Diagnostic Methods in Traumatic Brain Injury

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Gothenburg 2017

Cover illustration: Diffusion tensor imaging of the corpus callosum, by Johan Ljungqvist.

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ISBN 978-91-629-0166-0 (PRINT)
ISBN 978-91-629-0165-3 (PDF)

Printed in Gothenburg, Sweden 2017
INEKO AB

To Christina, Astrid and August

“Fibres as delicate as those of which the organ of mind
is composed are liable to break.”
– Gama, 1835[1]

ABSTRACT

Background
Traumatic brain injury (TBI) is a major cause of death and disability worldwide. Early detection and quantification of TBI is important for acute management, for making early accurate prognoses of outcome, and for evaluating potential therapies. Diffuse axonal injury (DAI) is a distinct manifestation of TBI that often leads to cognitive and neurologic impairment. Conventional neuroimaging is known to underestimate the extent of DAI, and intracranial hematomas can usually be detected only in hospitals with radiology facilities. In this thesis, studies I and II were longitudinal investigations using a magnetic resonance diffusion tensor imaging (MR- DTI) technique to quantify DAI. Study III tested whether a novel blood biomarker, neurofilament light (NFL) could identify DAI. Study IV tested whether a microwave technology (MWT) device, designed for use also in a prehospital setting, could detect intracranial hematomas.

Patients and methods
In study I, MR-DTI of the corpus callosum (an anatomical region prone to DAI) was performed in eight patients with suspected DAI in the acute phase and at 6 months postinjury. Clinical data and 6-month outcomes were also examined. In study II, MR-DTI was performed in 15 patients with suspected DAI, 6 and 12 months postinjury. Clinical data and 6- and 12-month outcomes were also examined. In study III, nine patients with DAI were tested for serum NFL-levels in the acute phase. The results were compared with those of healthy controls as well as with the DTI-parameters and outcomes of the patients at 12 months. In study IV, 20 patients with intracranial hematomas (chronic subdural hematomas, cSDH) and 20 healthy controls were tested with a MWT device.

Results
In studies I and II, differences were observed in the diffusion parameters (fractional anisotropy, FA, and diffusivity, here referred to as trace) of the patients. FA was decreased for patients in the acute phase and at 6 and 12 months compared with controls but did not change significantly between the investigations. Trace was unchanged in the acute phase compared with controls but increased at 6 months and continued to increase at 12 months. The findings correlated with clinical outcome as patients with worse outcomes had more evident changes in their diffusion parameters. In study III, the mean NFL concentrations among the patients displayed a 30-fold

increase compared with controls and NFL completely discriminated between the groups. There was also a relationship between NFL and the MR-DTI parameters. In study IV, the MWT device could identify all hematomas at a cost of 25% false positives.

Conclusions
The longitudinal MR-DTI studies (I-II) contribute to a growing body of knowledge about the natural course of DAI and the possible underlying pathophysiological mechanisms. MR-DTI can also quantify DAI which is important for making accurate early prognoses and prerequisite the evaluation of other diagnostic tools (such as the biomarker NFL) and potential therapies. A conspicuous finding was that the DTI parameters and the clinical outcomes changed between 6 and 12 months postinjury, which means that further studies are needed to determine if or when a stable state occurs. The serum marker NFL (study III) was found to be a potential early biomarker for DAI reflecting the severity of the injury. Finally, the results from a new approach to detect intracranial hematomas using a MWT device (study IV) yielded promising results for use in the early triage of patients with head injury.

Keywords
Traumatic brain injury, diffuse axonal injury, diffusion tensor imaging, serum biomarkers, microwave technology.

SAMMANFATTNING PÅ SVENSKA

Bakgrund
Traumatisk hjärnskada är en viktig orsak till dödsfall samt till olika typer av neurologiska funktionsnedsättningar. Att tidigt kunna kartlägga skadan är viktigt för att styra den akuta handläggningen samt för att kunna ge en säker prognos. Diffus axonskada (från eng. diffuse axonal injury, ofta betecknad ”DAI”) är en särskild typ av skada som drabbar den vita substansen och som ofta leder till omfattande kognitiva begränsningar. Konventionella radiologiska undersökningar som skiktröntgen (datortomografi, DT) och magnetkameraundersökning (magnetresonanstomografi, MRT) underskattar omfattningen av DAI, men de kan effektivt och med hög säkerhet påvisa blödningar innanför skallbenet. Det finns emellertid ingen tillgänglig utrustning som kan användas utanför sjukhus för att påvisa dylika blödningar. Denna avhandling omfattar två delarbeten (I och II) där patienter undersöks med en särskild diffusions-tensor MRT (betecknad MR-DTI) för att upptäcka och mäta DAI. Delarbete III undersöker om ett blodprov (neurofilament light, NFL) kan påvisa DAI. Delarbete IV undersöker om ett medicintekniskt instrument som nyttjar mikrovågsteknik och kan användas utanför sjukhus kan påvisa blödning innanför skallbenet.

Patienter och metod
I delarbete I-II inkluderades patienter med traumatisk hjärnskada och misstanke om DAI, eftersom DT av hjärnan inte kunde ge någon annan förklaring till det observerade kliniska tillståndet. I delarbete I undersöktes åtta patienter med MR-DTI av hjärnbalken (ett område som ofta drabbas vid DAI) i akutskedet samt 6 månader efter skadetillfället. Resultaten jämfördes med patienternas kliniska tillstånd efter 6 månader. I delarbete II undersöktes
15 patienter med MR-DTI, 6 och 12 månader efter skadetillfället, och resultaten jämfördes patienternas kliniska tillstånd efter 6 respektive 12 månader. I delarbete III jämfördes värden på blodprovet neurofilament light (NFL) taget i akutskedet, med värden från friska kontrollpersoner samt med resultaten från patienternas MR-DTI och det kliniska tillståndet 12 månader efter skadetillfället. I delarbete IV undersöktes 20 patienter med blödning innanför skallbenet och 20 friska kontrollpersoner med ett instrument som använder mikrovågsteknik.

Resultat
I delarbete I och II uppmättes förändringar i patienternas DTI-parametrar (fractional anisotropy, FA, och diffusivitet, betecknat trace). FA var lägre för patienterna i akutskedet samt efter 6 och 12 månader jämfört med friska

kontrollpersoner. FA var däremot väsentligen stabilt mellan undersökningarna. Trace var oförändrat i akutskedet men högre efter 6 månader jämfört med friska kontrollpersoner och fortsatte sedan att stiga fram till 12 månader. Det noterades även ett samband mellan utfallet av MR- DTI (FA och trace) och patienternas kliniska tillstånd genom att förändringarna var tydligare för patienter med sämre kliniskt tillstånd vid 6 och 12 månader efter skadetillfället jämfört patienter med bättre kliniskt tillstånd. I delarbete III uppmättes ett 30 gånger högre värde av NFL för patienterna jämfört med de friska kontrollpersonerna. Det observerades också ett samband mellan värdet på NFL och resultaten från MR-DTI- undersökningen. I delarbete IV kunde mikrovågstekniken upptäcka 100% av blödningarna men till en ”kostnad” av 25% falskt positiva undersökningar.

Slutsatser

Studierna som utvärderar MR-DTI (delarbete I och II) bidrar till ökad kunskap om naturalförloppet vid DAI och om de mekanismer som tros ligga till grund för skadan. Detta är angeläget för att kunna ge en säkrare prognos om patienternas kliniska tillstånd i efterförloppet samt för att möjliggöra att nya behandlingar och diagnostiska verktyg (som exempelvis NFL) kan utvärderas. Ett viktigt fynd var att såväl resultaten från MR-DTI som patienternas kliniska tillstånd förändrades mellan 6 och 12 månader. Detta innebär att ytterligare studier behövs för att avgöra om eller när skadan har uppnått en stabil fas. Blodprovet NFL (studie III) kunde användas för att påvisa DAI och uppvisade också ett samband med skadans omfattning. Instrumentet som använder mikrovågsteknik (studie IV) visade lovande resultat för att påvisa blödningar innanför skallbenet, och det har förutsättning att få bred användning såväl inom som utanför sjukhus för att skilja ut patienter som behöver akut neurokirurgi.

LIST OF PAPERS

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

I. Ljungqvist J, Nilsson D, Ljungberg M, Sörbo A, Esbjörnsson E, Eriksson-Ritzén C, and Skoglund T. Longitudinal study of the diffusion tensor imaging properties of the corpus callosum in acute and chronic diffuse axonal injury. Brain Injury. 2011; 25(4): 370-378.

II. Ljungqvist J, Nilsson D, Ljungberg M, Esbjörnsson E, Eriksson-Ritzén C, and Skoglund T. Longitudinal changes in diffusion tensor imaging parameters of the corpus callosum between 6 and 12 months after diffuse axonal injury. Brain Injury. 2017; 31(3): 344-350.

III. Ljungqvist J, Zetterberg H, Mitsis M, Blennow K, and Skoglund T. Serum Neurofilament Light Protein as a Marker for Diffuse Axonal Injury: Results from a Case Series Study. Journal of Neurotrauma. 2017; Mar 1;34(5): 1124-1127.

IV. Ljungqvist J, Candefjord S, Persson M, Jönsson L, Skoglund T, and Elam M. Clinical Evaluation of a Microwave-Based Device for Detection of Traumatic Intracranial Hemorrhage. In press: Journal of Neurotrauma. 2017 Mar 13. doi: 10.1089/neu.2016.4869.

CONTENT

ABBREVIATIONS

BNIS	Barrow Neurological Institute Screen for Higher Cerebral Functions
CT	Computed tomography
cSDH	Chronic subdural hematoma
CSF	Cerebrospinal fluid
DAI	Diffuse axonal injury
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
GCS	Glasgow Coma Scale
GOSe	Glasgow outcome scale, extended version
HC	Healthy controls
MR-DTI	Magnetic resonance diffusion tensor imaging
MRI	Magnetic resonance imaging
MWT	Microwave technology
NFL	Neurofilament light
RLS	Reaction level scale
ROC	Receiver operating characteristics
ROI	Region of interest
TBI	Traumatic brain injury

1 INTRODUCTION

Traumatic brain injury (TBI) is a major cause of death and disability around the world with an estimated 10 million people affected annually[2]. Intracranial hematomas comprise an important group of TBI, because early surgical evacuation significantly improves outcome[3]. Hence, it is important to detect hematomas as early as possible, but currently, this can be done only in hospitals with radiology facilities. This investigation tests whether a recently developed diagnostic device using microwave technology (MWT), designed for use also in a prehospital setting, can detect intracranial hematomas. Diffuse axonal injury (DAI) is another distinct manifestation of TBI, but for this condition, surgery does not improve the clinical course. However, the detection and quantification of DAI in the acute phase is important to make an accurate prognosis and to optimize early care and rehabilitation. Quantifying DAI also prerequisites the evaluation of other diagnostic tools and potential therapies. This investigation applies a magnetic resonance diffusion tensor imaging (MR-DTI) technique to quantify DAI and tests whether a new blood biomarker (neurofilament light, NFL) can identify DAI.

1.1 Traumatic brain injury

1.1.1 Epidemiology of traumatic brain injury

Traumatic brain injury (TBI) is a major public health and socio-economic burden throughout the world. The incidence of hospital-admitted TBI has been estimated at ~262 cases per 100,000 individuals in a meta-analysis from 16 European countries[4]. A meta-analysis comparing international studies on epidemiology found a lower proportion of TBI in Europe (228 per 100,000) compared with North America (331 per 100,000), Asia (380 per 100,000), and Australasia (415 per 100,000), but studies were diverse with respect to inclusion criteria, and the true incidence of TBI is probably considerably higher[5]. In a prospective cohort study of children and adolescents in New Zeeland, records from both in-patient and out-patient visits were gathered, and self-reports were obtained, yielding and incidence of 1750 per 100,000[6].
The epidemiology of TBI is also different between continents. In growing economies, road traffic accidents (RTA) become more frequent whereas in most Western countries, head injury due to RTA is decreasing[7]. The aging population, however, has shifted the epidemiology of TBI toward more frequent falls at home, mainly involving the elderly[7].

1.1.2 Subtypes of traumatic brain injury

TBI results from a mechanical load to the head leading to dysfunction and/or structural failure[8]. 'Dysfunction' refers to the clinical state after TBI and is associated with 'loss of consciousness' or 'coma'; however, symptoms also include cognitive and neurologic impairment. The level of consciousness after TBI relates to severity and is measured by coma scales[9,10]. Most TBI are mild "concussions", defined by a transient disturbance in consciousness or loss of memory, and do not lead to sequelae or structural changes. However, some mild injuries lead to persistent symptoms, called post-concussive syndrome, and although the pathophysiology is not entirely clear, it may be related to the neurodegenerative condition chronic traumatic encephalopathy (CTE) that results from repetitive concussive and subconcussive head injuries[11]. Moderate and severe TBI are associated with structural failure that include intracranial hemorrhages, cerebral contusions, edema and diffuse axonal injuries. Traditionally, TBI has often been categorized into 'focal' and 'diffuse' in terms of focal neurologic deficits or diffuse clinical symptoms (i.e. coma), and conventional imaging often reveals focal or localized damage in the first case and diffuse or no pathology in the latter. For patients with severe injuries, however, different pathologies often coexist, and Adams and colleagues have found that 76% of patients have more than two pathologies[12].

Intracranial hematomas are defined by their location in relation to the dura. Epidural and subdural hematomas are caused by mechanical deformation and vascular disruption that lead to brain compression and may cause focal ischemia, reperfusion injury, vasogenic edema and reduced cerebral blood flow. Brain contusions are intra-axial hemorrhagic lesions that give rise to local edema and ischemic change. The contusions are often located in the frontal and temporal lobes and they progress during the first days after the injury. The morbidity of brain contusions is directly associated with their size, depth and potential for bilateral involvement[13].

Diffuse axonal injury is a distinct manifestation of TBI, caused by stretching and shearing of white matter fibers in the brain due to rapid acceleration and deceleration. These mechanisms often leads to poor clinical outcome including physical and cognitive impairment[14]. The injuries will be discussed in sections 1.1.3 and 1.1.4.

1.1.3 Background to diffuse axonal injury

Diffuse axonal injuries were first described by the neuropathologist Sabina Strich in 1956[15]. Strich examined a series of five patients who died after "prolonged coma or other severe disturbances of consciousness following head injury".[15] These patients had a similar degeneration of the white matter of the cerebral hemispheres[15]. Gennarelli and colleagues were able to produce DAI in primates by accelerating the head without impact[16]. They found that "the duration of coma, degree of neurologic impairment, and amount of diffuse axonal injury (DAI) in the brain were directly related to the amount of coronal head motion used"[16]. Lateral or side-to-side motion was associated with more severe injury. Adams and coworkers made neuropathological examinations of 45 cases of DAI in humans and compared the results with 132 cases of fatal injury without DAI as well as with the study by Gennarelli and colleagues[17,18]. The main conclusion was that DAI was presented as a "distinct clinicopathological group and that their brain damage is sustained at the moment of injury" because there was a significantly lower rate of raised intracranial pressure, severe contusions and intracranial hematomas in the DAI group compared to the non-DAI cases[16]. These findings have also been confirmed in later studies[12].

Diffuse axonal injuries have a widespread distribution, and the damaged structures are found within regions with intact neuronal and vascular components although microbleeds may be associated with the injury[13]. The pathophysiological changes that take place in DAI can be categorized into "disruptive axonal injury" where axons are damaged at the time of impact, and "nondisruptive axonal injury" where there is a "perturbation" of the axolemma that leads to a cascade of biological changes that result in a loss of axons over the first 24 hours after injury[19,20]. In disruptive axonal injury or primary axotomy, the axon is thought to break, retract and swell at the end of the axonal axis, forming an axonal retraction ball. This histologic feature of DAI is characterized microscopically, as shown by Meythaler and coworkers[14] (Figure 1). Within a few days after impact, irregular swellings of axons appear as rounded or oval bulbs at the ends of axons. Within a few weeks, small clusters of microglia replace the axonal bulbs, and eventually the microscopic features correspond to Wallerian-type axonal degeneration as the axon disintegrates, indicating a loss of membrane integrity[14,21].

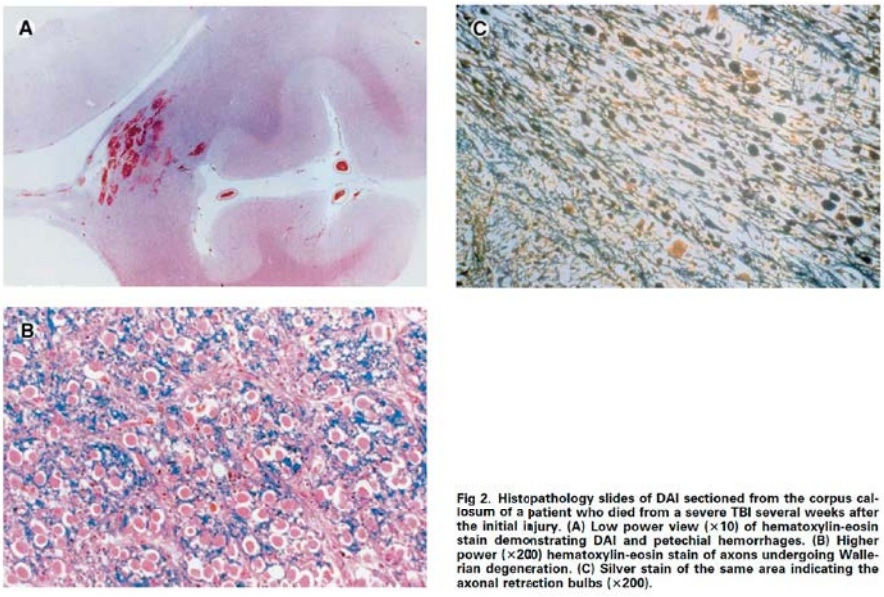


Fig 2. Histopathology slides of DAI sectioned from the corpus callosum of a patient who died from a severe TBI several weeks after the initial injury. (A) Low power view (×10) of hematoxylin-eosin stain demonstrating DAI and petechial hemorrhages. (B) Higher power (×200) hematoxylin-eosin stain of axons undergoing Wallerian degeneration. (C) Silver stain of the same area indicating the axonal retraction bulbs (×200).

Figure 1. Histopathology of DAI. Reprinted from Arch Phys Med Rehabil 82(10). Meythaler et.al. Current concepts: Diffuse axonal injury–associated traumatic brain injury. 1461-1471. Copyright (2001), with permission from Elsevier.

Nondisruptive- or secondary axonal injury results from some lesser tensile strain than primary injury and causes structural alterations of the membrane (axolemma), termed "perturbation"[19]. The permeability of the perturbated membrane changes, to allow for large amounts of calcium ions to enter the cells, leading to a series of steps which degrade the cytoskeleton network and cause mitochondrial damage[20,22]. Damage to the intraaxonal cytoskeleton has been pointed out as the predominant cause of DAI[14]. Subsequently, there will be loss of axonal transport, axonal swelling, and formation of axonal bulbs that will eventually lead to disconnection or secondary axonal injury[20,23].

Similar to primary axonal injury, the axonal retraction balls are replaced by clusters of microglia within a few weeks, and still later, astrocytosis occurs at sites of axonal damage and demyelination[24]. It is not clear, however, for how long this process of astrocytosis continues after injury.

There is currently no specific therapy for DAI, but a better understanding of the complex pathophysiology is prerequisite for the development of therapeutic interventions[23].

1.1.4 Grading of diffuse axonal injury

Diffuse axonal injury may be classified into three grades depending on its appearance and distribution. The grading scale, introduced by Adams and coworkers, was based on post mortem anatomical and histological findings[25]. In grade 1, there is histological evidence of axonal injury in the white matter of the cerebral hemispheres, the corpus callosum, the brain stem and, less commonly, the cerebellum; in grade 2 there is also a focal lesion in the corpus callosum; and in grade 3 there is in addition a focal lesion in the dorsolateral quadrant or quadrants of the rostral brain stem. The focal lesions can often only be found microscopically, but grades 2 and 3 can be considered severe if the focal lesions are apparent macroscopically[25]. The Adams' grading system is now also used for grading DAI in MRI, and an extended version of the Adams' grading system has recently been proposed that could further improve the ability to predict outcome[26,27].

1.1.5 Chronic subdural hematoma

A chronic subdural hematoma, cSDH, is a collection of blood and blood breakdown products between the surface of the brain and the dura. Figure 2 shows a CT scan of a patient with a cSDH. Contrary to acute subdural hematomas, caused by the tearing of bridging veins or arterial rupture that lead to acute dysfunction, cSDH expand slowly and symptoms can take weeks to appear. Most acute subdural hematomas that are not operated upon (either because they are small or do not cause symptoms or because there are contraindications to surgery) will resolve into a liquefied clot and be absorbed after a few weeks, but some hematomas will evolve into cSDH[28]. Another mechanism behind cSDH is that they originate from a subdural hygroma which consists of an accumulation of cerebrospinal fluid in the subdural space, due to a separation of the dura-arachnoid interphase, often in patients with brain atrophy[28,29]. Common to both aetiologies of cSDH, a neomembrane forms on the outer side of the effusion[28,30]. The pathophysiology of cSDH is not entirely clear, but current belief is that cSDH is caused by recurrent bleeding into the subdural space, caused by a cycle of local angiogenesis, inflammation, coagulation and ongoing fibrinolysis, and efforts are made to replicate this in animal models[31]. Patients with small or asymptomatic hematomas are usually managed conservatively while patients with large or symptomatic hematomas receive surgery[32].

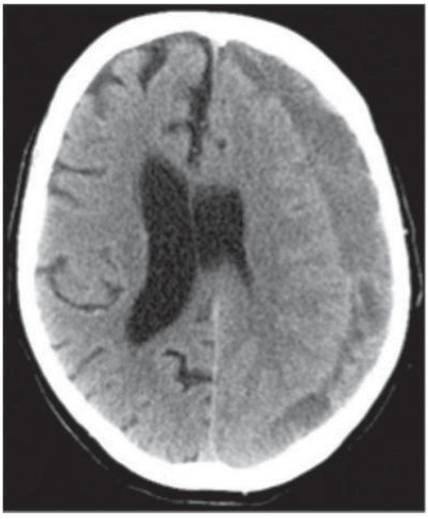


Figure 2. CT-scan of a chronic subdural hematoma on the left side. Reprinted with permission from JOURNAL OF NEUROTRAUMA, March 2017, by Ljungqvist et al, published by Mary Ann Liebert, Inc., New Rochelle, NY.

1.2 Imaging in traumatic brain injury

The roles of neuroimaging in TBI are; first, to identify conditions that require immediate neurosurgical attention; second, to identify other treatable injuries and to prevent secondary damage; and third, to provide useful prognostic information. Bruce and colleagues have identified four criteria for the "ideal" imaging technique: "(1) accessible and safe for use in acute injury in those with altered consciousness; (2) equally sensitive to all injury severities, (3) equally sensitive to the acute through chronic time course, and (4) appropriate for identification of the earliest of pathological changes in the transition to neurodegenerative disease"[33]. Unfortunately, there is currently no single technique that fulfil these criteria, so often several techniques must be applied. Computed tomography (CT) consists of rotating X-ray equipment and is fast, readily available and sensitive to blood (and bone) which makes it the primary imaging modality in the acute management of TBI. However, CT lacks the ability to identify DAI in all but the most significant cases. Magnetic resonance imaging (MRI) involves the interaction between a static magnetic field, local magnetic fields, and radio waves, to discriminate between tissues or structures of different proton densities. Conventional MRI is more sensitive to structural alterations than CT, and can better identify microbleeds associated with DAI. Nevertheless, it still known to underestimate the extent of white matter damage after TBI[34]. Advanced MRI techniques including diffusion tensor imaging (DTI, discussed in section 1.2.1 and 1.2.2), susceptibility weighted imaging (SWI), magnetic resonance spectroscopy (MRS) and functional magnetic resonance imaging (fMRI), have all been tested to improve the detection of brain abnormalities (i.e. diagnosis), particularly in mild TBI, but neither is commonly used in clinical routine[35]. A recent review by Studerus-Germann and colleagues, identified DTI as a valuable tool to identify DAI but emphasized the need for longitudinal studies[36].

1.2.1 Background to diffusion tensor imaging

Diffusion tensor imaging (DTI) is a magnetic resonance technique that can indirectly evaluate the integrity of white matter tracts by measuring the diffusion of water. Water is the dominating diffusing molecule in the human body, and if the medium is homogenous and without barriers (such as in the ventricles in the brain), the displacement is random. In the body, however, biological tissue is heterogeneous, consisting of structures that restrict the mobility of the water molecules[37]. These differences in diffusion provide the basis for DTI-measurements.

1.2.2 Principles of diffusion tensor imaging

Diffusion tensor imaging measures water diffusion and its directionality in three dimensions, using six or more gradient directions[38]. From this information, not only the mean diffusivity, but also the magnitudes of the diffusivities in the three different dimensions may be calculated. The diffusion pattern of a voxel is presented as a tensor, based on three orthogonal principal eigenvectors that are ordered by the magnitudes of their corresponding eigenvalues, i.e. $\lambda_1>\lambda_2>\lambda_3$[37]. The diffusion tensor may also be represented three-dimensionally as a diffusion ellipsoid (Figure 3)[38].
Fractional anisotropy (FA) is a rotationally invariant parameter that represents the ratio of the anisotropic component of the diffusion tensor to the whole diffusion tensor[38]. FA values range from 0 to 1, where 0 represents maximal isotropic diffusion as in a perfect sphere and 1 represents maximal anisotropic diffusion as in an indefinitely elongated ellipsoid[39].

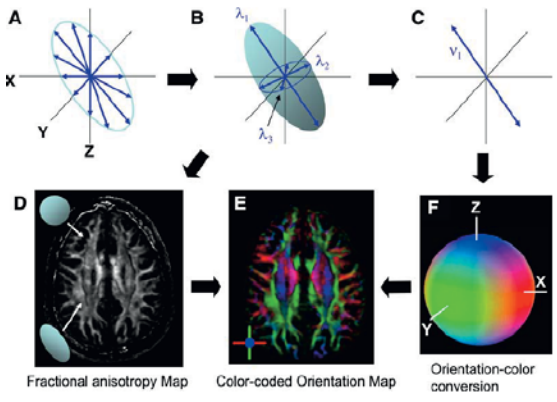


Figure 6. The Principle of DTI and Contrast Generation

From diffusion measurements along multiple axes (A), the shape and the orientation of a "diffusion ellipsoid" is estimated (B). This ellipsoid represents what an ink stain would be if ink were dropped within the pixel. An anisotropy map (D) can be created from the shape, in which dark regions are isotropic (spherical) and bright regions are anisotropic (elongated). From the estimated ellipsoid (B), the orientation of the longest axis can be found (C), which is assumed to represent the local fiber orientation. This orientation information is converted to a color (F) at each pixel. By combining the intensity of the anisotropy map (D) and color (F), a color-coded orientation map is created (E).

Figure 3. The principle of DTI. Reprinted from Neuron 51(5): Mori, S. and J. Zhang. Principles of diffusion tensor imaging and its applications to basic neuroscience research. 527-539. with permission from Elsevier.

The magnitude of the principal diffusion direction λ_1 or λ_{major}, corresponds to diffusion parallel to the axons: λ_1 = *axial diffusivity* or *parallel diffusivity* ($\lambda_{||}$)

The mean of λ_2 and λ_3 corresponds to diffusion perpendicular to the axons:

($\lambda_2 + \lambda_3$)/2 = *radial diffusivity* or *perpendicular diffusivity* ($\lambda_{\perp}$)

The mean of all eigenvalues corresponds to the mean diffusivity:

($\lambda_1 + \lambda_2 + \lambda_3$)/3 = *mean diffusivity* (MD)

The sum of all eigenvalues corresponds to *trace*: $\lambda_1 + \lambda_2 + \lambda_3$

Fractional anisotropy (FA) is a measure of the level of anisotropy on a scale from 0 to 1:

$$FA = \sqrt{\frac{3}{2}} \frac{\sqrt{(\lambda 1 - \mathrm{MD})_+^2 + (\lambda 2 - \mathrm{MD})_+^2 + (\lambda 3 - \mathrm{MD})_+^2}}{\sqrt{\lambda_-^2 + \lambda_+^2 + \lambda_-^2}}$$

Mean diffusivity and trace provide an overall evaluation of the magnitude of diffusional motion in a three-dimensional volume (voxel) or region[39]. Fractional anisotropy and the direction of λ_{major} is often presented as colour-coded FA-maps, where the intensity represents the FA-value and the colour the direction of λ_{major} in each voxel (Figure 3). Diffusivity and FA vary within the different regions of the normal brain (Figure 3).

In white matter regions with a regular parallel fiber arrangement, such as the corpus callosum, the diffusion of water molecules in the direction of the fiber is high compared to the water diffusivity in the perpendicular direction (high anisotropy, FA), whereas in less coherent structures, such as in regions where fibers of different bundles merge, the anisotropy is low (low FA)[40]. Thus, the diffusion characteristics vary between different structures, and such variations could also imply structural changes because of disease or trauma[41].

1.2.3 Background to tractography

Tractography is a technique that applies data from DTI to visualize white matter tracts by connecting voxels based on their principal diffusion direction and their levels of anisotropy. The underlying assumption is that the principal diffusion direction is aligned with the direction of the axons. Thresholds for the minimum FA and the maximal change of directions between the adjacent voxels must be specified to delineate the tracts. The voxel size used in DTI, however, is generally in the order of 1-2mm in each direction, and will therefore contain hundreds of thousands of axons. Hence, there may be different white matter tracts with different directions in each voxel, and this must be considered by technical or manual corrections.

However, tractography remains the only way to delineate white matter tracts in vivo. The technique is frequently applied in neurosurgery for preoperative mapping in tumor or epilepsy surgery[37].

1.3 New approaches for detection and quantification of TBI

1.3.1 Serum markers – NFL

Some of the limitations of imaging techniques such as CT and MRI have previously been discussed, i.e. that they are unable to detect or underestimate DAI.

MR-DTI is still mainly used in research studies and not in clinical routine. Further, it is not known how sensitive MR-DTI is in detecting DAI in mild TBI cases, and the technique has not been cross-validated against postmortem histology measures of axonal injury in humans. The analysis often needs time-consuming manual calculations, different hospitals use different methods for analysis, and it is difficult to compare DTI parameters among research groups. These limitations have led to the search for a useful biomarker as an alternative way to diagnose and quantify DAI.

A useful biomarker would be alternative way, or an adjunct, to diagnose and quantify DAI. An ideal blood biomarker should have increased serum levels in the acute stage related to TBI-induced DAI and persistent brain dysfunction. Further, because DAI by definition involves damage to the long myelinated white matter axons, neuronal proteins enriched in these structures are top candidates as fluid biomarkers. A number of proteins have been evaluated as candidate blood biomarkers for DAI but none has so far proved to be of prognostic value regarding DAI. Neurofilament light (NFL) protein is an important structural protein of the axon skeleton[42]. Upon axonal injury, NFL leaks from disrupted axons into the brain interstitial fluid, cerebrospinal fluid (CSF), and blood. Until recently, NFL has only been possible to detect in CSF. The biomarker calcium-binding protein B (S100B) often used in head injury algorithms was also tested[43,44]. S100B is not specific to the brain, but a low value also indicates low risk of developing severe TBI[45].

1.3.2 Microwave technology

Imaging techniques such as CT and MRI, also require that the patient is transported to a hospital with a radiology department. However, the time from injury to surgery for patients with intracranial hematomas is of the essence. Seelig and associates showed that in patients with traumatic acute subdural hematomas, the delay from injury to operation was the factor of greatest therapeutic importance[3]. Diagnosis and removal of the hematoma within four hours of impact considerably reduced mortality, i.e. from 90 % to 30 % mortality rate (n = 82, p < 0.0001). Any further delay in hematoma evacuation severely increased mortality and worsened functional outcome in the patients who survived[3]. A key to improve outcome for patients with TBI is also to reduce the time to definitive care by achieving a high triage accuracy. A compact system that can detect intracranial hemorrhage in a prehospital setting, e.g. in road and air ambulances, could improve triage accuracy and reduce the time between injury and surgery, resulting in reduced mortality rates and improved functional outcomes.

Microwave technology (MWT) for biomedical applications has been explored for over three decades[46]. Driving forces include the potential to realize portable devices at low cost to convey diagnostic information in a fast, non-invasive and safe manner. MWT can detect lesions such as cancer and internal bleedings due to the dielectric contrast between tissue types[47,48].

For detection of intracranial hemorrhage the contrast between blood and brain matter is utilised[49]. In neurosurgical care, MWT could be used to monitor patients with conservatively managed hematomas, to monitor patients postoperatively, and to monitor trauma patients in neuro-intensive care who are at risk of progressing contusions or extracerebral hematomas. Recently, Persson and colleagues showed that MWT can differentiate hemorrhagic and ischemic stroke[49]. They used two MWT prototype systems to measure 20 and 25 stroke patients, respectively, in the first two proof-of-principle clinical studies. The ability of a device to differentiate hemorrhagic and ischemic stroke in the prehospital setting could allow for rapid thrombolytic therapy in patients with ischemia, and more adequate care for patients with intracerebral hematomas.

2 AIM

Study I.
The aim was to evaluate the changes in the diffusion tensor imaging parameters of the corpus callosum in the acute phase and 6 months after TBI with suspected DAI, and to examine the relationship between DTI parameters, global and cognitive outcome.

Study II.
The aim was to evaluate the changes in the diffusion tensor imaging parameters of the corpus callosum 6 and 12 months after TBI with suspected DAI, and to examine the relationship between DTI parameters, global and cognitive outcome.

Study III.
The aim was to test NFL as a potential blood-based biomarker for DAI in a cohort of patients with DAI and to compare the results with the outcome at 12 months and the DTI parameters.

Study IV.
The aim was to test if microwave technology, using the first portable device enabling prehospital measurements, could be used as a medical screening tool to differentiate patients with a traumatic intracranial hematoma from a healthy control (HC) group.

3 PATIENTS AND METHODS

3.1 Subjects

3.1.1 Subjects study I-III.

The studies were approved by the Regional Ethical Review Board at the University of Gothenburg, and informed consent was obtained from all participants or their next of kin. All patients were referred to Sahlgrenska University Hospital during the period June 2006 through September 2009, and had sustained TBI. Patients were included based on the criterion that DAI had been suspected because of affected consciousness and/or focal neurological symptoms without an obvious explanation seen on the CT scan of the brain.

In total, 23 patients were included in the research project on DAI. Because of technical problems with saving of the raw data for DTI as well as loss of blood samples for analysis of NFL, subgroups of the 23 patients were included in the three studies presented here (study I, eight patients; study II, fifteen patients; and study III, nine patients). An overview of the patients that were included is presented in table 1.

3.1.2 Subjects study IV.

This study was approved by the Regional Ethical Review Board at the University of Gothenburg and reviewed by The Medical Products Agency - Sweden. The study was registered at Clinical- Trials.gov (Identifier: NCT02282228) before the recruitment of the first study participant. All patients were referred to Sahlgrenska University Hospital for operation of cSDH, during the period September 2015 to January 2016. The HC group was recruited in the same period as the patients and matched to the patient cohort for age and sex. Written informed consent was obtained from all participants before any study-related procedure was initiated. Safety follow- up for participants was performed >12 h after the diagnostic procedure.

Patient	ID Study I	ID Study II	ID Study III	RLS	GCS	Adams	GOSe 6m	GOSe 12m
A	1	1		7	4	2	6	6
B			1	6	5	3	3	3
C			2	7	4	1	0	0
D	2	2	3	8	3	3	4	5
E		3		2	14	0	7	7
F			4	2	14	2	6	6
G	3	4	5	3	7	2	6	6
H			6	8	3	3	3	3
I	4	5	7	5	6	3	3	3
J	5	6		1	14	0	6	6
K		7	8	4	6	3	3	3
L	6			2	14	2	8	8
M	7			5	6	3	4	5
N	8	8		7	5	2	5	7
O		9		1	15	1	5	6
P		10		2	12	2	4	4
Q		11		3	8	2	4	4
R			9	5	6	3	4	4
S		12		6	5	3	6	6
T				1	15	3	5	5
U		13		4	6	0	6	6
V		14		3	13	3	7	8
W		15			14			

Table 1. Overview of the patients (n=23, labeled A-W), their clinical characteristics, grades of diffuse axonal injury, and outcomes. RLS=reaction level scale, GCS=Glasgow coma scale, Adams=Adams' classification of diffuse axonal injury, GOSe=Glasgow outcome scale.

3.2 Methods

Common methods for studies I-III

Image acquisition

MR-DTI was performed on a Philips Gyroscan Intera 1.5 T, release 9. The software was upgraded to an Achieva release 1.5 during the study. Before the upgrade, the SENSE head coil utilized six channels, and after the upgrade eight channels. The DTI method used was HARDI (high angular resolution diffusion imaging; Philips, Eindhoven, the Netherlands).

DTI was performed using a single shot spin-echo echo-planar imaging (SE-EPI) sequence with SENSE factor of 3.2 and a halfscan factor of 0.712. A b=0 s/mm^2 and 15 diffusion-sensitizing directions with b=800 s/mm^2 were acquired.

For six of the controls and for patient 2, the imaging parameters were: TE= 69 ms, NSA=6, BW=33.8 Hz/pixel in AP direction, isotropic voxels of 2.2 mm^3 reconstructed to 1.9 x 1.9 x 2.2 mm^3 resulting in a scan time of 16 minutes. For the remaining 10 controls and for all patients (except patient 2),
the imaging parameters were: TE= 66 ms, NSA=3, BW=46.8 Hz/pixel in AP direction, isotropic voxels of 2.5 mm^3 reconstructed to 1.9 x 1.9 x 2.5 mm^3 resulting in a scan time of 7.5 minutes.

Data analysis

Post processing of diffusion tensor metrics and white matter fiber tracking was carried out using the software DTIStudio V 2.4 (Johns Hopkins Medical Institute, Laboratory of Brain Anatomical MRI, http://lbam.med.jhmi.edu/)[50]. To minimize artefacts due to subject motion, all diffusion images were co-registered to the b=0 image using the Automated Image Registration (AIR) included in DTIStudio[51].

Analysis of the corpus callosum

The corpus callosum was chosen for study in this investigation as it is prone to DAI and it is anatomically easy to define using MR-DTI[52,53]. The mid- sagittal slice through the corpus callosum was identified on the color-coded FA maps. Polygonal regions of interest (ROIs) were manually placed in the
corpus callosum in the two slices immediately paramedian to the middle slice. Fiber tracking was performed using the fiber assignment by continuous tracking (FACT) algorithm in DTIStudio[50]. The tracking propagation was terminated when the tract trajectory reached a voxel with an FA<0.2 or when the angle between two consecutive steps was >50^o. Only fibers passing through both ROIs were displayed and used for analysis.

From the data provided in DTIStudio, we extracted FA, λmajor, λmedium, λminor and trace for the tracked voxels in the mid-sagittal section of the

whole corpus callosum. From λmajor, λmedium and λminor the parallel and perpendicular diffusivities were calculated. The procedure was applied and tested for inter-rater reliability in study I.

Methods study I.

MR-DTI was performed in eight patients with suspected DAI within 11 days and at 6 months post-injury. Six controls were also examined. Fractional anisotropy (FA), trace and parallel and perpendicular diffusivity of the corpus callosum were analyzed. Clinical data including the initial level of consciousness, age and mechanism of injury was also recorded. The main outcome was the extended Glasgow Outcome Scale score, assessed at 6 months. The Barrow Neurological Institute Screen for Higher Cerebral Functions (BNIS) was also used for cognitive screening on a basic level.

Methods study II.

MR-DTI was performed in 15 patients with suspected DAI, 6 and 12 months post-injury. Sixteen controls were also examined. Fractional anisotropy (FA) and diffusivity (trace) in the corpus callosum were analyzed. Clinical data including the initial level of consciousness, age and mechanism of injury was also recorded. The outcome measures were the extended Glasgow Outcome Scale and the Barrow Neurological Institute Screen for Higher Cerebral Functions, assessed at 6 and 12 months.

Methods study III.

Nine patients, 7 men and 2 women, were included. Blood samples to measure serum NFL and S100B were obtained within six days postinjury. Blood samples for NFL were also taken from 22 healthy age-matched controls. The first MRI was performed within 9 days (range 4-9 days) postinjury, and sequences including T1, T2, T2* and FLAIR were analysed by a radiologist, and the presence of signs of DAI were classified according to Adams et al.[25]. The follow-up MRI was performed at 12 months postinjury and for these examinations, the DTI parameters were analysed. Fractional anisotropy (FA) and diffusivity (trace) in the corpus callosum were analyzed. Clinical data including the initial level of consciousness, age, and mechanism of injury. The outcome measure was the extended Glasgow Outcome Scale. The relationships between NFL concentrations and DTI parameters were then analyzed.

Methods study IV.

Eligible patients were included upon arrival to the neurosurgical unit and measured with the microwave device prior to surgery of cSDH. Twenty patients with cSDH were included and 20 healthy controls were measured with the MWT device within the same time frame as the inclusion of the patients. The patients' CT-scans were reviewed and used for comparison with the MWT data. Volume and attenuation of the hematomas, and midline shift was measured.

3.3 Statistical methods

Statistical methods study I.

Age in the patient group and the control group was compared using a t-test. For comparison between groups, Fisher's non-parametric permutation test was used[54]. For comparison over time within groups, Fisher's non-parametric permutation test for matched pairs was used[54]. Intra-class Correlation Coefficient (ICC) was used to assess inter-rater agreement[55].

Statistical methods study II.

Age in the patient group and the control group was compared using a t-test. For comparison between groups, Fisher's non-parametric permutation test was used[54]. For comparison over time within groups, Fisher's non-parametric permutation test for matched pairs was used[54].

Statistical methods study III.

Age in the patient group and the control group was compared using a t-test, NFL concentrations were compared using the Mann-Whitney U-test, and DTI-parameters were analysed using Fisher's non-parametric permutation test. $p<0.05$ was considered significant. The relationship between NFL concentrations and DTI parameters was explored using simple linear regression, and the goodness of fit is presented as R^2.

Statistical methods study IV.

Age in the patient group and the control group was compared using a t-test. The diagnostic performance was evaluated using the receiver operating characteristics (ROC) and the area under the curve (AUC). An AUC of ~0.5 represents a useless diagnostic test, equal to rely on chance. An AUC of 1.0 represents a perfect test that classifies all subjects correctly. The specificity at 100 % sensitivity was derived from the ROC.

4 RESULTS

Study I.
A significant reduction in FA in the corpus callosum was seen in the acute phase in patients compared with the healthy controls. There was no significant change in the parallel or perpendicular eigenvalues or trace. At 6 months, a significant reduction in FA and a significant increase in trace was noticed compared with controls. The results of FA and trace are presented in table 2. The significant increase in trace was mainly driven by the four patients with the worst outcomes.

	Controls	Patients acute	Patients 6 months	Differences controls/acute	Differences controls/6 mon.	Differences acute/6 mon.
Fractional anisotropy	0.66 (0.04)	0.58 (0.03)	0.55 (0.05)	**	**	NS
Trace	2.21 (0.15)	2.20 (0.44)	2.63 (0.27)	NS	**	*

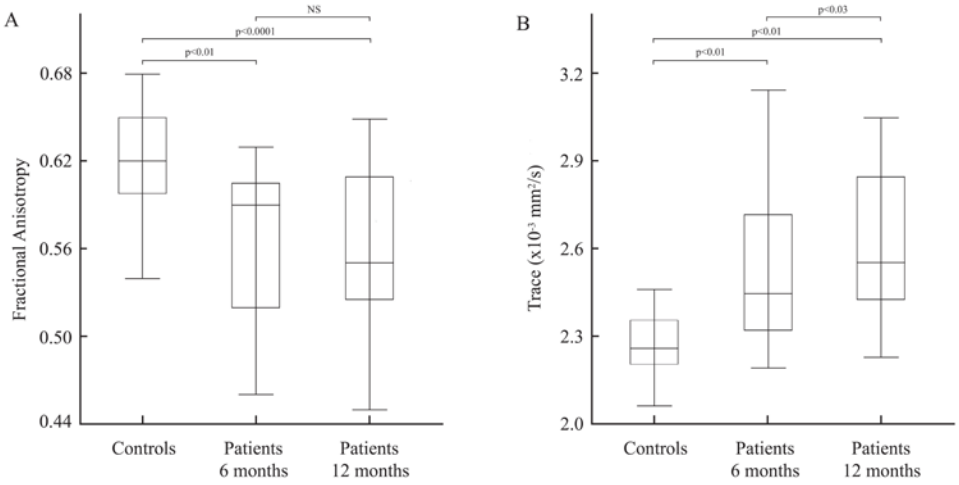
*Table 2. Results of DTI for controls and for patients in the acute phase and at 6 months postinjury. NS=no significance; *=significance (p <0.05); **=significance (p <0.01).*

Six months after the injury, one patient had 'good recovery' (GOSE 7-8), four patients had 'moderate disability' (GOSE 5-6) and three patients had 'severe disability' (GOSE 3-4). Seven of eight patients scored below the cut- off level (47) for BNIS, indicating cognitive dysfunction.

The methods of fiber tracking and placing regions of interest (ROI) before measuring the DTI-parameters of the corpus callosum was tested for interrater reliability and Inter-observer reliability. There were no significant differences ($p<0.01$) when limits of agreement were assessed for the measurements of the two observers for the parameters FA (ICC 0.99), trace (ICC 0.99), parallel diffusivity (ICC 0.98) and perpendicular diffusivity (ICC 0.97).

Study II.

FA decreased and trace increased at 6 and 12 months compared to controls. Trace continued to increase even further between 6 and 12 months, while FA remained unchanged (Figure 4 and Table 3). Patients with the worst outcomes had lower FA and higher trace compared to patients with better outcomes.



controls and the patients. Whisker bars represent range of data; top and bottom of boxes represent first and third quartile, respectively; midline through box represents median. NS = non-significant.

	Controls	Patients 6 months	Patients 12 months	Differences controls / 6 mon.	Differences controls / 12 mon.	Differences 6 mon. / 12 mon.
Fractional anisotropy	0.62 (0.04)	0.57 (0.06)	0.56 (0.06)	p<0.01	p<0.0001	p=0.66
Trace	2.28 (0.12)	2.53 (0.28)	2.62 (0.29)	p<0.01	p<0.01	p=0.03

Table 3. Results from DTI for controls and for patients at 6 and 12 months postinjury.

Study III.

The mean NFL serum concentrations among the patients displayed a 30-fold increase compared with controls, and NFL completely discriminated between patients and controls. We also found a relationship between serum NFL and MR-DTI parameters, with higher NFL concentrations in patients with higher trace ($R^2 = 0.79$) and lower fractional anisotropy (FA) ($R^2 = 0.83$).

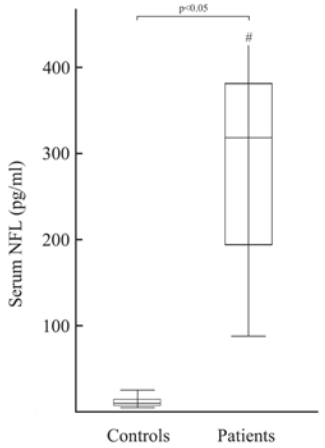


Figure 5. Diagram showing the levels of serum neurofilament light (NFL) for the control group and the patients. Whisker bars represent range of data; top and bottom of boxes represent first and third quartiles, respectively; midline through box represents median. # the whisker bar representing the maximum NFL level among the patients has been truncated (NFL for this patient was 852 pg/mL).

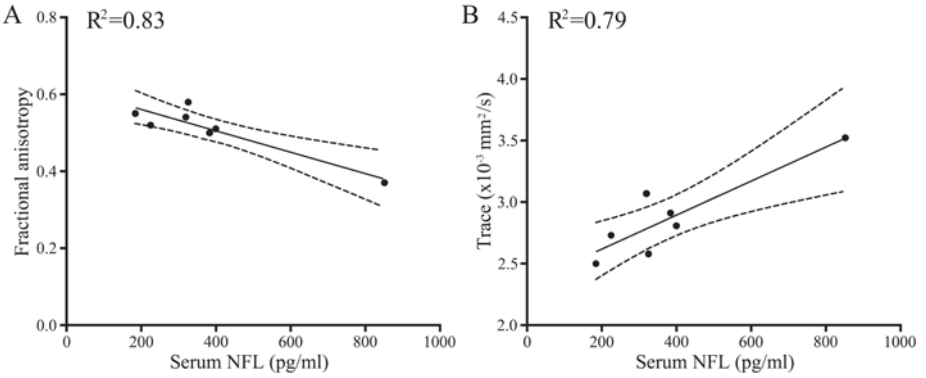


Figure 6. DTI parameters at 12 months plotted versus the acute serum levels of NFL. Dotted lines indicate 95% confidence interval. (A) fractional anisotropy vs NFL (R^2=0.83) and (B) trace vs NFL (R^2=0.79).

Study IV.

The MWT device was able to differentiate patients with cSDH from HC, yielding an AUC of 0.94, with a specificity of 75 % at 100 % sensitivity.

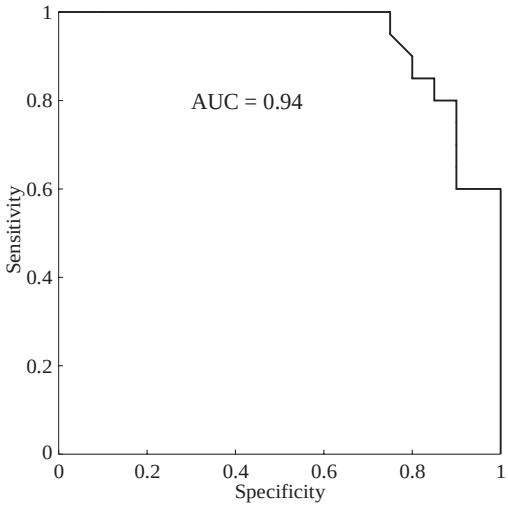


Figure 7. The receiver operating characteristic curve and area under the curve (AUC) value for the leave-one-out cross-validation procedure.

5 DISCUSSION

Three methods for detection and quantification of TBI were investigated in this thesis: MR-DTI in studies I-III; a serum marker (NFL) in study III; and a MWT device in study IV. The longitudinal MRI studies of DAI (studies I-II), contribute to a growing body of knowledge about the natural course of TBI and the possible underlying pathophysiological mechanisms, important for making accurate early prognoses. A conspicuous finding was that the diffusion tensor imaging parameters and the clinical outcome changed between 6 and 12 months after the injury, which implies that further studies are needed to determine if or when a stable state occurs. The new serum marker NFL (study III) was found to be a potential early biomarker for DAI, reflecting severity of the injury. The results from a new approach to detect intracranial hematomas using a MWT device showed promising results for use in the early triage of patients with head injury, and further studies are warranted to verify these results as well as to test the device for use in other applications in TBI.

5.1 Studies I-III.

5.1.1 Development of diffusion parameters

In study I, eight patients were examined, and FA in the corpus callosum was reduced in the acute phase in patients compared with the healthy controls. At six months, a significant reduction in FA was also noted compared with controls, but there was no significant further decrease between the acute phase and 6 months. In study II, including 15 patients, FA was reduced at 6 and 12 months compared with controls, but there was no significant decrease between the two follow-up examinations, and the interval between the acute phase and the follow-up examinations was not studied.

In study I, there was no significant change in trace between patients in the acute phase and controls. At 6 months, however, a significant increase in trace was found. In study II, trace was also higher at 6 months compared with controls, and trace continued to increase between 6 and 12 months after the injury.

While the diffusion parameters appear to be sensitive to DAI, they are not specific to reliably inform about different pathophysiological processes. However, the reduction in FA compared to controls, and the increase in trace that was observed at both 6 and 12 months and that continued to increase

further in the follow-up period, may be discussed in relation to other studies as it was done in study I. There, the results were compared to those form Concha and coworkers who studied patients before and after corpus callosotomy, a procedure that causes axonotomy and gives the opportunity to study the development of Wallerian degeneration in the tract connected to the induced lesion[56]. Concha found that anisotropy was reduced one week after surgery due to a reduction in parallel diffusivity (consistent with axonal fragmentation), whereas at 2-4 months, it was due to an increase in perpendicular diffusivity (consistent with myelin degradation). An increase in total diffusivity (trace) was also found 6 months after callosotomy[56]. The myelin degradation in the central nervous system (CNS) is a slow process, and according to Vargas and Barres, probably because oligodendrocytes cannot phagocytose myelin debris, and there is no influx of peripheral macrophages in the CNS to speed up the degradation process[57]. These findings provide a theoretical pathophysiological explanation to our finding that diffusivity (trace) continues to increase 12 months postinjury, and other longitudinal investigations have reported similar results[58-60].

5.1.2 Diffusion parameters and outcome

In study I, it was not possible to use the diffusion properties in the acute phase to predict clinical outcome at 6 months. However, the DTI characteristics at 6 months appeared to allow for differentiation between patients with worse vs. better outcomes using GOSE (c.f. study I, figure I, where patients with the worst outcomes are grouped in the upper left area of the diagram), and a similar pattern of DTI-parameter changes were observed when these patients were analyzed separately.

In study II, the aim was to investigate the changes in diffusion parameters between 6 and 12 months and to compare these findings with clinical outcome rather than prediction. Not surprising because of the findings in study I, patients with unfavorable outcome at 6 months (GOSE 3-5) had significantly lower mean FA and higher trace compared to patients with favorable outcome (GOSE 6-8). At 12 months, the difference in FA was not significant between the groups, but trace was significantly higher in patients with unfavorable outcome compared to patients with favorable outcome (cf. study II, figure 2, where patients with unfavorable outcomes are situated in the upper left corner of the graph). Neither the FA value nor the trace value could totally discriminate between patients with favorable and unfavorable outcomes, however, as there was an overlap between the groups. This overlap could be due to the limitation that the DTI was limited to the corpus callosum. If the brainstem was mainly affected, the patient would be likely to

have a poor outcome even though the diffusion changes in the corpus callosum were limited.

Outcome, as measured by GOSE is presented in table 1, and BNIS was presented in table 1 in studies I and II. Changes were found, both for GOSE and for BNIS, between the two follow-up investigations. A longitudinal study of the neuropsychological outcomes of a subset of the patients included in study II has shown that "recovery occurred in most cognitive functions for the majority during the first 6 months, but that there was then a reversion, which seemed to appear between 6–12 months, where cognition and reaction speed deteriorated in more than half the group."[61]. Björkdahl et.al. also studied a subgroup of the patients included in the present investigation, and found that the decline in cognitive function did not necessarily imply a corresponding decline in ability to perform activities[62].

Taken together, the changes in DTI-parameters and outcome between 6 and 12 months postinjury, imply that DAI should be considered a continuous process that probably reflects the structural changes of demyelinisation and Wallerian degeneration.

5.1.3 Diffusion parameters and NFL

In study III, serum NFL was tested as biomarker for DAI. The acute serum NFL concentrations were significantly higher for patients compared with controls, and we found a correlation between the increased NFL concentrations in the acute phase and the affected MR-DTI parameters 12 months postinjury. For the diffusion parameters, a significant reduction in FA in the corpus callosum was seen compared with the controls as well as a significant increase in trace. These findings were the same as in studies I and II and have been discussed in the previous section.

S100B concentrations were not significantly increased for the patients; however, blood samples were taken 4–9 days post-injury, and it is possible that S100B could have normalized.

5.1.4 Strengths studies I-III.

The method of studying the diffusion properties of the corpus callosum combining a ROI approach with fiber tracking seems to be stable, with very little inter-observer variation. The ROI technique also offers the possibility of a detailed analysis of diffusion properties in a defined white matter area or a

tract. Another technique of DTI, often used to assess white matter integrity after TBI, is whole-brain voxel-based analysis (VBA). The advantage of using VBA is that it is less operator-dependent and allows consideration of entire brain volumes[63]. This technique, however, is based on voxel-by-voxel comparison and requires normalization of the brain volumes to a common space of the brain. In patients with TBI, large structural abnormalities in the brain such as hydrocephalus, edema or decompressive craniectomy are common, seriously affecting the normalization required for VBA-based assessment. The ROI technique is not affected by such abnormalities.

5.1.5 Limitations studies I-III.

The combined ROI and fiber tracking approach allows for the possibility of a detailed analysis of diffusion properties in a defined white matter area or a tract. However, the analysis often needs time-consuming manual calculations, different hospitals use different methods for analysis, and the technique is mainly used in research and not in clinical routine. It also may be difficult to compare DTI parameters among research groups because of the different ROI methods used.

The technical limitations of the DTI approach are further discussed in study I, and include the risk of partial volume effect (i.e. including tissue outside the tract) and underestimating the extent of FA reduction by using FA both to define the ROIs and as the dependent measure. It is not thought that this has affected the results, and it has previously been shown that the described approach using tractographic ROI-based analysis for quantitative analysis of diffusion properties of the corpus callosum seems to be a stable method with very good inter-observer reliability[63]. The corpus callosum was chosen for investigation because it is prone to DAI, easy to examine with high inter-observer reliability, is associated with cognitive function, and often studied in patients with DAI [25,58,64-66]. The corpus callosum may also be affected by lesions in the white matter of the hemispheres. These lesions (usually classified as 'DAI grade I') may involve commissural tracts[52]. Both primary and secondary axonotomy in these lesions will lead to Wallerian degeneration, thus affecting the diffusion properties of the corpus callosum at the 6 and 12 month investigations. However, because only the corpus callosum was selected for examination, we realize that the full extent of DAI may have been under-estimated. For comparison, the Adams' DAI classification from the patients' first conventional MRI are presented in table 1. Four of the patients did not have signs of DAI according to Adams' but none of these made a full recovery which implies that conventional MRI is likely to underestimates the injury. However, because a formal comparison

between conventional MRI and MR-DTI was not performed, the studies do not prove that one is more sensitive than the other.

Although the studies tried to include only patients with pure DAI, the patients constitute a heterogeneous group of TBI ranging from severe-to-mild (GCS range = 3–15). Despite the lack of findings on the CT scans that could explain the patients' impaired consciousness and/or focal neurological symptoms, there was probably a mixture of different patterns of brain injury, for example, axonal disruption and intra- and extra-cellular edema. As demonstrated by the paper by Wilde and coworkers, even patients with very mild TBI (GCS 15 and no findings on the CT scan) can have a significant disturbance of the diffusion properties in the acute phase[67]. Consequently, the variation in diffusion properties in the acute phase was interpreted as being due to different types and degrees of edema as well as axonal injury. Hence, for studies II and III, the DTI-parameters in the acute phase were not considered for analysis. Moreover, for study III it is likely that the increase in NFL was caused by DAI, but it is also possible that axonal injury caused by hypoxic ischemic change and/or swelling might be reflected by the marker.

A common limitation to studies I-III was the small the sample size. Trends were noted of the changes in DTI parameters in studies I and II which might have reached statistical significance with larger sample size. The suspected heterogeneity of the patients and the limited sample size was due to the nature of the study, i.e. making a prospective study in a single institution on a patient group that is not very common. However, we found that the strengths of the methods outweigh the limitations and believe that our results deem verification in larger series.

5.2 Study IV.

5.2.1 Main findings study IV – MWT device

This is the first clinical study testing the validity of MWT in identifying a traumatic intracranial lesion, cSDH. At 100% sensitivity, the specificity was 75%, which implies that the technique could be valuable for clinical triage of patients with suspected TBI.
Improving early triage could reduce the time for patients with intracranial hematomas to come to surgery and thereby improve their outcome. In study IV, the results from the MTW device tested were discussed in relation to other devices (near-infrared spectroscopy[68] and electroencephalography[69]) and were found to be superior. The method also carries the potential to be used to monitor patients with conservatively managed hematomas, to monitor

patients postoperatively, and to monitor trauma patients in neuro-intensive care who are at risk of progressing contusions or extracerebral hematomas. Study IV is the very first clinical study on the device in trauma patients and the limitations and the implications for further research are discussed in section 5.2.3.

5.2.2 Strengths study IV

Study IV was conducted as a clinical test of a medical device. Study design and protocols were reviewed, and there was strict adherence to these. All measurements were made in a single institution and were carried out by only two examiners. (All but one of the measurements were performed by the first author and one measurement was performed under the supervision of the first author). All controls were measured by the first author. The study period was relatively short, and taken together, a complete data set was achieved. The processing of the data was carried out by authors who did not have access to the clinical information at the time of processing.

5.2.3 Limitations study IV

Study IV was the first clinical study of this MWT device in patients with TBI. Therefore, the particular limitations of the current study will be discussed first, and thereafter the implications for further research. The sample size was the main limiting factor because the diagnostic algorithm was derived from the same patient cohort as it was later used to evaluate. However, tests of robustness and bias were performed and indicated that the results would be applicable to a new patient cohort. It is likely, though, that the performance of the classifier would improve with a larger sample size. Candefjord and colleagues, performed tests on a phantom of subdural hematoma and numerical simulations, and demonstrated that the classifier requires a training data set size in the order of 100 patients and 100 control subjects to achieve high accuracy, because of high patient inter-variability of factors such as head size[70]. This indicates that a larger clinical study would allow for developing a diagnostic algorithm with capacity to detect all clinically significant hematomas, without causing a large number of false positives.

Another limitation to the current study was that the healthy controls were not subjected to imaging. CT could not be justified because of radiation and MRI was not feasible. Therefore, it was not possible to exclude intracranial pathology in the control group, although, there were no signs or symptoms of disease.

In study IV, the first proof-of-concept study of use of the MWT device in TBI, patients with cSDH were studied because this is a condition that usually does not require immediate surgery. In the emergency setting however, it is essential to find patients who require immediate surgery (i.e. those with acute intracranial hematomas). Although it is reasonable to believe that the results from this investigation would also apply to acute hematomas, this has not been studied, and such investigations are underway. As discussed in study IV, acute hematomas also have different composition and dielectric properties compared with chronic hematomas, and may be easier for the instrument to identify, but this remains to beshown.

Patients with known intracranial pathologies such as hydrocephalus or tumors were excluded in this investigation and so were also patients with foreign implants such as titanium screws, aneurysm clips or shunts. Before the MWT device can work properly in a clinical setting, these conditions must first be tested.

6 CONCLUSIONS

- Magnetic resonance diffusion tensor imaging (MR-DTI) is a method that allows for quantification of DAI; important for making accurate prognoses and prerequisites the evaluation of other diagnostic tools (e.g. the blood biomarker NFL) and potential therapies.

- The diffusion properties of the corpus callosum had not reached a stable level at 6 months after DAI, but continued to change at least until 12 months, probably reflecting an incessant microstructural alteration of the white matter.

- The blood biomarker serum neurofilament light (NFL) may be a valuable blood biomarker for TBI, reflecting the severity of DAI.

- A portable microwave technology (MWT) device could be used to identify patients with intracranial hematoma, which carries the potential to improve early triage and thereby save lives, but further studies are warranted to verify the results under various conditions.

7 FUTURE PERSPECTIVES

The global incidence of TBI is rising sharply, mainly because of an increase of motor-vehicle use in low-income and middle-income countries; and in high-income countries, it is the leading cause of death and disability among young individuals[71]. Increased efforts must therefore be taken to prevent these injuries and to optimize care and rehabilitation. Improvements in prehospital triage, by detection of intracranial hematomas at the scene of the trauma, as outlined by the MWT device tested in this thesis, carries the potential to reduce the time to surgery and to save lives and resources by selecting patients that require the resources of a neurotrauma centre. Hence, further research must be done to evaluate whether this diagnostic method can help patients in the acute phase after injury.

The complex pathophysiology of TBI continues to be an important field of research, and understanding this is prerequisite for the development of therapies[13,72]. One of the aims of this thesis was to improve the detection and quantification of DAI using a blood biomarker (NFL) and MR-DTI, and to investigate how the diffusion characteristics would evolve over time. It was recently pointed out by Smith and coworkers, who discussed possible therapeutic strategies in DAI, that "it is essential that neuroimaging techniques are further advanced and validated in anticipation of using them to non-invasively measure therapeutic efficacy in TBI treatment trials"[72]. Hence, continued efforts must be taken to further evaluate DTI- and other imaging techniques, especially beyond 12 months after injury, as well as to find methods that are sensitive and reliable, and allow for standardized imaging that can be implemented in the clinical routine.

ACKNOWLEDGEMENT

I would like to express my gratitude to everyone who has been involved and contributed to this thesis. Especially, I would like to thank:

Thomas Skoglund – my main supervisor, to whom I owe the greatest gratitude for all the time and effort that you have spent organizing and guiding me through this DAI research project. For helping me with any aspect of research, many aspects of neurosurgery, and quite a few other aspects of life in general as well.

Hans Silander – my co-supervisor, for introducing me to neurosurgery and for being the most sincere mentor one could ever ask for. For thoughtful advice and for always having the time and patience to show me what makes a good doctor.

Daniel Nilsson – my co-supervisor, for introducing and teaching me about DTI as well as for constructive discussions.

Daniel Stålhammar – my late supervisor, for introducing me to neurotrauma and for sharing your vast knowledge and genuine enthusiasm for research.

Bertil Rydenhag – for continuous support, encouragement and advice.

Maria Ljungberg, Ann Sörbo, Eva Esbjörnsson and **Catherine Eriksson-Ritzén,** co-authors of studies I and II, for collaboration and support.

Henrik Zetterberg, Kaj Blennow and **Marios Mitsis,** co-authors, of study III, for excellent collaboration, enthusiasm and e-mail replies within the hour.

Stefan Candefjord, Mikael Persson, Lars Jönsson, and **Mikael Elam,** co-authors of study IV, for the opportunity to work with this exciting microwave project. Stefan, for support, collaboration and joyful scientific discussions, and Mikael Elam, for enthusiasm and encouragement, and both of you, for taking care of the correspondence with the Medical Products Agency.

Lars Lindström, for all time spent teaching me about mathematical models.

Gudrun Barrows, for keeping track of all the data for study IV …and for keeping track of most other things at the department of neurosurgery as well.

All of my **colleagues and friends** at the Sahlgrenska University hospital, department of Neurosurgery, for encouragement, friendship and support.

All of my **colleagues and friends** at the Sahlgrenska University hospital, departments of Anesthesiology and Intensive Care and the Neurointensive Care Unit, for encouragement and friendship.

All **patients and subjects** for making these studies possible.

My wonderful family and most precious supporters, whose encouragement and help throughout the process made it all possible. And worthwhile!
My parents, **Eva** and **Lars**, and my brother **Martin**, for love, encouragement and support throughout the years.

My wife **Christina** and children **Astrid** and **August**, for love, encouragement, patience and understanding. I love you, and I promise,
I won't write another thesis!

This thesis and the included papers received financial support from the Health and Medical Care Committee of Västra Götaland, the Foundation for Medical Imaging in Memory of Erik Lysholm, the Swedish Research Council, the European Research Council, the Knut and Alice Wallenberg Foundation, the Torsten Söderberg Foundation, the Gothenburg medical faculty and the Chalmers Innovation Office.

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