results corroborate findings which have previously been reported by others (22-23), their implication in clinical practice has not been fully realized. It is currently not a standard procedure to analyze serum S-100 levels in advanced melanoma patients.

The role of serum markers in monitoring disease evolution during chemotherapy is an area of controversy. In patients who responded to treatment, our results showed a good correlation between disease progression and serum levels of S-100. In contrast, in patients who experienced disease progression, changes in serum levels of MIA and β2-microglobulin, but not S-100 levels, showed a good correlation with clinical response. Another study has previously found that response and relapse after treatment correlates well with changes in MIA and S-100 serum levels (24), but the different sensitivity of melanoma markers for response and for progression has not been previously reported. Confirmation of our results would underscore the advantages of using multiple prognostic markers rather than a single marker.

Our results contrast with the results of others groups with regard to sensitivity of the markers used. In our study, which used higher cut-off values for serum markers than were used in earlier studies (7, 13), LDH as a serum marker showed higher sensitivity for disease detection in metastatic melanoma patients (57%) than did MIA (50%) or S-100 (50%). The higher cut-off values were selected because these serum markers are not melanoma-specific. Increased S-100 serum levels have been seen in patients with strokes or brain malignancies (40% and 90%, respectively) (25) or with liver or renal injury (26). Elevated MIA values have been shown elevated in up to 17% of patients with epithelial neoplasms (12).

Increasing the cut-off values for MIA 14 ng/ml and S-100 0.20 μg/l increases the specificity to 98.9% and 96.8%, respectively, in melanoma patients without clinical signs of recurrence (data not shown). Others have found similar results, suggesting that higher cut-off values of MIA and S-100 than those previously recommended (MIA 6.50 ng/ml and S-100 0.12 μg/l) should be used and could be of clinical value (27).

In conclusion, S-100 has a prognostic value as a serum marker in metastatic melanoma, which may exceed that of LDH. The real value of serum markers for monitoring therapy in metastatic melanoma, including cost-effective parameters, should be further investigated.

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