Value of Serum S-100B for Prediction of Distant Relapse and Survival in Stage III B/C Melanoma

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Abstract. Background: The biochemical marker serum S-100B has been proven to reflect the stage of melanoma and to be useful for disease monitoring and prediction of survival, mainly in stage IV disease. For stage III melanoma, limited data are available and its predictive value for relapse is unknown. Serum S-100B was evaluated prospectively for monitoring response and its predictive value for relapse and overall survival in stage III B/C melanoma patients. Patients and Methods: Treatment consisted of one cycle of neoadjuvant and adjuvant chemo(immuno)therapy, around surgery. S-100B was measured at enrollment and prior to and following surgery. The levels of S-100B in serum were compared to the pattern and intensity of the expression of S-100B in the melanoma tissue. Results: Some patients with normal initial S-100B values (n=18) showed responses (3 complete remission and 2 partial remission), in contrast to patients with elevated S-100B values. Distant relapse within one year was found in 11/23 (48%) patients with increased S-100B versus 2/18 (11%) patients with a normal value (p=0.01). Overall survival was decreased in patients with increased S-100B compared to those with normal S-100B (p=0.02). Correlations between the pattern and intensity of S-100B expression in the tumor specimen and the value of serum the S-100B did not reach statistical significance. Conclusion: Serum S-100B is a valuable biomarker for the evaluation of response to treatment and prediction of early distant relapse and survival in stage III B/C melanoma. The marginal correlation between serum S-100B values and expression of S-100B in the tumor specimens needs further study.

The S-100-protein is a dimeric, 21 kDa acidic, calcium-binding protein found in tissue of neuroectodermal origin. It was first isolated from bovine brain (1). The protein consists of an α- and β-subunit, which can combine in three isoforms: the αα-dimer, ββ-dimer and αβ-dimer (2). S-100αα is found in striated muscle, heart and kidney; the ββ-dimer in glial and Schwann cells; and the αβ-dimer in glial and melanoma cells (3). Within cells, the S-100 proteins mostly exist as dimers. S-100B (S-100β) monomers form homodimers, S-100BB (S-100ββ), and are also found as heterodimers (S-100A1B or S-100A1β) when coupled with S-100A1 (S-100α) (4). Since the introduction of a new nomenclature for S-100-binding proteins, S-100α is known as S-100A1 and the previously known S-100β as S-100B (5). S-100B as a potential tumour marker for melanoma patients was first described by Fagnart et al. in 1988 (6). An immunoluminometric assay to the β-subunit has been developed and as a result serum S-100B is an increasingly used biochemical marker in the follow-up of patients who are treated for melanoma (7).

In general S-100B has proven to reflect the stage of melanoma and to be useful for disease monitoring and prediction of survival, mainly in stage IV disease (7-10). For stage III melanoma, limited data are available and its predictive value for relapse is unknown. In predicting micrometastatic disease and during the follow-up of patients treated for micrometastatic disease, S-100B seems not to be useful, with a sensitivity of 44% regarding recurrence (11, 12). In this current series, the value of serum S-100B for patients with clinically detectable regional lymph node and/or in-transit metastases was studied. These patients suffer from stage III B/C disease, according to the American Joint Committee of Cancer (AJCC) classification (13). The standard treatment for these patients is surgical resection, or regional isolated perfusion with high-dose chemotherapy in cases of extensive in-transit metastases (14). Adjuvant high-dose interferon alpha (IFNα) has remained controversial because of significant toxicity and debate regarding its antitumor effect continues (15-18). Based on promising results with neoadjuvant chemo- and chemobiotherapy, one
neoadjuvant cycle of a relatively mild chemo(immuno)therapy regimen was administered in a homogeneous patient population (19-21).

Before the introduction of S-100B as a potential biomarker in the serum of melanoma patients, the protein was a long-used target for the immunohistochemical and immunocytochemical detection of melanoma on pathology slides (22). However, limited data exist regarding a possible relationship between S-100B in immunohistochemical analysis and the S-100B values in serum. Banfalvi et al. found a diffuse staining pattern of S-100 (>90% of tumor cells stained positive) to be the predominant form in primary and metastatic tissue. They also found significantly higher serum S-100B concentrations in patients with heterogeneous or diffuse staining patterns compared to patients with focal staining patterns (23).

The aims of this study were to evaluate S-100B for monitoring response to chemoimmunotherapy followed by surgery and its predictive value for relapse and overall survival in patients with clinically detectable localized disease. Another aim was to analyze the expression of S-100B in the lymph nodes and in-transit metastases of the included patients and to compare these findings with the values of S-100B in the serum.

Patients and Methods

Schedule of treatment. Forty-one patients with locoregional lymph node or in-transit metastases received neoadjuvant temozolomide (Temodal®; Schering-Plough, Maarssen, The Netherlands) 150-200 mg/m2 orally, for five days or the same drug followed by twelve days of subcutaneous administration of combined immunotherapy (Table I). The combination consisted of granulocyte monocyte colony-stimulating factor (GM-CSF) (Molgramostim, Leukomax®; Schering-Plough) 2.5 μg/kg, interleukin-2 (Aldesleukin, Proleukin®; Chiron BV, Amsterdam, the Netherlands) 4 MIU/m2 and IFNα (Intron-A®; Schering-Plough) 5 MIU fixed dose. About four weeks from the start of treatment, surgical resection of the regional nodes or in-transit metastases or regional perfusion was performed. Four to eight weeks following surgery, a similar cycle of chemoimmunotherapy was administered.

The protocol was approved by the local ethical committee and all the patients provided written informed consent. Eligibility criteria included cytologically proven, clinically detectable regional metastasis of melanoma without evidence of distant metastases. The latter were excluded by radiography of the thorax and ultrasound of the liver and of abdominal lymph nodes in cases of regional metastasis in the lower trunk. The size of the regional lymph nodes was measured by ultrasound at the start of treatment and immediately prior to surgery. In-transit metastases were measured by physical examination and, if located subcutaneously, by ultrasound as well.

S-100B. The serum S-100B levels were determined by a two-site immunoluminometric assay (Liaison, DiaSorin, Bromma, Sweden) and measured at enrollment in the protocol, just prior to surgery and postoperatively prior to the start of the adjuvant cycle of chemoimmunotherapy. The normal range in our laboratory has been established as less than 0.16 μg/l (7).

The serum values of S-100B were compared with the intensity of the S-100B expression in immunohistochemical staining. S-100B expression in the tissue was analyzed in surgical resection specimens of the lymph node or in-transit metastases, using a murine anti-human S-100B monoclonal antibody (DakoCytomation Clone DAK-S100B/2, Dako B.V. Eindhoven) at a dilution of 1:100, for one hour at room temperature. Antigen retrieval was achieved by heating the sections in citrate buffer at pH 6.0 in a dedicated microwave oven. Antibody binding was detected by means of a DakoCytomation EnVision kit and negative controls consisted of omission of the primary antibody. The sections were analyzed for staining pattern and intensity. This analysis was done in a blind setting where the observer was not informed about the value of serum S-100B. The staining patterns were defined as A: diffuse when there was a positive reaction in over 90% of the tumor cells, B: heterogeneous if there was balanced positive/negative staining in different parts of the tumor (10-90%) and C: focal if less than 10% of the tumor cells were positive. The intensity of staining was further subdivided by using the following scoring system: strong (++); weak (+) or no staining (–).

Follow-up. The patients were monitored every three months for relapse by history, physical examination, serum S-100B determination, laboratory tests and X-ray of the thorax. Additional

Table I. Toxicity and response to temozolomide (TMZ) and immunotherapy in neoadjuvant treatment.

<table>
<thead>
<tr>
<th>Treatment schedule</th>
<th>N/a</th>
<th>Thrombocytopenia (grade)</th>
<th>Leukocytopenia (grade)</th>
<th>Liver function disturbances (grade)</th>
<th>Toxicity</th>
<th>Leukocytopenia</th>
<th>Liver function disturbances</th>
<th>Toxicity</th>
<th>Leukocytopenia</th>
<th>Liver function disturbances</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 3 4</td>
<td>2 3 4</td>
<td>2 3 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMZ 200 mg/m² + immunotherapy</td>
<td>16</td>
<td>2 1 1</td>
<td>1 0 1</td>
<td>8 1 0</td>
<td>CR c</td>
<td>PR</td>
<td>SD</td>
<td>PD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMZ 150 mg/m² + immunotherapy</td>
<td>11</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>2 5 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMZ 150 mg/m²</td>
<td>15</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

42 patients received neoadjuvant chemoimmunotherapy and were available for evaluation of toxicity according to the National Cancer Institute Common toxicity criteria; 3response was evaluable in 41 patients as one patient died during the neoadjuvant treatment cycle; 4CR: complete remission; PR: partial remission; SD: stable disease; PD: progressive disease.

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imaging was performed in cases where distant metastases were suspected. Regional recurrences were removed immediately by surgery. The patients were removed from the study when distant metastases became apparent.

Statistics. All the analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows (version 11.5, Microsoft Corporation). The Chi-square test was used to evaluate the association between elevated serum S-100B and distant relapse within the first year of follow-up. Survival curves were constructed by the Kaplan-Meier method and analysed by the log-rank procedure. Survival was measured from the date of surgery and the event was death. In the absence of an event, censoring took place at the last follow-up. The one-way ANOVA test was used for comparison of serum S-100B levels and the pattern and intensity of S-100B expression in the tumor samples. A p-value <0.05 was considered to be significant.

Results

Prior to treatment, serum S-100B was elevated in 23/41 (56%) patients. In seven patients, the increased S-100B levels normalized after the neoadjuvant cycle (Table II). Out of the remaining sixteen patients, fourteen (88%) showed normalized values following the surgical procedure. The other two patients developed distant relapses within four weeks after surgery. One patient with normal values prior to neoadjuvant treatment and surgery showed elevation of S-100B following surgery, accompanied by progressive disease (PD). Only two patients with increased serum S-100B also had increased serum lactate dehydrogenase (LDH) (≥450 kU/l).

Distant relapse within one year after surgery was found in 11/23 (48%) patients with increased S-100B prior to treatment versus 2/18 (11%) patients with normal initial values (p=0.01). There was no relationship between pre-treatment S-100B levels and locoregional relapse (2/23 with increased S-100B, 3/18 with normal S-100B). None of the twenty-three patients with increased S-100B showed a clinical response, in contrast to 5/18 (3 complete remission, 2 partial remission) patients with normal S-100B prior to surgery. There was no significant difference (p=0.68) in S-100B levels between stage IIIB patients and stage IIIC patients (increased serum S-100B: 10 stage III B patients, 13 stage III C patients; normal serum S-100B: 9 stage IIIB patients, 9 stage III C patients). The Kaplan-Meier curves of overall survival showed a significant difference between patients with normal S-100B versus those with increased S-100B prior to surgery (Figure 1, p=0.02).

Immunohistochemical staining with monoclonal anti S-100B was possible on the slides of 35 patients. Four patients did not undergo surgery, three patients had a CR and in one

### Table II. Median serum S-100B values (μg/l) and relation to relapse.

<table>
<thead>
<tr>
<th>S-100B</th>
<th>N</th>
<th>S-100B Before</th>
<th>Response to</th>
<th>Relapse</th>
<th>d</th>
<th>Regional</th>
<th>Distant 1st year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neoadjuvant CITb</td>
<td>Surgery</td>
<td>Adjuvant CITb</td>
<td>neoadjuvant CITb,c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Decreased to normal</td>
<td>7</td>
<td>0.29</td>
<td>0.11</td>
<td>0.07</td>
<td>6 SD; 1PD</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Decreased to above normal</td>
<td>5</td>
<td>0.43</td>
<td>0.32</td>
<td>0.09</td>
<td>5 SD</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Stable elevated</td>
<td>3</td>
<td>0.17</td>
<td>0.18</td>
<td>0.07</td>
<td>3 SD</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Increasing</td>
<td>8</td>
<td>0.23</td>
<td>0.37</td>
<td>0.07</td>
<td>5 PD; 3SD</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Normal</td>
<td>18</td>
<td>0.09</td>
<td>0.09</td>
<td>0.07</td>
<td>3CR, 2PR, 11SD, 2PD</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

*S-100B: serum level prior to treatment, elevated if ≥0.16 μg/l. The 23 patients with elevated serum S-100B were subdivided according to the change in values from before to after neoadjuvant chemoimmunotherapy; CIT: chemoimmunotherapy; CR: complete remission; PR: partial remission; SD: stable disease; PD: progressive disease.*

![Figure 1. Overall survival in relation to serum S-100B in 41 patients treated with neoadjuvant and adjuvant chemoimmunotherapy. Eighteen patients with normal S-100B (dotted line) prior to surgery compared favourably (p=0.02) to the other twenty-three patients with increased S-100B at that time (solid line).](image-url)
patient the partially regressed tumor after regional perfusion was not resected. In two other patients, only cytological material was available in the laboratory and no immunohistochemical staining with S-100B was performed. All but four of the 35 analyzed specimens showed S-100B expression (Figure 2). Fourteen out of twenty patients (70%) with intense (++) staining had an elevated serum S-100B, whereas none of the four patients without immunohistochemical S-100B expression (intensity –) had elevated serum values of S-100B. One of these four developed an elevation of her serum S-100B later that was related to recurrent disease. The other three did not develop elevated S-100B levels; two without recurrent disease at fourteen and 21 months respectively and one with stable in-transit metastases at fourteen months. Among the patients with weak (+) staining samples, 4/11 (36%) had an elevated serum S-100B level. There was no statistically significant relationship between a more intense staining pattern and the level of serum S-100B, although there was a trend (p=0.05) (Figure 2).

Most of the specimens showed diffuse staining (22/35=63%), fifteen of these (15/22=68%) had elevated serum S-100B prior to resection. Among the five patients with specimens showing focal staining, only one patient (1/5=20%) had elevated serum S-100B. Again, no significant relationship could be found between increased serum S-100B levels and diffuse staining patterns (p=0.10) (Figure 3).

Discussion

In this study, the serum S-100B values reflected the response to the neoadjuvant cycle of chemoinmunotherapy and the removal of the tumor by surgery in nearly all the cases where this marker was elevated. However, whether or not the S-100B level has a predictive value for rapid distant relapse is more relevant and has not previously been addressed. Almost half (11/23=48%) of the patients with elevated marker levels prior to surgery developed distant metastases within the first year following surgery, compared to 11% (2/18) of the patients with a normal initial S-100B value. Previously, we found decreased overall survival in relation to increased S-100B in a group with stage III B/C melanoma treated by surgery alone (7). In the current series, overall survival was also significantly decreased in patients with increased serum S-100B prior to treatment. These data support the concept that serum S-100B is a predictor of distant relapse, and that this marker can be used for risk-adapted management in a selected group of patients with stage III B/C melanoma.

Increased serum S-100B may reflect regional tumor load, actual hematogenous dissemination or both. The normalization of serum S-100B following surgery in nearly all patients suggested regional metastasis as the source of the S-100B elevation. On the other hand, there was no correlation of S-100B with tumor load in stage III melanoma (IIIB: limited number of nodes versus IIIC >3 lymph nodes or combination with in-transit metastases). The clear correlation between serum S-100B levels and distant relapse might indicate hematogenous dissemination as a source for the elevated marker values. Rapid surgical resection can limit the period in which hematogenous dissemination takes place, which would be an argument not to postpone surgical resection for more than one cycle of neoadjuvant chemotherapy in cases of increased serum S-100B.

Interestingly, only 2/23 patients with elevated serum S-100B also had increased LDH, a marker of high tumor load in stage IV melanoma. LDH has been described as the foremost blood parameter for stage IV melanoma patients.
and is included in the new AJCC staging system (13). LDH is elevated in late stage melanoma, predominantly in patients with liver metastases, but regardless of its prognostic value in (advanced) stage IV melanoma, its value in the other stages is debated due to low sensitivity (24-26).

Concerning the other aspect of this study, focal or absence of immunohistochemical staining intensity were hardly seen among the patients with elevated serum S-100B and the four patients with normal serum S-100B all showed no staining; however, in this relatively small number of samples, this difference did not reach statistical significance. As the percentage of elevated S-100B increases with melanoma stage, tumor burden is suggested as the source of S-100B in the serum. On the other hand, melanoma is highly heterogenous for S-100B expression in the tumor tissue (27). Accordingly, if in any way there is a relationship between S-100B in tumor tissue and in the serum, a focal pattern and low level of S-100B expression in melanoma metastases could only release a small amount of the marker into the circulation.

Conclusion

In stage IIB/C melanoma patients, pre-treatment serum S-100B values have a predictive value for overall survival. Stratification for normal serum S-100B versus increased S-100B in future trials therefore seems to be of importance and should be further studied.

The correlation between serum S-100B values and expression of S-100B in the tumor specimens was only marginal and needs further study.

References


