Hormones, Biomarkers, Genetics and Prognostication of Patients Suffering Severe Traumatic Brain Injury

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The cover:
The Edwin Smith Papyrus is an Ancient Egyptian medical text on surgical trauma. It dates from about 1600 BCE. It describes 48 cases of injury. The treatise is systematically organized in an arrangement of cases, which begin with injuries of the head and proceed downwards through the body. The prognosis of each case is classified as one of three different verdicts: (1) favorable, (2) uncertain, or (3) unfavorable.
“No head injury is so serious that it should be despaired of nor so trivial that it can be ignored.”

Hippocrates 460 BC – c. 370 BC
Abstract

Severe traumatic brain injury (sTBI) is a significant cause of mortality and mobility worldwide. In Umeå University Hospital, at the department of Neurosurgery, patients with sTBI are treated by the Lund concept, which can be characterized as an intracranial pressure (ICP) targeted therapy.

In recent decades, there has been an increasing interest in trying to understand why some patients recover better and survive after sTBI, and why some do not. Also, improving the instruments of prognostication is becoming increasingly important both for relocating health resources and for the benefit of patients and relatives.

The main goal of the work described in this thesis was to explore factors influencing clinical outcome and improve the prognostication of outcome after sTBI. The ultimate goal is to improve the clinical outcome in patients suffering sTBI.

It has been proposed that the outcome after sTBI is influenced by genetic variability, including variability in apolipoprotein E (APOE). We therefore examined the relationship between the presence of APOE ε4 allele and the outcome. Except for 1 dichotomization of Glasgow outcome scale (GOS) at 3 months, the presence of the allele did not influence the outcome.

The biochemical markers of brain injury, S-100B and NSE, can be used to quantify the tissue lesion in sTBI. We investigated whether the levels of the biomarkers were associated with the APOE ε4 allele. Patients expressing the APOE ε4 allele had significantly higher levels of S-100B than non-ε4 subjects. The temporal course of S-100B differed between the APOE groups. Similar, but not statistically significant results were observed for NSE. The results suggest that variations in genetics have to be considered when interpreting the biochemical markers.

We also found that serum levels of S-100B and NSE were correlated with ICP max, CPP min, and radiological findings on brain CT quantified by CT scoring systems and that S-100B and NSE may predict mortality.

The pituitary gland is vulnerable for traumatic events. This may be reflected in acute hormonal deviations, which can influence the clinical outcome. We found dynamic changes in hormone levels after sTBI. A large number of the patients had low cortisol levels, which were not however associated with an unfavourable outcome. We also found that a preserved capacity to a mutable hormonal response, i.e. fast and strong repression of the pituitary-gonadal axis and a capacity to re-establish activity in the pituitary-thyroid axis, was associa-
ated with less severe injury according to CT-findings and to a more favourable outcome after sTBI.

It is concluded that the presence of the APOE ε4 allele did not indicate worse long-term outcome in our patient group. Patients expressing the APOE ε4 allele, had significantly higher levels of S-100B than non-ε4 subjects, indicating that in some cases the genetics have to be considered when interpreting the biochemical markers. The biomarkers were also correlated to intracranial pressure and radiological findings, and may predict for mortality at 3 months. Profound hormonal changes in the acute phase occur. However, low levels of cortisol are not associated with a worse clinical outcome.
List of original Papers


III The association of ICP, CPP and CT findings on the levels of S-100B and NSE in severe traumatic head injury and the prognostic value of the biomarkers. Olivecrona Z, Bobinski L, Koskinen LOD. Submitted.

## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE ε4</td>
<td>Apolipoprotein E (allele)</td>
</tr>
<tr>
<td>ApoE</td>
<td>Apolipoprotein E (protein)</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood Brain Barrier</td>
</tr>
<tr>
<td>BR</td>
<td>Bulk Release</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral Blood Flow</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CPP</td>
<td>Cerebral Perfusion Pressure</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebral Spinal Fluid</td>
</tr>
<tr>
<td>DAI</td>
<td>Diffuse Axonal Injury</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle stimulating Hormone</td>
</tr>
<tr>
<td>GCS</td>
<td>Glasgow Coma Scale</td>
</tr>
<tr>
<td>GFAP</td>
<td>Glial Fibrillary Acidic Protein</td>
</tr>
<tr>
<td>GH</td>
<td>Growth Hormone</td>
</tr>
<tr>
<td>GOS</td>
<td>Glasgow Outcome Scale</td>
</tr>
<tr>
<td>ICP</td>
<td>Intracranial Pressure</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like Growth Factor 1</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing Hormone</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean Arterial Blood Pressure</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>NSE</td>
<td>Neuronspecific Enolase</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver Operated Characteristics</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
</tr>
<tr>
<td>SHBG</td>
<td>Sexhormone-binding Globulin</td>
</tr>
<tr>
<td>sTBI</td>
<td>Severe Traumatic Brain Injury</td>
</tr>
<tr>
<td>tSAH</td>
<td>Traumatic Subarachnoid Haemotoma</td>
</tr>
<tr>
<td>T3</td>
<td>Triiodothyronine</td>
</tr>
<tr>
<td>T4</td>
<td>Thyroxine</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroidstimulating Hormone</td>
</tr>
</tbody>
</table>
Table of Contents

Background 1
   Epidemiology 1
   Traumatic brain injury mechanisms and brain volume regulation 1
   Brain volume regulation 2
   Prognostic factors and prognostication of clinical outcome 3
   Definition of Traumatic Brain Injury 4
Evaluation of clinical outcome after severe traumatic brain injury 7
   Treatment protocol 8
   Measuring the intracranial pressure 9
   Biomarkers for severe brain injury 9
   Genetics in severe brain injury 12
   Acute neuro-endocrine changes 14
Aims 17
Materials and methods 18
   Patients and general methods 18
   Treatment Protocol 19
   Monitoring 20
   Assessment of outcome 21
   Papers I-II 21
   Papers II-III 21
   Paper III 21
   Paper IV 22
   Statistics 22
Results 23
   Paper I 23
   Paper II 24
   Paper III 26
   Paper IV 28
Discussion 33
   Association of APOE allele to clinical outcome and biomarkers 34
   Relation of biomarkers to physiological parameters, CT-findings and outcome 37
   Neurohormonal status, physiological parameters, CT-findings and outcome 38
Conclusion 42
Summary of the Thesis 43
Sammanfattning på svenska 44
Acknowledgements 46
Grants 48
References 49
Background

Epidemiology
Worldwide, TBI is recognized as one of the major causes of death and disability (Ghajar, 2000). In the Nordic countries, about 200 per 100,000 inhabitants are admitted to hospital each year due to a head injury (Ingebrigtsen et al., 2000). The rate of TBI mortality varies from approximately 10 per 100,000 residents in Sweden, Denmark and Norway, to around 20 per 100,000 in Finland (Sundstrom et al., 2007b). The main causes of TBI are traffic accidents, falls, violence, and sport-related injury. The demographic groups at high risk for TBI include males and the elderly (Maas et al., 2008).

It is estimated that the incidence of traumatic brain injury will increase, mainly in low and middle-income countries (Menon and Zahed, 2009). In recent decades, there has been a decrease in traffic related mortality, which could be attributed to preventive measures such as the use of seat-belts, safer roads and cars etc. rather than improved medical treatment (Sundstrom et al., 2007b).

Traumatic brain injury mechanisms and brain volume regulation
TBI is a complex condition that can be caused by focal or diffuse primary injuries that may initiate complex secondary neurochemical processes that proceed over hours and days. The major secondary events include ischemia, excitotoxicity, mitochondrial failure, oxidative stress, oedema, inflammation and neuronal death (Miller et al., 1978, Miller and Becker, 1982), Figure 1.

![Figure 1. A schematic illustration of some of the events following brain injury](image)
Background

Brain volume regulation

One of the main factors influencing the final clinical outcome after TBI is high intra-cranial pressure (Miller et al., 1977, Narayan et al., 1982, Eisenberg et al., 1988, Marmarou, 1992, Jiang et al., 2002). In fact, it is not the high ICP itself but the consequences of high ICP that are detrimental for the patient. There are several causes of a raised ICP, such as contusions, haematomas, oedema, disturbance in CSF resorption and outflow and instability in the regulation of cerebral blood flow. The volume regulation of the brain is more exact than in most other organs in the body. This is essential as the brain is enclosed in a rigid skull and thus the maximum volume is given. Approximate volumes within the skull are: blood 100ml, parenchyma 1400ml, CSF 125ml and extracellular fluid 125ml. The regulation of the volume is mainly due to a strict fluid exchange over the capillaries of the brain, which are different from most other capillaries in the body. They are distinguished by the capillary membrane, which is permeable only to water and almost impermeable to other molecules, ions such as sodium and chloride, and proteins. Specific energy dependent pores and pumps transport molecules over the brain capillaries. The tight capillary membrane forms the blood-brain-barrier (BBB). After sTBI, the BBB is focally disrupted after TBI, and this enables other molecules to pass readily over the BBB (Moody, 2006, Alves, 2014). CSF is generated at quite a constant pace, so that the absorption of the CSF is an essential feature in the volume regulation of the brain. The principle for removing CSF is based on the Monro-Kellie doctrine giving space for haematoma or diffuse cerebral oedema etc (Monro, 1783, Kellie, 1824).

There is a physiological compensation mechanism striving to keep the ICP as normal as possible. However, some volume changes (such as oedema) disturb the balance. Thus, if a volume \( x \) increases, the blood volume (mainly venous blood) will redistribute and CSF resorption will increase to compensate. This equation is demonstrated as the pressure – volume curve (Fig 2). Initially, a relatively large increase in volume (CSF, blood, oedema) results in a rather small increase in ICP, but when the capacity of compensation is fully utilized, a relatively small increase in volume \( \Delta x \) will cause a large increase in ICP. This relationship can thus be expressed as \( \Delta V / \Delta P \) which is equal to the compliance. The treatment of a patient with sTBI must aim to keep the patient as far to the left on the curve as possible, which is to strive for a high compliance. This is achieved by surgically removing volume (haematoma, CSF, decompressive cranietomy, etc) when indicated, but also by treating the patient non-surgically, aiming to bring oedema under control, diminish the venous blood-pool and thus the ICP under control.
**BACKGROUND**

Figure 2. The relation of the intracranial volume and the ICP. The pressure – volume curve

\[ V_{\text{intracranial}} = V_{\text{brain}} + V_{\text{cerebrospinal fluid}} + V_{\text{blood}} \]

Several other factors may influence the ICP and should be taken into account during the treatment of sTBI. Such factors include e.g. the respiration (mainly \( CO_2 \)), central venous pressure, intra-abdominal pressure, fever and pain.

**Prognostic factors and prognostication of clinical outcome**

Despite a major improvement in the outcome of TBI in the acute setting, the assessment, therapeutic interventions and prevention of long-term complications remains a challenge. The challenges today are primarily related to a rapid diagnosis, identification of patient’s pathophysiological heterogeneity (such as specific gene alleles that may be involved in the patients regenerative capacity) and to limit the secondary injuries.

The clinical uncertainty regarding the probable outcome after traumatic brain injury (TBI) is summarized in the Hippocratic aphorism: “No head injury is so serious that it should be despained of nor so trivial that it can be ignored.” Doctor’s estimates of prognosis are often excessively optimistic, needlessly pessimistic, or incorrectly dubious (Perel et al., 2007, Honeybul et al., 2012). Despite great medical advances during recent decades, it is still impossible to foretell what the prognosis is in the individual patient, but intensive research in the last two decades has made some degree of prognosis, at least on a group level (Hukkelhoven et al., 2005, MRC CRASH Trial Collaborators et al., 2008, Steyerberg et al., 2008, Nelson et al., 2010, Nelson et al., 2012, Olivecrona and Koskinen, 2012a, Olivecrona and Olivecrona, 2013).
It is important to understand and further explore predictors of outcome. Among the most difficult tasks for a physician is to try to predict for a patient or their family the outcome after severe traumatic brain injury. For many individuals, the most pressing question becomes ‘Will my loved one (I) return to their (my) pre-injury level of functioning, or will there be a persistent disability? There is no prognostic factor or association of prognosis factors that with certainty can tell the treating clinician not to initiate treatment. The development of such a prognostic factor to facilitate the decision to treat or not to treat would be of great benefit. Prediction of outcome would not only facilitate answering questions like this, but also clinical management and allocation of health resources (Honeybul et al., 2011a, Honeybul et al., 2011b, Honeybul et al., 2012, Olivecrona and Koskinen, 2012a, Olivecrona and Olivecrona, 2013).

Only a few factors have been found to be robust when assessing outcome. These are correlations found on a group level, such as patient age, GCS, hypotension, pupillary abnormalities, ICP and mass lesions on CT (Chesnut, 1993, Servadei et al., 2000, Hukkelhoven et al., 2005, Maas et al., 2005, Butcher et al., 2007, Marmarou et al., 2007, Steyerberg et al., 2008, Husson et al., 2010). Even though there is little doubt of the importance of these features from a clinical experience, none of these factors alone, together or in clinical calculators, can prognosticate the outcome at the individual level (Olivecrona and Koskinen, 2012a, Olivecrona and Olivecrona, 2013). There are still debates about the precise nature of their connections and about precisely how the different features should be judged. In fact, some of these clinical elements have been questioned as good prognostic factors in sTBI (Brorsson et al., 2011).

**Definition of Traumatic Brain Injury**

TBI can be classified based on the basis of severity, anatomical features of the injury, or mechanism. Mechanism-related classification divides TBI into closed and penetrating head injury. A closed (also called non-penetrating, or blunt) injury occurs when the brain is not exposed. An open, or penetrating head injury, occurs when an object penetrates the skull and tears the dura mater, the outermost membrane surrounding the brain.

Brain injuries can also be classified as mild, moderate, and severe (Stein and Spettell, 1995). The most commonly used system for classifying TBI severity, the Glasgow Coma Scale (GCS) (Table 1), grades a person’s level of consciousness on a scale of 3–15 based on eye-opening, verbal and motor reactions to stimuli (Teasdale and Jennett, 1974). It is generally agreed that a TBI with a GCS of 13 or above is mild, 9–12 is moderate, and 8 or below is severe (Stein and Spettell, 1995). Similar systems exist for young children. However, the ability of the GCS grading system to predict outcome is limited.
The Swedish Reaction Level Scale (RLS) (Table 2) is a simpler scale than the Glasgow Coma Scale (GCS), but it is less often used outside Sweden (Starmark et al., 1988). However, the RLS is focused on the consciousness of the patient and is considered to have fewer pitfalls than the GCS. Furthermore, the motor scale of GCS, which has been suggested for use instead of the full GCS, much resembles the RLS scale. From a historical point of view it is interesting to note that a TBI scale resembling the RLS and GCS motor score was developed in Beirut, Lebanon already in 1975 (Haddad, 1978).

Systems also exist to classify TBI by its pathological features. Lesions can be described radiologically as extra-axial, (occurring within the skull but outside the brain) or intra-axial (occurring within the brain tissue). Brain tissue damage from TBI can be focal or diffuse, confined to specific areas or distributed in a more general manner, respectively. However, both types of injury are often found in the same patient.

<table>
<thead>
<tr>
<th>Response</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best Eye Opening Response</td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>4</td>
</tr>
<tr>
<td>To verbal command, speech</td>
<td>3</td>
</tr>
<tr>
<td>To pain only</td>
<td>2</td>
</tr>
<tr>
<td>No response</td>
<td>1</td>
</tr>
<tr>
<td>Best Verbal Response</td>
<td></td>
</tr>
<tr>
<td>Oriented</td>
<td>5</td>
</tr>
<tr>
<td>Confused, conversation</td>
<td>4</td>
</tr>
<tr>
<td>Inappropriate words</td>
<td>3</td>
</tr>
<tr>
<td>Incomprehensible speech</td>
<td>2</td>
</tr>
<tr>
<td>No response</td>
<td>1</td>
</tr>
<tr>
<td>Best Motor Response</td>
<td></td>
</tr>
<tr>
<td>Obeys commands</td>
<td>6</td>
</tr>
<tr>
<td>Localizes Pain</td>
<td>5</td>
</tr>
<tr>
<td>Withdraws in response to pain</td>
<td>4</td>
</tr>
<tr>
<td>Flexion in response to pain</td>
<td>3</td>
</tr>
<tr>
<td>Extension in response to pain</td>
<td>2</td>
</tr>
<tr>
<td>No response</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1. The Glasgow Coma Scale.
GCS = Best Eye Opening + Best Verbal Response + Best Motor Response i.e.
GCS = 3 – 15 (Teasdale and Jennett, 1974)
<table>
<thead>
<tr>
<th>Mentally Responsive</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alert, no delay in response</td>
<td>1</td>
</tr>
<tr>
<td>Drowsy or confused, Response to light stimulation</td>
<td>2</td>
</tr>
<tr>
<td>Very drowsy or confused, Response to strong stimulation, Wards off pain</td>
<td>3</td>
</tr>
<tr>
<td>Unconscious</td>
<td></td>
</tr>
<tr>
<td>Localizes but does not ward off pain</td>
<td>4</td>
</tr>
<tr>
<td>Withdrawing movements on pain stimulation</td>
<td>5</td>
</tr>
<tr>
<td>Stereotype flexion on pain stimulation</td>
<td>6</td>
</tr>
<tr>
<td>Stereotype extension on pain stimulation</td>
<td>7</td>
</tr>
<tr>
<td>No response to pain stimulation</td>
<td>8</td>
</tr>
</tbody>
</table>

**Table 2.** The Reaction Level Scale (RLS 85) Starmark et al., Acta Neurochir 1988;91:12-20

A broader type of trauma scale is the Injury Severity Score (ISS), which is an established medical score to assess trauma severity and measures the total anatomical damage to the body of a patient with multiple injuries after severe trauma (Baker et al., 1974).

It is based on the AIS score, which grades six body regions (face, head, chest, abdomen, pelvis and extremities and external)(Safety., 1971). The scores are from 1 to 6 where 1 is minor and 6 is fatal. The scores of the three most injured regions are squared and then summed, ranging from 0 to 75. If an injury is assigned an AIS of 6 (a fatal injury), the ISS score is automatically assigned to 75. The ISS score is the only anatomical scoring system in use and it correlates with mortality, morbidity, hospital stay and other measures of severity (Baker et al., 1974).
Evaluation of clinical outcome after severe traumatic brain injury

There are several outcome measures in TBI research, and the Glasgow Outcome Scale (GOS) (Table 3) is the most widely used (Jennett and Bond, 1975, Wilde et al., 2010). The GOS is determined by a clinician at some point in the patient’s recovery. Generally the time after injury is reported together with the GOS. The GOS attempts to generalize and categorize the outcomes of patients suffering traumatic brain injury. In general, unlike the Glasgow Coma Scale, this scale is not used in the clinical care of the patient, but it is often in research to estimate the recovery patients have achieved. The scale is significantly correlated with more complex scales (Wilson et al., 2000). Other, more specific, complex and detailed grading systems have also been developed. One of these is an adaptation of the GOS, called the Extended GOS (Table 3)(Wilson et al., 1998).

<table>
<thead>
<tr>
<th>Clinical summary</th>
<th>Glasgow Outcome Scale</th>
<th>Point</th>
<th>Extended Glasgow Outcome Scale</th>
<th>Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead</td>
<td>Dead</td>
<td>1</td>
<td>Dead</td>
<td>1</td>
</tr>
<tr>
<td>Sleep / awake nonsententient</td>
<td>Persistent vegetative state</td>
<td>2</td>
<td>Persistent vegetative state</td>
<td>2</td>
</tr>
<tr>
<td>Conscious but dependent</td>
<td>Severe disability</td>
<td>3</td>
<td>Lower severe disability</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Upper severe disability</td>
<td>4</td>
</tr>
<tr>
<td>Independent but disabled</td>
<td>Moderate disability</td>
<td>4</td>
<td>Lower moderate disability</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Upper moderate disability</td>
<td>6</td>
</tr>
<tr>
<td>May have mild residual effects</td>
<td>Good recovery</td>
<td>5</td>
<td>Lower good recovery</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Upper good recovery</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 3. Glasgow Outcome Scale and Extended Glasgow Outcome Scale (Jennett and Bond, 1975, Wilson et al., 1998)

These scales are often used divided into favourable (GOS 4-5, GOSE 5-8) and unfavourable outcome (GOS 1-3, GOSE 1-4). Most improvements occur during the first few months after injury, therefore GOS and GOSE are often scored at 6 months.

There are also several other scales that assess quality of life, like HRQOL, SF-36 and QOLIBRI (Flanagan, 1978, Ware and Sherbourne, 1992, Burckhardt and Anderson, 2003, von Steinbuechel et al., 2005). However, these scales are often difficult to use early after trauma, and are preferably used in the early rehabilitation phase (< 1 year) and later (Ridley, 2001). Most of the cognitive recovery occurs within the first 6-18 months after injury, even though some improvement may also occur after 18 months (Ruttan et al., 2008).
**Treatment protocol**

The Lund concept for the treatment of severe head trauma, developed at Skåne University Hospital in Lund, Sweden, was introduced in 1992 and the last update was in 2006 (Grande, 1992, Asgeirsson and Grande, 1994, Grande, 2006). It is used in modified versions in Sweden. It is based on hypotheses originating from basic physiological principles of brain volume and cerebral perfusion regulation. Its main features have been of value in clinical and experimental studies (Asgeirsson and Grande, 1994, Eker et al., 1998, Naredi et al., 1998, Kongstad and Grande, 1999, 2001, Naredi et al., 2001, Elf et al., 2002, Elf et al., 2005, Wahlstrom et al., 2005, Olivecrona et al., 2007, 2009a, Stenberg et al., 2013)

The therapy has two primary goals: to reduce or prevent an increase in ICP and to optimise perfusion and oxygenation around contusions. The Lund therapy considers the consequences of a disrupted blood-brain-barrier for the development of brain oedema and the specific consequences of a rigid extrinsic shell for general cerebral haemodynamics. It emphasizes the importance of improving perfusion and oxygenation of the injured regions of the brain. This is attained by normal blood oxygenation, by keeping up normovolemia with normal haematocrit and plasma protein concentrations, and by counteracting vasoconstriction by minimizing stress and circulating catecholamine levels. The therapeutic measures mean normalization of all essential haemodynamic parameters (blood pressure, plasma oncotic pressure, plasma and erythrocyte volumes, PaO2, PaCO2) and the early use of enteral nutrition (Grande, 2006).

There are several other guidelines available, such as the Rosner protocol, the Brain Trauma Foundation guidelines, the European guidelines and the Adenbrooke (Rosner et al., 1995, Bullock et al., 1996, Maas et al., 1997, Brain Trauma Foundation et al., 2007, Menon and Zahed, 2009). All guidelines except the Lund concept can be characterized as CPP targeted. The brain trauma foundation guidelines are based on clinical studies, especially meta-analytic surveys. The Lund concept is instead based on physiological principles, and also finds support in experimental studies. Recently, a shift in the treatment paradigm has emerged, in centers not using the Lund concept. There has been an acceptance of a lower CPP, indicating a narrowing gap between the guidelines. There is no evidence supporting that any of the guidelines is superior to another. However, in a recent study of ICP and CPP management strategies, it was found that some patients responded better to CPP-based management and that others did better given ICP-based treatment (Howells et al., 2005). No randomized controlled study on the effect of Lund concept has so far been performed, but outcome studies with the Lund concept (and modified versions thereof) have been promising (Eker et al., 1998, Naredi et al., 1998, Naredi et al., 2001, Wahlstrom et al., 2005, Olivecrona et al., 2007, 2009b, Liu et al., 2010, Dizdarevic et al., 2012, Olivecrona et al., 2012)
It has also been shown that Lund Concept can lessen the pulmonary complications in ICU patients (Robertson et al., 1999, Fletcher et al., 2010).

**Measuring the intracranial pressure**
The intracranial pressure (ICP) was measured predominantly with an intraparenchymal pressure monitor (Codman MicroSensor™). This method has been shown to be reliable and with very low complication rates, and to agree very well with other methods (Koskinen and Olivecrona, 2005, Koskinen et al., 2013). In some cases, ICP was monitored with a ventricular drainage in the right frontal horn. When measuring with a ventricular drainage, the zero point was set at the level of external meatus. The mean arterial pressure (MAP) was measured invasively, commonly in the radial artery at the level of the heart, and the cerebral perfusion pressure (CPP) was calculated as MAP minus ICP. The patients were treated in a flat position without head elevation and no correction for a difference between zero points of blood pressure and ICP was therefore needed in the calculation of the CPP. Zero points are not always defined, but it is very important to define them, since there will otherwise be a difference in the measured values. ICP was targeted to be less than 20 mmHg and CPP was targeted at 60 mmHg (not lower than 50 mmHg).

**Biomarkers for severe brain injury**
In most medical fields there is a variety of serum markers available for analysis. Serum markers are essential elements for the diagnosis of diseases of many organs such as lipase for pancreatitis, creatinine for renal function or troponin for myocardial infarction. They are relatively specific for the cells of an organ system and may provide essential information about the diagnosis, the severity and the time course of the disease, the effect of treatment and the prognosis of the patient.

When it comes to the morbidity of the central nervous system, there is no broad choice of serum markers with sufficient specificity and sensitivity. This is especially unfavorable since clinicians who treat head injuries could definitely gain from the advantages provided by biomarkers. In the case of patients with mild head injury without salient neurological deficits, it would advance the determination of a diagnosis, by indicating whether or not tissue has been injured. This information would also assist the clinician in determining whether the patient should be hospitalised for observation, if it is vital to obtain CT or MRI scans, and if so when such examinations should take place. The use of biomarkers could lead to rapid diagnosis in the case of sedated, unconscious, or多traumatized patients even before the neuroimaging techniques can be utilized.

The results of laboratory tests may help to clarify the mechanism of injury and may also serve as a cost-cutting end point. Such laboratory tests could have a
prominent prognostic value in the severely injured with regard to rehabilitation, the mild and moderately injured with respect to health care benefits, and knowing whether or not the patient should be admitted for radiology. Expectations regarding biomarkers have changed recently. They should be easily traceable in the blood and correspond to the mechanical strength and the magnitude of the injury. Their sensitivity is as important as their specificity. They should also occur rapidly in the blood and show a striking distribution in time. It is also important that they should mirror the differences between genders and age groups. Preferably, they should indicate the pathophysiological mechanism. Unfortunately there is no such biomarker today. Currently, the most extensively studied biomarkers are S-100B, NSE and GFAP.

**S-100B**

S-100B is the most extensively studied biomarker in human TBI (Rothermundt et al., 2003, Unden et al., 2007, Vos et al., 2010). It was identified as a protein in the mid-1960s, detectable only in the brain and not in non-neural extracts. It was named S-100 because of its solubility in a 100% saturated solution of ammonium sulphate (Moore, 1965).

S-100B protein is a small Ca-binding protein, occurring mostly in glial cells of the central and peripheral nervous system (predominately astrocytes and Schwann cells) but also in chondrocytes, melanocytes and adipocytes. The concentrations in these cells are however very low (100-200 ng/mg of protein), compared to their concentrations in glial and Schwann cells (3500 ng/mg of protein) (Haimoto et al., 1987).

S-100B regulates enzyme activity and calcium homeostasis, and is involved in the signal transduction through inhibition of protein phosphorylation (Kleindienst and Ross Bullock, 2006). S-100B is also involved in the regulation of the cell morphology by affecting the cytoplasmatic cytoskeleton (Kleindienst and Ross Bullock, 2006). It is metabolized in the kidney and excreted in the urine, is not affected by haemolysis, and remains stable for hours without the need for immediate sample centrifugation and freezing (Jonsson et al., 2000, Kleindienst and Ross Bullock, 2006, Korfias et al., 2006).

It can be measured in arterial, capillary and venous serum, although the different tests are not interchangeable (Astrand et al., 2012).

After sTBI, serum S-100B increases promptly, and may rise up to 5–20 µg/L in the first minutes. This highly abnormal value is thought to be caused by the release of S-100B from primary damaged brain cells and an opening of the blood-brain-barrier (BBB) that occurs in the first minutes after trauma (Bar-

In mild and moderate head injury the serum concentration of S-100B decreases rapidly over the next few hours as expected by the short half-life of approximately 30–120 min (Jonsson et al., 2000, Townend et al., 2006).

In sTBI, S-100B levels also decreases rapidly, but they do not usually return to baseline over the next hours. This is explained by the more severe brain damage and a continuing release of S-100B from the injured brain (Kleindienst and Ross Bullock, 2006).

This continuing release is responsible for the steadiness of the S-100B level for a time longer than that expected by the half-life (Kleindienst and Ross Bullock, 2006). Up to now, there have been no reports of any rapid fluctuations of S-100B levels after 6–12 hours. Therefore, daily sampling seems to be sufficient for assessment of the temporal course of S-100B after a severe head injury, except for the first 6–12 hours.

With multiple traumas, patients without TBI have elevated serum S-100B levels, but 6 h after injury the level of S-100B decreases (Anderson et al., 2001). Thus, early serum sampling (0–6 h after injury) should be avoided because the observed increase in S-100B is probably due to extracerebral sources and does not reflect developing brain injury. This was confirmed by da Rocha and coworkers, who found no differences in S-100B levels (mean time 10.9 h after injury) between patients with isolated head injury and polytrauma victims with severe TBI (da Rocha et al., 2006).

Similarly, Thelin et al demonstrated that S-100B should probably be sampled 12–36 h posttrauma, as early samples have little predictive value and may reflect extracranial sources of S-100B (Thelin et al., 2013). These results are in line with results previously reported from our research group (Olivecrona et al., 2009b).

In mild traumatic brain injury, an important application of serum S-100B testing is in the selection of patients who do not need further neuro-radiological evaluation, since studies comparing CT scans and S-100B levels have demonstrated that S-100B values below 0.10 ng/mL are associated with a low risk of obvious neuroradiological changes (such as intracranial hemorrhage or brain swelling) or significant clinical sequelae (Unden and Romner, 2010). Indeed, this has now been suggested in an algorithm for evaluating patients suffering minimal, mild and moderate head injury (Astrand et al., 2013, Unden et al., 2013).
**NS**

NS is a glycolytic enzyme, located in the cytoplasm of neurons (Marangos and Schmechel, 1987). It is involved in increasing neuronal chloride levels during the onset of activity (Johnsson et al., 2000). At first, it was thought that NSE was found only in neurons, but later it was found that NSE is also found in neuroendocrine cells, platelets and red blood cells (Johnsson et al., 2000). NSE has been used as a biochemical marker for neuroblastoma and small-cell carcinoma of the lung (Cooper, 1994).

NSE can be detected in both serum and CSF and has therefore been a potential brain injury marker under examination.

Levels above 7-10 μg/L are considered to be abnormal (Nygaard et al., 1998). The half-time is estimated to be 30 hours (Johnsson et al., 2000). Previous studies have shown hopeful results that there is a correlation between NSE, pathological findings on CT and the outcome after severe brain injury (Guzel et al., 2008, Meric et al., 2010). There are however conflicting results. Some studies have shown no correlation with the outcome or other clinical parameters such as radiology (Bellander et al., 2011, Yokobori et al., 2013).

**Genetics in severe brain injury**

It is becoming increasingly clear that genetic factors affect the outcome after traumatic brain injury (McAllister, 2010). The most extensively studied gene in TBI patients is the apolipoprotein gene (APOE) encoding for the cholesterol carrier protein, apolipoprotein E (ApoE) (Kovesdi et al., 2010).

In the CNS, the ApoE protein acts as the major lipid-carrying protein between neural cells (Pitas et al., 1987). Apolipoprotein E is produced within the CNS mainly by astrocytes, although neurons and microglia may take part in apoE synthesis (McAllister, 2010). APOE is polymorphic and exists in three forms e2, e3, and e4, where the e4 allele has been identified as a susceptibility gene for late onset familial and sporadic Alzheimer’s disease (McAllister, 2010). The allele frequencies for the APOE forms are different in different parts of the world, but APOE3 seems to be the most common variant (Corbo and Scacchi, 1999). Several neurobiological functions are associated with APOE such as antioxidant activity, neurofibrillary tangle formation, mitochondrial damage, neuro-protection, neuronal repair, synaptic plasticity and memory function (McAllister, 2010). TBI patients with the APOE e4 allele had larger intracranial haematomas, longer hospital stays, and unfavourable outcome defined as severe disability, vegetative state or death (Teasdale et al., 1997, Friedman et al., 1999, Liaquat et al., 2002, Chiang et al., 2003). Furthermore, several studies shows that the presence of the gene may influence cognitive functioning after TBI (Crawford et al., 2002, Liberman et al., 2002, Sundstrom et al., 2004). These important
studies support the hypothesis that APOE plays an important role in the CNS response to acute and chronic injury, in a manner not yet fully understood.

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse injury I (no visible pathology)</td>
<td>No visible pathology seen on CT scan</td>
</tr>
<tr>
<td>Diffuse injury II</td>
<td>Cisterns are present with midline shift of 0–5 mm and/or</td>
</tr>
<tr>
<td></td>
<td>Lesion densities present</td>
</tr>
<tr>
<td></td>
<td>No high- or mixed-density lesion &gt; 25 ml</td>
</tr>
<tr>
<td></td>
<td>(may include bone fragments and foreign bodies)</td>
</tr>
<tr>
<td>Diffuse injury III (swelling)</td>
<td>Cisterns compressed or absent with midline shift 0–5 mm,</td>
</tr>
<tr>
<td></td>
<td>No high or mixed-density lesion &gt; 25 ml</td>
</tr>
<tr>
<td>Diffuse injury IC (shift)</td>
<td>Midline shift &gt; 5 mm,</td>
</tr>
<tr>
<td></td>
<td>No high- or mixed, density lesion &gt; 25 ml</td>
</tr>
<tr>
<td>Evacuated mass lesion</td>
<td>Any lesion surgically evacuated</td>
</tr>
<tr>
<td>Non-evacuated mass lesion</td>
<td>High- or mixed, density lesion &gt; 25 ml, Not surgically evacuated</td>
</tr>
</tbody>
</table>


In 2005, Maas and co-workers developed the Rotterdam classification system (Table 5), which seems to be superior to the Marshall classification, and also allows for comparison of CT scans over time (Maas et al., 2005). They combined different CT features (midline shift, presence of subarachnoid haemorrhage (tSAH), epidural haematoma, compression of basal cisterns) and found a greater prognostic value of clinical outcomes than the Marshall system (Maas et al., 2005). The presence of subarachnoid hemorrhage seemed to be the strongest prognostic factor (Maas et al., 2005).

<table>
<thead>
<tr>
<th>Predictor / Structure</th>
<th>Finding</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cisterns</td>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Compressed</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>2</td>
</tr>
<tr>
<td>Midline shift</td>
<td>No shift or shift ≤ 5 mm</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Shift &gt; 5 mm</td>
<td>1</td>
</tr>
<tr>
<td>Epidural mass lesion</td>
<td>Present</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>1</td>
</tr>
<tr>
<td>Intraventricular blood or tSAH</td>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>1</td>
</tr>
<tr>
<td>Sum score</td>
<td></td>
<td>+1</td>
</tr>
</tbody>
</table>

Table 5. Rotterdam classification. Maas 2005 (Maas et al., 2005).
The Morris-Marshall classification (Table 6) is a system describing the tSAH (Morris and Marshall, 1997). This method can also be used for prognostication of outcome.

<table>
<thead>
<tr>
<th>Description of CT findings</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CT evidence of traumatic tSAH</td>
<td>0</td>
</tr>
<tr>
<td>tSAH present only in one location</td>
<td>1</td>
</tr>
<tr>
<td>tSAH present at only one location but quantity of blood fills that structure or tSAH is at any two sites, filling neither of them</td>
<td>2</td>
</tr>
<tr>
<td>tSAH is present at two sites, one of which is the tentorium filled with blood</td>
<td>3</td>
</tr>
<tr>
<td>tSAH present at 3 or more sites, any quantity</td>
<td>4</td>
</tr>
</tbody>
</table>


**Acute neuro-endocrine changes**

Acute changes in pituitary hormone levels are frequently encountered in the acute phase after severe TBI. The relevance and therapeutic implications are still debated, with poor agreement among experts (Klose and Feldt-Rasmussen, 2012).

The pituitary gland is located within the sella turcica in the base of the skull. It is separated from the supracellar cistern by the diaphragma sella and is connected to the hypothalamus by the pituitary stalk which enters on its superior surface (Rhoton, 2002). The internal carotid arteries supply both the adeno-hypophysis and the neuro-hypophysis. The anterior pituitary gland receives 70-90% of its blood supply from the long hypophyseal portal vessels, which arise from branches of the internal carotid artery and anterior circle of Willis. The short hypophyseal portal vessels arise from branches of the intracavernous internal carotid artery, which enter the sella from below the diaphragma sella and supply the anterior gland with less than 30% of its vascular supply, predominantly in the medial portion of the gland including the pars intermedia (Gorczyca and Hardy, 1987, Rhoton, 2002).

Due to the pituitary glands confinement within the bony sella and its connection to the hypothalamus by the pituitary stalk, it has been hypothesized that the pituitary gland may be sensitive to mechanical trauma at the moment of impact (Dusick et al., 2012, Munoz and Urban, 2013). Fractures through the sella turcica and skull base as well as shearing and rotational injuries of the brain stem and hypothalamic-pituitary axis may directly injure the hypothalamus, pituitary stalk and pituitary gland. Secondary insults such as hypotension, hypoxia, anemia and brain swelling can lead to an ischemic injury to the gland, also seen in Sheehan’s post-partum pituitary necrosis (Sheehan, 1971). Because the long portal vessels pass through the diaphragma sella, these vessels
and the pituitary stalk are thought to be especially vulnerable to intracranial hypertension, mechanical trauma, low cerebral blood flow and brain swelling (Dusick et al., 2012, Munoz and Urban, 2013).

The somatotroph and gonadotroph axes have been found to be more sensitive to dysfunction following sTBI due to their location. The somatotrophs are located mainly in the lateral parts of the anterior pituitary, and the gonadotrophs are spread throughout the pars distalis and tuberalis. These areas are irrigated by the long hypophyseal portal vessels. The corticotrophs are predominantly located in the pars intermedia and the central part, and the thyrotrophs are mainly found in the anteromedial part of the gland, thus they are in less susceptible short hypophyseal portal territory (Dusick et al., 2012, Munoz and Urban, 2013).

Previous autopsy studies of fatal head injuries and imaging studies of TBI survivors have shown injuries to the hypothalamus, pituitary stalk and pituitary gland (Dusick et al., 2012, Munoz and Urban, 2013).

Several mechanisms may cause altered pituitary function in the acute phase of TBI: (1) the primary brain injury event itself; (2) secondary insults such as hypotension, hypoxia and high intracranial pressure; (3) critical illness-related hormonal changes; and (4) medication effects. It is probable that these factors individually and in varying combinations determine which hormonal axes are affected, the severity of the abnormality and whether the deficits are reversible or permanent.

The pituitary gland responds to critical illness, including acute traumatic events with both increased and decreased secretory patterns. Thus, cortisol, prolactin (PRL) and growth hormone (GH) levels increase, while luteinising hormone (LH), follicle-stimulating hormone (FSH) and thyroid axis (TSH, T4, T3) levels may either decrease or remain unchanged, associated with a lower activity of their target organ. Changes in the circulating hormone levels become apparent during the first hours or days after trauma, and may persist for the duration of the acute critical illness (Woolf, 1992, Dusick et al., 2012). These alterations mainly represent part of the adaptive response to the injury, and may be influenced by the type of injury and pharmacological therapy used to treat the critical illness (glucocorticoids, narcotic analgesics or dopaminergic agents) (Woolf, 1992). The changes are not disease-specific but a countermark in acute and critical illness, and they seem to be a part of adaptive mechanisms regulating the inflammatory response, caused by cytokine activation of many factors (Dusick et al., 2012, Munoz and Urban, 2013) also reported in other severe stressful situations such as subarachnoid haemorrhage (Zetterling et al., 2011, Zetterling et al., 2013).
The interpretation of hormonal changes involves several problems, and today there are no reliable diagnostic cut-offs for hormone deficiencies in critically ill patients.

The most important hormonal deficiency to consider in any critical illness situation, including TBI, is an untreated deficiency of the hypothalamic-pituitary-adrenal (HPA) axis, which could lead to a potentially fatal cortisol insufficiency.

During critical illness, profound and variable changes occur in the HPA axis, including HPA activation, decreased levels of cortisol-binding globulin leading to a larger amount of free cortisol, altered CBG-cortisol-binding affinity, and a greater tissue sensitivity to glucocorticoids (Molijn et al., 1995, Annane and Bellissant, 2000, Van den Berghe, 2000, Beishuizen et al., 2001, Venkatesh and Cohen, 2011). The actual tissue cortisol bioactivity in vital target organs in a critical illness situation is thus very difficult to assess using total serum cortisol above or below a set cut-off level (Venkatesh and Cohen, 2011).

Several studies have assessed the acute pituitary changes that occur after TBI, looking at potential correlations with clinical parameters, often aiming to use hormone levels as predictors of the outcome (Dusick et al., 2012, Munoz and Urban, 2013, Vespa, 2013). However, most of them are mixed materials (eg mild, moderate and severe TBI), mixed diagnoses (trauma and non-trauma, such as SAH) and only a few describe how the patients are treated (protocol used etc).
Aims

– To characterize and evaluate patients suffering sTBI treated in accordance with an ICP targeted therapy based on the Lund concept with the following emphases:

– To evaluate the influence of the APOE ε4 allele on the outcome after sTBI.

– To investigate whether the APOE ε4 allele is associated with the serum levels of brain injury biomarkers, S-100B and NSE, and to investigate whether ICP, CPP and MAP are associated with the genetic alteration.

– To explore whether the biochemical markers of brain injury (S-100B and NSE) might be related to ICP, CPP, radiological characteristics and clinical outcome.

– To investigate whether the clinical outcome can be related to biomarker levels and whether mortality can be predicted by the bulk release of the biomarkers.

– To examine and characterize early neuroendocrine changes in patients suffering sTBI and to explore possible associations between hormone levels and clinical parameters in the acute phase, including ICP, CPP, GCS and radiological findings.

– To see whether the hormone levels are associated with clinical outcome and could be used in the prediction of outcome.
Materials and methods

Patients and general methods

The patients in papers I-IV were part of a prospective, randomized, blind, placebo-controlled study of the effect of therapy with the addition of prostacyclin in sTBI patients during 2001–2005, (Olivecrona et al., 2009a). All patients studied have been treated at the Department of Neurosurgery and Intensive Care Unit of Umeå University Hospital.

Inclusion criteria were a verified head injury, GCS score ≤ 8 at intubation and sedation, age between 15 and 70 years, and arrival at our department within 24 hours of trauma. Exclusion criteria were an initial CPP ≤ 10 mm Hg (dead on arrival) and penetrating head injury. Pregnant and lactating women were also excluded. Patients who could leave the intensive care unit alive within 72 hours were excluded, as their head injury could not be regarded as severe, despite an initial GCS score ≤ 8. Patients with a GCS score of 3 and/or dilated, fixed pupils were included in the study as long as their initial measured CPP was more than 10 mm Hg.

During the study period, all patients aged 70 or younger were admitted if there was a suspicion of a severe traumatic brain injury regardless of clinical presentation at the primary receiving hospital. A total of 89 patients were admitted with a suspected sTBI and evaluated for participation in the study. Of these, 48 were included. The cause of trauma is shown in Figure 3.

Figure 3. Type of trauma as a percentage of the total material (n=48).
Treatment Protocol
We used an ICP-targeted therapy based on the Lund concept (Grande, 1992, Asgeirsson et al., 1994, Grande, 2006, Olivecrona et al., 2009a). This concept is based on the basic physiological principles of volume regulation of the brain and of the fluid fluxes over the blood-brain barrier. The over-all goal is to prevent an extravasation of fluid, optimising the micro-vasular blood flow and the delivery of oxygen and glucose. Another goal is to reduce the stress response mediated by the sympathetic nervous system and thus prevent or diminish the inflammatory effect following the initial brain impact. This is achieved by an aggressive neurosurgical approach and is based on four cornerstones: reduction of the energy metabolism and stress response, control of the capillary hydrostatic pressure, control of the colloid osmotic pressure and fluid balance, and a reduction of the cerebral blood volume. The goal is to keep the ICP<20mmHg and not to allow the CPP to fall to < 50 mmHg.

A schematic flow-chart of the treatment is shown in Figure 4. All the patients were sedated and ventilated using midazolam and fentanyl. The treatment protocol included adequate sedation and analgesia with midazolam and fentanyl, mechanical ventilation targeting a $P_O2 \geq 12$ kPa and $P_CO2 \leq 5.5$ kPa, flat head position and the arterial baseline level set at the heart level. Maintenance of normovolemia, normotension, normoxia and normal colloid osmotic pressure was achieved by infusion of red blood cells, albumin, glucose solution and Ringer’s acetate (S-Hb >110g/l, S-alb >40 g/l). The fluid balance was kept neutral and furosemide was used if indicated. When normovolemia and cardio-vascular stability had been achieved, continuous intravenous infusions with metoprolol and clonidine were given in order to normalize the blood pressure and to reduce the transcapillary hydrostatic pressure. These drugs also lower the stress level and response mediated by the sympathetic nervous system. Mass lesions were surgically removed when indicated. For episodes of ICP > 20 mmHg, additional sedation with a low dose of thiopental was used. The second tier therapy for intractable ICP was ventriculostomy and third tier therapies included unilateral or bilateral hemicranieectomy with duraplasty.
Figure 4. Schematic depiction of the treatment protocol

Monitoring
Multimodal monitoring was applied including frequent standard chemical samples, continuous arterial oxygen saturation, exhaled carbon dioxide partial pressure, frequent arterial blood gases, and continuous central venous pressure. The ICP was continuously monitored in all patients using a Codman MicroSensor (Johnson & Johnson Professional, Inc). The blood pressure was monitored by an arterial line with zero reference at the right atrium. No head elevation was allowed and the patients were all treated in the supine position. The mean hourly ICP and CPP values were calculated based on values recorded every minute. The hour with the highest mean ICP value was defined as ICP-max (ICP_{max}) and the hour with the lowest mean CPP value was defined as CPP-min (CPP_{min}). Continuous EEG was used in order to follow the sedation level and to observe any epileptic activity. In addition, cerebral and sub-cutaneous microdialysis were used as well as continuous brain tissue oxygen pressure. However, the results from these measurements are not a part of the present study.

Data were digitally stored using the LabPilot system (CMA Microdialysis, Solna, Sweden) and the Picis system (Picis, Inc., Wakefield, MA, USA).
Assessment of outcome
Clinical outcome was estimated by independent staff, performing structured interviews based on a protocol, and reported as GOS and GOSE (Jennett and Bond, 1975, Wilson et al., 1998). The results were also divided into deceased (GOS 1, GOSE 1) / alive (GOS 2-5, GOSE 2-8), and favourable (GOS 4-5, GOSE 5-8)/ non-favourable outcome (GOS 1-3, GOSE 1-4).

Papers I-II
In these papers, blood samples for analysis of the APOE alleles were drawn after admission and inclusion in the study. The samples were analyzed at the Department of Clinical Chemistry, Skåne University Hospital, Malmö, Sweden. Parts of the gene were amplified using the polymerase chain reaction from extracted genomic DNA. Allele-specific fluorescence-marked probes were hybridized with specific products creating fluorescence at a specific wavelength, which was analyzed in an automatized system.

Papers II-III
In these papers, blood samples for S-100B and NSE were collected twice daily (at 12 hour intervals) during the first 5 days. The serum was frozen and stored in a -70°C freezer until analysed. Three different analysis methods are currently available and these are not interchangeable (Erickson and Grenache, 2011).

The method adopted for the analysis was the fully automatized LIAISON® system, using the LIAISON Sangtec 100 assay for the S-100B and the LIAISON NSE assay for the analysis of NSE (AB DiaSorin, Sangtec Medical, Bromma, Sweden). The immunoluminometric method for NSE has a detection range of 0.04-200 µg/l and an intra- and inter-assay variation below 3% and 6% respectively. The corresponding immunoluminometric method for S-100B analysis has a detection range of 0.02-30 µg/l with intra- and inter-assay variations below 5% and 10% respectively.

The highest values ($S_{100B\text{max}}$, $NSE_{\text{max}}$) during the first five days were identified and the bulk release (area under the curve for $S_{100B_{\text{AUC}}}$, $NSE_{\text{AUC}}$) during the first three days was calculated using the KaleidaGraph software (Synergy Software, Reading, PA, USA). The $S_{100B_{\text{AUC}}}$ and $NSE_{\text{AUC}}$ are considered to represent the bulk release of the markers better than single values.

Paper III
In this paper, the initial CT scan ($CT_{\text{init}}$) and the CT scan taken at 24 hours after injury ($CT_{24}$) were classified according to the Marshall, Rotterdam and Morris-Marshall scoring systems (Marshall et al., 1991, Morris and Marshall, 1997, Maas et al., 2005). ISS was used for evaluation of the overall severity of the injury (Baker et al., 1974).
**Paper IV**

In paper IV, serum samples were collected in the morning (08-10 am) on day 1 and day 4 after sTBI for the analysis of serum cortisol, growth hormone (GH), prolactin, insulin-like growth factor 1 (IGF-1), thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone and sex-hormone-binding globulin (SHBG) (men). Serum for cortisol and GH was also obtained in the evening (5-7 pm) on day 1 and day 4. The samples were immediately centrifuged and stored at -70°C until analysis at the accredited clinical chemistry laboratory at Umeå University hospital. Electrochemiluminescence immunoassay (ECLIA; Modular Analytics E170, Roche, GmbH, Hannheim, Germany) was used for the analysis of serum cortisol, TSH, fT3, fT4, FSH, LH, prolactin and SHBG. Coat-a-count RIA (Siemens) was used for the analysis of serum testosterone and DPC Immulite 2000 (Siemens) for serum GH and IGF-1. Using total testosterone, SHBG and albumin levels, the free testosterone (fc-testosterone) levels were calculated according to the calculator at http://www.issam.ch/freetesto.htm.

Information regarding the women’s menstrual status was not obtained, which means that levels of LH, FSH and estradiol in the women could not be properly interpreted, and these were therefore omitted from the final analyses.

The severity of the trauma was determined by ISS and the extent of brain tissue damage by the Marshall classification.

**Statistics**

Continuous variables are reported as the mean ± standard error of the mean (SEM) and discrete variables are reported as the median and range. Groups were compared using the two-tailed paired Student’s t-test (continuous data) and, when appropriate, the Bonferroni correction was applied. The Wilcoxon rank sum test was used to assess the significance of differences between groups, and the χ²-test was used to assess the significance of differences between proportions. Correlations between continuous variables were calculated using the bivariate Pearson method, and Spearman’s rho test was used when at least one parameter was discrete. Logistic regression analysis and receiver operator characteristics curve analysis (ROC) were used to evaluate prognostic factors. P ≤ 0.05 was considered statistically significant.
Results

Paper I
The presence of the APOE ε4 was determined in 46 patients. The patient age (mean) was 35.0 ± 2.2 years, and the GCS score (median) at intubation and sedation was 6 (range 3–8). None of the patients were lost to follow-up. 39.1% (n=18) of the patients exhibited the presence of the APO ε4 allele (Figure 5).

![Figure 5. Histogram showing the percentages of APOE alleles in 46 patients with severe TBI.](image)

There were no statistically significant differences between the APOE ε4 and non-ε4 groups considering age, gender, ISS, GCS score or GOS score at any point in time (Table 7).
### RESULTS

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (46 patients)</th>
<th>Non-ε4 (28 patients)</th>
<th>ε4 (18 patients)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCS median at intubation and sedation</td>
<td>6 (3-8)</td>
<td>6 (3-8)</td>
<td>5 (3-8)</td>
<td>n.s</td>
</tr>
<tr>
<td>GCS mean at intubation and sedation</td>
<td>5.3 ± 0.25</td>
<td>5.3 ± 0.3</td>
<td>5.2 ± 0.4</td>
<td>n.s</td>
</tr>
<tr>
<td>Age (mean yrs)</td>
<td>35.0 ± 2.2</td>
<td>33.0 ± 2.5</td>
<td>38.7 ± 4.0</td>
<td>n.s</td>
</tr>
<tr>
<td>ISS median</td>
<td>29 (9-50)</td>
<td>31.5 (14-43)</td>
<td>25 (9-50)</td>
<td>n.s</td>
</tr>
<tr>
<td>ISS mean</td>
<td>28.7 ±1.48</td>
<td>29.7 ±1.6</td>
<td>27.3 ± 2.6</td>
<td>n.s</td>
</tr>
<tr>
<td>ICP max (mean mmHg)</td>
<td>29.1 ± 1.9</td>
<td>27.6 ± 2.5</td>
<td>31.5 ±3.1</td>
<td>n.s</td>
</tr>
<tr>
<td>CPP min (mean mmHg)</td>
<td>43.9 ± 2.0</td>
<td>43.9 ± 2.6</td>
<td>44.0 ± 3.5</td>
<td>n.s</td>
</tr>
<tr>
<td>GOS score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months median</td>
<td>3.5 (1-5)</td>
<td>4 (1-5)</td>
<td>3 (1-5)</td>
<td>n.s</td>
</tr>
<tr>
<td>3 months mean</td>
<td>3.5 ± 0.2</td>
<td>3.7 ± 0.3</td>
<td>3.1 ± 0.3</td>
<td>n.s</td>
</tr>
<tr>
<td>12 months median</td>
<td>4 (1-5)</td>
<td>4 (1-5)</td>
<td>3.5 (1-5)</td>
<td>n.s</td>
</tr>
<tr>
<td>12 months mean</td>
<td>3.6 ± 0.2</td>
<td>3.9 ± 0.3</td>
<td>3.2 ± 0.4</td>
<td>n.s</td>
</tr>
<tr>
<td>24 months median</td>
<td>4 (1-5)</td>
<td>4.5 (1-5)</td>
<td>4 (1-5)</td>
<td>n.s</td>
</tr>
<tr>
<td>24 months mean</td>
<td>3.7 ± 0.2</td>
<td>3.9 ± 0.3</td>
<td>3.3 ± 0.4</td>
<td>n.s</td>
</tr>
</tbody>
</table>

**Table 7.** Basic features of the 46 patients in which the APOE ε4 was investigated. Mean values are given as mean ± SEM. Median values are presented with the range in parentheses. N.s = not statistically significant. Calculated by the unpaired 2-tailed t-test (age, ICP, CPP) and Wilcoxon rank-sum test (GCS, ISS, GOS).

In a logistic regression model, APOE ε4 did not contribute to the prediction of outcome at 12 and 24 months.

The outcome at 3 months divided into favorable (GOS 4 and 5) and unfavorable groups (GOS 1–3) demonstrated a statistically significant worse outcome in the APOE ε4 group (p< 0.05, Wilcoxon rank sum test). When the outcome was divided into dead (GOS 1) and alive (GOS 2–5), or dead (GOS 1), unfavorable outcome (GOS 2 and 3), and favorable outcome (GOS 4 and 5), no statistically significant difference was found. Regardless of the division used, there was no statistically significant difference in outcome between the non-APOE ε4 and APOE ε4 groups at 12 and 24 months after trauma.

**Paper II**

Forty-eight patients were enrolled in the study. Two APOE alleles were lacking and the study is therefore based on 46 subjects (15 females, 31 males). The mean age was 35.0 ± 2.2 years and the mean GCS at intubation and sedation was 6 (3–8). The median GOS at 3 months was 3.5 (1–5), and at 12 and 24 months 4 (1–5). 39.1% of the patients had the APOE ε4 allele.
Figure 6. Histogram showing the maximum and mean ICP, as the minimum and mean CPP in the APOE groups.

Figure 7 shows the levels of the two biomarkers in the non-APOE ε4 group and in the APOE ε4 group. The levels of S-100B and NSE were higher in the APOE ε4 group and the difference was statistically significant in S-100B_{max} and S-100B_{AUC}.
The temporal profile of S-100B decay differed between non-APOE ε4 and APOE ε4 groups (Figure 8). The difference in the decay profile of NSE between the APOE groups was less obvious (Figure 7).

![Figure 8. The temporal profiles of S-100B and NSE decay in the non-APOE ε4 and APOE ε4 groups.](image)

To investigate whether the GCS differed between the APOE groups, GCS was divided into GCS 3-5 and GCS 6-8 groups and the biomarker concentrations in the two groups were compared. No statistically significant difference was found.

**Paper III**

The study included forty-eight patients (17 women, 31 men). The mean age was 35.5 ± 2.2 years, the median GCS at intubation and sedation was 6 (3 – 8), and the ISS was 29 ± 1. No gender-specific consequences were seen on the biomarker levels, ICP and CPP. The median GOSE at 3 months was 4 (1-7) and 6 patients had died.

The levels of the biomarkers are shown in Figure 9.
Figure 9. The levels of the biomarkers measured as initial, at 72 hrs, maximum and area under the curve (AUC).

There was a positive correlation between $ICP_{\text{max}}$ and the biomarker measurements and the strongest correlation was with $S-100B_{\text{max}}$ ($r=0.69, p<0.001$) and with $NSE_{\text{max}}$ ($r=0.57, p<0.0001$) (Figure 10).

Figure 10. Correlation between the maximal concentration of the biomarkers and $ICP_{\text{max}}$. 
A similar but negative correlation was observed between $\text{CPP}_{\text{min}}$ and $S-100B_{\text{max}}$ \((r=-0.63, \ p<0.0001)\) and $\text{NSE}_{\text{max}}$ \((r=-0.56, \ p<0.0001)\) (Figure 11).

**Figure 11.** Correlation between the maximum concentration of the biomarkers and $\text{CPP}_{\text{min}}$.

A versatile relation among different measurements of the biomarkers and the CT classifications was demonstrated. Generally, the Marshall scoring correlated to the biomarkers at 72 h, and the Rotterdam scoring correlated to the bulk release. The Morris-Marshall scoring correlated to all of the $S-100B$ values, but to none of the NSE values. The $S-100B$ values, $\text{NSE}_{72h}$ and $\text{NSE}_{BR}$ correlated negatively to GOSE at 3 months. A ROC curve demonstrated that the biomarkers were fairly good in prediction of the mortality. When two of the most powerful parameters ($S-100B_{BR}$ and Morris-Marshall$_{init}$) were combined in a ROC curve analysis, the power of prediction improved.

**Paper IV**

45 patients were included in the study. The mean age was 35.7±2.2, (range 15-64), the median ISS was 29 (9-43) and the median GCS at intubation and sedation was 6 (3-8). The patients were hospitalized at the neuro-intensive care unit for a mean period of 12.5 ± 0.6 days, median 12.3 days (3.5-23.7). Two patients died during the neuro-intensive care treatment, due to a therapy-refractive high ICP. At 3 months, the mortality was 8.9\% (4/45), the median GOS was 4 (1-5) and favorable outcome (GOS 4-5) was found in 53.3\% of the patients.

Major changes were found in most pituitary-dependent hormones in the acute phase after sTBI, i.e low levels of thyroid hormones, a strong repression of the pituitary-gonadal axis and high levels of prolactin (Figure 12).
RESULTS

Figure 12. Percentage of patients (n = 45) demonstrating hormone values above or below reference levels day 1 and day 4 after sTBI.

The essential findings of this study were:

1) A substantial proportion (54% day 1 and 70% day 4) of the patients had morning s-cortisol levels below the proposed cut-off levels for critical illness-related corticosteroid insufficiency (CIRCI), i.e. <276 nmol/L (=10 ug/dL). Low s-cortisol (<276 nmol/L) was not associated with a higher mortality or worse outcome at 3 months.

2) There was a significant association between early (day 1) and strong suppression of the pituitary-gonadal axis and improved survival and favorable functional outcome 3 months after sTBI (Figure 13-14). Furthermore, patients with a poor outcome at 3 months had significantly lower levels of fT3 and TSH at day 4 after sTBI (Figure 15-16).

3) Day 4 fT3 and TSH levels were shown to be predictive factors of outcome. LH and FSH (day 1) were predictive factors of outcome and LH was the main predicting factor. A ROC analysis showed a high AUC for LH day 1. The probability of worse outcome increased with increasing levels of LH day 1.
RESULTS

**Figure 13.** Day 1 and day 4 levels of serum LH, FSH, testosterone and fc-testosterone in patients with favorable (GOS 4-5) and unfavorable (GOS 1-3) outcome 3 months post injury. The value of fc-testosterone is divided by 10. Wilcoxon sign rank test between groups. Values are means ± sem.

**Figure 14.** Day 1 and day 4 levels of serum LH, FSH, testosterone and fc-testosterone in survivors and non-survivors 3 months post injury. The value of fc-testosterone is divided by 10. Wilcoxon sign rank test between groups. Values are means ± sem.
Figure 15. Day 1 and day 4 levels of serum fT3 and TSH in patients with favorable (GOS 4-5) and unfavorable (GOS 1-3) outcome 3 months post injury. Wilcoxon sign rank test between groups. Values are means ± sem.

Figure 16. Day 1 and day 4 levels of serum fT3 and TSH in survivors and non-survivors 3 months post injury. Wilcoxon sign rank test between groups and paired Student's t-test between day 1 and day 4 results. Values are means ± sem.
RESULTS

A higher Marshall CT score was associated with higher day 1 LH/FSH- and lower day 4 TSH levels.

Except for a significant correlation between $ICP_{\text{max}}$ and LH day 1 in men no significant correlation between GCS, ICP or CPP and hormone levels was detected.
Discussion

The early assessment of injury severity and the consequent prognosis are of major concern for physicians treating patients suffering from TBI.

Standard methods to prognosticate the severity of initial brain injury and anticipate the onset of secondary injury have included a neurological examination, neuroimaging studies, intracranial pressure monitoring and neurophysiological testing. These tests have limited reliability in critically ill patients who are frequently given sedatives, analgesics, and muscle relaxants, or are not sufficiently stable to leave the NICU for frequent neuroimaging studies. Therefore, over the past decades, there has been a search for other tools which may reliably reflect the severity of injury in order to predict the outcome. Genes, biomarkers and hormones are factors that may provide assistance in the establishment of diagnosis, in the selection of therapeutic intervention, and in the prediction of outcome.

The complex series of events that neurotrauma sets in motion and genetic influences on the brain’s response to brain injury, repair processes after injury, and neural plasticity may play a role in determining some aspects of the outcome and may contribute to the observed clinical variation after seemingly similar injuries. Of the many potential genes, there is a surprisingly small number that have been studied to date and even fewer that have been confirmed. At this time there is reasonable evidence that the APOE allele type affects the outcome, although the mechanism, to what extent and to whom is yet to be clarified.

In our first paper, we demonstrated an early (at 3 month) worse outcome in patients with the APOE ε4 allele who had suffered a severe brain injury. Similarly, in the second paper, the APOE ε4 had significantly higher levels of the biomarkers S-100B and NSE, which was interpreted as an indirect sign of a worse clinical status. In our third paper, it was demonstrated that higher levels of the biomarkers were also clearly associated with a higher ICP and lower CPP, as well as worse radiological findings. Also, in a prognostic model, they were fairly good in predicting the outcome. This homogeneous patient group were, despite its severity of illness and stressful situation was shown to have a "normal" hormonal adaptive response to trauma, and low levels of cortisol without an association with a worse outcome.

One can speculate that some of our results concerning biomarkers, radiological findings, hormone response etc. may diverge from other reported results in sTBI as the clinical outcome in the present study was fairly good. This can be due to several factors such a good treatment protocol in an environment as unstressful as possible and with anti-stressful therapy (β-blockers, clonidine,
midazolam) and no wake-up tests in the ICU. Furthermore, the treatment regime has aimed to avoid pressure drugs. These factors may contribute to a lower stress-response and less inflammation (Grande, 2006). The influence of the treatment given may be reflected in our results in the APOE allele study. At 3 months, we report a worse outcome, but at later time-points it cannot be demonstrated. We also demonstrate significantly lower levels of the biomarkers than most earlier studies. Whether or not this is due to the above-mentioned factors is only a matter of discussion. It is known that infusion of mannitol, which is not included in our protocol, opens the BBB, and promotes higher leakage of the biomarkers and this matter may affect our results (Rapoport, 2000, Brown et al., 2004). Another possibility is that the reparation mechanisms are so robust in this patient group, that the late clinical outcome is not affected.

The present study is unique in that it reports on a very well-defined controlled group of patients all of whom have been treated according to a strict ICP-targeted protocol and only with verified severe TBI.

**Association of APOE allele to clinical outcome and biomarkers**

Patients with the APOE ε4 allele had a significantly worse clinical outcome, divided into unfavorable (GOS 1–3) and favorable (GOS 4 and 5) at 3 months, than those lacking the APOE ε4 allele. Different divisions of GOS at 3 months showed no statistically significant difference in clinical outcome between patients with or without the APOE ε4 allele, nor was any worse clinical outcome seen at any later time. We also investigated whether the ICP and CPP were different in the groups. If so, this could be an explanation. However, no such difference was seen. In a logistic regression model, the APOE ε4 allele could not predict outcome.

A worse clinical outcome has been reported in patients with the ε4 allele after TBI in several, but no correlation in others, and even the opposite (Teasdale et al., 1997, Nathoo et al., 2003, Teasdale et al., 2005, Sundstrom et al., 2007a, Willemse-van Son et al., 2008). The literature is thus inconsistent with regard to the relation between the presence of ε4 and the outcome.

In this setting and population we did not find that the presence of the APOE ε4 allele influenced the outcome except at 3 months after injury and in one particular outcome dichotomization. In the literature, results have been reported on mixed patient populations in which the majority of the patients did not have a severe TBI; these patients were followed up at ≥ 6 months. The reported unfavorable outcome (GOS Scores 1–3) ranged from 19 to 35 %, and the corresponding favorable outcome (GOS Scores 4 and 5) ranged from 64 to 80 %. We found only two papers in the literature that reported exclusively on severe TBI, outcome, and the presence of the APOE ε4 allele. In both papers, the au-
thors described the treatment protocol (Alexander et al., 2007, Ost et al., 2008). Alexander et al. seem to have used a CPP-targeted therapy. They found no association between the APOE ε4 allele and the outcome, but they reported a slower recovery trajectory in the ε4 group (Alexander et al., 2007). Öst and associates used a protocol based on the Lund concept (Ost et al., 2008) and they found statistically higher mortality rates among men with APOE ε4 allele than those without APOE ε4 allele; however, this was not observed among women. Apart from this, there was no statistically significant difference in outcome between those with and those without the APOE ε4 allele.

We reported the occurrence of the APOE ε4 allele in 39.1% of the investigated patients. This is the highest reported frequency of APOE ε4 allele in a study population for severe TBI. In a study of mild TBI in a northern Swedish population, Sundström et al. reported an APOE ε4 allele frequency of 38.7% (Sundstrom et al., 2007a). Thus, the frequency of the APOE ε4 allele in this study is in concordance with the findings of Sundström and coworkers. In the literature, the frequency of APOE ε4 allele patients has been reported to vary between 11 and 41% with an approximate mean occurrence of 25% (Corbo and Scacchi, 1999).

We found a very high frequency of occurrence of the APOE ε4 allele in our study population and observed no significant influence of the APOE ε4 allele on the late clinical outcome, and this could be interpreted as an indirect sign that the presence of the APOE ε4 allele does not influence the clinical outcome after severe TBI.

The literature regarding the importance of the presence of the APOE ε4 allele is thus diverse. Some authors reported worse outcomes in the presence of the allele, some found no correlation, and others even found better outcomes in the presence of the allele.

We also showed that the ICP_{max} and CPP_{min} did not differ between the groups, and that the treatment goals, i.e. ICP < 20 mmHg and CPP > 50 mmHg, were reached in 97% of all registered ICP values during the first 120 h of treatment measured (once a minute). The treatment protocol as such could influence the findings as can the remarkably high frequency of ε4 in the patient cohort. What is less clear is how the different “adverse” alleles interact with each other. Largely unexplored are the questions of genetic contributors to the increased burden of mental diseases associated with TBI, and whether there are certain candidate genes/alleles that put an individual at increased risk for sustaining a TBI.

Patients with the APOE ε4 allele had significantly higher levels of S-100B_{max} and S-100_{AUC}, and an inclination towards higher levels of NSE_{max} and NSE_{AUC} com-
pared to non-ε4. The decline of the biomarkers toward normal values tended to be tardier in the APOE ε4 group.

Earlier studies have identified several roles for APOE in the central nervous system, which are possibly of importance after brain injury (Sundstrom et al., 2007a, McAllister, 2010). It is verified that APOE has an extensive role in preserving the integrity of the cerebral vasculature. Support for the hypothesis that vascular integrity and coagulation are relevant to the association of APOE ε4 allele with outcome after head injury has come from several clinical studies (Leung et al., 2002, Liaquat et al., 2002, Lanterna et al., 2005). From our data, one may speculate that there may be a gene-induced sensitivity to severe traumatic brain injury, and that patients with the APOE ε4 allele may be more receptive to brain cellular damage, or, a lack of repair ability, measured as S-100B and NSE. Therefore, it seems to be important to consider the APOE genotype when interpreting the levels of the biomarkers.

Kofke et al investigated whether NSE and S-100B increased after cardiac surgery in patients with the APOE ε4 allele (Kofke et al., 2004). They found significantly higher values of both brain injury markers in patients with the APOE ε4 allele. They concluded that patients with the APOE ε4 allele may be more susceptible to perioperative neural insults. McDonagh et al found no association between biomarker levels and the APOE ε4 allele in patients undergoing major non-cardiac surgery (McDonagh et al., 2010), but in this study none one of the patients had traumatic brain injuries.

The APOE ε4 allele has also been investigated regarding other CSF biomarkers than S-100B and NSE, and related to Alzheimer’s disease (AD). Kim et al found that the existence of one or more APOE ε4 alleles, affected the levels of the CSF biomarker Aβ, diagnostic for AD (Kim et al., 2011).

These studies demonstrate that the presence of the APOE ε4 genotype, may be associated with further pathologic processes in addition to its influence on S-100B and NSE in sTBI.

The conclusion is that patients suffering sTBI and expressing the APOE ε4 allele seem to release more S-100B than non-APOE ε4 allele carriers. Similar but not statistically significant results were observed for NSE. We speculate that these observations may be one of the explanations for the difficulty in interpreting the value of these biomarkers as a prognostic tool in s TBI.

Some studies report that S-100B and NSE are good predictors for worse outcome (Bloomfield et al., 2007, Murillo-Cabezas et al., 2010) while others report the opposite (Piazza et al., 2007, Bouzet et al., 2009, Olivecrona et al., 2009b).
Other authors suggest that NSE may not offer the same sensitivity and specificity as S-100B (Ross et al., 1996, Raabe et al., 1999). In this study, we used the AUC for the first three days, which we consider to be a measure of bulk marker release. This seems to be superior for the prediction of clinical outcome than to use the first measured value as earlier reported (Olivecrona and Koskinen, 2012b).

**Relation of biomarkers to physiological parameters, CT-findings and outcome**

We have reported different relationships between biomarker release and clinical parameters such as ICP and CPP and radiological findings. A clear correlation between ICP$_{\text{max}}$ and CPP$_{\text{min}}$ on the one hand and the S-100B and NSE levels was found. There were also significant correlations between the Rotterdam and Morris-Marshall CT scores and the S-100B$_{\text{max}}$ and S-100B$_{\text{BR}}$ values. More variable results were found between the CT scores and NSE. The clinical outcome assessed as GOSE at three months correlated negatively with the biomarker levels. We also observed that it was fairly possible to predict the three months survival using S-100B$_{\text{BR}}$ and NSE$_{\text{BR}}$ in the acute phase.

Our results imply that ICP$_{\text{max}}$ and CPP$_{\text{min}}$ clearly correlate with the biomarker levels measured as first value, maximum concentration and bulk release. The maximum concentrations and the bulk release of the biomarkers were better correlated to ICP$_{\text{max}}$ and CPP$_{\text{min}}$ than the first biomarker concentration in all cases. Our research group has previously shown that time-adjusted biomarker values and mean ICP were correlated (Olivecrona et al., 2009b). However, our present results show a greater degree of correlation.

An experimental animal study has shown that an increase in the ICP level results in an increase in the S-100B level (Ytrebo et al., 2000). This is partly in contrast to the results demonstrated in patients with sTBI (Woertgen et al., 1997).

We found complex correlations between S-100B and NSE with Marshall, Rotterdam and Morris-Marshall CT classifications. The presence of tSAH is a known negative prognostic factor with regard to the progression of pathological changes and their impact on the outcome. The Morris-Marshall classification score that describes the tSAH was only correlated with the S-100B levels. Since Morris-Marshall classification describes only the severity of tSAH, an explanation of this correlation could be that the S-100B and NSE levels reflect different types of pathophysiological changes after the sTBI. Our results confirm that the severity of the injury described by the CT classification systems is strongly correlated with the bulk release of both S-100B and NSE (Herrmann et al., 2000, Babcock et al., 2012). The initial and 24h Rotterdam scores were strongly correlated strongly with S-100B$_{\text{72h}}$, S-100B$_{\text{BR}}$ and NSE$_{\text{BR}}$. 
The correlation between the S-100B and NSE levels and the clinical outcome and prognostication of outcome is controversial. In this study, we found a statistically significant correlation between GOSE and the biomarker measures. $S_{100B_{br}}$ followed by $S_{100B_{72h}}$ showed the strongest correlation with the clinical outcome. Similar results were found for NSE. Furthermore, the highest AUC in the ROC analysis was found with the bulk release and the level at 72 hrs after the sTBI. It seems that these measures of biomarkers are superior for the prediction of clinical outcome than the first measured value, which was previously reported from our research group (Olivecrona et al., 2009b).

**Neurohormonal status, physiological parameters, CT-findings and outcome**

Severe TBI may affect the hypothalamic-pituitary hormone system during the acute phase, and it may also lead to long-term effects in terms of persistent posttraumatic hypopituitarism (Vespa, 2013). Many factors have been shown to influence the normal adaptive hypothalamo-pituitary reaction to acute critical illness, including actions affecting hormone production and binding (Hassan-Smith and Cooper, 2011, Dusick et al., 2012). There is also a risk for structural damage in TBI, causing temporary or permanent impairment (Munoz and Urban, 2013). Differentiating between these causes of altered hormone levels in the acute phase after TBI is difficult.

Anterior pituitary hormone alterations are often encountered in the acute phase after TBI (Dusick et al., 2012). The pertinence and therapeutic implications of such endocrine changes are still being discussed. The temporal course between TBI and hypopituitarism is poorly understood. Studies investigating TBI patients at different time after the acute phase to the years after the trauma have reported passing permanent and de novo deficiencies throughout this time span (Agha et al., 2004b, Tanriverdi et al., 2006, Klose et al., 2007, Kleindienst et al., 2009). This variation may be due partly to diagnostic difficulties, including those caused by stress or severe illness, but it may also be associated with the effect of medication such as anesthetics and analgesics.

As in many previous studies on acute TBI and acute critical illness, we found extensive changes in most pituitary-dependent hormones, i.e. low levels of thyroid hormones, strong suppression of the pituitary-gonadal axis and increased levels of prolactin.

In our study, s-cortisol levels were below 276 nmol/L in more than 50% of the sTBI patients. Nevertheless, we found no association between low cortisol levels and increased mortality or unfavorable outcome. In contrast to our results, the Dublin group found an increased mortality in patients with the lowest s-cortisol (Hannon et al., 2011). We found that in patients deceased at 3 months, there
was a tendency towards a higher serum cortisol in the acute phase than in survivors. This agrees with previous studies of cortisol levels in various types of critical illness and they may be attributed to a more severe critical illness and correspondingly higher stress response (Christ-Crain et al., 2007). Deviations in cortisol dynamics after severe traumatic brain injury are inconsistent, and correlations between serum cortisol levels and clinical outcomes have varied, since both high and low serum cortisol levels have been associated with poorer outcomes (Savaridas et al., 2004).

In agreement with a previous study, we found low levels of fT4 and fT3 in the acute phase of sTBI, with a significant decrease from days 1 and 4 (Woolf et al., 1988). FT3 levels were significantly lower at day 4 in patients who died within 3 months after sTBI than in survivors.

We found significantly lower fT3 levels in survivors with an unfavorable outcome at 3 months than in patients with a favorable outcome. Earlier studies have suggested an association between a stronger suppression of the hypothalamic-thyroid axis in more severe injuries and poor outcomes (Chiolero et al., 1988, Woolf et al., 1988). Accordingly, we found a negative correlation between day 4 TSH-levels and the Marshall CT grade score, i.e. more severe radiological findings were associated with a lower s-TSH on day 4 after sTBI. The low serum concentrations of both thyroid hormones and TSH found are consistent with a central repression of the hypothalamic-pituitary-thyroid (HPT) axis. This is corroborated by post mortem studies showing a decreased expression of thyrotropin-releasing hormone in the hypothalamic paraventricular nucleus of patients with a low serum T3 level (De Jongh et al., 2001). We found that a more prolonged and pronounced suppression of TSH was associated with an unfavorable outcome and worse CT-findings, suggesting that a less pronounced suppression and earlier recovery of the HPT-axis may be a marker of a less severe TBI and/or a more potent capacity to adapt and retrieve hypothalamic-pituitary function after TBI, with a more normal TSH-response (i.e. higher) to low thyroid hormone levels.

We found increased levels of serum prolactin after TBI, with a surge from day 1 to day 4. In line with previous studies, we have demonstrated hyperprolactinemia in more than 50% of our patients in the early, acute phase post-TBI, from mild to severe traumatic head injury (Bondanelli et al., 2002, Agha et al., 2004a, Klose et al., 2007). Day 1 serum prolactin levels were significantly negatively correlated with CPP_{min} and positively correlated with ICP_{max}, suggesting a higher prolactin release in more severe TBI. (Chiolero et al., 1988). Apart from this, we found no correlation between serum prolactin levels on day 1 or day 4 and the severity of TBI, which was reported in some previous studies (Matsuura et al., 1985, Chiolero et al., 1988, Agha et al., 2004a).
Gonadotropin (LH/FSH) and testosterone secretion is inhibited by elevated levels of prolactin, e.g. in patients with prolactinoma. However, in this study the elevated prolactin levels in acute TBI did not appear to contribute significantly to the strong suppression of the pituitary-gonadal axis, in agreement with previous studies (Spratt et al., 1993).

Also in accordance with previous studies, we found a strong central inhibition of the pituitary-gonadal axis already on day 1, with further suppression on day 4, when 100% of the male patients demonstrated testosterone levels under the reference range. Moreover, nearly all the men had low levels of LH and FSH both on day 1 and on day 4. Consistent with our results, a previous study showed a high incidence of hypogonadism in the early post-TBI period (Wagner et al., 2010). Previous studies have explicitly demonstrated that testosterone levels in males and oestrogen levels in females significantly decrease within the first 24 hours following TBI and remain low for 7–10 days (Agha et al., 2004a, Wagner et al., 2010).

The low levels of LH and FSH on day 1 were significantly related to more severe brain injury according to the Marshall CT grade score. This is in accordance with previous reports on associations between severe TBI (often lower GCS) and strong repression of the hypothalamus-pituitary-gonad-axis (Hannon et al., 2011). Male patients alive 3 months after TBI demonstrated significantly lower LH, FSH and testosterone levels on day 1 than non-survivors. Day 1 serum LH was also lower in male patients with a favorable outcome 3 months after TBI than in these with an unfavorable outcome, i.e. GOS 1-3. These observations indicate that very severe brain injury may impede the adaptive, physiological suppression of the pituitary-gonadal axis and that this incapacity is a poor prognostic sign.

Attention to sub-normal and supra-normal GH levels may be misguiding since GH is normally released in a highly pulsatile fashion. We found a strong variation in GH levels at all times. There was no significant diurnal variation, in mean serum GH levels between morning and evening. There was a trend towards a higher serum GH in patients who survived for 3 months than in those who died of their injuries. We also found a temporary decrease in serum IGF-1 with low levels on day 1, which were normalized on day 4 after sTBI. A low IGF-1 with elevated GH levels has been shown in the acute post-traumatic phase, as well as a normalization of GH and an increase in IGF-1 in the weeks after trauma (Wildburger et al., 2001). This has been attributed to a state of acquired peripheral GH resistance in critical illness (Van den Berghe, 2000). Contradictory literature is available on the GH levels following severe traumatic brain injury reported by various authors.
To investigate whether hormone levels may predict the outcome at 3 months post-injury we used a ROC curve. However, in our opinion the prediction power is still not sufficiently high and the clinical value is limited. ICP<sub>max</sub> had the strongest power to predict dead/alive in the whole study group. We believe that a combination of several predictors may be used together to provide more solid consistency in the prediction process.
Conclusion

The presence of the APOE ε4 allele did not indicate a poorer long-term outcome, but it was associated with significantly higher levels of S-100B than in non-APOE ε4 allele subjects, indicating that in some cases the genetics have to be considered when interpreting the biochemical markers. The biomarkers were also correlated with intracranial pressure and radiological findings and may predict mortality at 3 months. Profound hormonal changes occur in the acute phase. However, low levels of cortisol are not associated with a worse clinical outcome.
Summary of the Thesis

Patients with sTBI treated by an ICP targeted therapy based on the Lund concept (Asgeirsson et al., 1994, Grande, 2006) have been studied regarding different aspects of the prediction of outcome. Several factors influence the outcome after sTBI. By studying different aspects of genetics, biomarkers, radiology and hormone levels we try to come closer to some of the clues which may lead to further knowledge about how to predict the outcome after sTBI.

Except for one division of the GOS score at 3 months, the presence of the APOE ε4 allele does not influence the clinical outcome in patients with severe TBI treated with an ICP-targeted therapy based on the Lund concept.

Patients suffering sTBI who express the APOE ε4 allele seem to release more S-100B and in some cases more NSE as than non-ε4 subjects. The temporal profile of the biomarkers seems to differ between the APOE groups.

The bulk release of S-100B and NSE during the first three days after sTBI and the level at 72 hrs after trauma seems to be most sensitive in prediction of mortality. A combination of the bulk release of S-100B and the CT classification according to Morris-Marshall further improve the prediction of clinical outcome. The biomarkers have a clear correlation with the ICP_{max} and CPP_{min}. There is a complex correlation between the biomarker levels and the CT scoring systems.

Profound dynamic changes in hormone levels are found in the acute phase of sTBI. This is consistent with previous findings in different groups of critically ill patients, most of which can probably be attributed to physiological adaptation to acute illness. Low cortisol levels were a common finding, and these were not associated with unfavorable outcome. A retained ability to show a dynamic hormonal response, i.e. fast and strong suppression of the pituitary-gonadal axis and the ability to restore activity in the pituitary-thyroid axis was associated with a favorable outcome and less severe injury according to CT-findings.
Sammanfattning på svenska

Svår skallskada är i Sverige den vanligaste traumatiska orsaken till morbidityt och mortalitet. Vid Neurokirurgiska kliniken vid Umeå Universitetssjukhus behandlas patienter med svår traumatisk skallskada med en intrakraniell tryckstyrde terapi, baserad på en aggressiv neurokirurgi och fysiologiska principer som syftar till att kunna optimera mikrocirkulationen i hjärnan och därmed minska risken för sekundära hjärnskador.

Data är hämtade från en prospektiv randomiserad dubbel-blind läkemodelsstudie av prostacyklin vid svår skallskada.

Trots allt mer sofistikerade mediciner, diagnostiska instrument och teknologiska hjälpmedel såväl intensivvårdsmässigt som operativt, är det fortfarande mycket vanskligt att under den akuta skedet korrekt kunna prognosticera kliniskt utfall. Genom att studera en allel variant, biomarkörer, radiologisk bild, hormonella nivåer i det akuta skedet, har vi mått detta arbete försökt att angripa dessa problem.

Förekomsten utav genvarianten APOE ε4 allelen har diskuterats kunna ge ett sämre utfall vid svår traumatisk hjärnskada. Vi har visat att förekomsten av APOE ε4 allelen är vanligare hos patienter med dåligt (GOS 1-3) utfall vid 3 månader i jämförelse med bra utfall (GOS 4-5). Däremot sågs ingen skillnad mellan grupperna vid 12 och 24 månader. APOE ε4 allelen bidrog inte till förmågan att prognostisera outcome vid svår traumatisk hjärnskada.

Vi har även kunnat visa att APOE ε4 allelen är associerad till högre nivåer utav biomarkörerna S-100B och i vissa fall även NSE i jämförelse med individer som ej har APOE ε4.

Vi har också visat att S-100B och NSE kan associeras till ICP, CPP och till radiologiska fynd. S-100B och NSE har även en medelgod förmåga att diskriminera mellan död/ levande.

Neuroendokrins rubbningar har förknippats med negativa resultat både i den akuta och kroniska fasen efter svår skallskada. Dessa uppgifter understryker behovet av identifiering av hormonbrister, för att optimera patientens återhämtning från skallskada, förbättra livskvaliteten och undvika de långsiktiga negativa konsekvenserna av obehandlad hypofysisinsufficiens. Vi har visat att det sker kraftiga förändringar av hormonnivåerna i det akuta skedet. Låga kortisol nivåer var vanligt men inte associerat till dåligt utfall. Lågere nivåer av gonadhormoner liksom högre nivåer av thyroidea hormoner akut associerades till ett bättre utfall.
Slutsatsen är att APOE ε4 allelen inte kan användas för att prognosticera utfallet efter sTBI. Däremot har APOE ε4 allelen associerats till hjärnskademarkörv-inåerna, varför förekomsten av allelen bör tas i beaktande vid bedömning av hjärnskademarkörerna. Hjärnskademarkörerna är korrelerade till det intrakraniella trycket liksom till radiologiska fynd. Den totala mängden av hjärnskademarkörerna under de första 3 dygnen liksom nivåerna vid 72 h har högst förmåga att prediktera för utfallet. Låga nivåer av kortisol i akutskedet kan inte korreleras till sämre utfall.
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