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VALIDITY OF S-100 B IN PATIENTS AFTER BRAIN RADIATION

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Abstract

Background: S-100B is a calcium binding acute phase protein and a potential biomarker for brain injury. In prior studies elevated plasma S-100B levels were detected in stroke and severe head trauma. The aim of this study was to evaluate whether S-100 B is elevated during cerebral radiotherapy and whether that is associated with adverse outcomes.

Material and Methods: In this prospective pilot study, 45 patients (25 males, 20 females, median age 58 [17-81]) underwent cerebral radiation therapy because of a primary or metastaic cerebral malignancy. 39 patients were included in the evaluation. 6 patients died during the study period. S-100 plasma concentrations were measured with an electrochemiluminescence immunoassay on admission and weekly during radiation therapy for the duration of 6 weeks. In 10 healthy young volunteers (5 males, 5 females, median age 32 [28-36]) S-100 B plasma levels were measured weekly for 6 weeks as a negative control. Furthermore, in an active control 10 patients (4 males, 6 females, median age 68 [64-76]) with stroke (7 = major stroke, 3 = lacunar infarct) S- 100 B plasma levels were measured for 7 consecutive days after the event.

Results: During radiotherapy S-100 B plasma concentrations increased from median baseline values of 0.030 μg/l to 0.044 μg/l. For the time of radiation therapy most patients showed a mild increase, but absolute plasma values were still within the normal range. In the control group of healthy volunteers S-100 B remained unchanged. In stroke patients S-100 B increased to maximum values of 1.7 μg/l three days after the event. In the 3 patients with lacunar infarcts no increase of S-100 B levels could be detected.

Conclusion: Brain irradiation leads to a mild increase of S-100 B plasma levels. However, the absolute rise was far weaker compared to that seen in major brain injuries.

Implication Statement: Location: University of Düsseldorf, Structure: S-100 is a sensitive and early marker for brain damage.

Key words: S100 B, cerebral radiation therapy, acute phase response, neurological complications

Abbreviations: CNS = central nervous system, S = soluble, TBI = traumatic brain injury, ECLIA = electrochemiluminescence immunoassay, NCCLS = National Committee for Clinical Laboratory Standards, MCA = media cerebral artery, TIA = transitory ischemic attack

INTRODUCTION

Despite improvements in radiotherapy techniques , central nervous system (CNS) complications such as memory loss and fatigue remain a problem for patients undergoing cerebral radiation therapy. The incidence of CNS complications depends on risk factors including radiation dose, patient age, comorbidity and the type of radiation [2]. For neurological complications, no prognostic parameter has yet to be established.

In 1965, Moore detected a protein in bovine brains which was soluble in 100% ammoniumsulphate with a pH of 7 [9]. He named this protein S-100 (s= soluble). The S-100 family includes 17 monomers, all exhibiting tissue specific expression patterns. Among these, S-100 A1 and S-100 B are expressed in the CNS of vertebrates [10, 11]. Both monomers form either homodimers or heterodimers. S-100 B is an acidic calcium binding protein with a molecular weight of 21 kDa. It is present in high concentrations in glial and Schwann cells, whereas S-100 A1B is expressed by glial cells. Structural damage to the glial cells causes leakage of S-100 BB protein into the extracellular compartment and plasma. With a serum sample a monoclonal two-sided immunoradiometric assay can measure the B-subunit of protein S-100 [16, 20]. Several studies suggest that the S-100 B level is a plasma marker for CNS injury [10-12, 19]. A controversy remains regarding the

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specific release of S-100 B due to neurological damage. In 55 severely injured patients with and without traumatic brain injury (TBI), S-100 B levels increased in all patients within the first 48 hrs, regardless of whether they suffered TBI or not [14]. Accordingly, S-100 B was increased in rats undergoing hemorrhagic shock even in the absence of demonstrable CNS injury [15]. The increased S-100 B levels remained high for 24 hrs after shock. S-100 B levels were positively associated with shock severity.

During induced liver ischemia, S-100 B plasma levels increased. The elevation encountered occurred prior to removal of the ischemia causing ligature and levels remained largely unchanged throughout reperfusion. In contrast, S-100 B plasma levels did not increase during ischemia of the gut or kidney before ligature removal, but only increased 8 hrs later, achieving levels similar to those seen during reperfusion of the liver. Increased S-100 B levels have also been detected following myocardial infarction [13]. Perhaps tissue ischemia and reperfusion results in increased S-100 B plasma levels, independent of the type of organ, which has been affected. Never the less, even if S-100 B is relatively non-specific (but otherwise sensitive) for CNS it is hypothesized that S-100 B could be used as an early marker for brain damage if one suspects the possibility of CNS injury [8]. Since ionizing radiation to the brain may damage neuronal cells as well as tumor cells we hypothesized that S-100 B plasma levels would rise with cerebral radiation. Moreover we hypothesized that S-100 B levels may provide early warning of disturbances in brain homeostasis and may be predictive of complications (confusion, memory loss and fatigue).

Material and Methods

The following protocol was reviewed and approved by the institutional human subjects committee. All patients were informed, had questions answered and gave formal written consent prior to inclusion in this study.

Study design

45 patients (25 males, 20 females, median age 58 [17-81]) with brain tumors or cerebral metastasis (glioblastoma n= 14, astrocytoma grade III n = 5, meningeoma n = 2, brain metastasis of bronchial carcinoma n = 11, oligoastroctoma grade II n = 1, other brain metastasis n= 7, oligodendroglioma n = 1, prophylactic whole brain irridation n = 4) were enrolled in the study. Peripheral venous blood was drawn from all patients prior to and weekly during cerebral radiation therapy. S-100 BB levels from 10 healthy volunteers were analysed as a negative control weekly for 6 weeks. Furthermore, in 10 patients with a confirmed stroke (7 = major stroke, 3 = lacunar infarct), S-100 BB plasma levels were measured on a daily basis after hospital admission for a period of 10 days.

Assays

Venous blood samples were collected in vacutainor tubes and centrifuged to separate plasma. S-100 B plasma concentrations were measured with an electrochemiluminescence immunoassay (Roche Diagnostics Mannheim, Germany). The Elecsys S-100 B is a one-step sandwich electrochemiluminescence immunoassay (ECLIA) based upon streptavidin-biotin technology. Two monoclonal antibodies directed against the β-subunit of S-100 protein (S-100 homodimer ββ and heterodimer α1β) are utilized in the assay. Plasma samples of 20 µl were incubated with the antibodies (1st incubation) and with streptavidin coated microparticles (2nd incubation) resulting in a total incubation time of 18 min. The S-100 B sandwich complex was bound to the microparticles (solid phase) via the biotin-streptavidin interaction. The reaction mixture was then aspirated into a measuring cell, where the microparticles were magnetically captured onto an electrode surface. After removing unbound substances and the addition of tripropylamin, the application of voltage induced a chemiluminescent emission which was measured by a photomultiplier. The Elecsys S-100 B immunoassay was calibrated against weighed-out S-100 BB protein. The Elecsys S-100 B assay was performed on the Roche MODULAR ANALYTICS E170 immunoassay analyzer according to the manufacturer's instructions. Serum was collected using tubes containing separating gel (Becton Dickinson, Franklin Lakes, USA), centrifuged and stored at –20 °C according to the manufacturer's instructions. Before measurement, plasma specimens were thawed and analyzed at an ambient temperature (20-25 °C). To avoid evaporation effects, all measurements were performed within two hours. The measuring range of the S-100 B assay has been approved from 0.005 - 39 µg/L (defined by the lower detection limit and the maximum of the calibration curve). The 95th percentile of measurements in serum samples from 206 healthy adults was determined to be ≤ 0.105 µg/L. Imprecision of the immunoassay was determined using a modified protocol of the NCCLS (National Committee for Clinical Laboratory Standards). Calculated total imprecision CV's ranged between 2.5% (S-100 B 0.26 µg/L) and 3.1% (S-100 B 0.09 µg/L).

Statistical methods

Correlations between the clinico-pathological data and S-100 B plasma levels utilized the Fishers's exact test and whenever appropriate the Chi Square test. Candidate variables were evaluated, including neurological status, depth of tumor, tumor size, grading, histological subtype, tumor localisation and size of the brain injury by MRI of the brain. The level of significance was set at $p<0.05$. Statistical data analysis was carried out using SPSS software (SPSS Inc, Chicago, IL).

Results

39 patients (19 male, 20 female) completed the study. 6 male patients died during radiation therapy due to progression of their metastatic disease during radiation therapy. 19 patients received whole brain and 26 received partial brain radiotherapy.

S-100 B plasma concentrations increased non-significantly during radiation therapy from median base-

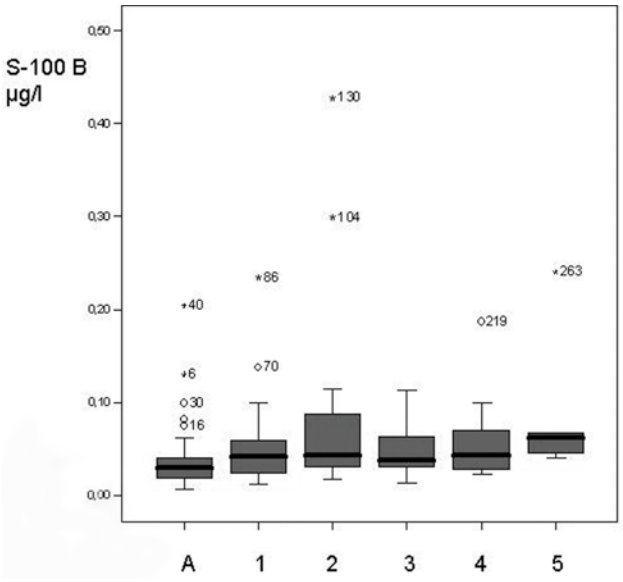

Fig. 1. S-100 B plasma levels during radiotherapy. Time sequence of the S-100 plasma levels in µg/l during radiotherapy. A= point of admission, 1 = 7 days after radiotherapy. 2 = 14 days after radiotherapy, 3 = 21 days after radiotherapy, 4 = 28 days after radiotherapy. 5 = 35 days after radiotherapy 6 = 42 days after radiotherapy. The lower and upper boundaries of the boxes represent the first and the third quartiles (25% and 75%), the middle line indicates the median. Vertical lines denote the maximum and minimum values. The numbers above the boxes denote the number of evaluable patients

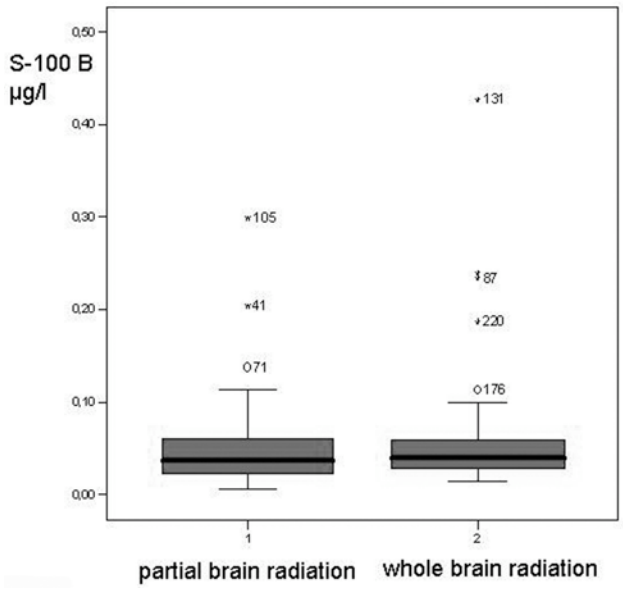

Fig. 2. S-100 B plasma levels in patients with partial and whole brain radiation Box and whisker plots of all S-100 plasma levels in patients with partial brain and whole brain radiation.

line values of 0.030 µg/l up to levels of 0.044 µg/l during radiaton therapy (Fig. 1). The majority of patients with radiotherapy showed a moderate increase of S-100 B from baseline values, though S-100 B plasma levels did not exceed the physiological upper limits of normal. Interestingly, no difference could be found between partial and whole brain radiation ($p>0.05$) (Fig. 2). Moreover, patients who died did not exhibit elevated S-100 B plasma levels compared to those that survived.

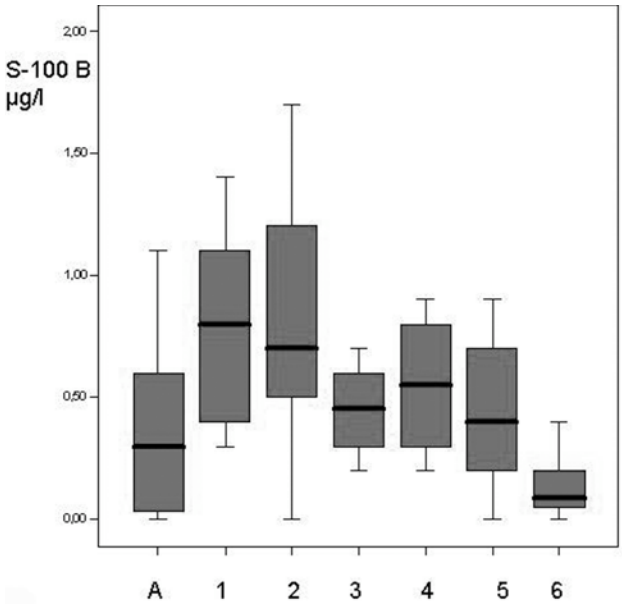

Fig. 3. S-100 B plasma levels in patients with cerebral ischemia and brain injury from stroke
Time sequence of the S-100 plasma levels in µg/l following cerebral stroke. A= on admission, 1-6= 1-6 day after stroke.

No increase of S-100 B plasma levels was found at any time the healthy volunteers. Conversely, all stroke patients demonstrated increased S-100 B plasma levels with values ranging up to 1.7 µg/l three days after the stroke (Fig. 3). S-100 B plasma levels slowly decreased to baseline values after stroke. Finally, patients with a small lacunar infarcts showed no elevated plasma S-100 B levels

Discussion

Our study demonstrates that partial- and total brain irradiation leads to a very mild increase of S-100 B protein plasma levels. Even patients exposed to whole brain irradiation had S-100 B within the physiological range. In contrast, patients with strokes showed levels of S-100 B greater than 1.5 µg/l. This is in accordance with other studies, demonstrating that S-100 B can serve as a biochemical marker for brain damage [3-6]. Recent studies demonstrated a continuous increase of S-100 B after a cerebrovascular stroke with peak values 48-72 hr after the onset of symptoms [7]. Herein, the time sequence and maximum values depend on the extent of the brain injury. In general, the most rapid rate of rise and highest peak values are observed if the stroke takes place in the distribution of the media cerebral artery (MCA). However, in cases of small lacunar infarcts or a transitory ischemic attack (TIA), measured S-100 B values are within the normal range. Furthermore, computed tomography recorded extent of injury demonstrates a positive correlation of S-100

B values and the size of the infarct region [7, 16, 18, 21]. Herrmann et al. and Wunderlich et al. demonstrated that the highest association of the two parameters is found on the third day after onset of stroke symptoms [3-6, 22]. In our study we did not find a correlation between S-100 B values and partial or whole brain irradiation. The role of S-100 B as a marker for the neuromonitoring , remains debatable . Most studies analyzing the topic were performed in cardiovascular surgery [1, 5, 17, 20]. Some investigators demonstrated an increase of S-100 B plasma concentrations with a doubling of peak values following cardiac surgery. In particular, an early increase after surgery (2-8 hr) was correlated with postoperative complications (CNS dysfunction or stroke). A later increase of S-100 B (24-72 hrs) was correlated with cognitive dysfunction [5]. One explanation for these results could be an extra-cerebral source of S-100 B. Although the largest amount is detected in the central nervous system, S-100 B is also found in fat tissue, skin and muscle tissue, but to markedly smaller degrees.

Contrary to results obtained from stroke patients and patients undergoing cardiac surgery, our study demonstrates, that radiation is not correlated with significant changes in S-100 B plasma levels. These results might be due to the fact, that in most of our patients the irradiation induced damage was not severe enough (no real cell lysis) to produce significant changes in acute phase markers like S-100 B. Correlations between elevated plasma levels and late side effects of the radiation therapy will be investigated in the near future.

Conclusion

Brain irradiation leads to a mild increase of S-100 B plasma levels. However, the absolute rise was far weaker compared to that seen in major brain injuries.

Key messages:
S-100 B is good marker for detecting the amount of brain damage.

S-100 B plasma levels are not significant elevated during radiation therapy.

Competing interests: The authors declare that they have no competing interests.

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