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Traumatic axonal injury in traumatic brain injury

Conventional and advanced MRI from
early to chronic phase and relation to
outcome

Thesis for the degree of Philosophiae Doctor

Trondheim, September 2014

Norwegian University of Science and Technology
Faculty of Medicine
Department of Neuroscience



NTNU – Trondheim
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**”A difference is only a difference
if it makes a difference”**

NORSK SAMMENDRAG

Traumatisk aksonal skade ved hodeskade: konvensjonell og avansert MR fra tidlig til kronisk fase og relasjon til funksjon.

Ved St.Olavs Hospital har vi en pågående prospektiv studie hvor alle pasienter som innlegges med moderat og alvorlig traumatisk hjerneskade (TBI) fortløpende inkluderes. I dette ph.d.-prosjektet har vi studert traumatisk aksonal skade (TAI) med MR hos disse pasientene både med konvensjonelle og avanserte MR-teknikker, i tidlig fase og longitudinelt. Vi har relatert funnene til både global og nevropsykologisk funksjon etter skaden.

I de to første studiene studerte vi TAI-lesjoner longitudinelt hos 58 pasienter hvor konvensjonell MR ble utført innen 4 uker, samt ved 3 og 12 måneder. Antall og / eller volum av TAI-lesjoner i de ulike sekvensene ble estimert og diffusjonsanalyser ble utført i normalt utseende områder av hjernebjelken (corpus callosum). Både antall av lesjonene og diffusjonen ble fulgt longitudinelt over de tre undersøkelsene og funnene ble relatert til både global og nevropsykologisk funksjon.

I den tredje studien undersøkte vi et større utvalg på 128 pasienter med tidlig MR innen 4 uker, hvor vi så på betydningen av den totale mengden av TAI-lesjoner i hjernebjelke, hjernestamme og thalamus. Funnene ble relatert til global funksjon, og diffusjonsanalyser ble utført i normalt utseende områder av hjernebjelken på samtlige pasienter.

I den siste studien i avhandlingen valgte vi ut de områdene med TAI lesjoner fra MR bildene tatt i akutt fase og sammenlignet med tilsvarende områder i diffusjonstensor avbildning (diffusion tensor imaging, DTI) som ble tatt i kronisk fase. Fraksjonell anisotropi (FA)-verdiene, som gir et uttrykk for omfanget av den mikrostrukturelle skade, ble hentet ut fra disse TAI lesjons-områdene. Vi hentet også ut FA-verdiene i en frisk kontroll gruppe, samt av TBI-pasienter uten enten påviselige mikrohemoragiske eller ikke-hemoragiske TAI-lesjoner. Hos de to sistnevnte gruppene hentet vi ut FA-verdiene fra interesseområder (ROIs) hvor man ofte finner synlige lesjoner hos pasienter. På denne måten kunne vi sammenligne mikrostrukturen mellom disse gruppene, og relatere graden av diffusjonsendring i lesjonene til de kognitive testresultatene til pasientene.

Hovedfunnene i denne avhandlingen er:

- ✓ Ikke-hemoragiske TAI-lesjoner på konvensjonell MR forsvinner ofte i løpet av de første tre månedene etter TBI, særlig gjelder dette lesjoner i hjernestammen. Hemoragiske TAI-lesjoner reduseres først etter tre måneder. I multivariable analyser med justering for andre prognostiske faktorer, var det bare funn på tidlig MR som predikerte det kliniske utfallet. Således kan viktig klinisk informasjon gå tapt, dersom MR tas for sent i pasientforløpet.
- ✓ Ved bruk av MR diffusjon fant vi i løpet av første året etter moderat og alvorlig TBI en gradvis økning i diffusjon i hjernebjelken (apparent diffusion coefficient, ADC), noe som kan tyde på en gradvis ødeleggelse av mikrostrukturen også i normalt utseende områder. Forandringene var størst i bakre deler av hjernebjelken. De gjennomsnittlige ADC-verdiene var assosiert både med globalt utfall og evnen til å gjennomføre hurtige, kompliserte sensomotoriske oppgaver.
- ✓ Den totale byrden av synlige lesjoner i hjernebjelken, hjernestammen og thalamus på diffusjonsvektede MR bilder (diffusion weighted imaging, DWI) og fluid-attenuated inversion recovery (FLAIR) var uavhengige prognostiske faktorer ved alvorlig TBI. Ved moderat TBI var imidlertid CT funn og antallet kontusjoner på MR av større betydning enn TAI-lesjoner med tanke på prognose.
- ✓ Vi fant en signifikant lineært økende ødeleggelse av mikrostrukturen i hvit substans etter TBI. Det var en gradvis fallende gjennomsnitts FA-verdi fra ROIs i hvit substans i friske kontroller til ROIs hos TBI pasienter enten uten mikrohemoragiske eller ikke-hemoragiske TAI-lesjoner og videre til lesjoner som forsvinner. Laveste FA-verdier ble funnet i ROIs i lesjoner som persisterer.

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Ovennevnte avhandling er funnet verdig for offentlig forsvar for graden Doctor Philosophiae i klinisk medisin. Disputas vil finne sted i audiotriet på MTFs, NTNU Fredag 26.09.14 kl 12.15.

ENGLISH SUMMARY

Traumatic axonal injury in traumatic brain injury: Conventional and advanced MRI from early to chronic phase and relation to outcome

At St. Olav University Hospital we have an ongoing prospective study of all patients admitted with moderate and severe traumatic brain injury (TBI) consecutively included. In this PhD project, we have studied traumatic axonal injury (TAI) with MRI in these patients using both conventional and advanced MRI techniques in early stages and longitudinally. We have related the findings to both global outcome and cognitive outcome after the injury.

In the first two studies, we studied TAI lesions longitudinally in 58 TBI patients for whom conventional MRI was obtained within four weeks, at 3 and at 12 months. The number and / or volume of TAI lesions in the different sequences were estimated and we performed analyzes of diffusion in the normal appearing corpus callosum. Both lesions and diffusion were followed longitudinally across the three acquisitions and findings were related to both global and neuropsychological outcomes.

In the third study, we examined a larger sample of 128 patients with early MRI within 4 weeks and we specifically wanted to study the importance of the total lesion load of TAI lesions in the corpus callosum, brain stem and thalamus. The findings were related to global outcomes and we also performed analyzes of the diffusion in the normal appearing corpus callosum for all patients.

In the last study of the PhD thesis, the areas with TAI lesions from the early MRI were compared to the corresponding areas in the diffusion tensor imaging (DTI) obtained in the chronic phase. Fractional anisotropy (FA) values, indicating the degree of microstructural damage, were extracted from these TAI lesion areas. We also estimated the FA values from DTI in the healthy control group and in TBI patients without either non-hemorrhagic lesions (NHLs) or microhemorrhagic lesions (MHLs). In the latter two groups, we extracted the FA values from regions of interest (ROIs) where we often find visible lesions in TBI patients. In this way we could compare the microstructure across the groups and relate the findings to cognitive outcome.

The main findings of this thesis are:

- ✓ NHLs in conventional MRI attenuate during the first 3 months after TBI; most importantly we found that brain stem lesions disappear. MHLs attenuate first after 3 months. Only early MRI findings predicted clinical outcome after adjustment for other prognostic factors. Hence, valuable clinical information may be missed if MRI is performed too late after TBI.
- ✓ During the first year after moderate and severe TBI we demonstrated a slowly evolving disruption of the microstructure in normal appearing corpus callosum by analyses of the diffusion in the apparent diffusion coefficient (ADC) map, most evident in the posterior truncus. The mean ADC values were associated with both global outcome and ability to perform speeded, complex sensory-motor action.
- ✓ The total load of visible TAI lesions in corpus callosum, brain stem and thalamus in diffusion weighted imaging (DWI) and fluid-attenuated inversion recovery (FLAIR) sequences were independent prognostic factors in severe TBI. Interestingly, CT findings and number of cortical contusions in MRI seemed to be more important for patients with moderate TBI.
- ✓ We found a significant linear decline in the integrity of white matter microstructure following TBI with gradually decreased mean FA values from ROIs in white matter in healthy controls to ROIs in TBI patients without either NHLs or MHLs and further to transient lesions. Lowest mean FA was found in persistent lesions.

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NTNU

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LIST OF PUBLICATIONS

PAPER I

A longitudinal MRI study of traumatic axonal injury in patients with moderate and severe traumatic brain injury.

Kent Gøran Moen, Toril Skandsen, Mari Folvik, Veronika Brezova, Kjell Arne Kvistad, Jana Rydland, Geoffry T. Manley, Anne Vik

J Neurol Neurosurg Psychiatry 2012. Dec;83(12):1193-200. doi: 10.1136/jnnp-2012-302644.

PAPER II

A longitudinal magnetic resonance imaging study of the apparent diffusion coefficient values in corpus callosum during the first year after traumatic brain injury.

Kent Gøran Moen, Asta Kristine Håberg, Toril Skandsen, Torun Gangaune Finnanger, Anne Vik

J Neurotrauma 2014. Jan 1;31(1):56-63. doi: 10.1089/neu.2013.3000.

PAPER III

Traumatic axonal injury: the prognostic value of lesion load in corpus callosum, brain stem, and thalamus in different magnetic resonance imaging sequences.

Kent Gøran Moen, Veronika Brezova, Toril Skandsen, Asta Kristine Håberg, Mari Folvik, Anne Vik

J Neurotrauma. 2014 Apr 28. [Epub ahead of print]

PAPER IV

Traumatic axonal injury in moderate and severe head injury: relationship between conventional MRI findings in the early phase and diffusion tensor imaging findings in the chronic phase.

Kent Gøran Moen, Anne Vik, Alexander Olsen, Toril Skandsen, Asta Kristine Håberg, Kari Anne I. Evensen, Live Eikenes

Neuroimage: Clinical. 1st revision submitted 13.05.14

ABBREVIATIONS

TBI	Traumatic brain injury
MRI	Magnetic resonance imaging
TAI	Traumatic axonal injury
DAI	Diffuse axonal injury
GCS	Glasgow coma scale
HISS	Head injury severity scale
LOC	Loss of consciousness
CT	Computed tomography
AIS	Abbreviated injury scale
ISS	Injury severity score
SE	Spin echo
FLAIR	Fluid-attenuated inversion recovery
T2*GRE	T2* gradient echo
SWI	Susceptibility weighted imaging
DWI	Diffusion weighted imaging
NHL	Non-hemorrhagic lesion
MHL	Microhemorrhagic lesion
ADC	Apparent diffusion coefficient
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
MD	Mean diffusivity
ROI	Region of interest
GOS	Glasgow outcome scale
GOSE	Glasgow outcome scale extended
IMPACT	International mission for prognosis and clinical trial
CRASH	Corticosteroid randomization after significant head injury
TMT	Trail making test
SDMT	Symbol digit modality test
D-KEFS	Delis kaplan executive function system
CWIT	Color-word interference test
GP	Grooved pegboard

1 GENERAL INTRODUCTION

1.1 Incidence and mortality

Traumatic brain injury (TBI) is not a disease, but a brain injury caused by an event or a sequence of events that can, in some instances, lead to a diagnosable neurological or neuropsychiatric disorder [1]. The consequences of TBI due to the loss of work years, medical care and rehabilitation have been estimated to cost the U.S \$76.5 billions annually [2]. Due to the tremendous costs and the fact that TBI is caused by an event, prevention has been stated to be the most important action from a socio-economic point of view. The incidence of TBI is known to differ between different countries and in a study from Western Europe including the Nordic countries, incidence rate of fatal and hospitalized TBI was 235 per 100 000 [3]. The mortality rates varies considerably between different countries from 9.4 to 24.4 in the EU, with an average of 15 per 100 000 [3]. A later study by Sundström et al. (2007) found a sizeable mortality reduction in the time period from 1987 – 2001 among all the Nordic countries except for Finland [4].

1.2 Definitions and classifications of traumatic brain injury

Since head injury refers to injury to the head, without necessarily involvement of the brain, we will throughout this thesis use the notion TBI. TBI is defined as an alteration in brain function, or other evidence of brain pathology, caused by an external force [5]. There exist various classification systems of TBI, but most of them are based on the patient's level of consciousness assessed by e.g. the Glasgow Coma Scale (GCS). The description and introduction of the GCS from Teasdale and Jennet, has been described as one of the most important neurosurgical findings [6]. The scale is based upon the response to stimuli regarding motor response, verbal performance and eye opening, and ranges from 3 (no response) to 15 (fully alert and oriented).

From a pathophysiological point of view, the reversible and permanent tissue injury following TBI will be displayed in a continuous specter, rather than a categorical one. Clinically it is advantageous to divide the patients with TBI into different categories based on the severity of the injury. The Head Injury Severity Scale (HISS) classification is the most widely used and is based on the post-resuscitation GCS score and the presence of different symptoms or signs [7].

The scale defines mild TBI as GCS score 14-15, plus amnesia or brief loss of consciousness (LOC) < 5 minutes or impaired alertness or memory. Patients with moderate TBI are defined with either GCS score 9-13 or focal neurological deficit or LOC \geq 5 minutes. Rather than subdividing “serious” head injury into “severe” (GCS score 5-8) and “critical” (GCS score 3-4) TBI, we used the term “severe” TBI for all patients with GCS score 3-8. In this thesis only patients with moderate and severe TBI were examined.

1.3 Definition and neuropathology of traumatic axonal injury (TAI)

The traumatic axonal injury (TAI) or diffuse axonal injury (DAI) is considered to be a very important component in all severities of TBI [8, 9]. It is histopathologically characterized by axonal swelling and, dependent on the severity, a subsequent axotomy [10]. The process was already described in 1956 by Strich et al., where five patients without increased intracranial pressure still had a fatal outcome [11]. Pathological, a diffuse degeneration of the white matter in the hemispheres was found, characterized as a “shearing injury” or primary / mechanic axotomy. The notion DAI was first introduced in 1982 by Adams et al. after they had performed autopsy and microscopic examination of patients with TBI [12, 13]. They also proposed a method for staging the severity of the TAI lesions (as described in section 1.6 Conventional MRI).

During the last two decades, after the papers from Povlishocks` group, it has been proposed that the axonal injury following TBI mostly is not characterized by a primary axotomy. Electron microscopic studies revealed that rather the injury was initiated by a biochemical cascade that led to a subsequent swelling of the axon that was either reversible or resulted in a secondary axotomy if the injury was severe enough (Fig.1.3.1.) [14, 15]. Much of the details behind the cellular biology are still unknown, but proteolytic enzymes like calpains or caspases that disturb the axonal transport are believed to be involved in the process [16]. Even though neuroimaging techniques are evolving and have improved the sensitivity of the “in vivo” diagnostics of TAI, the definite diagnosis is still only made at autopsy by immunostaining for β amyloid precursor protein (β -APP) [17].

The axonal swelling in the acute phase following a TBI, demonstrated by Singleton et al. [15], has also been found in neuroimaging studies since there is a reduction in diffusion properties, most prominent after one week [18]. In addition to this cytotoxic edema, there is also a varying degree of vasogenic edema [19]. In the subacute to the more chronic stages, the edema gradually resolves, and the demyelination assumes and may continue for at least 1-2 years post-injury [20]. This gives rise to increased diffusion, and together with the demyelination there is also an axonal loss to some degree [21], which facilitate the increased diffusion. A few longitudinal DTI studies exist with a limited number of TBI patients. These papers have evaluated the diffusion properties from the early phase and longitudinally in white matter without visible lesions [22-24]. However, there exists no DWI study even though DWI is easily accessible and routinely implemented in all modern MRI protocols of TBI patients.

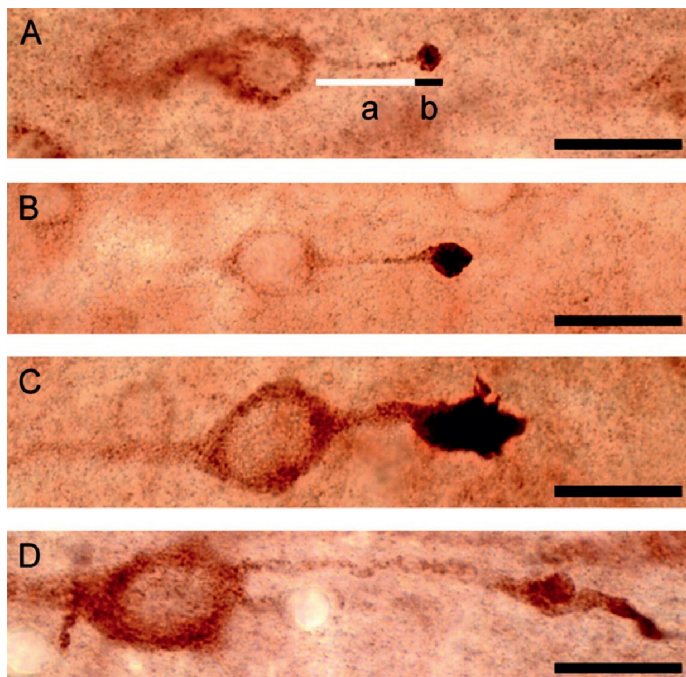


Figure 1.3.1

Electron microscopic picture of axonal swelling to animals surviving a TAI mechanism. After surviving 30 min (A), 4 hours (B), 12 hours (C), and 48 hours (D) after injury. Representative progressive changes in the length of the unswollen portion of the axon (indicated in A by white line a), length of swollen portion of the axon (indicated in A by black line b), and total axonal length (sum of a and b in A) are illustrated. Reprinted with permission from Singleton et al. (2002) [15] and *J. Neurosci.*

Even though Adams et al. introduced the DAI notion in the eighties, it is now also common to use the term TAI. The term is defined differently in the clinical, radiological or pathological arenas, and the use of the terms have therefore been confusing. With the purpose of clarifying the use of the two different terms, the Common Data Elements Neuroimaging Working Group in 2010 defined DAI as including more than three separate foci of signal abnormality,

while TAI was defined as 1-3 foci of signal abnormality [25]. In practice the two terms have been used interchangeably, but the use of the TAI term is increasingly used since it denotes the mechanism behind the injury (a traumatic mechanism). The DAI term is also to some degree misleading since the axonal injuries are found in predilection sites and are not as diffuse as initially assumed [20].

1.4 Injury severity variables and secondary events

The degree of injury could be assessed both with regard to the TBI in itself, but also the extracranial injuries should be assessed since they can influence the patient outcome [26]. The classification of the TBI has already been mentioned with the GCS and the HISS classifications. The abbreviated injury scale (AIS) values could also be used to classify the injury to the head [27]. The AIS score is one of the most common anatomic scales evaluating the severity of specific individual injuries in general traumatology, and is based on the score in 6 different body regions including the AIS – head. In the Injury Severity Score (ISS), the three AIS severity codes in each of the three most severely injured body regions, are squared and then added together to get the ISS value ($A^2+B^2+C^2$) [28].

A secondary event is also an important injury related variable. In the studies of this thesis, it was defined as having suffered either one or more episode(s) with hypoxia and / or hypotension in the time before or at admission to the study hospital. Moreover we also know, that such events can occur after admission to the hospital [29]. Both hypoxia and hypotension have severe impact on the prognosis following TBI [30].

1.5 Computed tomography in traumatic brain injury

In the acute phase of moderate and severe TBI, CT is the most important neuroimaging modality, since it can be performed quickly and it corroborates or rules out the need of urgent neurosurgery [31]. The prognostic value of CT has been shown in many studies [32, 33]. The International Mission for Prognosis and Clinical Trial (IMPACT) study also advocated that both the CT classification status and individual CT characteristics are important predictors for outcome in patients with TBI [34].

One of the most widely used CT classifications so far, is the Marshall classification, which is based on the Trauma Coma Data Bank [35]. However, the proposed Rotterdam classification is increasingly used in the prognostication of patients with moderate and severe TBI (Fig. 1.5.1) [36]. Both of these two classifications are based upon many of the similar CT features indicating increased intracranial pressure (ICP) like size of mass lesions, midline shift and degree of compression of basal cisterns. However, the Rotterdam classification was developed to increase the prognostic value of the CT findings, by adding the presence of traumatic subarachnoid or intraventricular hemorrhage as a negative prognostic sign. If an epidural hematoma is treated with urgent craniotomy and evacuation of hematoma, this type of bleeding is associated with a favorable prognosis and therefore it is considered as a positive prognostic sign in the Rotterdam score. In research purposes it is recommended to use the worst CT scan in the prognostic models [37].

Figure 1.5.1 Rotterdam CT score (Maas et. al 2005)

Rotterdam CT score – prognostic score chart for moderate and severe TBI	
Predictor value	Score
<i>Basal cisterns</i>	
Normal	0
Compressed	1
Absent	2
<i>Midline shift</i>	
No shift or shift ≤ 5 mm	0
Shift > 5 mm	1
<i>Epidural mass lesion</i>	
Present	0
Absent	1
<i>Intraventricular blood or tSAH</i>	
Absent	0
Present	1
<i>Sum score*</i>	+1

CT, computer tomography; TBI, traumatic brain injury;
tSAH, traumatic subarachnoid hemorrhage

* To the sum score plus 1 is added to make the grading numerically consistent with the grading of the motor score of the GCS and with the Marshall CT classification.

1.6 Conventional Magnetic Resonance Imaging in traumatic brain injury

Even though CT is important in the acute phase, it is important to know that the sensitivity of CT is limited when it comes to TAI diagnostics, since only the “tip of the iceberg” of lesions is detected (Fig. 1.6.1). Different magnetic resonance imaging (MRI) sequences are superior in detecting TAI [38], which will be further discussed in this and the following section.

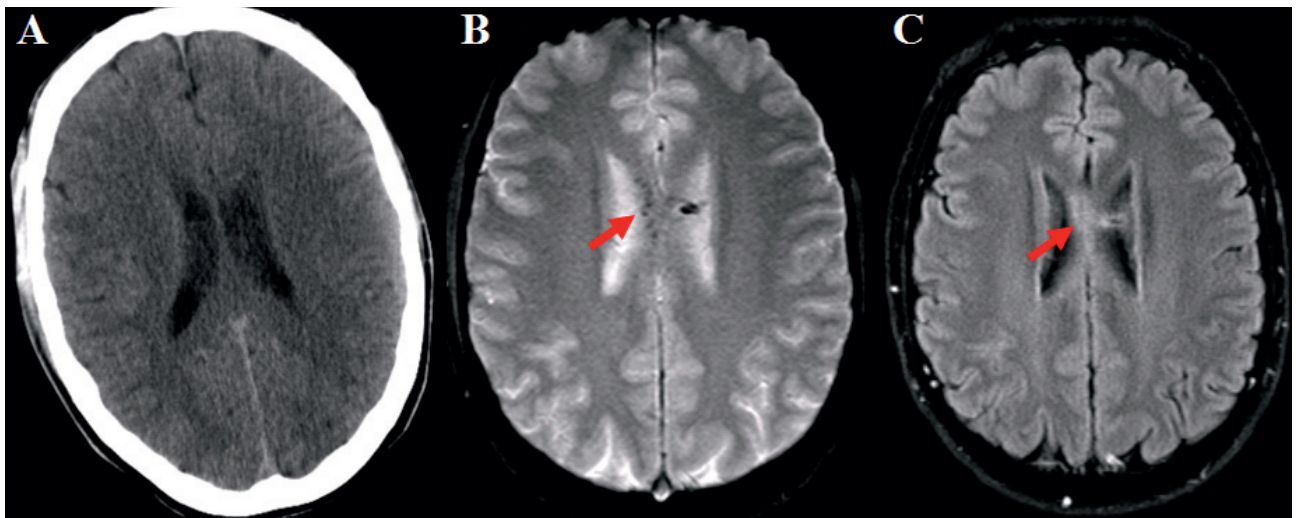


Figure 1.6.1

This 44-year old male was involved in a road traffic accident and was admitted to hospital with a GCS score of 9. The admission CT (A) did not reveal any parenchymal injuries to the brain, but the conventional 1.5 T MRI showed multiple microhemorrhages in the corpus callosum in the T2*GRE image (B) and a confluent lesion in the corresponding area in the FLAIR image (C).

MRI was already used sporadically in TBI in the eighties, but a study by Jenkins et al (1986) has been referred to as the first MRI study of TBI patients [39]. A later MRI study characterized the distribution of the TAI lesions based on the pathological appearance [12], which was later used as a basis for the modified staging into three TAI stages [40] that are shown in detail in Fig. 1.6.2.

Figure 1.6.2 Traumatic axonal injury (TAI) classification [40]

Traumatic axonal injury (TAI) classification in conventional MRI	
Stage 1	Lesions in the hemispheres White matter hyperintensities or micro hemorrhages in the hemispheres. Includes lesions in internal capsula, external capsula and cerebellum
Stage 2	Lesions in corpus callosum Most common in posterior truncus and splenium
Stage 3	Lesions in the brain stem Most common in dorsolateral aspects of midbrain and upper pons Includes lesions in the cerebellar peduncles

1.6.1. The MRI protocol for traumatic brain injury patients

The typical MRI protocol for TBI patients is performed without contrast on a 1.5 T MRI scanner. In addition to the T1 and T2 sequences, the protocol consists of a T2* weighted gradient echo (T2*GRE) sequence, a fluid-attenuated inversion recovery (FLAIR) sequence and diffusion-weighted imaging (DWI) including an apparent diffusion coefficient (ADC) map.

T2-weighted gradient echo (T2*GRE) sequence*

The T2*GRE sequence was first used in 1986 [41] and is essential for the detection of microhemorrhagic lesions (MHLs) in white matter. The technique utilizes the paramagnetic properties of the degradation products of hemoglobin, which appears as small hypointense foci in the T2*GRE sequence [42]. The size of these foci will be strongly influenced by properties of the imaging sequence and the MRI system, particularly the magnetic field strength [43]. If the T2*GRE image is missing, the b0 image from the diffusion sequences (see below) can also be used to detect MHLs. However this sequence is inferior compared to the T2*GRE sequence [44].

The prognostic value of MHLs is still unclear, since some studies have shown a relationship between number of lesions and either severity of the TBI [45] or directly outcome [46], while others have not found such a relationship [47, 48].

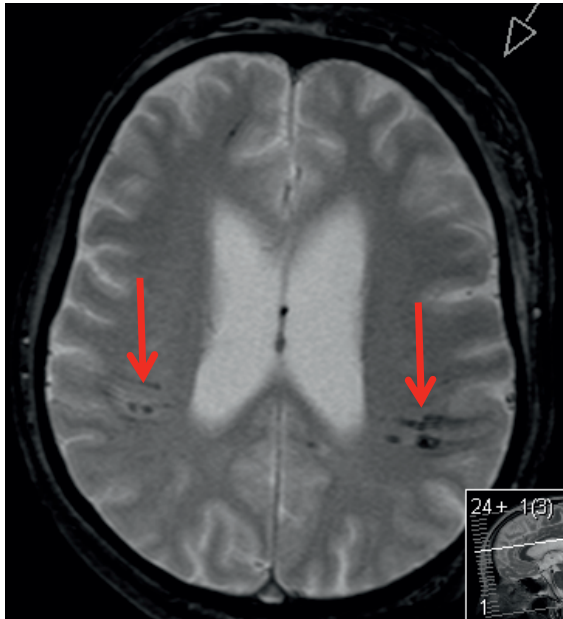


Figure 1.6.3

This is a 1.5 T transversal T2*GRE image of a 55 year old female patient who suffered a road traffic accident and was admitted to the hospital with GCS score 13. The T2*GRE image showed multiple MHLs in the parietal lobes bilateral (shown with red arrows). The MHLs clearly indicate the lateral direction of the forces involved in this trauma.

Fluid-attenuated inversion recovery (FLAIR) sequence

The FLAIR sequence was first introduced in 1992 [49] and is a T2 weighted spin echo (SE) sequence where the high signal from the cerebrospinal fluid (CSF) is suppressed by use of a long inversion time. Thus, lesions adjacent to the CSF will have remarkable improved image quality, making them easier to detect [50]. White matter hyperintensities or non-hemorrhagic lesions (NHLs) depicted in the FLAIR sequence following TBI represent edema in the acute stage and encephalomalacia or gliosis due to scarring in the chronic stage [31].

In contrast to the diverging studies on MHLs, earlier studies on volume of NHLs have demonstrated a correlation to clinical outcome in both the early and chronic phase [51, 52]. However these studies did not fit predictive models with adjustment for other important prognostic factors. Also the time from injury until the MRI scanning is performed will probably influence the prognostic value of the NHLs. There is a clinical experience that NHLs attenuate over time, but no good quality studies have examined this issue systematically. A Japanese study examined TAI lesions in FLAIR sequences in 29 mild-moderate TBI patients and subjectively concluded that the NHLs tended to be absent within 3 months without doing any statistical analysis [53].

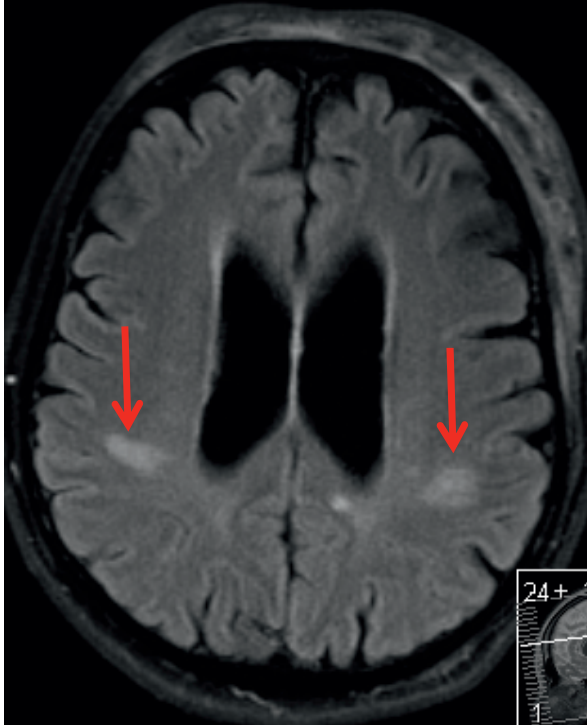


Figure 1.6.4

This is a FLAIR image of the same patient as mentioned in Fig. 1.6.3. NHLs were found on both sides of the parietal lobes (shown with red arrows) corresponding to the MHLs detected in Fig. 1.6.3.

Diffusion weighted imaging (DWI) sequence

The diffusion weighted imaging (DWI) sequence was first used in TBI patients in 1999 [54] and is a T2-weighted SE sequence where diffusion gradients are applied before and after the 180 degree pulse [55]. The b-value indicates the magnitude of the diffusion weighting provided by the diffusion gradients and is the basis for computing the ADC map. In standard MRI protocols two sets of diffusion images are acquired; the first with b0 (i.e. without diffusion gradients applied) and the second with higher b-value (usually b1000). The ADC map is automatically computed from these two images. The higher b-value scan is acquired in three directions and the diffusion changes along all the three axes are then averaged to get the “trace” diffusion image, where the anisotropic white matter tract effects are averaged out. The signal intensity in the DWI scan does not only depend on the ADC value, but also the T2 signals which could cause a “T2 shine through” effect. The ADC map must therefore be used simultaneously with the DWI scan to determine if there are reduced or increased diffusion (Fig. 1.6.5).

In research there exist different approaches to evaluate the diffusion sequences and their prognostic value. The sensitivity of detecting TAI lesions with the DWI scan and the ADC map has in earlier studies shown to be adequate compared with the other conventional sequences [44,

56]. A study by Schaefer et al. (2004) found a strong correlation between number of DWI lesions and modified Rankin Scale score [46]. Otherwise there are no studies examining the prognostic value of lesion load of visible DWI lesions.

Another common approach in research is a region of interest (ROI) approach, where the diffusion is quantitatively measured in lesions or various parts of normal appearing brain tissue. Corpus callosum is one of the most ideal white matter tracts for performing such measurements of the diffusion, since it is the largest tract in the brain. It is also the most defined tract and because of its immobility it remains one of the most important predilection sites of TAI [20, 38]. These factors together make corpus callosum suitable for TAI studies.

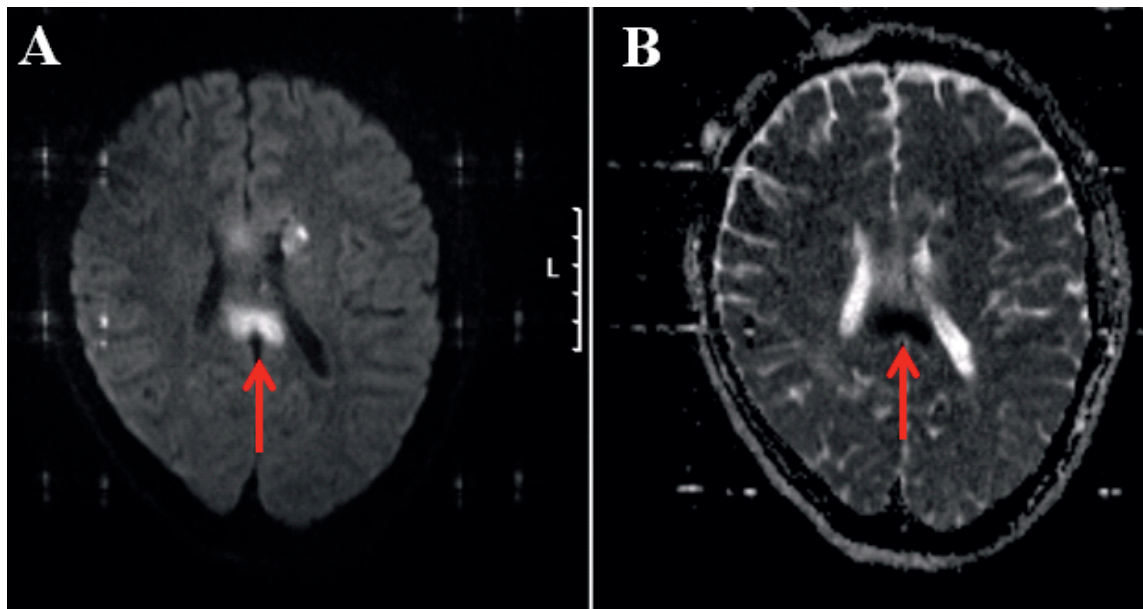
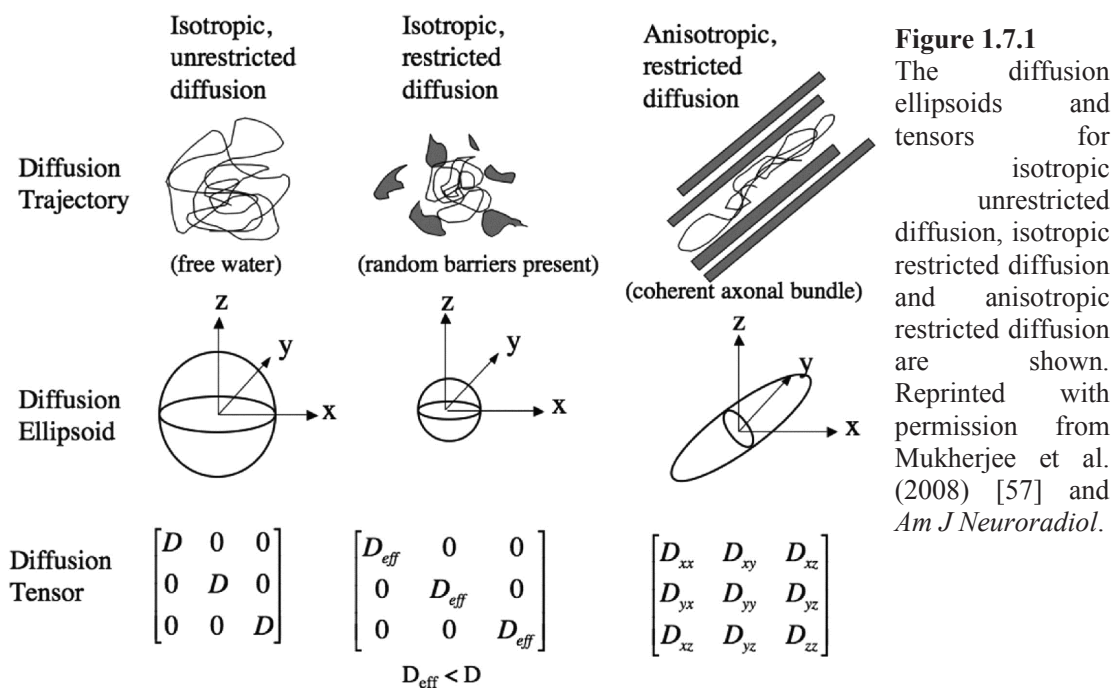


Figure 1.6.5

This is the 1.5 T transversal DWI (A) and ADC map (B) of a 29 year old male who suffered a severe fall injury and was admitted to the hospital with a post-resuscitation GCS score of 3. The MRI was obtained 4 days post-injury and in the DWI sequence a hyperintense non-hemorrhagic lesion (red arrow) was detected in the splenium part of the corpus callosum. In the ADC map a corresponding hypointense lesion was found (red arrow), indicating reduced diffusion (cytotoxic edema).

1.7 Diffusion Tensor Imaging (DTI) in traumatic brain injury

In clinical DWI the anisotropy of the white matter tracts disturbs the interpretation, and as described earlier, a trace image is therefore computed. However, the anisotropic diffusion could be utilized to get a quantitative characterization and also an anatomic mapping of the white matter tracts. This is utilized in diffusion tensor imaging (DTI) where images are acquired in multiple directions (at least six, but due to image noise often 20 – 60 directions are used), instead of three directions as in the conventional DWI [57]. The diffusion tensor, a 3x3 matrix of vectors, is a mathematical model of the 3D pattern of diffusion anisotropy of white matter tracts which is easiest to understand when viewed geometrically (Fig. 1.7.1).



For isotropic diffusion, the diffusion ellipsoid is a sphere, because the ADC in every direction is equal. Anisotropic diffusion is modeled with an elongated ellipsoid. Every voxel in the image is described by the ellipsoid, which is characterized by its three eigenvalues and their eigenvectors, which defines the length and direction of the axes, respectively. Thus, the largest eigenvector (primary eigenvector) and its associated axial eigenvalue (λ_1) indicate the direction and magnitude of the greatest water diffusion. The second and third eigenvectors are orthogonal

to the primary eigenvector and their associated eigenvalues (λ_2 and λ_3) and give the magnitude of the diffusion in the transverse plane of the axonal bundles. The mean of λ_2 and λ_3 is also known as the radial diffusivity [57].

The mean diffusivity (MD) is the mean of the three eigenvalues ($(\lambda_1+\lambda_2+\lambda_3)/3$) and describes the averaged diffusivity of water within a voxel and is mathematically equivalent to the ADC value [58]. The fractional anisotropy (FA) describes the degree of directionality of the diffusion and is derived by algebraic manipulation of the eigenvalues [59]. FA ranges from 0, i.e. isotropic diffusion corresponding to the situation in cerebrospinal fluid, to 1, i.e. maximal anisotropic diffusion, which corresponds to diffusion along one axis with full restriction in the other directions [57, 60].

DTI has demonstrated more extensive damage to the white matter than what could be revealed by conventional MRI in TBI patients [9, 56]. FA is considered a more sensitive biomarker than other DTI metrics such as MD [61]. Most previous DTI studies have found decreased FA in several white matter areas in the chronic stage following all severities of TBI [9, 22, 62, 63]. However, the lesion areas from the acute phase have never been examined by using DTI in the chronic phase. The reductions in FA are thought to be caused either by a reduction in the axial eigenvalue (representing axotomy and/or a disorganization of the axons) or by an increase of the radial diffusion (that could be caused by post traumatic edema) [59].

1.8 Outcome prediction and statistical models in TBI research

Since TBI is a leading cause of disability and death, especially among young adults, the clinicians often meet the question of outcome from the relatives already in the acute stage after the injury [64]. It is impossible to summarize the current knowledge in outcome among TBI patients, mostly due to difficulties comparing studies due to differences in patient selection, outcome measures, follow-up time, loss to follow-up and methodological weaknesses [65]. Already in a consensus conference in 1998 it was stated that mild, moderate and severe TBI differ greatly in symptoms, signs, recovery and outcomes, and that each category should to be studied specifically [66]. A review some years later (2006) concluded that "the natural history of mild, moderate and severe TBI had still not been adequately investigated" [67].

1.8.1 Global outcome

A range of different tools are used to assess outcome after TBI, but the most important ones are the Glasgow outcome scale (GOS) [68] and the Glasgow outcome scale extended (GOSE) classifications (Fig 1.8.1) [69]. Other alternatives for assessment of the global outcome are the Disability Rating Scale (DRS) and the modified Rankin Scale (mRS) [70, 71]. Mostly the evaluation of outcomes has been performed during the first year after the injury, since most of the improvement takes place during this period [72]. During the last years the IMPACT study group has published many interesting papers on different outcome aspects in moderate and severe TBI, but with analyses of the injury severity groups together [73].

Figure 1.8.1 Global outcome assessment scales

The Glasgow Outcome Scale (GOS) and the Glasgow Outcome Scale Extended (GOSE)			
GOS	GOSE	Description	Interpretation
1	1	Death	
2	2	Vegetative State	
3		Severe Disability	Conscious, but dependent on personal assistance.
	3	Lower Severe Disability	Dependent on personal assistance more often than every eight hour.
	4	Upper Severe Disability	Partial independence in ADLs, and the patient can be left alone for more than 8h at home.
4		Moderate Disability	Independent, but disabled
	5	Lower Moderate Disability	Not able to resume previous activities (work/social)
	6	Upper Moderate Disability	Post-traumatic signs present, but resumption of most former activities either full-time or part-time
5		Good Recovery	The person is able to resume all activities, including work, at the same level as before the injury although minor physical or mental deficits or complaints may be present.
	7	Lower Good Recovery	Minor physical or mental deficits or complaints that affect daily life.
	8	Upper Good Recovery	Full recovery without symptoms or signs or minor physical or mental deficits or complaints that do not affect daily life.

Among patients with severe TBI, older age is a strong predictor of poor outcome in most TBI studies [26, 74, 75]. In both the IMPACT [75] and the Corticosteroid Randomisation After

Significant Head Injury (CRASH) [26] studies, the GCS score and the pupil reactivity were also found to be strong predictors. In the CRASH study extracranial injury was a significant predictor, while in the IMPACT study the CT findings were important for outcome prediction. Also conventional MRI findings (both lesion location and lesion type) have shown to be important predictive variables [51, 52, 76], but there exist no studies where the conventional MRI is performed in the early phase where multivariable analyses with adjustment for other important prognostic factors also are performed.

Outcomes after moderate TBI are more uncertain than after severe TBI, since the predictive value of different variables to global outcome measures as GOS or GOSE scores have been difficult to find. This is mostly because there are few studies on only moderate TBI patients. Older age, lower GCS scores and CT findings is for certain associated with poorer outcomes [77-79]. Interestingly, the predictive value of TAI lesions detected in conventional MRI sequences has so far never been examined in a group of only patients with moderate TBI.

1.8.2. Cognitive outcome

Patients that have suffered a moderate or severe TBI often show impairments across a range of cognitive abilities including executive functions, processing speed, memory and attention [80-82]. These disabilities may be subtle and not always well understood by relatives or employers. Many of the studies evaluating the neurocognitive function post-injury, have not differentiated between moderate and severe TBI despite the fact that patients with TBI comprise a heterogeneous group [83]. Therefore, these studies may have overestimated impairments for moderate TBI and underestimated them for severe TBI. In an earlier study from our research group 43 % of the patients with moderate TBI had cognitive impairment according to norms on nine selected sensitive tests, while 65 % of severe TBI patients had such impairments [84]. It also could be that moderate and severe TBI have different rates of improvement over time. Even though severe TBI significantly affects cognitive function more than 6 months post-injury, such evidence is less clear for patients with moderate TBI [85].

Among the neuropsychological domains, the executive function is the one most often affected in TBI [86, 87]. Review studies have indicated that executive function is the most reliable neuropsychological indicator of reduced general global function [88, 89]. Information processing speed and attention [90] and memory [91] are other neuropsychological functions that

are often affected in TBI patients. Even though there exist many studies on TBI and different cognitive outcome measures, there exist only one study that has examined the load of TAI lesions (MHLs) in relation to different neuropsychological tests [48].

1.8.3 Statistical models in outcome prediction

The famous statistician George E.P. Box wrote that “essentially, all models are wrong, but some are useful”. This quote in particular applies to the predictive models used in TBI research. The computations of these models and the model assumptions have always been questionable and recommendations have changed with time. A rule of thumb in multivariable regression models has been that number of observations should be 10 times as many as number of covariates [92] to avoid oversaturated models. Such requirements have been eased, and the new trend is, as in accordance to the recommendations from the IMPACT group, to do multiple covariate adjustments [93]. This group has also advocated to use ordinal dependent variables in the analyses rather than dichotomizing the outcome measure in a binary logistic regression [94]. When raw data is converted to a binary outcome, you always lose some of the variance in the data since it will be assigned to only two different groups.

2 AIMS OF THE THESIS

The overall aim of this thesis was to study TAI lesions in conventional MRI and injuries to the microstructure in diffusion sequences of patients with moderate and severe TBI, and relate the findings to global and cognitive outcome.

2.1 Paper I

A longitudinal MRI study of traumatic axonal injury in patients with moderate and severe traumatic brain injury

In this prospective study of patients with moderate to severe TBI, we aimed to detect TAI lesions in conventional MRI sequences obtained longitudinally at different time points during the first year post-injury and relate these findings to the patient outcome.

2.2 Paper II

A longitudinal magnetic resonance imaging study of the apparent diffusion coefficient values in corpus callosum during the first year after traumatic brain injury

In this study we wanted to prospectively and longitudinally explore apparent diffusion coefficient (ADC) values in normal appearing tissue of the corpus callosum in patients with moderate and severe traumatic brain injury (TBI) and relate the findings to global and neuropsychological outcome.

2.3 Paper III

Traumatic axonal injury: the prognostic value of lesion load in corpus callosum, brain stem, and thalamus in different magnetic resonance imaging sequences

In this prospective study we aimed to explore the independent prognostic value of visible TAI lesion loads in corpus callosum, brain stem and thalamus in different conventional MRI sequences obtained from the early post-injury phase.

2.4 Paper IV

Traumatic axonal injury in moderate and severe head injury: relationship between conventional MRI findings in the acute phase and diffusion tensor imaging findings in the chronic phase

In this study we aimed to follow TAI lesions from the acute to the chronic phase and compare fractional anisotropy (FA) values in transient and persistent lesions, and relate the findings to cognitive outcome.

3 MATERIAL AND METHODS

3.1 Setting

3.1.1 The health region of Mid-Norway and St.Olav University Hospital

Norway has a scattered population and consists of four health regions. In this thesis we have included patients from the health region of Mid-Norway. This health region serves about 680 000 inhabitants (2010) and encompasses three counties (Sør-Trøndelag, Nord-Trøndelag and Møre-og Romsdal). St.Olav University Hospital is the only level I trauma center and the only hospital with a neurosurgical service in the health region. There are seven local hospitals in the region and decisions for transfer of patients with TBI are made by the on-call neurosurgeon. A telemedicine system allows the neurosurgeon to review the CT scan obtained at the local hospital before the decision of transfer is made. For severe TBI, all patients who are not deemed unsalvageable due to the TBI, age, premorbidity and / or other injuries will routinely be transferred as fast as possible to the University Hospital. Also some of the moderate TBI patients will be transferred, especially if CT shows injuries that possibly could progress or the patient has other injuries in other organ systems that warrants observation and treatment at a level I trauma center. St.Olav University Hospital also serves as a local hospital for 287 000 patients from the region and all patients with moderate head injury from this region will be admitted to the University Hospital.

3.2 Patients and the inclusion process

3.2.1 The Head Injury Project and the inclusion and exclusion process

The Head Injury Project is a prospective cohort study where all patients admitted to St.Olav University Hospital with moderate or severe TBI according to the HISS classification are consecutively included. The inclusion started in October 2004 and is still on-going. Patients admitted with severe TBI with bilaterally fixed dilated pupils (BFDP), GCS score of 3 and a CT scan showing signs of herniation, were deemed unsalvageable in the emergency room and not eligible for inclusion in the present studies. Likewise, patients that died within 24 hours of extra cranial injury or patients that did not consent to follow-up, was a foreigner with communicative difficulties or had other (psychosocial) reasons were not considered eligible for inclusions.

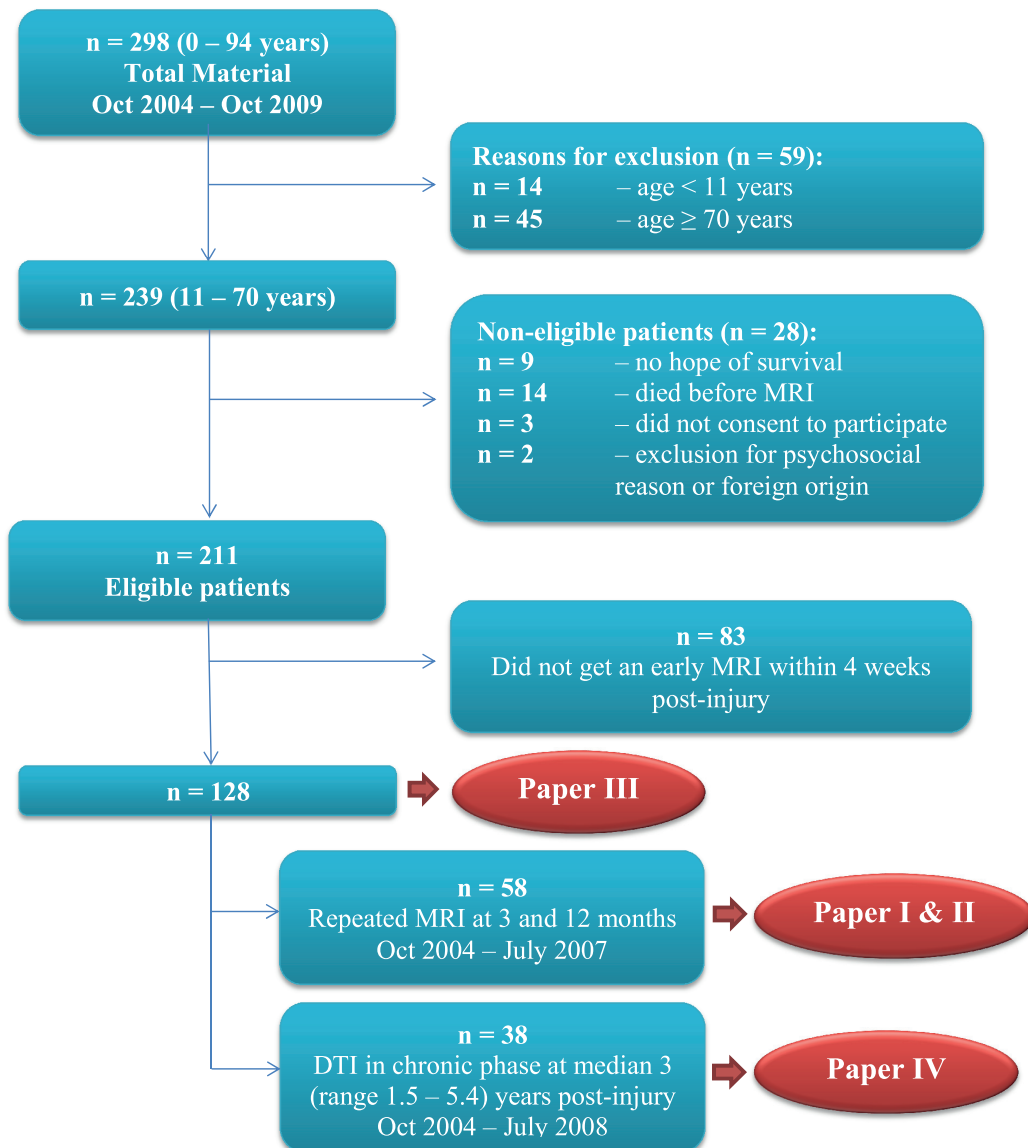
According to the HISS classification, all patients admitted with $GCS \leq 13$ should be included. However, the GCS score could be falsely low due to non-TBI factors i.e. intoxication or major extra cranial injury [95]. Accordingly, those patients whose GCS score had returned to 14-15 during the first 6 hours after admission and whose initial low GCS score could be explained by other factors, were diagnosed with mild head injury and excluded. Still, the patients were included if they had established focal neurological deficit(s) or they had certain and observed unconsciousness lasting for more than five minutes. Likewise, patients whose GCS score rose to 9-13 during the first 6 hours and whose initial low GCS score could be explained by other factors were upgraded from initially severe to moderate TBI [95, 96].

Hypoxia was defined as one episode with apnea, cyanosis or oxygen saturation $< 92\%$ and a hypotensive episode was defined as an episode with systolic blood pressure < 90 mmHg.

3.2.2 The patient cohorts of this thesis

In this PhD project we restricted the inclusion to a 5-year period (October 2004 – October 2009). Thus, all the patients in the four different papers are individuals from this cohort or a restricted part of it. The specific exclusion criteria are listed in the material and methods section in each paper. Since all papers in this thesis included one or more MRI scan post-injury, only patients that could receive active treatment and were cooperative, were included. We have excluded the pediatric population below 11 years [97], and also excluded the older patients > 65 years since the MRI scans could be influenced by age related changes (both NHLs and MHLs) [98]. The selection of patients for the different studies is indicated in detail in Fig. 3.2.1.

Figure 3.2.1



3.3 Healthy control subjects

In paper II-IV we have included a healthy control group. In the two first studies (paper II and III) the control group was used to obtain control ADC values from the different ROIs used. For these two studies we performed diffusion MRI (DWI including ADC map) in a total of 54 healthy controls. From this pool of control subjects we randomly selected a sample for each study that matched the study population with regard to age and sex. In both studies we ended up with a total of 47 control subjects.

In paper IV we also included a control group both to compare the FA values from the different patient groups and also to compare the neuropsychological test results. This control group consisted of 42 healthy individuals that underwent a DTI scan and neuropsychological testing in the years 2009 and 2010.

3.4 Neuroimaging

3.4.1 Computed Tomography

CT examination was obtained in the acute phase in all patients included in the papers of this thesis. For paper I, II and IV all the CT scans were reviewed by a specialist in radiology (I.H.S.). In paper III K.G.M. also classified a sample of the CT scans (n=24) in cooperation with three specialists in radiology (K.A.K., M.F., J.R.). All CT scans were classified according to the Rotterdam CT score as indicated in Fig. 1.5.1 [36].

3.4.2 Conventional Magnetic Resonance Imaging (MRI)

The conventional MRI was performed at the study hospital or at one of the local hospitals in the region. At the study hospital either a 1.5 T Siemens Symphony or a 1.5 T Siemens Avanto system was used, while at the local hospitals also a 1.5 T Philips Gyroscan NT Intera was used in some cases. Before study start the study hospital and the involved local hospitals received the MRI protocol with standardized MRI sequences and parameters for use in the early conventional MRI scanning of the patients. The scan protocol consisted of:

- 1) Sagittal turbo SE T2-weighted imaging: 20 slices, TR 4300 msec, TE 110 msec, echo train length 14, number of excitations 4, FOV 23 cm, acquisition matrix 291×512 , slice thickness 5.0 mm, gap 1.0 mm, in plane resolution 0.6×0.7 mm.
- 2) Sagittal, transverse, and coronal T2-weighted FLAIR imaging: 24 slices, TR 9000 msec, TE 109 msec, TI 2500 msec, number of excitations 4, FOV 23 cm, acquisition matrix 291×512 , slice thickness 5.0 mm, gap 1.0 mm, in plane resolution 0.6×0.7 mm.
- 3) Transverse T2*GRE imaging: 24 slices, TR 830 msec, TE 25.8 msec, number of excitations 4, FOV 21 cm, flip angle 20° , acquisition matrix 291×512 , slice thickness 5.0 mm, gap 1.0 mm, in plane resolution 1.0×0.8 mm.
- 4) Transverse SE T1-weighted imaging: 24 slices, TR 430 msec, TE 7.8 msec, number of excitations 4, FOV 23 cm, acquisition matrix 291×512 , slice thickness 5.0 mm, gap 1.0 mm, in plane resolution 0.9×0.9 mm.
- 5) Transverse DWI: single-shot, SE planar imaging sequences with following parameters: 19 slices, TR 3300 msec, TE 110 msec, number of excitations 4, FOV 23 cm, bandwidth 1240 Hz, acquisition time 1:44 min, slice thickness 5.0 mm. Baseline images were obtained ($b = 0$ second/mm²) and varying diffusion gradient strength along each of 3 orthogonal directions ($b = 500$ and 1000 second/mm²). Diffusion trace maps were computed from the isotropic diffusion image and were used to estimate the ADC map.

The conventional MRI scans were reviewed by K.G.M. in cooperation with two experienced neuroradiologists (K.A.K., J.R.). All image analyses were performed blinded for ID and clinical information. The image findings were characterized by visual inspection and number of TAI lesions were counted in the T2*GRE, FLAIR and DWI sequences, including the ADC map. The lesions were characterized on each side of the midline in different locations of the brain (hemispheres, corpus callosum, brain stem, thalamus, basal ganglia and cerebellum). TAI lesions were classified according to a modified staging based on the neuropathological appearance (Fig 1.6.2) [12, 40]. If there were more than 10 lesions in one location, they were automatically assigned the number 15. Superficial lesions in the cerebral cortex were defined as cerebral contusions [31], and the sequela after insertion of external ventricular drainage was not counted as a TAI lesion. Isolated injuries in the fornix were counted as TAI stage 2 lesions. Periventricular hyperintensities (“caps and bands”) were not counted as lesions since they were considered an age related normal phenomenon [99, 100]. In cases where the white matter

hyperintensities were attributable to other comorbid non-symptomatic disease [101], the patients were excluded for further analyses involving the FLAIR sequences.

3.4.3 Volumetric analyses of TAI lesions in FLAIR sequences in the conventional MRI

Since TAI lesions in FLAIR images turned out to be difficult to count due to extensive changes in some patients, we decided to also quantify the lesion load of the FLAIR lesions. We considered different programs for evaluating the volumes of the FLAIR lesions. The most widely used software at NTNU is Brain Voyager QX software, version 1.2. This is a software where you manually segment the lesion in all slices, and the software automatically corrects for the gap between the slices. Such a manual method is time consuming, so we considered using a semi-automatic or fully automatic method. However, these methods are not much validated and there is a need to manually control the results. We also experienced that these softwares had problems interpreting the diseased brain, while the registration for normal brains i.e. the control group was considerably better. We considered using FreeSurfer, a software freely available for download online (<http://surfer.nmr.mgh.harvard.edu/>) against registration specifications. This program demands powerful computers (8 hours processing time for each scan), the output gives a lot of information and it is mainly used for T1 sequences. We also considered using Software for Neuro-Image Processing in Experimental Research (SNIPER) [102], an automatic volumetric software that has been used measuring brain volumes in T2 sequences. A validating study concluded that the software was promising with a rapid assessment of the brain volumes, but they recommended improvements and concluded that a manual segmentation still should be the reference method [103]. Due to this we have used the manual method with Brain Voyager QX in all of our papers.

Since the hypointense foci identified in T2*GRE sequences represent magnetic susceptibility effects due to degradation products of hemoglobin [42], and the size of the microhemorrhages thus dependent on the MRI parameters, the volumes of these foci were not evaluated.

3.4.4 Inter-rater reliability

In paper I we extracted a total of 89 conventional MRI scans which were reviewed by an independent neuroradiologist (M.F.) not otherwise involved in the study. Numbers of TAI lesions in the hemispheres, corpus callosum, brain stem, thalamus, basal ganglia and cerebellum were counted on each side of the midline in the FLAIR, T2*GRE and DWI sequences. The TAI stage was also estimated. Since we also performed analyses of volume of the FLAIR lesions, we also randomly selected 37 of the FLAIR sequences that were evaluated by V.B. in cooperation with the neuroradiologist (M.F.). The analyses were done blinded for patient identification and clinical information.

3.4.5 Diffusion MRI

Since a DWI acquisition is routine in the conventional MRI used for all TBI patients admitted to our hospital, this sequence was evaluated in all studies of this thesis. In paper I and III the prognostic value of number of DWI lesions was evaluated, and in paper II and III a more detailed examination of the ADC values in different parts of corpus callosum was examined. In paper IV the anisotropic diffusion represented by FA from lesions in the early MRI was thoroughly evaluated. Different diffusion sequences have therefore played an important role in all four papers of this thesis.

3.4.5.1 Diffusion Weighted Imaging (DWI)

In paper II and III detailed analyses of the ADC map of normal appearing corpus callosum was performed. The DWI sequence parameters are listed in 3.4.2 Conventional MRI. Region of interest (ROI) analyses were performed in PACS using the Sectra Workstation IDS5 11.4.1. Circle shaped ROIs (radius 1.8 mm) were positioned in normal appearing corpus callosum in the ADC map. The DWI scan was used clinically to screen for lesions in the ADC map. The SD of the mean ADC values was by manual placement kept as low as possible within each ROI. The ADC measurements were computed in 10 different predefined ROIs on each side of the midline of the corpus callosum in the five regions based on the Hofer and Frahm scheme [104]. The fibres in region I (genu) project into the prefrontal region, while regions II, III and IV (truncus)

contain fibres projecting to premotor, motor and sensory cortical areas, respectively. Posterior parietal, temporal and occipital fibres cross the corpus callosum in region V (splenium) [104].

3.4.5.2 Diffusion Tensor Imaging (DTI)

In paper IV a MRI protocol including a DTI sequence for both the patients and the healthy controls was performed. All examinations were acquired on the same 3 T Siemens Trio scanner with Quantum gradients (30 mT/m) and a Head Matrix Coil (12 element receive only coil).

The DTI sequence was a single-shot balanced-echo EPI sequence acquired in 30 non-collinear directions with $b = 1000 \text{ s/mm}^2$ using the following parameters: 55 transversal slices, TR 6800 ms, TE 84 ms, FOV 240 x 240 mm, acquisition matrix 96 x 96, slice thickness 2.5 mm, no gap, in plane resolution 2.5 x 2.5 mm. For each slice, six images without diffusion weighting ($b=0$), and 30 images with diffusion gradients were acquired. The DTI sequence was repeated twice for increased signal-to-noise-ratio. In order to correct for image distortion caused by magnetic susceptibility artifacts two additional b_0 images were acquired with opposite phase-encode polarity [105].

The remainder of the scanning protocol is described in detail in the paper. DTI analyses were performed with tools from the FMRIB software library (FSL, Oxford Centre for Functional MRI of the Brain, UK; www.fmrib.ox.ac.uk/fsl) and a B_0 -unwarping method developed by Holland et al. [106]. The brain was extracted using Brain Extraction Tool (BET, part of FSL). FMRIB's Diffusion Toolbox (FDT) was used to fit a diffusion tensor model to the raw diffusion data in each voxel. Voxelwise maps of FA were calculated for the TBI patients and healthy controls. Detailed methodological description on how we extracted the FA values from the ROIs in persistent and transient TAI lesions and compared them with ROIs in TBI patients without different types of TAI lesions and healthy controls is described in paper IV. Briefly, the FLAIR / T2*GRE images from the early phase were linearly registered to the FA images from the chronic phase, and lesions manually segmented on the FA registered FLAIR / T2*GRE images from the chronic phase.

3.5 Outcome assessments

Outcome assessments were performed in all of the papers in this thesis. In paper I and II both a global outcome measure (GOSE score) and neuropsychological outcomes were evaluated. In paper III prognostic models with only GOSE score as dependent variable were done, while in paper IV only correlations with neuropsychological outcome was evaluated.

3.5.1 Global outcome

Global outcome was assessed by the GOSE score at 12 months post-injury. This scale has turned out to have good discriminating abilities [107], and it is also possible to use in telephone interviews [108]. Mainly prognostic models with multiple ordinal logistic analyses were done, where the GOSE score was treated as an ordinal variable. But in paper II and III, we did dichotomized analyses and GOSE score of 7-8 was defined as good recovery and GOSE score < 7 was defined as disability.

3.5.2 Neuropsychological outcome

In paper I and II the neuropsychological assessments were performed by neuropsychologists, two trained master level students, and one test technician at St. Olav University Hospital. The testing was performed at 3 and 12 months post-injury. Since we had no control group for the neuropsychological testing in these two studies, the raw scores were converted to standard scores by use of normative data provided by the manufacturers of the tests, except for the Symbol Digit Modality Test (SDMT), where a normative sample quoted by Lezak was used [81]. Standard scores were given as T-scores, and the individual neuropsychological tests were collapsed into composite-scores reflecting the overall function on each cognitive domain:

1. Motor function: Grooved Pegboard (GP) test using both the dominant and non-dominant hand, where time to place 25 keyhole pegs in a 5x5 matrix was measured [109, 110].
2. Information processing: Trail Making Test (TMT), part 2 (number sequencing), and part 3 (letter sequencing); Color-Word Interference Test (CWIT), condition 1 (color naming) and 2 (word reading) from the Delis Kaplan Executive Function System (D-KEFS) [111] and SDMT, oral and written versions [112].

3. Executive functions: Category Test computer version [113]; Verbal Fluency Test, condition 1 (letter fluency), and condition 3 (category change) from D-KEFS; TMT, condition 4 (Number-Letter Sequencing) from D-KEFS; CWIT, condition 3 and 4 (Inhibition and Inhibition/Switching) from D-KEFS; Tower Test from D-KEFS.

In paper IV a total of 37 of the 38 included patients had neuropsychological test scores. The testing was performed in the chronic phase at a median 3 (range 1.5 – 5.4) years post-injury. Both patients and the 42 healthy control subjects were tested. Executive function was assessed with the abovementioned TMT, consisting of five subtests; visual scanning (part 1), number sequencing (part 2), letter sequencing (part 3), number letter sequencing (part 4) and motor speed (part 5). Fine motor skills were assessed with the GP test. The raw scores (time in seconds required to finish) were used for both TMT and GP test in paper IV, since we had a healthy control group and transformation of the raw scores to normative data also is controversial among neuropsychologists [114].

3.6 Statistical methods

The statistical analyses are described in detail in each paper. In both paper I and II we have multiple measurements per subject and multilevel models were considered in the analyses [115]. In these cases, the units of analyses were measurements at different time points nested in each individual. These models represent an alternative to repeated data analyzed in fixed effects models such as linear regression models or ANCOVA that follow this model (Fig. 3.6.1 A):

The diagram shows the linear regression equation $Y = \alpha + \beta x + \varepsilon$. A box labeled "Slope" has a downward-pointing arrow to the coefficient β . A box labeled "Intercept" has an upward-pointing arrow to the coefficient α . A box labeled "Error term" has an upward-pointing arrow to the term ε .

$$Y = \alpha + \beta x + \varepsilon$$

In the abovementioned model both the intercept and the slope is fixed, and within subjects variability are not taken into consideration. In some data sets, especially when an individual is measured at multiple time points, the chance that this individual gets a score closer to his previous score is often much higher compared to scoring like one of the other individuals in the dataset. In such instances a mixed effect model is a better fit to the data since such a model takes these assumptions into consideration. There exist different types of mixed effects models, and a chi square likelihood ratio (LR) test determines which one you ought to use.

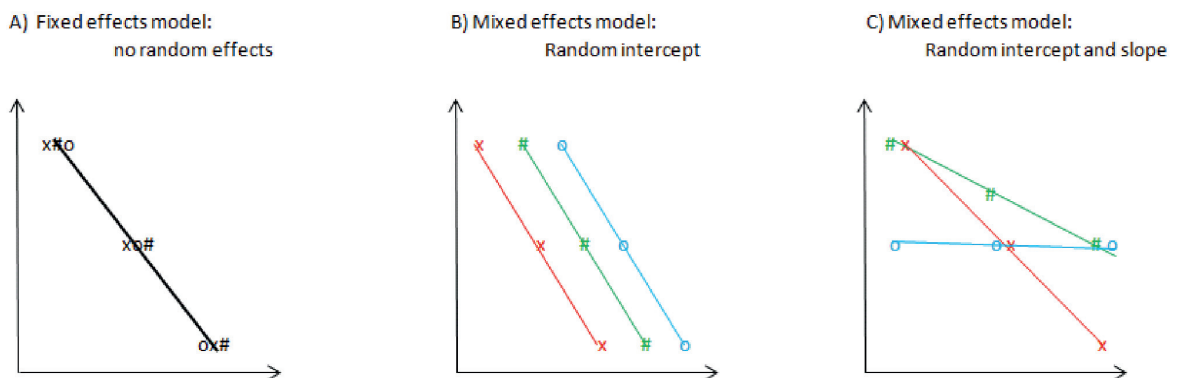
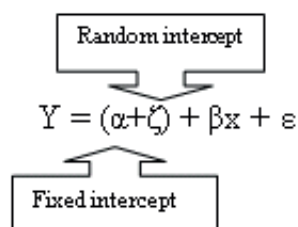


Figure 3.6.1

The figure shows three different models for interpreting and modelling multiple measurements data. If the data is clustered linearly a fixed effects model (A) could be adequate, but sometimes the data is clustered differently and it would be advantageous to take the within subject variability into consideration (B and C).

In a random intercepts model the intercepts are allowed to vary across groups, while the slopes are fixed. These analyses follow this model (Fig. 3.6.1 B):



In a random slopes model the slopes are allowed to vary, while the intercepts are fixed, and in a random intercepts and slopes model both intercepts and slopes are allowed to vary across groups. This is often the most realistic type of model, but it is also the most complex one. These analyses follow this model (Fig. 3.6.1 C):

$$Y = (\beta_1 + \zeta_1) + (\beta_2 + \zeta_2)x + \varepsilon$$

3.7 Ethical considerations

All studies were approved by the Regional Committee for Medical Research Ethics in Health Region Mid-Norway. All patients were prospectively included based on informed consent from the patients and if below the age of 16 or incapacitated, by their next of kin.

4 SUMMARY OF RESULT

4.1 Paper I

A longitudinal MRI study of traumatic axonal injury in patients with moderate and severe traumatic brain injury

Kent Gøran Moen, Toril Skandsen, Mari Folvik, Veronika Brezova, Kjell Arne Kvistad, Jana Rydland, Geoffrey T. Manley, Anne Vik

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The objective of this study was to follow the TAI lesions one year post-injury and relate the findings to global and neuropsychological outcome. Thus, the study could also indicate an optimal time point for when the MRI acquisition should be performed after TBI.

We included 58 patients with TBI (Glasgow Coma Scale score 3-13), and they were examined with MRI at three time points (median 7 days, 3 and 12 months) post-injury. The TAI lesions were evaluated blinded for patient information and categorized into three stages based on their location; hemispheres, corpus callosum and brain stem. Lesions in T2*-weighted gradient echo (T2*GRE), fluid-attenuated inversion recovery (FLAIR) and diffusion weighted imaging (DWI) were counted and FLAIR lesion volumes were estimated using a manual segmentation method (Brain Voyager QX). Inter-rater reliability score was calculated. Global outcome was assessed 12 months post injury by Glasgow Outcome Scale Extended (GOSE). In a supplement to the original paper we also examined the predictive value of MRI variables to executive function (Trail Making Test, TMT) and information processing speed (Symbol Digit Modality Test, SDMT) evaluated 12 months post injury.

We found that 31 % of the patients had brain stem lesions in the early MRI compared to 17 % in the MRI scan performed at 3 months ($p=0.008$). Number of lesions in the FLAIR and DWI sequences and volume of the FLAIR lesions, were all significantly reduced from early to 3 months MRI ($p<0.001$). In contrast to this, we found that the number of T2*GRE lesions persisted at 3 months, but was reduced in the 12 month MRI scan ($p=0.007$). Number of lesions in DWI and volume of FLAIR lesions in early MRI predicted worse clinical outcome in adjusted analyses ($p<0.05$). In the supplemental adjusted analyses of neuropsychological outcome, all

early MRI variables predicted TMT scores ($p < 0.004$), while only number of FLAIR ($p = 0.042$) and DWI lesions ($p = 0.001$) in early MRI predicted SDMT scores.

In conclusion, this study quantified the attenuation of non-hemorrhagic TAI lesions in conventional MRI during the first 3 months after TBI; most importantly the disappearance of brain stem lesions. Hemorrhagic TAI lesions attenuated first after 3 months. Only early MRI findings predicted clinical outcome after adjustment for other prognostic factors. Hence, valuable clinical information may be missed if MRI is performed too late after TBI.

4.2 Paper II

A longitudinal magnetic resonance imaging study of the apparent diffusion coefficient values in corpus callosum during the first year after traumatic brain injury

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The objective of this study was to explore the evolution of apparent diffusion coefficient (ADC) values in MRI in normal appearing tissue of the corpus callosum during the first year after TBI and relate findings to outcome.

We examined 57 patients (mean age 34 [11-63] years) with moderate to severe TBI with diffusion weighted MRI at three time points (median 7 days, 3 and 12 months), and a sex –and age matched control group of 47 healthy individuals were examined once. The corpus callosum was subdivided and the mean ADC values computed blinded in 10 regions of interests (ROIs) without any visible lesions in the ADC map. Outcome measures were Glasgow Outcome Scale Extended (GOSE) and neuropsychological domain scores at 12 months.

We found a gradual increase of the mean ADC values during the 12 month follow up, most evident in the posterior truncus ($r=0.19$, $p<0.001$). Compared to the healthy control group, we found higher mean ADC values in posterior truncus both at 3 months ($p=0.021$) and 12 months ($p=0.003$) post-injury. Patients with FLAIR lesions in corpus callosum in the early MRI and patients with disability (GOSE score ≤ 6) showed evidence of increased mean ADC values in genu and posterior truncus at 12 months ($p=0.001-0.032$). Mean ADC values in posterior parts of the corpus callosum at 3 months predicted the sensory-motor function domain score ($p=0.010-0.028$).

During the first year after moderate and severe TBI we demonstrated a slowly evolving disruption of the microstructure in normal appearing corpus callosum in the ADC map, most evident in the posterior truncus. The mean ADC values were associated with both global outcome and ability to perform speeded, complex sensory-motor action.

4.3 Paper III

Traumatic axonal injury: the prognostic value of lesion load in corpus callosum, brain stem, and thalamus in different magnetic resonance imaging sequences

Kent Gøran Moen, Veronika Brezova, Toril Skandsen, Asta K. Håberg, Mari Folvik, Anne Vik
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In this study we wanted to explore the prognostic value of visible TAI lesion loads in corpus callosum, brain stem and thalamus in different MRI sequences from the early phase after adjusting for established prognostic factors. We also wanted to explore the prognostic role of early apparent diffusion coefficient (ADC) values in normal appearing corpus callosum.

In this prospective study a total of 128 patients (mean 33.9 [range 11-69] years) with moderate (n=64) and severe TBI were examined with MRI at median 8 (range 0-28) days post-injury. TAI lesions in fluid-attenuated inversion recovery (FLAIR), diffusion-weighted imaging (DWI) and T2*-weighted gradient echo (T2*GRE) were counted and FLAIR lesion volumes estimated in different brain locations, blinded for clinical information. In patients and also 47 healthy control subjects, mean ADC values were computed in 10 regions of interests in normal appearing corpus callosum. Outcome measure was Glasgow Outcome Scale Extended (GOSE) at 12 months.

A total of 66% of severe and 42% of moderate TBI patients had TAI lesions in corpus callosum and/or brain stem. For severe TBI patients, the number of DWI lesions and volume of FLAIR lesions in corpus callosum, brain stem and thalamus predicted outcome in analyses with adjustment for age, Glasgow Coma Scale score, and pupillary dilation (OR 1.3-6.9, $p < 0.001-0.017$). For moderate TBI patients, only number of cortical contusions ($p=0.089$) and Rotterdam CT score ($p=0.065$) tended to predict outcome. Number of T2*GRE lesions did not affect outcome ($p=0.17-0.32$). Mean ADC values in normal appearing corpus callosum did not differ from controls.

In this large prospective study, we concluded that the loads of visible lesions in corpus callosum, brain stem and thalamus in DWI and FLAIR were independent prognostic factors in severe TBI patients. Interestingly, number of cortical contusions in MRI and CT findings seemed to be more important for moderate TBI patients.

4.4 Paper IV

Traumatic axonal injury in moderate and severe head injury: relationship between conventional MRI findings in the early phase and diffusion tensor imaging findings in the chronic phase

Kent Gøran Moen, Anne Vik, Alexander Olsen, Toril Skandsen, Asta Kristine Håberg, Kari Anne I. Evensen, Live Eikenes

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The aim of this study was to follow TAI lesions depicted in conventional MRI from the early to the chronic phase and compare fractional anisotropy (FA) values in transient and persistent lesions with those from healthy controls and patients without either NHLs or MHLs. We also wanted to relate the findings to cognitive outcome.

In this prospective study 38 patients (mean 24.7 [range 13-63] years) with moderate-severe TBI and 42 age and sex matched healthy controls were enrolled. Patients underwent 1.5 T MRI in the early phase (median 7 days) including fluid-attenuated inversion recovery (FLAIR) and T2* gradient echo (T2*GRE). TAI lesions were characterized as non-hemorrhagic lesions (NHLs) and microhemorrhagic lesions (MHLs). In the chronic phase (median 3 years) patients and controls were imaged at 3 T with FLAIR, T2*GRE, T1 and DTI, and the FLAIR/T2*GRE images were used to decide whether the lesions were persistent or transient. The FLAIR/T2*GRE images from the early phase were linearly registered to the FA images from the chronic phase, and lesions manually segmented on the FA-registered FLAIR/T2*GRE images with a ROI (region of interest) based approach. Cognitive outcome was measured with trail making test (TMT) and grooved pegboard test (GP).

We detected 61 NHLs in 25 patients and 97 MHLs in 29 patients in the early phase MRI. 25% of NHLs and 89% of MHLs were persistent to the chronic phase. In persistent NHLs, histogram analyses of individual voxel values demonstrated significantly reduced FA both in hemispheres and corpus callosum compared with transient NHLs ($p < 0.001$). For MHLs there were no such differences between individual FA voxel values in persistent and transient lesions. A significant linear decrease in mean FA of both NHLs and MHLs in the hemispheres and corpus callosum was demonstrated; mean FA in ROIs of healthy controls > mean FA in ROIs of patients without either NHLs or MHLs > mean FA in ROIs of transient lesions > mean FA in ROIs of

persistent lesions. Mean FA in persistent NHLs in the hemispheres correlated negatively with TMT 4 scores and mean FA in transient NHLs in corpus callosum correlated negatively with TMT 4-5 scores.

There was a significant gradual decline in FA from ROIs in white matter in healthy controls to ROIs in TBI patients without either NHLs or MHLs and further to transient lesions and finally lowest FA in persistent lesions. Mean FA in persistent NHLs in the hemispheres and in transient NHLs in the corpus callosum correlated with cognitive performance.

5 GENERAL DISCUSSION

5.1 MRI findings in a longitudinal perspective

5.1.1 The natural course of TAI lesions in different conventional MRI sequences

In paper I we examined different conventional MRI sequences at median 7 days, 3 months and 12 months. We found that NHLs detected in FLAIR and DWI sequences mainly disappeared at 3 months, while MHLs detected in the T2*GRE sequence first attenuated after 3 months and then to a lesser degree compared to NHLs. Our study is the first one that longitudinally evaluated number and volume of TAI lesions in these three important sequences of a conventional MRI protocol in TBI patients.

In a study by Shibata et al. (2000), NHLs in the brain stem detected in early acquired (< 7 days) 0.5 Tesla T2 sequences, were evaluated among 17 patients with mild-severe TBI [116]. 11 of these patients were reevaluated with a later MRI (10 days – 1.5 years) and in 5 of the patients the lesions could not be found, and for two of them the lesion size was decreased. However, this study was retrospective, included few patients and the time point for follow-up MRI was very wide. At the same time as we published paper I, Chung et al. (2012) examined the longitudinal course of NHLs detected in 32 patients with moderate and severe TBI examined in the early phase at mean 12 days post-injury, and they concluded that these lesions resolved almost completely during the follow-up period [117]. However, they did not mention their follow-up time that according to the figure texts seems to be very wide and differs from 1 month until 2 years post-injury.

The evolution of MHLs was evaluated in a prospective longitudinal study by Messori et al. (2003) in 127 TBI patients. In 61 % of the patients, number of lesions decreased either between 4-6 months and 1 year and / or between 1 and 2 years post-injury [42]. The authors concluded that lesions detected in T2*GRE sequences appeared less conspicuous with time, and our prospective study is in accordance with these results.

No later studies in TBI patients have reevaluated the longitudinal appearance of different TAI lesions in all the different conventional MRI sequences. In our study only 60 % of patients with TAI stage 3 were classified likewise at 3 months post-injury, and due to this attenuation of NHLs the MRI should preferably be performed as early as possible to avoid misclassification of

the patients' TAI stage. Lesions in the brain stem are strongly associated with outcome, especially if they are bilateral [118]. A misclassification of the TAI stage could therefore have implications for the prognosis, rehabilitation assessment and the information given to the family. We warrant a study where conventional MRI is acquired at multiple time points during the first weeks, to clarify at what time point the attenuation of NHLs is most prominent. In the upcoming large scale study "CENTER TBI" (<https://www.center-tbi.eu/>) this research question will be examined since MRI will be performed within 72 hours post-injury and repeated at 2-3 weeks post-injury.

5.1.2 The statistical approach with mixed effects models in longitudinal data

The repeated measurement data in our study was statistically treated with mixed effects longitudinal models [115, 119]. Such models are particularly appropriate when patient data are organized at more than one level i.e., nested data. When developing a multilevel model, you start with fixed coefficients (slopes and intercepts), and gradually compare your new model to the previous by using the LR-test until you fit the best model to your data [115]. However you should always consider the complexity and use the simplest model if the more complex one does not add considerably to your present model. In the medical literature after year 2000 the use of such models has increased [120]. These models have become more popular after the evolvement of computers with sufficient power and the development of suitable software, and we think it is a strength that our study used such analyses in the longitudinal data. In paper I our longitudinal data was best fitted to a random intercept and slope model, while in paper II a fixed effects model turned out to give the best fit.

5.2 The predictive value of lesions in early conventional MRI

Surprisingly, the predictive value of lesions in conventional MRI used in clinical practice has been little explored in good quality studies, while the more advanced neuroimaging methods as DTI have been extensively studied during the last decade [121]. In conventional MRI it is still an ongoing discussion on which MRI findings that are most important for outcome prediction. There is general agreement that the presence of no injuries in MRI, is the best predictor for a good outcome [122, 123].

In paper I and III we explored better answers to which conventional MRI findings that have highest impact on outcome after TBI. The type of lesions (MHLs or NHLs), the location of the lesions (hemispheres, corpus callosum, brain stem and thalamus) and the lesion loads (number or volume of the lesions) were all assessed in these studies. We also computed advanced statistical models where the contributions of the different MRI findings were examined with adjustments for other important prognostic variables. Since earlier studies have shown that there are considerable differences in the predictive value of different variables in moderate and severe TBI [77, 118], these analyses were also done separate based on injury severity.

5.2.1 The predictive value of conventional MRI findings in severe TBI

In paper III we found that number of DWI lesions and volume of FLAIR lesions in corpus callosum, brain stem and thalamus in the early MRI predicted GOSE category, even after adjustment for other well-known prognostic factors. The prognostic model that included number of DWI lesions in corpus callosum had highest explained variance (R^2) and the odds ratios were highest in the DWI sequences, indicating the importance of these lesions in outcome prediction.

Few studies have evaluated the visible lesions detected in DWI and their relationship to outcome. Schaefer et al. did a retrospective study of 26 TBI patients, in which most lesions were detected in the DWI sequences and volume of the DWI lesions had the highest correlation to the modified Rankin Scale score [46]. But no predictive models were proposed, and the location of the lesions was not specified. A study by Matsukawa et al. (2012) has emphasized the importance of lesion load in corpus callosum for outcome prediction [124]. In both paper I and III we detected fewer lesions with DWI compared to the FLAIR sequences. But even though the DWI lesions numerically were few, they seemed to be particularly important for prognosis. Thus, we could speculate if these visible diffusion changes indicate a more severe injury to the micro structure in the brain compared to the FLAIR lesions.

The importance of FLAIR lesion volumes on patient outcome was also highlighted in both paper I and III. These findings were in accordance with earlier studies, since both FLAIR lesions in corpus callosum [125] and brain stem [52, 126] have been found to have high impact on prognosis. However, thalamic lesions in conventional MRI sequences are barely studied so far. In a recent study describing outcome in patients with disorders of consciousness after TBI, thalamic lesions were found in five of the seven patients [127]. Since paper III also suggests a

very important contribution of NHLs in thalamus, we suggest that future outcome studies on severe TBI should be focused on this structure since it seems to be strongly linked to TAI. Anatomically it is also plausible that TAI injuries can occur in thalamus. First it serves as a relay station with multiple synaptic connections that may undergo a perisomatic axotomy [128, 129]. Second, it contains long white matter bundles since it is covered with a sheet of white matter on the superior (stratum zonale) and lateral surface (external medullary layer) and also divided vertically into three nuclear groups by a Y-shaped sheet of white matter (internal medullary layer) [130].

Even though we found that NHLs in the brain stem had high impact on prognosis, we did not further subclassify these lesions. Earlier studies have shown that brain stem lesions in MRI are associated with a poor outcome [76, 131]. Since a sub group of patients with brain stem lesions seemed to have a better outcome, it has been speculated if different pathophysiological mechanisms exist and especially two main mechanisms have been proposed [116, 132]. One type appears more dorsal and superficial in the brain stem which has been hypothesized to result from an impact against the free edge of the tentorium cerebelli. This type of lesion, possibly representing a contusion, is more often unilateral and has been related to a better prognosis than the second type that is found deeper in the brain stem [123, 133, 134]. The latter lesion type could be more consistent with a TAI mechanism, and thus more often bilateral and consequently associated with a poor outcome [118, 126].

Based on the multivariable prediction models both in paper I and III, we concluded that the lesion load of MHLs had no prognostic value. Two earlier studies have reached the same conclusion [47, 52], while two other studies found that early lesion loads of MHLs were of prognostic value [46, 135]. Hilario et al. evaluated 108 patients with severe TBI with MRI < 30 days post-injury and found that MHLs in the brain stem was a weak, but still a significant prognostic factor [133]. The T2*GRE sequence will in the future be replaced with a more sensitive 3D velocity-compensated gradient echo sequence named susceptibility weighted imaging (SWI) [136]. With this sequence the contrast in the image is considerably improved, which increases the sensitivity to detect microhemorrhages. However, as for the T2*GRE sequence, the predictive role of these microhemorrhages detected with the SWI sequence is also uncertain [52], even though a recent SWI study in pediatric TBI concluded that total number of SWI lesions correlated with global outcome [137].

However, multivariable analyses were not performed in any of the abovementioned studies. In the univariable analyses, we also found a predictive value of the number of MHLs. Anyway, these effects disappeared in the adjusted analyses, while the FLAIR and DWI lesion loads made an independent contribution. Based on this, we can conclude that the effect of MHLs on outcome is uncertain and probably less important, compared to lesions detected in FLAIR or DWI sequences.

In paper I we did not separate the lesions into different locations, but used the whole brain lesion loads in different sequences. In this longitudinally study we found that number of DWI lesions and volume of FLAIR lesions were particularly important in outcome prediction. But more important, the predictive value was only present in the early stage. Thus, by delaying the MRI acquisition to 3 months or later post-injury, the predictive value is lost. MRI should therefore be performed during the first weeks post-injury to improve lesion detection and outcome prediction.

5.2.2. The predictive value of conventional MRI findings in moderate TBI

In a previous study from our research group, no relation to outcome was found for the MRI based variable “depth of lesion” in patients with moderate TBI [118]. In that study, however, no quantification of lesion load was performed. In Paper I and III, we therefore applied a broader and more quantitative set of MRI variables. We detected many TAI lesions in the different conventional MRI sequences of the moderate TBI patients, even in the corpus callosum and brain stem. To our surprise, not even these predicted outcome. We found that age, Rotterdam CT score and contusions in MRI were the only variables with a predictive value on GOSE scores for the patients with moderate TBI. We believe this is a notable finding. Interestingly, Yuh et al. (2013) also found that CT findings and contusions on MRI predicted 3 months GOSE scores in mild TBI patients (including GCS score 13) with TAI lesions in conventional MRI [138]. Generally, research in the moderate TBI group has been sparse so far, and this particularly applies to early conventional MRI studies which are warranted in the future.

In paper I we also included more sensitive outcome measures, such as different cognitive outcome scores, and found that number of FLAIR lesions significantly predicted information processing speed in multivariable analyses. Executive function and information processing speed are the two cognitive domains strongest related to moderate-severe TBI [84, 139]. Even in a

study of 35 patients with mild TBI, the authors revealed impaired information processing [140]. Studies evaluating the cognitive function exclusively in patients with moderate TBI in relation to conventional MRI findings are warranted to better shed light on the importance of MRI findings in cognitive outcome. The cognitive function in relation to different findings in diffusion MRI will be further discussed in the next section.

5.3 The diffusion in corpus callosum evaluated with ADC values

5.3.1 How does the diffusion in corpus callosum change over time?

In paper II we found an increase of diffusivity that unfolded over time with significantly increased ADC values through the subacute to the chronic phase in the posterior truncus.

Light microscopic studies have shown that the density of thin diameter fibers reach a minimum and large myelinated fibers a maximum in the posterior truncus [141, 142]. Increased ADC values could therefore result from loss of myelin sheaths, a phenomenon that neuropathological review examinations have found to continue for 1-2 years post-injury [20]. This supports our finding of increasing ADC values evolving into the chronic phase. Since the posterior truncus is especially enriched in large myelinated fibers, it is reasonable that the demyelination would be more notable in this region, which is in accordance with our findings. Another study has shown that the density of fibers is highest in the genu and posterior parts of the corpus callosum [143], which were both areas with significant increase of the mean ADC values in paper II. The significantly more pronounced increase in ADC values in patients with atrophy further supports the notion that these fiber dense callosal regions suffer from an overall axonal loss. Kraus et al. concluded that the findings of increased radial and axial diffusivity in ROIs obtained in chronic moderate and severe TBI patients result from a combination of both myelin and fiber loss [144]. However, our study suggests that the effect of myelin loss on ADC values appears before the loss of fibers since the myelinated fibers are most numerous in the posterior truncus.

We found that ADC values in posterior corpus callosum predicted grooved PEG board performance at 12 months, and this is in line with the current knowledge of the topography of the corpus callosum, as these regions contain fibers converging to motor, sensory and occipital areas [104], all involved in the execution of this kind of task. The grooved PEG board test is a test of

higher order motor function and depends on visuo- motor coordination, manipulative dexterity and speed. It is a sensitive test in patients with TBI [84]. The proposed scheme from Hofer et al (2006) mapped the callosal motoric fibers to the posterior midbody [104]. This is in contradiction to the original Witelson scheme which mapped the motoric fibers to the anterior midbody [145]. Two other studies have also localized the callosal motoric fibers to the posterior midbody [146, 147]. Even though there is interindividual variability of the exact topography of the callosal motoric fibers along the anterior-posterior [148], we think the predictive value of mean ADC values in posterior parts of the truncus to the sensory-motor function is an interesting finding, since it corresponds to the location of the motoric callosal fibers.

5.3.2 The role of ADC values in corpus callosum in the early phase post-injury

In paper III we could not reveal any statistically significant differences in the mean ADC values in the different regions of normal appearing corpus callosum in early MRI performed between 0-28 days post-injury. But noteworthy, we observed a wide variation in the mean ADC values since some patients had very low and others had very high values. We believe this reflects different pathology of the patients in this stage of the injury, making it difficult to demonstrate any group differences. In the early stage of TBI, diffusion can be affected by axotomy and myelin degradation associated with increased ADC values [144], as well as post-traumatic cytotoxic edema with reduced ADC values [38]. Moreover, post-traumatic edema is present for a variable time after injury [149], and an earlier DTI study indicated that in the early phase reversible edema is dominating rather than traumatic axotomy [60]. The balance between these processes can result in the wide range of ADC values as we observed in paper III, not demonstrated so clearly in previous studies.

5.4 Chronic DTI findings in different lesions from the early conventional MRI

In paper IV we found that the mean FA gradually decreased from ROIs in healthy controls to ROIs in TBI patients without either NHLs or MHLs, and further to ROIs in transient NHLs and MHLs, with the lowest mean FA in ROIs of persistent NHLs and MHLs. FA changes in MHLs were more subtle compared to the FA changes in NHLs.

5.4.1 Mean FA values in different non-hemorrhagic lesions (NHLs)

Only one other study has examined FA values in white matter lesions depicted in conventional MRI from the chronic phase [150]. In that study FA in NHLs was only compared to FA in regions of normal appearing white matter in the contralateral side. There was no control group and since the study was conducted in chronic patients, all the NHLs were persistent. However, the results are still relevant and comparable to the results in paper IV, since they found lower FA values in the NHLs in the chronic phase when compared to normal appearing white matter in TBI patients.

Several previous DTI studies from the chronic phase have reported reduced FA in most parts of the brain after TBI when compared to healthy controls [62, 151], and an association between injury severity and degree of reduction in FA has also been found [152]. However, the previous DTI studies have not studied FA directly in lesion ROIs from the early phase and therefore not differentiated between areas with or without early lesions as we did in paper IV.

5.4.2 Mean FA values in different microhemorrhagic lesions (MHLs)

No other study has directly compared FA in persistent versus transient MHLs, and no other study has compared DTI metrics in MHLs to controls. In one DTI study of 8 patients with MHLs, lower FA values in corpus callosum in early MRI were found when compared to controls, and the reduced FA persisted at 6 months [22]. In 10 patients with mild-moderate TBI, DTI in the chronic phase showed reduced FA in regions which included most of the MHLs detected by T2*GRE in the chronic phase [153]. Also, Benson et al. reported that T2*GRE and FLAIR lesion areas depicted in MRI from the chronic phase were co-localized to areas with reduced FA in DTI indicating more severe injury to the visible lesion areas [154, 155]. However, all these studies differ from paper IV, since lesion areas were not directly studied. The studies also included very few patients.

5.4.3 Neuropathological origin and the predictive value of microstructural injury within lesion areas

As demonstrated in paper I, MHLs develop differently over time compared to NHLs. MHLs more often persist and are more often depicted in the hemispheres [42]. Since MHLs represent a

paramagnetic phenomenon caused by microhemorrhages in the tissue, they probably affect the microstructure of axons to a less degree compared to NHLs, which represents edema in the early phase and later gliosis. This could explain the findings that FA changes in MHLs were more subtle compared to the FA changes in NHLs. Theoretically, the blood breakdown products could also affect the FA value due to their magnetic properties. The differences in diffusion may also be more easily detected in NHLs, since these lesions are more extensive.

In paper IV we restricted the neuropsychological testing only to a test of executive function and one for fine motor skills. In a recent study, executive function and information processing speed were found to be the two cognitive domains most strongly associated with reduced FA values in the white matter tracts [139]. Moreover, the executive function seemed to have the strongest effect on functional outcome after moderate to severe TBI [156]. In two recent papers, our research group showed that executive function was most important for the ability to resume independent living, leisure activities and employment, and this was found even after adjusting for injury severity [84, 157].

In paper IV we found that FA within lesion areas was related to cognitive outcome, although the study design and the sample size did not allow us to make any firm conclusion. However, we think it was interesting that reduced FA values in transient NHLs of corpus callosum and persistent NHLs in the hemispheres correlated with poorer outcome in these cognitive tests. Due to the low numbers we could not analyze the correlation of persistent NHLs in corpus callosum, though we would expect an even stronger relationship to cognitive outcome. We warrant future studies investigating FA within lesion areas from early to chronic stage, which could further shed light on the functional significance of these microstructural injuries.

5.5 Strengths of the studies

One of the main strengths of this thesis is the prospective data collection. All patients with moderate and severe TBI were consecutively assessed for inclusion after admission to the study hospital and MRI was performed if possible at least within 4 weeks, but preferably before two weeks post-injury. Thus, we have an overview of the whole patient cohort admitted during the study period. Another strength is the thorough outcome assessments, with few lost to follow-up

(about 2 %). We also had sufficient power to perform multivariable ordinal regression outcome analyses with multiple covariate adjustments.

The image analyses of the conventional MRI scans were done blinded for patient ID and clinical information, and we also conducted inter-rater assessments of a random and representative sample both with regard to TAI classification, number and / or volume of TAI lesions in different sequences. For the two longitudinal conventional MRI studies, we managed to collect MRI at three different time points, during the first year, in a large number of TBI patients. The standardized time point of the second and third MRI is also a strength, and for a sample of these patients we also performed a comprehensive neuropsychological testing both at 3 and 12 months post-injury. For the analyses of the ADC values we collected a large, age -and sex matched control group.

In paper IV we performed complex analyses where DTI parameters in different lesions from the early phase were compared to typical TAI lesion areas in TBI patients without either NHLs or MHLs and to such areas in healthy controls. This novel approach has not previously been done. We also had a large, age –and sex matched control group both for the DTI analyses and also for the comparisons of cognitive outcome.

5.6 Limitations of the studies

It would have been preferable if time from injury to the first MRI scanning was equal and as early as possible post-injury. Even though we managed to do an early MRI in a high rate of patients, an even higher implementation rate in the potential eligible study population would have been advantageous. However, this is difficult to obtain in a clinical cohort due to both medical and logistic reasons.

The use of two different scanners at the study hospital (Siemens Avanto or Siemens Symphony), and also the fact that some were scanned at local hospitals with another scanner (15 patients in paper III) is a limitation. Even though we had an age limit of 70 years and sought to exclude the patients with obviously non-traumatic injuries, we cannot fully exclude that some non-traumatic lesions have been included in the analyses. We excluded a different number of patients due to the abovementioned reason in paper I and III, since we after paper I were criticized for the exclusion process. A study better describing the etiologies of different lesions would be warranted since some of the lesions probably are not traumatic. The placement of the

ROIs in the ADC maps in corpus callosum can vary slightly, since they were placed manually and we cannot exclude partially volume effects with the cerebrospinal fluid as some of the ROIs are placed near the ventricles [158].

In the DTI study it would have been preferable that the MRIs from the early phase also were performed in a 3 T scanner, and preferably the same scanner as used in the chronic phase [159]. Misregistration between the early MRI sequences and the chronic DTI images could introduce errors in the mean FA values, even though they were kept at a minimum since the early scan was used as a visual control in the registration process. Since MHLs are smaller than NHLs, they are probably more prone to registration errors. Differences in contrast in the FLAIR sequence could also introduce errors, since we had 3D acquisition in the chronic phase and 2D in the early phase. Some DTI studies advocate the use of absolute threshold masking to exclude voxels with FA values below a predetermined level, since these could be outside the target area [160]. It is, however, difficult to precisely determine a FA cut-off value, since the values could be substantially reduced due to injury in itself and not caused by being outside the white matter. In our study we also had a visual control that the ROIs were within the lesion.

The outcome assessments should preferably have been performed blinded for ID and clinical information in all papers [161]. In paper I and II, neuropsychological test results from the entire TBI group and the control group would have been preferable. The outcome analyses involved multiple testing in paper II and III, and we therefore applied corrections (Bonferroni). As earlier mentioned the study design and sample size of the outcome analyses in paper IV require that we interpret these results with caution.

6 MAIN CONCLUSIONS

- I. We found that NHLs in conventional MRI disappeared during the first 3 months after TBI; most importantly the brain stem lesions disappeared. MHLs attenuated first after 3 months. Only early MRI findings predicted clinical outcome after adjustment for other prognostic factors. Hence, valuable clinical information may be missed if MRI is performed too late after the TBI.
- II. During the first year after moderate and severe TBI, we demonstrated a slowly evolving disruption of the microstructure in normal appearing corpus callosum by analyses of the diffusion in the ADC map, most evident in the posterior truncus. The mean ADC values were associated with both global outcome and ability to perform speeded, complex sensory-motor action. Hence, corpus callosum showed evidence of microstructural injury after moderate and severe TBI, but this was first evident in the chronic phase.
- III. We found that the total loads of visible TAI lesions in corpus callosum, brain stem and thalamus in DWI and FLAIR were independent prognostic factors in severe TBI. Interestingly, number of cortical contusions in MRI and CT findings seemed to be more important for moderate TBI patients, even though many had TAI lesions in corpus callosum and / or brain stem.
- IV. We found a significant linear decline in white matter microstructural integrity following TBI with gradually decreased mean FA values from ROIs in white matter in healthy controls to ROIs in TBI patients without either NHLs or MHLs and further to transient lesions and finally lowest FA in persistent lesions. Hence, the detection of different TAI lesions from the early stage and their persistence into the chronic phase may indicate the degree of microstructural injury. In chronic DTI the addition of an early MRI for optimal lesion detection is therefore useful since the degree of microstructural injury differs from normal appearing tissue to different lesions.

7 FUTURE PERSPECTIVES

Even though both conventional and advanced MRI studies of TBI patients have evolved exponentially the last two decades, a lot of research questions remain to be answered in the future. Conventional MRI is still most used in the clinic, but surprisingly few longitudinal studies have been performed. It would clearly be interesting to know how FLAIR and DWI lesions develop during the short course of the first few weeks following TBI. The planned large scale CENTER TBI study (<https://www.center-tbi.eu/>) could give more answers on the prognostic significance of the different lesions. In our studies we found that both early MRI lesions in corpus callosum and thalamus in severe TBI had a high impact on prognosis, at least as comparable to TAI lesions detected in the brain stem.

It is also a remaining question if the standardized clinical classification of TAI should be revised to better reflect the prognostic significance of the different early MRI lesions. Should for instance thalamus lesions be included in such a classification, and what about unilateral versus bilateral injuries in different deep structures and their prognostic significance? Should brain stem lesions be further sub classified to better reflect the pathological mechanism behind the injury?

So far neither the Corticosteroid Randomisation after Significant Head Injury (CRASH) calculator (<http://www.trialscoordinatingcentre.lshtm.ac.uk/Risk%20calculator/index.html>) nor the IMPACT calculator (<http://www.tbi-impact.org/?p=impact/calc>) includes MRI variables when they estimate the prognosis following TBI. Probably will the most important prognostic acute or subacute MRI variables be included in such models in the future. Maybe the abovementioned EU multicentre study or the equivalent multicentre study from the United States, TRACK-TBI (<http://www.brainandspinalinjury.org/research.php?id=189>), could give some answers.

And what about the more advanced MRI techniques, such as DTI? During the last decade numerous DTI studies of TBI patients have been performed to detect TAI [121]. Even though some of these studies have small patient groups, they have found evidence of white matter injuries both in mild TBI [9] and even in minimal head injuries after repetitive blows to the head in contact sport athletes [162]. Due to the very high sensitivity of DTI for detecting TAI it may be questioned if clinically insignificant and / or reversible injuries are detected, and thus

contributing to medicalization rather than helping the patient. Numerous DTI studies have related their findings to different outcome measures, but multiple sources of errors makes it difficult to directly compare the different studies and therefore reach a clear conclusion [121]. In the future standardization across centers should be done, and this especially applies to study design, data acquisition parameters, type of MRI scanner, data analyzing techniques and use of specific and multiple outcome measures. Larger longitudinal studies with adequate statistical models that account for the complexity in TBI populations and the inter-individual differences will be essential for the evaluation of DTI as a prognostic biomarker in TBI in the future.

There exist multiple other new evolving imaging techniques in TBI research [163] and their significance will be interesting to follow in the future. The diffusion kurtosis imaging (DKI) that probably have even higher sensitivity compared to DTI [164], will be further evaluated in the future. The increased availability of different positron emission tomography (PET) techniques, such as PET/MR, will also be applied to TBI patients in the future. A recent study found that PET/MR could explain cortical dysfunction in patients with otherwise normal conventional MRI [165]. Since both of the two abovementioned techniques currently are available at our study hospital, our research group already has upcoming studies on these topics. So, there is a high potential for further exciting and hopefully clarifying research in the field of neuroimaging in TBI at our hospital.

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