Clinical Consequences of Axonal Injury in Traumatic Brain Injury

SAMi ABU HAMDEh
Traumatic brain injury (TBI), mainly caused by road-traffic accidents and falls, is a leading cause of mortality. Survivors often display debilitating motor, sensory and cognitive symptoms, leading to reduced quality of life and a profound economic burden to society. Additionally, TBI is a risk factor for future neurodegenerative disorders including Alzheimer’s disease (AD). Commonly, TBI is categorized into focal and diffuse injuries, and based on symptom severity into mild, moderate and severe TBI. Diffuse axonal injury (DAI), biomechanically caused by rotational acceleration-deceleration forces at impact, is characterized by widespread axonal injury in superficial and deep white substance. DAI comprises a clinical challenge due to its variable course and unreliable prognostic methods. Furthermore, axonal injury may convey the link to neurodegeneration since molecules associated with neurodegenerative events aggregate in injured axons.

The aim of this thesis was to study clinical consequences of axonal injury, its detection and pathological features, and potential link to neurodegeneration in severe TBI patients treated at the neurointensive care unit at Uppsala University Hospital. In paper I and IV DAI patients were studied for the relation of elevated intracranial pressure (ICP) and poor outcome to axonal injury on magnetic resonance imaging. In paper II, soluble amyloid-beta aggregates (oligomers and protofibrils), characteristic of AD pathology, were investigated in surgically resected brain tissue from severe TBI patients, using highly-selective Enzyme-Linked ImmunoSorbent Assays. In paper III, brain tissue biopsy samples from TBI patients with either focal injury or DAI were examined for differential proteome profiles using mass spectrometry-based proteomics.

The results provide evidence that axonal injury, located in the central brain stem, in substantia nigra and the mesencephalic tegmentum, is particularly related to poor outcome and increased ICP during neurointensive care of DAI patients. A novel classification system for prognostication after DAI is proposed. Furthermore, the thesis shows that severe TBI induces rapid accumulation of neurotoxic soluble amyloid-beta oligomers and protofibrils. In addition, DAI initiates unique proteome profiles different from that of focal TBI in structurally normal-appearing brain. These findings have implication for the clinical management of DAI patients, and provide new insight in the neuropathological consequences of axonal injury.

Keywords: Traumatic brain injury, diffuse axonal injury, intracranial pressure, magnetic resonance imaging, amyloid-beta, tau

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ISSN 1651-6206
urn:nbn:se:uu:diva-341914 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-341914)
To my family with love
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


* Contributed equally

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Additional publications not included in this thesis


Content

List of Papers ........................................................................................................... v
Abbreviations ........................................................................................................... ix

Introduction ............................................................................................................. 11
  1.1 Classification of traumatic brain injury ....................................................... 12
  1.2 Pathophysiology of traumatic brain injury .............................................. 15
  1.3 Multimodality monitoring in severe traumatic brain injury ..................... 16
    1.3.1 Monitoring of intracranial pressure .................................................. 17
    1.3.2 Monitoring of cerebral perfusion pressure ....................................... 17
    1.3.3 Monitoring of cerebral blood flow .................................................. 18
    1.3.4 Cerebral microdialysis .................................................................. 19
  1.4 Treatment algorithms in severe traumatic brain injury ......................... 19
  1.5 Outcome measures in traumatic brain injury .......................................... 20
  1.6 Diffuse axonal injury ............................................................................. 21
  1.7 Biomechanics of axonal injury ............................................................... 22
  1.8 Pathophysiology of axonal injury ........................................................... 22
  1.9 Impact of axonal injury ......................................................................... 24
  1.10 Detection of axonal injury ................................................................... 24
    1.10.1 Magnetic resonance imaging ....................................................... 25
    1.10.2 Neuromolecular imaging ............................................................. 27
  1.11 Classification of diffuse axonal injury .................................................... 28
  1.12 Consequences of axonal injury – clinical features during neurointensive care ................................................................. 29
  1.13 Consequences of axonal injury – a link to neurodegeneration? ...... 30
    1.13.1 Amyloid-beta pathology ............................................................... 30
    1.13.2 Tau pathology ............................................................................. 33

Aims ....................................................................................................................... 34
  2.1 Specific aims ............................................................................................. 34

Material and methods ......................................................................................... 35
  3.1 Patient population .................................................................................. 35
    3.1.1 Severe traumatic brain injury cohorts and timing of MRI ............ 35
    3.1.2 Control subjects ............................................................................. 36
  3.2 Image acquisition and analysis ............................................................... 36
  3.3 Clinical and multimodality monitoring data ......................................... 37
3.4 Sampling and preparation of brain tissue ........................................... 38
3.5 Immunohistochemistry for Aβ plaques .............................................. 38
3.6 Biochemical analysis of soluble Aβ species using ELISA .................. 39
3.7 Proteome analysis using mass spectrometry ..................................... 40
3.8 Outcome measures ............................................................................. 41
3.9 Statistical methods ............................................................................. 41

Results ........................................................................................................... 43
4.1 Paper I ................................................................................................ 43
4.2 Paper II ............................................................................................... 44
4.3 Paper III .............................................................................................. 45
4.4 Paper IV .............................................................................................. 49

General discussion ........................................................................................ 51
5.1 Paper I ................................................................................................ 51
5.2 Paper II ............................................................................................... 53
5.3 Paper III .............................................................................................. 54
5.4 Paper IV .............................................................................................. 56
5.5 Statistical considerations .................................................................... 57

Conclusions ................................................................................................... 59

Future perspectives ....................................................................................... 60

Summary in Swedish .................................................................................... 62

Acknowledgement ........................................................................................ 64

References ..................................................................................................... 67
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABP</td>
<td>Arterial blood pressure</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer's disease</td>
</tr>
<tr>
<td>APOE</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>Aβ</td>
<td>Amyloid-β</td>
</tr>
<tr>
<td>BACE-1</td>
<td>Beta-site APP-cleaving enzyme</td>
</tr>
<tr>
<td>βAPP</td>
<td>β-amyloid precursor protein</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood-brain barrier</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
</tr>
<tr>
<td>Cho</td>
<td>Choline</td>
</tr>
<tr>
<td>CPP</td>
<td>Cerebral perfusion pressure</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CTE</td>
<td>Chronic traumatic encephalopathy</td>
</tr>
<tr>
<td>DAI</td>
<td>Diffuse axonal injury</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
</tr>
<tr>
<td>DWI</td>
<td>Diffusion-weighted imaging</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked ImmunoSorbent Assay</td>
</tr>
<tr>
<td>EVD</td>
<td>External ventricular drain</td>
</tr>
<tr>
<td>FA</td>
<td>Fractional anisotropy</td>
</tr>
<tr>
<td>FLAIR</td>
<td>Fluid-Attenuated Inversion Recovery</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>GMT</td>
<td>Good monitoring time</td>
</tr>
<tr>
<td>GOS</td>
<td>Glasgow outcome scale</td>
</tr>
<tr>
<td>GOSE</td>
<td>Glasgow outcome scale extended</td>
</tr>
<tr>
<td>ICP</td>
<td>Intracranial pressure</td>
</tr>
<tr>
<td>iNPH</td>
<td>Idiopathic normal pressure hydrocephalus</td>
</tr>
<tr>
<td>ISF</td>
<td>Interstitial fluid</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass-to-charge ratio</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>MLS</td>
<td>Midline shift</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------------------</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MRS</td>
<td>Magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>NAA</td>
<td>N-acetyl aspartate</td>
</tr>
<tr>
<td>NFL</td>
<td>Neurofilament-light</td>
</tr>
<tr>
<td>NFTs</td>
<td>Neurofibrillary tangles</td>
</tr>
<tr>
<td>NI</td>
<td>Neurologically intact</td>
</tr>
<tr>
<td>NIC</td>
<td>Neurointensive care</td>
</tr>
<tr>
<td>PBtiO₂</td>
<td>Brain tissue oxygen partial pressure</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PiB</td>
<td>Pittsburgh compound B</td>
</tr>
<tr>
<td>PRx</td>
<td>Pressure reactivity index</td>
</tr>
<tr>
<td>P-tau</td>
<td>Hyperphosphorylated tau</td>
</tr>
<tr>
<td>rCBF</td>
<td>Regional cerebral blood flow</td>
</tr>
<tr>
<td>RLS</td>
<td>Reaction level scale</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SjvO₂</td>
<td>Jugular venous oxygen saturation</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single-photon emission computed tomography</td>
</tr>
<tr>
<td>SWI</td>
<td>Susceptibility-weighted imaging</td>
</tr>
<tr>
<td>T2* GRE</td>
<td>T2*-weighted gradient echo</td>
</tr>
<tr>
<td>TBI</td>
<td>Traumatic brain injury</td>
</tr>
<tr>
<td>TSAH</td>
<td>Traumatic subarachnoid hemorrhage</td>
</tr>
</tbody>
</table>
Introduction

Traumatic brain injury (TBI) is a leading cause of mortality and morbidity. Annually, 262 per 100 000 inhabitants are admitted for TBI in Europe and it accounts for one third of all injury associated deaths in the United States (1, 2). In Sweden, TBI accounts for 9.5 deaths per 100 000 inhabitants (3). It affects all ages and income groups and survivors are frequently left with debilitating deficits in motor, sensory and cognitive functions with marked impact on quality of life (4-7). TBI is considered a silent epidemic since the incidence is not seldom underestimated (1). The consequences following TBI persist long after the trauma and are not always immediately recognized (5). Since its incidence is particularly high among children and young to middle-aged adults, TBI has profound socioeconomic impact (8). In young and middle-aged adults, TBI is most frequently caused by motor-vehicle accidents, while in the pediatric and the elderly population falls account for the majority of TBI cases (1, 4, 9).

Historically, TBI has attracted the interest and fascination of ancient scientists, including Hippocrates (460–377 BC) who in his treatise “On Wounds in the Head” covered the contemporary knowledge of TBI thoroughly and extensively (10). In his treatise he described trepanation, a surgical method still in use today, for the treatment of skull fractures. Testimonies for this technique have been seen on trephined skulls from as far back as 10 000 BC, and in ancient times it was widely practiced in Western Europe, as well as in South America and Asia (11). Centuries later, Aulus Aurelius Cornelius Celsus (25 BC – AD 50), a scientist from the Alexandrian school of ancient Egypt, wrote one of the earliest known descriptions of severe TBI symptomatology, mentioning unconsciousness, focal signs, post-traumatic seizures and signs of elevated intracranial pressure (ICP) such as vomiting and blurred vision (11). However, the relation between altered level of consciousness and injury to the brain was not generally accepted until the 18th century (12). Modern neurosurgical management of TBI begun with the observations of Harvey Cushing on ICP (13) and the evolution of ICP monitoring techniques (14). Subsequently, TBI care evolved in combination with advances in neuroradiology (15). During the last two decades, progress in the management of TBI patients has been achieved mainly with the introduction and successive refinements of neurointensive care (NIC) and multimodality brain monitoring (16-19).
TBI is defined as an alteration in brain function, or other evidence of brain pathology, caused by an external force (20). Nonetheless, TBI may be considered “the most complex disorder in the most complex organ in the body” (21). Therefore this definition, unified only by the externally afflicted brain damage, encompasses a markedly heterogeneous entity. The injury spectrum ranges from mild to severe and commonly, TBI is categorized into either focal or diffuse injuries. While focal injuries include contusion and epidural, subdural and intracranial hemorrhage, diffuse injuries encompass concussion and diffuse axonal injury (DAI), with widespread damage to axons mainly in the subcortical white matter, the corpus callosum and the brainstem.

The theme of this thesis, DAI, is a particularly challenging subtype of TBI. Typically, patients with severe TBI and DAI are deeply unconscious at the impact site, and when admitted to hospital care, their initial radiological images using computed tomography (CT) display minimal findings. Furthermore, DAI has a variable clinical course both in the acute as well as in the chronic phase, and in addition, existing prognostic methods are unreliable. Therapeutic options for DAI are non-existing. Therefore, increased understanding of this condition is urgently needed.

1.1 Classification of traumatic brain injury

Traumatic brain injury is most often classified according to clinical severity indices, pathoanatomical type or physical mechanism (7). The Glasgow coma scale (GCS), a scale based on clinical assessment of level of consciousness using verbal and motor response and eye opening in response to stimuli (Table 1), is commonly used to classify TBI patients into broad categories of mild (GCS 15 – 13), moderate (GCS 12 - 9) or severe injury (GCS 8 or less) (22). The GCS has high inter-observer reliability, has proven to have excellent prognostic capabilities and is widely used in clinical trials (23). In most of Sweden, the similar Reaction Level Scale-85 (24) is used (Table 2). A comparison between the two scales is provided in Table 3.

Since scales assessing clinical severity of symptoms do not provide any information about the pathoanatomical background for the deficits (25), additional classification of location and anatomy of lesions using radiological characteristics is needed. Frequently, such classification is performed using the Marshall CT classification of TBI based on the degree of compression of the mesencephalic cisterns, the degree of midline shift, and the presence or absence of one or more surgical mass lesions (26) (Table 4). The Marshall classification has shown good prediction of ICP elevations and outcome. More recently, the Rotterdam CT classification has been developed to better enable classification using a combination of findings (27) (Table 5), which overcomes some
limitations of the Marshall classification, including difficulties in classifying patients with multiple injuries (7). Furthermore, newer CT scoring systems include the Stockholm and the Helsinki CT scores (28, 29) and seem to provide a more accurate outcome prediction (30).

Table 1. Glasgow Coma Scale (GCS)

<table>
<thead>
<tr>
<th>EYE OPENING RESPONSE</th>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPONTANEOUSLY</td>
<td>4</td>
</tr>
<tr>
<td>TO SPEECH</td>
<td>3</td>
</tr>
<tr>
<td>TO PAIN</td>
<td>2</td>
</tr>
<tr>
<td>NO RESPONSE</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VERBAL RESPONSE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ORIENTED</td>
<td>5</td>
</tr>
<tr>
<td>CONFUSED</td>
<td>4</td>
</tr>
<tr>
<td>INAPPROPRIATE WORDS</td>
<td>3</td>
</tr>
<tr>
<td>INAPPROPRIATE SOUNDS</td>
<td>2</td>
</tr>
<tr>
<td>NO RESPONSE</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MOTOR RESPONSE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>OBEYS COMMAND</td>
<td>6</td>
</tr>
<tr>
<td>LOCALIZES PAIN</td>
<td>5</td>
</tr>
<tr>
<td>WITHDRAWAL TO PAIN</td>
<td>4</td>
</tr>
<tr>
<td>ABNORMAL FLEXION</td>
<td>3</td>
</tr>
<tr>
<td>ABNORMAL EXTENSION</td>
<td>2</td>
</tr>
<tr>
<td>NO RESPONSE</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. Reaction Level Scale (RLS-85)

<table>
<thead>
<tr>
<th>CLINICAL RESPONSE</th>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALERT</td>
<td>1</td>
</tr>
<tr>
<td>DROWSY OR CONFUSED</td>
<td>2</td>
</tr>
<tr>
<td>VERY DROWSY OR CONFUSED</td>
<td>3</td>
</tr>
<tr>
<td>LOCALIZES PAIN</td>
<td>4</td>
</tr>
<tr>
<td>WITHDRAWAL TO PAIN</td>
<td>5</td>
</tr>
<tr>
<td>ABNORMAL FLEXION</td>
<td>6</td>
</tr>
<tr>
<td>ABNORMAL EXTENSION</td>
<td>7</td>
</tr>
<tr>
<td>NO RESPONSE</td>
<td>8</td>
</tr>
</tbody>
</table>
TABLE 3. COMPARISON BETWEEN REACTION LEVEL SCALE (RLS-85) AND GLASGOW COMA SCALE (GCS) (31)

<table>
<thead>
<tr>
<th>RLS-85</th>
<th>GCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCORE</td>
<td>SCORE</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>13-14</td>
</tr>
<tr>
<td>3</td>
<td>9-12</td>
</tr>
<tr>
<td>4</td>
<td>7-8</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

TABLE 4. MARSHALL CT CLASSIFICATION

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>DEFINITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIFFUSE INJURY I</td>
<td>No visible pathology</td>
</tr>
<tr>
<td>DIFFUSE INJURY II</td>
<td>Present cisterns, MLS 0-5 mm, No lesion &gt; 25 ml</td>
</tr>
<tr>
<td>DIFFUSE INJURY III</td>
<td>Compressed/absent cisterns, MLS 0-5 mm, No lesion &gt; 25 ml</td>
</tr>
<tr>
<td>DIFFUSE INJURY IV</td>
<td>MLS &gt; 5 mm, No 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>Lesion &gt; 25 ml, not surgically evacuated</td>
</tr>
</tbody>
</table>

CT = Computed Tomography, MLS = Midline shift
TABLE 5. ROTTERDAM CT CLASSIFICATION

<table>
<thead>
<tr>
<th>BASAL CISTERNS</th>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL</td>
<td>0</td>
</tr>
<tr>
<td>COMPRESSED</td>
<td>1</td>
</tr>
<tr>
<td>ABSENT</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MLS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NO SHIFT OR SHIFT ≤ 5 MM</td>
<td>0</td>
</tr>
<tr>
<td>SHIFT &gt; 5 MM</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EPIDURAL MASS LESION</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PRESENT</td>
<td>0</td>
</tr>
<tr>
<td>ABSENT</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>INTRAVENTRICULAR BLOOD OR TSAH</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSENT</td>
<td>0</td>
</tr>
<tr>
<td>PRESENT</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SUM SCORE</th>
<th>Total + 1</th>
</tr>
</thead>
</table>

CT = Computed tomography, TSAH = Traumatic subarachnoid hemorrhage

Additionally, TBI may be classified by the physical mechanism of injury, i.e., whether the head strikes an object (impact loading) or injury develops following brain movement inside the skull (inertial loading). Impact loading typically results in focal injuries, whereas inertial loading leads to subdural hemorrhage and diffuse injuries such as concussion, and DAI.

1.2 Pathophysiology of traumatic brain injury

Traumatic brain injury is not regarded as one single event, but rather a disease process initiated at time of impact and subsequently exacerbated by a series of complicated events leading to secondary injuries, and ensuing progressive deterioration (5). The impact results in the primary injury caused by mechanical deformation of brain tissue, with direct damage to neurons, glial cells and cerebral vessels (6). In its mildest form, the primary injury will result in biochemical and molecular alterations without visible lesions, but as the severity of the brain injury increases, the primary injury can encompass damage to brain structures incompatible with life. Unfortunately, this initial injury is not possible to treat, but preventive measures such as improved traffic safety, helmet use and prevention of falls in the elderly population can reduce individual suffering and related socio-economic cost (32-35).

Secondary injury processes may be initiated by the primary injury but may also be caused by a multitude of intracranial pathological events, secondary insults including hemorrhage expansion, cerebral edema, raised ICP and resulting ischemia. Furthermore, secondary insults may be due to systemic dis-
turbances such as electrolyte imbalance, hypo/hyperglycemia, pyrexia or hypoxia and hypotension secondary to respiratory distress or circulatory failure (36). On the cellular and molecular level, the initial impact offsets a cascade of events, including influx of calcium ions, mitochondrial damage and increase in free radical production leading to major disturbances in energy metabolism and extensive damage to the cytoskeleton (37, 38). These processes continue over the course of hours to days, and additionally, evidence suggests that neuroinflammatory and neurodegenerative processes continue for years following TBI (39-42).

Secondary injury leading to neurological deterioration was recognized in reports of trauma victims who succumbed to TBI, but had talked at some time after injury (43). This raised awareness of preventive measures and surveillance to avoid secondary insults, and has eventually led to the monitoring and treatment algorithms used today in the management of TBI patients, focusing mainly on the avoidance of secondary injury (16-19).

1.3 Multimodality monitoring in severe traumatic brain injury

Monitoring in severe TBI patients is aimed at avoiding clinical deterioration due to focal or diffuse neuronal distress (18). The brain is dependent on a continuous supply of oxygen due to high consumption and limited storage capacity. Although the brain only comprises a small portion of the body weight, it utilizes 15% of the cardiac output. Under normal circumstances, the cerebral blood flow (CBF) ranges between 20ml/100g/min in the white matter and 70ml/100g/min in the grey matter. Decreased CBF < 18ml/100g/min causes neuronal ischemia and reversible neuronal dysfunction, while CBF values < 10ml/100g/min results in neuronal death. However, cerebral oxygenation depends not only on CBF, but also on the arterial content of oxygen and the cerebral metabolic rate of oxygen (CMRO₂) (44). In TBI, ischemia plays a key role, and ischemic damage is observed in the vast majority of TBI patients at autopsy (45). Most commonly, cerebral ischemia following TBI is caused either by increased ICP or significantly decreased cerebral perfusion unable to match the brain’s metabolic demand. In addition to CBF reductions below critical values, neuronal distress may ensue despite seemingly adequate CBF. This occurs in situations where the brain’s metabolic demand is increased (e.g. seizures or hyperthermia) and not met by the cerebral perfusion or when mitochondrial dysfunction is a key feature, causing reduced oxidative metabolism (46). To aid the management of severe TBI patients, numerous techniques exist to monitor the different aspects of intracranial dynamics, in addition to standardized monitoring of systemic parameters.
1.3.1 Monitoring of intracranial pressure

In the awake patient, monitoring of neurological status using the GCS score, pupillary light reflex, motor and sensory assessment is sufficient to determine if there is improvement or worsening in the patient’s clinical status. However, in severe TBI, where depressed level of consciousness mandates sedation and mechanical ventilation to secure the patient’s airway (47), monitoring of ICP is regarded as fundamental in patient care to guide medical and surgical decisions (48). Refractory ICP elevation is strongly related to mortality and poor neurological outcome (49, 50), and management of ICP elevations above a threshold of 20 – 25 mmHg is widely considered standard of care. Nonetheless, in a trial comparing ICP monitoring to monitoring using clinical evaluations and neuroimaging to guide clinical decisions, similar outcomes were demonstrated (51). However, this study, conducted in Latin America where ICP monitoring is not regarded as standard of care, has insufficient external validity to be generalized (52) and ICP monitoring remains imperative to guide the treatment in severe TBI. Monitoring of ICP, usually performed via either an intraparenchymal monitoring device or an external ventricular drainage (EVD), is considered safe with low frequency of complications (53). In contrast to intraparenchymal ICP monitors, the EVD also provides a therapeutic option by allowing drainage of cerebrospinal fluid (CSF) for ICP control (54).

1.3.2 Monitoring of cerebral perfusion pressure

Cerebral perfusion pressure (CPP) is a simple and established measure of cerebral perfusion. Calculated by subtracting ICP from the mean arterial pressure (MAP), it provides continuous monitoring of the pressure driving the blood flow in the brain (55). For accurate CPP, MAP should be measured with the blood pressure transducer referenced at the level of the foramen of Monroi (56). The preferred threshold level for CPP is controversial but current recommendations are between 60 – 70 mmHg (55). Under normal circumstances, the cerebral pressure autoregulation, a process involving vasoconstriction and vasodilatation of cerebral vessels, maintains CBF unchanged although the MAP may vary considerably, ranging between 50 – 150 mmHg. Outside these limits, caliber changes of the cerebral vascular bed cannot withstand the effect of the significantly reduced or increased CPP and the CBF will rise or fall in accordance with the CPP (57). In the severely brain injured, cerebral autoregulation may be impaired, leading to inadequate CBF even with MAP well above 50 mmHg. Conversely, with elevations of MAP, CBF will rise sharply, causing hyperemia, increased cerebral blood volume and increased ICP. This may be aggravated by a disrupted blood-brain-barrier (BBB), causing leakage from the capillaries in the dilated vasculature, leading to brain edema (36), further ICP elevations and escalated cerebral ischemia (58). For this reason, it
is desirable to measure the status of cerebral pressure autoregulation to enable individualized treatment thresholds. By correlating the arterial blood pressure (ABP) to fluctuations in the ICP curve, measurement of the individual status of pressure autoregulation is possible (59). Additionally, by using the moving correlation coefficient between fluctuations in ICP and ABP, the pressure reactivity index (PRx) can be calculated and provides continuous monitoring possibilities of pressure autoregulation (60). The PRx, although strongly associated to outcome, has not been widely adopted into clinical routine.

1.3.3 Monitoring of cerebral blood flow

Direct measurements of CBF are feasible using perfusion CT or xenon-CT (61). Perfusion CT is rapid and widely available, however transfer of the patient from the NIC unit is usually a prerequisite (62). The xenon-CT utilizes inhalation of a gas mixture containing 28 % or 33 % xenon, a highly lipid soluble and radio opaque gas that rapidly diffuses into tissues, including the brain through the BBB (62). The xenon-CT has the advantage of being possible to perform with a mobile CT scanner, allowing for bedside measurements of CBF in ventilated patients. On the other hand, disadvantages include low signal-to-noise ratio, large slice thickness and xenon’s tendency to raise CBF, as increases of 30-40 % have been recorded (63). Additional imaging techniques providing measurements of CBF include perfusion magnetic resonance imaging, positron emission tomography (PET), and single-photon emission computed tomography (SPECT). These are however usually impractical in the critically injured.

Noteworthy is that imaging techniques provide intermittent CBF measurements and transient ischemia may be difficult to reveal. Methods for continuous monitoring of CBF exist in the form of a thermal diffusion method and a laser Doppler method, both requiring the insertion of an intraparenchymal probe to allow CBF monitoring. These methods measure CBF in only a small volume of brain tissue and the experience in the NIC is still limited (64, 65). Apart from these direct CBF measuring devices, indirect methods to quantify CBF adequacy include jugular venous oxygen saturation (SjvO2) and brain tissue oxygen partial pressure (PBtiO2). The SjvO2 can be quantified using a fiberoptic probe placed in the jugular bulb to measure oxygen saturation. In the uninjured brain, SjvO2 ranges between 55 – 75 %. Low Sjvo2 values indicate hypoperfusion and ischemia and episodes of desaturation are correlated with poor outcome (66). Additionally, high values > 75 % represent hypereemia of brain tissue, but can also occur after large brain infarctions, since oxygen is not extracted from irreversibly injured brain tissue (67). PBtiO2 requires a cerebral probe to be inserted, and allows regional measurements of cerebral oxygenation. Under normal circumstances, PBtiO2 values are > 20 mmHg and
critical hypoxia occurs with values < 10 mmHg. Also reduced PBtiO₂ is associated to poor outcome in TBI (68), and current treatment recommendations suggest interventions when PBtiO₂ falls < 15 mmHg (55).

1.3.4 Cerebral microdialysis

Cerebral microdialysis, utilizing a double lumen catheter with a semipermeable membrane inserted in the brain tissue, enables continuous neurochemical monitoring of the cerebral interstitial fluid (ISF). By perfusing the microdialysis catheter with a dialysate of artificial CSF, a concentration gradient is developed over the semipermeable membrane allowing for metabolites to diffuse through the catheter. It was introduced as a neurochemical tool in the NIC in 1992 (69), is extensively studied in TBI and currently widely used (70). Applications exist for bedside monitoring of different brain metabolites. Through the measurement of neurochemical biomarkers, assessment of energy metabolism (e.g. glucose, lactate and pyruvate including the lactate/pyruvate ratio), excitotoxicity (e.g. glutamate) and cell membrane degradation (e.g. glycerol) is possible. In addition, cerebral microdialysis allows for sampling and quantification of protein biomarkers of e.g. axonal injury, including amyloid-β and tau, thoroughly reviewed elsewhere in this thesis (71).

1.4 Treatment algorithms in severe traumatic brain injury

The Brain Trauma Foundation guidelines for severe TBI summarize the contemporary evidence regarding treatment of the severely brain injured (55) and provide recommendations strongly supported by well conducted and documented studies. However, it is noteworthy that many of the recommended therapies in severe TBI lack sufficient evidence from randomized controlled trials and are based on observational studies or expert consensus (72). In the developed world where resources exists, standardized treatment algorithms with focus on secondary injury prevention are the mainstay of TBI management in the NIC setting and have improved patient outcomes during the last decades (16, 73). With few exceptions, treatment algorithms are mainly based on ICP- and CPP-guided protocols, where stepwise escalation of medical or surgical therapeutic efforts is employed when the ICP is not sufficiently controlled, in accordance with the Brain Trauma Foundation guidelines (55).

In the NIC at the neurosurgical unit at the Uppsala University Hospital, treatment algorithms for severe TBI are based on the following principles; severe TBI patients presenting with GCS ≤ 8 are mechanically ventilated and sedated using a combination of intermittent intravenous (i.v.) morphine analgesia and
continuous i.v. propofol infusion. A CT scan is done at admission and repeat imaging is generously performed when needed. An ICP monitoring device is inserted for continuous measurements of ICP and CPP in all patients with severe TBI. All patients are initially mildly hyperventilated (PaCO₂ 30–35 mm Hg; 4.0–4.5 kPa), kept with head of bed elevated 30°, and treated with volume expansion to normovolemia. Normoventilation is applied as soon as possible as determined by the ICP. Secondary brain injury is minimized by keeping ICP at < 20 mmHg and CPP at > 60 mmHg, using an organized secondary insult program. Surgical evacuation of mass lesions is rapidly performed. Intracranial pressure elevations not controlled by standard therapy or CSF drainage are treated with a pharmacologically-induced coma, using continuous propofol infusion or continuous sodium pentobarbital infusion therapy, and/or decompressive craniotomy (16).

1.5 Outcome measures in traumatic brain injury

When assessing outcome after TBI, care must be taken to choose measures that are clinically relevant. To gain statistical power, it is preferable to use end-points that are frequently distributed. One previously common end-point in TBI research is mortality. However advances in the care of TBI patients in developed countries have made mortality less prevalent. Instead the Glasgow Outcome Scale (GOS), an ordinal scale with five categories objectively assessing the degree of functional recovery, is the most widely used outcome measure (74) (Table 6). In addition, an extension of GOS with eight categories, the Glasgow Outcome Scale Extended (GOSE), exists to overcome limitations of broad categories. The extended scale divides the good, moderate and severe categories of GOS into an upper and a lower class. The use of GOSE allows for potentiation of statistical power, as the increased number of categories in the ordinal scale theoretically provides increased sensitivity. Unfortunately, statistical power is lost when dichotomization is performed, a commonly used approach in clinical research. The practice of dichotomization has the advantage that it allows for the division of patients into equally sized groups, but at the same time it underuses the information gained by the ordinal scale (75).

The GOSE evaluation is performed via a standardized questionnaire based on structured interviews with the patient or the patient’s closest relative (76). The interview covers symptoms including altered consciousness, independence inside and outside home, ability to travel or shop, work status, social activities and relationships to family and friends as well as degree of return to pre-TBI lifestyle (77). The questionnaire approach enhances reliability and offers objectivity with a high degree of agreement between assessors. The time-point when the outcome is assessed should preferably be at least 6 months post-
trauma, to allow for sufficient improvement to occur. This is especially rele-
vant in patients with diffuse brain injuries, since recovery is considered to oc-
cur over long periods of time. However, further extending the period post-
trauma at which outcome is assessed carries the risk of causing patient drop-
outs from follow-up. In addition, although some improvement is possible be-
yond 6 months, it is likely the result of rehabilitative measures rather than true 
recovery (77).

**TABLE 6.**
GLASGOW OUTCOME SCALE
(GOS) (74)

<table>
<thead>
<tr>
<th>GOS CATEGORY</th>
<th>FUNCTIONAL STATUS</th>
</tr>
</thead>
</table>
| GOOD RECOVERY | Resumption of normal life, there may be minor neurological and/or psycho-
logical deficit. |
| MODERATE DISABILITY | Independent in 'daily life'. Able to maintain self-care and 'activities of daily living'. Considerable family dis-
ruption possible. |
| SEVERE DISABILITY | Dependent on daily support because of physical and/or mental causes. |
| PERSISTENT VEGETATIVE STATE | Unresponsive and speechless for weeks or months after acute brain damage. Sleep-wake cycles return after 2-3 weeks. |
| DEATH | Ascribable to a particular incident and due to original brain damage. |

1.6 Diffuse axonal injury

Widespread axonal injury was first reported by Sabina J. Strich in 1956 (78). In her paper, Strich described five victims of severe dementia following TBI who at autopsy 5-15 months post-injury revealed diffuse degeneration of cerebral white matter. Some decades later, the clinical entity of DAI was charac-
terized by the work of Adams, Genarelli and colleagues (79, 80), linking rotational acceleration-deceleration forces to diffuse shear injuries of white matter structures. By duplicating findings in human TBI victims suffering from prolonged posttraumatic coma, it was concluded using primate models that widespread axonal damage is the major cause of posttraumatic unconsciousness without focal lesions (79). Specifically, axonal injury was frequently found in predilection sites involving mainly subcortical white matter of the frontal and temporal lobes, the body and splenium of the corpus callosum and in the most severe cases, the upper rostral region of the brainstem (81). Today, DAI is considered a frequent finding in patients suffering from TBI, existing in approximately half of closed TBI cases in combination with focal lesions (82). Diffuse axonal injury in isolation, however, is less common (83). Still, DAI has a particularly negative impact on outcome, as patients frequently are left with severe motor, cognitive and behavioural impairments (84-86). Characteristically, severe TBI patients with DAI present with deep unconsciousness but no focal lesions on the initial admission CT scan. Advanced neuroimaging, using mainly magnetic resonance imaging (MRI), has enabled detection of axonal injury dispersed diffusely in white matter structures (87-89).

1.7 Biomechanics of axonal injury

Under normal circumstances, brain tissue is compliant and resilient to mechanical stretch, and can reshape to its original form (90). However, in DAI the principal force applied is rotational acceleration-deceleration leading to dynamic shearing, tensile and compressive deformation of the brain tissue (79, 91). The severe strain is instantly applied, causing the brain tissue to act stiff and brittle (92). The normally pliable axons will not withstand the uniaxial stretch or elongation, which cause damage to the axonal cytoskeleton. Additionally, the large size of the human brain and the variable densities of gray and white matter cause mass effects at the moment of injury, resulting in tension in the gray/white matter interface (91, 93). Although the deformations seldom lead to axonal disconnections at impact, these events trigger axonal pathology that eventually may lead to delayed axotomy (94). Additionally, damage is more profound in unmyelinated axons which seem vulnerable to injury and less likely to recover (95, 96).

1.8 Pathophysiology of axonal injury

Histologically, DAI is characterised by β-amyloid precursor protein (βAPP) accumulations in injured axons as either a single large axonal swelling, the classical axonal bulb, or as periodic localised swellings, axonal varicosities (Figure 1) (97). βAPP is involved in axonal transport and it moves down the
axon via anterograde axonal transport. As the axon is injured, the axonal transport is interrupted, and thus βAPP accumulates. This produces reactive axonal swellings appearing as bead-like structures along the axonal length, representing disturbed but not totally interrupted axonal transport (94, 98).

Axonal stretch causes local mechanical dysregulation of sodium channels, resulting in calcium influx (99). The excess intracellular calcium leads to microtubular loss and neurofilament impaction with damage to the cytoskeleton. In addition, neuronal depolarisation ensues as the cell membrane permeability is disrupted (100), while local mitochondrial damage leads to oxidative stress and disturbed energy metabolism (101). Calcium-mediated proteolysis of the cytoskeleton results in impaired axonal transport, swelling and ultimately disconnection. In the most severe axonal injury, axonal poration leads to an uncontrolled calcium surge, rapid breakdown of the axonal cytoskeleton and distal axonal fragmentation and disconnection (94).

Figure 1. Immunohistochemical image from a TBI patient included in paper II of the thesis. Axonal injury is evident, here as pathological accumulation of beta-amyloid precursor protein (βAPP) in injured axons (*), and axonal swelling (**).

These neuropathological features may appear already within the first few hours and progress in size during 1-2 days following injury (81, 94, 97). Distal to the disconnection, Wallerian degeneration of the remaining axon occurs. Of interest, the βAPP can subsequently be cleaved by mainly presenelin-1 and
beta-site APP-cleaving enzyme (BACE-1) to yield β-amyloid (Aβ), the main component of insoluble aggregates observed in Alzheimer's disease (AD) (102). Since TBI is a known environmental risk factor for AD (103-105), βAPP accumulations have caused DAI to be particularly appealing as a potential link between the two diseases.

1.9 Impact of axonal injury

In TBI, structural lesions visualized with CT or conventional MRI fail to explain the full extent of cognitive, emotional and behavioral symptoms observed (87, 106), mainly caused by disconnections in crucial brain networks due to axonal injury (107-109). In the human brain, neighboring and remote cortical regions are interconnected by a complex network of cortico-cortical axonal pathways (110), producing large-scale functional units. Patients suffering from DAI, in particular, tend to present severe deficits in cognition such as memory, attention and executive functions. Such complex cognitive functions are dependent on the integrity of widely distributed functional connections (111). The brain displays a small world architecture, where local hubs are highly connected and integrated with spatially remote areas through long-distance white matter tracts, typically damaged in DAI. These large-scale connectivity networks are tightly coupled with high-level cognition. Consequently, axonal injury disrupts connectivity between brain structure and function (108, 112). Cognitive deficits in TBI are associated with injuries in specific white matter connections. Damage in white matter tracts connecting frontal to posterior regions, such as superior longitudinal fasciculus, corona radiata, internal capsule, and cingulum, leads to executive dysfunction (107, 113). Impaired connections in the thalamo-frontal network associate with attention and executive function deficits (114), while disruption in interconnecting fibers from the hippocampus, through the fornix to the basal forebrain and diencephalon relates to memory dysfunction and learning difficulties (107). In addition, damage to limbic fibers interconnecting temporal lobe structures to the medial frontal lobe is linked to memory deficiencies (108). Only recently have these disconnections been possible to visualize, with the development of advanced MRI methodology, particularly diffusion tensor imaging (DTI).

1.10 Detection of axonal injury

Generally in TBI, CT is the preferred initial imaging modality, due to its wide availability, rapid acquisition and sensitivity in visualizing acute hemorrhages. However, its utility in DAI is limited, since CT poorly detects subtle axonal lesions in deep white matter structures (115, 116). Findings on CT are
mainly restricted to traumatic edema of the brain and petechial hemorrhage in the white matter suggestive of DAI. For diagnostic purposes, surveillance of axonal injury progression and adequate prognosis, other imaging modalities are more suitable.

1.10.1 Magnetic resonance imaging

Magnetic resonance imaging can detect microscopic amounts of blood (117) as well as non-hemorrhagic lesions in white matter associated with DAI (89), making this modality the primary method for detecting axonal injury in TBI patients. Additionally, MRI allows visualization, assessment and neurochemical analysis of critical white matter tracts.

1.10.1.1 Conventional MRI

Two MRI sequences sensitive to hemorrhagic lesions are widely available, the T2*-weighted gradient echo (T2*GRE) and susceptibility-weighted imaging (SWI). These sequences detect microhemorrhages from injury of small vessels running alongside axons. Paramagnetic properties of hemoglobin degradation products from the microhemorrhages produce distortion in the magnetic field, and thus hypointense lesions appear (118). Susceptibility-weighted imaging is more sensitive than T2*GRE in detecting hemorrhagic lesions in deep seated white matter regions (119, 120). Taking advantage of susceptibility differences between tissues (121, 122), it provides means to study microhemorrhages in deep brain regions previously difficult to detect. Importantly, both sequences sensitive to blood products depict lesions larger than their true size, and the lesion volume is strongly influenced by the MRI scanner’s properties, in particular, the magnetic field strength (123). In addition, particularly the SWI sequence, may be difficult to interpret as deoxygenated blood in veins can mimic hemorrhagic lesions, and enlarged veins may result in areas of signal loss (124).

Non-hemorrhagic lesions can be visualized with Fluid-Attenuated Inversion Recovery (FLAIR) and diffusion-weighted imaging (DWI). The FLAIR sequence adds a long inversion time to a T2-weighted spin echo sequence to suppress CSF, causing it to appear dark in the images. Therefore, FLAIR aids in the detection of axonal injury near CSF spaces, particularly in the periventricular white matter, the corpus callosum and the brainstem (125, 126). The FLAIR sequence is however, unspecific to axonal injury and timing of the MRI acquisition strongly influences its ability to detect injury. Following TBI, lesions may in the acute phase represent tissue edema in white matter, but may also be due to old scars, since a similar signal can be caused by encephalomalacia or tissue gliosis (127). In addition, lesions seem to diminish with time (126), limiting its usefulness to some extent in DAI. The DWI sequence is acquired by adding sequential gradient pulses to 90° and 180° spin-
echo sequences, and is sensitive to the microscopic motion of water molecules (128). It can therefore detect non-hemorrhagic lesions due to microstructural abnormalities following axonal shearing. The DWI sequence, in similarity with FLAIR, is influenced by the timing of the MRI, as the signal may evolve over time (129) and be significantly reduced already after 3 months post-TBI (126).

1.10.1.2 Diffusion tensor imaging

Diffusion tensor imaging is a technique where diffusion weighting is applied in different directions to estimate the amount of anisotropic water diffusion. This provides a quantitative measurement of axonal injury and enables precise anatomical reconstruction of white matter (130). Diffusion tensor imaging has enhanced sensitivity for axonal injury in comparison with conventional MRI, and accurately detects ultrastructural changes. By using post-processing techniques, DTI can be utilized to create detailed three-dimensional images of white matter tracts (131, 132). Parameters acquired with DTI include the mean diffusivity, which corresponds to the average diffusivity of water molecules, and the diffusion anisotropy or the fractional anisotropy (FA), measuring water molecules degree of directionality (130, 133). Additionally, the radial diffusivity measures water diffusion in two directions perpendicular to axons, while the axial diffusivity quantifies the diffusion along axons (133, 134). In DAI, reduced FA and increased diffusivity are observed (87, 135, 136), correlate to TBI severity (137, 138) and associate with cognitive and behavioral deficits (107, 139-144). Alterations in DTI parameters persist and may progress until the chronic phase post-TBI, with continuously decreasing FA and increasing diffusivity over time, and measurable deterioration of axonal integrity beyond 24 months post-injury (136, 145-148). Diffusion tensor imaging is an extensively studied technique to visualize axonal injury. However, DTI is to some extent impractical in the acute phase in severe TBI, as post-processing statistics are required to produce the images. Additionally, variations in data acquisition and analysis techniques as well as the location of structures investigated in previous studies prevent generalized conclusions of the clinical utility (149).

1.10.1.3 Magnetic resonance spectroscopy

The chemical shift is a phenomenon caused by variations of proton resonance frequency due to the local chemical environment. This can be utilized to detect and quantify neurochemical alterations in the brain by a technique called magnetic resonance spectroscopy (MRS) (150). The most studied metabolites in TBI are N-acetyl aspartate (NAA), a marker for neuronal and axonal integrity extensively found in neurons (151), and choline (Cho), which is increased after damage to cell membranes (152). In TBI, a decrease in NAA is generally seen, whereas Cho is typically increased (153-159), and these findings associate with neurocognitive deficits (160-162) and global outcomes (154, 155,
Magnetic resonance spectroscopy can detect subtle axonal injury, not possible to visualize using conventional MRI. Furthermore, measurements of glutamate can indicate early excitotoxic injury (163), while lactate elevations can imply hypoperfusion and anaerobic metabolism (164, 165).

1.10.2 Neuromolecular imaging

Neuromolecular imaging techniques include SPECT and PET. These techniques can be used to assess the neurochemical and neurophysiological environment in the brain, either by assessing variables related to brain functional activity and energy metabolism, or by assessments of specific neurochemical metabolites (166).

1.10.2.1 Single-photon emission computed tomography

In SPECT, radiopharmaceutical agents are used to produce images of physiological or pathological processes. Most commonly in TBI, \(^{99m}\text{Tc}\) Hexamethylpropylenamine oxime (HMPAO) and \(^{99m}\text{Tc}\) Ethylcisteinate dimer (ECD) are used to measure the regional cerebral blood flow (rCBF) and indirectly, the regional cerebral metabolism (167). SPECT provides an adjunctive measure to anatomical imaging modalities of white matter injury and has the advantage of being both affordable and widely available. Specifically, SPECT allows an objective visualization of functional brain pathology, and thus is helpful in situations where deficits are discordant with visible structural injuries by MRI (168). Studies of rCBF following DAI have shown decreased blood flow in the frontal lobe and dysfunction in deep areas including the brainstem. Particularly, the cingulate gyrus is commonly involved despite a lack of visible abnormalities (169-171), a finding that is associated with neuropsychological deficits (170). Plausibly, this could be due to deafferentation of interconnecting white matter caused by widespread axonal damage (172, 173). In addition, research is currently focused on developing radiotracers for imaging of aggregates of A\(\beta\) and tau in AD (174, 175), efforts with potential implication also after DAI.

1.10.2.2 Positron emission tomography

In PET, radiopharmaceutical agents labelled with positron-emitting radioisotopes, most commonly fluorine-18 \(^{18}\text{F}\), carbon-11 \(^{11}\text{C}\) and oxygen-15 \(^{15}\text{O}\) are used to image neurochemical and neurophysiological processes. The \(^{15}\text{O}\)-radioisotope can be used to measure CBF, CBV and CMRO\(\text{2}\) with high resolution (61). The commonly used \(^{18}\text{F}\)-labeled fluorodeoxyglucose (FDG) PET can measure local glucose metabolism, and thus regional neuronal activity. Similar to SPECT, FDG PET studies have revealed regional hypometabolism in medial frontal lobe structures including the cingulate gyrus in DAI (172, 176), findings associated with neuropsychological and cognitive symp-
In addition $[^{11}\text{C}]$PK11195, reflecting microglial activation, can be used to study neuroinflammation in the white matter following TBI (178). Furthermore, PET provides a method for imaging of substrates of axonal injury, particularly Aβ and tau, of special interest with regards to neurogenerative aftermath following DAI. Imaging of Aβ using the $[^{11}\text{C}]$ labeled Pittsburgh compound B (PiB) have shown increased retention signals in the cortex and striatum in TBI, a finding similar to what is commonly observed in AD (179, 180). With regards to tau imaging, two small studies of retired contact sport athletes have revealed tau elevations in vivo (181, 182), however, the radiotracer used, $[^{18}\text{F}]$FDDNP, binds both to tau tangles and Aβ aggregates. New tracers such as $[^{18}\text{F}]$T808, binding specifically to paired helical fragments of tau, are currently being developed (183).

1.11 Classification of diffuse axonal injury

As previously mentioned, diffuse injuries can be classified using the Marshall CT classification, taking the degree of compression of the mesencephalic cisterns, the degree of midline shift, and the presence or absence of one or more surgical mass lesions into account (26). However, this grading system is not specific for DAI. In 1989, Adams and colleagues proposed a histopathological grading for DAI based on autopsy findings in fatal TBI (81). The grading consisted of three grades and was based on the presence of axonal injury in the cerebral hemispheres with a predilection for the grey-white interface (Grade 1), the corpus callosum (Grade 2) and in the brainstem (Grade 3) (Table 7). With the development of advanced MRI, surrogate markers for the lesions found histopathologically in DAI became possible to visualize and therefore the Adams grading system has been adopted to neuroimaging (115). Today, this grading system is widely used. In severe TBI patients evaluated with MRI, at least 50% are grade III DAI using this grading system, with evident lesions in brainstem structures (83). Nonetheless, it is important to recognize that MRI cannot appreciate the full extent of the histopathological findings.
### TABLE 7.
**ADAMS DAI GRADING (81)**

<table>
<thead>
<tr>
<th>GRADE</th>
<th>PATHOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Widespread axonal damage in white matter of cerebral hemispheres</td>
</tr>
<tr>
<td>II</td>
<td>White matter damage extending to the corpus callosum with tissue tear hemorrhages</td>
</tr>
<tr>
<td>III</td>
<td>Pathology of grade I-II and in addition, tissue tear hemorrhages in the brainstem</td>
</tr>
</tbody>
</table>

#### 1.12 Consequences of axonal injury – clinical features during neurointensive care

Patients with severe TBI and DAI in isolation exhibit highly variable clinical characteristics, distinct from patients with predominantly focal injuries. Although patients with focal injuries may present initially with a markedly depressed level of consciousness, this clinical feature is more commonly encountered following DAI (93). In DAI, the deep coma seen at impact is caused mainly by damage to axons in diencephalic and brainstem structures (93, 184, 185). In addition, axonal injury in the pyramidal tract can cause motor deficit (186), without obvious radiological correlates on the initial CT scan. To date, methods used to prognosticate outcome following DAI in the acute setting are unreliable, and it is challenging to predict which patient will eventually regain consciousness and the time-point when this will occur (93, 184, 185). Fortunately, the advanced neuroimaging techniques previously described herein show promise in providing adequate prognostication in the near future. Some efforts have already been made to improve the prognostic accuracy with an increasing number of studies, and DAI-associated lesions in the corpus callosum, thalamus, and particularly the brainstem seem coupled to poorer outcomes (84, 125, 184, 187).

Moreover, the risk of developing elevated ICP in DAI is difficult to predict and studies evaluating the prevalence of high ICP show contradictory results (188-192). Already in 1982, Narayan and colleagues presented their experience from ICP monitoring in 207 severe TBI patients, of which 61 had a normal initial CT scan and were suspected to have DAI. Increased ICP was uncommon in this patient category unless the patient was aged > 40 years, had unilateral or bilateral motor posturing or episodes of systolic blood pressure <
90 mmHg (189). Similarly, a low incidence of increased ICP has been observed by other authors (188, 192), although short elevations of ICP still exist and in some cases require treatment (188). On the contrary, one study found increased ICP in the majority of severe TBI patients, despite the absence of mass lesions, midline shift or compressed basal cisterns on the initial CT scan (190). This study has limitations however, due to the low sample size. Common to all these studies, is that they were conducted before MRI was widely adopted in severe TBI management, and relied on CT scans with poorer quality than today’s standards. Studies evaluating ICP in relation to MRI findings in DAI are scarce, however an association between the maximum ICP and the number of hemorrhagic lesions on T2*GRE has been implicated (191).

1.13 Consequences of axonal injury – a link to neurodegeneration?

Compelling epidemiological evidence has linked TBI to the development of various neurodegenerative disorders (104, 105, 193-195). An episode of severe TBI has been observed to cause a fourfold increased risk for developing Alzheimer’s disease (AD) (193) and may also result in an earlier age at disease onset (196, 197). There is evidence suggesting that the risk of dementia rises with increased TBI severity (104, 193). Furthermore, large recent epidemiological studies have indicated an association between TBI and future onset of Parkinson’s disease (PD) (194, 195). Additionally, repetitive TBI has since long been linked to the development of cognitive and behavioral symptoms, a syndrome previously described as dementia pugilistica, but now termed chronic traumatic encephalopathy (CTE) (198, 199).

In AD, the hallmark histopathological findings are insoluble Aβ aggregates/plaques and neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau (P-tau) protein (200). Aβ pathology has been strongly linked to the etiology of AD, and intracellular fibrillary deposits of tau are characteristic of a large variety of neurodegenerative disorders including AD (201). Accumulation of P-tau into NFTs is also the characteristic finding in CTE and has been observed in a majority of symptomatic athletes where this condition was diagnosed post-mortem. In addition, concomitant Aβ pathology is frequently seen also in CTE (40, 199), albeit with a different distribution than in AD (202).

1.13.1 Amyloid-beta pathology

Following cleavage of βAPP, monomeric Aβ peptides of various lengths are produced. These peptides can either be N-terminally intact molecules or be
cleaved at the N-terminal, yielding N-terminally truncated forms of various lengths (203, 204). The Aβ monomers assemble as large soluble oligomers, defined as Aβ aggregates of variable molecular weights < 100 kDa, which may then assemble into protofibrils with a molecular weight > 100 kDa (205).

Figure 2. Evolution of Aβ aggregates begins with the enzymatic processing of βAPP that yields both N-terminally intact and truncated Aβ monomers, of which Aβ40 and Aβ42 are the most frequently found in Alzheimer’s disease related plaques. Monomers can then aggregate into soluble intermediary Aβ species, with potential toxic properties, and eventually aggregate further into insoluble Aβ plaques.
Further assembly of the soluble protofibrils can then form insoluble fibrils that constitute the Aβ plaques (Figure 2). Of particular interest for neurodegenerative development are the monomeric Aβ40 and Aβ42 peptides, which are prone to aggregate and are the major Aβ species found in Aβ plaques. It is however not verified whether the Aβ plaques confer the neurotoxicity in AD, and more attention is currently attracted towards soluble intermediary Aβ species such as oligomers and protofibrils, which have been assigned neurotoxic properties (206-209).

In TBI, accumulation of βAPP is a feature observed in injured axons (97, 210), and detected within hours (211), where βAPP may co-accumulate with its Aβ converting enzymes presenelin-1 and BACE-1 (97). Furthermore, deposition of Aβ, both as soluble monomeric peptides and insoluble Aβ plaques have been consistently reported in brains of TBI patients (211-215). By using microdialysis to analyze monomeric Aβ in ISF, it was also suggested that accumulations are more pronounced in patients with DAI, in comparison to focal injuries (213). Notably, immunohistochemical studies, as well as PiB PET imaging, have shown that these accumulations may persist over time in a subset of patients (41, 180).

Aβ pathology is a general finding following TBI, however Aβ plaques seem restricted to about 30 % of patients across all age groups (211, 215, 216). Plausibly, this is due to genetic predispositions. Epidemiological data implicate the lipid transporter protein Apolipoprotein E ε4 (APOE ε4) allele as a risk factor for the development of AD pathology, particularly in APOE ε4 allele carriers who previously sustained a TBI (104, 105, 217, 218). The APOE allele distribution varies substantially among populations with ranges of 0-20 % for APOE ε2, 60-90 % for APOE ε3 and 10-20 % for APOE ε4 (219). In Caucasians, homozygote APOE ε4 genotype is prevalent in 1.2 – 3.2 % of the population while heterozygote APOE ε4 genotype ranges between 1.5 % in Italy to 30.6 % in Finland (220). Although the exact mechanism by which APOE ε4 conveys the increased risk of AD is unclear, APOE regulates Aβ proteolysis and clearance in an isoform-dependent manner and may modulate Aβ–induced oxidative stress (APOE ε4< APOE ε3< APOE ε2) (221-224). In early mild cognitive impairment, APOE ε4 may cause increased cortical Aβ deposition (225), implying an early role in Aβ dysregulation in AD progression. Several meta-analyses show that the APOE ε4 allele is associated with a worse outcome following TBI, including an increased risk of seizures and dementia (226-228). Furthermore, the APOE ε4 allele has been associated with increased Aβ burden in TBI post-mortem brains and in CTE patients (202, 229). Experimental evidence also suggests that clearance of monomeric Aβ is impaired in APOE ε4 carriers following TBI (230).
1.13.2 Tau pathology

The microtubule-associated protein tau is an important structural element in the axonal cytoskeleton. It exists in six isoforms and under normal circumstances its biological activity is regulated by phosphorylation. However, hyperphosphorylation of tau may lead to aggregation in NFTs, which is associated with a number of neurodegenerative diseases (201). In TBI, tau release is particularly linked to axonal injury (41, 231). Tau has been found elevated both in CSF and in ISF monitored by microdialysis in TBI patients (231-235), and ISF tau correlates to axonal injury depicted using DTI (232). Initial CSF tau levels seem to reflect injury severity and are associated with patient outcomes (236). In addition, high serum tau levels after TBI are associated with a worse outcome, probably reflecting a more severe axonal injury (237). Although tau deposited into NFTs has not been observed in the early phase post TBI, experimental evidence implies that P-tau develops rapidly after trauma (238). In young athletes who died within 6 months from a concussion, NFTs were observed in a subset (239). Furthermore, widespread NFTs were found in one-third of long-time survivors following a single TBI at autopsy (41), suggesting that NFTs take time to develop. The distribution of NFTs following moderate to severe TBI is similar to that seen in AD, however in TBI, NFTs were primarily seen in superficial cortical layers, as opposed to in deep layers in AD (41). This distribution of tau pathology is similar to the distribution of NFTs in CTE following repetitive mild TBI/concussion observed in athletes (199). With the implementation of novel PET ligands specific for tau, further knowledge of temporal patterns of tau deposits will be acquired in the future (181, 182).
Aims

The general aim was to study axonal injury following severe TBI in humans, its detection in the clinical setting and its pathological features, with particular emphasis to neurodegeneration and potential long-term consequences.

2.1 Specific aims

I. To quantify the anatomical distribution of axonal injury using MRI, evaluate its impact on DAI patient outcomes and propose a novel grading system based on the widely used Adams histopathological grading (81).

II. To analyze Aβ deposition in surgically resected brain tissue post-TBI, including monomeric Aβ and soluble Aβ oligomers/protofibrils, and their relation to the APOE genotype.

III. To analyze the proteome profile of biopsies from structurally normal-appearing cortical tissue post-TBI, and compare proteome alterations in DAI vs focal TBI.

IV. To investigate the occurrence of increased ICP in DAI patients and to analyze whether anatomical distribution of axonal injury on MRI associates with ICP elevations.
Material and methods

All research included was approved by the Regional Research Ethics Committee at Uppsala University and was conducted in accordance with the ethical standards given in the Helsinki Declaration of 1975, as revised in 2008. For studies including human brain tissue (papers II and III), written informed consent was obtained. For patients with severe TBI, written informed consent was obtained from the patients’ closest relatives and from the patients themselves when they had sufficiently recovered from their injury at > 6 months post-injury. Informed consent was also obtained from the idiopathic normal pressure hydrocephalus (iNPH) patients and neurologically intact (NI) subjects and AD patients (or their relatives) for post-mortem brain donations (paper II).

3.1 Patient population

3.1.1 Severe traumatic brain injury cohorts and timing of MRI

All TBI patients included in the papers had suffered a severe TBI, defined as post-resuscitation GCS scores ≤ 8. Patients were > 16 years of age, did not have any known neurological disorder and had a decreased level of consciousness mandating mechanical ventilation and ICP monitoring. Patients were treated in the NIC unit at the Uppsala University Hospital using the standardized protocol for severe TBI patients, previously described in the introduction.

The DAI cohorts (papers I, III and IV) included severe TBI patients with post-resuscitation GCS scores ≤ 8, but with no mass lesion ≥ 25 ml. There were no ischemic/vascular lesions explaining the decreased level of consciousness and the patients had DAI-associated lesions depicted on conventional MRI. In paper I, the aim was to study DAI-associated lesions in the early phase post-TBI. Timing of the MRI scan is of particular importance for non-hemorrhagic lesions, since DWI lesions may become isointense to brain tissue after 5-7 days (129). To reduce the risk of missing lesions on DWI, the post-injury time to the MRI scan was chosen within one week. Preferably, the MRI scan should be performed within the first days post-injury. However, severe TBI patients are typically unstable during the first post-injury days and this would have caused the exclusion of a significant number of patients.
In papers III and IV the MRI was performed at 1 day – 8 weeks post-injury. In paper III, the objective was to confirm the DAI diagnosis. In paper IV, the rationale to also include patients who had performed MRI in the subacute phase was to decrease the risk of selection bias, since ICP instability may restrict an early MRI in some patients. In paper II, severe TBI patients all suffered from life-threatening elevations of ICP and/or the presence of a space-occupying brain swelling or hemorrhage, mandating surgical focal decompression. Thus, the evacuated brain tissue could be stored, following informed consent, in an established tissue bank at our department (Uppsala Brain Bank-Trauma). Subsequently, tissue was extracted to allow the biochemical analysis performed in the study.

3.1.2 Control subjects

For obvious ethical reasons, fresh brain tissue from healthy control subjects was not possible to obtain. To allow comparison of TBI brain tissues with tissues lacking post-mortem alterations in papers II and III, we included patients with iNPH, planned for ventriculoperitoneal (VP) shunt insertion. Prior to VP shunt insertion, these patients were evaluated clinically and lumbar CSF was obtained and analysed for Aβ42, total tau, P-tau and neurofilament-light (NFL). Care was taken to not include patients with clinical symptoms suggestive of AD and no iNPH patient had a CSF biomarker profile indicative of AD or any other neurodegenerative disease. In addition to the iNPH patients in paper II, post-mortem temporal cortical tissue from five deceased patients with AD-related dementia, and post-mortem temporal cortex from four NI subjects were included. These tissues were obtained from the Uppsala Brain Bank at the Department of Pathology and Cytology.

3.2 Image acquisition and analysis

Patients with severe TBI were subjected to an admission CT, which was scored using the Marshall classification. When the clinical status had stabilized, patients with diffuse injury and suspected DAI underwent an MRI with a 1.5T scanner (papers I, III and IV). Imaging included three sequences, T2*GRE, DWI and SWI.

In paper I and IV, images were independently assessed by two raters, graded using Adams histopathological classification (81) and the number of DAI-associated lesions were counted in different anatomical locations in the brain. In previous studies, anatomical description of DAI-associated lesions in the brainstem was not well defined in relation to visible anatomical structures (81), resulting in difficulties when comparing study findings. Therefore, an
anatomical division of brainstem structures was performed to facilitate localization and grading (Figure 3). Anatomically, the mesencephalon was divided in three regions; (1) crus cerebri, (2) substantia nigra and mesencephalic tegmentum, and (3) tectum including the superior and inferior colliculi. The pons and the medulla oblongata were divided into a ventral and a dorsal tegmental portion. Following the independent assessment of images by a neurosurgeon and a neuroradiologist, the inter-rater agreement was good in all assessed sequences.

Figure 3. Definition of brainstem and mesencephalic structures. The mesencephalon was anatomically divided into the three regions; (1) crus cerebri, (2) substantia nigra and tegmentum, and (3) tectum including the superior and inferior colliculi, Mes = Mesencephalon, Med = Medulla oblongata, Crus = Crus cerebri, SN = Substantia nigra, Teg = Mesencephalic tegmentum, Tec = Mesencephalic tectum. Reprinted with permission from Journal of Neurotrauma.

3.3 Clinical and multimodality monitoring data

In all studies, clinical and demographic data were collected using the electronic patient record system, the TBI NIC database, and the Uppsala TBI registry, a registry established at our department in collaboration with Uppsala Clinical Research Center (UCR, www.ucr.uu.se, Uppsala University), including all severe TBI patients treated at the NIC (240). In papers I and IV, physiologic monitoring data were acquired using a computerized multimodality monitoring system collecting minute-by-minute average values (241). Data were assessed and validated manually and invalid data were withdrawn from the total monitoring time. Interruptions of data collection occurred when the patient was taken to the operating room or for radiologic evaluation and during network or software failures. The remaining monitoring data gave the good
monitoring time (GMT), which was extracted for critical monitoring parameters including ICP and CPP. Based on the treatment goals for the evaluated parameters, thresholds for secondary insults were defined and secondary insults were then calculated as percent of GMT. In addition, in paper I, the mean PRx and mean ICP amplitudes were calculated to evaluate the status of cerebral pressure autoregulation and intracranial compliance respectively (60, 242).

3.4 Sampling and preparation of brain tissue

Following surgical decompression in paper II, brain tissue from severe TBI patients was stored in -80°C freezer until analysed. In nine out of twelve patients, brain tissue was also placed in 4 % buffered formalin (HistoLab Products AB, Gothenburg, Sweden) and fixed for 24-72 hours for immunohistochemical analysis. Subsequently, samples of approximately 5 mm² were taken from the surgically evacuated fresh frozen brain tissue for biochemical analysis.

In paper III, brain tissue biopsies were sampled in conjunction with the insertion of an ICP monitor in the same corticotomy. The technique for the biopsy sampling is well established at our department and has been used for several years in > 500 iNPH patients with minimal complications. The corticotomy is performed with a sharp syringe to avoid thermal injury and the biopsy needle has a 14 gauge (2.11 mm) diameter and an 8 mm side cutting window (Elekta Instrument AB, Innsbruck, Austria). This allows for minimally traumatizing biopsy sampling. The biopsies included both cortical and subcortical brain tissue and were stored in -80°C freezer until analysed. In the TBI cohort, one patient had a minimal hematoma (1.1 ml) without clinical significance at the ICP monitoring site following the biopsy sampling. This is a hematoma rate in line with previous observations after insertion of ICP monitors (53). The brain biopsies sampled from iNPH patients (papers II and III) were taken in conjunction with the VP shunt insertion using the same technique, and tissue handling thereafter followed the same protocols for TBI and iNPH patients as well as for the AD and the NI subjects in paper II.

3.5 Immunohistochemistry for Aβ plaques

Brain tissues in paper II and biopsies from iNPH patients in paper III were analysed using immunohistochemistry for Aβ plaques. Immunohistochemistry is a well-established technique for protein detection in fixed tissues, based on antigen-antibody interactions. The protocol used is tailored to suit the specific tissue where the analysis is undertaken. Steps include tissue processing,
antigen retrieval, blocking of unspecific binding, antigen-antibody interactions and finally visualization, where various detection systems are available. Here, Aβ plaques were stained using Aβ antibodies (6F/3D, M0872; dilution 1:100, Dako, Glostrup, Denmark). For visualization, Dako Autostainer plus with Dako EnVision FLEX detection system was used.

3.6 Biochemical analysis of soluble Aβ species using ELISA

Enzyme-Linked ImmunoSorbent Assay (ELISA) is a commonly used plate-based biochemical assay to detect and measure antigen-antibody interactions in solutions. The antigen is immobilized either by direct adsorption to the plate, or via a capture antibody attached to the plate surface. Detection of the antigen can then be accomplished by direct detection using a labeled primary antibody, or indirectly using a labeled secondary antibody. The latter technique, including indirect capture and detection, is usually referred to as sandwich ELISA and allows for potentiation of ELISA signal intensities and thus increased sensitivity of the immunoassay. The signal is further amplified using a streptavidin-biotin approach. In paper II, sandwich ELISA assays were performed using highly-selective antibodies for various forms of soluble Aβ species (Figure 4). The antibodies (mAb82E1 and mAb158) used for detection of Aβ oligomers and protofibrils have previously been well characterized. The mAb82E1 assay ELISA detects both smaller (< 100 kDa) and larger soluble aggregates of Aβ (243), whereas the mAb158 assay detects large Aβ protofibrillar species and has high sensitivity for detecting soluble protofibrils (244, 245). The mAb158 assay can also detect amyloid fibrils, however with an ap-

![Figure 4. Sandwich ELISA utilizes indirect capture and detection, in paper II using the mAb82E1 for Aβ oligomers and mAb158 for Aβ protofibrils. TMB = Tetramethylbenzidine, Strep-HRP = Streptavidin-Horseradish Peroxidase.](image-url)
proximately 15 times lower sensitivity than for protofibrils (246). Still, by including a centrifugation step prior to analysis, the amyloid fibrils could be removed from the samples.

3.7 Proteome analysis using mass spectrometry
The proteome refers to the total set of proteins produced by an organism, in similarity to the genome, which is the total set of DNA (247). In contrast to the static genome however, the proteome is dynamic and may change in response to external factors such as TBI. Proteomics, the study of the proteome, was applied on brain biopsies of TBI and iNPH patients in paper III using mass spectrometry (MS), a technique to identify proteins or peptides in biological samples (Figure 5). MS separates ionized molecules based on their mass-to-charge ratio ($m/z$). Briefly, the basic concepts of MS include vaporization of samples by a heater, ionization of molecules to convert them into gas phase ions using an ion source, accelerations into a focused beam through an ionization chamber, deflection by a magnetic field, where the amount of deflection is dependent on the $m/z$, and finally detection by a detector able to record different ions at specific $m/z$ values. MS enables quantification of molecules either by absolute or by relative quantification (248).

Figure 5. Steps in mass spectrometry-based proteomics include homogenization of the tissue samples, enzymatic digestion of proteins to peptides, vaporization and ionization of samples, ion acceleration into a focused beam, deflection by the magnetic field and lastly detection by a detector able to record different ions at specific $m/z$ values.

In absolute quantification, signal intensities in the experimental samples are compared to a known concentration of a reference peptide, while relative quantification involves comparison of protein alterations between different
samples. The latter thus allows for analysis of proteome profiles in large sets of samples.

To optimize the detection of the proteome in biological samples, proper extraction of proteins is imperative. In paper III, the tissue biopsies analyzed with MS were small, and to minimize the tissue loss, extraction was performed using a combination of sonication probe, ultrasonic bath and detergent lysis buffer. Subsequently, the biopsy samples were analyzed in two separate studies. In *Study A*, a cohort of six severe TBI patients including both diffuse and focal injuries was compared to six iNPH patients. In *Study B*, five patients with DAI, five patients with focal TBI and five patients with iNPH were analyzed and compared (Figure 6).

![Study Design](image)

Figure 6. Study design in paper III.

### 3.8 Outcome measures

In papers I and IV, long-term outcome was assessed at > 6 months after injury according to the GOSE. The GOSE score was assessed by an intensive care specialist nurse. Evaluation was performed via a standardized questionnaire based on structured interviews (76) to be filled by patients or the closest relative. If supplementary information was required, patients or their relatives were contacted by telephone.

### 3.9 Statistical methods

The data were analyzed using the Kolmogorov-Smirnov test and when the data were non-normally distributed, non-parametric statistics were used. When statistic comparison involved multiple groups, the Kruskal-Wallis test
was first performed to analyze if there existed statistically significant differences between groups, and it was followed by Mann-Whitney U test if significant group differences were indicated. To compare related variables the Wilcoxon signed-rank test was used, and Spearman’s rank-order correlations were used for analyses of relationships. When data was normally distributed, Student’s t-test was used for continuous or Fisher exact test for categorical variables. Two-tailed P-values were used and a P-value < 0.05 was considered statistically significant.

Inter-rater agreement between the two raters of MRI images (paper I and IV) was analyzed using linear weighted Cohen’s κ statistics for categorical values and the intra-class correlation coefficient (ICC) was calculated with two-way random single measures (consistency/absolute agreement) for continuous values. Values of ≤ 0.2 were considered poor, 0.21 – 0.4 fair, 0.41 – 0.6 moderate, 0.61 – 0.8 good and ≥ 0.81 a very good agreement for both κ and ICC values.

In paper I, the data were analyzed using ordinal logistic regression for associations between potential MRI-related and clinical prognostic factors and long-term outcome. The GOSE category after 6 months post-injury was used as the dependent variable. This model was chosen to maximize statistical power by using the ordinality of the data. Factors found significantly associated with outcome on univariate analysis were further analyzed in multivariate logistic regression. In paper IV, linear regression was used to analyze the association between the proportion of GMT with ICP > 20 mmHg and clinical and MRI-related variables. Variables with significant or near-significant association with the proportion of increased ICP > 20 mmHg were further analyzed in a multivariate linear regression model.
Results

4.1 Paper I

Thirty patients (mean age 31.2 years +/- 14.3 SD; 26 males, 4 females) with DAI, who had underwent an MRI within one week of trauma, were included in the study. Median admission GCS motor score was 5 (range 1-6) and median discharge GCS motor score was 6 (range 4-6) respectively. After > 6 months nine patients (30 %) had a good recovery (GOSE 7-8), 11 patients (36.7 %) were moderately disabled (GOSE 5-6), eight patients (26.7 %) were severely disabled (GOSE 3-4) and one patient (3.3 %) was in a vegetative state (GOSE 2). One patient (3.3 %) was lost to follow-up.

Lesions were frequently found in the cerebral hemispheres, corpus callosum, brainstem, and in deep-seated grey substance. When comparing MRI sequences, the SWI sequence identified more lesions than T2*GRE ($P < 0.05$) and DWI ($P < 0.05$). Lesions in the brainstem (DAI grade III) were common, existing in 70 % of patients. Of the clinical factors, age, GCS motor score on admission, GCS motor score at discharge and low proportion of GMT with CPP < 60 mmHg were associated with long-term outcome in univariate analysis ($P < 0.05$). The MRI-related factors associated with long-term outcome in univariate analysis included the number of DWI lesions in the thalamus, basal ganglia and internal capsule as well as the number of SWI lesions in the mesencephalon and the dorsal pons ($P < 0.05$). Further analysis using multivariate logistic regression revealed an independent relation with poor outcome at > 6 months for higher age (OR 1.18, 95 % CI 1.05 – 1.32, $P < 0.05$) and lesions in the mesencephalic region corresponding to the substantia nigra and mesencephalic tegmentum on SWI (OR 4.51, 95 % CI 1.48 – 13.78, $P < 0.05$) (Table 8).
TABLE 8

<table>
<thead>
<tr>
<th></th>
<th>Regression coefficient</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>p-value</th>
</tr>
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<tr>
<td>Age</td>
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<td>1.05 – 1.32</td>
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<td>Lesions in SN and mesencephalic tegmentum on SWI</td>
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<td>4.51</td>
<td>1.48 – 13.78</td>
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<td>Adams DAI grade</td>
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<td>Marshall score</td>
<td>N.S</td>
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<tr>
<td>Admission GCS motor score</td>
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<tr>
<td>Discharge GCS motor score</td>
<td>N.S</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CPP &lt; 60 mmHg*</td>
<td>N.S</td>
<td></td>
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</tbody>
</table>

Odds ratios in multiple ordinal logistic regression with GOSE as the dependent variable. In addition to factors found significantly associated with long-term outcome in univariate analysis Adams DAI grade and Marshall CT score were included to the model. ‡ = Basal ganglia, † = Internal capsule, * = Proportion of good monitoring time during first 96 hours, GOSE = Glasgow outcome scale extended, SN = Substantia nigra, DWI = Diffusion-weighted imaging, SWI = Susceptibility-weighted imaging, DAI = Diffuse axonal injury, GCS = Glasgow coma score, CPP = Cerebral perfusion pressure

4.2 Paper II

Twelve severe TBI patients (mean age 49.5 years ± 19 SD; 11 males, 1 female) who underwent surgical evacuation of cerebral contusions were included in the study, and the extracted brain tissue was used for the analysis of soluble Aβ aggregate as well as Aβ immunohistochemistry. The TBI cohort was compared to AD patients and a control group consisting of iNPH and of NI subjects. The TBI cohort was significantly younger than the AD (P < 0.05) and the combined control group (P < 0.05). Insoluble Aβ aggregates/plaques were visualized in three out of nine TBI patients where brain tissue was available for immunohistochemical analysis (Figure 7).

Soluble Aβ oligomers were detected in 12/12 TBI patients and were significantly higher in TBI than in the control group (P < 0.05). Soluble Aβ protofibrils were detected in 7/12 TBI patients and in none of the controls (P < 0.05) (Figure 8). Levels of monomeric Aβ40 and Aβ42 were not elevated in TBI patients.
Four TBI patients carried the AD risk genotype APOE ε3/4 (APOE ε3/4+), whereas seven were APOE ε3/3 and one was APOE ε2/4 (collectively referred to as APOE ε3/4-). In the APOE ε3/4+ patients, levels of Aβ oligomers and prototofibils were higher than in APOE ε3/4- (P < 0.05 for both comparisons) and higher than in controls (P < 0.05 for both comparisons). In addition, APOE ε3/4+ patients had higher levels of both Aβ1-42 and Aβx-42 than the APOE ε3/4- patients (P < 0.05 for both comparisons), indicating increased levels of both N-terminally intact and N-terminally truncated monomeric Aβ42 species.

Figure 7. (A) An example of a patient with a focal left frontal contusion injury following severe traumatic brain injury (TBI). (B-D) Immunohistochemical image from the three TBI patients demonstrating accumulation of diffuse (*) and compact (**) insoluble Aβ plaques/aggregates. Reprinted with permission from Brain Pathology.

4.3 Paper III

Sixteen patients with severe TBI (mean age 43.7 years ± 20.7 SD; 12 males, 4 females) were included (n=6 in Study A, n=10 in Study B). The TBI patients
were younger than the patients with iNPH (mean age 73.7 years ± 5.2 SD; 7 males, 4 females), used as controls.

In Study A, the proteomics approach identified a total number of 692 unique proteins. Of these, 45 proteins were found to be significantly increased (n=11) or decreased (n=34) in TBI in comparison to iNPH, of which 20 % are involved in neurodegeneration or cytoskeletal function, 9 % in cell death or survival functions, 24 % in cell signaling, transport or repair pathways, 9 % in oxidation/reduction pathways and 11 % in energy metabolism. The remaining

![Figure 8](image-url)

Figure 8. (A) Amyloid-β oligomers were detected in all TBI patients, using mAb82E1 sandwich ELISA. (B) Aβ oligomers were elevated in TBI when compared to controls (P < 0.05). (C) Aβ protofibrils were detected in 7/12 TBI patients. (D) Aβ protofibrils were elevated in TBI when compared to controls (P<0.05). TBI=traumatic brain injury, AD=Alzheimer’s disease, iNPH=idiopathic normal pressure hydrocephalus, NI=neurologically intact, CTRL=controls, #=indicates Aβ aggregates/plaques on immunohistochemistry. Reprinted with permission from Brain Pathology.

24 % are involved in a variety of cell functions including neurite outgrowth, protein metabolism and regulation of nucleic acid (Figure 9).
In Study B, the proteomics approach identified a total of 1844 unique proteins. Of these, 51 proteins were significantly increased (n=27) or decreased (n=24) in DAI in comparison to focal TBI, of which 24% are involved in neurodegeneration or cytoskeletal function, 12% in cell death or survival functions, 22% in cell signaling, transport or repair pathways, 8% in oxidation/reduction pathways and 12% in energy metabolism. The remaining 24% are involved in neurite outgrowth, protein metabolism, regulation of nucleic acid and lysosomal function (Figure 9).

In comparison to iNPH, 41 proteins were found significantly increased (n=25) or decreased (n=16) in DAI. Of these, 17% are involved in neurodegeneration or cytoskeletal function, 12% in cell death or survival functions, 14% in cell signaling, transport or repair pathways, 10% in oxidation/reduction pathways and 10% in energy metabolism. The remaining 36% are involved in neurite outgrowth, protein metabolism, regulation of nucleic acid, immune response and lysosome function. Twenty proteins with significantly increased or decreased expression were found in DAI both when compared to focal TBI and when compared to iNPH (Figure 9).

As to focal TBI in comparison to iNPH, 29 proteins were significantly increased (n=20) or decreased (n=9). Of these, 10% are involved in neurodegeneration or cytoskeletal function, 14% in cell death or survival functions, 21% in cell signaling, transport or repair pathways, 3% in oxidation/reduction pathways and 14% in energy metabolism. The remaining 38% are involved in neurite outgrowth, protein metabolism, regulation of nucleic acid and immune response (Figure 9).
Figure 9. (A) Tissue biopsy, obtained with a biopsy needle with 14 gauge (2.11 mm) diameter and an 8 mm side cutting window in comparison to an external ventricular drain (outer diameter 2.5 mm) (B) Diagram demonstrating number of differentially expressed proteins in different cellular mechanisms in patients with severe TBI when compared to patients with iNPH (Study A). Positive values represent upregulated proteins. Negative values represent downregulated proteins. (C) Diagram demonstrating number of differentially expressed proteins among groups in different cellular mechanisms in Study B. (D) Venn diagram demonstrating overlap in differentially expressed proteins in the analysis of DAI vs focal TBI, DAI vs iNPH and focal TBI vs iNPH in Study B. ND/CS = neurodegeneration/cytoskeletal, CD/CS = cell death/cell survival, TR/SI/RE = transport/signaling/repair, ROS = reactive oxygen species, ENM = energy metabolism, MISC = miscellaneous.
To validate the results from the MS proteomic analysis, Western blot analysis of glial fibrillary acidic protein (GFAP) was performed on the tissue extracts from Study A. As in Study A, the Western blot analysis revealed a decrease of GFAP expression in TBI ($P < 0.05$) (Figure 10).

![Western blot analysis](image)

**Figure 10.** Validation of glial fibrillary acidic protein (GFAP) expression in Study A by Western blot analysis in TBI vs iNPH samples. Similar to the results from the MS proteomic analyses, the level of GFAP was decreased (*) in TBI as compared to iNPH ($P < 0.05$).

### 4.4 Paper IV

Fifty-two patients (median age 24 years, range 9-61, 40 males, 12 females), were included in the study. Episodes of ICP > 20 mmHg were present in all patients and the mean proportion of GMT with ICP > 20 mmHg was 5 % ± 7 SD of monitoring time. Fourteen patients (27 %) had more than 5 % of GMT with ICP > 20 mmHg. Thirty-four patients (65.4 %) were managed by basal ICP treatment only, while the remaining 18 patients (34.6 %) needed escalation of the ICP management. Of these, nine patients (17.3 %) required drainage of CSF via an EVD, seven patients (13.5 %) were treated with propofol coma, and two patients (2.8 %) required addition of barbiturate coma to manage increased ICP.

After > 6 months post-injury 16 patients (30.8 %) had a good recovery (GOSE 7-8), 12 patients (23.1 %) were moderately disabled (GOSE 5-6), 15 patients (28.8 %) were severely disabled (GOSE 3-4) and four patients (7.7 %) were either in a vegetative state or dead (GOSE 1-2). Five patients (9.6 %) were lost to follow-up. The proportion of ICP > 20 mmHg was associated with an unfavorable outcome measured by GOSE at > 6 months post-injury ($P = 0.05$).
MRI was performed at a mean of 7 days (range 1-37 days; MRI 1st week n=37, MRI 2nd week n=10, MRI late n=5) post-injury. The univariate analysis of clinical factors including Marshall CT score showed that GCS motor score on admission had a significant association with the proportion of ICP > 20 mmHg ($P < 0.05$). A near-significant association was found for gender ($P = 0.07$) and motor deficit on admission ($P = 0.06$). MRI variables found associated with the proportion of increased ICP were lesions in the region of the substantia nigra and mesencephalic tegmentum on DWI images ($P < 0.05$). The total number of lesions in the brainstem on DWI images had a near-significant association ($P = 0.06$). In the multivariate linear regression analysis (Table 9), lesions in the substantia nigra and mesencephalic tegmentum on the DWI images (8 % of GMT with ICP > 20 mmHg, 95 % CI 3 – 13 %, $P < 0.05$) and younger age (-0.2 % of GMT with ICP > 20 mmHg, 95 % CI -0.07 – -0.3 %, $P < 0.05$) were independently associated with the proportion of GMT with ICP > 20 mmHg (Table 9).

**TABLE 9.**

<table>
<thead>
<tr>
<th>Regression coefficient, 95 % CI</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td><strong>SN-T DWI lesions</strong></td>
<td>8 (3 – 13) % of GMT with ICP &gt; 20 mmHg</td>
</tr>
<tr>
<td>Age</td>
<td>-0.2 (-0.07 - -0.3) % of GMT with ICP &gt; 20 mmHg</td>
</tr>
<tr>
<td>Marshall score</td>
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<td>Brainstem DWI lesions</td>
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</tr>
<tr>
<td>Gender</td>
<td>N.S</td>
</tr>
<tr>
<td>Admission GCS motor score</td>
<td>N.S</td>
</tr>
<tr>
<td>Motor deficit (yes/no)</td>
<td>N.S</td>
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</tbody>
</table>

Multivariate linear regression with univariate variables with significant or near-significant association to proportion of GMT with ICP > 20 mmHg. In addition, age and Marshall CT score were included in the model. The regression coefficient is expressed as the increase (positive value) or decrease (negative value) of percent of GMT with ICP > 20 mmHg for every unit increase of the predictive variable. SN-T = Substantia nigra and mesencephalic tegmentum, DWI = Diffusion-weighted imaging, GCS = Glasgow coma score, N.S = Not significant, GMT = Good monitoring time, ICP = Intracranial pressure.
General discussion

The four papers herein study different consequences of diffuse axonal injury (DAI) following severe TBI. Axonal injury is believed to be a key contributor to cognitive, emotional and behavioral symptoms following TBI (87, 106). Particularly, impaired memory, information processing speed and executive functions are frequently encountered in DAI (249-251). By disrupting vital motor tracts, axonal injury may also lead to severe motor deficits (186). Thus, axonal injury has profound impact on patient outcome after severe TBI. In addition, axonal injury is believed to be intimately related to the increased risk of neurodegenerative diseases post-injury (39-41). This highlights the need for studying white matter alterations following severe TBI. In recent years the understanding of the underlying pathological mechanisms, the possibilities of visualizing lesions using advanced neuroimaging techniques, and the prognostic methods have evolved. Still, DAI remains a challenging condition, due to difficulties in its detection, the unpredictable post-injury clinical course in both the short- and long-term perspective and to date, limited therapeutic options.

5.1 Paper I

In paper I, the anatomical localization of DAI-associated lesions on conventional MRI was studied. Histopathologically, DAI is characterized by widespread axonal injury, detected by accumulations of βAPP in injured axons (97). These accumulations cannot explicitly be detected with MRI, instead the lesions seen in DAI predilection sites should be considered as surrogate markers for axonal injury. In the evaluation of DAI patients with MRI, timing of the MRI scan is of importance, as both non-hemorrhagic and hemorrhagic DAI lesions appear less conspicuous over time (118, 126). This holds true especially for non-hemorrhagic lesions, since DWI studies in stroke patients show that MRI signals can change significantly already during the first week following a brain insult (129), while hemorrhagic lesions may take longer to diminish. In paper I, the MRI was performed within the first week post-injury, and plausibly this potentiates the quantification of anatomical distribution of DAI-associated lesions. However, this may also have caused selection bias, as patients with DAI in isolation that were prevented from an early MRI scan due
to instability from intracranial or systemic causes, were excluded. An additional limitation of this study is the time span in which the data were gathered. Despite the long time span of data acquisition, all patients were treated using the same ICP- and CPP-guided protocol, physiologic data were collected using the same computerized multimodality monitoring system, and images were acquired with a 1.5T MRI scanner using the same protocol, limiting the effect of recall bias.

In this study, imaging included three sequences, T2*GRE, DWI and SWI. In addition to these sequences, FLAIR is commonly applied in studies of DAI patients (125, 126). The reason to refrain from this sequence was its limited specificity for DAI-associated lesions. One objective of the study, was to utilize MRI sequences useful for neurosurgeons in the clinical setting. Therefore only sequences considered specific for traumatic axonal lesions were used.

Adams grading is widely used for classification of DAI (81). In our study, 70% of patients had lesions in the brainstem and thus were graded as the most severe form of DAI (grade III). This was not surprising given that the study was conducted on severe TBI patients, admitted to the NIC due to prolonged unconsciousness, plausibly attributed to lesions in the diencephalon and the brainstem (184). Consequently, Adams DAI grading lacked enough sensitivity to separate patients with a worse outcome from those that eventually recovered. Brainstem lesions have been reported to accompany half of severe TBI cases (83). The higher incidence in paper I is probably due to the use of the SWI sequence, with increased sensitivity to hemorrhagic lesions in regions of the brain previously difficult to visualize.

In multivariate ordinal logistic regression, advanced age and hemorrhagic lesions visualized on the SWI sequence in the substantia nigra and mesencephalic tegmentum were independently associated with a worse outcome, measured with GOSE at > 6 months. Plausibly, lesions in these central brainstem structures may represent a true traumatic axonal injury mechanism with stronger relation to poor long-term outcome than more superficial lesions in the crus cerebri or the tectum (252, 253). The results were used to create an extended anatomical classification system for DAI, based on Adams pre-existing grading (81). The extended MRI classification system is based on four stages (Stage I – Hemispheric lesions, Stage II – Corpus callosum lesions, Stage III – Brainstem lesions and Stage IV – Substantia nigra or mesencephalic tegmentum lesions); all subdivided by age (≥/< 30 years). This classification system needs further validation in larger studies. However, the independent associations between outcome and advanced age, and lesions in the substantia nigra and mesencephalic tegmentum justify its preliminary use.
5.2 Paper II

In paper II, a detailed analysis of Aβ aggregation following TBI was performed, using surgically removed brain tissues due to space-occupying brain swelling or hemorrhage with resulting life-threatening ICP elevations. We found increased levels of soluble Aβ aggregates in the form of oligomers and protofibrils. Additionally, insoluble Aβ plaques were found in 30% of patients, in consistency with previous observations (211, 215, 216). These findings may have important implications in the search for a link between TBI and an increased risk of AD development (104, 105, 193, 254). The clinical significance of these aggregates is however still unknown. Nonetheless, both low-molecular weight (i.e. dimers to oligomers) and high-molecular weight (i.e. protofibrils) soluble Aβ aggregates have been attributed with neurotoxic properties (207-209, 255-257). In AD research, there are currently a number of on-going trials targeting these compounds (258). Thus, future research will elucidate whether these soluble aggregates, found in the majority of the TBI patients in paper II, also cause secondary injury and confer an increased risk of AD post-TBI. In addition, the same pharmacological agents used in AD may be relevant even for TBI patients, should they prove effective in reducing AD-related dementia.

Interestingly, carriers of the AD risk allele APOE ε4 displayed higher levels of soluble Aβ aggregates and Aβ plaques were only observed in APOE ε4 carriers. In addition, the production of Aβ monomers in APOE ε4 carriers seemed shifted towards increased N-terminally truncated forms, believed to be more prone to oligomerize (203, 204, 259). The genetic susceptibility of AD in carriers of APOE ε4, especially pronounced among patients who previously have sustained a TBI, may be due to a synergistic effect where Aβ is produced in high quantities but inadequately cleared from the brain tissue (217, 227, 229).

The use of surgically removed brain tissues has its inherent limitations, of which blood products may be one cause of false positive findings. Blood products in such severely injured brain tissues cannot be avoided, and especially platelets contain βAPP and carry the enzymatic capacity to generate Aβ species (260, 261). However, we did not observe an excess of blood in those samples that displayed the highest levels of Aβ aggregates (Figure 11), and the levels of Aβ40, mainly produced by platelets, were low in TBI patients. In addition, false positive signals may occur due to cross-linkage of antibodies in a sandwich ELISA in the presence of human anti-mouse antibodies (HAMA) (262). However, no such activity was detected.

Furthermore, it was not possible to ascertain whether aggregated Aβ was present already prior to the injury or to assess temporal changes in Aβ levels.
Studies analyzing the temporal patterns of Aβ are scarce but by PET imaging, increased amyloid deposition following TBI with time was noted. Using PiB-based imaging, a peak within the first week post-injury (179) and increased signals with longer time from initial injury were observed (180). Unfortunately, PiB binds only insoluble fibrillar Aβ deposits, but temporal assessments of soluble Aβ oligomers and protofibrils will become possible with the development of new antibody-based PET imaging ligands (263).

Figure 11. Homogenates from each TBI tissue sample contained amounts of blood, with no apparent excess of blood in samples with the highest Aβ oligomer and protofibril as well as the highest Aβ1-42 and Aβx-42 levels (indicated with a circle).

5.3 Paper III

In paper III, we acquired biopsies from structurally normal-appearing cortex in patients with severe TBI, which were analyzed with MS-based proteomics. The study revealed fundamental molecular differences between DAI and focal TBI, suggesting that widespread axonal injury rapidly induces cascades which may be linked to secondary injury pathways. The heterogeneity of TBI and the complex cellular and molecular differences involved in the TBI subtypes form a major challenge in TBI research, and may be a cause as to why all pharmacological compounds evaluated in TBI have failed to provide benefit,
to date (264, 265). The approach introduced in this paper, with sampling of cortical biopsies in conjunction with insertion of an ICP monitoring device, has the potential of adding broader understanding of cellular and molecular pathways distinct between TBI subtypes. Thus current TBI classifications, which still cannot fully appreciate the diversity in TBI, could be refined by direct molecular evaluations of cortical tissue.

Of imperative significance is the safety profile of the sampling procedure. The biopsies were obtained using a minimally traumatizing technique established at our department as a routine procedure for iNPH, in conjunction with VP shunt placement (266). In this paper, one small hematoma without clinical significance in the area of the brain biopsy and ICP monitor was observed, in line with the low reported hemorrhage risk from ICP monitoring per se (53). The technique is rapid, simple and inflicts no additional injury to the brain, apart from the unavoidable when inserting an ICP monitoring device. Importantly, tissue biopsies eliminate the caveats of using post-mortem tissues as controls, including tissue alterations caused by prolonged post-mortem time.

Our main findings include altered levels of 51 proteins in DAI vs focal TBI, in structurally normal-appearing cortex remote from radiologically evident injury sites. This illustrates early onset of molecular pathways in response to the brain trauma, which previously has not been possible to assess, and seem especially pronounced after axonal injury. Of particular importance is the observed elevations of tau in patients with DAI, as tau has an important role in various neurodegenerative diseases (201). Since tau is associated with the axonal cytoskeleton, its accumulation in CSF and ISF is tightly linked to axonal injury (267, 268), and the elevated levels are plausibly due to microscopic axonal damage. Thus, cortical biopsy sampling could be an approach to validate surrogate markers for axonal injury, such as DTI metrics (231). Additionally, the elevated tau levels in DAI were observed significantly increased in 4/4 MS platforms used, providing validity of the findings.

Monomeric Aβ40 and Aβ42 were also analyzed in this paper, however due to their hydrophobicity, high mass (> 4 kDa), and low abundance, they are difficult to detect using conventional MS proteomics (269). Therefore, we analysed Aβ species using highly-sensitive ELISA. Similar to the result in paper II, no elevations of monomeric Aβ were detected in TBI. However, we did not include an analysis of Aβ oligomers and protofibrils and thus cannot ascertain whether oligomerization of Aβ, similar to the observations made in paper II, is the plausible cause of low Aβ levels.

Importantly, this study provides evidence that axonal injury, although not visualized with the neuroimaging methods used, still is detectable at the cellular
and molecular level, through direct analysis of brain tissue. Biomarkers of axon- 
only injury, such as tau and Aβ observed to be widely distributed in the brain 
post-TBI (41), may have important implications for neurodegenerative alterations. In addition, the findings in this study may aid in future individualization of therapeutic efforts.

5.4 Paper IV

In paper IV, the prevalence of increased ICP in patients with DAI was investiga- 
ted. The study shows that elevations of ICP were not uncommon in DAI 
patients, as approximately one third of patients had ICP > 20 mmHg during a 
substantial proportion of time despite escalated ICP treatment. The results indi- 
cate that increased ICP is an important contributor to the risk of secondary 
injury development in DAI patients, although not to the same extent as in focal 
injuries. In accordance with expert consensus, the study reinforces that sus- 
ppected or confirmed DAI argue against refraining from ICP monitoring in un- 
onscious patients even with no or minimal findings on the initial CT scan 
(270).

Non-hemorrhagic lesions in the region of the substantia nigra and mesence- 
phalic tegmentum, thus in the same region as identified to be associated with 
outcome in paper I, were independently associated with increased ICP. We 
cannot ascertain by this study that lesions in this region preceded the ICP ele- 
vation in all patients. However, brainstem lesions due to elevated ICP are most 
commonly observed with persistently intractable intracranial hypertension de- 
spite treatment (271). Although stepwise escalated ICP therapy was needed in 
a subset of patients, ICP could eventually be controlled and intractable ICP 
crises did not occur in this patient cohort. Thus, it is unlikely that the lesions 
observed by MRI were caused by increased ICP. The combined findings of 
paper I and IV indicate that these lesions are of particular importance in the 
management of severe TBI patients with DAI, which is not surprising consid- 
ering the numerous vital structures located in this area (Figure 12).

In addition to DWI lesions in the substantia nigra and mesencephalic tegmen- 
tum, younger age was independently associated with increased ICP after ad- 
justment for other factors. Previous studies imply that age may influence ICP 
and brain compliance (272, 273), where lower ICP levels are noted in older 
patients (274). DAI patients are usually younger than patients with focal inju- 
ries. The absence of brain atrophy in the young facilitates the strains between 
the grey and white substance underlying the axonal damage seen in DAI (92). 
Of note, no significant association with age was observed in the univariate 
analysis. The lack of association in univariate analysis may be explained by
the occurrence of patients with relatively high age with non-hemorrhagic lesions in the substantia nigra and mesencephalic tegmentum, confounding the effect of age on ICP. This is not an uncommon phenomenon in clinical research (275, 276). Therefore, care must be taken not to disregard significant associations when building statistical models based only on results from univariate statistical testing.

Limitations of this study include the extended time span for data gathering, and the shift from T2*GRE to SWI as the preferred imaging sequence for hemorrhagic lesions during this time. This resulted in some inconsistency in the used MRI sequence which may have confounded the results. However, the majority of patients (42/52) were still examined with the more sensitive SWI sequence.

![Anatomical illustration of brainstem and mesencephalic structures](image)

Figure 12. Anatomical illustration of brainstem and mesencephalic structures. Cross section of the mesencephalon is through the superior colliculi. Central brainstem lesions associated with increased intracranial pressure are located in the region of the substantia nigra and mesencephalic tegmentum. As depicted in the figure, this region includes numerous vital structures.

### 5.5 Statistical considerations

Clinically, DAI in isolation is rare and most commonly axonal injury is encountered in combination with focal injuries (82). Additionally, two of the studies were performed on surgically excised brain tissues, with limited availability. Generally in Western societies, severe TBI is becoming less common
due to preventive measures such as traffic safety regulations (4). In addition, modern neurosurgical management, utilizing decompressive craniectomy for the treatment of uncontrolled ICP elevations have made the surgical removal of contusions (the prerequisite for the studies performed in paper II) less frequent (277). Furthermore, sampling of brain biopsies in TBI patients has not been previously performed. Due to the rarity of the TBI subtype studied and the nature of the evaluated tissues, the papers in this thesis include a relatively small number of patients. This is commonly encountered in clinical research and causes some important limitations. The small sample size may result in over-inflation of the effect size and risks false-positive findings (type 1 error). Simultaneously, the small sample size may lead to a reduced capacity to detect a true difference (type 2 error). These two inherited statistical risks are closely coupled, and the risk of a type 1 error can be reduced by reducing the preset significance level, however not without an accompanied increase in the risk of a type 2 error. In addition, multiple testing increases the risk of making type 1 errors. Therefore, the chosen statistical methods need to be robust, and these limitations must be considered when conclusions are drawn. At the same time, the rarity of isolated DAI and the difficulty of obtaining the tissues studied warrants further investigation not to disregard highly relevant associations. Although a type 1 error may be considered a more serious problem in many instances, correction for multiple testing was not performed in the included papers. In addition, the patient samples were collected during several years and it is not likely that a larger sample size could have been achieved in the near future. However, assumption of effect sizes is generally conservative, and statistical analysis was performed with awareness of these limitations.
Conclusions

The general conclusions of this thesis are that axonal injury in severe TBI patients may lead to a multitude of clinically relevant consequences, from changes in protein expression with a potential link to neurodegeneration, to intracranial hypertension. Axonal injury seems to offset biological cascades rapidly producing soluble Aβ aggregates and tau, proteins with pathological link to AD. Furthermore, we have identified axonal injury in the region of the substantia nigra and mesencephalic tegmentum as particularly important for both the development of intracranial hypertension and long-term outcome.

**Paper I**
In severe TBI patients with DAI, MR lesions are widely distributed and brain-stem lesions exist in a majority. Higher age and hemorrhagic lesions on MRI in the substantia nigra and mesencephalic tegmentum indicate poor long-term outcome.

**Paper II**
Soluble intermediary Aβ oligomers and protofibrils form rapidly after severe TBI and are especially pronounced among APOE ε3/4 carriers.

**Paper III**
In severe TBI, DAI initiates unique biological pathways in comparison to focal TBI, with regulatory differences in proteins involved in a multitude of cellular functions, at sites remote from radiologically visible brain injury.

**Paper IV**
Increased ICP is present in about one third of severe TBI patients with DAI and associates with young age and non-hemorrhagic lesions in the region corresponding to the substantia nigra and mesencephalic tegmentum.
Future perspectives

In this thesis, axonal injury following severe TBI has been evaluated using both neuroimaging and techniques for biomarker discovery. However, many unresolved issues remain in DAI. Novel neuroimaging techniques such as DTI and PET may shed more light on the evolution of DAI-associated lesions and their impact on white matter connections between brain areas, advancing the understanding of the cognitive, emotional and behavioral symptoms seen following DAI. Injury to white matter tracts has only recently become possible to visualize with the development of DTI and tractography techniques. However, the clinical significance of lesions observed with these techniques remains to be established. To facilitate research on DTI in DAI, future studies need to homogenize data acquisition, analysis techniques and spatial location of investigated structures. Nonetheless, this field is highly interesting and rapidly evolving. A limitation of DTI, is still the requirement for post-processing techniques, which is time-consuming and impedes broader use in the acute setting in the neurointensive care unit. In this context, the usefulness of conventional MRI techniques is still superior. Surprisingly, there is a paucity of studies evaluating lesions on conventional MRI following DAI, and these studies suffer from differences in terminology and definition of studied structures, making comparisons between study results difficult. The most frequently used DAI classification system is based on histopathological findings and appears suboptimal when adopted to advanced neuroimaging in severe TBI. In our studies, the aim was to use and elaborate on already existing anatomical nomenclature and classification. Other future research efforts, such as the multicenter Center-TBI study, will possibly provide more answers in the search for an optimal DAI classification.

One important but unresolved issue in DAI research is the evolution of MR lesions during the first two weeks post-injury. Longitudinal studies display disappearance of non-hemorrhagic lesions when repeat imaging is performed in the chronic phase (126). However to date, no clinical longitudinal study have analyzed how they evolve during the first two weeks. Although suggested by animal studies (278), it is not established in humans whether there is a blooming effect similar to what is seen following cerebral contusions. The major difficulty of performing such studies is the transfer of critically injured patients to the radiological department for a time-consuming MRI scan. This
will be facilitated with future development of MRI scanners with shorter image acquisition times as well as logistic solutions with neuroimaging hardware in close proximity to the NIC or the operating theatre.

Severe TBI is an exceedingly heterogeneous disease and the brain trauma field is moving towards individualized treatment decisions. The refinements of multimodality monitoring aid patient-oriented decision making and wider implementations of neuromonitoring tools such as microdialysis and \( \text{PbtiO}_2 \) among others, will facilitate this process. As more attention is directed towards the significance of brain connectivity, it is increasingly recognized that DAI constitutes a distinctive subgroup of TBI. Not only do symptoms arise at impact, but may also evolve and progress over long periods of time, making the quest for pharmacological therapeutic options of particular importance in these patients. However, for pharmacological therapy to be successful in DAI, more knowledge about biological processes underlying the progressive deterioration of axons is needed. Innovative techniques for protein detection such as sandwich ELISA, proximity ligation assays (PLA) and MS, allowing detection and quantification of biomarkers in minute amounts of biological samples, will be more used and integrated in the patient’s care. This will enable more accurate risk stratification of patients for both short- and long-term development of secondary axonal injury.

The association between TBI and future neurodegeneration needs to be further evaluated. Studying the development of A\( \beta \) and tau aggregates as well as other biomarkers such as \( \beta \text{APP} \) and neurofilaments, is one logical continuation in the field of axonal injury, considering their importance in AD. In addition, vast ongoing efforts exist at present to intervene pharmacologically against aggregated proteins in neurodegenerative disorders (279-282). More longitudinal studies using recently developed ligands for selective PET imaging of A\( \beta \) and tau can provide clues on the mechanisms of neurodegenerative development and the temporal and spatial patterns of such aggregates in relation to DAI. However, advancements in neuroimaging still require validation in biological samples. Therefore, continued sampling and investigation of biomarkers from CSF, ISF and brain tissue are essential, albeit their invasive nature. This thesis shows that sampling from the end organ in TBI, the brain tissue, is possible and safe using minimally invasive technique in conjunction with clinically indicated procedures such as insertion of an ICP monitor or an EVD. The information gained from such approaches is invaluable in the research field on axonal injury and ultimately to the patient.
Traumatisk hjärnskada (THS) är en vanligt förekommande orsak till död och funktionsnedsättning. THS, som orsakas främst av trafikolyckor och fall, leder till fler dödsfall och förlorade arbetsår i den yngre populationen < 40 år än cancer, stroke och HIV/AIDS tillsammans. THS är dessutom en etablerad riskfaktor för neurodegenerativa åkommor som Alzheimer's sjukdom. Vanligtvis kategoriseras THS i fokal eller diffus skada samt utifrån de kliniska symptomens svårighetsgrad, i mild, måttlig och svår THS. Diffus axonal skada (diffuse axonal injury, DAI), uppkommer efter rotationsvåld med samtidig acceleration följt av plötslig hastighetsminskning. Detta ger upphov till utbredda skador i nervcellernas utskott, axonerna, på grund av slitningar och sträckskador när hjärnans grå och vita substans rör sig i förhållande till varandra. Skador ses oftast i yttlig vit substans, samt i djupare strukturer som hjärnbalken och hjärnstammen.


Syftet med doktorsavhandlingen var att studera kliniska konsekvenser av axonal skada vid svår THS, med fokus på detektion, neuropatologiska egenskaper samt potentiell länk till neurodegenerativa processer. Studierna omfattar patienter med svår THS som vårdats inneliggande på neurokirurgiska intensivvårdsavdelningen (NIVA) på Akademiska Sjukhuset i Uppsala och i avhandlingen ingår fyra delarbeten. I delarbete I och IV inkluderades patienter med DAI och förhållandena mellan lokalisationsen av axonal skador på MRT och uppkomst av förhöjt intrakraniellt tryck samt klinisk prognos studerades.
I delarbete II undersöktes med en högselektiv antikroppsbaserad detektionsmetod (ELISA) förekomsten av potentiellt neurotoxiska lösliga amyloid-beta aggregat, karaktäristika för Alzheimers sjukdom, i kirurgiskt utrymd hjärnvävnad från patienter med svår THS. I delarbete III studerades hjärnbiospies från patienter med fokala skador och DAI, där proteinuttrycket i hjärnvävnad som radiologiskt förefaller vara oskadad undersöktes med masspektrometri, en känslig detektionsmetod som påvisar förekomst av proteiner utifrån deras massa och laddning.

Avhandlingsens resultat visar att axonal skada vid DAI, i området kring substantia nigra och mitthjärnans vita substans (lat. tegmentum mesencephali) i hjärnstammen ger ökad förekomst av förhöjt intrakraniellt tryck och försämrad klinisk prognos. Ett nytt klassifikationssystem för DAI med korrelation till det kliniska utfallet föreslås. Avhandlingen visar även att svår THS resulterar i ansamling av lösliga och potentiellt neurotoxiska amyloid-beta aggregat hos människa redan efter några timmar från skadetillfället. Därtill visar avhandlingen att DAI, i jämförelse med fokala skador, initierar unika proteinuttryck i hjärnvävnad som radiologiskt förefaller vara oskadad. Resultaten från avhandlingen är värdefulla för att förbättra handläggningen av DAI-patienter samt ger ny insikt i de neuropatologiska processer som initieras av den axonala skadan, vilket kan ha betydelse för uppkomsten av neurodegenerativa sjukdomar i framtiden.
Acknowledgement

This thesis would not have been possible without the help and support of many talented people, to whom I wish to express my great appreciation.

Niklas Marklund, my main supervisor, for all your lessons and inspirations throughout this thesis work. Your knowledge, meticulousness and enthusiasm is truly admirable.

Per Enblad, my co-supervisor, for all your help and support and for teaching me how to combine high-class academic research with excellence in clinical skills and judgment.

Lars Hillered, my co-supervisor, for sharing your vast knowledge in neuroscience and for your inspiring joy while working with research.

Johan Wikström, thank you for teaching me how to properly assess MRI images and for all your important contributions to this thesis.

Raili Raininko, for your meticulousness and for your challenging, constructive and intelligent critique.

Timothy Howells, for your vast contribution in making the NIC unit in Uppsala the invaluable research platform it is and for always having time to explain what is not easily understandable to neurosurgeons.

Olafur Gudjonsson, my head of department, for giving me time, advice and support and for your quiet Icelandic sense of humor and our discussions on world changing topics.

Nils Wesslén, my former head of department, for hiring me to become a neurosurgeon and for pushing me into research with the words “research is not an absolute requirement but you have to!”

Konstantin Salci, my clinical supervisor, for being a world class all-round neurosurgeon and for always telling it as it is.
Kristina Cesarini, for your guidance and your energy, your kind words of support and immense aid in providing control tissue biopsy samples.

Anders Lewén, for your excellent clinical and research skills in neurotrauma and neurointensive care and for providing the invaluable TBI registry.

Elisabeth Ronne-Engström, for guiding me through my first publication and for introducing me to the exciting new world of multivariate regression.

Hans Eriksson, for your teachings and support, and for showing me the pleasure in working with pain.

Pelle Nilsson, for your encouragement and intelligent advice for neurosurgery, research and life in general.

Göran Hesselager, for teaching me the art of microsurgery and for sharing my interest in obsolete indie pop.

Mats Ryttlefors, for your inspiring, tireless pursuit for excellence of neurosurgical technique.

The late Anders Holtz, for your surgical lessons and humble appearance and for our many Champions League evenings together.

All my colleagues at the department of neurosurgery in Uppsala for providing everyday top of the line care to neurosurgical patients and for continuously calling me in the darkest of hours to collect the tissue which made this thesis possible.

Irina Alafuzoff, for you expertise in neuropathology and your contributions in establishing the Uppsala Brain Bank – Trauma biobank.

My co-authors Erik Rollman Waara, Christer Möller, Linda Söderberg at BioArctic AB, and Martin Ingelsson, Hans Basun and Lars Lannfelt at the department of Public Health/Geriatrics which made paper II possible. It has been a pleasure to collaborate with such a talented group with great attention to all details of research.

My co-authors Jonas Bergquist, Ganna Shevchenko, Sravani Musunuri and Jia Mi at Analytical Chemistry, Department of Chemistry-BMC which made paper III possible. I hope to be able to continue our fruitful collaboration in the future and learn more about your innovative techniques.
Johanna Flygt and Fredrik Clausen for keeping up the great work in the TBI lab.

Lena Nyholm for your helpful outcome assessments of severe TBI patients.

The staff at the NIC unit (NIVA) and the operating theatres at the department of neurosurgery in Uppsala for your important help during this thesis work.

All my friends both on the Island and on the Mainland for always being there and for all the fun times.

My family in the Homeland, I miss you a lot and wish you could have taken part of my dissertation day.

My parents-in-law Lars and Mona, for making me a part of your beautiful, eccentric family.

My brothers Rami and Adel and my sister Sanna with families, for all our shared moment in the past, today and in the future. One cannot have better siblings.

My parents Rabah and Samira, for always supporting my choices and decisions, for being excellent loving parents and for pushing us to strive for things you did not have the opportunity to do.

My children Loa and Luna, for being the coolest kids around, for making me laugh and almost cry every day and for giving my life a purpose.

My wife Sara, for putting up with me despite my compulsions and my lack of planning, for being my solid point, for your love and support and for everything we create together. I love you, you are the best I know!
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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)