ON THE PATHOPHYSIOLOGY OF IDIOPATHIC ADULT HYDROCEPHALUS SYNDROME
Energy Metabolism, Protein Patterns, and Intracranial Pressure

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## Contents

Abstract ........................................................................................................ 4
List of original papers ................................................................................. 5
Abbreviations .............................................................................................. 6
Introduction .................................................................................................. 7
  Idiopathic adult hydrocephalus syndrome ............................................. 7
  Diagnosis – and prognosis? ................................................................. 8
  Pathophysiology ..................................................................................... 9
  Differential diagnosis – or comorbidity? ................................................ 13
  Approaching the enigma - selected paths ............................................. 15
  CSF biochemical markers ................................................................... 15
    Glucose supply and metabolism ....................................................... 16
    Brain tissue oxygen tension ............................................................ 20
    ICP oscillations .................................................................................. 21
Aims .............................................................................................................. 23
Patients and Methods .................................................................................. 24
  Patients .................................................................................................. 24
  Controls ................................................................................................. 25
  Methods ................................................................................................ 25
    CSF biochemical markers ................................................................. 25
    Evaluation of gait ............................................................................... 25
    Surgical procedure and patient management .................................... 26
    Intracranial pressure ......................................................................... 27
    CSF hydrodynamics ........................................................................... 28
    Microdialysis ..................................................................................... 29
    Brain tissue oxygen tension ............................................................. 30
    B-wave analysis ................................................................................ 30
Results and Discussion ............................................................................... 32
  The pattern of CSF biochemical markers ........................................... 32
  Effect of ICP changes on glucose metabolism in deep white matter .... 37
  B waves – its value in diagnostic workup can now be evaluated ......... 42
  IAHS – the diagnostic challenge in view of pathophysiology ............. 46
Conclusions ................................................................................................. 47
Acknowledgements ..................................................................................... 49
References ................................................................................................... 50
Original papers ........................................................................................... 65
Abstract

The symptoms in Idiopathic Adult Hydrocephalus Syndrome (IAHS) – gait disturbance, incontinence, and cognitive deficit – correlate anatomically to neuronal dysfunction in periventricular white matter. The pathophysiology is considered to include a cerebrospinal fluid (CSF) hydrodynamic disturbance, including pressure oscillations (“B waves”), in combination with cerebrovascular disease. IAHS and Subcortical Arteriosclerotic Encephalopathy (SAE) show clinical similarities, which constitutes a diagnostic problem.

The aim of this thesis was to investigate biochemical markers in CSF, possibly related to the pathophysiology, and their usefulness in diagnosis, to investigate the effect of ICP changes on glucose supply and metabolism in periventricular deep white matter, and to present criteria for objective, computerised methods for evaluating the content of B waves in an intracranial pressure (ICP) registration.

CSF samples from 62 IAHS patients, 26 SAE patients, and 23 controls were analysed for sulfatide, total-tau (T-tau), hyperphosphorylated tau (P-tau), neurofilament protein light (NFL), and beta-amyloid-42 (Aβ42). In ten IAHS patients, recordings of ICP, brain tissue oxygen tension (PtiO₂), and samplings of brain extracellular fluid from periventricular white matter by way of microdialysis were performed, at rest and during a CSF infusion and tap test. Microdialysis samples were analysed for glucose, lactate, pyruvate, glutamate, glycerol, and urea. Patterns before and after spinal tap were analysed and changes from increasing ICP during the infusion test were described. The long-term ICP registration was used to evaluate two computerised methods according to optimal amplitude threshold, monitoring time, and correlation to the manual visual method.

In CSF, NFL was elevated in both IAHS and SAE patients, reflecting the axonal damage. In a multinominal logistic regression model, the combined pattern of high NFL, low P-tau and low Aβ42 in CSF was shown to be highly predictive in distinguishing between IAHS, SAE and controls. Analysis of microdialysis samples for glucose, lactate, and pyruvate showed, in combination with PtiO₂, a pattern of low-grade ischemia. After the spinal tap of CSF, the pattern changed, indicating increased glucose metabolic rate. During the infusion test, there were prompt decreases in the microdialysis values of glucose, lactate and pyruvate during ICP increase, but no sign of hypoxia. The values normalised immediately when ICP was lowered, indicating that the infusion test is not causing damage. One of the computerised methods, with an amplitude threshold set to 1 mm Hg, was shown robust in evaluating B wave content in an ICP registration. At least 5 hours registration time was needed.

The highly predictive pattern of biochemical markers in CSF indicates a possibility of identifying simple tests in diagnosing and selecting patients for surgical treatment. The results of microdialysis and PtiO₂ indicate low-grade ischemia in the periventricular white matter, which is ameliorated from CSF removal, and that glucose supply and metabolism are sensitive to short-term ICP elevations, thus proposing a link between ICP oscillations and symptoms from neuronal disturbance. A computerised method for evaluation of B waves is a prerequisite for evaluating the impact of pressure oscillations in the pathophysiology of IAHS.

Key words: hydrocephalus, biochemical markers, microdialysis, brain tissue oxygen tension, cerebrospinal fluid, B waves.
List of original papers


**Abbreviations**

AD - Alzheimer’s Disease  
ADDTC - Alzheimer’s Disease Diagnostic and Treatment Centers  
AHS - adult hydrocephalus syndrome  
ATP - adenosin-tri-phosphate  
Aß42 - beta-amyloid 1-42  
B% - B wave content in an ICP registration  
CPP - cerebral perfusion pressure  
CSF - cerebrospinal fluid  
CT - computed tomography  
DSM-III - the Diagnostic and Statistical manual of Mental disorders, 3rd edition  
DSM-IV - the Diagnostic and Statistical manual of Mental disorders, 4th edition  
DWMH - deep white matter hyperintensity  
ELISA - enzyme-linked immuno-sorbent assay  
IAHS - idiopathic adult hydrocephalus syndrome  
ICD-10 - International Statistical Classification of Diseases, 10th revision  
ICP - intracranial pressure  
MMSE - minimental state examination  
MRI - magnetic resonance imaging  
NFL - neurofilament protein light  
NINDS-AIREN - National Institute of Neurological Disorders and Stroke – Association Internationale pour la Recherche et l’Enseignement en Neurosciences  
NPH - normal pressure hydrocephalus  
PET - positron emission tomography  
P-tau - hyperphosphorylated tau  
Pito2 - brain tissue oxygen tension  
PVH - periventricular hyperintensity  
R-out - resistance to outflow  
SAE - subcortical arteriosclerotic encephalopathy  
SD - standard deviation  
SPECT - single photon emission computed tomography  
T-tau - total tau  
Xe CT - xenon contrast computed tomography
Introduction

IDIOPATHIC ADULT HYDROCEPHALUS SYNDROME

In 1965, Hakim and Adams described a syndrome with the now classic symptom triad of gait disturbance, urinary incontinence, and mental deterioration, and the radiographic picture of enlarged ventricular system. They noted a positive effect of shunting cerebrospinal fluid (CSF), despite a “normal”, i.e., not markedly increased, intracranial pressure (Hakim & Adams, 1965), and thus, named the syndrome normal pressure hydrocephalus (NPH). During the 40 years passed, the definition of NPH has been expanded to include any form of chronic, communicating hydrocephalus, and sometimes even a few non-communicating forms such as aqueductal stenosis (Bradley 2000). Since patients with hydrocephalus of different aetiology may present with a similar clinical picture, and they may all be treated with a ventriculoperitoneal shunt, this expansion of the definition seems inappropriate, and from a pathophysiological context, confusing.

As the pathophysiology differ in various forms of hydrocephalus, it is appropriate to distinguish the idiopathic NPH from communicating hydrocephalus with known aetiology. For example, the idiopathic form of NPH tends to present in the elderly (Krauss et al 1997), whereas patients with chronic communicating hydrocephalus from prior subarachnoid haemorrhage, meningitis, neurosurgery, or head trauma present in relation to the causing event. Some authors also state that shunting seems to be less successful (30-50%) in patients with the idiopathic form than in patients with a known cause of communicating hydrocephalus (50-80%) (Vanneste 1994), although this has never been confirmed in a clinical study.

The term NPH is misleading since the intracranial pressure (ICP) is slightly elevated compared to healthy individuals. The alternative term Adult Hydrocephalus Syndrome (AHS) was introduced by Ekstedt in 1989 as a more correct description (Malm et al 1991). However, the old name is still widely used in the literature. In this book, the term AHS is consequently used, also when referring to other works, where the term NPH is used. When referring to idiopathic hydrocephalus, the term Idiopathic Adult Hydrocephalus Syndrome (IAHS) is used.

A hallmark in the pathophysiology of AHS is the CSF hydrodynamic disturbance (Stein & Langfitt 1974, Børgesen & Gjerris 1982, Malm et al 1995), though the connection to the clinical picture is not elucidated. Several theories are based on the concept of white matter disease. The gait disturbance and the cognitive dysfunction are considered to be of subcortical origin (Bradley et al 1991a). The frontal horns are pathologically widened

IAHS as well as other hydrocephalic conditions are treated by insertion of a ventriculoperitoneal or ventriculoatrial shunt system. In IAHS, the rate of positive outcome after shunt surgery may reach as much as 75% with careful selection of patients (Malm et al 1995, Boon et al 1997, Bech-Azeddine et al 2001, Tullberg et al 2001).

The rate of response to shunt surgery reveals a diagnostic problem, in two ways. Several of the above described clinical and radiological properties of IAHS are also seen in cerebral white matter disorders of considered vascular origin, such as subcortical arteriosclerotic encephalopathy (SAE). In patients not responding to shunt surgery (under the condition of a functioning shunt), the problem can be a misdiagnosis, with SAE probably being the most frequent differential diagnosis. Other explanations are development of a concurrent disease, or that the patient at the time of surgery has reached an irreversible state (Malm et al 2004). On one hand, we wish, from thorough selection, to spare the non-responders from the risks associated with shunt surgery. On the other hand, with too strong selection criteria, we run the risk of denying possible responders their chance of improvement.

It is my hope that a better understanding of the underlying pathophysiology in IAHS, will lead to improved diagnostic and predictive measures in selecting patients for shunt surgery.

**DIAGNOSIS – AND PROGNOSIS?**

Despite more than 40 years of research, there is neither consensus on the definition of IAHS, nor on the diagnostic procedure. A recent German survey reveals a wide variability between different centers, which reflects the lack of consensus on the diagnosis of IAHS. Usually, computed tomography (CT) or magnetic resonance imaging (MRI) and CSF removal in some setting are involved in the diagnostic work-up in almost all centers. This reveals an agreement
on ventricular enlargement and CSF hydrodynamic disturbance as basic diagnostic prerequisites (Krauss and Halve, 2004).

Apart from diagnostic tests, great efforts are being made to find tests predicting outcome from CSF diversion by shunt surgery. Several of the methods being used are not clearly assigned as diagnostic or prognostic, and some are claimed to serve for both purposes. Clinically, a history of gait disturbance preceding symptoms of cognitive impairment and/or urinary incontinence has been noted as a positive predictive sign (Graff-Radford & Godersky, 1986, Vanneste 1994).

A typical CT scan should demonstrate an Evan’s ratio of minimum 0.32 (i.e., the maximal width of the frontal horns divided with the maximal transverse inner diameter of the skull), rounded frontal horns, flattening of the sulci on the convexity, and a low degree of periventricular and white matter lucencies (Wikkelsø et al 1986, Vanneste et al 1993 and 2000, Boon et al 2000). Utilising MRI, an increased velocity of pulsatile CSF in the aqueduct, “the flow voiding sign”, has been advocated as a supplementary test (Bradley et al 1986 and 1991b).

Demonstrating an increased CSF outflow resistance, or the inverse measure, a low conductance, is considered one of the hallmarks in diagnosis. Nevertheless, it is not used at all centra. In predicting outcome from shunting, both high predictability (Børgesen & Gjerris 1982, Tans & Poortvliet 1984, Boon et al 2000), and lack of predictive value are reported (Graff-Radford et al 1989, Malm et al 1995). In selecting patients for shunt surgery, some authors claim a positive tap-test, i.e., temporary improvement from a spinal CSF tap, at least in patients with non-typical clinical picture (Wikkelsø et al 1986). Others have shown that a negative tap test does not exclude positive response to shunting (Malm et al 1995).

The use of radionuclide-cisternography to demonstrate CSF outflow disturbance is also advocated. The picture of “ventricular filling and convexity block”, i.e., a persistent appearance of radionuclide in the ventricles and absence of radionuclide over the cerebral convexities at 24 hours after radionuclide application, is considered typical for AHS (Wikkelsø et al 1986, Larsson et al 1991). In a contradicting study, cisternography was no better than the combined clinical and CT criteria in predicting outcome to shunt surgery (Vanneste et al 1992).

**PATHOPHYSIOLOGY**

**Neuropathology**

Experimental studies in animal models support the theory of engagement of white matter damage in hydrocephalus. In a rat-model, with kaolin-induced hydrocephalus, progressive
damage to axons in the periventricular white matter, gradual death of oligodendroglial cells, astroglial hyperplasia, and microglial activation were seen (Del Bigio & Zhang 1998).

In an experimental study on kaolin-induced hydrocephalus and shunting in kittens, the white matter was found edematous, with reactive astrocytes and markedly reduced periventricular myelination. In shunted kittens, no edema was present. In these animals more myelination and greater recovery in the periventricular white matter was noted compared to the non-shunted kittens, but less than in the control animals. Histologically, decreased local cerebral glucose utilization in central white matter was seen in non-shunted hydrocephalic animals (Chumas et al 1994).

Neuropathologic studies in humans are mainly provided from cortical biopsies. These have shown arachnoid fibrosis in 50% and frequent pathological parenchymal changes (Bech et al 1997). Changes typical for Alzheimer’s disease (AD) and arteriosclerotic changes have been reported. However, these changes have not been shown to exclude positive response to shunt surgery (Bech et al 1997, Savolainen et al 1999, Golomb et al 2000, Silverberg et al 2002a). There is one comparative study showing significantly more changes of Alzheimer-type in biopsies from hydrocephalus patients than in age-matched autopsy controls (Del Bigio et al 1997).

The frequently cited autopsy study by Akai et al describes a rather heterogeneous group of seven patients with clinical and radiological signs of AHS, mostly secondary cases from various causes. The neuropathological findings included leptomeningeal thickening, vascular changes with multiple microinfarcts, arteriosclerosis, demyelination and loss of axons in white matter. Altogether, changes compatible with arteriosclerotic encephalopathy (Akai et al 1987).

The entry of MRI into the diagnostic arena yielded a need of defining the neuropathological correlates for the various changes noted in brain white matter. It has been

MRI image, revealing periventricular hyperintensities (PVI) as smooth halos around the enlarged ventricular horns, and moderate deep white matter hyperintensities (DWMH).
found feasible to divide the changes most frequently seen into two main categories: deep white matter hyperintensities (DWMH) and periventricular hyperintensities (PVH). In a comparative study of MRI and autopsy, the mildest changes of only a periventricular rim, were reported to include only moderate demyelination and no arteriosclerotic changes. In DWMH and more severe PVH, a varying extent of nerve fiber loss, reactive gliosis, lacunar infarctions, and arteriosclerotic vascular changes have been demonstrated (Fazekas et al 1993).

Several studies have reported more severe and more extensive DWMH and PVH in AHS patients compared to age-matched controls (Bradley et al 1991a, Kristensen et al 1996, Krauss et al 1997, Tullberg et al 2001, Corkill et al 2003).

The cerebrospinal fluid system

The main production site of CSF is the choroid plexus in the ventricles. CSF is actively secreted as ultrafiltration in the choroidal epithelium, flows through the ventricles and out through foramina into the subarachnoid space. The calculated mean formation rate in man is 0.35 ml/min or 500 ml/day (Ekstedt et al 1978). A smaller part arises as a bulk flow of the interstitial fluid in the white matter of the brain, probably secreted from blood plasma and produced by brain glucose metabolism. This fluid spreads mainly along axon tracts and perivascular spaces, and may play a role in clearing excretory products (Abbott 2004). The total production rate is considered constant and unaffected by age under normal conditions (Ekstedt et al 1978, Silverberg et al 2002b). Malm et al has found a normal production rate in patients with idiopathic intracranial hypertension and in AHS (Malm et al 1992 and 1995). Contradictory, downregulation of CSF production in AHS and in AD has also been described (Silverberg 2002b and 2003).

CSF is considered to be absorbed mainly through the arachnoid villi penetrating the superior sagittal sinus. However, a recent study has shown, that, in rest, 38% of CSF is assimilated in the spinal subarachnoid space. This portion is substantially increased from physical activity (Edsbagge et al, 2004). The rate of CSF absorption is proportional to the pressure gradient between the CSF compartment and the receiving compartment, where the resistance to CSF outflow (R-out) is the inverse of the proportionality coefficient between pressure and flow (Ekstedt et al 1978).

The physiology of entry to and clearance from the CSF space of macromolecules is poorly understood and the turnover mechanisms are probably variable. In healthy subjects, there is a rostra-caudal CSF gradient for albumin and monoamines (Blennow et al 1993, Malm et al 1994, Reiter 2003), but not for sulfatide (Freedman & Lekman 1997), tau protein, β-amyloidal (Blennow 2004), or neurofilament protein light (NFL) (Rosengren LE, personal communication).
In AHS, R-out is expected to be increased and the CSF outflow from the ventricles impaired, in spite of free communication through the aqueduct. A relation of the CSF outflow impairment to the ventricular enlargement is plausible, though the mechanisms for this have not been clarified.

**Cerebral blood flow**

The arterial supply in the white matter mainly consists of long medullar branches from the brain surface and, to a lesser extent, of perforating striate arteries from arterial cerebra media. These consist mainly of end-arteries, which explains why this tissue is sensitive to ischemia. Decreased cerebral blood flow in this area is frequently reported in IAHS, and a connection to the enlarged ventricles and increased R-out is proposed (Kristensen et al 1996, Momjian et al 2004, Tullberg et al 2004, Vorstrup et al 1987, Owler et al 2004).

The main methods to assess cerebral blood flow in white matter are based on labelled tracers as in single photon emission computed tomography (SPECT), positron emission tomography (PET), and xenon contrast computed tomography (Xe CT). In recent years, perfusion MRI has been developed for this purpose (Owler and Pickard 2001).

Several studies using SPECT have shown reduced global cerebral blood flow in IAHS (Vorstrup et al 1987, Kristensen et al 1996, Chang et al 1999 and 2000, Tullberg et al 2004), results that are confirmed in PET and MRI studies (Owler et al 2004, Momjian et al 2004, Corkill et al 2003). In studies of regional cerebral blood flow, the most affected areas were the frontal region (Graff-Radford et al 1989), frontal and temporal regions (Kristensen et al 1996), and frontal and basal regions (Tullberg et al 2004). On the contrary, Owler et al found in a PET study a decrease in cerebral blood flow in the deep gray regions of the thalamus, putamen, and caudate nucleus, but not in periventricular white matter (Owler et al 2004).

An influence of increased ICP on regional cerebral blood flow is demonstrated by a PET study during a CSF infusion test. From increasing ICP, cerebral blood flow in white matter was most reduced adjacent to the ventricles and progressively normalized with distance. The site of maximal reduction corresponded to a mean of 9 mm from the ventricular wall (Momjian et al 2004).

The attempts to show improvement in cerebral blood flow after CSF diversion are inconclusive, as are the attempts to correlate preoperative patterns of cerebral blood flow to response to shunt surgery. A reduced cerebral blood flow may also reflect the situation of reduced metabolic rate as a result of irreversible neuronal damage (Owler & Pickard 2001).
DIFFERENTIAL DIAGNOSIS – OR COMORBIDITY?

Vascular white matter encephalopathy

The terminology concerning syndromes of diffuse white matter lesions from vascular origin is confusing. The entity dates back to Binswanger’s original description from 1894, which designated the neuropathological picture. However, this condition was never expressed in more detail than white matter atrophy and hydrocephalus. Clinical criteria for Binswanger’s disease were later introduced by Bennett et al (Bennett et al 1990, Caplan & Schoene 1978). These include cognitive impairment and gait disturbance or incontinence in combination with vascular risk factors and radiological signs of vascular white matter changes.

The term subcortical arteriosclerotic encephalopathy (SAE) was introduced by Olszewski in 1962, as a neuropathological term, to describe “a form of cerebral arteriosclerosis in which vessels of the white matter and subcortical gray matter are affected predominantly”.

With the introduction of CT and MRI where white matter lesions were found more frequently than previously expected, in both symptomatic and asymptomatic subjects, a radiological description was needed. The terms leukoaraiosis or subcortical leukoencephalopathy were introduced to designate white matter areas of hypodensity on CT or hyperintensity on T₂-weighted MRI. Leukoaraiosis is reported to occur in 41-100% in patients with dementia of presumed vascular origin and in 21-100% in normal control subjects. The clinical significance of leukoaraiosis remains incompletely understood (Pantoni & Garcia 1995).

In the clinical context, the term vascular dementia has become widely used. At least five different systems of diagnostic clinical criteria are being used (DSM-III, ICD-10, NINDS-AIREN, DSM IV and ADDTC). These different criteria include different patient cohorts, which render heterogeneity in patient selection in studies (Pohjasvaara et al 2000). All include focal neurological signs on neurological examination. This may exclude patients with small-vessel subcortical vascular dementia, who frequently do not show clear-cut focal signs. From this reason, a modification of the NINDS-AIREN criteria for subcortical vascular dementia is proposed (Erkinjuntti 2002). The presence of extensive white matter changes, implying a co-existence of vascular disease and IAHS, has in several studies been reported in patients who benefit from shunt surgery (Bradley et al 1991a, Krauss et al 1997, Tullberg et al 2001 and 2002).

In this book, the term SAE is chosen for subcortical lesions of vascular origin. The term designates a clinical picture of cognitive impairment as the prominent symptom and a radiological picture of extensive white matter disease. Gait disturbances as well as other extrapyramidal symptoms are consistent with the diagnosis, but not necessarily focal neurological signs.
Reports of the neuropathological correlates of white matter changes from vascular disease are more numerous than those of AHS. The diffuse ischemic white matter disease is described as reduced number of oligodendroglial cells, reduced myelin content, fewer axons with fiber thinning and fragmentation, and increased number of reactive astrocytes. Degenerative vascular changes and hyaline fibrosis were frequent. No deposition of mature amyloid is noted within white matter, even when the amyloid load is marked elsewhere in the brain (Erkinjuntti et al 1996, Tanoi et al 2000, Englund 2002).

Apart from the above-mentioned neuropathological changes, marked accumulation of axonal transport proteins in the axonal bundles, indicating compromised axonal transport, has been demonstrated (Akiguchi et al 1997). In an animal model of global incomplete ischemia, demyelination was found to precede axonal damage. This suggests that the primary event in cerebral hypoperfusion is changes in oligodendrocytes and that changes in neurofilament follow (Kurumatani et al 1998).

The neuropathological description of SAE is to great extent similar to that of AHS, which, in combination with similar symptomatology, indicates a final common path of white matter damage in these syndromes.

**Alzheimer’s disease**

The diagnosis of AD is only definite on autopsy, showing neurofibrillary tangles, neuropil threads, and amyloid-containing senile plaques (Goedert 1993, Clark et al 2003). In contrast to SAE, AD is considered a homogenous entity and diagnostic criteria are more widely accepted. It is considered a neurodegenerative disorder, clinically characterised by a decline in several areas of cognition. Gait disturbance and extrapyramidal symptoms are common in advanced disease.

Pre-morbid diagnosis can be supported from CSF analysis, where low values of beta-amyloid$_{1-42}$ (Aß42) and high values of hyperphosphorylated tau (P-tau) is considered typical (Andreasen et al 1999, Blennow 1995 and 2004).

In biopsy studies in AHS, changes typical for AD are frequently noted (Bech et al 1997, Del Bigio et al 1997, Savolainen et al 1999, Golomb et al 2000). The coexistence of AD and IAHS is shown not to affect the results from CSF diversion negatively (Golomb et al 2000) and a study including clinically pure AD patients showed a trend in favour of shunt treatment (Silverberg et al 2002a).
**CSF biochemical markers**

The rationale for analysing CSF in a pathophysiological study on cerebral white matter, is the assumption of a close connection between brain parenchyma and CSF, through the extracellular fluid.

Neurofilaments are structural elements of the neurons, which are found mainly in large myelinated axons. They are composed of neurofilament proteins of different molecular weight and are important in maintaining the shape and size of axons. Among these, the presence of the light neurofilament subunit (NFL) in the CSF has been found to be useful as a marker for axonal damage in various neurological conditions, such as amyotrophic lateral sclerosis, multiple sclerosis, and subcortical vascular damage (Rosengren et al 1996, Tullberg et al 1998, Malmeström et al 2003, Norgren et al 2003 and 2004).

Sulfatide is the major acidic glycosphingolipid in the oligodendrocytes, forming the myelin sheath (Fredman & Lekman 1997). An increased concentration of sulfatide in CSF has been found in vascular dementia and is considered to reflect a demyelination process (Fredman et al 1992, Tullberg et al 2000).

The cytoskeleton protein Tau promotes the assembly and stability of microtubules by binding to tubulin (Goedert 1993). The highest values of Total-tau (T-tau) in CSF are found in disorders with the most intense neuronal degeneration, such as Creutzfeldt-Jakob disease, whilst a moderate to marked increase is found in AD. Thus, the level of T-tau probably reflects the intensity of the neuronal damage and degeneration. In AD, increased levels of an abnormally hyperphosphorylated form of tau (P-tau) are found in CSF. CSF P-tau levels are normal in conditions like Creutzfeldt-Jakob, which suggest that P-tau is not a marker for general neuronal damage, but reflects the phosphorylation state of tau (Blennow 2004).

β-amyloid is generated continuously as a soluble protein during normal cellular metabolism, and is found in CSF in two variants – the shorter β-amyloid$_{(1,39)}$ with 39 amino acids, and a longer form, β-amyloid$_{(1,42)}$ (Aβ42), containing 42 amino acids. The latter form is predominating in neuritic plaques found in AD. The CSF levels of Aβ42 are decreased in AD. The mechanism for this reduction is unclear. Reduced levels has also been noted in disorders like Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis, and multiple system atrophy, but not in acute ischemic stroke, why it cannot be regarded as simply a marker for neurodegeneration (Andreasen et al 1999, Blennow 2004).
**Glucose supply and metabolism**

In closing up to the area of interest, the periventricular white matter and its vulnerable content of myelinated axons, oligodendrocytes, astrocytes, and microglia, a few minutes on the subject of energy supply is justified.

Glucose is the essential metabolic substrate of brain, taken up into cells by facilitated diffusion. The existence of the blood-brain barrier, which comprises tight junctions between the endothelial cells, necessitates the transcellular transport of glucose from the blood to the brain through the endothelial cells. This transport is mediated by glucose transporter proteins. These are in the brain located at the luminal and at the abluminal membranes of the endothelial cells, in the perivascular endfeet of the surrounding astrocytes, and in neurons. The continuous supply of glucose to the neurons is thus facilitated even at low interstitial glucose concentrations.

Normally, there is a fixed stoichiometric relationship between oxygen consumption and glucose utilization (6 mol oxygen/1 mol glucose). Downregulation of glucose transporter expression during chronic inactivation has been shown in an experimental model (Duelly & Kuschinsky, 2001).

Blood glucose is taken up by astrocyte endfeet linking to the intracerebral capillaries. In the astrocytes, glucose is subject to rapid and dynamic turnover of glycogen formation and glycogenolysis/glycolysis. Energy supplies for neuronal use consist of glucose and/or lactate released to the extracellular space. Extracellular concentration of glucose is shown to decrease during neuronal activity but also in situations of low energy demand, such as anaesthesia, sleep, and hypoxia (Forsyth1996, Fillenz et al 1999).
An outline of the metabolism of glucose is shown on the previous page. Glycolysis is the anaerobic metabolism of glucose to pyruvate and lactate. It results in the net production of only 2 mol of ATP for each mol of glucose. Pyruvate can enter the aerobic tricarboxylic acid (TCA) cycle and produce 30 mol of ATP per mol of glucose via the mitochondrial oxidative phosphorylation cascade (Clarke & Sokoloff 1998).

Like neurons, central myelinated axons are critically dependent on a continuous supply of oxygen and glucose. A large proportion of the ATP synthesized from glycolysis and oxidative phosphorylation is used to maintain membrane potential in neural cells (Leppanen & Stys, 1997).

Glutamate is the main excitatory amino acid in the brain. When released into synaptic areas, glutamate stimulates astrocytes to release glucose or pyruvate to the extracellular space, in response to increased neuronal energy demand. This mechanism is probably restricted to gray matter, as synapses are scarce or non-existing in white matter. This is supported by a microdialysis study of experimental global non-total ischemia, showing a 25-fold increase of glutamate in gray matter, but only less than five-fold increase in white matter (Shimada et al 1993). A similar pattern was seen in one of the first microdialysis studies in human brain (Hillered et al 1990).

Microdialysis

The principle of microdialysis was initially described by Bito (Bito et al 1966) and Delgado (Delgado et al 1972). The technique as we know it today was developed by Ungerstedt (Ungerstedt & Pycock 1974, Ungerstedt 1991), and the first report on its use in human brain came in 1990 (Hillered et al 1990, Meyerson et al 1990).

The idea of microdialysis is to implant an “artificial blood capillary” into a tissue. The probe is constructed as a double-lumen catheter, which is constantly perfused with a fluid physiologically mimicking normal extracellular fluid. The distal end consists of a semi-permeable membrane, which allows substances of molecular weights lower than the cut-off of
the membrane, typically 20 kD, to diffuse from the interstitial fluid into the perfusion fluid inside the catheter. This fluid, the dialysate, is collected in microvials and available for analysis of low-molecular-weight substances.

The extracellular compounds most studied are the redox-state substances glucose, lactate, and pyruvate, and the tissue-damage markers glutamate and glycerol. In clinical use, analysis of these substances, and urea, can be performed bedside in the colorimetric CMA 600 analyzer (CMA Microdialysis, Solna, Sweden). The correlation between the CMA 600 analyzer and the gold standard of high performance liquid chromatography is shown to be good. In research, samples are often frozen pending analysis. Freezing the samples in –70°C for three months does not significantly affect the results achieved from the CMA 600 analyzer (Hutchinson et al 2000).

To interpret the concentration values obtained when analysing the perfusion fluid, one must take into account the issue of recovery, i.e., the concentration in the dialysate expressed as percent of the true concentration in the interstitial fluid. Recovery is dependent on the properties of the membrane, its length, and the flow rate of the perfusion fluid. The longer the membrane and the slower the perfusion rate, the higher the recovery will be (Roslin et al 2003). Brain tissue factors affecting recovery include interstitial diffusion characteristics (tortuosity), size of the interstitial compartment, transport capacity over the cell membrane and blood-brain barrier, turnover rate of compounds measured, and temperature. These parameters may change during monitoring from changes in blood-brain barrier permeability, ICP, edema formation, and gliosis formation (Hillered & Persson 1999, Boutelle & Fillenz1996). In IAHS however, the blood-brain barrier is shown to be intact (Wikkelsø & Blomstrand 1982).

In attempt to determine in-vivo recovery, Hutchinson et al by using the extrapolation-to-zero-flow method, has shown an exponential relationship between flow and concentration, given that all other conditions are stable (Hutchinson et al 2000). Other methods used in attempt to determine recovery, are the no-net-flux method and the reference-substance method. These methods are not suitable for repeated calibrations in the clinical setting. The endogenous compound urea is evenly distributed in body compartments, why the stability of the ratio between the CNS and subcutaneous values can be used to continuously verify that the probes are performing correctly (Ronne-Engström et al 2001).

Normal values of extracellular substances collected by microdialysis are, from ethical reasons, difficult to achieve. Efforts are made, by sampling in frontal cortex in patients operated for posterior fossa tumours (Reinstrup et al 2000). Roughly similar values were found in SAH patients, where the microdialysis catheter was inserted in “better” side, 1.2-1.5 cm below the cortical surface (Schulz et al 2000). In our study, the catheter was placed in deep white matter. No results from sampling in this area have been reported previously, why normal values are unknown.

The complication rate from this invasive method is reported to be very low, but cannot be excluded. The implantation trauma is considered negligible, as levels of dialysate
metabolites are demonstrated to stabilise within approximately 30 minutes (Hillered et al 1990). The effects from anaesthesia and extubation are longer, between 2 and 6 hours (Reinstrup et al 2000, Schulz et al 2000). A pathology study on sheep brain showed small haemorrhages in the catheter tract, and minimal immunologic reaction after seven days use. These changes may be considered as clinically non-significant (Whittle et al 1998).


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<th>Mild ischemia or penumbra</th>
<th>“Hyper-glykolysis” or early reperfusion</th>
<th>Manifest infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td>↑</td>
<td></td>
<td>↑↑</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>↔</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>Lactate to pyruvate ratio</td>
<td>↑</td>
<td></td>
<td>↑↑</td>
</tr>
<tr>
<td>Glutamate</td>
<td>↔</td>
<td>↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Glycerol</td>
<td>↔</td>
<td>↔</td>
<td>↑↑</td>
</tr>
</tbody>
</table>


In studies of image-verified cerebral ischemia in various conditions, a uniform pattern has evolved. A rise in lactate to pyruvate ratio indicates ischemia, though not necessarily irreversible. In more severe ischemia or infarction, there is also a decrease in glucose and a rise in glutamate and glycerol, which may be seen with some time-delay after the rise in lactate to pyruvate ratio. In mild-to-moderate hypoxia with preserved oxidative phosphorylation, lactate and pyruvate will both increase as a consequence of increased glycolysis, with a less pronounced increase of the lactate to pyruvate ratio (Persson et al 1996, Enblad et al 1996 and 2001, Cesarini et al 2002, Hutchinson et al 2002). A correlation
between glycerol levels and LP-ratio suggests membrane phospholipid degradation in ischemic areas, and impending manifest infarction (Hillered et al 1998, Frykholm et al 2001).

The impact of cerebral perfusion pressure (CPP) on glucose metabolism has been investigated in patients with severe brain injury. Usually, a CPP > 70 mm Hg is desired in neurointensive care. In brain tissue close to an evacuated mass, an unfavourable microdialysis profile was seen only with CPP falling below 50 mm Hg. With the dialysis probe in unaffected hemisphere, no difference in lactate or lactate to pyruvate ratio was seen even with CPP-values below 50 mm Hg (Ståhl et al 2001a, Nordström et al 2003).

**Brain tissue oxygen tension (PtiO₂)**

Cerebral oxygenation is determined by the oxygen offered to the brain (arterial oxygenation), the cerebral blood flow, and the rate of cerebral oxygen metabolism (Zauner et al 2002). Attempts to assess brain oxygenation have been made by several methods. Jugular venous oxymetry and near-infrared spectroscopy have the drawback of being less specific for regional changes. Techniques to measure tissue oxygen tension based on the polarographic Clark-type cell were developed in the 60’s and 70’s (Clark et al 1956, Cater 1960). In the 80’s, catheters for implantation into the human brain were evolved. Two catheters for tissue oxygen tension are commercially available.

In our study, we have used the Licox® system (GMS, Kiel, Germany). The Licox® microprobe allows oxygen to diffuse from the tissue into the inner electrolyte chamber of the catheter tube. Here oxygen is transformed at the polarographic Clark-type cathode, resulting in a current change proportional to the oxygen tension in the tissue. Since the PtiO₂ value is temperature dependent, the use of a cerebral temperature catheter for continuous measurement, connected to the monitoring device is advised.

The oxygen sensitive area of the probe in brain tissue is 7-14 mm², but tissue destruction around the tip can enhance the sampling area. Probe insertion causes a microtrauma that temporarily influences the PtiO₂. When inserted without the use of an introducer, stable measures are reached within two hours. Absolute values are shown to be variable when using more than one probe in the same patient, indicating variations in oxygenation that can be due to vicinity to the capillary mesh (Dings et al 1998, van den Brink et al 2000).

Using the standard equipment, the oxygen sensitive surface of the probe lies in white matter, at a fixed position 22 to 27 mm below the dura mater. Stepwise removal of the probe has shown a gradient of PtiO₂, with the lowest values closest to the ventricle (23.8±8.1), gradually increasing to the highest values in cortical tissue (33.3±13.3) (Dings et al 1998).

Several authors have, in animal models and in clinical trials on severe brain injury, found a correlation between PtiO₂ and cerebral blood flow as judged by cerebral perfusion pressure (CPP) (Maas et al 1993, Rossi et al 2000, Lang et al 2003, Reinert et al
2003) and by xenon computed tomography (Doppenberg et al 1998). A similar response to increased arterial oxygenation by increasing inspired oxygen content is noted in both animal and clinical studies, with a generally greater rise of PtO$_2$ in damaged tissue (Meixensberger et al 1993, Rossi et al 2000, Reinert et al 2003). In animal experiment, PtO$_2$ is shown to decline in response to increasing ICP and to vasoconstriction due to hyperventilation (Zauner et al 1993, Rossi et al 2000). An elegant study on subarachnoid haemorrhage by Khaldi et al demonstrated a close relation between PtO$_2$ and cerebral vasospasm as measured by microdialysis nitric acid (Khaldi et al 2001).

In clinical practice, PtO$_2$ is most frequently used in monitoring severe brain injury and subarachnoid haemorrhage. 15 mm Hg is considered as the lowest critical value for favourable outcome, though both depth and duration of cerebral hypoxia have a relationship with mortality (van den Brink et al 2000).

PtO$_2$ is affected by anaesthesia. Inhalation preparations, like isoflurane, are shown to increase tissue oxygenation, probably through vasodilatation and abolished brain oxygen regulation. On the other hand, the intravenous drug propofol has no effect on brain tissue oxygen reactivity (Swartz et al 2003, Hoffman et al 2000).

The Licox system is proved to be relatively safe, with no infection and only two minor intracranial haemorrhages occurring in a material of 101 patients (Dings et al 1998).

**ICP oscillations**

It seems plausible to presume a link between impaired neuronal function, cerebral blood flow, impaired CSF circulation, and ICP in IAHS. Mean ICP is slightly increased in IAHS, and is not significantly decreased from implantation of a shunt device (Malm et al 1995, Lundkvist et al 2001). Instead, short-lasting ICP peaks has attracted interest as a factor in pathophysiology.

The concept of B-waves was introduced by Nils Lundberg in 1960, as slow and rhythmic oscillations of ICP. The initial definition of a B-wave was a period of 0.5 to 2 minutes with an amplitude from discernible to 50 mm Hg. In order to obtain a high resolution, visualising even small variations in amplitude, Lundberg magnified the printed ICP recordings but did never define any lower limit (Lundberg, 1960). B-waves have been
described in a variety of situations such as traumatic brain injury and subarachnoid haemorrhage as well as in healthy subjects (Mautner-Huppert et al 1989, Edsberg et al 2004). The origin of these pressure waves is unknown, but a relation to fluctuations in blood flow has been proposed (Auer et al 1983, Newell et al 1992, Droste et al 1999, Lang et al 1999). A close association of the amount of B waves and the mean ICP in the registration has been noted (Newell et al 1992, Stephensen et al 2005).

Throughout the years, the original definition of B waves, given by Lundberg, has been modified. Some authors state that an amplitude exceeding a certain limit above the baseline ICP should be required to define a B wave (Crockard et al 1977, Cardoso et al 1989, Raftopoulos et al 1992), whereas others require B waves to be present for more than 10 minutes to qualify (Fishman, 1992). Some authors have proposed that the appearance of a single wave, i.e., the morphology of the wave, could be of fundamental importance (Raftopoulos et al 1992 and 1994). The recording time considered necessary for a reliable evaluation has also varied, from a few hours to several days (Graff-Radford et al 1989, Symon et al 1975, Crockard et al 1977, Pickard et al 1980, Newell et al 1992, Krauss & Halve, 2004). Czosnyka et al has designed a model for computer detection of “slow waves”, detecting waves in the ICP registration within the frequency limits of 0.05-0.0055 Hz, which corresponds to periods of 20 seconds to 3 minutes (Czosnyka & Pickard 2004, Czosnyka et al 2004). This latter description does not fulfil the definition given by Lundberg.

Most studies on B waves in hydrocephalus have been small and descriptive, and the method has not gained widespread acceptance, where to the necessity of invasive ICP monitoring probably has contributed. Until today, evaluation of ICP curves has been manually performed.

Still, the presence of B-waves as a predictor for outcome of shunt surgery in IAHS has been advocated by several authors, though the B-wave content considered required to be predictive for positive outcome from shunt surgery has varied. Suggestions vary from two hours in an overnight recording to 50% of the recording time (Børjesen 1984, Graff-Radford et al 1989, Krauss et al 1995, Bech et al 1997, Vanneste 2000).
Aims

• To increase the understanding of the pathophysiology in IAHS by using different approaches to investigate changes in periventricular white matter related to the CSF hydrodynamic disturbance.

• To evaluate the pattern of lumbar CSF biochemical markers for neuronal damage and demyelination in IAHS patients compared to SAE and elderly healthy subjects.

• To evaluate any correlation of CSF concentrations of NFL, T-tau, P-tau, sulfatide, and Aβ42 to preoperative clinical properties in IAHS patients.

• To investigate the effect on lumbar CSF concentrations of these markers from insertion of a CSF shunt device in IAHS.

• To evaluate whether the techniques of intracerebral microdialysis and brain tissue oxygen tension are applicable in research in IAHS patients.

• To investigate glucose metabolism and cerebral oxygenation in the periventricular white matter in IAHS patients, before, during, and after a CSF infusion and tap test.

• To assess whether there is any sign of brain tissue damage from the CSF hydrodynamic investigation.

• To present objective computerised methods for the analysis of B wave content in ICP recordings.
Patients and Methods

PATIENTS

**Paper II-IV**

Ten patients with clinical, radiological, and CSF hydrodynamic evidence of IAHS were included. The mean age was 69 years (range 55-78), eight men and two women. They all presented with gait disturbance as the first and major problem, followed by development of mild cognitive decline and/or urinary incontinence. MRI was performed in all patients, which revealed a communicating hydrocephalus with dilated ventricles, narrow sulci, and an open aqueduct. Significant ischemic lesions or extensive leukoaraiosis in deep white matter were not seen in any case. Diagnostic workup included, apart from MRI, clinical characteristics, routine laboratory tests, Minimental State Evaluation (MMSE) (Folstein et al 1975), and gait test.

The patients were monitored by means of ICP, microdialysis, PtiO₂, and standard neurointensive care monitoring over 30-32 hours. A lumbar CSF infusion test and a tap test were performed in the morning on the day after implantation of probes and catheters. All patients subsequently received an adjustable shunt device after a time delay of 1-2 months in order to avoid contamination. At a follow-up visit 3-6 months postoperatively neuroradiology (CT or MRI), MMSE, CSF infusion test and video recording of the gait were repeated. The B wave analysis in paper IV includes the first eight of these patients.

**Paper I**

This retrospective study included patients who received a shunt device during the years 1992 to 2002 under the diagnosis IAHS. Inclusion was restricted to patients whose CSF samples from both the preoperative and the postoperative investigation remained stored, and who gave their written informed consent to the analysis, leaving a patient cohort of 62 patients. The diagnosis IAHS required a clinical picture with a gait disturbance as the first and major symptom. Urinary incontinence and cognitive decline were optional. CT or MRI scan showed dilated ventricles without marked atrophy or extensive white matter changes. A typical CSF hydrodynamic profile including an increased CSF outflow resistance with normal or slightly increased ICP, or a positive tap test, were required in patients not showing a typical clinical picture. Precipitating events and other diagnoses explaining the symptoms were ruled out.
Twenty-six patients with a clinical picture consistent with SAE were chosen out of the patients referred on the suspicion of IAHS during the same time period, (Bennett et al 1990). These patients followed the same investigation procedure as described above, but were not operated on. All had a history of mental deterioration, gait disturbance, vascular risk factors, and radiology revealed extensive deep white matter changes.

**CONTROLS**

**Paper I**

The control group included 23 patients, without known psychiatric or neurological disorder, undergoing hip or knee replacement surgery. CSF samples were collected when applying spinal anaesthesia.

**METHODS**

**CSF biochemical markers – Paper I**

Concentrations of NFL in CSF were analyzed using a sandwich ELISA (Rosengren et al 1996). CSF T-tau was determined using a sandwich ELISA constructed to measure total tau, that is, both normal tau and P-tau (Blennow et al 1995). CSF P-tau was determined using a sandwich ELISA, constructed to specifically measure tau phosphorylated at Thr181 (Vanmechelen et al. 2000). CSF A 42 was determined using a sandwich ELISA, constructed to specifically measure A 42 (Andreasen et al 1999). The concentration of sulfatide in CSF was determined by an immunoaffinity procedure (thin layer chromatography B immunostaining) (Davidsson et al 1991, Fredman et al 1992).

**Evaluation of gait – Paper I**

Two independent observers studied video recordings of the IAHS and SAE patients. The recordings were evaluated according to the following three items: A; Step length: 2 = normal step, 1 = shuffling, but step longer than footlength, 0 = step shorter than footlength. B; Walk 10 meters: 4 = steady walk without support, 3 = no support, but unsteady gait, 2 = unilateral support, 1 = bilateral support, 0 = cannot walk 10 meters. C; Turning: 2 = normal, 1 = no
more than 3 steps, but unsteady, 0 = more than 3 steps. A patient with no gait disturbance reaches the maximum score of 8.

For the gait test, subjects were asked to walk 25 meters as quickly as possible. The task was repeated three times and the mean gait velocity was calculated (Malm et al, 1995).

Patients were considered to have improved from surgery if gait speed was increased with at least 10% and/or if gait score from video review was ≥ 2 scores better postoperatively, or reached maximum score.

**Surgical procedure and patient management – Paper II-IV**

Surgery for implantation of catheters was performed under general anaesthesia induced by barbiturate (thiopental) and maintained by inhalation of either isoflurane or sevoflurane. Insertion of the different catheters was guided from the preoperative MRI imaging.

An ICP transducer (Codman MicroSensor, Johnson & Johnson Professional, Inc., Raynham, MA) was inserted into deep white matter close to the frontal horn of the right ventricle, at a depth of 20-35 mm from the cortical surface. A CMA 70 microdialysis catheter (CMA Microdialysis, Solna, Sweden) was inserted into the same canal and to the same depth. In patients no. 3-10 a brain tissue oxygen tension catheter (LICOX pO₂ probe, GMS, Kiel, Germany) was used. Since the PtIO₂ probe required a special bolt for fixation, it was inserted into a separate burr hole, located frontal to the two other probes. At the end of the observation time, a CT scan was performed to confirm the position of the catheters and to rule out any complications. The tip of the ICP transducer was radiologically verified to be located 0-7 mm from the ventricular wall.
Patients and Methods

(A) Location of probes for intracranial pressure and microdialysis, into the same canal, frontal view. (B) Lateral view. Brain tissue oxygen tension probe is located frontal to the two others.

Implantation of the probes was completed before 11:00 a.m. for nine of the patients, one was completed at 1:00 p.m. Microdialysis samples from the brain were collected every 30 minutes during approximately 30 hours. During the infusion and tap test in the next morning, samples were collected every three minutes. The patients were monitored at the neurointensive care unit, where they were kept supine in bed, given an intravenous solution of buffered 2.5% glucose, and not allowed to eat or drink. External oxygen supply was avoided if possible.

Intracranial pressure – Paper II-IV

ICP was continuously recorded throughout the observation time. From the bedside monitor, ICP data were transferred as an analogue signal and recorded to a personal computer (Power PC 7600, Apple, Inc., Cupertino, CA) by using a multimodal recording system including a data acquisition card, MIO16X50 (National Instruments, Inc., Austin, TX) and commercially available software (LabVIEW; National Instruments). The sampling rate was 100 Hz. The
samples were averaged over 1 second and subsequently analysed with a sampling rate corresponding to 1 Hz. The ICP recordings were analysed with a specially designed program, also developed in LabVIEW.

\textit{CSF hydrodynamics – Paper I-III}

The CSF hydrodynamic investigation was performed as a constant pressure infusion test followed by a tap test. Two needles were inserted in the L3-4 interspace while the patient was in the sitting position. Free passage was ascertained by aspiration of 5-10 mL CSF, constituting the sample saved for analysis. The amount was replaced with artificial CSF before start of registration. The patient was then placed supine with the zero-pressure reference level at outer meatus. Drainage of CSF and infusion of artificial CSF were performed with a peristaltic pump. The management of data acquisition and pressure regulation through pump control were performed with software and an electronic control unit developed at Umeå University.

The resistance to outflow (R-out) of the CSF pathways was determined by applying a pressure level to the CSF space while recording the resulting rate of inflow of artificial CSF into the patient. In clinical routine, equilibria of pressure and flow are obtained at six different levels of CSF pressure. The levels are increased in steps of 0.5 kPa and kept stable for approximately 5-10 minutes. There is a straight-line relationship between pressure and flow. The slope for the pressure-flow values (i.e., the regression coefficient) is equal to the conductance, and inverse to R-out.

In the studies described in Paper II-III, the infusion test was modified. Only two or three pressure levels were applied, including levels of 35 and 45 mm Hg. These pressure levels were kept stable for at least 10 minutes each, in order to ascertain that microdialysis samples could be linked to individual pressure levels. The procedure was completed by a tap test, where CSF was drained to a pressure level close to zero. This usually means drainage of approximately 40 mL CSF (Lundkvist et al 2001).

As clinical routine, the CSF infusion test is repeated 3-6 months after shunt surgery, in order to confirm that the shunt is working properly.
Patients and Methods

Simultaneous registrations of lumbar and intracranial pressure during the infusion and tap test.

**Microdialysis – Paper II-III**

A CMA 70 microdialysis catheter with a 10-mm semipermeable membrane and a cut-off of 20 kDa (CMA Microdialysis, Solna, Sweden) was used. The microdialysis system was perfused with Perfusion Fluid CNS (CMA Microdialysis, Solna, Sweden) at a flow rate of 2 μL/min.

Microdialysis samples from the brain were collected every 30 minutes from end of surgery on Day 1, until the infusion and tap test was performed in the morning Day 2. During this procedure, brain microdialysis samples were collected every 3 minutes. Afterwards, sampling every 30 minutes continued for another 5-7 hours.

Additionally, in patients 3-10, a subcutaneous microdialysis catheter CMA 60 (CMA Microdialysis, Solna, Sweden) was inserted in the abdominal wall as reference. This catheter was perfused with Ringer solution at a rate of 0.3 μL/min. Samples were collected every 60 minutes throughout the observation period.

In Paper II we chose to analyse and compare samples collected before and after CSF infusion and tap test. Samples collected at 2:00-4:00 p.m. on Day 1 were compared with samples collected at the corresponding time on Day 2. The same time intervals were chosen both days in order to avoid the influence of any diurnal variation. Every sample reflects the mean concentration of extracellular metabolites during the previous 30 minutes, delayed by 2.5 minutes, which is the dead-volume time needed for the dialysate to reach the microvial. The samplings collected at 2:00-4:00 p.m. thus reflects the brain metabolism during 1:28-3:58
p.m., which on Day 2 corresponds to the interval of approximately 3.5-5.5 hours after the removal of 40 mL CSF.

In Paper III, microdialysis samples collected during the CSF infusion test is analysed. During baseline, with the patient in supine position and before manipulating the CSF system, data are presented as mean values of samples collected. During raising and lowering of the intracranial pressure the last sample from each pressure level was analysed, and finally one sample 15-30 minutes after completing the hydrodynamic procedure. Dead-volume time from catheter tip to sample vial is calculated to 2.5 minutes. As each pressure level lasts 10 minutes or more, and samples were collected every 3 minutes, the samples analysed are considered to be representative for the pressure level in question.

The samples were frozen at –80°C pending analysis. After thawing, samples for all patients were analysed for glucose, pyruvate, lactate, glutamate, urea, and glycerol as one batch, using the enzymatic colorimetric method of CMA 600 microdialysate analyser (CMA Microdialysis, Solna, Sweden).

**Brain tissue oxygen tension – Paper II-III**

In patients 3-10, a brain tissue oxygen tension catheter (LICOX pO₂ probe, GMS, Kiel, Germany) was used. PtO₂ was continuously recorded throughout the observation time, and data was recorded in the above-described multimodal system. In Paper II, we compared the mean ICP and the mean PtO₂ during 1:28-3:58 p.m. on Days 1 and 2 in order to find any correlation to metabolic changes. In Paper III, PtO₂ data are presented as mean values during each pressure level in the CSF infusion test.

**B-wave analysis – Paper IV**

ICP was recorded continuously from approximately 12 a.m. to 8 a.m. the next day (range 17.5-20.8 hours). During the recordings the patients were lying in bed awake or asleep.

The B wave content (B%) was defined as the accumulated time with B waves divided by the total monitoring time. A manual estimation of B wave content (B%\textsubscript{VISUAL}) was performed according to the method of Lundberg (Lundberg, 1960), who defined B waves as oscillations in the ICP that had a period of 0.5 to 2 minutes and amplitudes from discernable levels to 50 mm Hg. The ICP recordings were printed on a paper with the axis corresponding to the same speed (5 mm/minute) and amplitude (4 mm = 1 mm Hg) as Lundberg used in his thesis. Two neurologists independently reviewed the recordings without knowing to which patient each recording was related. If there was any interpretative disagreement, the reviewers conferred and reached an agreement.
The $B\%_{VISUAL}$ was compared with the B wave content that had been determined using two different computerised methods. The technical aspects of each computerised method are described in detail in Paper IV, figure 1. Briefly, the computerised Method I was an individual wave analysis. The recording time with all waves that had a period of 0.5 to 2 minutes and that reached a defined threshold amplitude were accumulated (Paper IV, figure 1b). The threshold amplitude ($P_1$) was varied between 0.25 and 4 mm Hg. The corresponding $B\%_I$ (i.e., accumulated time with B waves divided with total monitoring time) was noted for each threshold value.

Computerised Method II was a power analysis. The software was used to assess the local B-wave content by calculating the RMS amplitude for 10-minute blocks of filtered ICP data (Paper IV, figure 1c). The $B\%_{II}$ for the total monitoring time was calculated as the sum of all time intervals in which the RMS amplitude was larger than the predefined $P_{II}$ divided by the total measurement time.

The $B\%_{VISUAL}$, $B\%_I$, and $B\%_{II}$ values were determined over the full monitoring time for all patients. In addition, $B\%_I$ and $B\%_{II}$ were determined at shorter monitoring times (1, 2, 5, and 10 hours). For each patient at each monitoring time, these assessments were repeated 20 times in randomly chosen starting points within the total monitoring curve. The mean values and SDs of $B\%_I$ and $B\%_{II}$ were calculated for each monitoring time. Additionally, $B\%_I$ was determined at 1, 2, 3, 4, 5, 10, and 15 hours, with the same starting point at the beginning of the ICP registration, for all eight patients, in order to analyse the effect of monitoring time.
Results and Discussion

We have used different approaches in trying to understand the pathophysiology of IAHS. One way to get in touch with the region of interest, the periventricular white matter, is by analysing CSF. As CSF is in close contact with brain tissue, its patterns of biochemical markers may reflect metabolic events in the brain. The technique of microdialysis allows us to proceed even further into this concealed area. We are now provided with a tool to investigate “the missing link” between CSF disturbance in terms of ICP peaks and the neural impairment causing the patients symptoms. Finally, we present a method for evaluating these pressure oscillations.

THE PATTERN OF CSF BIOCHEMICAL MARKERS

The diagnostic procedure in IAHS and the methods used to predict response to shunt surgery are inconsistent between different centra (Krauss & Halve 2004). Quite a number of clinicians and investigators, struggling with this difficult diagnosis, hope for a simple and highly predictive test. A biochemical marker, diagnostic for IAHS, and/or excluding patients that will not respond to surgery, would fulfil the criteria for such a test.

In Paper I, we studied CSF concentrations of selected biochemical markers for neural degeneration and demyelination in patients diagnosed as IAHS and SAE, as well as in elderly, neurologically healthy controls.

Preoperative levels in IAHS

The preoperative values of NFL was highly elevated in IAHS and SAE patients, with a significant difference between SAE patients and controls. We also found a trend of higher NFL concentrations in IAHS patients with more severe symptoms, a finding which also was made by Tullberg et al (Tullberg et al 1998).
T-tau, P-tau, and Aβ42 all showed the same pattern – significantly lower concentrations in IAHS patients vs both SAE patients and controls. No difference between SAE patients and controls was found.

The levels of sulfatide did not differ between the groups, thus being of no help in distinguishing between the diagnoses. Previous studies show divergent results, from significantly higher levels in SAE compared with a mixed population of idiopathic and secondary AHS (Tullberg et al 2000), and no difference in the concentrations of sulfatide between healthy subjects and patients with extensive leukoaraiosis (Tarvonen-Schröder et al 1997).

Our finding of lower tau values is in contrast to previous studies, that showed higher T-tau values for IAHS patients compared to healthy controls and SAE patients, respectively (Kudo et al 2000, Tullberg et al 2000).

The discrepant findings in different studies (see table next page) may in part be explained by difference in patient and control group selection. This in turn may reflect the lack of consensus in definition and diagnosis of IAHS, the mix of idiopathic and secondary cases found in most series, as well as the use of different criteria for vascular disease. In our AHS cohort, only idiopathic cases were included. In the control group, vascular risk factors were found in almost the same extent as in the IAHS group, which probably is representative for the general population of this age in the northern part of Sweden.

<table>
<thead>
<tr>
<th></th>
<th>IAHS patients, n=62</th>
<th>SAE patients, n=26</th>
<th>Controls, n=23</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFL, ng/L mean (SD)</td>
<td>854 (917)</td>
<td>1268 (1134)</td>
<td>395 (209)</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.01 (IAHS vs controls, SAE vs controls)</td>
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</tr>
<tr>
<td>Sulfatide, nmol/L mean (SD)</td>
<td>273 (106)</td>
<td>313 (94)</td>
<td>310 (100)</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-tau, ng/L mean (SD)</td>
<td>171 (68)</td>
<td>311 (105)</td>
<td>330 (179)</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.01 (IAHS vs SAE, IAHS vs controls)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-tau, ng/L mean (SD)</td>
<td>33 (10)</td>
<td>48 (22)</td>
<td>58 (29)</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.01 (IAHS vs SAE, IAHS vs controls)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-amyloid, ng/L mean (SD)</td>
<td>503 (103)</td>
<td>700 (127)</td>
<td>716 (170)</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.01 (IAHS vs SAE, IAHS vs controls)</td>
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### Results and Discussion

<table>
<thead>
<tr>
<th>NFL, ng/L</th>
<th>AHS/IAHS</th>
<th>White matter disease</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Tullberg et al 1998</td>
<td>864 (1538), n = 65 (IAHS = 21)</td>
<td></td>
<td>156 (81), n = 40</td>
</tr>
<tr>
<td>Rosengren et al 1999</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tullberg et al 2000</td>
<td>2252 (4084), n = 43 (IAHS = 21)</td>
<td></td>
<td>1111 (704), n = 19 (SAE)</td>
</tr>
<tr>
<td>Wallin &amp; Sjögren 2001</td>
<td></td>
<td>1316 (1218), n = 25 (SVD)</td>
<td>241 (166), n = 18</td>
</tr>
<tr>
<td>Sjögren et al 2001</td>
<td></td>
<td>1977 (1436), n = 9 (SVD)</td>
<td>156 (66), n = 20</td>
</tr>
<tr>
<td>Ågren Wilsson et al 2005</td>
<td>815 (893), n = 62 (all IAHS)</td>
<td></td>
<td>1268 (1134), n = 26 (SAE)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T-tau, ng/L</th>
<th>AHS/IAHS</th>
<th>White matter disease</th>
<th>Controls</th>
</tr>
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<tbody>
<tr>
<td>Blennow et al 1995</td>
<td>445 (195), n = 17</td>
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<td>185 (50), n=31</td>
</tr>
<tr>
<td>Tullberg et al 2000</td>
<td>265 (255), n = 43 (IAHS = 21)</td>
<td>139 (78), n = 19 (SAE)</td>
<td>138 (42), n = 13</td>
</tr>
<tr>
<td>Kudo et al 2000</td>
<td>391 (66), n = 20 (IAHS not reported)</td>
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<td>Wallin &amp; Sjögren 2001</td>
<td>530 (399), n = 25 (SVD)</td>
<td>350 (187), n = 20</td>
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<tr>
<td>Sjögren et al 2001</td>
<td>605 (423), n = 9 (SVD)</td>
<td>375 (176), n = 18</td>
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<tr>
<td>Andreasen et al 2001</td>
<td>461 (280), n = 23</td>
<td>264 (102), n = 18</td>
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</tr>
<tr>
<td>Ågren Wilsson et al 2005</td>
<td>168 (66), n = 62 (all IAHS)</td>
<td>311 (105), n = 26 (SAE)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sulfatid, nmol/L</th>
<th>AHS/IAHS</th>
<th>White matter disease</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fredman et al 1992</td>
<td>307 (118), n = 20 (VAD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tarvonen-Schröder et al 1997</td>
<td>Mean 157-159, n = 23 (moderate and severe LA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tullberg et al 2000</td>
<td>206 (99), n = 43 (IAHS = 21)</td>
<td>766 (957), n = 19 (SAE)</td>
<td></td>
</tr>
<tr>
<td>Ågren Wilsson et al 2005</td>
<td>273 (102), n = 62 (all IAHS)</td>
<td>313 (94), n = 26 (SAE)</td>
<td></td>
</tr>
</tbody>
</table>

Review of CSF values for NFL, T-tau and sulfatide in AHS/IAHS, vascular white matter disease and controls. SVD = subcortical vascular dementia, VAD = vascular dementia, SAE = subcortical arteriosclerotic encephalopathy (clinical diagnoses). LA = leukoaraiosis (radiological diagnosis).
Patterns of CSF markers predicting the diagnosis

The most striking finding was the power of the combined pattern of NFL, P-tau and Aβ24 in distinguishing between the diagnostic groups. In a multinominal logistic regression model using NFL, P-tau, and Aβ42, all three biomarkers were significant in discriminating between the diagnosis of IAHS, SAE, and controls from each other. No correlation was found between the individual biochemical markers used in the model.

<table>
<thead>
<tr>
<th>Observed diagnosis, n</th>
<th>Predicted diagnosis, n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IAHS</td>
</tr>
<tr>
<td>IAHS</td>
<td>57</td>
</tr>
<tr>
<td>SAE</td>
<td>4</td>
</tr>
<tr>
<td>Controls</td>
<td>4</td>
</tr>
<tr>
<td>Overall percentage</td>
<td></td>
</tr>
</tbody>
</table>

A pattern of biomarkers predicting outcome after shunt surgery could, however, not be found. Still, the clear relationship between this pattern of CSF markers and the clinical diagnoses indicates a connection between these markers and the current diagnostic methods for IAHS.

Taken together with the discrepant findings concerning different CSF markers in previous studies, it is plausible to assume that a single diagnostic CSF marker does probably not exist. Our results indicate that the search for a combination of CSF markers may be more fruitful, in the effort to add a powerful tool into the diagnostic battery.

The levels of CSF markers changes from CSF diversion

An increased CSF concentration of NFL, being a marker of ongoing axonal damage, is consistently found in IAHS patients. A great deal of these patients improves from shunt surgery and we wanted to explore whether this improvement could be mirrored by a change in postoperative CSF.

As seen in the figure on next page, the postoperative concentrations of sulfatide, NFL, T-tau, P-tau, and CSF-protein were all significantly higher than the preoperative concentrations, while the concentration of Aβ42 was unchanged. In three of the four patients with non-functioning shunts, there was no increase in CSF-protein, sulfatide, T-tau, or P-tau after shunt surgery.

These results indicate that CSF flow in the cerebrospinal system is changed after insertion of a shunt. Assuming that the major CSF outflow from the ventricles takes place
through the shunt system, a decreased turnover of CSF in the lumbar space can be anticipated, leaving the possibility of an accumulation of macromolecules at this spinal level. The normalisation of low values of spinal neuropeptides after shunt surgery found by Poca et al, may reflect the same change in CSF turnover, and not biochemical improvement as suggested by others (Poca et al 2001). There are also indications of a disruption of the blood-brain barrier caused by an inflammatory reaction from the shunt system (Wikkelsø & Blomstrand 1982).

Light bars = lumbar CSF concentrations before shunt surgery, dark bars = concentrations at the postoperative control.

However, the unchanged concentration of Aß42 is confusing. In AD, Aß42 accumulates in senile plaques in the brain, which explains its decrease in CSF. A true decrease in CSF masked by accumulation in the lumbar space is a possibility, but seems less plausible since AD is not suspected in our patients. Also, the concentration of Aß42 is shown to be stable over time in patients diagnosed as AD (Andreasen et al 1999), which contradicts this explanation.

Another sign of possible relation between CSF flow and concentrations of macromolecules, was the finding of a significant correlation between sulfatide and R-out (left). This is in concordance with a previous study, showing correlation between monoamines and R-out (Malm et al 1994), but
contradicted in another study, where indirect methods for determining flow were used (Reiber 2003).

We have to conclude that the knowledge of protein turnover in the CSF system is poor, and that the knowledge of how the turnover is affected from changes in CSF flow from pathological processes or from the insertion of a shunt system, is even poorer.

**EFFECT OF ICP CHANGES ON ENERGY METABOLISM IN DEEP WHITE MATTER**

The symptoms in IAHS – gait disturbance, incontinence, and cognitive deficit – are all considered to be of subcortical origin, and from anatomical point of view can be explained from axonal dysfunction in periventricular white matter (Graff-Radford et al 1986, Bradley et al 1991b, Kristensen et al 1996). White matter changes of vascular character are common findings in radiological investigations (Tullberg et al 2001). A decrease in cerebral blood flow has been noted by several authors, using different methods (Vorstrup et al 1987, Kristensen et al 1996, Chang et al 1999 and 2000, Corkill et al 2003, Tullberg et al 2004, Owler et al 2004, Momjian et al 2004). This decrease can be interpreted as a consequence of mechanical compression from the expanding ventricles, but also as blood flow regulation in accordance with regional metabolic demands, depending on neural activity, or a reduced metabolic rate from reduction of the number of axons. A persistent reduction in blood flow after shunting may reflect irreversible tissue damage as a result of the process of AHS (Chang et al 1999, Owler & Pickard 2001).

An elegant study by Momjian et al, showed a maximum decrease of cerebral blood flow at a mean distance of 9 mm from the ventricular wall, when increasing ICP during infusion test (Momjian et al 2003). This location corresponds perfectly to the area investigated by microdialysis in the present study.

*Microdialysis – a mirror of cellular reactions –
– in combination with tissue oxygenation*

We studied the changes in extracellular concentrations of glucose and its metabolites lactate and pyruvate, the excitatory amino acid glutamate, glycerol as the marker of cell membrane breakdown, and urea as an inert compound. In eight of the ten patients, PtiO$_2$ was recorded simultaneously. Two situations were studied – the response to CSF removal (Paper II), and the response to temporary increase in ICP (Paper III).
Response to CSF removal

The results in Paper II reporting mean concentrations of the metabolites before and after CSF infusion and tap test are presented below. The baseline values before manipulation of the CSF system showed an increased lactate to pyruvate ratio, which is consistent with a disturbed energy metabolism. This mild increase is in concordance with findings from other studies where mild to moderate ischemia has been demonstrated (Persson et al 1996, Enblad et al 1996 and 2001, Shulz et al 2000, Cesarini et al 2002, Hutchinson et al 2002). After the procedure, there were significant elevations of lactate (p < 0.01) and pyruvate (p < 0.01) (Wilcoxon sign rank test). The lactate/pyruvate ratio was unchanged. Glucose and glutamate showed a trend towards lowering, though not significant.

Above. Mean concentrations for all ten patients on the day before (black bars) and 3.5-5.5 hours after CSF tap test (grey bars). Below. Corresponding mean PtiO₂ for each patient.

PtiO₂ was measured in patients no. 3-10 (left). At Day 1, the PtiO₂ was below 20 mm Hg in seven of the eight patients. After the infusion and tap test at Day 2, an increase in PtiO₂ was noted in five of the eight patients. Patient number 4 had a chronic obstructive lung disease, requiring external oxygen supply and aggressive treatment with bronchodilating
agents throughout the observation time, which explains the high oxygen levels.

Baseline PtO₂ levels were low in our patients as compared to previously published studies from human and experimental animal studies. However, these studies are all performed during general anaesthesia, and, in humans, in situations of trauma or subarachnoid haemorrhage (Dings et al 1998, Rossi et al 2000). No PtO₂ studies in non-sedated and non-traumatised human brains have been previously performed, to my knowledge.

These results may mirror a state of incomplete ischemia, which is ameliorated after CSF removal. Brain-tissue PO₂, being an indirect measure of microcirculation, showed a trend to rise after CSF removal. An increase in lactate and pyruvate and an unchanged, still elevated lactate to pyruvate ratio, has previously been noted in patients with subarachnoid haemorrhage and good clinical outcome. Though the explanation is not clear, an increased glucose utilisation rate or “hypermetabolism” must be considered (Persson et al 1996, Ståhl et al 2001, Cesarini et al 2002).

Glutamate was lowered after CSF removal, which may be understood as decreased ischemic damage. However, as white matter contains few or no synapses, excitotoxic mechanisms are not considered to be of importance in the development of ischemia in this area (Del Bigio et al 1998, Waxman & Ransom 1998). Furthermore, time for glutamate to reach baseline after surgery has been found to be 4-6 hours, which is longer than for any of the other studied metabolites in this series (Schulz et al 2000). Therefore glutamate may not have reached true baseline levels in the first samplings on Day 1.

Concerning glucose and its metabolites, the extracellular concentrations may be affected by anaesthesia and awakening at the time of sampling on Day 1. However, the same rise in lactate and pyruvate was seen, when using samples collected between 6.00-8.00 in the morning, immediately before the infusion test, as reference level.

**Left:** Grey bars = morning. Black bars = after spinal tap.

A shortcoming of using a combined infusion and tap test in this study is the necessity of increasing the intracranial pressure in order to calculate the CSF outflow resistance. From Paper III, it was found that the increased ICP did not cause permanent effects on metabolism.
and that there was no fall in PtO$_2$ during infusion. Therefore it can be assumed that the changes in metabolism reported in Paper II is secondary to the spinal tap.

**Response to temporarily increased ICP**

Results from microdialysis during the infusion test (Paper III) are shown below. When raising the intracranial pressure through infusion of artificial CSF, we found a drop in the

![Bar charts showing changes in various metabolites during infusion test](image-url)
extracellular concentration of glucose, lactate, and pyruvate. The following CSF drainage resulted in a rise in the same analytes, and there was a tendency to overshoot, with values after drainage being slightly higher than during baseline. Comparing the values during baseline to values at the highest pressure level, the drop in glucose, lactate, and pyruvate was significant ($p < 0.05$). Concentrations after drainage were significantly higher than corresponding values at the highest pressure level for all three glucose-related analytes ($p < 0.01$). The lactate to pyruvate ratio did not change significantly during the increase in ICP, but was significantly lower ($p < 0.05$) when comparing the value at drain to that at baseline. The concentrations of glutamate, urea, and glycerol did not alter during any phase of ICP change.

There was no significant decrease in oxygen tension during ICP elevation, indicating that the procedure does not induce hypoxia (left). After drainage, there was a significant rise in $\text{PtiO}_2$ compared to both the highest pressure level and baseline ($p < 0.05$).

One straight-forward assumption, is that the infusion test does not cause any permanent damage to the neurons from this short period of raised ICP, as the energy metabolism normalises with no time delay when ICP is lowered.

These results can be viewed from different aspects. The simplest explanation would be the plain mechanical one – the raised ICP causing decreased cerebral perfusion and subsequently a lower glucose supply in the periventricular area. Oxygen supply should not be the first limiting step even if there were periods of low perfusion, since this could be compensated by a greater arterio-venous oxygen difference (Gjedde et al 2002).

Biochemical interactions are seldom as simple as that. Another hypothesis could be that the raised intracranial pressure leads to impaired axonal transport and subsequently impaired neural activity. This leads to lower metabolic demand and decreased release of glucose, and possibly lactate, from astrocytes (Dienel & Hertz 2001). As less glucose and/or lactate is oxidised, pyruvate decreases as well. This interpretation is supported by studies on experimental glaucoma, where compromised axonal transport in the optic nerve as a consequence of elevated intraocular pressure has been reported (Quigley et al 1980, Pease et al 2000). To fully explain our results, there would have to be a net export of glucose from the extracellular space. This has to my knowledge not been shown.

A third possible explanation involves disturbance of glucose delivery from capillaries to neurons. This active, transport-protein mediated, multi-step procedure, is dependent on the participation of astrocytes. Astrocytes are shown to be vulnerable to reactive oxygen species, hypoxia, and acidosis. Water is taken up in astrocyte endfeet through aquaporins by osmosis. Therefore, in brain edema, the astrocyte pericapillary endfeet are the
first cellular elements to swell. The raised ICP could theoretically lead to dysfunction in glucose uptake and release, with consequences for axonal function (Chen et al 2003). This hypothesis is supported by a newly published experimental study in a rat kaolin-model (Kondziella et al 2003).

A dilutional effect from increased fluid content in the extracellular space, caused by the infusion test, is a possibility. This seems less likely, since the significant reduction in microdialysis concentrations is seen only in glucose and its metabolites lactate and pyruvate, and not in glutamate, urea, and glycerol.

When interpreting microdialysis results, we must bear in mind that our knowledge of brain metabolism is based on in-vitro studies of whole parenchyma specimens. Several factors may have an influence on the extracellular concentrations of metabolites, like transport mechanisms over cell membranes and mitochondria, the condition of the blood brain barrier, and whether mild ischemia affects enzymatic regulation. The knowledge of these mechanisms is evolving, but is still insufficient.

The rate of catheter recovery in this study is expected to be rather low, since a high perfusion rate was used. Therefore are the individual concentrations lower than in previous studies (Hutchinson et al 2000, Reinstrup et al 2000, Schulz et al 2000, Cesarini et al 2002, Nordström et al 2003, Roslin et al 2003). There is also a possibility for the recovery in our study to be affected, by the close contact of the semipermeable membrane with the pressure monitoring probe. However, since the patients are followed longitudinally and no interindividual comparisons are made, we consider the absolute values to be of minor importance. Additionally, the concentration of metabolites and amino acids differ between different areas of the brain. As most previous studies are performed in patients with different pathological conditions and with a juxtacortical placement of the microdialysis catheter, comparable normal values of human brain metabolism in deep white matter are lacking.

**B WAVES – ITS VALUE IN DIAGNOSTIC WORKUP CAN NOW BE EVALUATED**

Apart form the clinical and radiological presentation, which most investigators agree on, there is a great variety in diagnostic and predictive tests. In Paper III we see that the glucose metabolism is affected by temporarily increased ICP. Short-term pressure peaks are frequently found in ICP registrations from IAHS patients. Whether these are more common in these patients compared with healthy individuals is not elucidated. Another coupling may be that the periventricular white matter in IAHS patients, from vascular risk factors, is more vulnerable from ICP variations.

An ICP registration for evaluation of B waves is advocated by many authors as an important preoperative investigation. A high proportion of B waves is considered to anticipate response from CSF diversion and, hence, should be useful in selecting patients for
surgery. With the availability of modern technology, the advantages of a computerised analysis of the B wave content in ICP recording are obvious. A broad consensus concerning the interpretation of ICP recording, and an objective method for its assessment, is necessary to finally evaluate the importance of ICP oscillations in the pathophysiology of IAHS.

**Criteria to be fulfilled**

The original definition of B waves by Lundberg describes ICP waves with a period of 0.5 to 2 minutes and an amplitude from discernible to 50 mm Hg (Lundberg 1960). This has been considered too vague to be useful even in clinical practice, why it has been modified in different ways. It is certainly not applicable for a computerised method – the establishment of a defined amplitude threshold is necessary.

Firstly, for a B wave analysis method to be predictive, the pathological limit for the B% should be well removed from the upper and lower ends (0% and 100%). At the same time we expect patients with IAHS to experience increased B wave activity, and to fall into the upper half – the choice of an optimal threshold was based on this prerequisite.

Secondly, the method should differentiate the population studied from other disease entities and from healthy subjects. A low variation within the target group will minimise overlapping with other groups. In this aspect, the lower threshold sensitivity and the corresponding lower variation of the individual wave analysis clearly favours Method I.

Third, the method should correlate with the subjective manual visual method of B wave activity estimation.

**Threshold dependence**

The two computerised methods described in this study are using fundamentally different ways of analysing data, though both are based on the calculation of B wave content in the same frequency range as in Lundberg’s definition.

The criteria of a minimum amplitude is explicitly fulfilled in the Method I, since variations are regarded as B waves, if the absolute value of its extreme point exceeds the chosen threshold value $P_1$. For the Method II, the threshold is set to a certain level of equivalent power in the frequency range. This is related to the root mean square of the waves amplitudes. The length of the wave package is not taken into account in the Method I; it investigates every single wave separately. In Method II the B wave content over a ten minutes interval is examined, and the mean value during that period is compared to a predefined threshold.
Below, the mean B%I and B%II are plotted against the different amplitudes (that is, P_I and P_II). With an increasing threshold, B% fell rapidly for all patients. The patterns of B%I and B%II as functions of the threshold values were similar but fell off faster for Method II. The threshold interval for B% (range between 75% and 25%) was 0.72 to 2.12 mm Hg for Method I and 0.83 to 1.63 mm Hg for Method II. The general patterns were quite similar for all patients. The differences between patients were largest in the threshold range of 0.75 to 2 mm Hg, with SDs of both B%I and B%II exceeding 10%.

**Manual methods compared with computerised analysis**

Even though the manual, eye-balling, method of evaluating B waves is vague and ill-defined, comparison with this “gold-standard” still has to be done.

Correlation analysis showed that the B%I correlated significantly with B%II for thresholds between 0.25 mm Hg and 3 mm Hg. The significant and highest correlation between the B%I and B%VISUAL was found at a P_I between 0.5 mm Hg and 1 mm Hg. A significant correlation was found between B%II and B%VISUAL for P_II of 0.5, 0.75, 3.0 and 3.25. However, B%II for the 3.0 and 3.25 thresholds was close to 0% for all patients.

Generally, the correlation between visual analysis and Method I was higher than the corresponding values for Method II.

**Monitoring time**

Another issue that has to be addressed, is the impact of total monitoring time. In Paper IV, we have calculated the standard deviations (SD) corresponding to a monitoring time of 1, 2, 5 and 10 hours for computerised Method I and II. The SD of B%I and B%II increased significantly (analysis of variance, p < 0.001) at shorter monitoring times. SD of the B% estimates was significantly lower for Method I than for Method II for monitoring times of 1, 2 and 5 hours (p < 0.05) and close to significant at 10 hours (p = 0.11). Due to the total monitoring time of 17.5-20.8, the 10 hour registrations are to a great deal redundant, why the true SD probably is wider than we calculated. An alternative way to determine the
shortest reliable monitoring time from our data is shown below, which points to the same result. The shortest monitoring time can thus not be properly established from our results, but we can conclude that samplings of shorter duration than five hours give a less reliable result.

We have shown that a computerised method for analysis of B waves can be made reliable and convenient. This allows an objective evaluation of ICP registration, and hence a possibility to compare results from different studies and centra. The two methods described here produced similar results. However, we consider Method I, set to a threshold value of 1 mm Hg, to be the most accurate method replacing a visual analysis performed ad modum Lundberg.

This method has been recently used in a clinical setting of 55 patients with communicating and non-communicating hydrocephalus, out of which there were only 13 patients with IAHS (Stephenson et al 2005). This study confirmed our results, showing a mean B% of approximately 60%. However, it was not possible to evaluate the method as a predictive test, as the response rate from shunt surgery was very high (82%). In combination with the high B% noted, a much greater population would be necessary to prove true predictability.
IAHS – THE DIAGNOSTIC CHALLENGE IN VIEW OF PATHOPHYSIOLOGY

The diagnostic challenge may not be restricted to discriminate IAHS from SAE, as these diagnoses to great deal reflect a similar condition. In patient selection, the challenge should be restricted not only to improve results from shunt surgery by excluding patients who will not respond, but also to identify those patients who would respond, if given the possibility. Hopefully, a more thorough understanding of the physiologic and metabolic interactions in brain deep white matter will take us closer to this goal.

The pathophysiology of IAHS is more and more recognised as caused by a complex interaction between a CSF hydrodynamic disturbance and cerebrovascular disease, mainly affecting periventricular deep white matter. The symptoms are related to a dysfunction of the axons in the white matter, as indicated by high levels of NFL in CSF and indications of decreased cerebral blood flow in white matter. However, the decreased cerebral blood flow can reflect both the cause of the syndrome, and the consequence from lower metabolic demand from lesioned nerve fibres. To elucidate this issue, a study including simultaneous registrations of regional cerebral blood flow, regional energy metabolism, and manipulation of intracranial pressure is needed.

We found indications of a change in CSF macromolecule turnover from the disturbed CSF physiology included in the syndrome, but also from insertion of a shunt system. Further studies are needed concerning entry into and removal of protein molecules from the CSF system, in healthy subjects and in disease conditions.

In spite of normal or close to normal mean ICP, oscillations with frequent pressure peaks (i.e., B waves) are seen in ICP registrations in IAHS patients. The origin of these oscillations is unknown, and also their possible connection to the pathophysiology of IAHS – cause, consequence, or both? Theoretically, frequent ICP peaks over a long time could eventually cause axonal dysfunction and, eventually, persisting damage. The improvement from CSF diversion may be explained from neutralising these ICP peaks, thereby ameliorating the strain on energy supply and metabolism in periventricular white matter.
Conclusions

• Concentrations of NFL were elevated in CSF in patients with IAHS and SAE as a sign of neuronal damage in periventricular white matter.

• A combined pattern of high levels of NFL and low levels of P-tau and Aß42 in CSF separated IAHS patients, SAE patients and controls from each other.

• The CSF concentration of sulfatide correlated to R-out, indicating that high R-out may lead to demyelination and subsequently permanent neuronal damage.

• Levels of macromolecules in lumbar CSF were generally elevated after insertion of a shunt device, indicating a changed CSF macromolecule turnover.

• Lactate to pyruvate ratio was increased in extracellular fluid in deep white matter in IAHS patients, in accordance with an incomplete glucose metabolism.

• There was a prompt decrease in glucose metabolism but not in brain tissue oxygenation when ICP was increased, and a similarly prompt normalisation when ICP was lowered.

• The shown decrease in glucose metabolism during a period of increased ICP may serve as the missing link between the CSF hydrodynamic disturbance and the symptoms in IAHS.

• After CSF removal, lactate and pyruvate were increased, indicating an increased glycolysis rate.

• The techniques of microdialysis and brain tissue oxygen tension proved to be useful methods in IAHS patients.

• There was no sign of brain tissue damage from the CSF infusion test, as assessed by our methods.

• The content of B waves in an ICP registration was reliably analysed in the proposed computerised methods, compared to the traditional visual evaluation.
• An amplitude threshold value of 1 mm Hg was calculated optimal in identifying an IAHS patient cohort and is suggested for computerised methods to be used in future studies.

• In evaluating B waves, the ICP registration time should not be shorter than five hours.
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