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# Prognostic value of serial blood S100B determinations in stage IIB–III melanoma patients: A corollary study to EORTC trial 18952

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ABSTRACT

S100B is a prognostic factor for melanoma as elevated levels correlate with disease progression and poor outcome. We determined its prognostic value based on updated information using serial determinations in stage IIb/III melanoma patients.

211 Patients who participated in the EORTC 18952 trial, evaluating efficacy of adjuvant intermediate doses of interferon α2b (IFN) versus observation, entered a corollary study. Over a period of 36 months, 918 serum samples were collected. The Cox time-dependent model was used to assess prognostic value of the latest (most recent) S100B determination.

At first measurement, 178 patients had S100B values <0.2 μg/l and 33 ⩾ 0.2 μg/l. Within the first group, 61 patients had, later on, an increased value of S100B (⩾0.2 μg/l). An initial increased value of S100B, or during follow-up, was associated with worse distant metastasis-free survival (DMFS); hazard ratio (HR) of S100B ⩾ 0.2 versus S100B < 0.2 was 5.57 (95% confidence interval (CI) 3.81–8.16), $P < 0.0001$, after adjustment for stage, number of lymph nodes and sex. In stage IIb patients, the HR adjusted for sex was 2.14 (95% CI 0.71, 6.42), whereas in stage III, the HR adjusted for stage, number of lymph nodes and sex was 6.76 (95% CI 4.50–10.16). Similar results were observed regarding overall survival (OS).

Serial determination of S100B in stage IIb–III melanoma is a strong independent prognostic marker, even stronger compared to stage and number of positive lymph nodes. The prognostic impact of S100B ⩾ 0.2 μg/l is more pronounced in stage III disease compared with stage IIb.



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## 1. Introduction

The S100B protein is an immunohistological marker for malignant melanocytes[1] that was first detected in melanoma cell cultures[2] and is overexpressed in most cells of neuroectodermal origin.[3] S100B is located in the cytoplasm and in the nucleus as a disulphide cross-linked homo- or heterodimers containing primarily $Ca^{2+}$.[4] It has many cell functions, mainly in cytoskeleton integrity, in cell cycle regulation[5] and apoptosis through its interaction with p53.[6] However, the mechanism by which the protein leaks to the blood is not fully understood but most probably related to cell damage or cell death.[7] This is strongly supported by the fact that immunoreactive S100B is found exclusively in the cytoplasm and nucleus; a limited secretion of the protein has only been reported in the brain, and its substantial release has been found in brain damage and stage IV melanoma. Additionally, serum S100B monitoring has been found useful in the latter disease.[8,9]

While many reports show that S100B blood levels provide a good indication of disease progression as well of response to chemotherapy in stage IV patients[10–12], it remains of little or limited usefulness in early stages (II and III) where the disease is most often confined to lymph nodes.[13] Guo and colleagues[14] assessed S100B serum levels in 126 melanoma patients and found positive levels in 1.3%, 8.7% and 73.9% of patients with stages I/II, III and IV disease, respectively. This illustrates its poor utility in stages I–III. However, the progression from the early stages to distant organ involvement is frequently accompanied by a significant increase in S100B levels.[15]

The utility of serial determinations of S100B serum levels in patients with high-risk melanomas (stages IIb–III), using a time-dependent Cox model, has not been reported on until now. As early elevations in S100B levels may precede clinically detectable relapses serial determinations may have a particular prognostic value in the setting of adjuvant systemic therapy. This study was designed to determine the prognostic value of serial serum S100B levels in stages IIb–III melanoma. Patients were enrolled in the randomised phase III EORTC 18952 trial evaluating the efficacy of adjuvant intermediate doses of interferon (IFNα-2b).[16] Serum S100B levels were measured during treatment and follow-up, to a maximum of 36 months.

## 2. Material and methods

### 2.1. Patients and sample collection

Patients aged 18–75 years with melanoma either stages IIb or III (N1, patients with microscopically involved lymph nodes on sentinel node biopsy, or N2, those with palpable tumour-involved nodes) were eligible to be included in the EORTC 18952 study. Patients were randomised between 13-month interferon (IFN), 25-month IFN or observation in a 2:2:1 fashion. IFN treatment comprised a 4-week induction period of 10 million units (MU) s.c. 5 d/week, followed either by 10 MU × 3/week for 1 year or 5 MU × 3/week for 2 years. Clinical/radiological evaluations have been scheduled every 3 months in year 1, every 4 months in year 2, every 6 months in years 3–5 and annually thereafter. In eight European institutions that participated in the EORTC 18952 trial, 211 patients entered this corollary study. Over a 36-month period 918 serum samples were collected (Table 1). According to the study protocol, when patients relapsed they went off study, treatment was discontinued and sampling was also stopped. Occasionally, some samples were collected after relapse. Pretreatment S100B levels were not determined since these are often false positive due to recent surgery.[7] Serum was separated, aliquoted and frozen at −18 °C until assayed.

### 2.2. S100B determination

S100B was measured in serum samples by an immunoluminometric assay LIA-mat (Sangtec Medical, Sweden), following the manufacturer's instructions. We have already estimated a threshold for pathological S100B levels to be of 0.2 μg/l also based on *in vivo* non-specific liberation by normal cells mainly endothelial cells.[7]

### 2.3. Statistical analysis

Distant metastasis-free interval (DMFI) was the time interval between the date of randomisation until the first appearance of distant metastases; the follow-up of patients who did not develop distant metastases has been censored at the latest visit/last contact. In this S100B study, as no patient died without having developed distant metastases, DMFI was identical to the distant metastasis-free survival (DMFS). Overall survival (OS) was computed from the date of randomisation until the date of death or until the last date of follow-up (censored observations). Time to S100B $\geqslant$ 0.2 μg/l (the considered pathological cutoff level) was the time from randomisation until the first date S100B level was $\geqslant$0.2 μg/l; the follow-up of patients for whom S100B did not reach the 0.2 μg/l level has been censored at the latest date of assessment of S100B.

Kaplan-Meier technique was used to estimate survival-type distributions and the standard errors (SE) of the estimates were obtained via the Greenwood formula.[17] Two-tailed log-rank test and generalised Wilcoxon test, which gives more weight to differences occurring at earlier time points, were used to test differences between curves. The landmark method was used to test the prognostic impact of S100B level at 6 months for the subsequent outcome. Since sampling was scheduled every 3 months during the first year we used a time window of 45 d not to have overlap. The serial measurements of S100B have been performed at several time points (Table 1) with the same schedule for IFN-treated as untreated patients. Since the samples were not exactly drawn at the time points according to study protocol, numbers in Table 1 reflect the closest time points. As mentioned before, for patients who developed distant metastases, generally their S100B levels have not been assessed subsequently after their relapse, therefore the sampling rate is lower towards the end of the study. To determine whether the latest (most recent) value of S100B, which was assessed during the course of the study, before or at the time of distant metastases, has a prognostic impact on the subsequent outcome, the Cox

**Table 1 – Number of patients analysed at the different time points.**

| Month | 1[a] | 3 | 6 | 9 | 12 | 16 | 20 | 24 | 30 | 36 |
|---|---|---|---|---|---|---|---|---|---|---|
| No. of patients | 211 | 113 | 134 | 107 | 104 | 84 | 61 | 49 | 44 | 11 |

a End of induction/observation.

time-dependent model was used.[18] In the Cox time-dependent model, for patients free of event (distant metastasis – for DMFS – or death – for OS) just before a time point $t$, the hazard ratio (HR) was set as;

$$HR = e^{\beta_1 \times S100B(t) + \beta_2 \times Stage + \beta_3 \times Number\ of\ positive\ lymph\ nodes + \beta_4 \times Sex}$$

with S100B($t$) = 0 if the latest S100B determined before or at time $t$ was <0.2 μg/l; or = 1, if the latest S100B value determined before or at time $t$ was ⩾0.2 μg/l. Thus, for purposes of risk assessment the most recent value of S100B at time $t$ (determined at that time or earlier if not available at time $t$) was used. In the Cox time-dependent model, all available samples/determinations were used, regardless of a time window.

Based on the data (serial measurements of S100B and the outcome of each patient), an estimate of HR along with its 95% confidence interval (CI) has been calculated; the Wald test (standardised coefficient) was used to determine the prognostic value of variables considered in the model, in univariate or multivariate setting (data coding: see Table 2). In stage IIb patients, variables stage and number of positive lymph nodes have not been considered. Analyses were censored at 4 years since thereafter only few distant metastases occurred and the time interval between the latest S100B evaluation and a possible event became too long.

SAS 9.1 software (SAS Institute Inc., Cary, NC, USA) was used to perform the statistical evaluation.

## 3. Results

### 3.1. *Baseline characteristics and S100B values*

The distribution of patients according to the treatment protocol, stage of the disease, number of positive lymph nodes, sex and presence of ulcerated primary melanoma, is summarised in Table 2. These characteristics were comparable with those of patients included in the entire 18952 study, and treatment distribution was in accordance with the randomisation scheme: 20% randomised in the observation arm and 40% in each IFN group. At the initial measurement time point, corresponding generally to the end of induction/observation period (median of 28 d from randomisation), 178 patients had S100B values below 0.2 μg/l and 33 above or equal 0.2 μg/l. A higher incidence of stage IIb patients has been found in those with an initial S100B ⩾ 0.2 μg/l than in those with a S100B < 0.2 μg/l: 39% versus 21%. The number of positive lymph nodes did not correlate with S100B increase.

**Table 2 – Patient distribution and treatment allocated at randomisation according to S100B levels at baseline (randomisation/end of induction).**

| Variable | S100B < 0.2 μg/l No. (%) | S100B ⩾ 0.2 μg/l No. (%) | All patients No. (%) |
|---|---|---|---|
| *Treatment* | | | |
| Observation | 30 (16.9) | 8 (24.2) | 38 (18.0) |
| 13-month Interferon (IFN) | 74 (41.6) | 13 (39.4) | 87 (41.2) |
| 25-month IFN | 74 (41.6) | 12 (36.4) | 86 (40.8) |
| *Stage of the disease* | | | |
| IIB | 38 (21.3) | 13 (39.4) | 51 (24.2) |
| III N1 | 40 (22.5) | 6 (18.2) | 46 (21.8) |
| III N2 | 100 (56.2) | 14 (42.4) | 114 (54.0) |
| *No. of positive lymph nodes* | | | |
| 0 | 38 (21.3) | 13 (39.4) | 51 (24.2) |
| 1 | 75 (42.1) | 5 (15.2) | 80 (37.9) |
| 2–4 | 49 (27.5) | 9 (27.3) | 58 (27.5) |
| 5+ | 16 (9.0) | 6 (18.2) | 22 (10.4) |
| *Sex* | | | |
| Male | 95 (53.4) | 16 (48.5) | 111 (52.6) |
| Female | 83 (46.6) | 17 (51.5) | 100 (47.4) |
| *Ulceration status of primary melanoma* | | | |
| Absent | 95 (53.4) | 23 (69.7) | 118 (55.9) |
| Present | 50 (28.1) | 8 (24.2) | 58 (27.5) |
| Unknown | 33 (18.5) | 2 (6.1) | 35 (16.6) |

A total of 116 (55.0%) of 211 patients developed distant metastases within 4 years, with a median DMFS of 2.7 years, and 97 (46.0%) of 211 patients died (median OS was not reached). As in this series IFN did not have an impact on the outcome (for DMFS: HR = 1.15 observation arm versus the 1-year IFN arm, HR = 0.92 observation versus 2-year IFN arm, overall *P*-value, 0.55, and for OS: HR = 1.49 observation arm versus the 1-year IFN arm, HR = 1.19 observation versus 2-year IFN arm, overall *P*-value, 0.34), in the subsequent analyses, patients from the three treatment groups were pooled together.

### 3.2. *Prognostic significance of S100B ⩾ 0.2 μg/l*

Among the 178 patients who initially had a S100B < 0.2 μg/l, 61 had, later on, an increased value of S100B (⩾0.2 μg/l). Therefore, overall, a total of 94 patients (33 + 61) had a S100B level ⩾ 0.2 μg/l. Time between random assignment and S100B level ⩾ 0.2 μg/l, according to the disease stage, is shown in Fig. 1. Within the first 3 months, the cumulative rate of patients with S100B level ⩾ 0.2 μg/l was higher in stage IIb patients than in stage III patients.

A total of 61 patients (65%) from the 94 patients reaching S100B ⩾ 0.2 μg/l developed distant metastases, of these, six patients (6%) had such a rise after the detection of the metastases. For the remaining 55 patients, median time between S100B ⩾ 0.2 μg/l and the development of distant metastases was 94 d (range 0–1580 d). Contrary, from the 117 patients with normal S100B levels, 62 (53%) patients developed distant metastases.

The impact of the *initial* S100B levels on DMFS and OS was especially seen within 1 year from randomisation, but thereafter the curves converged (Fig. 2). According to the Wilcoxon test, a test which gives more weight to differences occurring at earlier time points, there was a significant difference between initial S100B levels and DMFS (*P* = .03). However, when considering the overall curve using the Log-rank test, this difference was not significant (*P* = .21). This temporary effect of elevated S100B levels on prognosis is also illustrated in Fig. 3, showing Landmark methods for DMFS and OS at

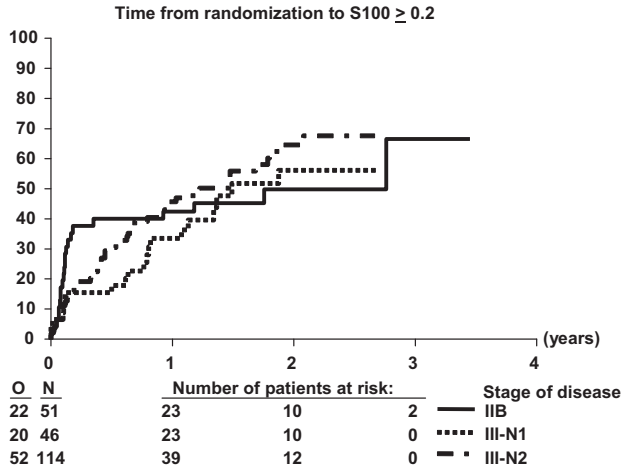


**Fig. 1 – Time to first S100B level ⩾ 0.2 μg/l by initial stage of disease.**

6 months from randomisation; patients with S100B serum level ⩾ 0.2 μg/l at 6 months after randomisation have, at a short term, a worse prognosis compared to patients with levels <0.2 μg/l.

In order to evaluate the prognostic value of S100B level ⩾ 0.2 μg/l for the different disease stages, we performed an analysis in which we selected the 94 patients with S100B level ⩾ 0.2 μg/l, excluding the six patients who developed distant metastases before their rise in S100B levels. In these 88 patients, outcome was very different according to the initial stage: stage IIb patients had a high 3-year distant metastasis free survival (76% at 3 years), whereas stage III–N1 and especially stage III–N2 patients had a very poor prognosis: 45% and 23% respectively (Fig. 4). This indicates that the effect of increased S100B levels on prognosis is most pronounced in more advanced disease (stage III–N2).

### 3.3. *Impact of serial S100B measurements on DMFS and OS*

An advantage of serial measurements is the availability of updated information during the course of study. Therefore, a more accurate evaluation of a potential biomarker and its effect on disease outcome is possible. Herein the Cox time-dependent model was used to evaluate the prognostic significance of the latest (most recent) S100B value, in univariate and multivariate setting, as well as according to the initial stage.

For the entire series of 211 patients, in univariate analysis, the following factors appeared to be of prognostic importance regarding the DMFS: initial stage (stage III–N2 versus stage III–N1 or stage IIb: HR = 3.18, 95% CI 2.13–4.75, *P* < 0.0001) (Fig. 5), number of lymph nodes (0 versus 1 versus 2–4 versus 5+: HR = 1.76, 95% CI 1.44–2.14, *P* < 0.0001) and sex (male versus female: HR = 1.77, 95% CI 1.22–2.58, *P* = 0.003).

For DMFS, according to the Cox time-dependent model evaluating all 211 patients, the estimated HR for S100B(*t*) ⩾ 0.2 μg/l versus S100B(*t*) < 0.2 μg/l comparison was 3.80 (univariate analysis) and, after adjustment for the variables stage, number of lymph nodes and sex, HR was 5.57 (95% CI 3.81–8.16; *P* < 0.0001, Table 3). Moreover, in the multivariate analysis, HR for disease stage (HR = 2.75) and number of positive lymph nodes (HR = 1.43) were both lower than for S100B (HR = 5.57). In stage IIb patients only, the estimated HR for S100B adjusted for sex was 2.14 (95% CI 0.71–6.42; *P* = .18), whereas in stage III patients, the HR for S100B adjusted for stage, number of lymph nodes and sex was 6.76 (95% CI 4.50–10.16; *P* < 0. 0001, Table 3). Similar results were observed in stage III–N1 and stage III–N2 patients (data not shown).

Regarding OS, the risk of death of those with S100B(*t*) ⩾ 0.2 μg/l was 3.8 higher than the one observed in those with a S100B(*t*) < 0.2 μg/l. In multivariate analysis, the estimate of the HR was 4.73 (95% CI 3.14–7.12), *P* < 0.0001; in stage IIb patients the HR was lower than in stage III patients: 2.73 (95% CI 0.79–9.44; *P* = .11) versus 5.46 (95% CI 3.52–8.45; *P* < .0001).

In multivariate analyses, ulceration (presence, absence, unknown) had a weak prognostic impact. Addition of ulceration, however, in the multivariate analyses did not change the prognostic importance of S100B (Supplementary Table).

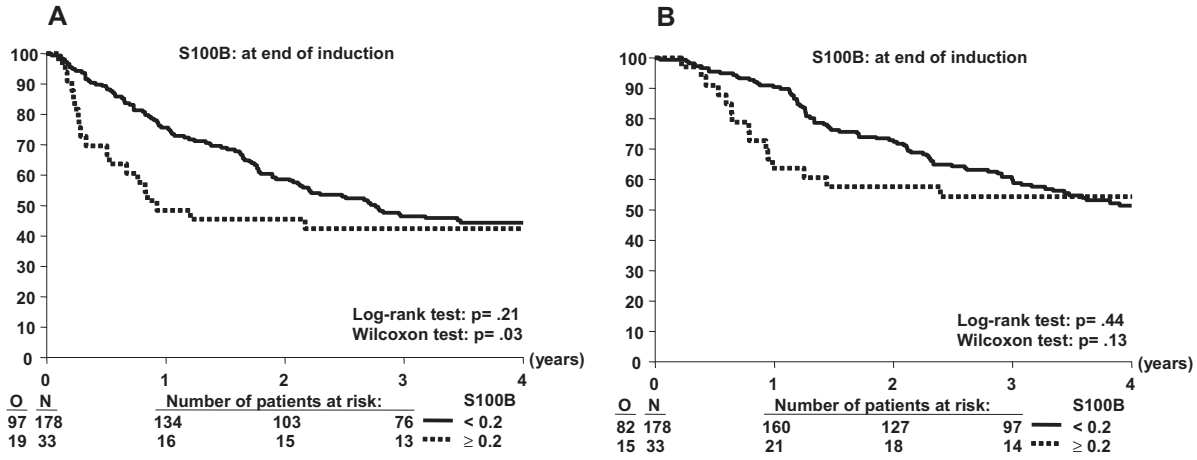


**Fig. 2 – Kaplan-Meier curves regarding distant metastasis-free survival (panel A) and overall survival (panel B) from randomisation according to initial S100B level (<0.2 versus ⩾0.2 μg/l). Analyses were censored at 4 years. N = number of patients at risk. O = observed number of patients who developed distant metastases (panel A) or who died (panel B).**

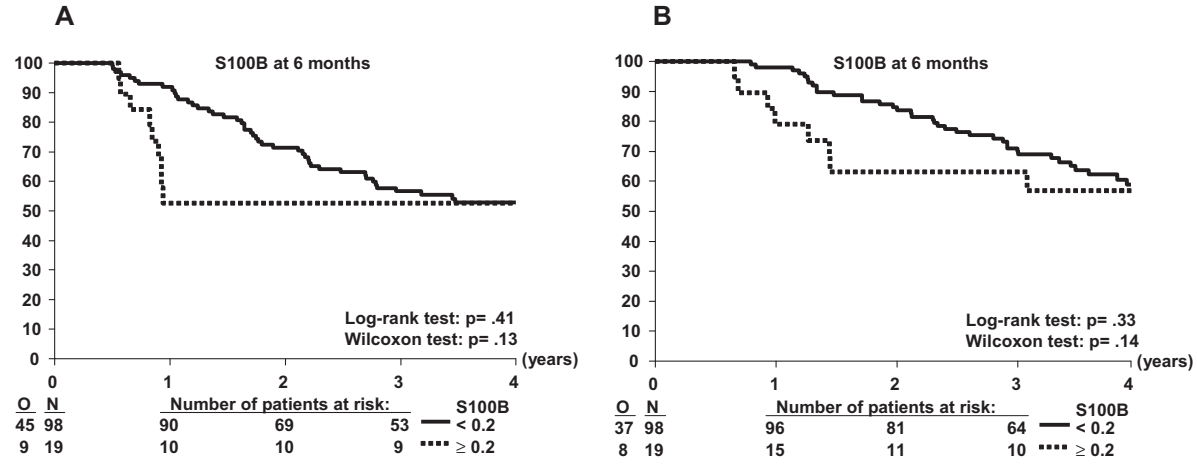


**Fig. 3 – Landmark method: Kaplan-Meier curves regarding distant metastasis-free survival (panel A) and overall survival (panel B) from randomisation according to S100B level (<0.2 versus ⩾0.2 μg/l) assessed at 6 months post-randomisation. Analyses were censored at 4 years. N = number of patients at risk. O = observed number of patients who developed distant metastases (panel A) or who died (panel B).**

In summary, according to the time-dependent Cox model, which uses the most recent S100B value (whether <0.2 μg/l or ⩾0.2 μg/l at that time point), S100B is in both the univariate as well as in the multivariate setting as an independent prognostic factor for worse DMFS and OS. This effect is most pronounced in stage III disease, and even stronger than the other important prognostic factors, disease stage and number of positive lymph nodes.

## 4. Discussion

We demonstrated in this corollary study to the EORTC 18952 trial in stages IIB–III melanoma patients that serial determinations of S100B serum levels strongly correlate with DMFS and OS. Strikingly, the hazard ratio (HR) for S100B determinations was higher and more significant than the one corresponding to stage, and number of positive lymph nodes, two strong prognostic factors in stage III melanoma.

The use of Cox regression with time-dependent covariates to assess the effect of S100B on the end-points provides new insights into the prediction value of the marker. Unlike previous studies, several determinations per patient over time could be thus considered and statistically taken into account. However, such an approach focusing on S100B level changes rather than independent single values in time has been suggested to monitor and predict treatment outcome.[10,11]

Another point to consider was the S100B cutoff level to choose in order to separate “pathological” from “normal” values. Based on previous studies, 0.20 μg/l was chosen also because it allows avoiding possible false positives due to S100B release by damaged vessels after surgery. This is clearly of relevance in this postoperative adjuvant therapy trial, where most patients entered the trial after a full regional lymph node dissection.

The prognostic impact of the baseline S100B on DMFS and OS has been observed, especially within the first year after

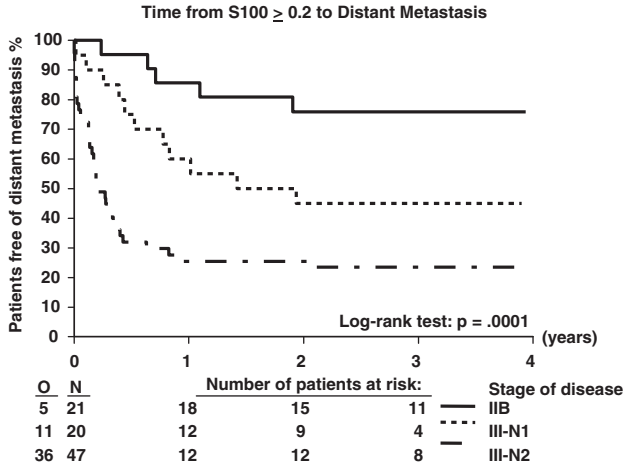


**Fig. 4 – Kaplan-Meier curves regarding distant metastasis-free survival from the first moment S100B level was ⩾0.2 μg/l, according to initial stage of disease. Analyses were censored at 4 years. N = number of patients at risk. O = observed number of patients who developed distant metastases.**

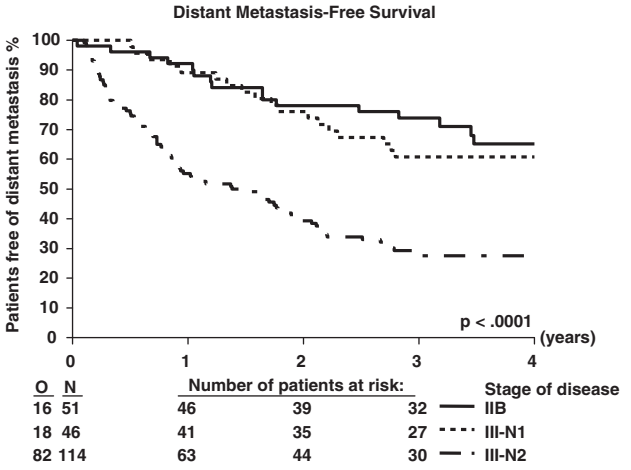


**Fig. 5 – Kaplan-Meier curves regarding distant metastasis-free survival from randomisation according to initial stage of disease. Analyses were censored at 4 years. N = number of patients at risk. O = observed number of patients who developed distant metastases.**

the assessment (Fig. 2). Landmark analysis at 6 months also showed short term (6–12 months) differences between the two groups for both DMFS and OS (Fig. 3). These results suggest that a time-window exists within a careful monitoring of S100B can be very helpful to assess the risk of distant metastasis or death. This is further substantiated by the finding that while stage and the number of positive lymph nodes correlated significantly and as expected with DMFS, S100B serial value appeared to be in a Cox time-dependent model not only as an independent prognostic factor from these variables but also to have a much higher prognostic importance than these variables. In stage III patients the estimated HR of S100B ⩾ 0.20 μg/l versus S100B < 0.20 μg/l, adjusted by these factors and by sex, was very high (6.76, $P < .0001$), but was lower and not significant (2.14, $P = .18$, adjusted by sex only) in stage IIb patients (Table 3). This finding is in accordance with previous studies that were all suggesting S100B to be a late progression marker but of little value when the disease is still confined to the lymph nodes. This is also consistent with the mechanism of release and the short biological half-life of this marker that is mostly related to a rather substantial cell death.[7] A recent paper by Tarhini et al. reported a

**Table 3 – Results of the Cox time dependent model.**

| | All patients | | | | Stage IIb | | | | Stage III | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hazard ratio | 95% CI | | P-value | Hazard ratio | 95% CI | | P-value | Hazard ratio | 95% CI | | P-value |
| *Distant metastasis-free survival*[a] | | | | | | | | | | | | |
| Univariate | | | | | | | | | | | | |
| S100B[b] | 3.80 | 2.62 | 5.51 | <0.0001 | 1.33 | 0.46 | 3.86 | .60 | 6.15 | 4.11 | 9.20 | <0.0001 |
| Multivariate | | | | | | | | | | | | |
| S100B[b] | 5.57 | 3.81 | 8.16 | <0.0001 | 2.14 | 0.71 | 6.42 | .18 | 6.76 | 4.50 | 10.16 | <0.0001 |
| Stage[c] | 2.75 | 1.68 | 4.48 | <0.0001 | | | | | 2.86 | 1.68 | 4.88 | 0.0001 |
| Nb of +LN[d] | 1.43 | 1.13 | 1.83 | .004 | | | | | 1.37 | 1.03 | 1.81 | 0.03 |
| Sex: male versus female | 1.94 | 1.32 | 2.85 | 0.0007 | 5.61 | 1.52 | 20.68 | 0.01 | 1.58 | 1.05 | 2.37 | 0.03 |
| *Overall survival*[a] | | | | | | | | | | | | |
| Univariate | | | | | | | | | | | | |
| S100B[b] | 3.80 | 2.54 | 5.67 | <0 .0001 | 1.36 | 0.41 | 4.54 | .61 | 5.48 | 3.56 | 8.46 | <0.0001 |
| Multivariate | | | | | | | | | | | | |
| S100B[b] | 4.73 | 3.14 | 7.12 | <0.0001 | 2.73 | 0.79 | 9.44 | .11 | 5.46 | 3.52 | 8.45 | <0.0001 |
| Stage[c] | 1.92 | 1.13 | 3.25 | 0.02 | | | | | 1.87 | 1.06 | 3.28 | 0.03 |
| Nb of +LN[d] | 1.51 | 1.17 | 1.95 | 0.002 | | | | | 1.48 | 1.10 | 1.98 | 0.01 |
| Sex: male versus female | 2.20 | 1.44 | 3.35 | 0.0003 | 15.77 | 1.93 | 129.10 | 0.01 | 1.77 | 1.14 | 2.75 | 0.01 |

[a] Time variable right censored at 4 years.
[b] S100B: 0 < 0.2, 1 ⩾ 0.2 μg/l (Cox time-dependent).
[c] Stage: 0 = stage IIb ($n = 51$) or stage III–N1 ($n = 46$), 1 = stage III–N2 ($n = 114$).
[d] Number of positive lymph nodes: 0 = 0, 1 = 1, 2 = 2-4, 3 = 5 or more.

weak association of S100B as a time-varying covariate and the risk for mortality.[19] The number of time points in this study was limited: baseline, 4–6 weeks and 12–14 weeks. The low impact of S100B on OS (HR 1.4) can be explained by the long lag-time between the latest evaluation of S100B and the moment a patient is considered in the analysis. Indeed, the S100B has a short-term prognostic value (e.g. Figs. 2 and 3), so, when obsolete information on S100B is provided, this leads to a drastic loss in its prognostic value.

Results from the EORTC 18991 trial, evaluating long-term treatment with pegylated IFNα-2b[20], suggested that treatment with PEG-IFN was more beneficial in stage IIb and stage III–N1 as compared to stage III–N2 patients. Our study of S100B within the framework of the 18952 trial also raises the question whether S100B could identify patients benefiting IFN treatment or not. One could argue that patients receiving IFN should discontinue therapy once S100B levels of $\geqslant 0.2$ μg/l have been reached, indicating disease progression and no treatment response. Unfortunately we have no guidance by biomarkers to determine use, dose or duration of adjuvant systemic therapy in melanoma. Gogas and colleagues[21] have reported that patients treated with adjuvant IFN who developed autoantibodies or clinical signs of autoimmunity had a significantly better outcome than patients who did not develop these signs of autoimmunity. The development of markers that might predict who will mount a host immune response could be extremely important. The markers could be used to determine which patients to treat with IFN and for how long. An evaluation of the presence or emergence of autoantibodies in patients who participated in the EORTC 18952 and Nordic IFN trial did not confirm Gogas' observations.[22] Nor did a subsequent similar study in the large EORTC 18991 trial, showing no prognostic or predictive value of autoimmune antibodies in PEG-IFN treated patients.[23]

In conclusion, time-dependent evaluation of serial blood measurements of S100B showed a very significant prognostic value of S100B, which was even stronger compared to stage and number of positive lymph nodes. Stage III patients with increased S100B levels ($\geqslant 0.2$ μg/l) should more frequently be screened for the occurrence of distant metastases.

## Conflict of interest statement

None declared.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2010.10.005.

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