May 06, 2020

Protocol S-100B Determination in Melanoma

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1 Works for me dx.doi.org/10.17504/protocols.io.bf4cjqsw

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ABSTRACT

This protocol describes the implementation of a dummytube system in AJCC stage III melanoma patients, to investigate whether serum S-100B levels can be falsely elevated by adipocyte contamination in the first drawn serum separation tube.
A dummy tube system was introduced for performance of venipunctures during regular follow-up in all AJCC stage III melanoma patients, with no evidence of disease.After venipuncture, a dummy tube was drawn first, and subsequently the regular tube was drawn. The first tube was anonymously coded and stored, while the second tube was registered in the patients' medical results. After performing the assay, S-100B levels between the two consecutively drawn samples were compared.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Double Venipuncture is not Required for Adequate S-100B Determination in Melanoma Patients.

GUIDELINES

Background

Patients with AJCC stage I / II melanoma have a 20-30% chance of a positive sentinel node.[1,2]Regional gland metastases develop in 20-60% of patients with stage I / II melanoma[1,3]and 10-50% of these patients eventually develop distant metastases.[3,4,5]

Patients with clinical stage III melanoma are staged using an FDG-PET and / or CT scan. Distant metastases appear to be present in 32% of these patients with palpable lymph nodes and are therefore stage IV.[6,7]The surgical treatment of regional gland metastases is a complementary or therapeutic lymph node dissection. The surgical techniques for the various gland dissections have been defined.[8]The indication for additional radiotherapy is also defined.[9]

Research in stage III melanoma has shown that the biomarker S-100B has a predictive value with respect to disease-free and melanoma specific survival; at normal values (<0.20µg / l) 38% disease-free and 47% 5-year survival, at elevated values 7% disease-free and 28% 5-year survival, respectively.[5,10]Early detection of distant metastases and, if possible, surgical treatment of these, contributes to an improved 5-year survival (15-28% 5-year survival with surgical treatment, as opposed to 5-10% with system therapy, based on data from the years 1993 to 2008).[11]

The biomarker S-100B appears to be more reliable than LDH in the early detection of melanoma metastases.[10]In recent years, there has been a real breakthrough in the treatment of metastatic melanoma in the field of "targeted therapy" and in the field of "immunotherapy".[12]Early diagnosis of melanoma metastases is generally associated with lower tumor load and therefore potentially more successful treatment.[13]

National guideline melanoma follow-up

The Dutch Melanoma Guideline does not, except for the frequency of follow-up, contain any advice regarding additional diagnostics to be carried out during the follow-up, in contrast to other National guidelines.[14,15]As outlined earlier, a biomarker is available, S-100B, which can indicate the presence or absence of metastatic

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disease.[5,16]This biomarker has been implemented in the German Melanoma Guideline, due to a higher sensitivity for disease progression in melanoma than LDH.[17]

In anticipation of a possible future change of the Dutch Melanoma Guideline, the Department of Surgical Oncology at the University Medical Center Groningen has decided to amend the Protocol Follow-up Melanoma stage III, given the successful breakthroughs that have been achieved in the systemic treatment of the melanoma. These treatments are available for recognized melanoma centers in the Netherlands, including the UMCG.

Protocol Follow-up Melanoma Stage III UMCG

	Clinical visit + LDH + S-100B
1st year	**4 times per year**
2nd year	**3 times per year**
3rd – 5th year	**2 times per year**
> 5th year	**1 times per year**

The laboratory results of S-100B and LDH are registered in the Electronic Patient Record. The reference value of S-100B is <0.20µg / l and of LDH <250U / l.

At a marginally increased value of S-100B (0.20-0.30µg / l) and / or LDH (250-400U / l), the S-100B and LDH will be reassessed after 4 weeks. At an absolutely increased value of S-100B (> 0.30 µg / l) and / or LDH (> 400U / l) an FDG-PET / CT will be performed and an MRI scan of the brain in case of FDG positive hearths. In addition, standard mutation analyzes will be requested in stage III melanoma patients: BRAF, MET, C KIT. Patients with elevated biomarkers are discussed in the multidisciplinary melanoma discussion in accordance with the SONCOS guideline.[18]

BEFORE START

The study was conducted in accordance with the Declaration of Helsinki, and conforms to the guidelines of the central medical ethics committee (**METc2015.215**).

MATERIALS TEXT

Determination of S-100B in a second blood tube (dummytube)

Recent research by the UMCG has shown that drawing a second tube of blood before the S-100B tube can prevent possible incorrect values.[19] For the careful introduction of this extra serum tube, we ask the patients' permission to determine the S-100B value in this tube as well. This extra result will be processed anonymously and will not be disclosed to the patient. By handing over the extra tube to the puncture lab, the patient agrees to the additional determination.

If patients do not want to participate, they can return the tube to the outpatient clinic.

Patient ID number	
Puncture date	
Project number	
S-100B result project	
Routine number	
S-100B result routine	
>1 cm subcutaneous	**Yes / No**

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Traumatic puncture	**Yes / No**

S-100B analysis

S-100B concentrations were determined by performing the S-100B assay (Diasorin, Saluggia, Italy) on an ELISA Robot platform (DS2, Dynex Technologies, Magellan Biosciences, Worthing, United Kingdom).After routine centrifugation, serum was separated from the tubes, aliquoted and stored at -80 °C. The two tubes of all individuals were analyzed after thawing the samples, using the S-100B ELISA according to manufacturer's protocol.

The intra-assay Coefficient of Variation of the S-100B assay is 7% at levels of 0.04 µg/L (0.0028 µg/L). The Limit of Blank was determined to be 0.0034 µg/L, whereas the Limit of Quantitation (20 % CV) was determined to be 0.092 µg/L.

The reference interval was determined by analysis of S-100B values in 120 healthy individuals (median 0.07; range 0.01-0.59) and calculating the 95% confidence interval according to the Clinical and Laboratory Standards Institute EP28-A3c guideline (formerly C28-A2), resulting in a reference cut-off value for the healthy population of 0.20µg/L.[20]

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