Full title
Serum neurofilament light protein as a marker for diffuse axonal injury - results from a

case series study

Running title
Serum NFL as a marker for DAI

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Case series study of serum neurofilament light protein (NFL) as a marker for DAI

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Keywords

Abstract

Diffuse axonal injury (DAI) is an important cause of morbidity in patients with traumatic brain injury (TBI). There is a lack of a simple and reliable technique to early identify patients with DAI and to prognosticate long-term outcome in this patient group.

In the present study we examined acute serum concentrations of NFL (neurofilament light) in nine patients with severe TBI and DAI using a novel ultrasensitive Single molecule array (Simoa) assay. The relationships between the NFL concentrations and MRI in the acute stage as well as clinical outcome and MR-DTI parameters at 12 months were analysed.

We found that the mean NFL concentrations among the patients displayed a 30-fold increase compared to the controls and that the NFL completely discriminated between the patients and the controls. We also found a relation between the increased concentration of NFL and the MR-DTI parameters.

Although the number of patients in the study is small our results indicate that NFL may be a useful blood biomarker of TBI and that serum NFL might be an indicator of the severity of DAI.

Introduction

Diffuse axonal injury (DAI), caused by stretching and shearing forces of the white matter axonal fibers in the brain, is the primary neuropathological change in patients with traumatic brain injury (TBI).[1] The rapid inertial forces of acceleration and deceleration initiate a progressive process that leads to axonal failure and disconnection.[2,3] DAI is a common feature of severe TBI but is also considered to be important in mild TBI and might be involved in the development of post-concussive syndrome (PCS) with lasting and commonly disabling effects including headache, concentration difficulties, insomnia, mood disturbances, and dizziness.[4] There is a lack of reliable techniques to identify and quantify DAI in TBI patients. Conventional neuroimaging such as computerized tomography (CT) is considered inadequate for the evaluation of DAI, and standard magnetic resonance imaging (MRI) techniques lack sensitivity and are thus criticized for underestimating the extent of injury.[5] Magnetic resonance diffusion tensor imaging (MR-DTI) is increasingly used to detect DAI. DTI can indirectly evaluate the integrity of white matter tracts by measuring water diffusion, its directionality in three dimensions, and the diffusion anisotropy.[6] A large number of studies have shown that DTI can be used to detect alterations in the white matter ultrastructure after TBI (for a recent review see Studerus-Germann et al.[7]). However, MR-DTI is still mainly used in research studies and not in clinical routine. Further, it is not known how sensitive MR-DTI is to detect DAI in mild TBI cases, and the technique has not been cross-validated against post-mortem histology measures of axonal injury in humans. The analysis often needs time-consuming manual calculations, different hospitals use different methods for analysis and it is difficult to compare DTI parameters between research groups.

The search for a useful biomarker has been an alternative way to try to diagnose and quantify DAI. An ideal blood biomarker should have increased serum levels in the acute stage related to TBI-induced DAI and persistent brain dysfunction. Further, since DAI by definition

involves damage to the long myelinated white-matter axons, neuronal proteins enriched in these structures are top candidates as fluid biomarkers. A number of proteins have been evaluated as candidate blood biomarkers for TBI but none has so far proved to be of prognostic value regarding DAI.

NFL (neurofilament light) protein is an important structural protein of the axonal cytoskeleton.[8] Upon axonal injury, NFL leaks from disrupted axons into the brain interstitial fluid, cerebrospinal fluid and blood. In the present prospective longitudinal study we have examined NFL as a potential blood-based biomarker for DAI using an ultrasensitive Single molecule array (Simoa) assay in a cohort of patients with DAI and compared it to the 12-month outcome and MR-DTI parameters.

Methods

This study was approved by the Regional Ethical Review Board at the University of Gothenburg, and informed consent was obtained from all participants or their next of kin. All patients were referred to Sahlgrenska University Hospital during the period June 2006 – September 2009 and had sustained TBI. Patients were included based on the criteria that a suspicion of DAI was raised due to affected consciousness and/or focal neurological symptoms without an obvious explanation seen on the computerized tomography (CT) scan of the brain. On admission, the initial level of consciousness was assessed using the Reaction Level Scale (RLS).[9] The RLS can be translated to the Glasgow Coma Scale (GCS) and the translated GCS is presented here.[10] The type of trauma was also recorded.

The extended Glasgow Outcome Scale (GOSE) was used for global evaluation of outcome.[11] The assessment of GOSE scores was based on interviews with the patients or their next of kin by a nurse at 12 months postinjury.

The first MRI was performed within 9 days (range 4-9 days) postinjury and sequences including T1, T2, T2* and FLAIR were analysed by a radiologist (MM) and the presence of signs of DAI were classified according to Adams et al.[12] The follow-up MRI was performed at 12 months postinjury and for these examinations the DTI parameters were analysed. MR-DTI was performed on a Philips Gyroscan Intera 1.5 T, release 9. The DTI method used was HARDI (high angular resolution diffusion imaging; Philips, Eindhoven, the Netherlands). For the DTI analysis the corpus callosum was chosen for study in this investigation as it is prone to DAI[12] and it is anatomically easy to define using MR-DTI. Fiber tracking was performed using the fiber assignment by continuous tracking (FACT) algorithm in DTIStudio V 2.4 (Johns Hopkins Medical Institute, Laboratory of Brain Anatomical MRI, http://lbam.med.jhmi.edu/).[13] From the data provided in DTIStudio, we extracted FA and trace for the tracked voxels in the mid-sagittal section of the whole corpus callosum. Data was

analysed for 7 patients at 12 months postinjury as well as for 16 healthy, age-matched controls. The technique for DTI acquisition and data analysis has been described in detail elsewhere.[14]

Blood samples from the patients were obtained within six days postinjury as well as from 22 healthy age-matched controls. Serum NFL concentrations were measured using an ultrasensitive Simoa assay as previously described.[15] S100B concentrations were measured on the Cobas e601 instrument using the Elecsys S100 test (Roche Diagnostics, Mannheim, Germany).

The results are expressed as mean ± standard deviation. Age in the patient group and the control group was compared using a t-test, NFL concentrations were compared using the Mann-Whitney U-test, and DTI-parameters were analysed using Fisher's non-parametric permutation test. $P < 0.05$ was considered significant. The relationship between NFL concentrations and DTI parameters was explored using simple linear regression, and the goodness of fit is presented as R^2.

Results

Nine patients, 7 men and 2 women, were included. The mean age of the patients was 40.4 years (range 19-69 years). The initial GCS scores of the patients ranged from 3 to 14. None of the patients had any intracranial mass lesions requiring evacuation that could explain their loss of consciousness.

One patient died and the outcomes of the surviving patients, measured by the GOSE at 12 months, ranged from 3 to 6. In summary, five patients had a severe disability and three patients a moderate severity. Using conventional MRI in the acute stage, all patients had signs of DAI, with a majority of patients classified as Adams' 2-3 and displaying signs of axonal injury in the hemispheres, the corpus callosum as well as in the brain stem. Clinical characteristics and the outcome scores of the patients are presented in table 1.

[Insert Table 1 about here]

Serum NFL concentrations of the patients ranged from 87.5 to 851.6 pg/ml and were markedly higher than serum levels in controls (10.8 ±5.4; Figure 1). Serum S100B concentrations of the patients ranged from 0.03 to 0.10 µg/L (reference value <0.10 µg/L).

[Insert Figure 1 about here]

For seven patients, it was possible to analyse the MR-DTI performed at 12 months (two examinations were missing since one patient died and one examination was lost due to technical problems). For the diffusion parameters, a significant reduction in FA in the corpus callosum was seen compared to the controls (0.51 ±0.07 vs. 0.62 ±0.04) and a significant increase in trace (2.87 ±0.34 vs. 2.28 ±0.12).

When dichotomizing the patients into two groups based on their outcome at 12 months, five patients had a severe disability (GOSE 3-4) and three patients a moderate severity (GOSE 5-6). The mean serum NFL concentration in the patients with severe disability was 411 ±263 and in the patients with a moderate severity it was 277 ±80. The difference was not significant.

When plotting the acute serum NFL concentrations versus the DTI parameters at 12 months postinjury we found a linear relationship. A positive relation was found between trace and the serum NFL (R^2=0.79) and negative relation between the FA and the serum NFL (R^2=0.70; Figure 2).

[Insert Figure 2 about here]

Discussion

In the present prospective longitudinal study we have examined a novel biomarker, serum NFL, in a cohort of patients with DAI. The acute serum NFL concentrations were significantly higher for patients compared to controls, and we found a correlation between the increased NFL concentrations and affected MR-DTI parameters at 12 months postinjury. S100B concentrations were not significantly increased for the patients, but the blood samples were taken 4-9 days postinjury, and it is possible that S100B could have normalized.

cerebrospinal fluid (CSF) NFL is a well-established biomarker for axonal injury and clearly elevated concentrations are seen in patients with cerebrovascular insults and mild to severe traumatic brain injury.[16,17] However, an obvious limitation of CSF NFL is the requirement for lumbar puncture. For this reason, a sensitive and reliable blood biomarker of CNS injury would be preferred. Recent developments in ultrasensitive measurement techniques have allowed us to establish a sensitive and specific NFL assay for serum and plasma measurements[15,18] and we have shown that serum/plasma NFL correlates with CSF NFL with a correlation coefficient of 0.89.[15]

In the current study we used this NFL assay on a cohort of TBI patients with a clinical suspicion of DAI, due to loss of consciousness that could not be explained by CT findings and signs of DAI classified as Adams' grade 2-3 on the acute MRI. The outcomes at 12 months with GOSE scores ranging from 1 to 6 indicate that the patients were severely injured.

We found that the mean NFL concentrations among the patients displayed a 30-fold increase compared to the controls and that NFL concentrations completely discriminated between the patients and the controls. However, while it is likely that the increase in NFL is caused by DAI, it is also possible that axonal injury due to hypoxic ischemic change and/or swelling

might be reflected by the marker. Although the number of patients in our study is small our result indicates that NFL may be a useable biomarker of TBI.

A recent study showed that measurement of another axonal protein, microtubule-associated protein tau, in interstitial fluid collected by microdialysis catheters correlates with DTI measurements.[19] However, not only does collection of interstitial fluid require craniotomy, but tau protein also seems to be a less sensitive biomarker for mild TBI than NFL.[20,21] In the present study, we analysed NFL in blood samples and found a relation between the increased serum concentration and the DTI parameters. A number of studies have shown a relationship between the severity of TBI, affected DTI parameters and clinical outcome. TBI, and especially DAI, reduces FA and increases trace, and the affected DTI parameters correlate to clinical outcome.[7,14] The positive relation between NFL and trace, and the negative relation between NFL and FA, indicate that serum NFL might be an indicator of the severity of the DAI.

We currently plan studies with larger number of patients to further investigate the possible role of serum NFL as a valuable biomarker in TBI. Further studies will show if NFL also is increased in patients with mild TBI, if it is specifically sensitive to DAI and if it is directly correlated to outcome.

Acknowledgements

Declaration of Interest

HZ and KB are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg.

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