Original Research Article

S-100B in the follow-up of patients with stage III and IV melanoma: pursuit of the Holy Grail

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ABSTRACT

Background: Patients with stage III-IV melanoma are at considerable risk for disease recurrence. Early detection of recurrence is important to optimize immunotherapy administration and improving progression-free and overall survival. S-100B can be used as tumor marker to evaluate whether patients are at risk for developing disease recurrence or progression. It could be a promising tool to determine whether patients need to receive additional diagnostic procedures. However, implementation in routine clinical setting is limited. Hence, this study aimed to evaluate the value of S-100B as a decision tool for the need to perform an ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography with computed tomography (PET/CT) to confirm disease recurrence or progression in stage III-IV melanoma patients.
Methods: Data of 51 stage III-IV melanoma patients, presenting for follow-up after surgery with curative intent, was retrospectively extracted from a single-center electronic patient record system. ^{18}FDG-PET/CT was performed based on clinical signs or elevated S-100B levels. S-100B measurements were treated as independent data points.
Results: Fifteen out of 303 S-100B levels were elevated. Six elevated levels were causes by disease progression; 4/6 measurements were noted with concurrent clinical signs. Twenty-four events of disease progression were confirmed. The sensitivity and specificity of S-100B serum test were 25.0% [95% CI (9.8-46.7)] and 96.8% [95% CI (94.0-98.5)]. The positive predictive value (PPV) and negative predictive value (NPV) of S-100B were 40.0% [95% CI (20.6-63.2)] and 93.8% [95% CI (92.2-95.0)].
Conclusions: Elevated S-100B levels did not exclude nor indicate metastatic or recurrent disease in this study. Using S-100B in routine clinical setting does not seem to be of additional value.

Keywords: Melanoma, S-100B marker, Follow-up, Recurrent disease

INTRODUCTION

Patients with stage III and IV melanoma are at considerable risk for disease recurrence.[1,2] As knowledge on cancer immunology has improved, early detection of disease recurrence is of great importance in order to improve progression-free survival (PFS) and overall survival (OS) rates.[1,3,4] Hence, several biomarkers have been studied to early diagnose disease recurrence in malignant melanoma. However, none of the evaluated biomarkers have been proven to be sufficiently accurate. Among the studied biomarkers, lactate dehydrogenase (LDH) has proved to have a limited sensitivity and, moreover, it is non-specific for melanoma as LDH levels are elevated in various benign and malignant diseases.[5] Additionally, serum S-100 calcium-binding protein B (S-100B) has been widely studied, as the fluctuation of serum concentration has been associated with processes

in melanocytes and tumors such as melanoma. Serum S-100B seems to be a promising prognostic biomarker, and is even recommended as the most accurate serologic test in the follow-up of melanoma patients by the European society of medical oncology (ESMO). Implementation in the routine clinical setting, however, is limited.[6-9]

Currently, the combination of positron emission tomography and computed tomography (PET/CT) is implemented in most clinical settings to accurately detect residual disease or distant metastasis in melanoma patients and is implemented in the Dutch medical guideline.[10] PET/CT combines morphologic and metabolic information and is regarded as a superior imaging technique for staging and monitoring in malignant melanoma.[11] PET/CT is superior to CT or magnetic resonance imaging (MRI) alone to assess metastasis.[7] As S-100B levels are associated with melanoma specific disease, it is routinely measured during follow-up of immunotherapy.[6] Studies have investigated the importance of the combination of S-100B and (^{18}F-FDG) PET/CT during follow-up of patients with melanoma, and more specifically, the value of S-100B as a screening or selection tool before performing a PET/CT scan.[7-9,12] However, no consensus has been reached with regard to implementing serum S-100B testing in daily practice as a screening tool. Moreover, PET/CT imaging as a routine surveillance technique may not be appropriate for all melanoma patients as this poses a burden on imaging facilities and increases healthcare costs substantially. Since serum S-100B is used as a surveillance technique in other fields than oncology, it is easily available in hospitals. Since many hospital laboratories already have the ability to test for S-100B, routinely testing for melanoma patients will not encumber health care facilities. Therefore, we aimed to investigate diagnostic accuracy of serum S-100B testing to assess whether it could be used as a selection tool on when to perform PET/CT imaging in stage III and IV melanoma patients in order to confirm or exclude the presence of recurrent or metastatic disease.

METHODS

Patients

In this single-center retrospective study, data of stage III and IV melanoma patients who underwent surgery were extracted from the electronic patients' record at Zuyderland Medical Center (Sittard/Heerlen, the Netherlands). Wide local excision (WLE) of the melanoma, sentinel node biopsy (SNB) and follow up took place between January 2014 and July 2021. Patients were included when above 18 years, without signs of active disease after surgical treatment and at least one reported S-100B value. Patients with stage I or II melanoma, or patients with stage IV melanoma with non-curative metastasis were excluded. Sample size was not calculated in this retrospective design. All patients treated between January 2014 and July 2021 were assessed for inclusion. This is a true representation of the current clinical practice

population. Data collection was conducted according to the declaration of Helsinki ethical principles for medical research involving human subjects. The medical ethical committee of Zuyderland Medical Center granted approval (Z2019018) and informed consent was waived.

Treatment and follow-up

All patients underwent at least one whole body PET/CT scan at baseline for staging III/IV disease. Experienced surgical oncologists executed wide local excision (WLE) and sentinel node biopsy (SNB) and, if indicated, completion lymph node dissection or resection of distant metastasis. After surgery, outpatient clinic visits were held at 3, 6, 9, 12, 18, 24 and 36 months. During follow-up, the surveillance program included physical examination, taking medical history into consideration. S-100B tests were not routinely requested by all surgical oncologists, therefore, S-100B tests filed by medical oncologists were included as well. Only S-100B values with corresponding follow-up details were used. Patients could be subjected to multiple S-100B measurements. Each S-100B value was analyzed at the laboratory for diagnostics at Radboud UMC (Nijmegen, the Netherlands) or Klinikum Aachen (Aachen, Germany). Serum concentrations of S-100B were determined by the use of enzyme-linked immuno sorbent assay (ELISA) and analyzed on the Liaison immunoassay analyzer.

Based on clinical signs, suspicion of disease progression and/or elevated S-100B levels, patients were appointed to whole body ^{18}FDG PET/CT. According to local reference values, the S-100B cut-off value was set at 0.11 µg/l.

Pathological reports

All histopathologic reports of the diagnosis of stage III or IV disease were reviewed to confirm staging of patient groups. Histologic data retrieved from primary tumors and metastases were retrospectively retrieved from PALGA, the nationwide network and registry of histology and cytopathology. Architectural and cytological features were described in the pathological report and histological features as asymmetry, dermal mitosis, ulceration, cytological atypia, and cell maturation were used to confirm the diagnosis.

Statistics

Patient and histological characteristics were described in detail. Continuous variables were reported as means and standard deviations (SD) or, in case of severe skewness, as median and interquartile range (IQR). The distribution of continuous variables was assessed by visual inspection of histograms. Categorical variables were reported as counts and percentages.

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using contingency tables, treating all S-100B values as

independent data, although patients generally contributed more than one S-100B value. Additionally, a sub-analysis was performed on patient-level data.

Secondly, a receiver operating characteristic analysis was performed to evaluate the diagnostic performance of S-100B. IBM statistical package for social sciences (SPSS) for Windows (version 26) was used for the statistical analysis.

RESULTS

Patient and tumor characteristics

A total of 51 patients with fully resected stage III or stage IV melanoma were included in the study. Twenty-three of 51 (45.1%) patients were male. The mean age of the patient population was 59.8±13.9 years. Thirty-seven (72.5%) patients were diagnosed with stage III melanoma and 14 (27.5%) with stage IV melanoma. All patients underwent diagnostic excision of the primary tumor, except for 8 (15.7%) patients who were diagnosed with stage III or IV disease with an unknown primary tumor. Forty-four (86.3%) patients received additional wide local excision of the biopsy wound. A sentinel node procedure was performed in 37 (72.5%) patients, of which 22 (59.5%) underwent subsequent completion lymph node dissection. Other patients underwent direct lymph node dissection without a prior sentinel node procedure due to prior cytological proven lymph node metastasis. Patient characteristics and pathological characteristics of primary tumors are presented in Tables 1 and 2.

Disease progression was defined as any histologically proven recurrence or metastasis during follow-up.

S-100B measurements

A total of 303 S-100B values were obtained after surgical treatment, a median of 6.0 (4.0-8.0) measurements per patient. An elevated S-100B level was found in 15 measurements (5.0%), 86.8% in stage III and 13.3% in stage IV patients. The median value of elevated S-100B measurements was 0.19 (0.14-0.84) µg/l. Recurrent disease was confirmed in six (40.0%) cases. Clinical signs and/or symptoms of recurrent disease were noted concurrently in four (26.7%) patients with elevated S-100B levels. All four cases were selected for PET/CT, of which three (75.0%) revealed disease recurrence.

PET/CT

Overall, 55 [18]FDG PET/CT scans were performed. Twenty-four (43.6%) PET/CT scans were solely indicated based on clinical signs without elevated S-100B levels. Four (7.3%) scans were indicated based on clinical signs combined with elevated S-100B levels, and 10 (18.2%) scans were performed based on elevated S-100B levels only.

In 15 out of 24 (62.5%) cases with normal S-100B levels, disease recurrence was diagnosed. Recurrent disease was diagnosed in 6 out of 14 (42.9%) cases with elevated S-100B levels.

Table 1: Patient characteristics at baseline.

Patient characteristics	N	%
Gender		
Male	23	45.1
Female	28	54.9
Age		
Mean, SD	59.8±13.9 years	
TNM stage		
III unspecified	9	17.6
IIIA	10	19.6
IIIB	11	21.6
IIIC	7	13.7
IV	14	27.5
Pathology		
Superficial spreading	23	45.1
Nodular	11	21.6
Metastasis	8	15.7
Lentiginous	1	2.0
Unknown	8	15.7
Wide local excision		
Yes	44	86.3
No	7	13.7
SN procedure		
Yes	37	72.5
No	13	25.5
Unknown	1	2.0
Lymph node dissection		
Yes	31	60.8
No	19	37.3
Unknown	1	2.0

Disease recurrence or metastasis

Disease recurrence or metastasis was either confirmed with [18]FDG PET/CT and/or CT guided biopsy. A total of 24 (7.9%) events were confirmed out of 303 S-100B measurements. Some patients experienced disease recurrence or metastasis more than once. Disease progression was histologically confirmed in 17 (33.3 %) out of 51 patients after a median follow-up time of 19.1 (9.3-38.0) months. A flowchart of S-100B measurements, clinical signs, imaging and occurrence of disease progression is presented in Figure 2.

Sensitivity, specificity, positive predictive values and ROC curve

Treating serum S-100B tests as independent data points (303 data points), the sensitivity and specificity of the S-100B serum test were 25.0% [95% CI (9.8-46.7)] and 96.8% [95% CI (94.0-98.5)], respectively. The PPV and

NPV of S-100B were 40.0% [95% CI (20.6-63.2)] and 93.8% [95% CI (92.2-95.0)], respectively.

Table 2: Tumor characteristics at baseline.

Tumor characteristics	n	%
Pathology		
Superficial spreading	23	45.1
Nodular	11	21.6
Metastasis	8	15.7
Lentiginous	1	2.0
Unknown	8	15.7
Tumor location		
Upper extremities	7	13.7
Head or neck	7	13.7
Anterior trunk	6	11.8
Posterior trunk	15	29.4
Lower extremities	12	23.5
Occult primary	2	3.9
Metastasis	2	3.9
Breslow (mm)		
≤1	5	9.8
1.1-2.0	11	21.6
2.1-4.0	17	33.3
>4.0	9	17.6
Unknown	9	17.6
Ulceration		
Yes	14	27.5
No	25	49.0
Unknown	12	23.5
Intradermal mitosis		
Yes	28	54.9
No	11	21.6
Unknown	12	23.5
Microsatellites		
Yes	1	2.0
No	40	78.4
Unknown	10	19.6
Regression		
Yes	6	11.8
No	35	68.6
Unknown	10	19.6

Analyses on patient level data (51 patients) revealed a sensitivity and specificity 23.5% [95% CI (6.8-49.9)] and 88.2% [95% CI (72.6-96.7)], respectively. The PPV and NPV of S-100B were 50.0% [95% CI (22.1-77.9)] and 69.8% [95% CI (63.3-75.5)], respectively.

All values are reported in Tables 3 and 4.

The receiver operating characteristic (ROC) analysis demonstrated an area under the curve (AUC) of 0.613 [95% CI (0.468 to 0.757)] for S-100B to discriminate between disease recurrence or metastasis, or no recurrence or metastasis (Figure 1). The ROC curve graphically indicates the diagnostic ability of serum S-100B.

Table 3: Diagnostic values S-100B measurement (independent data).

Parameters	Recurrent disease or metastasis	No recurrent disease or metastasis	Total
Elevated S-100B	6	9	15
Normal S-100B	18	270	288
Total	24	279	303

Sensitivity: 25.0% [95% CI (9.8-46.7)]; PPV : 40.0% [95% CI (20.6-63.2)]; specificity: 96.8% [95% CI (94.0-98.5)]; NPV: 93.8% [95% CI (92.2-95.0)]

Table 4: Diagnostic values S-100B measurement (patient data).

Parameters	Recurrent disease or metastasis	No recurrent disease or metastasis	Total
Elevated S-100B	4	4	8
Normal S-100B	13	30	43
Total	17	34	51

Sensitivity: 23.5% [95% CI (6.8-49.9)]; PPV: 50.0% [95% CI (22.1-77.9)]; specificity: 88.2% [95% CI (72.6-96.7)]; NPV: 69.8% [95% CI (63.3-75.5)]
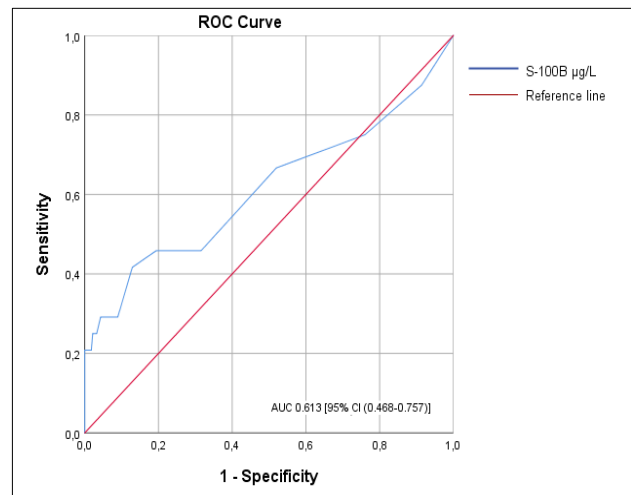

Figure 1: ROC curve analysis of serum S-100B.
ROC=Receiver operating characteristics, AUC=area under the curve

All patients with disease progression were discussed in a multidisciplinary team and received appropriate treatment. Treatment comprised surgery, radiation and/or palliative systemic therapy. Patients who had recurrent disease were suffering from either lymph node metastases, lung metastases, abdominal metastases, bone metastases or cerebral metastases.
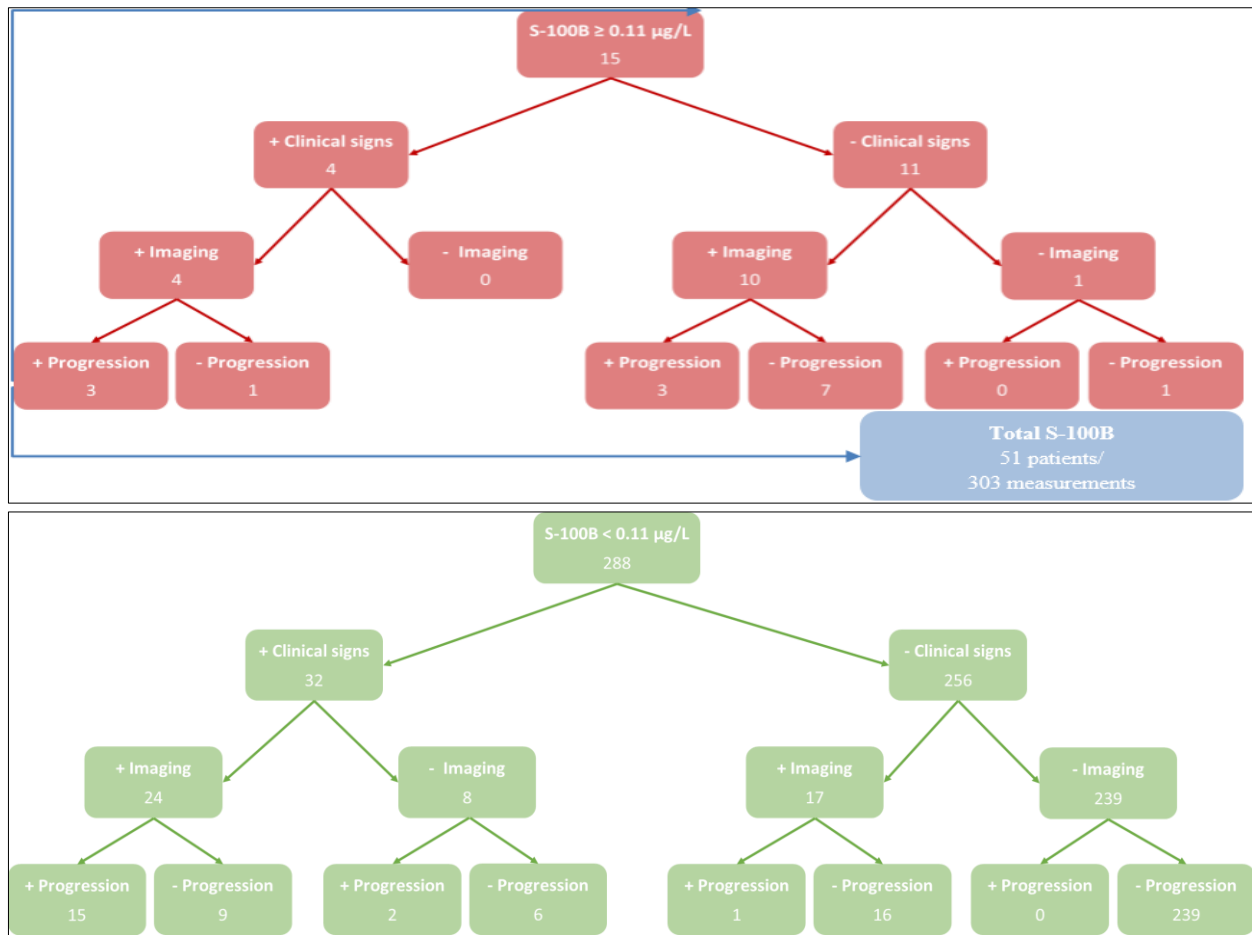
Figure 2: Flowchart of events of S-100B during follow-up.

DISCUSSION

This study aimed to evaluate serum S-100B testing as a screening or selection tool to perform PET/CT in stage III/IV melanoma patients to confirm or exclude disease progression. In this study 17 (33.3%) stage III or IV melanoma patients developed disease progression during follow-up. Of these 17 patients, five had an elevated S-100B value; these findings are therefore consistent with the findings of Madu et al. They showed that in patients with stage IIIB and IIIC melanoma, all recurrences detected by PET/CT, had a normal range of S-100B at the time.[13] Furthermore, Deckers et al recently showed that S-100B could not exclude recurrent disease during follow-up of stage III melanoma.[12] In this study, recurrent disease only occurred in a minority of asymptomatic patients whose serum S-100B level was the only reason to execute an [18]FDG PET-CT scan.

Disease progression was detected in 24 out of 55 (43.6%) PET/CT scans that were performed, which is in line with the recurrence rate reported by Lewin et al.[14] In this study, the authors looked into stage III melanoma only and used substage- specific schedules for routinely using [18]FDG PET-CT scanning.

Detection of recurrent disease remains a controversial topic in melanoma research. Are current surveillance strategies too much or not enough?[15] It has become clear in the literature that routine imaging is not appropriate for all melanoma patients, as it results in an overuse of healthcare facilities due to false positive findings. This in itself leads to higher costs in healthcare, not to mention patient distress and exposure to radiation. Current guidelines are mainly based on expert opinions which results in different imaging strategies, it is clear that imaging is an important tool to identify melanoma recurrences, particularly those that are distant and asymptomatic.[16] Early detection of recurrent melanoma or distant metastasis may have a survival benefit as additional treatment can be instituted as soon as possible. Accurate risk stratification is therefore essential to target surveillance imaging to high-risk patients. Freeman et al reviewed current guidelines on melanoma (AAD, NCCN, ESMO and NICE) and provide a thorough overview of published evidence on surveillance imaging in the detection of melanoma recurrences.[16,17] They do not, however, provide any information on potential markers and their role in surveillance. S-100B seemed to be a promising tool in melanoma follow-up in recent years.[17] It has previously been shown to have a prognostic value in metastatic disease, but it has not led to implementation of

S-100B serum measurements in the standard follow-up recommendation.[18-22]

Due to the fast evolving non-surgical treatment of melanomas, current practice is changing. Since the introduction of adjuvant treatment with immunotherapy and targeted therapies, PET/CT scans are generally performed routinely in the first two years after treatment. These new developments could very likely make the use of biomarkers or tumor markers redundant in the follow-up of stage III/IV melanoma patients. Moreover, our patient cohort was a combination of both stage III and IV melanoma, making it a rather small heterogeneous group to directly compare to current literature, which is one of the limitations of this study.

Another limitation is its retrospective design. Findings are based on a retrospective analysis of a period in which no standardized follow-up protocol was executed in the hospital. As S-100B values were not determined at every visit, the number of values could be considered low, negatively affecting precision of the results. Selection bias and verification bias could not be excluded from this study as PET/CT scans were not performed if S-100B measurements were normal. Hence the rate of false negatives cannot be predicted. This is however a true representation of the dilemmas in current clinical practice.

The current study shows that the correlation between S-100B and recurrent melanoma or distant metastasis is less promising than previously expected.

CONCLUSION

In conclusion, a normal S-100B marker in the follow-up of stage III/IV melanoma patients does not exclude nor indicate metastatic or recurrent disease. Clinical suspicion still seems to be the cornerstone on which the decision for additional imaging should be based.

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