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Normal values of serum S-100B predict prolonged survival for stage IV melanoma patients

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Abstract

The value of normal S-100B levels to predict survival was evaluated in 145 patients with stage IV melanoma. Treatment consisted of temozolomide given alone or was followed by combined cytokine immunotherapy, given every three to four weeks, with an evaluation of response following two treatment-cycles. S-100B values were measured prior to and following each cycle of systemic therapy and regularly thereafter. Patients with normal initial S-100B values ($n = 32$) had higher response rates and fewer and more favourable metastatic sites with better overall survival rates than patients with elevated S-100B levels (median 14.0 versus 6.6 months). Normal S-100B values increased in nearly all patients (28/31) after a median of 7.9 months. In addition, patients with rapid normalisation of their serum level ($n = 12$) following systemic treatment experienced prolonged survival. However, upon multivariable analysis S-100B prior to treatment lost its independence as a prognostic factor, whereas lactate dehydrogenase (LDH) remained. When measured after treatment, both markers had independent value.

Keywords: S-100 protein; Melanoma; Metastases; Lactate dehydrogenase; Survival

1. Introduction

In the current American Joint Committee of Cancer (AJCC) classification of melanoma, stage IV disease is divided into subgroups that reflect the better survival rates for patients with (sub)cutaneous and lymph node metastases beyond the regional basin (stage IVa) than for patients with lung metastases (stage IVb). Patients with other visceral metastases (stage IVc) have an even worse prognosis [1]. Serum lactate dehydrogenase (LDH) is introduced in this new AJCC staging system as a predictive factor of survival, even in multiple analyses and after accounting for the number and site of metastases [2,3].

Serum S-100B has been reported to be a reliable tumour marker in metastatic melanoma [4], reflecting the tumour load [5,6] and predicting response to chemoimmunotherapy [7,8]. S-100B was also described as a good marker for monitoring response to therapy in stage IV disease. Although the pre-treatment level of LDH serves as a good prognostic predictor [2,3], elevation of LDH is not restricted to metastatic melanoma. S-100B proved to be a more sensitive marker [5,7]. From a

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recent prospective study of stage IIIB/C disease, S-100B levels reflected actual haematogenous dissemination rather than tumour load, as increased values correlated with early distant metastasis and not with the tumour load. This was reflected by an absence of a correlation of the S-100B level with stage IIIB versus stage IIIC melanoma [9].

We noticed that a considerable portion of patients with stage IV melanoma have a normal serum S-100B level, which seems to contrast with our previous observations. We were curious as to what this observation signified. Could a normal S-100B level reflect an absence of actual progressive dissemination and, as a result, identify patients with a better survival rate? To address this question, we prospectively evaluated the prognostic relevance of serum S-100B levels at the start of chemo(immuno)therapy as well as serial values during therapy with regard to treatment response and overall survival. The prognostic relevance of the S-100B level for survival was compared with the level of LDH and other known prognostic factors (number and type of metastases) in the univariate and multiple analyses.

2. Patients and methods

From October 1998 to November 2002, 145 patients with stage IV melanoma (according to the AJCC classification) were included in two studies. These studies were approved by the Protocol Review Committee of the Netherlands Cancer Institute, which dealt with the ethical aspects. Patients were treated by temozolomide (200 mg/m^2, five days orally) with cytokine-based immunotherapy in a phase II trial ($n = 69$) or treatment consisted of temozolomide with ($n = 37$) or without ($n = 39$) twelve days of subcutaneous (s.c.) combined immunotherapy granulocyte macrophage-colony stimulating factor (GM-CSF) Molgramostin 2.5 μg/kg, low dose interleukin-2 4 MIU/m^2 and interferon-α 5 MIU flat dose, in a phase III trial. A number of the patients were part of an earlier publication reported in [10]. All patients were monitored at regular intervals at The Netherlands Cancer Institute.

The patients' S-100B levels were measured in serum using the LIAISON® Sangtec® 100 (Diasorin, Saluggia, Italy). The normal range in our laboratory has been determined to be below 0.16 μg/l (mean + 2 standard deviations(+2SD)) [4]. Serum samples used to measure S-100B levels were taken prior to the first cycle of treatment and at regular times during therapy and follow-up. LDH measurements were done using a Hitachi 917 device (Roche Diagnostics, Mannheim, Germany). The upper limit of the normal range in our laboratory has been established as 450 kU/l.

Response to therapy was analysed after two cycles of treatment following the Response Evaluation Criteria in Solid Tumors [11]. Stable normal levels and rapid normalisation of serum S-100B level were defined as two serial normal values six to eight weeks following the start of treatment, with an interval of at least three weeks. Increases in the S-100B level were defined as repeated elevations without normalisation.

3. Statistical analysis

The χ^2 test was performed for statistical analysis of the correlation between S-100B values and the number and type of metastatic sites.

Univariable and multivariable analyses of survival were performed using the Cox regression proportional hazards model to investigate the value of S-100B in relation to other known prognostic factors for stage IV melanoma (LDH, number and type of metastatic sites). S-100B and LDH were investigated as continuous variables using natural splines with knots as suggested by Harrell in [12] to account for possible non-linearity. As the frequency distributions of S-100B and LDH were highly positively skewed, we performed a logarithmic transformation.

In the survival analysis, the onset of the therapy was used as the starting point. The relevance for survival of serum S-100B values following therapy was also studied and the S-100B measurement 6–8 weeks following chemo(immuno)therapy was used as the starting point for this purpose.

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows (Version 11.5) and S+ (Version 6.2) for multivariable analyses. Statistical significance was assumed at $P < 0.05$. Kaplan–Meier plots were used for the survival graphs.

4. Results

4.1. Patients' characteristics

Thirty-two of the 145 patients (22%) had normal S-100B values at inclusion and 113 patients (78%) had pre-treatment values of 0.16 μg/l and above. Their characteristics concerning gender, age, response to treatment and LDH levels are summarised in Table 1.

Twenty-seven of the 32 patients (84%) with normal values of S-100B prior to systemic treatment had oligometastatic disease (1–2 metastatic sites) compared with 56 of 113 patients (50%) with elevated values (Fig. 1(a), $P < 0.001$). Only thirteen patients (41%) with normal S-100B values had visceral – non-lung – metastases, compared with 76 (67%) of the other 113 patients (Fig. 1(b), $P = 0.023$).

Table 1
Patients' characteristics

Variable	S-100B < 0.16 μg/l *N* (%)	S-100B ⩾ 0.16 μg/l *N* (%)	Total *N* (%)
Total	32 (22)	113 (78)	145 (100)
Gender			
Male	21 (66)	73 (65)	94 (65)
Female	11 (34)	40 (35)	51 (35)
Age (mean ± SD) in years	51 ± 10.4	47 ± 12.0	48 ± 11.8
Response			
CR	3 (9)	4 (3)	7 (5)
PR	12 (38)	19 (17)	31 (21)
SD	7 (22)	18(16)	25 (17)
PD	10 (31)	72 (64)	82 (57)
LDH			
<450 kU/l	28 (88)	56 (49)	84 (58)
⩾450 kU/l	1 (3)	53 (47)	54 (37)
Missing	3 (9)	4 (4)	7 (5)

SD, standard deviation; LDH, lactate dehydrogenase; CR, complex response; PR, partial response; SD, stable disease; PD, progressive disease.

4.2. S-100B values during follow-up

One of the 32 patients with normal initial values did not undergo any further tests of their S-100B level because they died due to progressive disease after 4.3 months. In 28 of the remaining 31 patients (90%), at a median follow-up of 7.9 months, S-100B values increased above 0.16 μg/l and this was accompanied by progression of their disease. A rapid increase in S-100B levels during systemic treatment accompanied by progressive disease was observed in only a few patients ($n = 3$) who started with normal values. Three patients showed no elevation during the follow-up period. One had a durable complete response (CR) (48 months at the last follow-up) and two had a partial response (PR), one of whom was alive without progression after 15.9 months. The other died after 8.3 months and their S-100B level was measured only once after four months.

When S-100B was elevated at the outset, a CR was only reached if rapid normalisation was seen. Rapid normalisation of serum S-100B levels was observed in twelve of the 113 patients (11%). More detailed information is given in Table 2.

4.3. Overall survival

No effect on survival was found for the addition of immunotherapy between the treatment groups ($P = 0.9$) and also in various international studies [18]. Therefore, analysing both treatment groups together is justified. Overall survival data are presented in Fig. 2. Median overall survival was 14.0 months (95% CI 7.0–21.0) for patients who started with normal values of S-100B and 6.6 months (95% CI 5.9–7.4) for patients with elevated initial values.

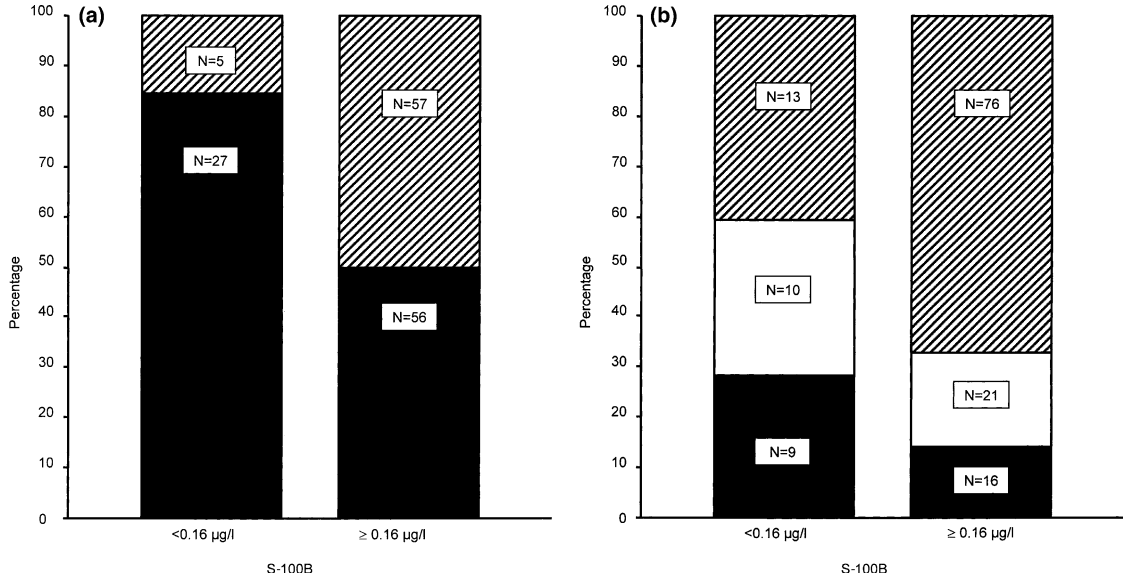

Fig. 1. (a) Number of metastatic sites in relation to S-100B. Bars: black, 1–2 metastatic sites; striped, three or more metastatic sites. A lower number (1–2) of metastatic sites is significantly correlated to a normal serum S-100B (<0.16 μg/l): $P < 0.001$. (b) Metastatic sites in relation to S-100B. Bars: black, distant (sub)cutaneous or lymph node metastases; white, lung metastases with or without distant (sub)cutaneous or lymph node metastases; striped, other visceral metastases. An elevated serum S-100B was significantly correlated to visceral – non-lung – metastases: $P = 0.023$.

Table 2
Patients with (rapid) normalisation of serum S-100B levels following one to two cycles of systemic treatment ± surgical intervention for metastatic remnants

Patient	Gender/age (years)	S-100B pre-treatment/following 1–2 cycles (±surgery) (mg/l)	Metastatic sites[a]	Clinical response	Time to progression (months)[d]	Latest follow-up	Latest S-100B level (μg/l)	Overall survival (months)
1	M/37	0.20/0.07	LN	CR	NP	CR	0.08	16+
2	M/65	0.34/0.12	Cut, SC	CR	NP	CR	0.07	50+
3	F/60	0.29/0.11	lung	CR	NP	CR	0.11	23+
4	F/42	0.22/0.12	LN	CR	10.4	Dead	1.01	15
5	M/55	0.37/0.08	LN	PR	9.4	Dead	7.07	10
6	M/30	0.18/0.15	LN	PR	12.4	Dead	24.80	16
7	M/41	0.43/0.08	LN, lung	PR (CR)[b]	NP	CR	0.05	28+
8	F/35	0.29/0.18 (0.09)	lung, adr	PR (CR)[c]	NP	CR	0.07	13+
9	M/41	0.16/0.13	lung, brain	SD	9.7	PD	4.64	21+
10	M/37	0.33/0.09	SC, lung	SD	9.8	PD	6.30	14+
11	F/51	0.28/0.81 (0.08)	lung	SD (CR)[c]	NP	CR	0.06	54+
12	M/51	0.64/0.08	LN, adr, brain	PR	3.7	Dead	0.41	15
13	F/35	1.88/0.13	lung, cut, brain	PR	5.6	Dead	0.16	9
14	M/35	0.53/0.22 (0.08)	lung	SD (CR)[c]	35.6	Dead	1.68	48
15	F/58	0.55/0.06	LN, lung	PD	0	Dead	0.11	5

M, male; F, Female.
[a] LN, lymph node; Cut, cutaneous; SC, subcutaneous; adr, adrenal gland.
[b] One patients achieved a CR after extra chemotherapy (total of six cycles).
[c] Three patients achieved a CR following surgical removal of their residual metastases.
[d] NP, no progression.

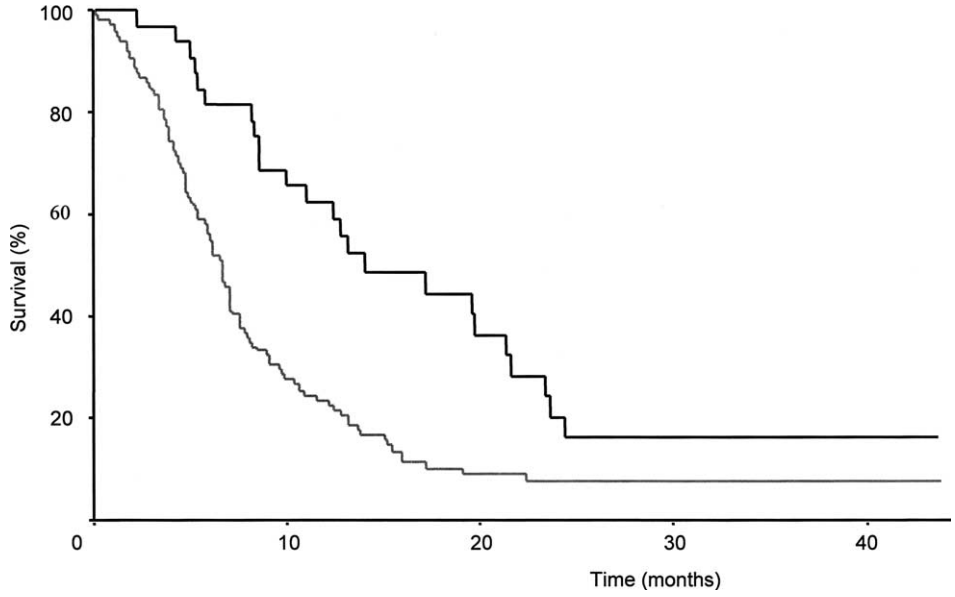

Fig. 2. Overall survival for patients with normal versus elevated S-100B levels. Survival was significantly better ($P = 0.0002$) for patients who had normal S-100B values (<0.16 μg/l) prior to treatment (black line) versus patients who started with elevated values (grey line).

The clinical implications of stable normal S-100B levels or rapid normalisation regarding the overall survival rates were also studied. Median survival in this combined group ($n = 40$) was 13.7 months (95% CI 8.8–18.6) versus 4.8 months (95% CI 4.1–5.5) in the other 105 patients (Fig. 3). Fig. 3 also illustrates that long-term survival was limited to patients in the favourable group.

4.4. Multivariable analysis

The significance of known prognostic factors in stage IV melanoma, as described in the AJCC-staging system (LDH, number and type of metastatic sites), and that of serum S-100B levels was studied. All four factors influenced survival, i.e. survival was worse with increasing

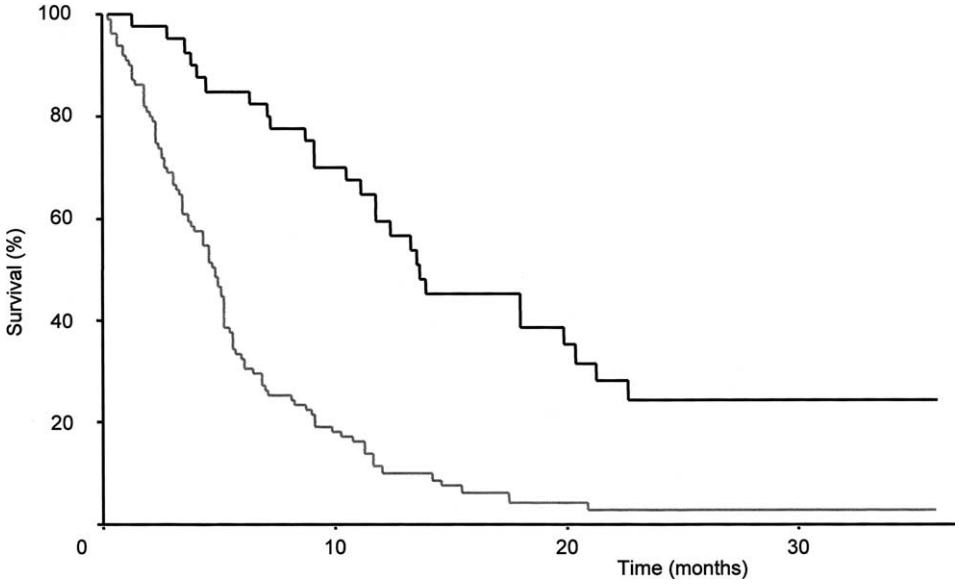

Fig. 3. Overall survival for patients with stable normal and rapidly normalised S-100B levels following treatment (black line) was significantly better ($P < 0.0001$) compared with patients with S-100B-values that remain elevated or normal values that had increased above normal (grey line).

number and sites of metastases and increasing level of serum S-100B and LDH ($P \leqslant 0.0001$).

A Cox regression analysis was performed to determine multivariantly the influence of the afore mentioned factors on survival. In the final Cox model with number and type of metastases, LDH and S-100B (the latter two non-linearly modelled), there was an independent relationship between the survival and LDH ($P = 0.0002$). However, there was no longer any evidence for an independent relationship between the S-100B level and survival ($P = 0.098$) and there was no longer an independent association with survival for the number ($P = 0.57$) and type of metastases ($P = 0.52$). The relationship between the log(hazard) for survival and log(LDH) was markedly non-linear ($P = 0.0001$), with a constant, or even decreasing, hazard between 145 and 380 kU/l, a sharply increasing hazard between 380 and 500 kU/l and a constant hazard above 500 kU/l, resulting in a maximum hazard ratio of approximately 7. The Spearman's correlation coefficient between the S-100B and LDH levels was high (0.71). A Cox regression analysis was performed following chemo(immuno)therapy. Both LDH and S-100B levels appeared to be independent factors influencing survival, $P < 0.0001$ and $P = 0.0001$, respectively. There was no independent association between survival and the type of metastases ($P = 0.16$), but weak evidence for a relationship between survival and the number of metastases ($P = 0.043$). This confirms that both LDH and S-100B levels following treatment are good indicators for potentially long-term overall survival/prognosis. Again, both markers correlated well with one another (Spearman's correlation coefficient 0.78).

5. Discussion

In this study, a normal serum S-100B level in stage IV disease, prior to treatment, identified a group of patients with oligometastatic disease and more favourable metastatic sites. Our previous study in stage IIIB/C patients hypothesised that increased levels of S-100B reflect ongoing haematogenous dissemination rather than tumour load, because of the relationship with early distant metastases and the absence of a correlation with tumour load [9]. However, this has to be confirmed in larger studies. If this hypothesis is true, a normal initial S-100B level may reflect stable, localised stage IV disease.

The possibility that the absence of an elevated serum S-100B level was due to genetic differences was excluded by data obtained during follow-up. Nearly, all of the patients with normal initial values had increased serum S-100B levels associated with progression of their disease. The other patients had a follow-up period that was probably too short to measure increased values or alternatively remained progression-free .

As observed previously [7,8], a rapid normalisation of the serum S-100B level following chemo(immuno)therapy identified a second patient group with prolonged survival and better response rates.

In the present study, the S-100B level before treatment did not achieve statistical independence in multivariable analysis. However, our findings suggest S-100B is a good 'stand-alone' prognostic marker for overall survival in metastatic melanoma. The LDH level before the start of the chemo(immuno) therapy was the only independent statistically significant marker for a poorer survival, in multivariable analysis. The S-100B level in this study did not provide additional significant

information. A possible explanation might be the high correlation between S-100B and LDH levels. Thus, we postulate that another similar group of patients may show results in favour of serum S-100B levels.

Despite the variety of approaches used, such as intensive chemotherapy and chemo-immunotherapy, to improve the outcome for stage IV melanoma patients, results are still disappointing [13,14]. It is therefore of great interest to define a subgroup of patients with an increased likelihood of prolonged survival. A biochemical marker that could be used as a guide when contemplating more intensive treatment rather than just standard chemotherapy would be very useful. Elevated marker levels usually correlate with advanced disease and predict a reduced survival. LDH has been described as the foremost blood parameter and is included in the new AJCC staging system [1]. Keilholz and colleagues [15] the combination of performance status and LDH level be used as a prognostic factor to determine further treatment.

Although the prognosis of stage IV patients is usually poor, long-term survival is occasionally achieved. Surgical resection of distant metastases yields survival rates as high as 20%. It appears that other therapies (in addition to chemotherapy), such as resection of (residual) metastases with or without adjuvant immunotherapy, may yield more durable CRs in selected patients with stage IV melanoma [16–21]. As the serum S-100B level was a marker of prolonged survival, independent of the number and site of metastases, selection of patients with stable normal or rapidly normalised serum S-100B levels following chemo(immuno)therapy would be helpful if additional therapies are considered. It is also crucial that oncologists are as informed as possible about the extent of the disease, as important treatment decisions have to be made. Positron emission tomography (PET) has proved useful for this purpose [22]. PET scanning permits assessment of the whole body. With these new technologies, recurrence can be identified early and more accurate staging can be accomplished to enable tailor-made treatment.

In conclusion, normal values of S-100B are correlated with favourable locations and numbers of metastatic sites and with better overall survival of stage IV melanoma patients. The improved prognosis for these patients is possibly due to less haematogenous spreading. Although this study did not show the S-100B level to be independent prognostic factor prior to treatment, the importance of the S-100B level following treatment was clear. In our opinion, a selected group of stage IV melanoma patients, based on stratification by their serum S-100B level, have a better chance of having responsive disease and might benefit from multimodal treatments. If several cycles of chemotherapy are successful, and the serum S-100B level remains stable or decreases to normal, one may consider removing the remaining metastases by surgery to achieve a CR. This approach is currently being applied at The Netherlands Cancer Institute.

Conflict of interest statement

None of the authors who contributed to this article have any financial or personal relationships with people or organisations that could inappropriately influence the data published.

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