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Serum Levels of S-100B Protein and Neuron-specific Enolase in Glioma Patients: A Pilot Study

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Abstract. *Background: Serum levels of S-100B protein (S-100B) and neuron-specific enolase (NSE) are elevated after various cerebral injuries and are considered markers of central nervous system damage. In brain tumor patients, literature data on the prognostic value of serum S-100(B) and NSE levels are scarse and conflicting. Patients and Methods: We assessed serum S-100B and NSE levels in 20 consecutive cerebral glioma patients, and evaluated serum levels in relation to survival to determine their prognostic value. Kaplan-Meier survival curves were constructed for patients with "high" (> median value) versus "low" (≤ median value) serum S-100B and NSE levels. Results: A statistically significant shorter survival was found in patients with high serum S-100B levels, whereas a similar classification of patients based on serum NSE levels demonstrated no statistically significant difference in survival between the two groups. Conclusion: These preliminary data suggest that serum S-100B might be a prognostic variable in cerebral glioma patients. Further study is warranted to evaluate whether serum S-100B is an additional, independent prognostic variable.*

The S-100 protein family belongs to the calcium binding proteins, of which the ββ isoform (S-100B) is found in high concentrations in glial and Schwann cells, the αβ isoform in melanocytes and the αα isoform (S-100A1) in striated muscle, heart and kidney. Neuron-specific enolase (NSE) is the dimeric isoenzyme of the glycolytic enzyme enolase and is found predominantly in neurons and cells with neuroendocrine differentiation. In normal subjects, S-100B and NSE levels are very low in cerebrospinal fluid (CSF) and serum, though NSE is more concentrated in serum while S-100B is predominantly present in CSF.

Serum release patterns of S-100B and NSE as markers of brain damage have been investigated in many different clinical and experimental settings, such as traumatic brain injury, subarachnoid haemorrhage, stroke and cardiac surgery (1-4). In these studies, serum S-100(B) and NSE levels frequently correlated with clinical status and outcome. Serum S-100B is also used as a tumor marker for cutaneous malignant melanoma, (5) whereas serum NSE is used as a tumor marker for neuroblastoma (6) and small cell lung cancer (7). In summary, both serum S-100B and NSE levels reveal cellular (brain) injury and appear to provide information on the severity and prognosis of brain damage. Regarding the potential prognostic value of serum S-100(B) and NSE levels in brain tumor patients, literature data are scarse and conflicting (8-11).

The aim of this pilot study was to assess the serum levels of S-100B and NSE in patients with cerebral glioma and to evaluate whether S-100B and NSE might have clinical value in terms of prognosis.

Patients and Methods

Patients. Serum samples were obtained in 20 consecutive glioma patients who visited the Neurology out-patient clinic of our hospital, and who gave informed consent to participate in this pilot study. These patients suffered from different types of gliomas and were in different stages of their disease and treatment. Clinical data and survival data were obtained from patient files.

Analysis. Serum samples were obtained by venous puncture, centrifuged and stored at -20°C. All samples were assayed within the same run to exclude inter-assay variation. Serum S-100B was measured by immunoluminometric assay (LIAISON Sangtec 100), a test based on three monoclonal antibodies which specifically bind to the S-100B isoprotein in serum. The detection limit of this assay is 0.02 μg/L. Serum NSE was measured by immunoluminometric assay (LIAISON NSE), a test based on two monoclonal antibodies which specifically bind to the Y-subunit of NSE in serum. The

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Table I. *Patient characteristics and descriptive data on serum S-100B and NSE levels.*

male / female	15 / 5
median age * (years) (range)	42 (27 - 64)
primary tumor histology: GBM / A / AA / O / AO / AOA	6 / 3 / 2 / 5 / 2 / 2
previous cranial surgery: yes / no	20 ** / 0
previous radiotherapy: yes / no	17 / 3
previous chemotherapy: yes / no	3 / 17
median time from diagnosis to blood sampling (months) (range)	25 (2 - 245)
median survival since blood sampling (months) (range)	13 (2 - 21)
median overall survival *** (months) (range)	35 (10 - 256)
median / mean S-100B serum level ± SD (μg/L) (range)	0.09 / 0.10 ± 0.07 (0.05 - 0.27)
median / mean NSE serum level ± SD (μg/L) (range)	6.35 / 7.55 ± 2.62 (4.21 - 14.43)

Abbreviations: GBM = glioblastoma multiforme; A = astrocytoma; AA = anaplastic astrocytoma; O = oligodendroglioma; AO = anaplastic oligodendroglioma; AOA = anaplastic oligo-astrocytoma; SD = standard deviation

* age at the time of blood sampling

** craniotomy (n=18); biopsy (n=2)

*** overall survival = duration of survival since primary diagnosis

detection limit of this assay is 0.04 μg/L. All samples were analyzed without knowledge of the clinical status of the patient.

Statistical analysis. Kaplan-Meier survival curves were calculated for patients with serum S-100B levels > 0.09 μg/L (median value) and ≤ 0.09 μg/L, and for patients with serum NSE levels > 6.35 μg/L (median value) and ≤ 6.35 μg/L. Differences among the survival curves were assessed by the log-rank test.

Results

Patient characteristics. Patient characteristics and descriptive data on serum S-100B and NSE levels are shown in Table I. All patients had previously undergone cranial surgery. Seven patients were re-operated once and three were re-operated twice. Seventeen patients had undergone prior radiotherapy and three had previously been treated with PCV (the combination of procarbazine, CCNU and vincristine) chemotherapy. One patient received (conventional) radiation therapy and two patients were treated with PCV chemotherapy at the time of blood sampling.

Analysis. The median time from primary diagnosis to the time of blood sampling for all patients was 25 (range 2-245) months. The median survival since blood sampling was 13 (range 2-21) months and the median overall survival (since primary diagnosis) was 35 (range 10-256) months, with seven patients being alive at the time of assessment. The median serum level of S-100B in all patients was 0.09 μg/L (range 0.05-0.27), while the median NSE serum level was 6.35 μg/L (range 4.21-14.43).

There is no consensus with respect to limits of normality for both serum S-100B and NSE (5-7,9,10,12). Since our study aimed at evaluating the prognostic value of serum S-100B and NSE as such, instead of detecting subjects with elevated levels, we analyzed our data based on a "median-split" of all serum S-100B and NSE values in two groups. Because S-100B and NSE values may change over time, we focussed on the duration of survival since blood sampling and not on overall survival. Kaplan-Meier survival curves, based on the dichotomy serum level S-100B > 0.09 *versus* ≤ 0.09 μg/L, demonstrated that classification according to this cut-off value leads to patient groups that differ significantly with regard to survival since blood sampling (see Figure 1A) (p=0.015); S-100B levels above 0.09 μg/L were correlated with shorter survival. Kaplan-Meier survival curves, based on the dichotomy serum level NSE > 6.35 and ≤ 6.35 μg/L, demonstrated no statistically significant difference in the duration of survival since blood sampling (see Figure 1B) (p=0.531).

Analyzing protein levels in individual patients, remarkably high serum S-100B levels were found in three patients (0.27; 0.27; 0.21 μg/L), of which one demonstrated a high serum NSE level as well (14.43 μg/L). These three patients all had a glioblastoma multiforme (GBM) and two of them demonstrated the shortest survival since blood sampling (2 and 4 months) and the shortest overall survival (10 and 12 months) of all 20 patients. Additionally, two of these three patients received treatment at the time of blood sampling (radiotherapy; PCV chemotherapy).

Discussion

This pilot study demonstrated that, with a cut-off point of 0.09 μg/L for serum S-100B, the difference in survival since blood sampling between patients with levels above and

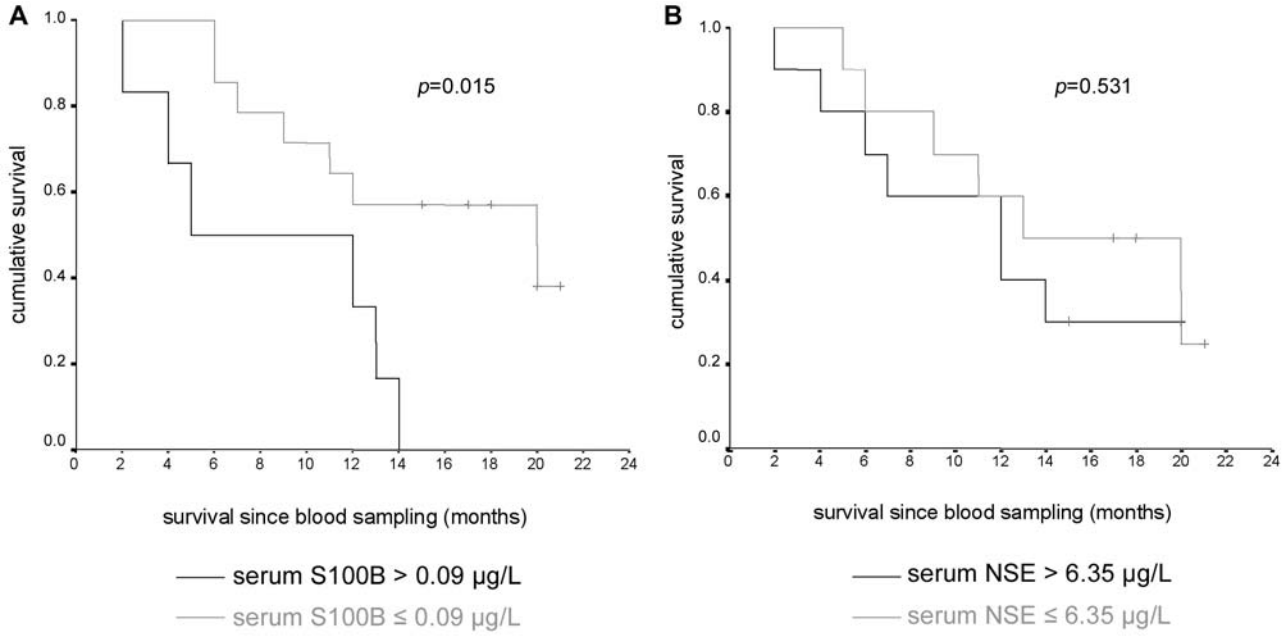

Figure 1. *Kaplan-Meier survival curves based on (A) the dichotomy serum level S-100B > 0.09 µg/L versus ≤ 0.09 µg/L (p=0.015) and (B) the dichotomy serum level NSE > 6.35 µg/L versus ≤ 6.35 µg/L (p=0.531).*

levels equal to or below 0.09 µg/L was significant, with high serum S-100B levels being associated with shorter survival. With respect to serum NSE, classification of patients based on dichotomisation around the median (6.35 µg/L) demonstrated no statistically significant difference in survival between the two groups. Both the median survival since blood sampling and the median overall survival were considerably shorter for patients demonstrating high serum S-100B levels (> 0.09 µg/L) than for patients demonstrating low serum S-100B levels (≤ 0.09 µg/L) (9 *versus* 15 months and 25 *versus* 38 months, respectively).

These preliminary findings suggest that serum S-100B might be valuable as a prognostic variable in glioma patients. However, when we focus on the group of patients demonstrating high serum S-100B levels, this subgroup appears to be older than the patients demonstrating low serum levels; median age 54 (range 39-64) *versus* 37 (range 27-63) years, with a relatively high proportion of patients with GBM (half *versus* one-fifth of patients). As both old age and high malignancy tumor grade are well-known independent unfavourable prognostic variables in glioma and as we, in addition, studied a heterogeneous and only small group of patients, the results of this exploratory study should be interpreted with caution. In this respect, however, an important finding is that serum S-100B levels in healthy adults do not seem to be age-dependent (12). With respect to the time-interval between (last) surgery and blood sampling, and between completion of (last) radiotherapy and blood sampling, there are some (non-significant) differences between the group of patients demonstrating high serum S-100B levels and the group of patients demonstrating low serum S-100B levels: the median interval between last surgery and blood sampling and between completion of last radiotherapy and blood sampling was 6 (range 1-66) and 15 (range 0-252) months, respectively, for patients with high levels, *versus* 13 (range 1-84) and 11 (range 5-108) months, respectively, for patients with low levels.

Further study is warranted to evaluate whether elevation of serum S-100B in glioma patients is tumor- or therapy-related, and whether S-100B is an additional, independent prognostic variable in cerebral glioma patients. Therefore, the longitudinal dynamics of serum S-100B levels have to be evaluated. Currently, we are planning to perform additional analyses in patients with primary GBM, by serial blood sampling at different stages of the disease.

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