

Rapid release of AMPA receptor (AMPA) peptide correlates with concussion and mild traumatic brain injury (TBI)

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Background: We investigated clinical feasibility of a rapid magnetic-particle (MP) ELISA to detect AMPAR degradation peptide in blood of patients with concussion and mild TBI to help improve diagnostic certainty of posttraumatic stress disorder (PTSD).

Materials and Methods: Double-blind blood samples were collected from 100 adolescents at Pavlov's State Medical University and analyzed in two groups: concussion (n=38) and mild TBI (n=62). AMPAR peptide levels were measured within 24-72 hours after brain injury. All patients were assessed using EEG and cognitive testing.

Results: Increased AMPAR peptide levels were observed within 24 hours of injury. Compared with nontraumatic controls (n=23; 0.7 plus/minus 0.08 ng/mL), AMPAR peptide was 1.51-1.72 ng/mL in the concussion group and 2.33-3.12 ng/mL in the mild TBI group. EEG registered nonspecific paroxysmal discharges in 18% of cases and epileptiform activity in 6%. Reduced cognitive abilities were observed in 30% of cases of multiple concussion and mild TBI. Assay sensitivity and specificity for concussion were 95% and 94%, respectively, at a cutoff of 1.0 ng/mL; positive predictive value was 91%. A posttest probability of 96% was calculated from the prior test's probability of 63% defined by cognitive testing (MMSE scale) and EEG monitoring.

Conclusion: Elevated levels of AMPAR peptide indicates acute mild TBI within 72 hours following brain trauma and is associated with alterations in EEG spiking activity and reduced MMSE scores. Use of the AMPAR peptide assay in conjunction with results of EEG and cognitive testing may improve diagnostic certainty of PTSD after concussion and mild TBI.

Role of targeted quantitative Metabolomics in biomarker discovery of perinatal brain injury

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Introduction

Brain injury in newborns is one of the major causes of lifelong neurological sequelae. Although potential neuroprotective strategies are arising, there is a significant gap in early diagnosis of brain injury.

Hypothesis: Quantitative metabolomics is able to characterize animals models on a functional level, to gain new knowledge of the pathophysiology as a fundament for drug development and to help in the development of biomarkers for brain injury. The aim of the study is to characterize the impact and time course of HI brain injury on amino acids, biogenic amine, acylcarnitine, sphingomyeline and glycerophospholipid metabolism in newborn rat brain and plasma.

Methods: 7-day old rat pups were anesthetized with isoflurane, the right carotid artery was ligated, after 2 h recovery pups were exposed to hypoxia. Sham-operated animals underwent only neck incision. Control animals had no damage. Animals were euthanized i) immediately after hypoxia ii) after 24hrs, iii) at P12. Brains were collected, immediately frozen and stored at -80°C. We used a multi-parametric, highly robust, sensitive and high-throughput targeted metabolomic LC-MS/MS method for the simultaneous quantification of endogenous intermediates (amino acids, biogenic amines, acylcarnitines, sphingomyelins and glycerophospholipids) in brain and plasma samples.

Results: HI brain injury led to a significant specific time-dependent change in the pattern of amino acid, biogenic amine, acylcarnitine, sphingomyelin and glycerophospholipid and eicosanoid levels.

Conclusion: Quantitative targeted metabolomics is a promising tool for biomarker discovery as well as for the evaluation and improvement of pre-clinical models and thereby for acceleration of drug development.

ApoE-epsilon4 allele influences the levels of S-100B and NSE in severe head injury

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Background: The ApoE-epsilon4 allele (e4) has been associated with worse outcome after brain injury. The relation between ApoE-epsilon allele and the biochemical markers S-100B and NSE in patients with severe head injury strictly treated by an ICP targeted therapy based on the Lund concept was studied.

Material and Methods: 46 subjects with severe head injury (GCS \leq 8) were included. S-100B and NSE were collected twice daily for five days. AUC for the 3 first days was calculated. Blood samples for ApoE-epsilon gene were collected. Results are given as mean \pm sem or median (range). Wilcoxon rank sum test was used for analysis of statistical significant differences.

Results: Age 35.0 \pm 2.2 yrs, ISS 28.7 \pm 1.5, median GCS 6(3-8). The e4 was present in 39% of the subjects. The first S-100 value was 0.71 \pm 0.27 in the non-epsilon4 (non-e4) group and 1.56 \pm 0.34 ug/l in the e4 group (p=0.07). The corresponding values for NSE were 15.93 \pm 3.02 and 24.09 \pm 3.87 ug/l (p=0.12). The highest values were for S-100B 0.80 \pm 0.09 in the non-e4 group and 2.32 \pm 1.18 ug/l in the e4 group (p=0.05). The corresponding values for NSE were 20.89 \pm 4.57 and 32.96 \pm 10.55 ug/l (p=0.23). AUC for S-100B in the non-e4 group was 2.29 \pm 1.81 and 7.08 \pm 2.25 ug/l (p=0.03) in the e4 group. The corresponding values for NSE were 66.85 \pm 13.15 and 75.09 \pm 24.34 ug/l (p=0.9).

Conclusion: Subjects expressing e4 seems to release more S-100B and in some cases NSE as compared with non-e4 subjects. This can influence the interpretation of the existing results on S-100B and NSE as biomarkers for brain injury.

Serum S100B in mild traumatic brain injury

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Objective: The aim of the study was to correlate early serum S100B measurements and initial CCT findings in the patients who have sustained mild traumatic brain injury (MTBI).

Methods: The prospective study included patients of all ages with a history of MTBI. CCT scans and blood sampling for S100B analysis were performed within 6 hours after injury. Levels of S100B above 0.1 ng/ml (S100B+) and any CCT detectable trauma-relevant intracranial lesions were considered positive (CCT+). Blood alcohol concentration, ISS, time between injury and blood sampling were also recorded.

Results: A series of 102 patients were involved in the study. CCT+ scans were present in eighteen (17.6%) and CCT- scans in 84 (82.4%) patients. There were 74 (72.5%) patients in S100B+ and 28 (27.5%) in S100B- group. Sensitivity of S100B assay attained 83.3% with a negative predictive value of 89.3%. Three patients from CCT+ group had negative plasma level of S100B with mean injury to blood sampling time 3.17 hours. Two of them, a case of epidural haematoma and a case of acute subdural haematoma, required surgical treatment during their hospital stay. Significant association between S100B concentration and ISS was found (Correlation coefficient $r = 0.44$ (95% CI 0.26 to 0.58), $P < 0.0001$).

Conclusion: S100B serum protein marker seems to be an unreliable screening tool for determination of an intracranial injury risk group due to low sensitivity and negative predictive value seen from samples taken greater than 3 hours after an MTBI.

From cerebrospinal fluids to brain microdialysates: discovery of brain damage markers through quantitative proteomics

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Background: To discover innovative markers of brain damage, the quantitative comparisons of human cerebrospinal or brain interstitial fluid proteome were carried out using isobaric tagging and tandem mass spectrometry.

Materials and methods: Ante- and post-mortem cerebrospinal fluids (n = 4 and 4) and brain interstitial fluid samples from ischemic stroke patients (sampled either from the infarct core and penumbra (n = 2), infarct core and controlateral (n = 2) or penumbra and controlateral brain regions (n = 2)) were used respectively in these studies. In both cases, a shotgun approach combining isobaric tandem mass tags, multi-dimensional separation, mass spectrometry and bioinformatics identification and quantitation was performed.

Results: In the first proteomic study, post-mortem cerebrospinal fluid was considered as a model of massive brain damage injury. The comparison with cerebrospinal fluid from living patients showed 78 proteins with increased concentration in the cerebrospinal fluid of deceased patients. Several of these proteins (NDKA, GSTP1, UFD1, PARK7) are already being assessed as markers of brain damage pathologies (see presentations by N. Turck et al.). The second study focused on brain microdialysates from patients suffering an ischemic stroke. Proteins such as GFAP, H- and B-FABP and S100B were shown to be increased amongst others in the infarct core microdialysate samples with respect to the penumbra and controlateral brain regions.

Conclusion: Both quantitative studies were complementary. They demonstrated the validity of the working hypothesis and pointed out the relevance of the achieved quantitative proteome maps, prior further validation of marker candidates.

Neurofilament ELISA validation

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Background: Neurofilament proteins (Nf) are highly specific biomarkers for neuronal death and axonal degeneration. As these markers become more widely used, an inter-laboratory validation study is required to identify assay criteria for high quality performance.

Methods: The UmanDiagnostics NF-light® enzyme linked immunosorbent assay (ELISA) for the neurofilament light chain (NfL, 68 kDa) was used to test the intra-assay and inter-laboratory coefficient of variation (CV) between 35 laboratories world-wide on 15 cerebrospinal fluid (CSF) samples. Critical factors, such as sample transport and storage, analytical delays, reaction temperature and time, the laboratories' accuracy and preparation of standards were documented and used for the statistical analyses.

Results: The intra-laboratory CV averaged 3.3% and the inter-laboratory CV 59%. The results from the test laboratories correlated with those from the reference laboratory ($R=0.60$, $P<0.0001$). Correcting for critical factors improved the strength of the correlation. Differences in the accuracy of standard preparation was identified as the most critical factor. Correcting for the error introduced by variation in the protein standards improved the correlation to $R=0.98$, $P<0.0001$ with an averaged inter-laboratory CV of 14%.

Conclusion: This multi-centre validation study identified the preparation of accurate and consistent protein standards as the main reason for the poor inter-laboratory CV. This issue is also relevant to other protein biomarkers based on this type of assay and will need to be solved in order to achieve an acceptable level of analytical accuracy.

Neuron specific enolase and protein S100B as markers for brain damage after traumatic brain injury (TBI)

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Background: Brain injury is a leading cause of death and disability in active population under 45 years of age. Mortality and unfavourable outcome after head injury ranges between 25% and 50%. Protein S100B is a marker of glial activation and has some neurotrophic as well as some neurotoxic properties. Neuron specific enolase (NSE) is a glycolytic enzyme mainly present in neurons. Clinical and radiological markers of brain injury have limited predictive value for outcome after brain injury. Determination of biochemical markers in serum seems to be very specific and sensitive method for isolated TBI detection.

Material and methods: Our study group comprised of 20 patients (15 men, 5 women) mainly injured in car accidents, who were admitted to trauma centre. In all patients GCS, pupils and ICU survival was assessed. CT scan was evaluated by neurosurgeon using 5-point scale. At admission blood sample was taken for the determination of S100B and NSE. S100B and NSE were performed with chemiluminescence method.

Results: All patients with ab-normal CT had elevated values of S100B and NSE upon arrival at emergency department. There was a significant correlation between the severity of injury assessed on CT scan and the serum concentrations of protein S100B and NSE. **Conclusions:** We found the statistical significance correlation between biochemical markers and GCS, which is as a standard clinical tool for grading severity of TBI. S100B and NSE are sensitive markers of brain injury as reliable as CT scan and even more accurate than GCS for assessment of isolated TBI.

A multiparameter panel method for outcome prediction following aneurysmal subarachnoid hemorrhage

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Purpose: Accurate early anticipation of long-term irreversible brain damage during the acute phase of patients with aneurysmal subarachnoid hemorrhage (aSAH) remains imprecise. Using a combination of clinical scores together with brain injury-related biomarkers (H-FABP, NDKA, UFD1 and S100B), this study aimed at developing a multiparameter prognostic panel to facilitate early outcome prediction following aSAH.

Methods: Blood samples of 140 aSAH patients from two separated cohorts (sets of 28 and 112 patients) were prospectively enrolled and analyzed with a 14-month delay. Patients were admitted within 48h following aSAH onset. H-FABP, NDKA, UFD1, S100B and Troponin I levels were determined using classical immunoassays. The World Federation of Neurological Surgeons (WFNS) at admission and the Glasgow Outcome Score (GOS) at 6 months were evaluated.

Results: In the two cohorts, blood concentration of H-FABP, S100B and Troponin I at admission significantly predicted unfavourable outcome (GOS 1-2-3). A multivariate analysis identified a 6-parameter panel, including WFNS, H-FABP, S100B, Troponin I, NDKA, and UFD-1; when at least 3 of these parameters were simultaneously above cut-off values, prediction of unfavourable outcome reached at least 70% sensitivity in both cohorts for 100% specificity.

Conclusion: The use of this panel, including four brain injury-related proteins, one cardiac marker and a clinical score could be a valuable tool to identify aSAH patients at risk of poor outcome.

Early elevation of serum GSTP and NDKA levels predict stroke events and outcome

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Purpose: Stroke is one leading cause of death and disability in industrialized countries. Diagnosis is based on physician evaluation and neuroimaging and no predictive tools exist for patient outcome. In this context, new biomarkers, detected early in blood, may help clinicians to assess diagnostic and prognosis of stroke patients. **Methods:** Home-made sandwich ELISAs were used to quantify serum GSTP and NDKA levels in two independent cohorts encompassing 91 control and 67 stroke patients (samples taken inferior to 12h after the stroke onset). GSTP and NDKA levels were normalized and merged. Student t-tests and ROC curves determined their clinical performances. Pearson correlation between biomarker levels and NIHSS at 12h and 3 days after symptom onset were calculated. **Results:** Serum GSTP and NDKA levels were significantly elevated in stroke patients (0.0001, Student t-tests). With a normalized cut-off value at 1.43, GSTP discriminated stroke patients with 65.7% sensitivity (SE) for 92.2% specificity (SP) whereas NDKA (at 1.19 normalized value) classified correctly patients with 43.3% SE for 92.2% SP. NDKA levels showed a significant correlation with the NIHSS at 12h and 3 days ($\rho = 0.30$ and 0.40 , respectively). In addition, its level appeared higher in patients who died in next 3 months (0.035, Student t-test).

Conclusion: These results support GSTP as a novel biomarker for the early diagnosis of stroke and NDKA as a potential predictor of patient outcome. These findings open new avenues for the management of stroke patients by establishing GSTP and NDKA as robust blood biomarkers, which may complement clinical assessment.

Does increasing glutamine signal boosting metabolism in hard working astrocytes?

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BACKGROUND: Glutamate and glutamine cycling between astrocytes and neurons is highly energy demanding. Interstitial glutamine correlate with pyruvate levels in subarachnoidal hemorrhage (SAH) patients.

METHODS: The clinical course and brain interstitial glutamine levels were explored in 33 SAH NICU patients where microdialysis (MD) probes were placed in non-injured frontal lobe cortex. MD-lactate, -pyruvate, -glutamate and -glutamine were related to computerized multimodality monitoring data, neurological ischemic deteriorations (DIND), CT-verified ischemia and admittance parameters.

RESULTS: 12 out of 13 periods with L/P>40 and glutamate>30 μ M related to CT-verified infarcts or DINDs. In 11 out of 15 DINDs L/P and glutamate elevations preceded the deterioration (n=6) and/or there were glutamine surges at DIND onset (n=8). Five out of 7 vasospasm treated patients had glutamine surges as the only DIND associated pattern (Fig.A). Glutamine and pyruvate were higher and glutamate, lactate and L/P were lower when ICP<10mmHg than when ICP>10mmHg. In three patients ICP was rapidly lowered from around 15-20mmHg to 10mmHg by increased CSF-drainage. This resulted in surging glutamine and pyruvate levels (Fig.B).

Poor WFNS score patients had lower glutamine at MD onset than good WFNS score patients. Glutamine levels increased over time. **CONCLUSION:** A MD pattern with L/P>40 and glutamate>30 is associated with ischemia. Glutamine surges appeared (1) during DIND in the absence of CT ischemia and MD ischemia (2) in recovery after either high ICP or after ischemic MD patterns. Increasing glutamine levels may signal augmented astrocytic metabolism with accelerated glutamate uptake and glutamine synthesis.

Explorative investigation of biomarkers of brain damage and coagulation system activation in clinical stroke differentiation.

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A simple and accurate method of differentiating ischemic stroke and intracerebral hemorrhage (ICH) is potentially useful to facilitate acute therapeutic management. Blood measurements of biomarkers of brain damage and activation of the coagulation system may potentially serve as novel diagnostic tools for stroke subtypes. **METHODS** : Ninety-seven stroke patients were prospectively investigated in a multicenter design with blood levels of brain biomarkers S100B, neuron specific enolase (NSE), glial fibrillary acidic protein (GFAP) as well as a coagulation biomarker, activated protein C - protein C inhibitor complex (APC-PCI), within 24 hours of symptom onset. **RESULTS** : Eighty-three patients (86 %) had ischemic stroke and fourteen patients (14 %) had ICH. There were no differences in S100B ($p = 0.13$) and NSE ($p = 0.67$) levels between patients with ischemic stroke or ICH. However, GFAP levels were significantly higher in ICH patients ($p = 0.0057$). APC-PCI levels were higher in larger ischemic strokes ($p = 0.020$). The combination of GFAP and APC-PCI levels, in patients with NIHSS score more than 3, had a sensitivity and negative predictive value of 100 % for ICH in our material ($p = 0.0052$). **CONCLUSION** : This exploratory study indicated that blood levels of biomarkers GFAP and APC-PCI, prior to neuroimaging, may rule out ICH in a mixed stroke population.

Prediction of time trends in recovery of cognitive function after mild head injury

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To investigate relations between predictors and outcomes, and especially to identify predictors influencing the time trend in recovery after a mild traumatic brain injury.

Methods: We included 59 patients with mild head injury (MHI) in a prospective study. They underwent extensive assessment with neurological and neuroradiological examinations, serum S100B analysis, and APOE genotyping. Neuropsychological testing was performed before discharge and after six months. Linear mixed models were used to assess the associations between baseline predictors and neurocognitive performance and its change.

Results: GCS score < 15, TBI demonstrated with computed tomography, magnetic resonance imaging (MRI), and Serum S100B > 0.14 µg/L predicted impaired cognitive performance both at baseline and after six months. APOE genotype did not. There was significant improvement of performance after six months. APOE ε-4 genotype was the only independent factor significantly predicting less improvement.

Conclusion: The presence of the APOE ε-4 allele predicts less recovery of cognitive function after MHI.

Neurofilament Biomarkers of Brain Damage and Disease

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We developed an ELISA which can sensitively detect the heavily phosphorylated axonal form of neurofilament subunit NF-H (pNF-H) in the CSF and blood of animals and patients suffering from a variety of CNS damage and disease states. The levels of pNF-H give a convenient measure of the degree of ongoing axonal injury or degeneration. We have recently shown that pNF-H is released into the blood of transgenic rodent models of ALS and the mouse EAE model of multiple sclerosis, but cannot be detected in the blood of control rodents. In both cases levels of blood pNF-H reflect the seriousness of the disease state, and pNF-H can be detected in blood prior to the onset of symptoms. Pharmacological amelioration of the EAE phenotype also greatly reduces blood pNF-H levels, suggesting that pNF-H blood levels may be used to screen for potentially neuroprotective drugs in animal models and to monitor drug efficacy in patients with MS, ALS and traumatic CNS injuries. Blood and CSF levels of pNF-H provide prognostic and clinical information on patients suffering from aneurysmal subarachnoid hemorrhage, ALS and several other damage and disease states. We developed more refined pNF-H assays based on novel monoclonal antibodies developed specifically for use in ELISA. We are currently characterizing the exact form of pNF-H released into CSF and blood and will describe our latest findings. We have also developed assays to detect several other potential brain injury biomarkers and will present a brief synopsis of the data obtained with each.

Two different assays for determining S-100B in serum and urine: a comparison

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Background: Brain injury after head trauma can be detected by S-100B measurements in serum. While the half life of serum S-100B is short (approximately 30 minutes) S-100B in urine, on the other hand, could be detected for many hours or even days after traumatic brain injury. This makes urine a potential suitable biological fluid for S100B analyses. The aims of the present study are two-fold: to compare serum measurements of two assays, the Liaison Sangtec®100 system and the Elecsys®S100 test, and to investigate to what extent they can detect and measure S-100B in urine.

Materials and methods: A total of 191 serum and 174 urine samples from 107 patients (children aged between 1 and 18 years following head trauma) were measured with both assays. The results were compared using correlation analysis and Bland-Altman difference plots.

Results: Comparisons between the Sangtec® and the Elecsys® test regarding S-100B in serum showed a correlation (correlation coefficient 0.80) but not an agreement between the methods. In urine, S-100B could only be detected in 20 out of 174 samples with the Sangtec® system (range 0.02-0.06 microg/L), whereas the Elecsys® test could detect S-100B in 171 samples (range 0.005-0.14 microg/L). No clear relation was observed between the two methods in urine analysis (correlation coefficient 0.60).

Conclusion: The Sangtec® and Elecsys® assays are not interchangeable methods when analyzing S-100B in serum or urine samples after head injury.

Does extracerebral trauma affect the predictive values of S100B regarding cerebral injuries?

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Background

Protein S100b has proven to be a useful marker for cerebral damage. The predictive ability of S100b may, however, be affected by extra cerebral injuries.

We sought to investigate serum levels of S100B in patients with either Isolated Head Injury (IHI), Multi trauma with Head Injury (MTHI), or No Head Injury (NHI). Primary aim was to assess if a significant difference in serum levels of S100b could be found between IHI and MTHI patients.

Methods

We included 233 patients primarily admitted to the trauma center. Serum levels of S100b were analyzed on admission and 6 hours after trauma.

Variables included Abbreviated Injury Scale (AIS) for head trauma, Injury Severity Score (ISS), and 30-day survival.

Results

Two patients could not be classified. IHI occurred in 28, MTHI in 102, and NHI was found in 101. The median S100b concentration on arrival was 0.47, 1.68, 0.49 microg/l, respectively ($P < 0.0001$). The corresponding values at 6 hours were 0.14, 0.31, and 0.15 microg/l, respectively ($P < 0.0001$). S100b was significantly higher in patients with MTHI than in patients with IHI at both time points (P values 0.0005 and 0.01). There was no significant difference in S100b between patients having IHI and patients with NHI ($P = 0.81$ and $P = 0.67$).

Conclusion

High serum concentration of S100b were found early after trauma. The highest concentrations of S100b was found in patients with multi trauma. This suggests that S100b serum concentrations are significantly affected by extra cerebral injuries.

NR2 Peptide Assay for Assessment of Cerebral Ischemic Events

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Introduction: NR2 peptide, the breakdown product of N-methyl-D-aspartate (NMDA) neuroreceptor, can be detected in blood. To reduce nonspecific binding and increase stability and turnaround time, a microtiter plate ELISA assay detecting NR2 peptide has been enhanced using magnetic particles (MP) and used to measure NR2 peptide and assess its concentrations in healthy persons.

Methodology: A sensitive, 1-step enzymatic MP-ELISA has been developed that measures NR2 peptide in 20 μ L of EDTA plasma sample against recombinant NR2 peptide calibrators (0-25 ng/mL). The antibody pair used in the assay measures free NR2 peptide. **Results.** The within-run and total precision for NR2 peptide in quality control material were <5%. Lot-to-lot consistently reproducible results were observed ($R^2=0.99$) using plasma samples (0.2-25.0 ng/mL). The average correspondent analytical and functional sensitivities were 0.1 and 0.2 ng/mL (20% CV). Dilution and spiking studies showed an average recovery of 96-110%. Median NR2 peptide value for a healthy population (n=154) was 0.4 ng/mL. NR2 peptide concentrations were within 10% of control in tests with triglycerides, creatinine, cholesterol, bilirubin, and human serum albumin but not with hemoglobin.

Conclusions. A sensitive and reliable MP-ELISA NR2 peptide assay has been developed to measure low levels of NR2 peptide in EDTA plasma. Using this assay, approximate median NR2 peptide levels in a healthy ambulatory population can be measured with <10% CV. These results suggest MP-ELISA NR2 peptide assay performance is acceptable for further investigation of the NR2 peptide as a biomarker of cerebral ischemic events.

Serum and urine S-100B after traumatic head injury in children

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Background

The clinical evaluation of children with head trauma is difficult and improved diagnostic tools are needed. Serum S-100B has been shown to be a sensible marker for brain damage after head injury in adults but few studies have focused on its use in children. Urine S-100B has not been fully explored previously and if detectable it could be useful in the evaluation of children with head trauma.

Methods

In the present study 111 children with head trauma were included. Venous blood and urine samples were taken at arrival (S1 and U1) as well as 6 hours later (S2 and U2). Clinical and radiological evaluations were performed according to hospital routine. Two groups were identified - Group 1: No CT-scan performed or a CT-scan without any sign of trauma-related intracranial pathology (n=105). Group 2: A CT-scan with signs of trauma-related intracranial pathology (n=6).

Results

In Group 1 the median (inter quartile range) serum S-100B value in S1-samples was 0.111 μ g/L (0.086-0.153), and in Group 2 it was 0.282 μ g/L (0.195-1.44), (p<0.01). Also S2 values differed significantly between the two groups. Urine S-100B was not significantly different between the groups.

Conclusions

Serum S-100B values within 6 hours after head trauma in children were significantly higher in patients with intracranial pathology compared to those without intracranial complications. Identification of these high risk patients in the emergency department is important, and we suggest that S-100B could be a valuable diagnostic tool in addition to those used in clinical practice today. Urine S-100B has to be more investigated.

S100B serum level predicts computed tomography findings after minor head injury

Muller Kay, Townend W, Biasca N, Uden J, Waterloo K, Romner B, Ingebrigtsen T

The present multicenter study was performed to investigate whether determination of protein S100B in serum could contribute to the selection of patients for CT scanning. **METHODS:** We included 226 patients with a history of head injury and a Glasgow Coma Scale (GCS) score of 13 to 15 at admission to hospital. Blood samples for S100B analysis and head CT were obtained within 12 hours after the injury. The diagnostic properties of S100B measurements for prediction of intracranial injury revealed by CT were tested with receiver operating characteristic (ROC) analysis and cross-table analysis at different cut-off levels. **RESULTS:** CT showed intracranial injury in 21 (9.3%) patients. S100B levels were significantly ($p < 0.001$) elevated in patients with intracranial injury (mean, 0.36; 95% CI, 0.21-0.50 microg/L) compared with those in patients without intracranial injury (mean, 0.18; 95% CI, 0.16-0.20 microg/L). ROC curve analysis showed a significant ($p = 0.001$) area under the curve (0.73; 95% CI, 0.62-0.84). Cross-table analysis showed that 20 of 21 (sensitivity 0.95) patients with intracranial injury were detected at a cut-off level of 0.10 microg/L, but 141 of 205 (specificity 0.31) patients with no such injury also had a S100B level above this limit. **CONCLUSION:** Determination of serum S100B cannot replace the clinical examination or use of CT for patients with minor head injury. Adding S100B measurement to the clinical evaluation might support selection of patients for CT scanning.

Clinical significance of serum S100B levels in neurointensive care

Johan Unden, Ramona Åstrand, Gunnar Andsberg, Peter Reinstrup, Knut Waterloo, Tor Ingebrigtsen, Johan Bellner, Bertil Romner

A total of 79 patients with confirmed or suspected head injury or cerebrovascular insults (CVIs) (based upon patient history, computed tomography (CT) and/or magnetic resonance imaging (MRI) and neurological examination including coma scoring) who required neurointensive care were included in the study. Sampling for S100B was performed at admission and daily until patients were discharged from the NICU. S100B measurements were statistically compared to occurrence of secondary complications and outcome according to Glasgow Outcome Scale (GOS), with focus on clinical prediction.

17 of 79 patients (22%) had secondary neurological complications. Mean S100B levels were found to be an independent parameter associated with these complications ($P=0.03$). Mean S100B levels were higher in patients with complications compared to those without on both the complication day ($P=0.033$) and the day after ($P=0.015$), but not the day prior to the complication ($P=0.62$). S100B did not predict secondary neurological complication. Neither mean ($P=0.182$) nor peak ($P=0.370$) S100B levels were associated with or predicted outcome according to dichotomised GOS.

Daily S100B measurements are associated with secondary complications but not to outcome. However, daily S100B levels do not predict secondary complications, which limit the usefulness of this brain biomarker in this setting.

Evaluation of serum S100B sampling in clinical head injury management

Olga Calcagnile, Bertil Romner, Johan Unden

Brain biomarker S100B has shown great promise in clinical studies as a potential tool for CT selection after mild head injury. This study evaluates 2 years of clinical usage of serum S100B in actual head injury management in a secondary care center. 400 patients have so far been followed up. S100B was <0.10 microgram/liter in 25% of patients compared to 32% in previous studies. No patients with low S100B showed any clinical/radiological signs of acute complication after mild head injury. Initially, doctors did not use the S100B value in decision-making but this gradually changed during the study period. A total of 40/400 patients were spared a CT scan. S100B seems to be both safe and cost-effective in this setting.

NSE predicts poor neurological outcome in hypothermia treated cardiac arrest.

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Background: Hypothermia treatment (TH) of cardiac arrest (CA) patients necessitates sedation during treatment. This renders prognostication more difficult, and obscures early neurological examination. Adjunctive prognostication tools are therefore needed. **Materials and Methods:** Between Aug 2003 and Dec 2007 Serum-NSE was collected at 2, 24, 48 and 72 h after CA in consecutive TH-treated patients. The NSE analysis was performed using LIAISON® NSE, (DiaSorin AB, Sundbyberg, Sweden). Patients were evaluated using the Cerebral Performance Categories (CPC) score. A CPC 1-2, compatible with independent neurological function, at any time during the 6 months follow up was considered a good outcome. The material was dichotomised in a good and a poor outcome group.

Results: 102 patients were included. Samples with visible hemolysis were discarded. 56/102 (55%) had a best CPC 1-2. S-NSE $\geq 28\mu\text{g/l}$ at 48h after the CA was the best predictor of a poor outcome (specificity 100%, sensitivity 67%). The development of NSE levels over time differed significantly between the good and the poor outcome groups ($p < 0.0005$, Friedmans test). A rise of $>2\mu\text{g/l}$ between 24 and 48h was strongly correlated to a poor outcome (Logistic regression, odds ratio 9.8, CI 3.5-27.7)

Conclusion: The result indicates that NSE is a valuable adjunct in the prognostication of neurological outcome in TH CA patients.

Blood Pressure Response to Anesthesia: A Retrospective Analysis of the Impact of Genetic Polymorphism in the Renin-Angiotensin System, Adrenergic System and Nitric Oxide System

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Background: Hypotension is frequently observed in patients undergoing anesthesia and may aggravate neurological outcome.

Objective: We tested the hypothesis that the level of hypotension following anesthesia with remifentanyl/propofol or remifentanyl/sevoflurane is associated with polymorphisms in genes involved in cardiovascular regulation. **Patients/methods:** 570 Caucasians undergoing neurosurgery were studied. End-point variable was the lowest mean arterial pressure (MAP) reached in each patient from the time of induction of anesthesia to beginning of surgery. Patients were genotyped for 31 polymorphisms in 12 genes involved in the renin-angiotensin system, adrenergic system and nitric oxide system: ACE, ADRA1A, ADRA2A, ADRA2C, ADRB1, ADRB2, AGT, DDAH2, GNAS, GNB3, NOS2A and NOS3. **Results:** No single polymorphism demonstrated association with degree of hypotension. One distinct haplotype pair in the NOS2A gene (ACGT/ACGT, N = 70, 12 %) was related to a lesser level of hypotension compared with other NOS2A haplotype pairs (ANCOVA, P = 0,044). The difference, however, was only three mmHg in average. The main factor determining the level of hypotension was MAP before anesthetic induction: The higher a MAP before induction, the higher the nadir value following induction (P < 10⁻⁵). Age, alcohol abuse and body mass index were associated with the percentage decrease in MAP following induction, but only because of their relation to a high MAP before induction. **Conclusion:** Patients with the ACGT/ACGT haplotype pair in the NOS2A gene had slightly higher nadir values of MAP following anesthesia than others. Present data indicate that perioperative level of MAP is primarily associated with MAP before anesthesia.

Age and gender distribution of capillary and venous serum S100B among healthy children

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Brain biomarker serum S100B is presently used for selection of computed tomography after minor head in adults. The majority of traumatically head injured patients arriving to the emergency room are children. Our primary aim was to investigate the age and gender distribution of capillary and venous serum S100B concentrations in healthy children.

Children (1-15 years of age) who underwent elective ear or throat surgery or otoscopic investigation during general anaesthesia were included. All children were neurologically healthy, did not have a known kidney disease, recent bone fracture or recent head injury requiring hospitalisation. Parental consent was obtained. Two venous S100B samples, one before and the other one during anaesthesia were collected from a peripheral vein. Two capillary S100B samples were collected during anaesthesia, the second saved as back-up.

198 children (114 males, 84 females) have been included so far. 158 venous samples before anaesthesia, 186 venous samples after anaesthesia and 154 capillary samples after anaesthesia have successfully been analysed. We present a preliminary reference curve for both capillary and venous S100B from cerebrally healthy children, divided into 7 age groups (age 1-14). Mean capillary S100B was significantly higher for all age groups than mean venous S100B. The mean difference was greatest at age group 1-2 years. We found no significant gender differences in either venous or capillary blood.

Our preliminary data present reference levels for both capillary and venous serum S100B related to age. These data are crucial before further clinical S100B studies can be performed in paediatric traumatic head injury.

Risk of aneurysmal subarachnoid hemorrhage and genetic polymorphism in the nitric oxide synthase system

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Background: Polymorphisms in the endothelial nitric oxide synthase genes have been associated with aneurysmal subarachnoid hemorrhage (SAH). Moreover, several lines of evidence implicate nitric oxide and its modulating enzymes as important pathogenetic factors in this disease. We conducted a preliminary case-control study to answer the question: Does genetic polymorphisms in the nitric oxide system increase risk of aneurysmal SAH?

Method: The genes encoding the endothelial and inducible nitric oxide synthases (NOS3 and NOS2A) and dimethylarginine dimethylaminohydrolase 2 (DDAH2) were analysed. The latter functions as a promotor of nitric oxide synthase activity through cleavage of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase. We then selected 15 polymorphisms through review of the literature and with the haploview software. 145 SAH patients from the neuro-intensive care unit were included and 444 elective neurosurgical patients without SAH served as controls. After genotyping, haplotypes were estimated using the PHASE software.

Results: No significant associations between SAH and the endothelial and inducible nitric oxide synthases were found. The -1020CC genotype of the DDAH2 gene showed significant association with SAH ($p < 0.05$; odds-ratio [95 % confidence interval]: 3.3 [1.21 - 9.07] versus controls).

Conclusion: The DDAH2 gene may contribute to the pathogenesis of aneurysmal SAH.

Minor traumatic brain injury in sports: a review in order to prevent neurological sequelae.

N Biasca, WL Maxwell

Minor traumatic brain injury (mTBI) is caused by inertial effects, which induce sudden rotation and acceleration forces to and within the brain. At less severe levels of injury, for example in mTBI, there is probably only transient disturbance of ionic homeostasis with short-term, temporary disturbance of brain function. Recent evidence has suggested that there may be two discrete pathologies that may develop in injured nerve fibers. In the TBI scenario, disturbances of ionic homeostasis, acute metabolic changes and alterations in cerebral blood flow compromise the ability of neurons to function and render cells of the brain increasingly vulnerable to the development of pathology. In ice hockey, current return-to-play guidelines do not take into account these new findings appropriately, for example allow returning to play in the same game. It has recently been hypothesized that the processes summarized above may predispose brain cells to assume a vulnerable state for an unknown period after mild injury (mTBI). Therefore, we recommend that any confused player with or without amnesia should be taken off the ice and not be permitted to play again for at least 72h.

Temporal profiles in serum concentrations of S100B after traumatic brain injury inflict on outcome.

BM Bellander, E Thelin

Retrospective study of patients suffering from TBI admitted to the neuro-intensive care unit at Karolinska University Hospital between 2001 and 2008 where the first serum sample of S100B was obtained within the first 48 hours after trauma and followed by at least three samples obtained within the first 72 hours. Out of 320 patients, 192 matched the inclusion criteria. Two different patterns of S100B levels were observed. Patients with increasing values ("Climbers") and patients with only decreasing values after the initial values ("Decliners") were recognized. There was no difference in time between trauma and first obtained serum sample between the groups ("Climbers": 12,1 vs "Decliners": 12,6 hours; Wilcoxon test: $p=0.41$). The final mortality was significantly higher among the Climbers compared to the Decliners (29% vs 8%; Wilcoxon test: $p=0.0004$). Climbers were less successful in reaching an independent life (GOS 4 or 5) compared to Decliners (37% vs 55%; Wilcoxon test: $p=0.019$). Conclusions: The temporal profile of s-S100B could be used for prognostic purposes. The biochemical rationale for these two different patterns should be further explored. Differences between "Climbers" and "Decliners" will be discussed.

Comparison between capillary and venous serum S100B in traumatically head injured patients

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Serum S100B has recently been introduced in clinical use as a negative predictive brain marker after traumatic head injury among adults. Presently, there is no such serum marker in use for paediatric head injuries. The aim of our study was to compare the relation between venous and capillary samples of serum S100B.

49 pairs of simultaneously drawn capillary and venous samples were obtained from 31 severely head injured patients admitted to the Neurosurgical unit at Lund University Hospital. Seven patients had more than one pairs of samples drawn.

We found a significant difference between capillary and venous samples of S100B, and with slightly increasing difference with increasing serum level. These results will be further presented and discussed.

Although it is encouraging to have found that serum S100B can be analysed from finger-stick samples, our results show that capillary serum S100B cannot be used as a direct replacement for venous serum S100B. Further studies will focus on investigating the possibility to use capillary S100B in the management of paediatric head injury.

Analysis of protein S-100B in serum: a methodological study

K Muller, A Elverland, B Romner, K Waterloo, B Langbakk, J Unden, T Ingebrigtsen

Dysfunction and damage of the human central nervous system can be detected with biochemical markers, and protein S-100B is the best-established such marker. The aim of this study was to evaluate whether the protein is stable during long-term storage, to establish reference values for the new Elecsys S100 test and to compare this new method with the Liaison Sangtec 100 test. **METHODS:** We analysed blood samples from 118 blood donors and 196 patients with subarachnoid haemorrhage or head injury. The long-term stability of S-100B in frozen serum samples was evaluated with repeated analysis in 1997 and 2003 using an immunoradiometric assay. Method comparison between the Liaison Sangtec 100 and Elecsys S100 tests was performed using Bland-Altman difference plots. **RESULTS:** Serum concentrations increased significantly during long-term storage (mean difference 0.15 microg/L; +/-2 SD, 0.55 microg/L). Serum measurements using the Elecsys S100 method in 118 healthy blood donors showed S-100B levels between 0.02 and 0.08 microg/L (mean 0.05). The 95th percentile was 0.07 microg/L. The Liaison Sangtec 100 test usually measured higher concentrations than the Elecsys S100 method, and the difference between the two methods increased with increasing concentrations. The mean difference between the methods was 0.14 microg/L (+/-2 SD, 0.39 microg/L). **CONCLUSIONS:** Protein S-100B is not stable during long-term storage and the two analytical methods are not interchangeable.