Letter to the Editor

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Adipocytes in venipunctures cause falsely elevated S-100B serum values

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To the Editor,

The calcium-binding protein S-100B is increasingly used in melanoma as a serum biomarker to reflect tumor load, but also as a prognostic tool in advanced melanoma [1, 2]. In neurology, S-100B in serum and cerebrospinal fluid is predominantly used to detect and quantify brain injury [3]. Previously, multiple studies have also described the presence of S-100B in adipocytes [4–6].

Determination of the serum S-100B concentration in patients is performed by drawing a blood sample by venipuncture and subsequent analysis of S-100B by immunoassay. With the increased clinical applications and use of S-100B, accurate analysis and interpretation of this biomarker becomes more important, especially in monitoring and predicting prognosis of melanoma patients where minor changes of serum S-100B might have important clinical consequences [2].

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As S-100B is present in adipocytes, the hypothesis was that damaged subcutaneous adipocytes, trapped in the needle before entering the vein during a venipuncture, could contaminate the serum used for S-100B analysis. False positive values of S-100B, caused by adipocytes in a blood sample, have not been reported before. The aim of this study was to investigate 1) the influence of adipocyte contamination in a blood sample on S-100B values, 2) whether difficult venipunctures could result in falsely elevated S-100B values, and 3) the difference in S-100B values of the first and second drawn serum separation tube. For clinical purposes, it seems to be of high importance to prevent contamination with adipocytes, as falsely high S-100B values might lead to potential hazardous over-staging and mismanagement, and potential wrongly informed patients regarding their prognosis [2, 3].

Two subsequent experiments were performed, in accordance with the Declaration of Helsinki and after written approval by the medical ethics review committee of the University Medical Center Groningen (METC ABR NL42601.042.12). Differences between the sample groups were assessed for statistical significance ($p<0.05$), using a one sample T-test for the normally distributed differences and Wilcoxon signed Rank test or Kruskal-Wallis for not normally distributed values (IBM SPSS statistics version 22, Chicago, IL, USA).

The first experiment was conducted to determine whether the presence of adipocytes would increase S-100B values in a serum sample. In two healthy men, aged 27 and 28 years, a single blood sample was drawn and divided into two tubes after centrifugation. Subsequently, subcutaneous adipocytes were obtained from the abdominal subcutis of the same individual using a 40 mm 18 G needle, and mixed with one of each individuals' blood samples. The four samples were stored at –20 °C overnight to induce lysis of the present adipocytes due to freeze thawing before the samples were analyzed, and to mimic the handling and storage conditions in a routine laboratory. After addition of the adipocytes, the samples were analyzed also in dilution to exclude high-dose hook effect.

The serum mixed with adipocytes showed extremely high S-100B levels: 73.8 µg/L and 55.1 µg/L, whereas the control tubes (serum only) both had S-100B values <0.01 µg/L.

In the second experiment, after informed consent and completion of a questionnaire, three subsequent serum separation tubes were drawn in 20 individuals by entering the vein after a 1.5 cm subcutaneous route, simulating a difficult venipuncture (Figure 1). The study group consisted of 11 female and nine male volunteers, median age 32 (range 22–63) years and median body mass index (BMI) of 23.6 (range 18.5–29.4) kg/m^2. None of the individuals reported particularities. Blood samples were collected by venipuncture in 8.5 mL Vacutainer tubes (Becton Dickinson, Plymouth, UK). After routine centrifugation, serum was separated from the tubes, aliquoted and stored at −80 °C. After thawing the samples, 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, UK).

The within-run assay coefficient of variation (CV) of the S-100B automated ELISA was 7.2%, 5.4% and 6.0% at 0.04 µg/L, 0.194 µg/L and 2.121 µg/L, respectively. The between-run CV of the assay was 11.8%, 13.4% and 5.6% at 0.05 µg/L, 0.209 µg/L and 2.066 µg/L, respectively. 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 first two tubes of all individuals were analyzed. The subsequent samples showed median S-100B values of 0.23 µg/L and 0.03 µg/L for the first and second tube, respectively, with a significant mean difference of −0.198 µg/L (95% CI: −0.257; −0.140, p<0.001) (Figure 2). This demonstrates a relatively high contamination effect, considering the reference cut-off of S-100B (0.20 µg/L) used at our institution. Theoretically, smaller quantities of adipocyte contamination associated with shorter subcutaneous tracks in uncomplicated venipunctures could also cause clinically relevant elevations of the S-100B level. S-100B reference values that were previously established by analysis of healthy individuals, which will be the case for most hospital laboratories, should probably be re-established from adipocyte-free venipunctures. This might lead to a lower cut-off point, making the biomarker more sensitive.

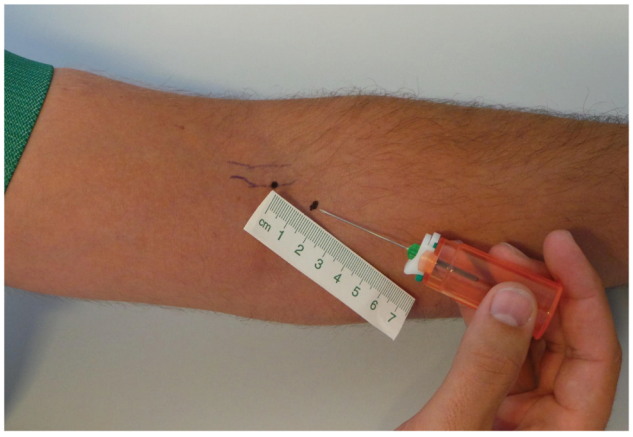

Figure 1: Measuring and marking the 1.5 cm subcutaneous route before venipuncture.

According to the literature, in vivo S-100B secretion from adipocytes is decreased by insulin, but increased by glucagon, stress, physical training or fasting [5, 7, 8]. Some studies reported a correlation between serum S-100B and BMI, whereas others did not find this association [6, 8]. In our study, no correlation was found, possibly due to the absence of weight loss or obesity in these apparently healthy volunteers.

A reanalysis was performed after 4 months, now also including the third drawn sample. This resulted in median S-100B values of 0.23 µg/L, 0.04 µg/L, and 0.04 µg/L for tubes one, two and three, respectively. The pre-analytical stability of S-100B is previously reported to be very high over a wide range of time periods (within 24 h) and temperatures [9]. However, the present study found a slight, although significant, elevation of S-100B in the second tube (0.01 µg/L, 95% CI: 0.002; 0.019, p=0.02) after longer storage time and an extra freeze-thaw cycle, in accordance with previous literature [10]. This elevation of S-100B could be the result of lysis of a larger quantity of adipocytes [5]. Nevertheless, the first tube still contained the highest value of S-100B after 4 months storage (p<0.001) (Figure 2).

Although adipocytes are the main cell type in subcutal tissue, it contains other molecules that can be measured during serum analysis, like triacylglycerol and free fatty acids [5]. The presented research setup could be used to test which other clinically relevant serum parameters suffer the same serum contamination during venipuncture caused by (sub)cutaneous molecules.

To our knowledge, this is the first study showing contamination of the first drawn blood sample with subcutaneous adipocytes to cause significant elevation of S-100B values in serum analysis. The risk of adipocyte induced elevated S-100B values is higher in difficult venipunctures, but might even be present in easy venipunctures. Therefore, we recommend to avoid the use of the first drawn blood sample for S-100B analysis, especially when used as a tumor marker in melanoma patients.

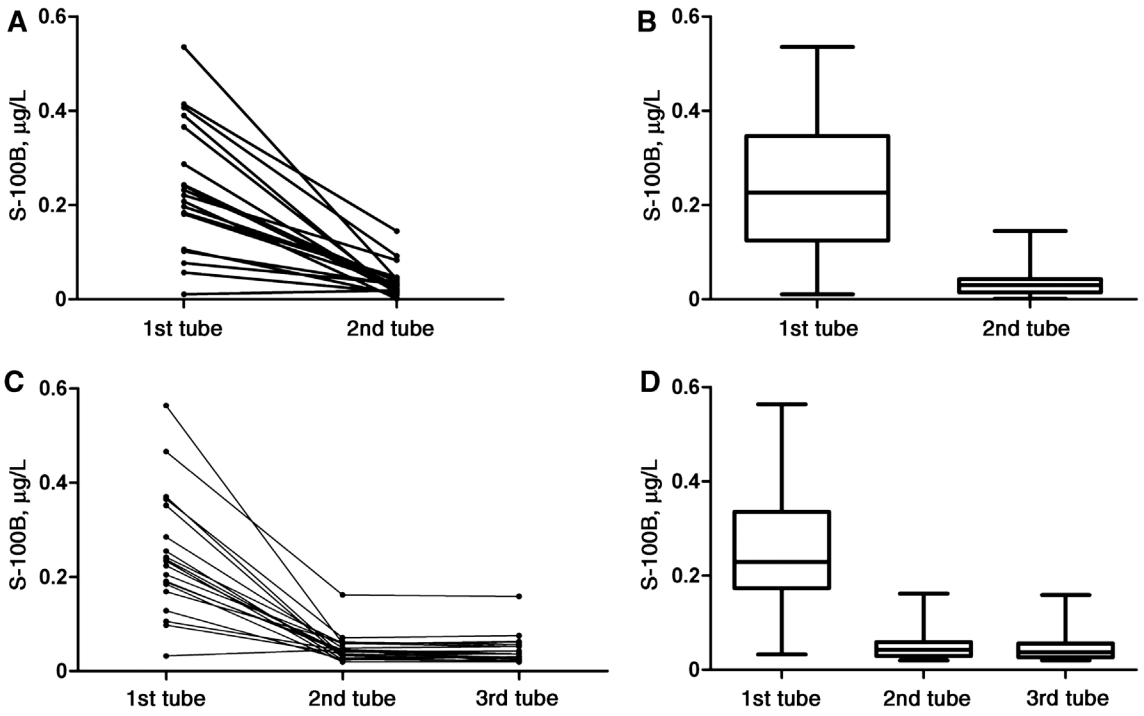

Figure 2: The effect of adipocyte contamination on S-100B values measured in three subsequent drawn tubes from 20 individuals, after venipuncture using a 1.5 cm subcutaneous route before entering the vein.
(A) First analysis; significant decrease in S-100B value in 2nd tube (median 0.03 µg/L, SD 0.03, range 0.001–0.15 µg/L) compared to 1st tube (median 0.23 µg/L, SD 0.13, range 0.01–0.54 µg/L), $p<0.001$. (B) Box plot summarizing the results of Figure 2A. (C) Second analysis; significant decrease in S-100B value in 2nd tube (median 0.04 µg/L, SD 0.03, range 0.01–0.16 µg/L) compared to 1st tube (median 0.23 µg/L, SD 0.13, range 0.03–0.56 µg/L), $p<0.001$. No difference between 2nd tube and 3rd tube (median 0.04 µg/L, SD 0.03, range 0.01–0.16 µg/L). (D) Box plot summarizing the results of Figure 2C.

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