Prediction value of genetic and neuromarkers in blood and liquor in patients with severe traumatic brain injury

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Till min älskade hustru
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Prediction value of genetic and neuromarkers in blood and liquor in patients with severe traumatic brain injury

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Background: Severe traumatic brain injury (sTBI) is the most common cause of mortality in young adults. sTBI induces variable brain damage, invisible in Computer Tomographic scans early post-trauma. Further, neurology is difficult to evaluate in sedated patients. Therefore, biochemical neuromarkers (BNMs) in blood or cerebrospinal fluid (CSF) may be valuable tools to both evaluate trauma and to prognosticate patient outcome.

Aims: The aim of the thesis was to evaluate if concentrations of the BNMs; Glial Fibrillary Acid Protein (GFAP, CSF, study IV), Neurofilament light (NFL, CSF, study IV), Tau (CSF, study II), β-amyloid (1-42) and amyloid precursor-proteins (CSF & plasma, study I) were enhanced after a sTBI. Further, we investigated if these levels were correlated to outcome, neurology and patient ability of daily living 1-year post-trauma. Finally, we explored if patient-genotype, specifically Apolipoprotein E, (*gene APOE*), influenced 1-year outcome in sTBI-patients, (plasma, study III).

Methods: Patients were consecutively included if; aged ≥7 years, < 9 in Glasgow Coma Scale, receiving an indwelling ventricular catheter allowing CSF sampling), were artificially ventilated and admitted to the Neurointensive care unit (NICU) within 48h post-trauma. NICU-care was performed according to a standardized protocol. CSF samples were collected on days 0-4, 6, 8 and once on days 11-18. Surviving patients were assessed at 1-year evaluating; 1) outcome by Glasgow Outcome Scale (GOS), 2. neurology and 3. activities of daily living. NFL, GFAP, Tau, β-amyloid (1-42) and amyloid precursor-proteins were all analyzed by ELISA-methods. *APOE* genotyping was performed by polymerase chain reaction & solid-phase mini-sequencing.

Results: During the inclusion period, patients (n=28-96) were included into studies I-IV for CSF and /or blood sampling. Study I; β-amyloid (1-42) and amyloid precursor-proteins increased from day 0 until day 11 in the CSF, but not in plasma. In study II we found enhanced levels of CSF-Tau on days 2-3 correlated to mortality (GOS 1) at 1-year. In study III we found that patients with *APOE allele 4* had worse outcome (GOS) at 1-year. Finally, in paper IV we found increased CSF levels of GFAP and NFL both correlating to outcome (GOS) at 1-year.

Conclusions; In this thesis we have found in sTBI-patients that genetic and BNMs in the plasma and/or CSF correlate to outcome at 1 -year post-trauma. The result may be clinically applicable to prognosticate outcome and influence treatment paradigms in these patients.

Keywords; Traumatic brain injury, outcome, NFL, GFAP, tau. β-amyloid, apolipoprotein E, biochemical neuromarkers.

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. A. Olsson, L Csajbok, M. Öst, Höglund, K. Nylén, L. Rosengren, B. Nellgård and K. Blennow.
Marked increase of β-amyloid $_{(1\text{-}42)}$ and amyloid precursor protein in ventricular cerebrospinal fluid after severe traumatic brain injury.
Journal of Neurology (2004) 251:870–876

II. M. Öst, K. Nylén, L. Csajbok, A. Olsson, O. Öhrfelt, M. Tullberg, MD, C. Wikkelson, P. Nellgård, L. Rosengren, K. Blennow and B. Nellgård.
Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury.
Neurology (2006) 67:1600–1604

III. M. Öst, Nylén, L. Csajbok, K. Blennow, L. Rosengren and B. Nellgård.
Apolipoprotein E polymorphism and gender difference in outcome after severe traumatic brain injury.
Acta Anaesthesiologica Scandinavica (2008) 52:1364–1369

IV. M. Öst, L. Csajbok, K. Nylén, K. Blennow and B. Nellgård.
CSF concentrations of glial fibrillary acidic protein and neurofilament light correlate with 1-year outcome after severe traumatic brain injury.
Submitted for publication (2015)

SAMMANFATTNING PÅ SVENSKA

En svår traumatisk skallskada (TBI; djupt medvetslösa med Reaction Level Scale (RLS) >4) är den vanligaste dödsorsaken hos barn och unga vuxna (<40 år) och står för fler dödsfall än alla andra diagnoser gör tillsammans i denna ålderskategori.

I dagsläget är det svårt att kliniskt prognostisera sjukdomsförloppet efter en TBI. Denna ger individ-relaterade kombinationsskador av blödningar, hjärnsvullnad (ödem) och syrgasbrist (hypoxi), där datortomografi (CT-hjärna) och initial neurologi i nuläget bara ger en prognostisk vägledning. Experimentellt finns i dagsläget ett flertal blod- och/eller hjärnvätske- (CSF) prover (neuromarkörer) som kan prognostisera överlevnad och restsymptom vid TBI. Avsaknaden av validerade kliniska neuromarkörer ger oss svårigheter att; 1) följa sjukdomsförlopp, 2) jämföra effekten av nya och gamla behandlingar, 3) prognostisera restsymptom, för att 4) kunna intensifiera vården och att lägga den på rätt vårdnivå. Redan nu finns en validerad ospecifik neuromarkör S-100B, som vid lättare TBI kan ge beslutsstöd för ev. CT-hjärna undersökning och inskrivning på sjukhus eller ej, men vid svår TBI saknas kliniska neuromarkörer.

Denna avhandling undersöker om neuromarkörer kan relateras till överlevnad och restsymptom (patientfunktionsnivå med Glasgow Outcome Scale (GOS)), efter 1 år, just hos patienter med svår TBI. Hos dessa patienter insamlade vi dagligen, under 2 veckor, grunddata som blodtryck, puls, intrakraniellt tryck (ICP), medvetandegrad (RLS), B-glukos, elektrolytstatus samt blodgaser. Vi har fokuserat våra studier på att undersöka CSF-markörer, eftersom koncentrationerna här är oberoende av blodhjärnbarriärs-genomsläppligheten.

I studie I påvisade vi ökade koncentrationer av neuromarkörerna 1) β-amyloid (1-42) och 2) amyloid precursorproteiner i CSF hos patienter med svår TBI. Dessa markörer är kopplade till Alzheimers sjukdom (AD).

I studie II undersökte vi ytterligare en neuronal (nervcells) markör, tau, kopplat till AD. Koncentrationen av CSF-tau ökar initialt under den första veckan efter traumat och denna ökning predikterar signifikant mortalitet och ofördelaktig neurologisk prognos 1 år efter traumat.

Sambandet mellan TBI och AD förstärks i studie III, där vi undersökt om patientgenotypen har betydelse för prognosen efter en svår TBI. I studien visar vi att TBI-patienter med en genetisk proteinvariant (lipoproteinet APO-E, 4) har sämre överlevnad och mer neurologiska restsymptom efter 1 år. Samma genetiska variant är också överrepresenterad hos patienter som insjuknar i AD.

I studie IV, har vi mätt CSF-koncentrationerna av neuromarkörerna glial fibrillary acid protein (GFAP, astrocytskada) och neurofilament light (NFL, neuronskada) under dag 0-14 efter en svår TBI. CSF-koncentrationerna av -GFAP, -NFL ökade kraftigt under första veckan efter TBI och dessa förhöjda värden korrelerar signifikant till överlevnad och neurologiska/funktionella restsymptom, enligt GOS, ett år efter traumat.

Således har vi påvisat att neuro- och genmarkörer i blod och CSF kan prediktera överlevnad och restsymptom hos patienterna ett år efter en allvarlig traumatisk skallskada. Neuromarkörerna kan inom en snar framtid användas för att styra behandlingar av patienter med svår TBI och likaledes tidigt i förloppet ge anhöriga information om prognosen.

CONTENT

ABBREVIATIONS

Aβ	β-amyloid
AD	Alzheimer's disease
APLP	Amyloid precursor-like proteins
APP	Amyloid precursor protein
BBB	Blood brain barrier
CT	Computed tomography
CNS	Central nervous system
GAD	Gracile axonal dystrophy
GCS	Glasgow Coma Scale
ICP	Intracranial pressure
MRI	Magnetic resonance imaging
VCSF	Ventricular cerebrospinal fluid
sAPP	Soluble amyloid precursor protein
TBI	Traumatic Brain Injury
DAI	Diffuse axonal injuries
MRT	Magnetic Resonance Tomography
CPP	Cerebral perfusion pressure
DND	Delayed neuronal death
NICU	Neuro-intensive care units
LC	Lund concept
ICP	Intracranial pressure
GOS	Glasgow Outcome Scale
GOSE	Glasgow Outcome Scale Exended
NIHSS	National Institute of Health Stroke Scale
ADL	Activities of Daily Living
RLS	Reaction Level Scale
MAP	Mean Arterial Pressure
ApoE	Apolipoprotein E
TP	Tumor proteins
CSF	Cerebral Spinal Fluid
NFL	Neurofilament light
GFAP	Glial fibrillary acidic protein
MBP	Myelin Basic Protein
NSE	Neuron-specific enolase
MABP	Mean arterial blood pressure
SD	Standard deviation
ROC	Receiving Operating Curve
CI	Confidence Interval
OR	Odds Ratio
TIA	Trans ischemic attack
BNM	Biochemical neuromarkers
BTFG	Brain Trauma Foundation Guidelines

INTRODUCTION

Traumatic brain injury is the most common cause of mortality and neurological morbidity in young adults. The entity may be subdivided into variable brain injury severities, but in this thesis only those with severe traumatic brain injury are studied.

The inclusion criteria in the studies have been strict to scientifically address formulated hypothesis. This strictness does not quite apply to the clinicians world, where an initially less severe brain injury may develop into a severe. This notion clearly addresses the problem where initial neurological investigation and initial brain computer scan (CT) has a relatively low impact in prognosticating long-term outcome in these patients. Therefore, the longitudinal measurement of biochemical neuromarkers may be a simpler and more robust way for the clinician to trail treatment paradigms as well as to early prognosticate outcome. The thesis explores different neuro- and genetic-markers measured in both blood and in cerebrospinal fluid (CSF) and then correlates them to outcome at 1-year.

Background

Traumatic Brain Injury (TBI) is subdivided into chronic and acute entities. Chronic TBI can be induced by repeated head-trauma as boxing and other contact sports, leading to early development of Alzheimers Disease (AD).(Jordan 2000) Acute TBI, from fall accidents, war, natural disasters and traffic injuries, leads to enhanced morbidity and, in severe cases, death.

In 1974 Teasdale and Jennet, introduced a brain injury severity score called Glasgow Coma Scale (GCS), including the assessment of motor, verbal and pupillary response on a scale from 3, (no response), to 15, (normal response). (Teasdale *et al.* 1974) Acute TBI can then be subdivided into mild (GCS 13-15), moderate (GCS 9-12) and severe (GCS 3-8) injury. (Table 1,2)

Table 1. Reaction Level Scale 85

Reaction Level Scale 85 (RLS)
1. Alert
2. Drowsy or confused
3. Very drowsy or confused
4. Localises pain
5. Withdrawing movements
6. Stereotype flexion movements
7. Stereotype extension movements
8. No response to pain stimulation

About 10% of the acute TBI´s are severe (sTBI) and among young adults and children sTBI is the most common cause of increased morbidity and mortality. In the group of young adults (< 40 years), TBI causes more death and mortality than all other diseases do together. TBI also causes more loss-of-years for the group of patients in working age, (18-65 years old), than cancer, heart diseases and HIV/AIDS do together. Traffic accident is the most common cause of sTBI (Tagliaferri *et al.* 2006) and among patients with sTBI 75% are male. In the papers included in this thesis, only patients with severe head injuries are included and studied.

Table 2. Glasgow Coma Scale

	Glasgow Coma Scale (GCS)	
Best motor response	**Best verbal response**	**Eye opening**
Obeys commands (6)	Oriented speech (5)	Spontaneous (4)
Localises pain (5)	Confused speech (4)	To command (3)
Flexor withdrawal (4)	Words only (3)	To pain (2)
Abnormal flexion (3)	Sounds only (2)	None (1)
Extension (2)	None (1)	
None (1)		

Mechanisms of Severe Traumatic Brain Injury

Severe traumatic brain injury (sTBI) causes different types of brain damage encompassing focal contusions, intra- and extradural hematomas and diffuse axonal injuries (DAI). Many of these injuries are not visible in Computer Tomographic scans (CT) the initial days post-trauma. Magnetic Resonance Tomography (MRT) is a more precise method describing injuries including DAI, but the technique is initially not clinically applicable in these unstable patients.

The sTBI starts a chemical cascade and disturbances of potassium-, sodium- and calcium-ion balances, as well as inducing hyperglycolysis, glutamate alterations, decreased tissue-oxygen delivery and apoptosis.(Giza *et al.* 2001)

The primary head injury, in most cases, develops into a secondary brain injury with edema and a subsequent increase in intracranial pressure (ICP) leading to a decrease of cerebral perfusion pressure (CPP) inducing cerebral ischemia.

Presently, we know some of the brain´s response to a mixed insult, like a sTBI, a heterogenic entity encompassing hypoxia as well as focal and global ischemia. sTBI induces several different injury mechanisms to neuronal and astroglial cells. Astrocytes exposed to ischemic injury, if not necrotic, react with gliosis, starting a reparation cycle. The astrogliosis may be activated by the JNK/c-Jun/AP-1 pathway through a calcium influx from the extracellular compartment.(Prochnow 2014)

sTBI may induce contusions with a necrotic core where both astroglial and neuronal cells rapidly succumb. Areas adjacent to the necrotic core are variably ischemic. If ischemia is severe, neurons die by delayed neuronal death (DND), histologically noted from day 4-5 in experimental investigations. Finally, watershed areas with compromised circulation surround ischemic areas. If circulation is not adequately restored in these areas, neuronal apoptosis starts after days and weeks. (Giza and Hovda 2001)

Treatment of sTBI

As previously described sTBI is a heterogenic injury. Experimentally, models mimicking concussion, focal cerebral ischemia as well as global cerebral ischemia have been developed. (Smith *et al.* 1984). These models have been utilized when exploring the neuroprotective effects of a multitude of pharmacological compounds. Although many of them have demonstrated promising positive effects in animal models, none have emerged as useful in clinical trials.

Therefore, the clinical interest has focused on improving pre-hospital care, developing dedicated neuro-intensive care units (NICU) and improving neuro-rehabilitation.

Although we have no "wonder drug" reducing neurological deficit after a sTBI, intensive care treaments adressing physiological intracranial changes have emerged. The treatment paradigm utilized on all patients included in the studies of the thesis is called "The Lund Concept for the Treatment of Patients with severe Traumatic Brain Injury" (LC). This concept developed by Grände and coworker (Asgeirsson *et al.* 1994) reduces high intracranial pressure (ICP) by reducing the high blood pressure (hydrostatic capillary pressure) concomitantly preserving the oncotic pressure. The LC includes a multitude of factors like; head-up tilt, avoiding fever and controling sodium, potassium, Albumin, Glucose and Hemoglobin within normal levels. The LC is not accepted in a large part of the world, (Sharma *et al.* 2011) although the results demonstrated, particularly in Sweden, have greatly improved patient outcome after utilizing the concept. (Koskinen *et al.* 2014)

Neurological instruments to examine outcome

Glasgow Outcome Scale

There are various outcome instruments that focus on different brain functions. The most commonly used outcome instrument for TBI is the Glasgow Outcome Scale (GOS). (Teasdale and Jennett 1974)

The GOS scale is divided into five outcome entities; Death (GOS 1), Vegetative state (GOS 2), Severe disability (GOS 3), Moderate disability (GOS 4) and Good outcome (GOS 5) (Table 3)

If the patient is not dead or vegetative the questionnaire covers five areas; Independence at home, independence outside home, employability and ability to engage in premorbid social and leisure activities and interpersonal relationships.

A patient with severe disability is dependent on daily support compared with a patient with moderate disability who is able to travel by public transportation and can work, although adjusted. A patient with good recovery can live a more or less normal life.

At which point the outcome assessment should be performed is somewhat unclear. The GOS scale is constructed to be utilized after hospital discharge

to examine post-traumatic neurological and psychological disability. (Wilson *et al.* 1998) As far as time is concerned, appraisal at one year seems appropriate in patients with severe TBI (Corral *et al.* 2007)

The GOS scale is focusing on how the patient is living and levels of independence, rather than actual symptoms or deficits caused by the injury. Statistically, GOS is often dichotomised into dead (GOS 1) or alive (GOS 2-5) or bad outcome (GOS 1-3) compared to good outcome (GOS 4-5). (Teasdale and Jennett 1974)

Table 3. Glasgow Coma Scale/Extended

GOS	GOSE	Definition
1	1	Dead
2	2	Vegetative state
3	3	Lower severe disability completely dependent on others
3	4	Upper severe disability dependent on others for some activities dependent on others for some activities
4	5	Lower moderate disability unable to return to work or participate in social activities
4	6	Upper moderate disability return to work at reduced capacity, reduced participation in social activities
5	7	Lower good recovery good recovery with minor social or mental deficits
5	8	Upper good recovery

Extended GOS

(Jennett *et al.* 1981) presented an extended GOS scale (GOSE) to allow a more divided and sensitive outcome instrument. However, this scale leads to lower patient's agreements. (Wilson *et al.* 1998) (Table 3)

Barthel´s Index

Barthel´s Index measures the ability of mobility and personal care. It evaluates patient independence when eating, bathing, dressing, walking and getting out of bed and chairs. The scale is normally utilized to describe patient ability of Activities of Daily Living (ADL). (Mahoney *et al.* 1965) (Table 4)

Table 4. Barthel´s Index

Activity	0 Points	5 Points	10 Points	15 Points
Feeding	Unable	Needs help	Independent	
Bathing	Dependent	Independent (or in shower)		
Grooming	Needs help with personal care	Independent		
Dressing	Dependent	Needs help but can do about half unaided	Independent	
Bowels	Incontinent	Occasional accident	Continent	
Bladder	Incontinent	Occasional accident	Continent	
Toilet use	Dependent	Needs some help	Independent	
Transfers (bed to chair and back)	Unable	Major help (one or two people	Minor help (verbal or physical)	Independent
Mobility	Immobile	Wheelchair independent	Walks with help of one people	Independent
Stairs	Unable	Needs help	Independent	

National Institute of Health Stroke Scale

National Institute of Health Stroke Scale (NIHSS) evaluates areas of language, dysarthria (speech) coordination, visual field, neglect eye movement, consciousness and motor- and sensory functions. (Barsan *et al.* 1989)

NIHSS is the most used scale in stroke intervention and recommended for the neurological classification in the American Heart Association Stroke Outcome Classification. The extended NIHSS also investigates limb paralysis. (Table 5)

Table 5. NIHSS

Category	0 Points	1 Points	2 Points	3 Points	4 Points
1a. Level of Consciousess	Alert	Drowsy	Stuporous	Coma	
1b. LOC Questions	Answers both correctly	Answers one correctly	Incorrect		
1c. LOC Commands	Obeys both correctly	Obeys one correctly	Incorrect		
2. Best Gaze	Normal	Partial gaze palsy	Forced deviation		
3. Visual Fields	No visual loss	Partial Hemianopia	Complete Hemianopia	Bilateral Hemianopia (Blind)	
4. Facial Paresis	Normal	Minor	Partial	Complete	
5a. Motor Arm - Left	No drift	Drift	Can't resist gravity	No effort against gravity	No movement
5b. Motor Arm - Right	No drift	Drift	Can't resist gravity	No effort against gravity	No movement
6a. Motor Leg - Left	No drift	Drift	Can't resist gravity	No effort against gravity	No movement
6b. Motor Leg - Right	No drift	Drift	Can't resist gravity	No effort against gravity	No movement
7. Limb Ataxia	No ataxia	Present in one limb	Present in two limbs		
8. Sensory	Normal	Partial loss	Severe loss		
9. Best Language	No aphasia	Mild to moderate aphasia	Severe aphasia	Mute	
10. Dysarthria	Normal articulation	Mild/ moderate slurring of words	Near to unintelligable or worse		
11. Extinction and Inattention	No neglect	Partial neglect	Complete neglect		

Variables retaled to outcome

A number of independent variables have significantly been correlated to outcome after a severe TBI. Among them CT classification, hypotension, fever, intracranial pressure (ICP), cerebral perfusion pressure (CPP), age, temperature, glucose levels, pupil reaction to light and neurological assessment (Glasgow Coma Scale) have all been utilized separately or combined. These factors have predominatly been in use prior to the development of biochemical assays of biochemical neuromarkers. However, they are important clinical tools for the clinician to evaluate clinical status and to assess eventual neurological deteriation.

GCS

This score has previously been commented upon. However, after its introduction investigations was performed correlating the GCS score, noted at the scene of trauma, to outcome. (Marmarou *et al.* 2007) A low GCS score correlated to severity of outcome. However, the initial motor score of the GCS seems to give an even better progostic correlation to outcome than GCS *per se*. (Healey *et al.* 2003) This fact was used when constructing the Reaction Level Scale (RLS) originating from Sweden. (Starmark *et al.* 1988) RLS has become the clincal assessment tool in patients with neurological deficits like TBI and subarachnoidal hemorraghe in Sweden. (Table 1,2)

ICP and CPP

Clinically, patients with sTBI receive an indwelling catheter for ICP measurement and eventual CSF drainage. However, more recent alternatives to ICP-recording is the insertion of a parenchymal catheter or the insertion of "bolt".

The critical level of ICP inducing treatment, in patients with a sTBI, differs worldwide. Thus, the American guidelines state 2010) that at an ICP > 20 mmHg reduction therapy should be started, while the Lund Concept states that ICP-lowering therapy should start at hospital admittance. (Koskinen *et al.* 2014) The level of CPP have been controversial, but now guidelines from both the Brain Trauma Foundation and the Lund concept are in consert aiming at a CPP > 50 mmHg in adults and > 40 mmHg in pediatric patients.

CT

Marshall (Marshall 1991) introduced a Computer Tomography (CT) scan classification bearing his name. This focused on cisternal shift and size and could differentiate particularly between fatality or not. Thus, Marshall et al. showed that 74% of the patients with bilaterally unresponsive pupils after resuscitation became vegetative or died. (Marshall *et al.* 1992) They also demonstrated higher mortality in patients with a cerebral CT demonstrating cisternal shift. (Table 6) Nelson *et al.* has suggested a CT scoring system and shown that the magnitude of midline shift is a better predictor than the Marshall score for predicting death and bad outcome (GOS) (Nelson *et al.* 2010)

Table 6. Marshall

Category	Definition
Diffuse injury I	No visible intracranial pathology
Diffuse injury II	Cisterns are present with midline shift of 0-5mm and/or lesion densities present, lesion densitets present, no high- or mixed- density lesion of > 25 mL
Diffuse injury III	Cisterns compressed or absent, with midline shift of 0-5 mm, no high- or mixed-density lesion of > 25 mL
Diffuse injury IV	Midline shift of > 5mm, no high or mixed density lesion of > 25 mL
Evacuated mass lesion V	Any lesion surgically evacuated
Non-evacuated mass lesion VI	High or mixed density lesion of > 25 mL, not surgically evacuated.

Hypotension

It has been demonstrated that hypotension correlates to outcome. Thus, hypotension, *i.e.* systolic blood pressure <90 mmHg has been associated with higher mortality (Chesnut *et al.* 1993)**,** mainly because by the importance of maintaining cerebral perfusion pressure (CPP = Mean Arterial Pressure (MAP) – Intra Cranial Pressure (ICP). The avoidance of hypotension does not however *per se* indicate that increasing the MAP to supra normal levels are advantageous.

Age

Age is an independent negative predictive factor for outcome in TBI patients, where both increased mortality and morbidity is noted in TBI patients > 65 years old. (Mosenthal *et al.* 2002)

Hyperglycemia

Persistent hyperglycemia affects outcome negatively in patients with TBI. (Salim *et al.* 2009) Glucose variability has also been correlated to worse outcome in similar patients' cohorts. (Matsushima *et al.* 2012) Therefore, the investigated patient cohort was under strict glucose control to mitigate a confounding factor.

Temperature

The importance of temperature regulation and avoiding hyperthermia comes from animal studies. (Nellgård *et al.* 2001) In humans the induction of slight hypothermia may be advantageous in patients with cardiac arrest. (Friberg *et al.* 2009) Recent studies demonstrated that active cooling may worsen outcome after sTBI. (Grände *et al.* 2009) However, the decrease of fever may be advantageous (Grände 2006)

Pupillary reactivity

Pupillary reactivity is a prognostic sign utilized for decades in patients with TBI. Although having a weaker prognostic value than GCS, the loss of pupillary reaction to light may prognosticate omnious outcome. (Majdan *et al.* 2015) If combining the evaluation of pupillary reaction at hospital admission with the initial motor GCS score, this combined assessment enhances the prognostic value more than they do separately.

Genetic susceptibilty to outcome

The cellular and molecular pathways that regulate neuron function are under complex polygenic control. However, the genetic variability in patient cohorts of sTBI is not adequately explored, mainly because of its novelity. The most investigated genetic susceptibility to outcome after a TBI are Apolipoprotein E (ApoE) variability, but also genes encoding for interleukins (IL) like IL-1 and IL-6 as well as tumor proteins (TP53) have been investigated (Davidsson J The Neuroscientist, 2014, 1-18). The *APOE* gene has *3 allelic* variants *ε2*, *ε3* and *ε4* encoding for the 3 isoforms of the protein (*ε2*, *ε3*, *ε4*). (Teasdale *et al.* 1997) The APOE isoforms differ in amino acids at positions 112 and 158: *ε2* (cysteine/cysteine), *ε3* (cysteine/arginine) and *ε4* (arginine/arginine). The *ε4 allele* is the most neurotoxic isoform and can induce neurodamage by proteolytic cleavage. (Mahley *et al.* 2012)

Biochemical neuromarkers

The lack of clinical and/or radiological applicable investigations available to prognosticate and give clinical support for intervention early after a sTBI makes the need for relevant biochemical neuromarkers great. Organ-specific proteins have been in clinical usage for many years, particularly those connected to myocardial infarction.

The ideal neuromarker has been defined already in the 1980´s as; Neuromarkers should appear rapidly in the Cerebral Spinal Fluid (CSF) and/or in the blood, being specific for brain tissue damage and correlate to both short- and long-term outcome. (Bakay *et al.* 1983) This ideal marker has not been found to be used in the clinical setting.

Larger BNMs do not penetrate the blood brain barrier (BBB) if it is intact. However, following a severe TBI the BBB disrupts, at least partially, making BNM-leakage possible. The amount varies probably with TBI severity, although this has yet to be proven. However, a small amount of BNM can be detected in the blood post-trauma if the detection level is very low. In the cerebral spinal fluid (CSF) these compunds are however readily measured.

Microdialysis

The history of microdialysis dates back to mid-sixties where membrane-lined sacks containing 6% dextran were inserted into the cerebral hemispheres of dogs. (Bito *et al.* 1966) In the 1970 the technique was modulated and developed into microdialysis (MD). In the early nineties commercially produced MD catheters became available. The principles of MD have been

reviewed in detail elsewhere. (Benveniste *et al.* 1990) Cerebral MD allows measurement of local tissue biochemistry and on-line cerebral MD monitoring is a reality. MD is the only method of measuring brain tissue biochemistry at the bedside and is a useful tool for the detection of biochemical changes associated with hypoxia/ischemia after TBI. Nelson *et al.* has shown, measuring glucose, lactate, pyruvate and glycerol, osmolality, creatinine and more, that MD data show weak correlation to ICP and CPP. This correlation between outcome and MD was noted when using univariate statistics, but only osmolarity and creatinine was correlated to outcome after adjusting for known predictors of TBI (age, GCS, pupil response and CT score). (Nelson *et al.* 2012)

Generally, the BNM´s can be didived by their source *i.e.* they originate either from neurons, astrocytes or other cells within the brain. Biochemical markers for brain damage originate from neuronal destruction such as Tau (Ost *et al.* 2006) and neurofilament light (NFL), (Nylen *et al.* 2006) from astroglial protein production like glial fibrillary acidic protein (GFAP) and S-100B,(Romner *et al.* 2000) or from subcellular components such as Myelin Basic Protein (MBP) a marker for myelin sheat damage.(Hu *et al.* 2004) Finally, different cytokines and complement factors are markers for both microglial and neuronal cytoplasmatic damage.(Neher *et al.* 2014)

Biochemical markers for TBI could be valuable as tools to grade the severity of trauma, predict prognosis, and identify minor TBI in cases with negative MRI or CT scans.(Blennow *et al.* 2012), (Zetterberg *et al.* 2013) Several studies have also suggested that biochemical neuromarkers are relevant prognostic factors after sTBI (Glenner *et al.* 1984), (Iwatsubo *et al.* 1994) and Subarachnoid Hemorrhage (SAH). (Seubert *et al.* 1992)

Specific BNMs

Different BNMs have been investigated in traumatic brain injury (TBI). Among them, S-100B and neuron-specific enolase (NSE), measured in blood, have most frequently been utilized. Although associations between concentrations of S-100B and outcome in TBI patients are reported, both S-100B and NSE have low sensitivity and specificity. (Pelinka *et al.* 2005) Focus has been oriented toward other BNMs. (Raabe *et al.* 1999)

GFAP is thought to be important in modulating astrocyte motility and structural stability. When exposed to trauma, astrocytes become reactive, (astrogliosis), and rapidly synthesize GFAP. (Eng *et al.* 2000) GFAP is an early indicator of brain damage initiating a reparation process in intact astrocytes.(Eng *et al.* 2000) In a previous paper, we demonstrated that sTBI induced and increased astrocytal GFAP production as reflected by increased

serum levels. (Nylen *et al.* 2007) Patients with unfavorable outcome had significantly higher serum-GFAP concentrations than those with favorable outcome. CSF-GFAP has not been investigated thouroghly after a sTBI correlating results to long-term outcome.

NFL is the light unit of the neurofilament protein being the primary component of the neurofilament core. We have previously demonstrated that lumbar CSF-NFL, obtained on day 11, correlated to outcome after aneurismal subarachnoid hemorrhage.(Nylen *et al.* 2006) No previous investigation of CSF-NFL and long-term outcome following sTBI has been performed.

BNMs connected to Alzheimer's disease

β-*amyloid*

Alzheimer's disease (AD) is characterized by synaptic and axonal degeneration together with neurofibrillary tangles and β-amyloid deposits called senile plaques. The β-amyloid peptides (Aβ(1–40), Aβ(1–42)) of 40–42 amino acid lengths are the major components of the amyloid deposits. The peptides are breakdown products of one of the two alternative proteolytic cleavage pathways of the amyloid precursor protein (APP). (Glenner and Wong 1984), (Iwatsubo *et al.* 1994)

Apart from TBI being considered a risk factor for AD, (van Duijn *et al.* 1992) severe head injury seems to initiate a cascade of molecular events that are also associated with AD. Aβ depositions following head trauma have been observed both in man, (Graham *et al.* 1996) and in experimental animal models. (Hamberger *et al.* 2003) Moreover, minor head trauma which frequently occurs in professional boxers, often results in dementia pugilistica, a disorder similar to AD in terms of Aβ deposition. (Roberts *et al.* 1990) Axonal impairment induced by TBI disrupts the normal fast axonal transport of APP 13 and elicits up regulation and accumulation of APP in damaged axons.(Gentleman *et al.* 1993)

In addition to the Aβ production, which also occurs in normal subjects,(Seubert *et al.* 1992) the sub-sequential β- and γ-secretase cleavage of APP also leads to a simultaneous release of soluble β-secretase cleaved APP (β-sAPP). In contrast, the alternative cleavage pathway prevents Aβ formation and the α-secretase cleavage of APP generates α- sAPP. (De Strooper *et al.* 2000)

In previous studies of ventricular CSF (VCSF) the first days after TBI an elevation of Aβ(1–42) has been noted, but no change of total sAPP. (Emmerling *et al.* 2000)

Knowledge of the APP metabolism during the early pathogenesis of AD and TBI is only fragmentary.

Tau

Tau proteins, (48-67kD) are mainly found in neurons. Here they have a role in the assembly of microtubule, a structure maintaining the cytoskeleton and axonal transport. Aggregation of tau proteins is noted in intra-neuronal tangles, seen on AD. (Goedert 1993) As previously noted AD and TBI have a connection, one would assume that if so, the learning and memory deficits noted after a sTBI may the same biochemical origin as AD. (Fig. 1)

Apolipoprotein E

The AD has a low hereditary incidence, but there are genetic risk-factors found. Among them the genetic variations which may influence outcome has been suggested in previous studies.(Wilson *et al.* 1998), (Chiang *et al.* 2003)

Apolipoprotein E (*ApoE* = protein, *APOE* = *gene*) is synthesized by astrocytes and, after binding to specific receptors on neurons, directs lipid transportation within the brain/CSF and may assist neural transmission. (Mauch *et al.* 2001) In humans, three common alleles of the *APOE gene (ε2, ε3, ε4)* code three protein isoforms (ApoE 2–4) with differences in action. *APOE*, particularly *APOE ε4*, promotes amyloid formation and is supposedly connected with Alzheimer's disease (AD), although this is now questioned. (Peacock *et al.* 1994), (Lambert *et al.* 2005)

Retrospective and prospective studies on the association of *APOE* genotype and outcome following TBI have been published.(Wilson *et al.* 1998), (Chiang *et al.* 2003) Particularly *APOE ε4* has been associated with unfavourable cognitive and functional recovery and deposition of β-amyloid after TBI.(Nicoll *et al.* 1995), (Crawford *et al.* 2002) However, these studies have either been small, evaluated short-term dichotomized by GOS, or included TBI of variable severity (Iwatsubo *et al.* 1994), (Chamelian *et al.* 2004)

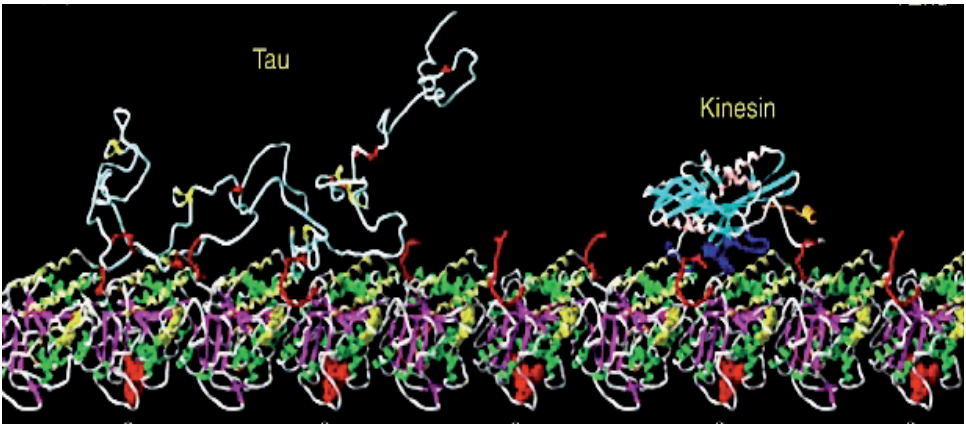


Fig. 1. *The picture visualises a Tau molecule and a kinesin motor domain bound to a microtubule. Printed with permission from Cold Spring Harbor Laboratory Press.*

AIM

The central aims were to study clinical findings and the levels of neuronal and genetic markers in blood and CSF in patients with Severe Traumatic Brain Injury (sTBI) the first two weeks after trauma. Further the aims were to evaluate if there is a correlation of the neuronal and genetic markers in CSF and serum to long term (1-Year) outcome in patients with sTBI.

Paper I

Analyse the levels of Aβ $_{(1–42)}$, β-sAPP and α-sAPP in CSF and Aβ $_{(1–42)}$ in plasma during the first two posttraumatic weeks in patients with sTBI compared to a control group with Normal Pressure Hydrocephalus (NPH).

Paper II

Study if total tau levels in serum or CSF during the first two posttraumatic weeks are associated with long-term outcome (1-Year after trauma) in patients with sTBI and if these levels are higher than in our age matched control-group of patients with NPH.

Paper III

Evaluate if *APOE* genotype has impact on short term (30 days) and long-term (1-Year) outcome in patients with severe Traumatic Brain Injury.

Paper IV

Investigate if the CSF levels of astrocytic (GFAP) and neuronal (NFL) neuromarkers during the first 2 weeks post-trauma in patients exposed to a sTBI correlates to 1-year outcome.

PATIENTS AND METHODS

Inclusion

The University Hospital Medical Ethics Committee, Gothenburg, Sweden approved the present study protocol. Informed consent was obtained from each patient or their relatives prior to inclusion in this study. The study was performed in accordance with the provisions of the Helsinki Declaration. The patients were admitted to the Neurointensive Care Unit at Sahlgrenska University Hospital Gothenburg, Sweden, between October 2000 and December 2002. Patients were included within 48 hours after trauma. Patients included had; 1) severe TBI, i. e. a Glasgow Coma Scale (GCS) score of < 8 at admission, 2) a need for artificial ventilation, and 3) living in >Sweden to secure the ability for a follow up of the patients and 4) a therapeutic indication to monitor ICP and in patients in the studies were CSF was collected (Paper I,II,IV) an intracranial ventricular catheter was operatively placed**.**

Regime

After radiologic and clinical assessments, the patients underwent neurosurgical interventions within hours (1 - 4 hours) after admission, to receive an indwelling ventricular catheter for intracranial pressure (ICP) monitoring and therapeutic CSF drainage. When appropriate, space-occupying lesions like hemorrhages and contusions were surgically removed. Patients were treated in accordance with the Lund concept, a standardized protocol. (Grände *et al.* 2002)

Laboratory and physiologic measurements were uninterruptedly recorded during the study period and concomitantly adjusted to be retained within the following limits: hemoglobin >120 g/L, serum albumin 35 - 50 g/L, serum sodium >135 - <150 mmol/L, serum potassium 4.0 - 5.0 mmol/L, core temperature 37 +/- 0.5 °C, mean arterial blood pressure (MABP) between 70 and 100 mm Hg, Blood glucose was preserved between 4 and 6 mmol/L ICP <20 mm Hg, cerebral perfusion pressure (= MABP - ICP) >60 mm Hg, PO_2

12 to 18 kPa, PCO_2 around 4.5 kPa, and normalized pH. NICU-care was performed according to a standardized protocol.

Data collection and analyze techniques

CSF serum samples were collected on the day of trauma, on days 1, 2, 3, 4, 6, 8 and once during days 11-18. Surviving patients were assessed at 1-year by a neurologist blinded to CSF and serum results. CSF and blood samples were centrifuged at x 2,000 g for 10 minutes at 4 °C and frozen at –70 °C until analyzed. Occasionally CSF samples could not be obtained because of unstable clinical status such as high ICP.

As a reference group, we examined CSF from age-matched patients with NPH. Details of this patient cohort has previously been presented. (Tullberg *et al.* 2000)

Paper I

The VCSF levels of α-sAPP and β-sAPP were determined using two newly developed sandwich ELISA methods. (Olsson *et al.* 2003) Briefly, in both ELISA methods, the monoclonal antibody 8E5 (Oltersdorf *et al.* 1990), (Games *et al.* 1995) recognizing an epitope within amino acids 444–592 of APP695, was used as capturing antibody. Biotinylated monoclonal 6E10 (Signet Laboratories, Inc, Dedham, USA), which is reactive to amino acids 1–17 of human β- amyloid peptide, was used as a detector of α-sAPP. An antibody termed 192, which is specific for amino acids 591–596 of APP695, (Seubert *et al.* 1993) was used as a detector of β-sAPP. The concentration of α-sAPP and β- sAPP in samples of VCSF was calculated from the linear interval of the standard curve using recombinant proteins as standards.

The VCSF-Aβ(1–42) and the plasma-Aβ(1–42) concentrations were determined by INNOTEST™ β-amyloid(1–42) HS ELISA (Innogenetics, Ghent, Belgium). The assay and its characterisation have previously been described in detail. (Vanderstichele *et al.* 2000)

APOE genotyping was performed by PCR followed by mini-sequencing as described previously. (Blennow *et al.* 2000)

Paper II

Total tau (normal tau and hyperphosphory-lated tau) was determined using a sandwich ELISA technique (Innotest hTAU-Antigen; Innogenetics N.V., Ghent, Belgium), as previously described in detail. (Blennow *et al.* 1995)

Paper III

Whole blood was taken once during the hospital stay to explore the APOE genotype. The samples were all analyzed in the same laboratory. APOE genotyping was performed by polymerase chain reaction and solid-phase mini-sequencing, which directly detects the six common *APOE* genotypes *ε2/ ε2, ε2/ ε3, ε2/ ε4, ε3/ ε3, ε3/ ε4* and *ε4/ ε4*. (Blennow *et al.* 2000)

The result of the genotypes was blinded to the neurologist performing neurological exams both during the patient's initial stay at NICU and at the 1-year follow-up.

As control group for the *APOE* genotype served a random population-based group (n=54) of sex- and age-matched population (mean age 38.7) in the same geographical area. The control group was selected from a larger control group, one individual at a time. The variables tested were age and three dichotomized dummy variables indicating different age intervals. In the statistical analysis involving the active and the selected control group the confounding bias from age was therefore eliminated.

Paper IV

CSF NFL was analyzed using the NFL ELISA method, (Uman Diagnostics, Umeå, Sweden), as described in detail elsewhere. (Norgren *et al.* 2003)

Analysis of CSF NFL was analyzed using the NFL ELISA method, (Uman Diagnostics, Umeå, Sweden), as described in detail elsewhere. (Norgren *et al.* 2003)

Analysis of CSF GFAP was performed according to a previously described ELISA method. (Rosengren *et al.* 1994)

Neurologic assessment

An experienced neurologist, unaware of neurochemical results, assessed neurology on all living patients, routinely at the hospital, but occasionally at the patient´s home, one year post TBI.

In papers II and IV Glasgow Outcome Scale (GOS) was utilized were 1= dead and 5 totally recovered and in paper III Glasgow Outcome Scale Extended (GOSE) was utilized, where 1 = dead and 8 = totally recovered.(Wilson *et al.* 1998) GOS could then be dichotomized to bad (GOS 1 - 3) *vs.* good (GOS 4-5) outcome and likewise GOSE was then dichotomized to bad (GOS 1 - 4) *vs.* good (GOS 5-8). The Barthel Index was used to rate activities of daily living (ADL), where 100 = fully independent and 0 = patient needs help with all ADL. (Table 4) The level of the neurologic deficit at 1-year after trauma was assessed with the NIH Stroke Scale (NIHSS), where 0 = no neurologic deficit and 36 = major deficits. The NIHSS contains 15 items including level of consciousness, eye movement, visual field deficit, coordination, language (aphasia), speech (dysarthria), neglect and motor and sensory involvement. The scale was criticised for not measuring distal limb strength and an extra item regarding this function was attached and used in some trials of thrombolysis, but it did not obtain general acceptance. The extended version, including distal limb strength, is used by us, as this was the leading form when we started the study. (Table 5)

Statistical methods in the different papers

Paper I

The distribution of the continuous variables is given as median (Q1 – Q3) and as mean, standard deviation (SD), minimum and maximum in the theses. For comparison between two groups Mann Whitney U-test was used, which is a special case of Kruskal-Wallis one-way analysis of variance with only two groups. For comparison of change over time Wilcoxon Signed Rank test was used. All significance tests were two-sided and conducted at the 5% significance level. (Table 7)

Paper II

The distribution of the variables is given as median and interquartile range and as mean, standard deviation (SD), Q1, Q3, minimum and maximum in the theses. For comparison between two groups Mann Whitney U-test was

used, which is a special case of Kruskal-Wallis one-way analysis of variance with only two groups. For all correlation analysis Spearman's rank correlation analysis was used. Univariable logistic regression analysis with mortality and morbidity (0-1) as dependent variable and tau levels in ventricular CSF as independent variable was used to calculate the ROC-curve and to detect the best sensibility and specificity for 1-year outcome. Area under the ROC-curve was used to describe the goodness of fit for the model. Positive likelihood ratio (LR+) was calculated as an overall measure of the test by dividing sensitivity with (100 – specificity). All significance tests were two-sided and conducted at the 5% significance level. (Table 7)

Paper III

For comparison between two groups Mann-Whitney U-test was used for continuous variables and Fisher's exact test for dichotomous variables. All survival analysis of time to death between two groups was analyzed with the log-rank test and described with Kaplan-Meyer curves. Positive likelihood ratio (LR+) was calculated as an overall measure of the test by dividing sensitivity with (100 – specificity). All significance tests were two-sided and conducted at the 5% significance level.

The age and sex matched control group was selected choosing controls one by one by choosing the control that minimizes the maximum t-values between the patients and the controls. Age was matched both by mean and in age-classes. In order to find a difference in 1-year survival of 30% in the patients and 10% in the controls with a power of 96% with two-sided Fisher's exact test at significance level 5% 45 patients and 45 controls were needed. (Table 7)

Paper IV

The distribution of the continuous variables is given as mean, minimum and maximum in the text and as minimum, 1:st quartile, median, 3:rd quartile and maximum in the boxplots. In the figures the original values are presented on a logarithmic scale and all analyses and tables are based on logarithms.

For comparison between two groups Mann-Whitney U-test was used for continuous variables. For analysis of change over time Wilcoxon signed rank test was used. Spearman's rank correlation was used for all correlation analysis.

For all rank analyses, like Spearman's correlation analysis and Mann-Whitney U-test the p-values were the same for original values and logarithms.

Univariable logistic regression was performed based on the dependent variables dead/alive and bad/good outcome *vs.* the independent continuous variables of NFL (ng/l) and GFAP (ng/l) in CSF and was presented with Odds Ratio (OR) with 95% Confidence Interval (CI), p-values and area under the ROC-curve. The ROC-curve from the logistic regression was also presented. In these logistic regression analyses the independent variables were analyzed in logarithm based 10 scale. All statistical analysis was performed with SAS system Version 9. All significance tests were two sided and conducted at the 5% significance level. (Table 7)

Table 7. Overview statistical methods paper I-IV.

Overview Statistical Methods	Paper			
	I	II	III	IV
Descriptiv Statistics Mean, Median and Interquartile range	X	X	X	X
Number and % for categorical variables	X	X	X	X
Boxplot	X	X		X
For description of survival curves: Kaplan-Meier curves			X	
Statistical Analysis For comparison between two groups: - Mann-Whitney U-test for continuous variables	X	X	X	X
- Fisher's exact test for dichotomous variables			X	
For analysis of changes over time continuous variables: Wilcoxon Signed Rank Test	X			X
For comparison of two survival curves: Log-rank test			X	
For correlation analysis: Spearman's correlation analysis		X		X
For prediction of dichotomous variables: Logistic regression analysis with area under the ROC-curve		X		X
Odds ratio with 95% confidence interval				X
Sensitivity, Specificity and Positive likelihood ratio (LR+) with 95% confidence interval		X	X	

RESULTS

Paper I

All patients sustained diffuse brain injury. 28 patients,7 women and 21 men, median (inter-quartile range) 41 [23–61] years (range 15–81) were included. The cause of trauma was in 14 patients road traffic accident, fall injury in 7 patients, 2 assault and miscellaneous in 5 patients. The severity of the injury was rated by initial computed tomography (CT) according to a modified Marshall score [18] in which we used a 10mm midline shift as a criterion for scoring. One patient was rated in category 1, 13 patients in category 2, 9 patients in category 3 and finally 5 patients in the most severe category 4. The mean score for the 28 patients was 2.64. Twenty patients had isolated brain injury including two patients with additional visceral trauma and 8 patients had a multitrauma. In the examined group 2 patients had a history of DM, 3 of heart diseases, 1 with hypertension and eight had neurologigical diseases, including 2 with the history of EP. At the time of TBI 5 patients had measurable concentrations of alcohol and 1 with narcotics. (Table 8)

Table 8. Background data paper I

Paper I	Numbers
Men	21
Women	7
Age (years)	
Mean	42
Median	41
Range	15-81
Cause of trauma	
Road traffic accident	14
Fall	7
Miscellaneous	5
Assault	2
Type of trauma	
Isolated brain injury	18
Visceral	2
Multitrauma	8
Measurable drug concentration at the time of TBI	
Alcohol	5
Narcotics	1
History of diseases	
Diabetes mellitus	2
Heart diseases	3
Hypertension	1
EP	2
Neurological diseases	6
Outcome	
GOS 1 = Dead	5
GOS 2 = Vegetative state	0
GOS 3 = Severe disability	8
GOS 4 = Moderate disability	10
GOS 5 = Good recovery	5

The CSF-Aβ (1–42) increased stepwise on day 3 vs. day 0–1 (573 %, $p \leq 0.01$), on day 4 vs. day 0–1 (+855 %, $p \leq 0.001$), on days 5–6 vs. day 0–1 (1173 %, $p \leq 0.001$), then CSF-Aβ(1–42) levels dropped on days 7–11 vs. day 0–1 (872 %, $p \leq 0.001$) (Fig. 2A)

Contrary, the plasma-Aβ (1–42) level stayed unchanged after TBI, (Fig. 2B). There was a significant stepwise increase of the VCSF-α-sAPP level, peaking at days 7–11 (2033 % of day 0–1, $p \leq 0.01$). There was also a rise of VCSF-β-sAPP on days 5–6 vs. day 0–1 (159 %, $p \leq 0.05$) and on days 7–11 vs. day 0–1 (157 %, $p \leq 0.05$).

The patient material was evaluated according to *APOE* genotype. Eight patients were *APOE ε4* carriers, while 20 patients had no *APOE ε4* allele. There was no significant change in maximal VCSF-Aβ(1–42) level between *APOE ε4* carriers (145 [93–178], n = 8) and *non-APOE ε4* carriers (158 [74–203], n = 20). Neither was there any significant difference in VCSF-α-sAPP or VCSF-β-sAPP change (data not shown).

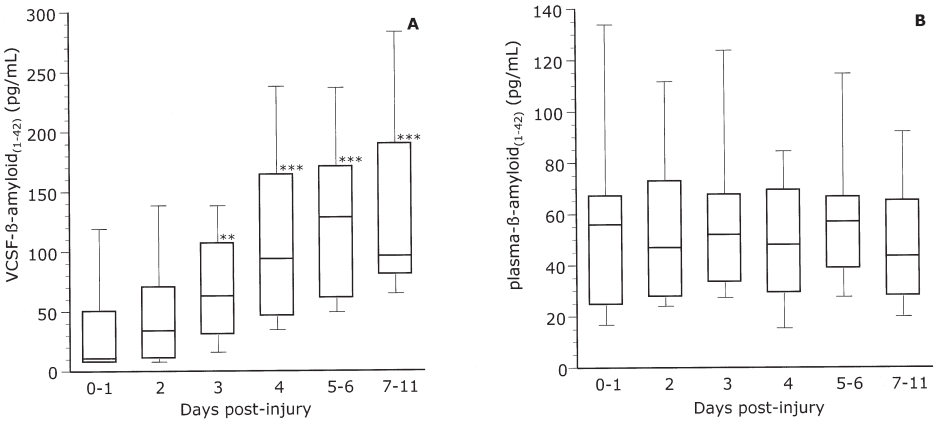


Fig. 2A+B *The β-amyloid(1–42) (Aβ(1–42)) levels (pg/mL) in ventricular cerebrospinal fluid (VCSF) (A) and blood plasma (B) from 28 patients on days 0–1, 2, 3, 4, 5–6, and 7–11 after traumatic brain injury. The lower, upper and line through the middle of the boxes correspond to the 25th percentile, 75th percentile and median respectively. The whiskers on the bottom extend from the 10th percentile and top 90th percentile. Several samples from the same patient are represented on days 0–1, 5–6, and 7–11. Significance levels * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$-elevated levels compared with day 0–1.*

Paper II

Fiftysix patients with TBIs were initially included, but unstable ICP only allowed ventricular CSF (vCSF) collection in 39 patients. These patients (women: n = 9; men: n = 30), with a median age of 49 years (range 16 to 82 years), are those presented.

Among all patients 3 patients had epilepsy, (including 1 operated for meningioma), and 3 patients had a history of other neurologic diseases like TIA, stroke or polio. Thus, the remaining 33 patients had no history of neurologic disorders before TBI. At admittance, in 8 patients ethanol and in 3 narcotics could be measured in the blood, respectively. The remaining 28 patients were drug free when exposed to the trauma. However, 2 of these patients had a previous history of alcohol addiction. The initial trauma was traffic accident in 16 patients, fall accidents in 13 patients, and miscellaneous causes like assault in 10 patients. Twenty-six patients had an isolated TBI, whereas in eight cases, other fractures than in the skull could also be detected. Finally, in five patients, internal organs were traumatized in combination with TBI. (Table 9)

Table 9. Background data paper II

Paper II	Number
Men	30
Women	9
Age (years)	
Mean	46
Median	49
Range	16-82
Cause of trauma	
Road traffic accident	16
Fall	13
Miscellaneous	8
Assault	2
Type of trauma	
Isolated brain injury	26
Visceral	6
Multi-trauma	7
Measurable drug concentration at the time of TBI	
Alcohol	8
Narcotics	3
History of diseases	
Diabetes mellitus	1
Heart diseases	4
Hypertension	2
EP	3
Neurological diseases	6
Outcome	
GOS 1 = Dead	7
GOS 2 = Vegetative state	0
GOS 3 = Severe disability	12
GOS 4 = Moderate disability	14
GOS 5 = Good recovery	6

At hospital arrival, the patients' GCS scores ranged from 3 to 15; subdivided as GCS 3 to 4 (n = 6), GCS 5 to 6 (n = 11), GCS 7 to 9 (n = 9), GCS 10 to 13 (n = 7), and GCS 14 to 15 (n = 6). All patients had GCS <8 within 48 hours after trauma. After arrival to NICU, the patients were taken to surgery for placement of an intracranial ventricular catheter within 4 hours. The mean hospital stay was 18.9 days, and the hospital mortality was 15% (6/39).

The concentrations of CSF total tau on days 2 - 3 post-TBI correlated to morbidity and mortality at 1-year ($R2 = 0.17$, $R = 0.42$, $p < 0.001$).

Higher levels of CSF total tau (8,500 pg/mL, IQR 7,638) on days 2 -3 post - trauma was shown in non-survivals (GOS 1) 1-year post trauma, compared with survivors (GOS 2 - 5), with levels of 682 pg/mL (IQR 1,155, $p < 0.001$) (figure 1). Patients with bad outcome (GOS 1 - 4) had higher levels of vCSF total tau (2,580 pg/mL, IQR 7,443) *vs.* those with good outcome (GOS 4 - 5) (504 pg/mL, IQR 1,256) on days 2 - 3 ($p < 0.01$). (Fig. 3)

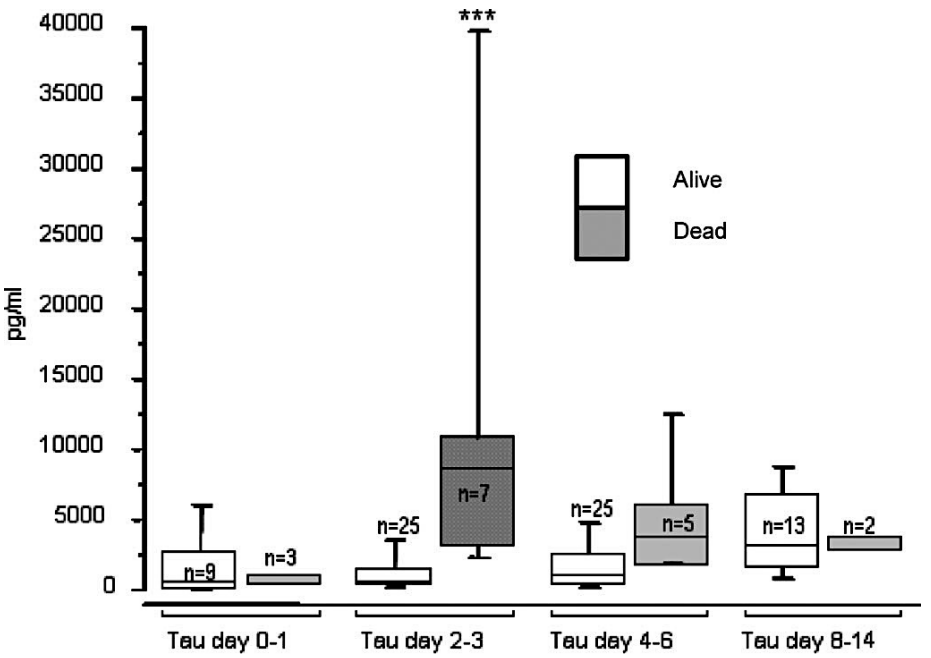


Fig. 3. *Tau levels in ventricular CSF days 0 - 14 in alive vs. dead patients related to 1-year outcome in traumatic brain injury patients. ***p < 0.001.*

Patients who died (GOS 1) demonstrated increased CSF total tau levels at all sample intervals, days 2 - 3 ($p < 0.001$), days 4 - 6 ($p < 0.001$), and days 8 - 14 ($p < 0.001$) compared to the levels (677 pg/mL (IQR 308)) in normal pressure hydrocephalus (NPH) patients (reference group).

TBI-survivors (GOS 2 - 5) had higher levels of CSF total tau *vs.* NPH - patients on days 4 - 6 ($p < 0.05$) and days 8 - 14 ($p < 0.01$). Patients with bad outcome (GOS 1 - 3) had increased levels of CSF total tau at all sample intervals, days 2 -3 ($p < 0.001$), days 4 - 6 ($p < 0.01$), and days 8 - 14 ($p < 0.001$) compared to patients with NPH. In patients with good outcome (GOSE 4 - 5) enhanced levels of CSF total tau could be detected on days 8 - 14 ($p < 0.001$).

One-year GOS outcome demonstrated that in the 39 patients included, 51% (20/39) had a good outcome (GOS 4-5), whereas 49% (19/39) had a bad outcome (GOSE 1 - 3). The 1-year mortality (GOS 1) was 18% (7/39).

With ROC, we detected a sensitivity of 100% and a specificity of 81.5% for 1-year mortality at a vCSF total tau level > 2,126 pg/mL on days 2 - 3 (table). The calculated AUC was 0.934. (Fig. 4, table 9) The specificity, sensitivity, and LR+ of different cut-off levels for mortality of vCSF total tau are depicted in the table. Patients with bad outcome (GOS 1 -3) had a cut-off level of CSF total tau of>702 pg/mL, with a sensitivity of 83.3% and a specificity of 69%, with a calculated AUC of 0.814 (Fig. 5)

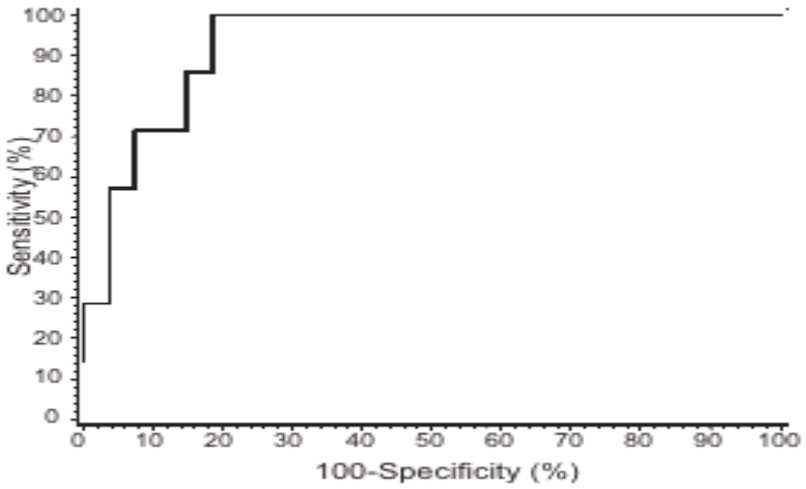


Fig. 4. *Receiver operator curve for 1-year mortality. Calculated sensitivity and specificity for tau levels in patients with traumatic brain injury on days 2 - 3. Area Under Curve of 0.934.*

Table 10. Calculated specificity and sensitivity and positive likelihood ratio (LR+) of different cut-off levels of ventricular CSF total tau on days 2 - 3 in patients with traumatic brain injury.

Tau, pg/mL	Sensitivity	Specificity	LR+
11,060	28.6	96.3	7.71
8,500	57.1	96.3	15.4
4,040	71.4	92.6	9.6
3,760	71.4	88.9	6.4
2,938	85.7	85.2	5.8
2,126	100	81.5	5.4

LR+ is a calculated value of sensitivity (100 – specificity).

When investigating neurologic status with NIHSS and Barthels scale 1-Year post trauma, no correlations were found.

Finally, throughout the study, there were no detectable levels of total tau in serum.

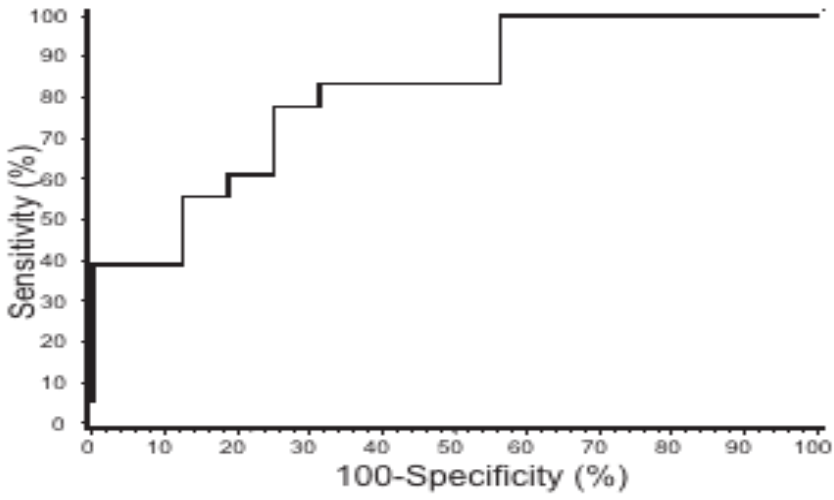
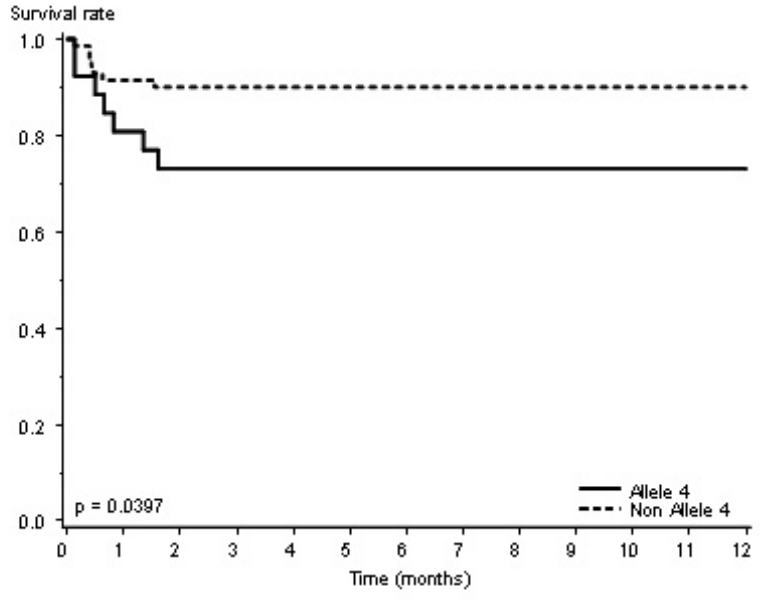


Fig. 5. *Receiver operator curve for 1-year bad (Extended Glasgow Outcome Scale [GOSE] score of 1 to 4) outcome. Calculated sensitivity and specificity for tau levels in patients with traumatic brain injury on days 2 - 3. Area Under Curve of 0.814*

Paper III

This study included 96 patients (women, n =26; men, n=70), with a mean age of 38 years (range 8–81). In this cohort 12 patients had a history of neurological diseases such as trans ischemic attack (TIA), brain tumour and epilepsy. Ten patients had a history of cardiovascular diseases like cardiovascular failure (n = 2), hypertension (n= 5), one patient with both atrial fibrillation and heart infarction, one patient with AV block III, and one with both angina and myocardial infarct. Twenty-six patients (27%) had blood contents of ethanol (n =19), narcotics (n=5) and benzodiazepines (n=2). Trauma aetiologies were fall accidents (n = 25), traffic accidents (n =

53) and miscellaneous causes (n = 18). Sixtyfour patients had an isolated TBI, while in 26 cases other fractures were found and finally in six patients traumatized internal organs in combination with the TBI were found. (Table



11)

Fig. 6. *Kaplan–Meyer figure expressing mortality in the whole patient cohort during the first year after trauma, showing worse outcome in patients with APOE allele ε4, vs. patients lacking APOE allele ε4, P<0.05.*

Table 11. Background data paper III

Paper III	Numbers
Men	70
Women	26
Age (years)	
Mean	38
Median	37
Range	8-81
Cause of trauma	
Road traffic accident	53
Fall	25
Miscellaneous	11
Assault	7
Type of trauma	
Isolated brain injury	64
Visceral	6
Multi-trauma	26
Measurable drug concentration at the time of TBI	
Alcohol	19
Narcotics	7
History of diseases	
Diabetes mellitus	6
Heart diseases	5
Hypertension	5
EP	6
Neurological diseases	12
Outcome	
GOS 1 = Dead	14
GOS 2 = Vegetative state	1
GOS 3 = Severe disability	31
GOS 4 = Moderate disability	31
GOS 5 = Good recovery	19

At hospital admittance the patients were subdivided according to level of conscience into three groups: (1) GCS 3-4 (n =24), (2) GCS 5–8 (n=43) and (3) GCS>8 (n=29). The latter cohort deteriorated within hours to a GCS<9. No difference (P =0.26) was noted in allele distribution among patients in these groups.

Of the 96 patients included 26 patients (women, n =5 and men, n=21) did expressed the allele *APOE ε4* and seventy patients (women, n =21; men, n =49) did not express allele *APOE ε4*.

Patients with *APOE ε4* had higher mortality at 1-Year (P<0.05) with LR 12.69 (CI 1.05–6.93), but not at 30 days (P=0.14). The mean mortality age of patients at both 30-days and 1-Year was 54 years. All patients who succumbed were either <30 or >45 years of age. Neither in gender nor in genotype age by itself differed. (Fig. 6)

In females the 30-day and 1-year mortality was 14.3% (n=3) in patients without *APOE e4*, while none succumbed in the *APOE ε4* cohort. Differences in outcome between allele groups could be detected neither (P= 0.37) at 30-days nor at 1-year.

The impact of neurosurgical interventions on APOE genotype and outcome was explored, but no correlation was found (data not presented).

In male patients, 7 died of those expressing *APOE ε4* at 1-year, while 4 died of those not expressing *APOE ε4*. At 30 days, in male patients not expressing *APOE ε4*, 6.1% died (n=3). In males with *APOE ε4,* 23.5% (n = 5) died. At 1-year, 8.2% (n = 4) died in those not expressing the *APOE ε4*, while in those expressing *APOE ε4*, 33.3% (n=7) died.

The mortality in men with APOE e4 was higher at 1-year (P<0.02) with LR14.08 [confidence interval (CI) 1.34–12.48] and at 30-days (P<0.05) with LR13.88 (CI 1.02–14.87).

There was no correlation between APOE alleles and Barthel or NIHSS index.

Paper IV

Patients were included when fulfilling the inclusion criteria as described in the method section. During the inclusion period of two years, 222 TBI patients were treated at NICU. Seventythree of these patients were considered having sTBI. Out of these, 20 patients were further excluded because of lack of informed consent (n=4), not residing in Sweden (n=4) or the inability to take the first sample within 48 h (n=12). Thus, the remaining patients (n=53) were consecutively included, subdivided into men (n=43) and women (n=10) with an age range of 8-76 years, (mean 41 years). Nine patients died during the first year post-sTBI, leaving 44 surviving patients.

No correlation could be found between NIHSS, investigating neurologic status 1-year post trauma, and initial CSF maxGFAP or maxNFL levels. Further, there was no correlation between initial CSF maxGFAP or maxNFL levels and ADL, assessed by Barthel's Scale, 1-year post trauma**.** (Table 12)

Table 12. Background data paper IV

Paper IV	**Numbers**
Men	43
Women	10
Age (years)	
Mean	41
Median	41
Range	8-76
Cause of trauma	
Road traffic accident	28
Fall	16
Miscellaneous	8
Assault	1
Type of trauma	
Isolated brain injury	33
Visceral	4
Multi-trauma	16
Measurable drug concentration at the time of TBI	
Alcohol	14
Narcotics	3
History of diseases	
Diabetes mellitus	4
Heart diseases	4
Hypertension	2
EP	7
Neurological diseases	11
Outcome	
GOS 1 = Dead	9
GOS 2 = Vegetative state	0
GOS 3 = Severe disability	17
GOS 4 = Moderate disability	16
GOS 5 = Good recovery	11

CSF NFL

The CSF NFL levels correlated ($R_{s=}$ -0,47992 p=0,0003) to 1-year outcome (GOS 1-5)

Overall NFL gradually increased from days 0-2 to days 11-18. Significant differences were found between days 0-2 (1,353 ng/L) *vs.* days 6-8 (7,030 ng/L; p=0.018) and between days 3-4 (2,920 ng/L), *vs.* days 6-8 (7,030 ng/L; p<0.0001) and finally between days 6-8 (7,030 ng/L) *vs.* days 11-18 (15,870 ng/L; p=0.014). (Fig. 7)

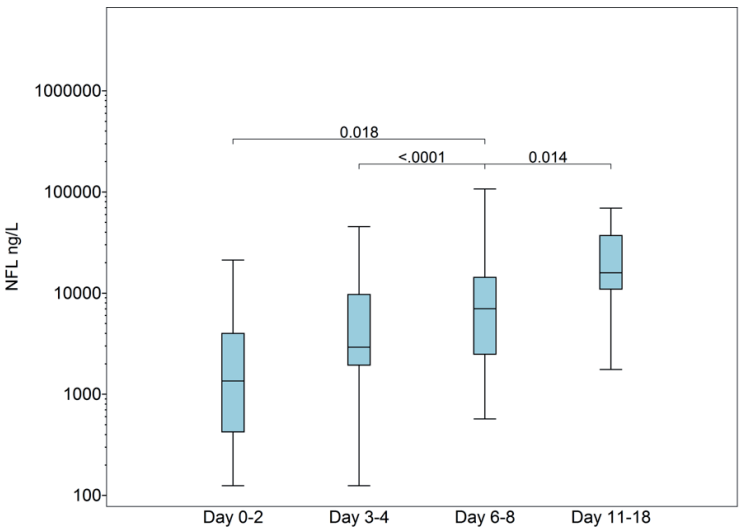


Fig. 7. *Cerebrospinal fluid concentrations of neurofilament light (NFL) were measured in patients on days 0-18 after severe traumatic brain injury (sTBI). CSF NFL gradually increased from days 0-2 until days 11-18. Significant differences were found between days 0-2 (Median 1,353 ng/L) vs. days 6-8 (7,030 ng/L; p<0.02) and between days 3-4 (2,920 ng/L), vs. days 6-8 (7,030 ng/L; p<0.0001) and finally between days 6-8 vs. days 11-18 (15,870 ng/L; p<0.02). Line within box is median. Whiskers is min and max. P-values, Wilcoxon Signed Rank test for paired observations, are based on log10 values of NFL ng/L.*

During the initial post-traumatic days CSF NFL levels during the investigated period, correlated to 1-year outcome (GOS 1-5; p=0.0003). After dichotomizing the results into dead (GOS 1) *vs.* alive (GOS 2-5) CSF maxNFL (OR 27.18; (CI 2.65-279.12), p=0.0054 AUC=0.86, (CI 075-0.97)) concentrations were significantly increased in patients who succumbed. The CSF maxNFL levels in the group that died (44,370 ng/L) were higher than in the group of survivals (6,205 ng/L; p=0.0007). (Fig. 8)

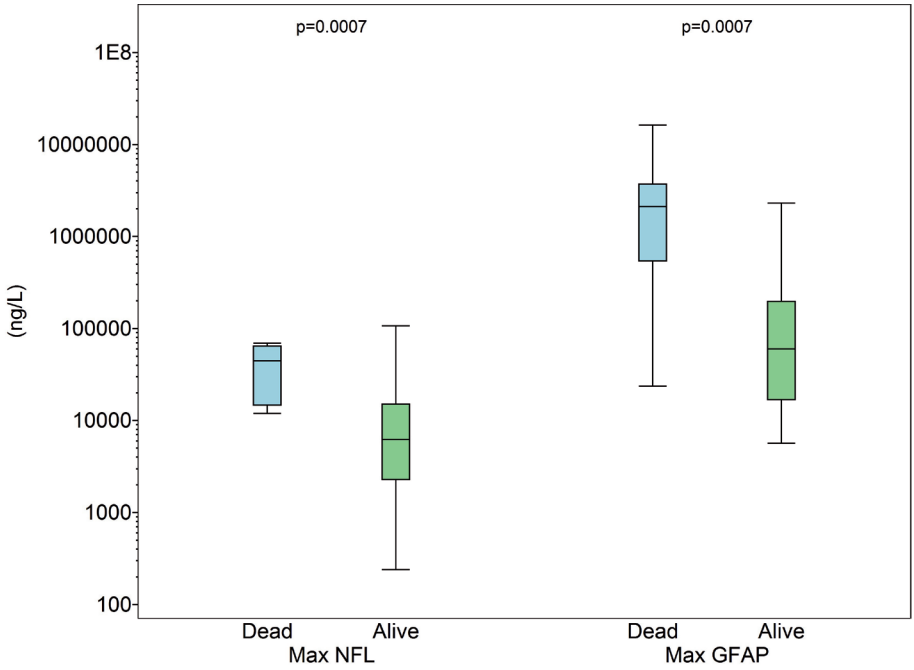


Fig. 8. *When dichotomizing the group into dead and alive, the succumbing patients had higher CSF maxNFL levels (44,370 ng/L) and CSF maxGFAP (2,116,000 ng/L compared to the patients who survived (CSF maxNFL 6,205 ng/L; p=0.0007) and (CSF maxGFAP 59,860; p=0.0007). Line within box is median. Whiskers is min and max.*

When subgrouping into days, non-survival patients (GOS 1) had higher levels of CSF maxNFL compared to those surviving (GOS 2-5), measured days 0-2 (p=0.0229), days 3-4 (p=0.0016), days 6-8 (p=0.0031), and on days 11-18 (p=0.0172). (Fig. 9)

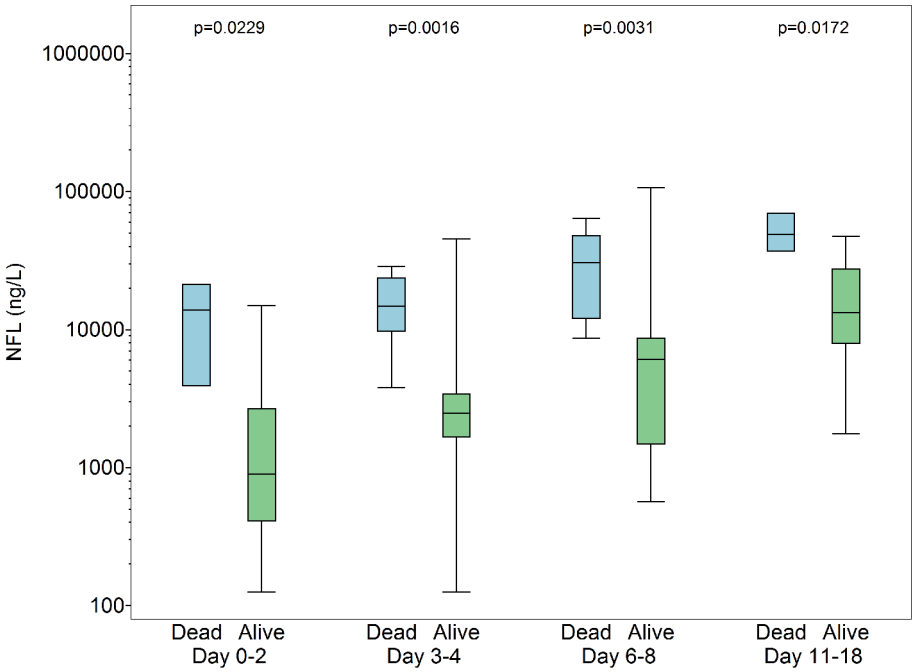


Fig. 9. *TBI patients who succumbed within 1-year after trauma (GOS 1) had higher levels of CSF maxNFL compared to those surviving (GOS 2-5), measured days 0-2 (p=0.0229), days 3-4 (p=0.0016), days 6-8 (p=0.0031), and on days 11-18 (p=0.0172). Line within box is median. Whiskers is min and max.*

All patients with CSF maxNFL concentrations below 12,000 ng/L survived as demonstrated in Receiving Operating Curve (ROC), (AUC= 0,861). (Fig. 10, Table 13)

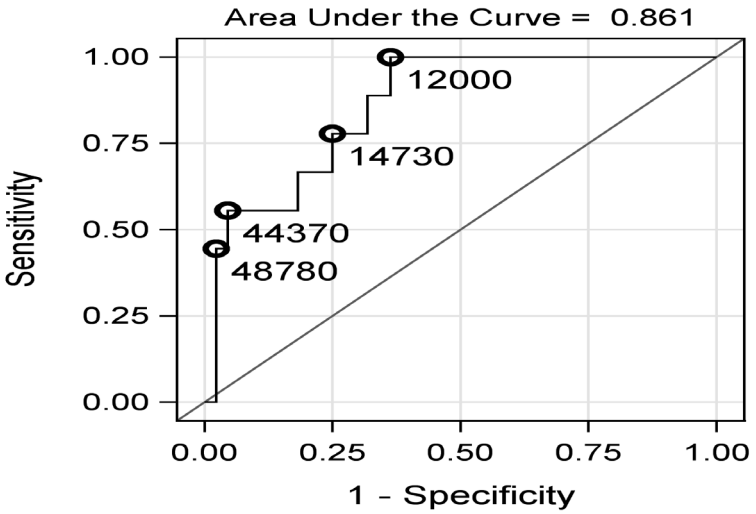


Fig. 10. *Receiver operator curve for 1-year mortality. Calculated sensitivity and specificity for CSF maxNFL levels in patients with sTBI.*

Table 13. Calculated specificity and sensitivity and positive likelihood ratio (LR+) of different cut-off levels of CSF max NFL over all.

NFL ng/L	Sensitivity	Specificity	LR+
48 780	44.4	97.7	19.56
44 370	55.6	95.5	12.22
14 730	77.8	75.0	3.11
12 000	100	63.6	2.75

When dichotomizing the results into bad outcome (GOS 1-3) and good outcome (GOS 4-5) CSF maxNFL in patients with bad outcome was higher (OR 4.19; 2.87-4.84); p<0.0016) compared to CSF maxNFL in patients with good outcome.

When subgrouping the groups into days the patients with bad outcome had higher levels of CSF maxNFL compared to those with good outcome days 0-2 (p=0.003) but there was no difference between the groups days 3-4 (P=0.1560) and days 6-8 (P=0.2548) and days 11-18 (P=0.0875).

Thus, we found higher concentrations of CSF maxNFL over the period in patients with bad outcome (15,445 ng/L) *vs.* patients with good outcome (3700 ng/L; p=0.0016).

CSF GFAP

The CSF GFAP levels correlate ($R_{s=}$ -0,42902 p=0,0013) to 1-year outcome (GOS 1-5)

Overall initial CSF GFAP levels on days 0-2, (median 36,622 ng/L), ranged from 30 ng/L to max 3,717,000 ng/L. CSF GFAP levels peaked on days 3 - 4 (100,000 ng/L, (range; 1,630 ng/L to 10,415,500 ng/L)) and then declined on days 6 - 8 (23,465 ng/L; (range; 1,830 to 2,234,000 ng/L); p=0.0001) and further declined on days 11-18 (8,560 ng/L; (range; 1,355 to 119,200 ng/L); compared to both days 3 - 4 (p=0.0078) and days 6-8 (p=0.037). (Fig. 11)

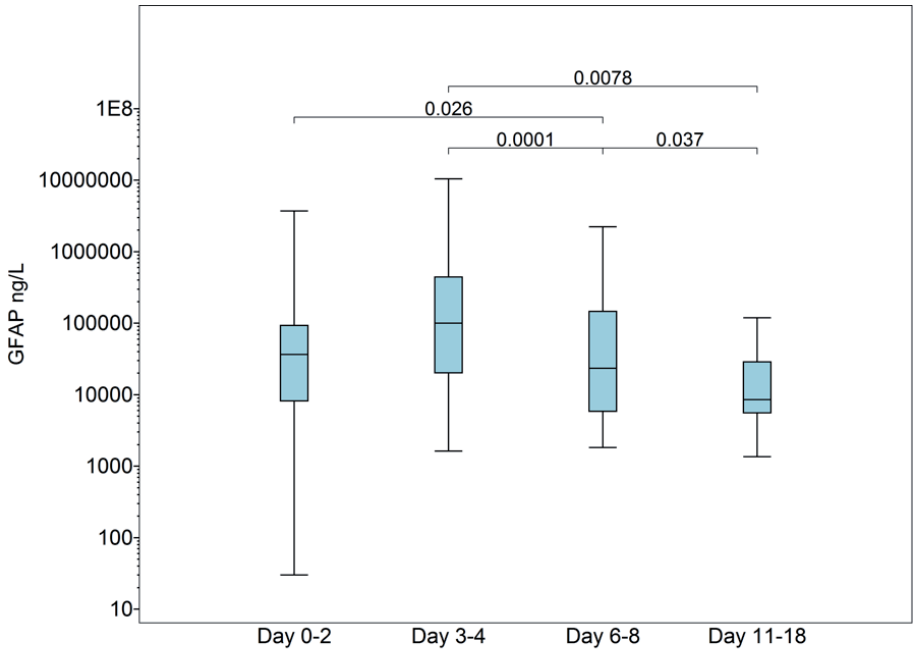


Fig. 11. *TBI patients who succumbed within 1-year after trauma (GOS 1) had higher levels of CSF maxGFAP compared to those surviving (GOS 2-5), measured days 0-2 (p=0.0229), days 3-4 (p=0.0016), days 6-8 (p=0.0031), and on days 11-18 (p=0.0172). Line within box is median. Whiskers is min and max. P-values, Wilcoxon Signed Rank test for paired observation, are based on log10 values of GFAP ng/L.*

After dichotomizing the results into dead (GOS 1) *vs.* alive (GOS 2-5) (OR 8.35 (CI 2.27-30.71) p=0.0014; AUC 0.86), CSF maxGFAP concentrations were significantly increased in patients who succumbed. (Fig. 8)

The CSF maxGFAP concentrations over the entire period in the group dying (GOS 1; 2,116,000 ng/L) were higher than in the group surviving (GOS 2-5; 59,860 ng/L; p=0.0007). (Fig. 8)

All patients with CSF maxGFAP concentrations below 23,740 ng/l survived as demonstrated in ROC, AUC=0.861. (Fig. 12, Table 14)

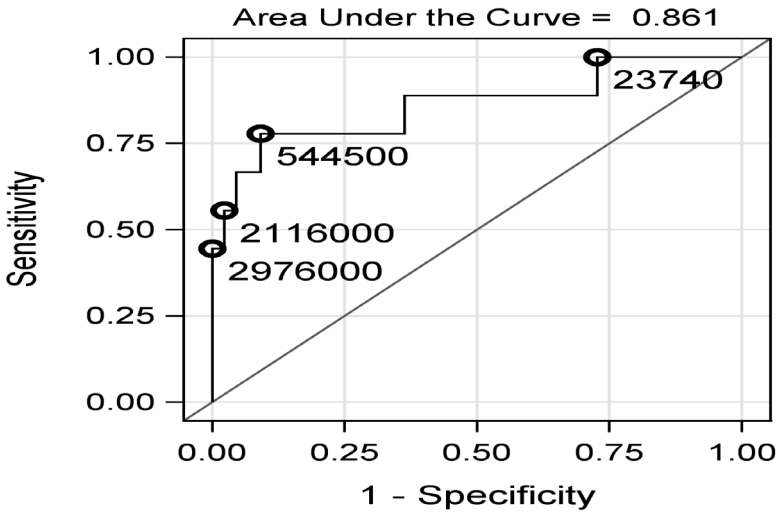


Fig. 12. *Receiver operator curve for 1-year mortality. Calculated sensitivity and specificity for CSF maxGFAP levels in patients with sTBI.*

Table 14. Calculated specificity and sensitivity and positive likelihood ratio (LR+) of different cut-off levels of CSF max GFAP during the investigated period. LR+ is a calculated value of sensitivity (100-specificity)

GFAP ng/L	Sensitivity	Specificity	LR+
2 976 000	44.4	100	
2 116 000	55.6	97.7	24.44
544 500	77.8	90.9	8.56
23 740	100	27.3	1.38

When subgrouping into days, non-survival patients (GOS 1) had higher levels of CSF maxGFAP compared to those surviving (GOS 2-5), measured days 0 - 2 (Median 1,118,000 *vs.* 21,240; p=0.0114), days 3 - 4 (Median 1,325,400 *vs.* 54,205; p=0.0208), and on days 6 - 8 (Median 312,125 *vs.* 19,805; p=0.0134), but not on days 11-18 (Median 28,820 *vs.* 6,730; p=0.0969).

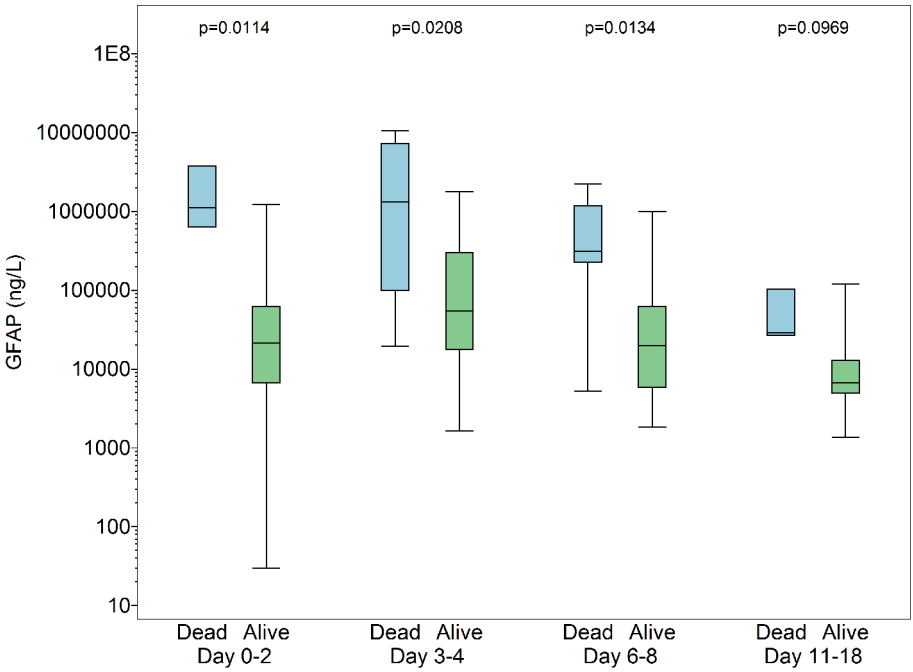


Fig. 13. *TBI patients who succumbed within 1-year after trauma (GOS 1), had higher levels of CSF maxGFAP compared to those surviving (GOS 2-5), measured days 0-2 (p=0.0114), days 3-4 (p=0.0208), days 6-8 (p=0.0134), but not on days 11-18 (p=0.0969) after trauma. Line within box is median. Whiskers is min and max.*

DISCUSSION

General remarks

Clinically, the neuro-intensivist neurologically examines the patient at NICU admission. Further, CT scan investigations of the brain are made and catheters may be placed extra- and intracranially to record ICP changes as well as metabolic changes. However, none of them give simple and robust patient outcome prognostication. Therefore, there is an urgent need for simple blood or CSF samplings to longitudinally discover patient deterioration and to early prognosticate outcome for rehabilitation purposes as well as giving information to next-of-kin.

Thus, this thesis includes four papers all reflecting genetic and biochemical methods of predicting outcome in patients with severe traumatic brain injury. Our primary hypothesis was that concentrations of biochemical neuromarkers (BNMs), collected early after a sTBI, by themselves correlate to patient outcome at 1-year. If so, we speculated to find concentration cut-off levels for mortality, survival, good and bad outcome. Finally, we speculated to find connections between BNMs and/or genetic variables associated to Alzheimer's disease (AD) with those found at sTBI.

Our result proved the primary hypothesis to be true, but we could not find significant cut-off levels, as hypothesized, for some BMNs. Further, we found connections between AD BNMs and sTBI.

sTBI´s impact on society

Historically, only about 10% of the traumatic brain injuries are severe (sTBI). Thus, only a small minority of TBI patients are called sTBI, but amongst young adults and children sTBI is the most common cause of increased morbidity and mortality. (Tagliaferri *et al.* 2006) These facts highlight the importance sTBI and its treatments. Placing the included patients into this scenario we found that they had an median/mean age of around 37-38 years and subsequently a successful treatment of these patients is utterly important for society and thus for the healthcare system.

Study design

To optimize the patient cohort and variability, the inclusion criteria were strict. Thus, patients with a sTBI were included only if they have a GCS < 9 (RLS >4) when admitted to NICU, or within 48 h post-trauma. Further, the patients had to be artificially ventilated and tracheal intubated within the same time-interval. Also, the patients had to reside permanently in Sweden to allow for the 1-year neurological assessment. Finally, because CSF-collection was a prerequisite for study enclosure, an indwelling intra-ventricular catheter had to be placed.

With these strict inclusion criteria the study size itself needs some clarifications. In our hands, as in many others, utilizing the Lund Concept of TBI treatment (LC) the overall 1-year mortality was between 15-20%**.** It was difficult to make a pre-study power analysis referring to our hypothesis, but we speculated that patients with high concentrations of neuromarkers had an increased mortality rate of 30%, while those with low concentrations had a mortality rate of 10%. When analyzing the numbers of historically admitted patients to our tertiary NICU, we found that about 100 patients yearly had a sTBI. But, as our inclusion criteria were more strict than just having a sTBI, we speculated that only 50% of patients could be included, an assumption that proved to be true. Thus, during this 2-year inclusion period we had 200 patients initially being considered for inclusion. However, as suspected 50% of the sTBI-patients did not fulfil the study criteria, leaving an inclusion group of around 100 patients, being the patient-cohort to be studied.

A comment on this patient-cohort seems appropriate, where the median age was 38 years and >70% of patients were men. To obtain a large inclusion group we included patients from the age of 7 with no upper limit age-wise and therefore the age range was from 8-81 years.

As some patients were elder, we also explored their previous medical history revealing that a third of the patients had a history of diabetes mellitus, hypertension, heart diseases, epilepsy and/or neurological diseases.

Nearly two thirds of patients had an isolated head injury, while the remaining cohort had other injuries *i.e.* they were multitrauma patients. This fact influenced the choice of biochemical neuromarkers, where we only analyzed those being highly specific to the brain, omitting for example S-100B having at least partial extracranial origin. (Nylen *et al.* 2006)

The majority of cases (> 50%) were involved in traffic accidents and between 20-30% of the patients had alcohol or drugs "on-board" when admitted to hospital. These facts are not different from other investigations. (Tagliaferri *et al.* 2006)

The entity sTBI develops over time. Thus, patients may be awake post-trauma, and then deteriorate within the next 48 hours to a GCS <9. This notion was evident in our studies were up to a third of patients had a GCS> 9 at the primary investigation, then deteriorating to a GCS <9, this being an inclusion criteria.

The mechanism for this phenomenon is unclear, but there is a general agreement that cerebral edema as well as contusions size-wise increase over time, climaxing on days 2-5 post-trauma, inducing an ICP increase. (Stocchetti *et al.* 2007), (Stein *et al.* 2013) In contrast, some patients become deeply unconscious at ictus without virtually any cerebral CT scan findings. We now know, from MRI investigations, that an "overstretching" of axons may be the cause of this deep coma, called diffuse axonal injury (DAI)**.** (Li *et al.* 2009)

Lund Concept of Brain Trauma Treatment

It was not the scope of the present investigations to change treatment paradigms of sTBI. However, as we utilize the Lund Concept (LC) for treating sTBI in Sweden, (Asgeirsson and Grände 1994), (Grande *et al.* 1997), while virtually all other centers utilize the Brain Trauma Foundation Guidelines (BTFG), it seems appropriate to briefly discuss LC.

The LC originates from a theoretical notion based on experimental physiological and pathophysiological principles of circulation in a "closed room" as the skull is. From these experimental investigations a simulation of brain perfusion, regulation and brain volume was achieved. (Asgeirsson and Grände 1994), (Grande *et al.* 1997) The principles described then clinically applied has been unchanged,(Asgeirsson and Grände 1994) (Asgeirsson and Grände), (Grande *et al.* 1997), (Grande 2011) except that dihydroergotamin, which reduces ICP via cerebral venous constriction is no longer used, because of possible side effects from peripheral vasoconstriction in high doses. However, at the time of our study this change of regime was at hand.

To our knowledge no large randomised clinical trials comparing TBI guidelines *i.e.* the LC *vs.* the BTFG have been performed. (Muzevic *et al.* 2013), (Koskinen *et al.* 2014) The Brain Trauma Foundation´s guidelines are now closer to LC today than 10 years ago, concerning cerebral perfusion

pressure (CPP) and the use of vasopressors. (Bullock *et al.* 1996) One difference is that the BTFG recommend that the ICP-reducing therapy should start when ICP is above 20 mmHg (Brain Trauma Foundation 2007),2010) whereas LC says that it should start immediately after arrival to hospital irrelevant of ICP, but having a sTBI. In LC patients are sedated continuously to reduce ICP and there is no need to daily explore GCS by wake-up evaluations, as it is common in the BTFG. (BTFG 2010)

Non-BNM variables

GCS/RLS

Prior to the evolution of adequate measurable neuromarkers neurosurgeons and –intensivist focused on physiological parameter and CT scanning in their search for factors correlating to outcome after a sTBI. The most common grading level of consciousness, the Glasgow Coma scale (GCS) includes best motor response, best verbal response and eye opening, with numerical values from 3 (no response) to 15 (fully alert).(Teasdale and Jennett 1974). In Sweden we use the RLS scale, a synopsis of the motor component of GCS. (Starmark *et al.* 1988) In our patients a RLS >4 was clinically equal to GCS <9, although GCS was calculated as part of the study design. Studies correlating low GCS to outcome have been published. (Lee *et al.* 2014) However, clinical knowledge states that the initial GCS may not always be the most important parameter as some patients with initially virtually normal GCS, also noted in studies I-V, may develop brain edema deteriorating on days 2-5. In contrast, patients with DAI may have normal ICP but very low initial GCS, as noted in a few patients in our patient cohort. Thus, initial GCS may prognosticate longterm outcome, but is far from valid in all cases. This was also clearly noted in our studies where about one third of patients were awake *i.e.* a RLS <4 at initial evaluation at primary hospital, then deteriorated within 48 h to become unconscious *i.e.* RLS>4.

Age

Age is an independent negative predictive factor for outcome in TBI patients, where both increased mortality and morbidity is noted in TBI patients > 65 years old. (Mosenthal *et al.* 2002) Age was an independent negative factor for outcome in our study population *i.e.* elder patients had relatively higher mortality. However, as both mean and median ages in the studies were around 40 years, the age predictively was low. An interesting finding appeared when scrutinizing our results; all patients dying were either < 30 or >45 years of age. When speculating on this fact it may be assumed that

younger persons have a larger brain volume, then decreasing by age. Thus, ICP increases more by a small volume loading like contusion expansion, as these brains have a lower compliance. In contrast, elder persons have smaller brain volume, but concomitant diseases like heart and lung disorders. Another confounding factor which we have not explored in our material is the eventual neuro-protective effect of oestrogen produced by younger females. Further, aggressive neurosurgical interventions by unilateral and/or bilateral craniektomies are/were more utilized in younger persons, including our patient cohort**.** (Skoglund *et al.* 2005) Finally, in the patient cohort studied the majority of patients had neurosurgical operations evacuating hematomas and contusions, speculatively more common in younger patients. Conclusively, an explanation on why none died in the age bracket of 30-45 years may be because of a combination of all factors discussed, but this has to be further investigated in the future.

CT/MRT

Marshall classified CT grading in patients with TBI from I to IV, where I noted no pathological changes while IV had; 1) cisternal absence, 2) midline shift lesions, and 3) > 25 ml of contusion volume. (Lawrence F. Marshall 1991)

Our results demonstrate, by Marshall Classification (MC) of the initial CT scan, that grades II, III and IV had a third of patients in each subgroup. The MC is not precise as sTBI may result in different types of brain damage including focal contusions, intra- and extradural hematomas and diffuse axonal injuries (DAI). ((DeKosky *et al.* 2013)). Some of these injuries are not visible in computerized tomography (CT) scans during the first days after trauma. Magnetic resonance imaging (MRI) is a more precise method to identify these injuries including DAI, but the technique is not initially clinically applicable in these unstable/ unconscious patients. However, only a very few patients (<5) of those studied in studie I-IV, did not reveal any CT scan findings on the primary investigation *i.e.* being MC I.

Hypotension

Hypotension, *i.e.* systolic blood pressure <90 mmHg has been associated with higher mortality. (Chesnut *et al.* 1993) This was a concern in all our patients studied, where we kept ICP and CPP within the LC limits except in those dying in NICU. As the blood pressure according to both BTFG and LC treatment paradigms is kept at a minimum of normal values, (*i.e.*) a systolic BP > 120 mmHg, hypotension is not applicable as a risk factor in our patient cohort.

Neurological tools to examine outcome

When validating patients after a neurological insults like sTBI it is important to consider; 1) optimum time for outcome validation, 2) what tools to utilize, 3) number of evaluators and if they are blinded.

When designing the studies I-IV we designated the experienced neurologist in our group to do all these procedures, thereby excluding the personal bias factor.

When is the appropriate time post-ictus to evaluate outcome/neurology after a sTBI? What tools are adequate?

We decided to use the GOS scale, (see introduction), being constructed to be used after hospital discharge to examine post-trauma neurological and psychological disability. (Teasdale and Jennett 1974) We found that GOS was considered to be optimal at 1 year. (Wilson *et al.* 1998) This notion was later confirmed by Corral and co-workers. (Corral *et al.* 2007) They could demonstrate that particularly those with higher GCS within the sTBI group *i.e.* GCS 6-8 improved significantly between 6 to 12 months. This improvement could also be noted in patients with initial lower GCS score of 3-5, although not so prominent. We plan to explore posttraumatic outcome at 10-15 years for the patient cohort, using telephone evaluated GOS examinations.

If a patient has intact intellectual brain function, but has a limb paresis the patient may be graded from GOS 3 to 5. To differentiate between these, we used the National Institute of Health Stroke Scale (NIHSS) recommended for neurological classification. (Brott *et al.* 1989) However, in our patient cohort we found no correlation between the BNMs concentrations and NIHSS at 1-year. This surprised us, but may be explained by the fact that sTBI is not a cerebrovascular stroke with complete occlusion of a cerebral artery giving necrosis to a complete anatomical area of the brain. Patients with sTBI did either recover mentally, and could by physical therapy recover physically, or they did not.

Further, to evaluate patient function more precisely we used Barthel´s Index (BI) evaluating mobility and personal care *i.e.* Activities of Daily Living (ADL). (Mahoney and Barthel 1965) Similarly, we found no correlation between BI and BNM concentrations, speculatively based on the same reasoning as above.

Conclusively, GOS at 1-year seems appropriate to evaluate outcome after sTBI.

Biochemical neuromarkers

To recapitulate from the introduction, the ideal BNM has been defined as being; 1) rapidly detectable in CSF and/or in blood post-trauma, 2) specific for brain tissues damage, 3) correlative to short- and longterm outcome, and 4) longitudinally measurable and correspond to treatment interventions. (Varelas *et al.* 2006)

The blood brain barrier (BBB) causes large differences in blood- *vs.* CSF concentrations of BNMs with several fold higher concentrations in the CSF. Therefore, highly sensitive bioassays, not always on hand, are needed to analyse brain derivate proteins in the blood. Conclusively, studies in ventricular or lumbar CSF may be advantageous, but the discussion of advantages of CSF *vs.* peripheral blood samples is ongoing. (Neher *et al.* 2014) The simplicity of collecting a venous or arterial blood sample makes the neuromarkers detectable in the blood more clinically relevant. There is also a trend in neurosurgery to utilize parenchymal instead of ventricular catheters arguing against the usage of CSF-BNMs in the clinical setting.

With this said, there are advantages of CSF-BNMs. First of all, the BBB function does not reflect BNM concentrations. Secondly, the concentration levels of BNM in the CSF is larger as the CSF volume is smaller than the blood volume, and finally timewise there may be a difference where increased levels of neuromarkers are noted in the CSF prior detectable to plasma levels. In papers I. II and IV CSF-BNMs were measured.

When studying sTBI-patients we have demonstrated in papers I, II and IV that CSF-concentrations of the BNM´s α-sAPP, β-sAPP, Tau, GFAP and NFL are all increased the first week after trauma. By longitudinal collection of CSF we have also found enhanced concentrations of the BNM´s Tau, GFAP and NFL during the first two weeks post-trauma. These increased levels then all correlated significantly to outcome at 1-year. Finally, in paper IV, we have demonstrated a correlation in patients expressing the allele *APOE e4* and enhanced 1-year mortality.

BNM non AD-connected

NFL

NFL is a selective neuromarker of cerebral neuronal damage. NFL is abundant in large myelinated axons and is an essential component of the neurofilament core. (Rosengren *et al.* 1994)

NFL, being a selective BNM of neuronal damage, seems to have difficulties to penetrate the BBB and has previously only been measured in the CSF as was done in this thesis. (Nylen *et al.* 2006) This in contrast to another neuronal BNM, neuron-specific enolase (NSE), a cytoplasmic glycolytic neuronal enzyme that has been measured in the blood. (Soetrisno *et al.* 2000)

However, very novel analytic technique has given us the opportunity to analyse blood levels of NFL. These analyses are on going but presently we do not have these results.

Thus, recent investigations analysing serum NFL have demonstrated this being a BNM of spinal cord injury and outcome, (Kuhle *et al.* 2014) and also a predictor of neurological outcome after cardiac arrest. (Rana *et al.* 2013)

CSF-NFL has not previously been investigated in sTBI. sTBI seems to be connected to AD, see this paragraph, but interestingly it seems that CSF-NFL is also connected to neurodegenerative diseases and may predict severity in survival in these diseases. (Shahim *et al.* 2014) Thus, future investigations may explore any connection between NFL and AD.

In this investigation we had CSF control values collected from patients normal pressure hydrocephalus. These values were 156 ng/l**.** (Tisell *et al.* 2004)

In paper IV we found that CSF-NFL increased over the first week and climaxed on days 11-18 post-trauma. These values started on days 0-2 at 1.353 ng/l to days 11 -18 of 15870 ng/l, *i.e.* 10 to 100 times higher than those seen in control patients. We then dichotomized the patients into dead (GOS 1) *vs*. alive (GOS 2-5) at 1-year we found significantly enhanced levels in those dying (20.770 ng/l) *vs.* those surviving (6.060 ng/l). We further divided the patients into good (GOS 4-5) *vs.* bad (GOS 1-3) outcome and found significantly higher CSF-NFL levels in those with bad (14.730 ng/l) than in those with good (3.440 ng/l) outcome

Interestingly, we found that all patients with max levels < 12.000 ng/l survived. Thus, a cut-off value during the first 2 weeks for survival after a sTBI has been found. The validity of this finding has to be confirmed by others and hopefully we will find similar cut-off levels when analysing the serum-NFL samples of the same patients in the near future.

GFAP

GFAP is an important part of the astroglial cytoskeleton. As previously described, a brain injury activates astrocytes responding with astrogliosis, followed by disintegration and CSF-GFAP leakage. (Vos *et al.* 2004)

GFAP can be measured in the blood, see below, but in the present investigation we wanted to explore the CSF-levels of GFAP during the first 2 weeks post-trauma in patients exposed to a sTBI and then correlate them to 1-year outcome. We hypothesized that CSF-GFAP was an early indicator (peaking after 3-4 days) of cerebral damage in patients with sTBI, a notion that turned out to be true.

We found in paper IV that CSF-GFAP levels peaked on days 3-5 (100.000 ng/l) then declining to days 11-18 (8.560 ng/l). Control patients (NPH-patients) had CSF-GFAP levels of 637 ng/l. (Tullberg *et al.* 1998) Thus, the concentrations found in our investigation were 12–150 times elevated than those normally found in the CSF.

We then dichotomized the patients into dead (GOS 1) *vs*. alive (GOS 2-5) and at 1-year we found significantly increased CSF-concentrations in those dying (2.729.164 ng/l) *vs.* those surviving (410.119 ng/l). We then further subdivided the patients into those with good (GOS 4-5) *vs.* bad (GOS 1-3) outcome and found significantly higher CSF-GFAP levels in those with bad (111.600 ng/l) than in those with good (4.548 ng/l) outcome.

Finally, we found that all patients with maxCSF-GFAP levels < 23.740 ng/l survived

We have previously demonstrated that elevated serum levels of GFAP are associated with bleak 1-year outcome in patients with TBI. (Nylén *et al.* 2006). In this investigation the serum levels did not exceed >25.000 ng/l, even among outliers. In the serum the GFAP concentrations peaked on days 0-2, then declining, while in the CSF the levels peaked on days 3-5, but with values 50-100 times higher than those found in serum. Our results also indicate that there might be a slight time-difference and evidently a concentration-difference when serum and CSF GFAP values peak post-trauma. The CSF-GFAP levels peaks similarly to those in the blood, found by previous studies. (Eng *et al.* 2000), (Nylen *et al.* 2006) The concentration on days 0-2 in the CSF was more than 50-fold higher than those found in the serum. The enormous concentration gradient between serum and CSF-GFAP concentrations found may have a two fold explanation; 1) CSF volume is a tenth of the blood volume, and 2) the BBB may have a variable leakage between serum and CSF. (Pelinka *et al.* 2004)

Interestingly, the CSF-GFAP levels stayed increased during the first week while serum GFAP decreased after a few days. Speculatively, the BBB closes after the initial insult combined with a continuous deterioration of astrocytes explaining this discovery.

Three research groups, including ours, have addressed sTBI and CSF-GFAP concentrations. The first study by Fraser et al. investigated CSF-GFAP concentrations in a small group of paediatric sTBI-patients, but found no outcome correlation. In the same study they found correlations between serum-GFAP concentrations (s-GFAP) and outcome. (Fraser *et al.* 2011)

The other study addressing CSF-GFAP was performed by Böhmer et al. (Bohmer *et al.* 2011) In a small pilot study (n=20) of sTBI in adult patients they found no difference in CSF-GFAP concentrations between those surviving and dying. In contrast, we have demonstrated a positive correlation between CSF-GFAP levels and outcome in adult sTBI-patients (paper IV).

Neurochemical markers in AD and connections between TBI and AD

How, and if repetitive mild/moderate TBI or even a single sTBI is associated with later development of AD/cognitive impairment is not fully investigated. Epidemiological evidence implicate TBI as such, as a risk factor for the later development of AD. (Lye *et al.* 2000) However, other studies fail to find this association. (Launer *et al.* 1999)

In longitudinal studies in patients with a previous history of TBI, an enhanced risk of AD development has been demonstrated. (Plassman *et al.* 2000) This notion is further confirmed in a retrospective autopsy study by Jellinger and co-workers who found a correlation between sTBI and the development of AD. (Jellinger *et al.* 2001)

Thus, there are connections between trauma to the head and the development of AD. We wanted to investigate this hypothesis by measuring BNMs connected to AD and to 1) investigate if they are at all found in the CSF after a sTBI, and 2) if so, if these concentrations correlate to outcome including neurology at 1 year.

Beta amyloid

In paper I we did not investigate outcome, but only the levels of the Alzheimer connected neuromarker β-amyloid. This was a preliminary study where we speculated that β-amyloid levels were enhanced in sTBI-patients *vs.* those noted in control patients and power-analyses were calculated from this assumption.

In consent with this speculation it has recently been found a connection between TBI and the AD connected factors tau and β-amyloid intracerebrally in patients several years after a single sTBI (Johnson *et al.* 2012)

Tajiri and co-workers investigated AD transgenic Mice *vs.* control rats, both exposed to a standardized TBI. (Tajiri *et al.* 2013) The results demonstrated an increase of extracellular β Amyloid deposits as well as cognitive impairment six weeks post-trauma in the AD transgenic Mice group.

Another study suggested that the biological marker of AD is Plasma Aβ(1–42) level, (Vanderstichele *et al.* 2000) although most other studies have shown unchanged plasma-Aβ(1–42) levels in AD compared with healthy controls. In our previous study we have demonstrated that plasma-Aβ (1–42) levels were unchanged after TBI, but that CSF-Aβ(1–42) levels after injury increased. (Olsson *et al.* 2004)

These findings support the suggestion that plasma-Aβ(1–42) does not reflect Aβ metabolism in the brain and thus is not likely to be a biological marker of AD or TBI. Further, the increased levels of CSF-Aβ(1–42) we found after sTBI implies that CSF-Aβ(1–42) may be a neuromarkers for TBI. (Olsson *et al.* 2004)

Tau

Tau is a small phosphoprotein found in the axonal compartments of neurons binding to microtubules promoting their stability and assembly. (Ohgami *et al.* 1992) Increased Tau levels are a sign of axonal injury and have been described in Alzheimer disease (AD). Supposedly TBI damage intracellular microtubules, with axonal injury and proteins like tau then being released into CSF. The axonal injury may mimic those seen in diffuse axonal injury (DAI), as previously discussed. Thus, tau may be a BNM candidate for DAI diagnosis. (Blennow *et al.* 2004)

In paper II we measured total tau including all sub-forms. This is more precise method and has been used by others and us. (Tisell *et al.* 2004) However, in this discussion we call total-tau for tau.

When discussing the tau results it is important to appreciate the fact that all CSF was collected from a ventricular catheter as lumbar CSF may give different results. (Blennow and Nellgård 2004)

CSF-tau found in control patients (NPH-patients (Tisell *et al.* 2004) was 677 pg/ml. In paper II we found that CSF-tau concentrations did correlate to 1-year outcome detected with GOSE. Thus, on days 2-3 CSF-(total) tau discriminated between dead (GOSE 1; 8.500 pg/ml) and alive (GOSE 2-8;

682 pg/ml). Interestingly, the values in patients surviving were very similar to those found in normal patients. At this time we also found cut-off levels of survival *i.e.* those having a CSF-Tau > 2.126 pg/ml died. When comparing CSF-tau concentrations in patients succumbing (GOSE 1) we found that these levels were increased *vs.* survivors on days 2 – 3 post-trauma. This difference was not noted from day 4 post-trauma.

Thus, it seems that patients dying within 1-year have a great early rise in CSF concentrations the first 2-3 days. This may indicate that more severely damaged patients have axonal injury (DAI-patients) measurable in the CSF during a small timeframe *i.e.* days 2-3 post-trauma.

Similarly, we also found on days 2-3 significant differences between those with good outcome (GOSE 5-8; 504 pg/ml) and those with bad outcome (GOSE 1-4; 2.580 pg/ml). When analysing these dichotomized cohorts we observed that significant differences were only noted on days 2-3 between groups. But overall an increase of CSF- tau concentrations with time is noted and on days 8-14 higher values are noted in patients with both good and bad outcome.

In another small retrospective study correlating CSF-tau to in-house mortality no difference between groups could be found. (Franz *et al.* 2003) In an experimental study with mice a TBI also induce enhanced levels of CSF-tau. (Genis *et al.* 2000) These values were more variable by time than those found in our study.

The connection between AD and tau is interesting. (Corkin *et al.* 1989) One of the most prominent neuro-pathological components of AD diagnosis is tangles composed of hyper-phosphorylated tau. Another protein, β-amyloid is also connected to AD promoting enzymatic cleavage by caspases of tau. The caspases are connected to apoptosis. (Blomgren *et al.* 2001) Thus, the increased tau noted in our investigation may reflect an intra-cerebral apoptosis induced by the sTBI.

Tau has previously not been measurable in blood samples. However, recent developments in ultrasensitive assays have allowed analysis of Tau in plasma, and pilot studies suggest a Tau increase after mild TBI and ischemic brain damage detectable in blood. (Randall *et al.* 2013), (Shahim *et al.* 2014) (Rosén *et al.* 2014)

Thus, penetration of the BBB by Tau has been demonstrated in very novel investigations. When the present study was performed we could not detect

any levels of Tau in the serum as the technique at the time did not allow detection of ultralow levels of tau, although they are now possible. However, we are presently repeating the serum investigations in the same patient cohort, but the results are not yet available. Hopefully, these results will confirm the CSF data in paper II and blood sampling will in the future simplify the usage of tau as BNM for sTBI.

Apo E

Briefly, Apolipoprotein E (ApoE = protein, *APOE = gene*) is synthesized by brain astrocytes binding to specific neuronal receptors. It then directs lipid transportation within the brain/CSF and assists neural transmission. (Mauch *et al.* 2001) In humans, 3 alleles of the *APOE gene (ε2, ε3, ε4)* code 3 protein isoforms (ApoE 2–4). *APOE*, particularly *APOE ε4*, promotes amyloid formation and is connected with Alzheimer's disease (AD). (Peacock and Fink 1994), (Lambert *et al.* 2005) There has been a connection of TBI and *APOE e4*, although it is unclear. (Chiang *et al.* 2003) and therefore the study in Paper II was initiated to evaluate whether the APOE genotype influences short- (30 days) and/or long-term (1-year) neurological outcome in a patient cohort of sTBI, treated in a NICU. We hypothesized that patients with *APOE e4* had worse outcome after sTBI than those without this allele.

We included 96 patients (women, n=26; men, n=70) and blood was collected during their NICU-stay to analyse *APOE allele* variations. If the patient had at least one *ε4* in the pair (heterozygote, like *ε4/ ε3*) the patient was characterized as expressing *APOE ε4*. The results demonstrated that 70 patients (women, n=21; men, n=49) did not express *APOE ε4*, while 26 patients did (women, n=5; men, n=21). Thus, 27% of the patients had *APOE e4* being in concert with the general southern Swedish population expressing *APOE ε4* in 28%. (Qiu *et al.* 2004)

We recorded outcome at 30 days and at 1-year by GOSE, (previously described). The results demonstrated that when dichotomizing patients into dead (GOSE 1) *vs.* alive (GOSE 2-8), patients with the *APOE ε4 allele* had significantly increased mortality, but only at 1-year, than those without this allele. When subdividing patients into gender, the difference persisted in the male cohort, but not among females. The mean mortality age was 54 years, while the average study cohort age was 38 years.

First of all, the results conclude that 30-days are a too short period for outcome evaluations. As previously discussed, even patients with very low GCS improve their status up until 1-year.

Secondly, the cohort of male patients was around 70% of the total number of patients, in-line with other investigations. (Tagliaferri *et al.* 2006) This could not explain the gender difference in outcome, when referring to *APOE genotype*. We assume that the female cohort was underpowered thus giving these disparate results. However, we found no mortality in female patients expressing *APOE ε4*, while 3 patients died in the *non-APOE ε4* group (average aged 59 years). When scrutinizing the mortality rate, we found ; 1) the average mortality age was 54, and 2) no mortality was noted in patients < 30 or >45. Years of age a far-fetched speculation connecting mortality age, gender and *APOE genetic* could be that pre-menopausal females have oestrogen neuroprotection and in these younger women the negative impact of *APOE genetic* is less prominent that the positive hormonal effects. This notion has been explored in *APOE* knock-out mouse where estrogen supplementation provided neuroprotection. (Horsburgh *et al.* 2002)

The effects of *APOE genetic* and TBI have been studied in a few other investigations. However, a mix of mild to severe TBI patients may explain the divergent results found. (Chamelian *et al.* 2004) Other studies have also divergent result probably because of smaller inclusion groups and/or variable trauma impact. (Jiang *et al.* 2006), (Alexander *et al.* 2007)

In paper II we have not measured CSF-levels of ApoE. Normally plasma concentrations are 10 times higher than those in the CSF. (Carlsson *et al.* 1991) It would be interesting to explore CSF-concentrations of ApoE in sTBI-patients.

The ApoE may play a role in the inflammatory response and neuronal repair following a TBI**.** (Lynch *et al.* 2002) However, it has been demonstrated that one of the genetic alleles *APOE ε4* is associated with neuropathological impairments like; decreased neurite outgrowth, cytoskeletal disruption, hyper-phosphorylation of tau, neuronal mitochondrial dysfunction and increased β amyloid concentration. (Huang 2010) (Mahley *et al.* 2006)

Conclusively, the presence of *APOE ε4* is disadvantageous to patients exposed to a severe sTBI and particularly in male patients this could render an enhanced treatment regime in the NICU.

CONCLUSION

In general our results show that CSF- Aβ (1-42), CSF-BSAPP, CSF-Tau, CSF-NFL and CSF-GFAP in patients after TBI are higher in CSF than in patients with NPH.

The levels of CSF-Tau, CSF-NFL and CSF-GFAP and patients with APOE allel $\varepsilon 4$ gene correlates to 1-Year outcome measured with GOS but not with Barthels or NIHSS.

The unchanged level of plasma Aβ (1-42) does not mirror Aβ metabolism in the CNS and therefore may not be a suitable marker for neither TBI nor AD.

The concentrations of CSF-Tau, CSF-NFL and CSF-GFAP are higher in patients who succumb over the period of two weeks after trauma, compared to those who survive.

Paper I

The unchanged level of plasma Aβ (1-42) does not mirror AB metabolism in the CNS and therefor may not be a suitable marker for neither TBI nor AD.

Reading the increase of CSF-Aβ (1-42) and CSF-BsAPP after sTBI the increase in CSF AB (1-42) indicates that AB expression may be reflecting a axonal damage in patients after TBI.

Paper II

CSF tau correlates to 1-year outcome measured with GOS but not with NIHSS or Barthels. The rise of CSF tau in TBI patients compared to patients with NPH suggests that Tau reflects axonal injury and that Tau may predict longterm outcome in sTBI

Paper III

Patients with *APOE allele ε4* have worse longterm outcome measured with GOS 1-year after TBI. These indicate that genetic predisposition may influence outcome after TBI.

Paper IV

CSF GFAP and CSF NFL concentration both increase significantly after sTBI and correlate to 1-year outcome measured with GOS. This may reflect neuronal and astrocytal damage in patients with TBI.

FUTURE PERSPECTIVES

The main results in this study were that BNM and genetic markers can supplement the clinician to predict long-term outcome in patients with sTBI. We have also shown that in some BNMs, a single CSF/blood sample during the first week post-trauma is sufficient to predict outcome. However, to capture secondary ischemic events sequential BNM concentration measurements during the first two weeks post-trauma is more appropriate. Further, we have shown that some BNMs were not detectable in serum when the analyzes in this thesis were performed. However, recent ultrasensitive ELISA techniques detect BNMs with 1000 time's higher sensibility. Thus, presently our research group has initiated serum investigations of Tau and other BNMs to explore if they correlate similarly to long-term outcome as we have demonstrated in the CSF. Therefore, routine BNM blood sampling in NICU and Trauma Centers may be available in the near future to be utilized in patients with severe sTBI. We speculate that BNM concentrations in the near future may predict long-term outcome, capture secondary ischemic events and guide treatment paradigms in this patient cohort.

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