Microdialysis monitoring of Amyloid β isoforms - potential biomarkers for diffuse axonal injury after severe traumatic brain injury

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Abstract

Severe traumatic brain injury (TBI), defined as patients with a Glasgow Coma Scale (GCS) score \( \leq 8 \), is a well-known cause of life-long disability and death. TBI is commonly observed in young adults although the incidence of elderly people is increasing. TBI has also been identified as an important environmental risk factor for the development of Alzheimer’s disease (AD) later in life. Amyloid-\( \beta \) (A\( \beta \)) peptide, believed to play a central role in the development of both familial and late-onset AD, can be detected in the human brain using intracerebral microdialysis (MD) in the early post-injury after TBI. The aim of this study was to further evaluate whether A\( \beta \)-40 and A\( \beta \)-42 could be detected after severe TBI by intracerebral microdialysis, since these peptides are of particular interest as potential biomarkers for diffuse axonal injury (DAI).

We evaluated 10 patients with severe TBI up to 4 days post-injury using MD to sample interstitial A\( \beta \) peptides later analyzed using the Luminex xMAP technique allowing analysis of two-hour fractions. The A\( \beta \) results were compared to markers for cerebral energy metabolism (glucose, lactate, pyruvate, glycerol, glutamate), CT-scans and clinical factors.

We found that interstitial A\( \beta \)-40 and A\( \beta \)-42 levels were consistently higher in the DAI group compared to the focal injury group day 1-6 post-injury, being significantly increased at 97-98 and 113-116 hours post-injury (\( p < 0.05 \)).

These results indicate that A\( \beta \)-40 and A\( \beta \)-42 might be important biomarkers for axonal injury and their detection using microdialysis can help us better understand the dynamics of axonal injury mechanisms in the injured human brain.
Introduction

Traumatic brain injury (TBI), most commonly caused by traffic accidents, falls, assault and contact sports is one of the main causes of motor dysfunction, cognitive impairment and mortality in the population under 50 years of age. Motor vehicle accidents are the leading cause amongst young adults while fall accidents top the list amongst the elderly. The incidence of TBI among men is close to twice that of women, likely caused by the risk-taking behavior and high-risk activities frequently engaged in by men [1, 2]. In general, TBI is a devastating disease which leads to a life-long disability that negatively affects the lives of survivors, their relatives and society [3]. It is also important to point out that neurointensive care has reduced the mortality and improved the outcome after severe TBI in the recent decades [4].

TBI is divided into primary and secondary brain injury. The primary injury occurs at the time of accident and cannot be treated, only prevented. The secondary injury consists of a complex cascade of events that goes on for hours, days or even months post-injury [5]. This secondary injury cascade can at least partially be treated and prevented at the Neurointensive care unit (NICU) where measures are taken to protect the patient from secondary insults such as hypoxia, hypotension, anemia, seizures and increased intracranial pressure (ICP) all known to exacerbate the primary injury. The only drugs currently available at the NICU are mannitol used to decrease emergent intracranial hypertension, pentothal or other sedative drugs for reduction of brain metabolism and lowering of ICP, and antiepileptic drugs [6]. Furthermore, surgical removal of life-threatening focal mass lesions may in some cases be required.

TBI can be categorized into focal and diffuse brain injury. Focal injuries are typically caused by direct impact to the head, that can result in extracranial hematomas (sub- and epidural hematomas) and cerebral contusions, whereas diffuse brain injuries are caused by rotational acceleration of the brain deforming the white matter leading to diffuse axonal injury (DAI) [7]. DAI appears throughout the deep and subcortical white matter especially located at the gray/white matter junction, corpus callosum, brainstem, and/or adjacent to the third ventricle [7, 8]. The most common and instant impairment associated with DAI is – despite the absence of mass lesions – coma, above all when axonal injury involves the brainstem and/or the thalamus. Thus the depth and duration of coma do not reflect the extent of axonal pathology in the cerebral hemispheres and predicting the post-injury outcome of the patient is difficult [7, 9].

In early literature, axons were described to be mechanically torn and disconnected at the moment of injury. However, contemporary studies consider DAI as a progressive process developing from focal axonal alteration to delayed axonal disconnection [10]. This process can be divided into primary and secondary axotomy. Primary axotomy is caused immediately by a severe mechanical force to the brain and disconnection of axons is relatively infrequent and observed mainly in patients dead from the initial impact. Secondary axotomy represents the delayed phase of axonal injury, characteristic of increased intraaxonal mechanisms, which lead to influx of Ca$^{2+}$ with the activation of proteolytic activity resulting in altered transport
and accumulation of axonal transport proteins within axonal swellings, finally leading to axon disconnection and bulb formation [7].

Amyloid precursor protein (APP), one of the accumulated proteins in the swollen axons [9, 11-15], is a transmembrane glycoprotein synthesized in the neuronal cytoplasm proteolytically cleaved to form Aβ [13, 16]. The cleavage of APP is made by different enzymes. The proteolytic cleavage of α-secretase generates a large soluble ectodomain fragment (α-APPs), released into the extracellular space, and an 83-residue COOH-terminal fragment (CTF), retained in the membrane. When not subjected to the α-secretase, APP-molecules can be alternatively cleaved by β-site APP cleaving enzyme (BACE), generating a smaller ectodomain derivate (β-APPs) and retaining a 99-residue CTF (C99) in the membrane. Both the C83 fragment and the C99 fragment can then undergo cleavage by γ-secretase to release a short peptide named p3 and Aβ peptides, respectively. Initial cleavage of APP by α-secretase rather than BACE prevents the generation of Aβ [17, 18]. According to an earlier study by Cirrito et al [19] there is a direct relationship between increased neuronal activity and increased interstitial fluid (ISF) Aβ, and on the contrary, a reduced neuronal activity decreases ISF Aβ levels. Furthermore their experiments suggested that synaptic vesicle exocytosis alone can drive changes in Aβ levels, independent of neuronal activity. It appears as if the APP processing is secondary and distinct from the rapid changes in extracellular Aβ levels that are due to synaptic vesicle exocytosis [19]. Recent studies have demonstrated soluble, intermediate Aβ assemblies such as Aβ-derived diffusible ligands (ADDLs) [20] and protofibrils (PF) [21] that can alter electrical activity of neurons albeit with different neurotoxic effects. PFs may also mimic the ability of mature amyloid fibrils to destabilize calcium homeostasis with increased influx of calcium across the plasma membrane leading to neurotoxic effects [22-24]. ADDLs and PFs have been suggested to cause neuronal death [20, 21]

The most clearly identified causes of Alzheimer’s disease (AD) are genetic, involving the overexpression or altered metabolism of APP [25]. Patients with trisomy 21 (Down’s syndrome) can develop classical AD neuropathology (neuritic plaques and neurofibrillary tangles) early in life because of an overexpression of structurally normal APP due to elevated gene dosage. Therefore it seems likely that an extended period of time with accumulation of APP in swollen axons, which is seen after severe TBI, increases the likelihood of generating Aβ peptides, triggering the cascade of Alzheimer’s pathology [15, 25]. In fact, TBI is considered the most important environmental risk factor for the development of AD later in life (see below).

In addition to APP, BACE has been found in injured and swollen axons [5, 26]. Increased APP production following TBI may saturate the normal α-secretase processing pathway, resulting in increased BACE processing and Aβ formation [13]. Moreover, it has been suggested that oxidative stress can upregulate BACE mediated by γ-secretase activity (also enhanced because of oxidative stress) [13].

Neurons are one of the cell types expressing the highest levels of APP in the body but other brain cells also express APP and release variable amounts of Aβ, including astrocytes,
microglia, and endothelial and smooth muscle cells. All of these different cell types contribute to the secreted pool of Aβ that eventually leads to extracellular deposition [16]. The cleavage of APP to Aβ peptides and their subsequent release in their normal, soluble form within the ISF, is in itself not a pathological process, occurring under normal cellular conditions throughout life [27]. Conversion of soluble Aβ into plaques or oligomers (see below) is concentration-dependant, meaning that elevated levels of ISF Aβ are likely to accelerate disease pathology [27].

Chronic traumatic encephalopathy (CTE) or dementia pugilistica (“punch-drunk syndrome”) is caused by repeated blows to the head. Postmortem histopathologic analysis of brains from boxers with CTE has revealed neurofibrillary tangles (particularly in the temporal lobe) and diffuse plaques containing Aβ [7, 25]. Initial symptoms are deterioration in concentration, attention, and memory, gradually affecting the pyramidal tract resulting in disturbed coordination and gait, slurred speech and tremors [28, 29]. Symptoms may develop late in a boxer’s career, and even years after retirement from the ring [30]. Subsequently, a single incident of severe head injury was shown to induce the formation of Aβ plaques in the cortex of 30% of patients, within days after injury [7, 25, 31]. In another study by Robert and colleagues [25] it was also demonstrated that one third of cases dying as a result of severe head injury had Aβ deposits in one or more cortical areas. This has, years later, been corroborated by Ikonomovic et al in their analysis of surgically resected temporal cortex from survivors of severe TBI, revealing that the formation of extracellular Aβ deposits can occur within hours after injury. Extracellular deposits of Aβ, APP, and apoE were documented in 30% of subjects examined [32]. In a study by Plassman and colleagues it was found that moderate or severe head injury in early adult life significantly increased the risk of AD or dementia. Also, interestingly, when those who reported to have had multiple head injuries were excluded, the risk of AD and dementia did not diminish [33].

Thus, TBI is an important risk factor for AD and accumulation of APP and Abeta may be an important contributor to both early and late post-traumatic exacerbation of the primary injury. We are interesting in evaluating AD-related pathology following severe TBI in vivo. Microdialysis (MD) is a unique bedside technique whereby the focal interstitial, extracellular neurochemistry (e.g. glucose, lactate, pyruvate, glutamate, glycerol etc) can be monitored within the injured human brain [8, 34]. To date, MD has contributed to a growing knowledge of secondary injury mechanisms following TBI [35]. The rationale for using microdialysis after TBI is to make possible an early detection of biochemical changes - low glucose and high lactate/pyruvate ratio (LPR) indicative of abnormal brain metabolism, correlating well with tissue ischemia [34, 36]. Initially, only low-molecular-weight compounds < 20 kDa could be sampled although the recent development of clinically approved and commercially available microdialysis catheters with a membrane pore size of 100 kDa has expanded the sampling to small proteins, such as cytokines, providing a tool for the evaluation of novel biomarkers in the injured brain [8, 37-39]. In the present study the sampling of Aβ peptides, Aβ40 and Aβ42, was our primary interest, since Aβ peptides, and especially Aβ42 (being more prone to aggregate), constitute the major components of amyloid plaques in AD. Since TBI has been shown to be an important risk factor for the development of AD [1, 7, 13, 25,
28, 33], microdialysis might help investigating how these peptides are involved in the pathogenesis of the disease.
**Methods**

**Patient population and neurointensive care treatment**

10 patients with severe TBI, GSC<8, were included in the study. All patients were mechanically ventilated and ICP was monitored in all patients, either with an intraparenchymatous ICP monitor (Codman, Johnson & Johnson, six patients), an external ventricular drain (three patients) or both an intraparenchymatous ICP-monitor and external ventricular drain (one patient). All patients were initially sedated with propofol. One patient (case 6) received several bolus doses of pentothal due to intermittent elevations of ICP. The neurologic state was assessed at a regularly basis, registering Glasgow Coma Scale (GCS) and Reaction Level Scale (RLS) scores and focal neurologic signs on computerized observation charts. Treatment was given at the NICU of Uppsala University Hospital according to an ICP-guided protocol with mild hyperventilation (PaCO$_2$ 30-35 mmHg; 4.0-4.5 kPa), head elevation 30°, and cautious volume expansion to obtain normovolemia [8, 40]. ICP, cerebral perfusion pressure (CPP), intraarterial blood pressure, central venous pressure, oxygen saturation, and temperature were continuously monitored. Arterial blood gases and blood glucose were checked regularly [4, 40]. Treatment goals for each of the variables are shown in table 1 [40].

**Table 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Goal</th>
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<tbody>
<tr>
<td>ICP</td>
<td>≤ 20 mmHg</td>
</tr>
<tr>
<td>CPP</td>
<td>60 mmHg</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>≥ 100 mmHg</td>
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<tr>
<td>CVP</td>
<td>0-5 mmHg</td>
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<tr>
<td>PCO$_2$</td>
<td>4,0 – 4,5 kPa</td>
</tr>
<tr>
<td>PO$_2$</td>
<td>≥ 12 kPa</td>
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<tr>
<td>SaO$_2$</td>
<td>≥ 96%</td>
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<tr>
<td>B-glucose</td>
<td>5-10 mmol/L</td>
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<tr>
<td>Temperature</td>
<td>≤ 38 °C</td>
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At approximately six months post-injury, patient outcome was assessed using the extended Glasgow Outcome Scale (eGOS; [41]).

**Radiological analysis**

The Marshall classification [42] was used when analyzing patients’ CT-scans, and the “worst” CT-scan - i.e., the CT-scan with the most pathological entities and mass effect during the course of disease - is presented for all 10 patients in table 2. Compression of basal cisterns was determined by the following scoring system: 0 = normal, 1 = compressed yet visible, 2 = compressed. The midline shift was calculated at the level of the thalami. The degree of compression of the basal cisterns combined with the midline shift provided a measure of the radiological mass effect. The volume of each mass lesion was calculated with the formula...
length x width x height / 2. The location of the microdialysis probe was evaluated in all patients and also checked for hemorrhages.

Associated injuries
When suffering from severe TBI other injuries are often present, scored according to the New Injury Severity Score (NISS) [43]. To calculate the NISS the body is divided into six body regions: head/neck (including cervical spine), face, chest (including thoracic spine), abdomen (including lumbar spine), extremity (including pelvis) and external. Each injury in the different body regions is then given an abbreviated injury scale (AIS) 1-6: 1 = minor, 2 = moderate, 3 = serious, 4 = severe, 5 = critical and 6 = unsurvivable. The three highest AIS scores are each squared and then added together to get the NISS, ranging from 1 to 75. If an injury is given an AIS of 6, unsurvivable, the NISS is automatically set to 75.

Microdialysis
In all cases the CMA 71 intracerebral microdialysis catheter with a membrane length of 10 mm and a membrane cut off of 100 kDa was used for the sampling of low-molecular-weight (LMW) substances. The catheter was inserted via a burr hole in connection with the insertion of an ICP monitoring device, 1 to 2 cm anterior to the coronal suture in seven cases. In the remaining three cases the microdialysis catheter was inserted in the pericontusional tissue following craniectomy for the removal of a mass lesion. Care was taken to insert the microdialysis probe obliquely in macroscopically non-lesioned cortex by using atraumatic techniques aiming at an intracortical positioning of the microdialysis membrane. The outflow hydrostatic pressure of the perfusion system was set at the zero midcranial reference level by taping the collecting vials next to the bandage on the patient’s head [8, 37]. Perfusion of the catheters was done by using 4% albumin in artificial CSF (Na\(^+\) 148 mM, Ca\(^{2+}\) 1,2 mM, Mg\(^{2+}\) 0,9 mM, K\(^+\) 2,7 mM, Cl\(^-\) 155mM) at a rate of 0,3 µl/minute by using a microdialysis pump (model 106, CMA Microdialysis). Sampling was delayed at least two hours after the insertion of the MD probe to allow for normalisation of changes caused by probe insertion. Samples were collected every 60 minutes and analyzed at bedside for glucose, lactate, pyruvate, and urea using a CMA model 600 Microdialysis Analyzer (CMA Microdialysis). After each bedside analysis, the samples were frozen on dry ice and stored at -70°C until further analyzed. Urea was monitored to ensure stable probe function. Glutamate and glycerol were analyzed retrospectively (CMA model 600). The analyzer was automatically calibrated when started, and every sixth hour thereafter by using standard calibration solutions from the manufacturer. Also, every weekday, quality controls at two different concentrations for each substance were performed. The imprecision value for between-assay coefficient of variation was <10% for all analytes.

Based on previous data [8, 44, 45] and personal experience, the following values for the LMW substances were considered critical, with potential pathological implications: glucose < 1 mmol/L; LPR > 30; pyruvate < 120 µmol/L; glutamate > 15 µmol/L; glycerol > 100 µmol/L.
Analysis of Abeta peptides
Aβ1-42 and Aβ1-40 levels in interstitial fluid were measured using the INNO-BIA plasma Aβ forms assay (Innogenetics, Ghent, Belgium), which is a multiplex microsphere-based Luminex xMAP technique. In this assay, the monoclonal antibodies 21F12 and 2G3, which specifically bind Aβ peptides ending at Ala42 and Val40, respectively, are used as capture antibodies, and the monoclonal antibody 3D6, which specifically binds Aβ peptides starting at Asp1, is used as detector antibody (Hansson, 2010).

Statistical Methods
The microdialysis data did not meet the assumption of normal distribution and pairwise comparisons between groups were analyzed using the Mann Whitney U-test using the Statistica software (StatSoft, Inc). Correlation analysis was performed using Microsoft Excel. Graphs and statistical analysis were performed using Microsoft Excel. The area under the curve (AUC) values were calculated from day 1-6 days post-injury. A p-value <0.05 was considered statistically significant.
Results
Patient characteristics and radiology
In the patient population of 10 patients, there were seven male and three female patients with a mean age of 38.5 ± 18 years [range 18-76 years]) When presented in the NICU, the patients had a post-resuscitation Glasgow Coma Scale (GCS) of 4-8 (RLS score of 3-8). Their median motor component of the GCS (GMS) score was 4, which corresponds to a median RLS of 4-5 (Table 2). Life-threatening focal mass lesions were surgically evacuated in three patients (case 1, 4 and 7). Based on the Marshall classification, the patients were divided into the two groups: focal/mixed and DAI. Radiological characteristics of DAI are diffuse brain swelling and/or small petechial hemorrhages located at the gray/white matter junction, corpus callosum, brainstem, and/or adjacent to the third ventricle. Five patients were categorized as having a DAI and five had a focal/mixed injury (includes focal lesions such as epidural hemorrhages (EDHs), subdural hemorrhages (SDHs) and lobar contusions). The degree of severity of the associated injuries using the NISS score is shown in Table 2.

Microdialysis protocol
The mean duration from time of accident to start of microdialysis sampling was 19.7 hours (range 7-75 hours), and the mean duration of microdialysis sampling was 179.9 ± 8 hours (range 45-329 hours) (Table 2). No signs of bleeding around the microdialysis probe were seen on any control CT scans.

Analyses of LMW substances using microdialysis
Urea levels in microdialysis samples were stable, thus indicating adequate probe function, with a gradually increasing trend during the course of the monitoring period. Only approximately one percent of the urea samples showed deviating levels from the expected trend leading to exclusion of all samples during that hour.

Glucose
In the DAI group, 42 (4.7%) out of 897 microdialysis samples showed a glucose value of < 1 mmol/L whereas in the focal injury group, 137 (20.0%) of the 685 microdialysis samples had a glucose value of < 1 mmol/L. The DAI group had consistently higher glucose values (mean 2.5 mmol/L) compared to the focal injury group (mean 1.6 mmol) between 30 and 185 hours post-injury of microdialysis sampling (data not shown).

The LPR
In the DAI group, 143 (16.1%) of the 886 samples had an LPR >30. In the focal injury group, 147 (21.6%) of all 681 microdialysis samples showed an LPR >30. The focal injury group had higher initial LPR values compared to the DAI group, although at later time points (> 95 hours post injury), there were no differences between the groups (Fig. 1).

Glutamate
In the DAI group, 285 (39.4%) out of 723 microdialysis samples had glutamate levels > 15 µmol/L compared to 85 (14.7 %) of 579 samples in the focal injury group. Throughout the
microdialysis monitoring the DAI group had somewhat higher (although non-significant) values than the focal injury group, particularly after 100 hours post injury.

**Glycerol**

In the DAI group, 95 (10.3%) of all 924 samples had glycerol values > 100 µmol/L compared to 223 (30.4%) of 733 microdialysis samples in the focal injury group.

From the start of microdialysis sampling, from 15 hours to 93 hours post-injury the focal injury group showed higher levels of glycerol compared to the DAI group. The DAI group only showed minor fluctuations and with occasional pathological values.

In conclusion, these results suggest that there was no significant difference between the DAI and focal injury group in energy metabolic measured by microdialysis.

**Aβ40 and Aβ42**

Aβ40 and Aβ42 were detected in all 10 patients. Sixty-four samples from 19-146 hours post-injury were analyzed in both the DAI and focal TBI groups. The mean interstitial Aβ-40 levels were 774.9 (range 457.0-971.3) pg/ml and 479.9 pg/ml (range 281.3-766.5) in the DAI and focal TBI groups, respectively (Fig. 2).

One patient in the DAI group, case no 8 (see below), had very high levels of Aβ40, ranging from 825-1995 pg/ml where 77 (92.8%) out of its 83 MD samples had values > 1000 pg/ml. Mean interstitial Aβ42 levels were 105.3 (range 62-127.5) pg/ml and 61.1 (range 46-90) pg/mL in the DAI and focal TBI group, respectively. Case no 8 in the DAI group (see below) had consistently high levels of Aβ42 ranging from 120-285 pg/ml.

Area under the curve (AUC) Aβ-40 and Aβ-42 values for each patient in the DAI (5 patients) and focal (5 patients) injury groups were calculated and compared between the hours 19-146 post injury. The total AUC in the DAI group was higher compared with the total AUC in the focal injury group (p=0.14, Fig. 3).

Statistical analysis was performed to examine if there was a correlation between Aβ-peptide levels (Aβ-40 and Aβ-42) and MD-pyruvate, MD-LPR, and patient age (Fig. 4-6 ). No correlations were found for any of these factors. The same analysis was also done for Aβ-peptides (Aβ-40 and Aβ-42) and MD-glutamate, MD-glycerol, MD-glucose, and the outcome using the extended Glasgow coma scale (eGOS). No correlations were found (data not shown).

Aβ40 and Aβ42 levels were also separately evaluated with regard to age (Fig. 7). The ten patients included in the study were divided into two groups, one containing five patients (case 4, 5, 6, 8, 10) under 40 years of age and the other containing five patients (case 1, 2, 3, 7, 9 ) over 40 years of age. Samples harvested between 30 and 46 hours post injury were twice as high in the younger patients, however not significant. Towards the end of microdialysis monitoring these differences between the groups became increasingly small. AUC Aβ-40 and
Aβ-42 values were also calculated and no significant difference was seen between the two age groups.

Illustrative cases

Case 8 (DAI group)
This patient, age 35, was thrown off during horseback riding and the horse fell over her. At the scene of the accident she had a GCS of 4 (RLS 5) with a dilated non-responsive right pupil. An emergency whole-body CT scan showed a few minor contusions, smaller quantities of traumatic subarachnoid hemorrhage with intraventricular extension and slightly compressed basal cisterns. No other injuries were detected. Of all 10 patients this patient had the highest Aβ40 (range 825-1995 pg/mL) and Aβ42 (range 120-285 pg/mL) levels (Fig 8 A and B). None of the MD-glucose values where pathological and only one LPR ratio was > 30 (Fig 8 B and C). There was a decline in MD-glucose and an increase in LPR at 83 hours post-injury and onwards. A modest decrease was observed at the same time period for the Aβ values. However this decrease was only temporary since Aβ levels increased at the end of monitoring. The worsening of energy metabolic status may be explained by infection (unknown focus), for which the patient was successfully treated. At the NICU ICP levels remained low and stable (< 20 mmHg), neurological status improved and when extubated on the day 10 post injury she had a GCS of 7 (RLS 3). The eGOS at three months follow-up was seven.

Case 1 (Focal/mixed group)
This patient, age 76, had a fall accident and at the site of injury he had a GCS of 14-15 (RLS 2) and normally reacting pupils. The emergency CT scan showed a midline shift of 7.5 millimeters, a large hematoma in the right frontal lobe and a right subdural hematoma. Shortly after the CT scan the patient deteriorated to a GCS of 7 (RLS 3b) and was immediately intubated and transported to surgery for evacuation of the mass lesion. An intraparenchymatous ICP monitor and microdialysis catheter was inserted via the craniectomy. Postoperatively, the patient had a GMS of 4 (RLS 5). At two days post-injury, the patient developed post-traumatic seizures successfully treated with fosphenytoin. At this time, increased brain swelling was evident on the CT scan. The temperature rose above 38°C with laboratory signs of infection and anemia, and there were difficulties controlling CPP and ICP. Neurological status was unchanged at GMS 4 (RLS 5). After nine days of monitoring and treatment at the NICU clinical and neurological status had improved and the patient was transferred from the neurosurgical department.

This patient had the highest Aβ peptide levels in the focal injury group (Fig. 9 A and B). The mean Aβ40 level was 854.3 pg/mL and Aβ42 level was 85.4 pg/mL. During the first two days of microdialysis monitoring energy metabolic status were critical with pathological glucose (Fig. 9 C), glycerol and LPR (Fig. 9 D) values. These values improved during the next few days but subsequently worsened at approximately seven days post injury. Aβ40 and Aβ42 values gradually increased between 32 and 138 hours post injury, then decreased to levels seen at the beginning of monitoring. At follow-up, this patient had an eGOS of 4 (moderate disability, capable of independent living).
Discussion

The aim of this study was to evaluate the significance of Abeta peptides, especially Aβ42, for axonal injury after TBI using intracerebral MD. The Aβ results were also compared to markers for cerebral energy metabolism (glucose, lactate, pyruvate, glycerol, and glutamate), CT-scans and clinical factors. Our results demonstrated a significant difference in Aβ levels in patients with DAI compared with focal injury early after TBI.

Aβ40 and Aβ42 levels were consistently higher in the DAI group compared with the focal injury group. Furthermore the focal injury group had four times more pathological glucose samples than the DAI group. The differences in LPR and glycerol values were not as remarkable as the glucose values, and glutamate levels were higher in the DAI group which to some part can be explained by one patient (case 10; data not shown) who had quite high glutamate levels throughout microdialysis monitoring. Over all, the DAI group had better brain energy metabolic status than the focal injury group. Aβ levels - both Aβ40 and Aβ42 - were rather stable with small alterations with changes in brain energy metabolic status and clinical status. For some of the patients (case 1, 2, 6, 8, 9, 10) decreasing glucose and increasing LPR values led to a decrease in Aβ levels. These results contradict previous suggestions that Abeta concentrations increase as neurological status improves and vice versa [36]. In addition, in that study, a positive correlation was seen with interstitial brain glucose and a negative correlation with LPR (which was not the case in our study). Since microdialysis is not inserted in connection with the accident it is impossible to know the Abeta concentrations immediately after the impact. What would help us even more in our understanding of the dynamics of Abeta is to know the concentration under the normal, uninjured condition and this is of course not feasible in humans. Based on our study we cannot make any conclusions of an eventual decline in Abeta directly after injury, which was done by Schwetye et al [46]. Using a mouse model for this purpose the authors showed that Aβ levels decreased by 25-50% following TBI compared with baseline values sampled 12 hours prior to the injury in PDAPP, Tg2576 and Tg2576-ApoE2 transgenic mice producing human Aβ plus wild-type animals.

CSF-tau has been examined in 26 patients up to 5 months after acute ischemic stroke demonstrating an increase in CSF-tau at days 2-3, with a peak at one week post-stroke but with no significant change detected for Aβ42 [47]. In yet another study ventricular CSF-tau and Aβ40 and -42 was analyzed in 32 patients (19 SAH patients and 13 controls), results showing a decrease in Aβ peptides at days 3 and 4 post-SAH compared to the control group and lower CSF Aβ42 correlated with an unfavourable outcome. In contrary to the Aβ peptide levels CSF-tau levels increased post SAH [48]. These studies support a previous microdialysis study where patients with a predominantly focal lesion had significantly higher tau levels than Aβ42 and patients with DAI had higher Aβ42 levels[8]. In a study by Raby CA [49] increased Aβ42 in the CSF of six patients with DAI was observed, similar to the results obtained by others.
Aβ42 is not only a desirable candidate biomarker for axonal injury but also as a diagnostic marker for AD which brought Sjogren and colleagues [51] to examine CSF with regard to Aβ42 and tau to obtain adequate reference values that can be used in clinical practice. 231 neurologically and psychiatrically healthy subjects 21-93 years old were included in the study and a reference value for Aβ42 was determined to be > 500 ng/L (mean 1040 ± 213 ng/L). No significant correlation was found between CSF-Aβ42 and age. Similar results were demonstrated by Hulstaert F et al [52] in a multicenter cooperation study with samples gathered and analyzed from patients with AD compared with samples from individuals without an AD diagnosis. Their results showed that CSF-Aβ42 levels in healthy adults were about twice as high as the values in AD patients (median 487 pg/mL). Additional studies have confirmed decreased Aβ42 levels in patients with AD [53-56]. In our study the mean value of Aβ42 in the DAI group was 103.3 pg/mL (range 37-293 pg/mL) and 73 pg/mL (range 37-408 pg/mL) in the focal injury group. None of our patients had an AD diagnosis but note that all of their values are under 500 pg/mL, which is a threshold suggested by the studies above when diagnosing patients with AD. However our Aβ values are due to a unique environment in the brain caused by TBI and it would be most interesting to know how these values develop years from now.

In a pig model of diffuse brain injury Aβ and tau were detected in axonal bulbs 3-10 days post injury and Aβ-containing plaques was found in approximately one third of the animals (representative of the group of animals with the highest amount of axonal injury) [57]. In humans axonal pathology post-TBI with the accumulation of Aβ, APP and BACE have been shown to persist for up to at least three years post injury. However, almost no Aβ plaques were found. Neprilysin, a member of zinc-dependent metalloproteases and a potent Aβ degrading enzyme, has been suggested to play a major role in degrading Aβ in vivo [58, 59]. In a study Iwata N et al Aβ40 and Aβ42 levels were elevated in a gene-dose dependent manner [60], that is, neprilysin-deficient mice (-/-) did not degrade the majority of Aβ injected and in the heterozygously deficient (+/-) mice the same process was decelerated. This also suggests that a partial downregulation of neprilysin activity, which may be the case with aging in humans, could act in favour of plaque formation. In one study patients with sporadic AD were shown to have up to a 50% reduction in neprilysin in the hippocampus and temporal gyri, which are areas in the brain vulnerable to plaque formation [61]. Neprilysin polymorphism has also been indicated to play a role in plaque formation in patients post TBI [62]. Except for neprilysin activity microglia has been shown to have the ability to clear Aβ both by phagocytosis and via secretion of neprilysin, insulin-degrading enzyme, matrix metalloproteinase 9 and plasminogen. Additionally, the anti Aβ properties of microglia were shown to change with reduced expression of their Aβ-binding receptors and Aβ-degrading enzymes in the aging mice [63].

At the present, although microdialysis is a bedside technique, if bedside analyses cannot be performed, its use as an acute diagnostic tool is limited. Despite these limitations, microdialysis is a unique and important tool that confers better understanding about ongoing processes in the injured human brain and may help us find novel biomarkers to evaluate and
monitor patients affected by axonal injury after TBI. It is clear that more research needs to be done to gain a deeper knowledge in this subject matter. Additional proteins, such as T-tau and neurofilament (NF) need to be sampled and evaluated.


