

S-100B Concentrations Predict Disease-Free Survival in Stage III Melanoma Patients

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ABSTRACT

Background. Elevation of the tumor marker S-100B in melanoma patients is a highly specific indicator of recurrence.

Materials and methods. The role of S-100B in disease-free survival (DFS) was evaluated in stage III melanoma patients (staged with fluorodeoxyglucose positron emission tomography [FDG-PET] and computed tomography [CT]) with palpable lymph node metastases who underwent therapeutic lymph node dissection. S-100B and LDH were measured on the day before surgery ($d = -1$) and on days 1, 2, and 7 postoperatively. Multivariate logistic regression was used to study factors associated with preoperative elevation of S-100B. Univariate (log-rank test) and multivariate (Cox regression) survival analyses were performed to identify factors associated with DFS.

Results. Between 2004 and 2008, 56 patients (median age 57, range 24–93) years, 27 males (48%) and 29 females (52%) entered the study. Preoperative S-100B elevation was found in 27 patients (48%) and elevated LDH in 20 patients (36%). No association was found between these two markers at any time. Multivariate analysis showed that elevated S-100B preoperatively (hazard ratio [HR] 2.7, $P = .03$) was associated with DFS. S-100B elevation was

associated with increased tumor size (odds ratio [OR] 3.40; $P = .03$).

Conclusion. Elevated S-100B preoperatively in patients with optimally staged clinical stage III melanoma is associated with decreased disease-free survival. S100-B could be used as a prognostic marker in the stratification of new adjuvant trials to select stage III melanoma patients for adjuvant systematic treatment.

The incidence of melanoma is still increasing in the Netherlands. Between 1989 and 2003, the incidence increased from 9.5 to 13.7/100,000 in men and from 13.4 to 18.5/100,000 in women.¹ About 90% of the patients had stage I or II melanoma.² After surgical treatment, 20–28% of melanoma patients present with locoregional recurrence or regional dermal metastases, 26–60% with regional recurrences, and 15–50% with distant metastases.³ Despite improved public awareness as well as early diagnosis and treatment, mortality from melanoma increased from 2.5 to 3.6/100,000 in men and from 2.0 to 2.4/100,000 in women in the Netherlands between 1989 and 2003.¹

It is impossible to predict the metastatic behavior of melanoma in individual patients. At present, the Multicentre Selective Lymphadenectomy Trial (MSLT II) is investigating whether sentinel lymph node biopsy reduces the risk of locoregional metastases and improves survival.⁴ In patients with clinical stage III melanoma (palpable lymph node metastases), 5-year survival varied between 26 and 67%.⁵ Recent staging studies with whole-body fluorodeoxyglucose positron emission tomography (FDG-PET) and/or spiral computed tomography (CT) in patients with clinical stage III melanoma have shown that about one quarter of these patients had distant metastases.^{6,7} Furthermore, the uptake of FDG in the lymph node metastases in patients with

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clinical stage III melanoma proved to be of prognostic value.⁸ FDG-PET or spiral CT improved the staging of clinical stage III melanoma and led to better selection of patients for therapeutic lymph node dissection.^{6,7}

Serum lactate dehydrogenase (LDH) had high specificity for melanoma, but low sensitivity. It was an independent prognostic factor in stage IV melanoma.^{9–12} Increased S-100B concentrations were first detected in melanoma patients in 1980.¹³ Later, S-100B was found to be a serological tumor marker for melanoma and is mostly increased in stage III and IV.^{14,15}

S-100B is a 21 kilodalton (kDa) protein that was first isolated from the central nervous system in vertebrates. The name is derived from its 100% solvency in saturated ammonium sulfate with neutral pH. It has calcium-binding properties and as a dimer, consists of two isomers α and β . All possible combinations can occur (S-100 $\alpha\alpha$, S-100 $\alpha\beta$, S-100 $\beta\beta$).¹⁵ The S-100B protein is of neuroectodermal and mesodermal origin and is expressed in various parts of the body. S-100B is chiefly found in glial cells and Schwann cells.¹⁶ Serum S-100B can be measured with an immunoradiometric assay (Sangtec 100 IRMA/RIA assay) or with an immunoluminometric assay (LIA Mat Sangtec 100).

This study addressed the question whether the perioperative measurement of the tumor markers S-100B has prognostic value in FDG-PET and spiral CT staged patients with stage III melanoma who are selected for therapeutic lymph node dissection.

MATERIALS AND METHODS

Patients

Melanoma patients, earlier staged AJCC I and II, now presenting with clinically and cytologically proven regional nodal metastases of melanoma (AJCC stage IIIb and IIIc) were staged with whole-body FDG-PET (Siemens ECAT EXACT HR + scanner) and with 64-slice spiral CT (Siemens Somatom Sensation). Sentinel node positive patients and patients with regional dermal metastases were excluded for this study. The patients whose test results were negative for distant metastases were eligible for therapeutic lymph node dissection with curative intent. Nodal metastases of ≥ 3 cm and/or ≥ 3 positive lymph nodes and/or extra nodal growth received adjuvant radiotherapy (20×2.4 Gy).^{17,18} LDH and S-100B were measured preoperative (day -1) and postoperative days 1, 2, and 7. The study was approved by the Medical Ethical Committee of the UMCG.

Methods

LDH was analyzed routinely by means of Roche Modular (Hitachi) with an enzymatic activity measurement;

normal values of LDH were considered to be <250 U/L. Concentrations of S-100B were measured using Sangtec 100 immunoassay on the Advantage (Nichols).

The S-100B half-life is estimated to be 30 min.¹⁹ Approximately 10 times the half-life after lymph node dissection, S-100B values should reduce to the minimum possible. When tumor cells are still present the release of S-100B will continue or may even increase. Therefore, S-100B levels were measured on days -1, 1, 2, and 7.

Levels were calculated on the basis of a calibration curve and checked against internal standards with a known concentration of S-100B. In this assay, a cut-off point of 0.15 g/L was set. The reference values for the S-100B assay (Liason Sangtec 100) were established by analysis of S-100B values of 120 healthy men and women according to the CLSI C28A2 guideline. Concentrations within this range were considered to be normal, whereas concentration above this range were considered to be elevated. A postoperative increase of S-100B was defined as any increase on the postoperative days 1, 2, or 7 (day 1 as baseline value, elevation on day 2 compared with day 1, and/ or elevation on day 7 compared with day 2).

Statistics

Data were analyzed with STATA version 10.0. Logistic regression analysis was used to assess factors associated with elevated S-100B concentrations. Factors associated with disease-free survival (DFS) were analyzed with standard univariate (log-rank test) and multivariate (Cox proportions hazard) survival methods.

RESULTS

Patients

In the period from January 2004 to January 2008, 56 patients (27 males [48%] and 29 females [52%]), median age 57 (range 24–93) years underwent therapeutic lymph node dissection (Table 1). Preoperatively, 27 patients had elevated S-100B concentrations (48%), while 20 patients had increased LDH levels (36%). The range on day -1, 1, 2, and 7 for S-100B was 0.03–23.3, 0.02–1.5, 0.03–0.82, 0.01–0.22 and for LDH 109–428, 106–816, 108–335, 115–802. No associations were found between S-100B and LDH levels on days -1, 1, 2, and 7 ($P = .45$, $P = .87$, $P = .84$, and $P = .90$, respectively). The S-100B concentration normalized postoperatively in 22 of the 27 patients (81.4%) and the LDH in 18 of the 20 patients (90%).

Preoperative S-100B elevation was associated with increased lymph node size (odds ratio [OR] 3.4, $P = .03$), however not significantly with extranodal growth ($P = .8$),

TABLE 1 Characteristics of 56 AJCC stage III melanoma patients

Variable	Patients	Percentage
<i>Gender</i>		
Male	27	48.2
Female	29	51.8
<i>Median age (years)</i>	57.0	
<i>Localization primary tumor</i>		
Trunk	24	42.8
Head and neck	3	5.3
Upper extremities	7	12.5
Lower extremities	22	55.8
<i>Breslow thickness primary tumor</i>		
T1 (<1.0 mm)	7	12.5
T2 (1.0–2.0 mm)	20	35.7
T3 (2.0–4.0 mm)	21	37.5
T4 (>4.0 mm)	8	14.2
<i>Clark level primary tumor</i>		
1–3	24	42.6
4–5	31	55.4
Unknown	1	1.8
<i>Localization lymph nodes</i>		
Neck	6	10.7
Axilla	20	35.7
Groin	30	53.5
<i>Nodes removed</i>		
<15	27	48.2
≥15	29	52.8
<i>Positive nodes</i>		
<2	36	64.3
≥2	20	35.7
<i>Extranodal growth</i>		
Yes	24	42.6
No	42	56.4
<i>Lymph node size</i>		
<3 cm	28	50.0
≥3 cm	28	50.0
<i>Ulceration primary tumor</i>		
Yes	13	23.2
No	43	76.8
<i>AJCC stage</i>		
IIIa	0	0.0
IIIb	30	53.6
IIIc	26	46.4

more than 2 positive lymph nodes ($P = .5$), age ($P = .6$), or gender ($P = .6$) (Table 2).

Disease-Free Survival

During a median follow-up of 14.2 months (range 0.6–40.9) months, tumor recurrence was diagnosed in 24

TABLE 2 Logistic regression analysis on elevated preoperative S-100B in stage III melanoma patients

Variable	Elevated preoperative S-100B (%)	OR	<i>P</i> value
<i>Sex</i>			
Male	51.9	Ref	
Female	44.8	0.8 (0.3–2.2)	.6
<i>Age</i>			
<55 years	44.0	Ref	
≥55 years	51.6	1.4 (0.5–3.9)	.6
<i>Positive lymph nodes</i>			
≤2	44.4	Ref	
>2	55.0	1.5 (0.5–4.6)	.5
<i>Extranodal growth</i>			
Yes	52.2	Ref	
No	48.4	0.9 (0.3–2.5)	.8
<i>Tumor size</i>			
<3 cm	35.7	Ref	
≥3 cm	65.4	3.4 (1.1–10.4)	.03

Elevated S-100B was defined as S-100B ≥ 0.15 g/L

Ref reference category

patients (43%), of which 17 patients were diagnosed with distant metastasis and 7 patients with a regional recurrence. As shown in Table 3, DFS was not associated with sex, localization of the lymph node, or the size of the lymph nodes. Two-year DFS in patients with elevated preoperative S-100B concentrations was 34% versus 61% for patients without elevated preoperative S-100B concentrations (hazard ratio [HR] 2.6, $P = .03$). In the patients whose S-100B increased postoperatively, 2-year DFS was 30% versus 51% for patients without a postoperative S-100B increase (HR 2.0, $P = .1$) (Fig. 1).

In multivariate analysis (model with positive lymph nodes, extranodal growth, preoperative and postoperative S-100B and substage IIIb or IIIc), extranodal growth (HR 0.4, $P = .05$), and elevated preoperative S-100B concentrations (HR 2.6, $P = .03$) were significantly associated with decreased DFS. Patients with increasing postoperative S-100B serum levels showed a trend toward statistical significance when correlated to DFS (HR 2.6, $P = .07$).

DISCUSSION

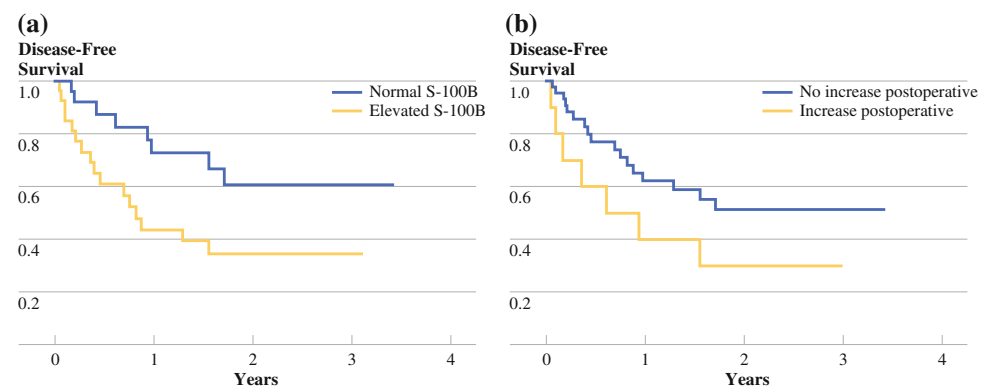
This is the first study that demonstrates a potential prognostic value of preoperative S-100B concentrations in patients who underwent a therapeutic lymph node dissection after preoperative staging with FDG-PET and CT. According to the literature, the proportions of patients with elevated S-100B concentrations were 0–9% in stage I/II,

TABLE 3 Disease-free survival (DFS) of AJCC stage III melanoma patients

Variable	2-year DFS (%)	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P value	HR (95% CI)	P value
<i>Sex</i>					
Male	45	Ref			
Female	47	1.2 (0.5–2.6)	.7		
<i>Localization lymph node</i>					
Neck	83	Ref			
Axilla	51	3.3 (0.4–26)			
Groin	36	4.9 (0.7–37)	.2		
<i>Positive lymph nodes</i>					
≤2	64	Ref		Ref	
>2	23	3.3 (1.5–7.5)	.004	1.6 (0.6–4.3)	.3
<i>Extranodal growth</i>					
Yes	28	Ref		Ref	
No	58	0.4 (0.2–0.8)	.02	0.4 (0.1–1.0)	.05
<i>Lymph node size (cm)</i>					
<3	53	Ref			
≥3	35	1.5 (0.7–3.3)	.4		
<i>Stage</i>					
IIIB	69	Ref		Ref	
IIIC	23	3.9 (1.6–9.5)	.003	1.8 (0.6–5.2)	.3
<i>Preoperative S-100B</i>					
Normal	61	Ref		Ref	
Elevated	34	2.6 (1.1–6.0)	.03	2.6 (1.1–6.1)	.03
<i>Postoperative S-100B</i>					
No increase	51	Ref		Ref	
Increase	30	2.0 (0.8–4.8)	.1	2.6 (0.9–7.2)	.07

Ref reference category

FIG. 1 Kaplan-Meier curve for preoperative S-100B and disease-free survival of AJCC stage III melanoma patients. **a** Elevated preoperative S-100B (34% 2-year DFS) versus not elevated (61% 2-year DFS) levels are associated with a decreased disease-free survival ($P = .03$). **b** Increasing postoperative S-100B levels (30% 2-year DFS) versus not increasing (51% 2-year DFS) ($P = .1$)



5–98% in stage III, and 40–100% in stage IV melanoma (Table 4). The highest S-100B concentrations were found in patients with liver and/or skeletal metastases.^{20,21} Serum S-100B concentrations are therefore correlated with the clinical stage of the disease.

The aim of this study was to evaluate the prognostic value of preoperative and postoperative concentrations of the tumor marker S-100B in clinically stage III melanoma patients who underwent a so-called “curative lymph node

dissection” after preoperative staging with whole-body FDG-PET and spiral CT. Our results showed that preoperative elevated concentrations of S-100B was statistically significantly associated with a decreased DFS. It is difficult to compare the studies investigating the value of S-100B in patients with melanoma, because various assays (IRMA or LIA-mat) and cut-off points and groups of patients with different stages were used (Table 4).⁸ Garbe et al. also found a relation between S-100B and disease-free survival

TABLE 4 Review literature S-100B values above cut-off point in melanoma patients

Reference	No.	Assay	Cut-off value	Stage I/II	Stage III	Stage IV
Guo et al. ¹⁴	126	IRMA	0.15	1%	9%	74%
Miliotes et al. ³⁸	67	IRMA	0.05	47.8%	–	–
Abraha et al. ³⁹	97	IRMA	0.2	9%	82%	–
Bosserhoff et al. ⁴⁰	112	IRMA	0.15	0%	62%	–
Buer et al. ²⁴	99	IRMA	3.00	–	–	22.2%
Henze et al. ²⁹	73	IRMA	0.3	4%	21%	79%
Tofani et al. ⁴¹	53	IRMA	0.5	0%	55%	–
Schultz et al. ⁴²	84	IRMA	0.3	0%	31%	69%
Bonfrer et al. ⁴³	251	LIA	0.16	I/II: 1%	–	III/IV: 79%
Seregini et al. ⁴⁴	438	IRMA	0.2	I: 4.2%, II: 5.3%	38.5%	–
Hauschild et al. ⁴⁵	412	IRMA	0.2	2%	19%	68%
Curry et al. ²²	147	IRMA/LIA	0.2/0.12	–	23%/47.5%	–
Berking et al. ⁴⁶	352	LIA	0.2	7%	8%	48%
Jackel et al. ¹⁶	276	LIA	0.12	6%	5%	48%
Kaskel et al. ⁴⁷	570	LIA	0.11	9%	94%	–
Brouard et al. ⁴⁸	122	LIA	0.09	0%	54%	84%
Vuoristo et al. ⁴⁹	50	LIA	0.12	–	–	64.0%
Jury et al. ⁵⁰	214	LIA	0.2	7%	29%	92%
Mohammed et al. ²¹	68	LIA	0.2	–	–	73.5%
Martenson et al. ²⁰	727	LIA	0.10	12%	–	III/IV: 72%
Juergensen et al. ⁵¹	50	LIA	0.12	–	–	I/II/III/IV: 50%
Rebmann et al. ⁵²	183	LIA	0.12	–	–	I/II/III/IV: 21%
Banfalvi et al. ³⁰	59	LIA	0.12	40%	25%	59%
Garbe et al. ³²	296	LIA	0.12	–	–	–
Banfalvi et al. ³¹	478	LIA	0.18	I: 29%, II: 19%	32%	48%
Andres et al. ⁵³	85	LIA	0.15	–	I/II/III: 15%	89%
Ugurel et al. ⁵⁴	300	LIA	0.12	–	–	I/II/III/IV: 20%
Smit et al. ¹⁵	145	LIA	0.16	–	–	78%
Schmidt et al. ⁵⁵	85	LIA	0.15	–	–	59%
Domingo et al. ³³	97	LIA	0.15	–	II/III: 21%	–
Cao et al. ⁵⁶	42	LIA	0.20	–	–	50%
Tarhini et al. ³⁷	670	LIA	0.15	–	IIb/III: 13%	–
Kruijff et al. 2009	56	LIA	0.15	–	48%	–

LIA lumino immunometric assay, IRMA immuno radiometric assay

in stage II and III melanoma patients. Postoperative elevated S-100B preceded detection of developing metastases by imaging techniques.² According to Martenson et al. and Curry et al., elevated S-100B in stage III melanoma after lymph node dissection may form an independent prognostic factor.^{20,22} Four studies demonstrated that elevated S-100B was associated with shortened (disease-free) survival.^{20,23–25} In stage IV patients with normal S-100B levels, survival was better for patients with low S-100B levels compared with those with elevated S-100B levels.^{15,26} Increasing concentrations of S-100B in stage IV patients who were receiving systemic treatment were associated with disease progression.^{14,27–29} In a study on

1007 melanoma patients, S-100B was not only correlated with the clinical stage of the disease, but it also appeared to be an independent prognostic marker in stages II and III. The 5-year survival of patients with S-100B values of <0.10 g/L was 91%, compared with 51% when the values were >0.10 g/L.²⁰ In two other studies, similar patterns were found.^{30,31} Two studies comparing 5-S-cysteinyldopa, LDH and S-100B as prognostic markers found that S-100B had the highest specificity.^{30,31}

Several studies have therefore recommended the use of S-100B as a tumor marker to monitor the course of disease in stage III melanoma patients and to evaluate the effect of therapy in stage IV patients. Successful treatment with

lymph node dissection, chemotherapy, or immunotherapy was associated with decreased S-100B concentrations, whereas increased concentrations were an expression of disease progression.^{14,27,28}

The prognostic value of the preoperative and postoperative course of S-100B concentrations in “true stage III” melanoma patients has not been studied before. In advance, we hypothesized that a significant decrease in S-100B concentration after therapeutic lymph node dissection would correlate with better DFS. In this way we assumed S-100B could be used as a quality-control marker for dissection. In our study, 27 patients (48.2%) had elevated S-100B preoperatively, and indeed in 22 of them (81.4%) the concentrations normalized postoperatively. However, 12 patients of these 22 (55%) developed tumor recurrence during a median follow-up of 1.18 years. Multivariate analyses showed that preoperative S-100B elevation was an important predictor of decreased DFS ($P = .03$).

Although the results indicated that therapeutic lymph node dissection did have a temporary influence on the postoperative course of S-100B, a more important predictor for shortened DFS seemed preoperative S-100B elevation. For AJCC stage III, postoperative increasing S-100B serum were associated with a decreased DFS. However, when adjusted for stage III substages, probably because of small numbers, only a trend toward statistical significance was observed.

S-100B is located in the cytoplasm of melanoma cells. Elevated serum S100-B occurs as the result of loss of melanoma cell integrity.¹⁹ Therefore, the hypothesis that elevation of S-100B should be interpreted as a process of subclinical microscopic metastatic disease seems to be suggested by these data. Preoperative elevated S-100B could be an expression of early dissemination not detected by standard imaging tests.³³ However, the tumor marker is not suitable for screening purposes or for the early detection of recurrence in the follow-up of melanoma patients.³⁴

The prognostic biochemical marker S-100B may be of value in the design of future trials on adjuvant systemic chemotherapy or immunotherapy. At present, LDH is the most prominent serum parameter in stage IV melanoma, and it has been included in the new AJCC staging system.⁵ Our study on optimally staged clinical stage III melanoma patients has now shown that preoperative S-100B elevation is associated with significantly poorer survival. These results are supported by studies from M.D. Anderson Cancer Center, Barcelona, and a recent meta-analysis and a pooled analysis of ECOG studies in high-risk surgically resected melanoma by Kirkwood.^{33,35–37} These studies showed that direct postoperative (baseline) S-100B is indeed a prognostic marker for disease-free survival (DFS) and overall survival (OS). Our results seem in accordance

with these data, but more importantly reveal that S-100B is a prognostic marker for DFS when determined before dissection. In our study, the 2-year DFS is slightly better than in the recently published ECOG, which is explained by the fact that the patients were staged with FDG-PET and spiral CT.³⁷ A more refined individualized risk assessment for adjuvant treatment is possible with serum marker S-100B. Therefore, S-100B could be included in future stratification research for stage III melanoma.

In summary, preoperative elevation of S-100B levels in patients with true (clinical) stage III melanoma is associated with decreased disease-free survival and might have a prognostic clinical value in high-risk stage III melanoma patients. Therefore S-100B could be used to select patients for adjuvant systematic treatment in the stratification for new adjuvant therapeutic trials as well as to provide information for stage III melanoma patients who want to be informed about disease prognosis.

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