CSF biomarkers in idiopathic normal pressure hydrocephalus

Diagnostics and pathophysiology

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For my mother. Because diagnosis makes a difference.

"The only reason for time is so that everything doesn´t happen at once"

Albert Einstein

ABSTRACT

Idiopathic normal pressure hydrocephalus (iNPH) is a disease of the elderly with enlarged ventricles despite a normal CSF pressure. Clinically, iNPH presents with gait- and balance disturbances, cognitive decline and incontinence. As the symptoms are reversed by shunt surgery, precise diagnostics is of essence. As of today, the etiology of the disease is largely unknown and specific diagnostic and prognostic tests are lacking.

The overall aim of this thesis project was to explore the diagnostic and prognostic potential of CSF biomarkers in iNPH. By measuring markers reflecting different pathophysiological aspects, we also wanted to elucidate underlying pathophysiologic mechanisms of iNPH.

In ***paper I***, we showed that NFL was elevated and amyloid precursor protein (APP)–derived proteins and tau proteins were lower in patients with iNPH than in healthy individuals (HI). Post-surgery, there was an increase of NFL, APP-derived proteins, p-tau, and albumin in ventricular CSF, whereas levels of MBP and T-tau decreased. In ***paper II*** the concentrations of all soluble forms of APP, all Aβ isoforms and APL1β28 were lower, whilst APL1β25 and APL1β27 were higher in CSF of iNPH patients compared to HI. No difference could be seen in biomarker concentrations between patients who improved after surgery and those who did not. In ***paper III***, iNPH patients had lower concentrations of tau and APP-derived proteins in combination with elevated MCP-1 compared to HI and the most important differential diagnostic disorders. A prediction algorithm consisting of T-tau, Aβ40 and MCP-1 was designed as a diagnostic tool showing high discriminating ability. In ***paper IV*** all soluble forms of APP and all Aβ isoforms were lower in both subcortical small vessel disease (SSVD) and iNPH in comparison to HI, albeit with a more pronounced reduction in iNPH. INPH and SSVD had elevated concentrations of NFL, MBP and GFAP compared to HI.

Our findings indicate that patients with iNPH have a CSF biomarker profile that distinguishes them from HI of the same age as well as from their mimics. The profile is characterized by a downregulation of APP-proteins, CSF biomarkers reflecting destruction to the white matter and astrocyte activation but no substantial cortical damage. Analysis of CSF biomarkers may provide an important tool for diagnosing patients with iNPH.

Keywords: Idiopathic normal pressure hydrocephalus, cerebrospinal fluid, biomarkers

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POPULÄRVETENSKAPLIG SAMMANFATTNING

Demenssjukdomar är ett växande problem såväl inom hälso- och sjukvården som för samhället i stort. De flesta demenssjukdomar är idag obotliga eller har mycket begränsad möjlighet till behandling. Normaltryckshydrocephalus (NPH), som ger drabbade patienter gång- och balanssvårigheter, kognitiv nedsättning och inkontinens, kan betraktas som ett demenstillstånd hos äldre där förloppet är potentiellt reversibelt. Patienterna har ökad mängd ryggvätska (hydrocephalus = vattenskalle) och kan behandlas genom insättandet av en shuntslang från hjärnans vätskefyllda hålrum till (vanligtvis) bukhålan, där överskottsvätskan kan tas upp av kroppen. NPH kan ibland förklaras av patientens sjukdomshistoria men en stor del uppkommer utan någon känd orsak, och benämns då idiopatisk NPH (iNPH). Hos den äldre befolkningen är iNPH vanligare än vad statistiken antyder och andelen som kommer till diagnos och får behandling med shunt är låg.

I denna avhandling har vi undersökt proteiner (= äggviteämnen) i ryggvätskan hos patienter med iNPH. Genom att studera dessa ville vi öka precisionen i diagnostiken och öka kunskapen om sjukdomsmekanismer för iNPH.

Vi har funnit att proteinerna i ryggvätskan karaktäriseras av ett specifikt mönster bestående av lägre halter av amyloid- och tauproteiner och ökning av vissa proteiner som speglar påverkan på hjärnans vita substans. Vi tror att detta kan förklaras av att den ökande mängden vätska bidrar till en försämrad cirkulation i hjärnvävnaden och som en följd av detta till en minskning av dessa proteiner. Det vita substansen och hjärnans stödceller är påverkade men hjärnbarken är enligt våra resultat inte påverkad i någon större grad. Vi tror att påverkan på hjärnans små kärl till viss del liknar den vid andra så kallade ”sub-kortikala” sjukdomar och detta pekar mot att det kanske finns fler individer som skulle kunna hjälpas av en shuntoperation än de som opereras idag. Proteinmönstret hjälper oss att skilja iNPH patienter från friska äldre och även från de viktigaste sjukdomarna som kan likna symptombilden vid iNPH och försvåra diagnostiken.

Det är vår förhoppning att resultaten kommer att bidra med nya pusselbitar för att förstå sjukdomsprocesserna vid iNPH och att denna kunskap kan hjälpa fler patienter till en säkrare diagnos, liksom till potentiell symptomlindring genom kirurgi.

LIST OF PAPERS

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

I. **Jeppsson, A**, Zetterberg, H, Blennow, K, Wikkelsø, C.

Idiopathic normal-pressure hydrocephalus- Pathophysiology and diagnosis by CSF biomarkers.

Neurology 2013;80:1385-1392.

II. **Jeppsson A**, Holtta M, Zetterberg H, Blennow K, Wikkelsø C, Tullberg M.

Amyloid mis-metabolism in idiopathic normal pressure hydrocephalus.

Fluids Barriers CNS 2016;13:13.

III. **Jeppsson, A**, Wikkelsø, C, Blennow, K, Zetterberg, H, Constantinescu, R, Remes A M, Herukka, S-K, Rauramaa, T Nägga, K, Leinonen, V, Tullberg, M.

CSF biomarkers distinguish idiopathic normal pressure hydrocephalus from its mimics.

Accepted for publication in Journal of Neurology, Neurosurgery & Psychiatry.

IV. **Jeppsson, A**, Bjerke, M, Hellström, P, Blennow, K, Zetterberg, H, Kettunen, P, Wikkelsø, C, Wallin, A, Tullberg, M.

CSF biomarkers highlight pathophysiological similarities and differences in idiopathic normal pressure hydrocephalus and subcortical small vessel disease.

Manuscript.

CSF biomarkers in idiopathic normal pressure hydrocephalus.

CONTENT

ABBREVIATIONS

Ab	Antibody
ACG	Anterior cingulate gyrus
AD	Alzheimer's disease
AED	Astheno-emotional disorder
ANOVA	Analysis of variance
APLP1	Amyloid precursor like protein 1
APP	Amyloid precursor protein
AQP-4	Aquaporin-4
ARWMC	Age related white matter changes
AUC	Area under the curve
Aβ	Amyloid beta
BACE1	β-site APP cleaving enzyme
BBB	Blood-brain-barrier
BD	Binswangers disease
BPH	Benign prostatic hyperplasia
CNS	Central nervous system
CV	Coefficient of variation
CSF	Cerebrospinal fluid
CSF TT	CSF tap test
CSF-OP	CSF opening pressure
CT	Computed tomography
DSI	Disease state index
ECF	Extracellular fluid
ECM	Extracellular matrix
ECS	Extracellular space of the brain
EI	Evans index
ELD	External lumbar drainage
ELISA	Enzyme-linked immunosorbent assay
EMD	Emotional-motivational blunting disorder
FLAIR	Fluid-attenuated inversion recovery

FTLD	Fronto-temporal lobar degeneration
GFAP	Glial fibrillary acidic protein
HI	Healthy individuals
IL	Interleukin
iNPH	Idiopathic NPH
ISF	Interstitial fluid
IQR	Interquartile range
LBD	Lewy-body dementia
LCSF	Lumbar CSF
LLOQ	Lower limit of quantification
LRG	Leucine-rich α2-glycoprotein
LTP	Long-term potentiation
MAb	Monoclonal antibody
MBP	Myelin basic protein
MCI	Mild cognitive impairment
MCP-1	Monocyte chemoattractant protein 1
MMP	Matrix metalloproteinase
MMSE	Mini mental state examination
MRI	Magnetic resonance imaging
MSA	Multiple systems atrophy
NFH	Neurofilament heavy chain
NFL	Neurofilament light chain
NFM	Neurofilament medium chain
NPH	Normal pressure hydrocephalus
OAB	Overactive bladder
PAG	Periaqueductal grey
PD	Parkinson´s disease
PD	Proton density
PDD	Parkinson's disease with dementia
PET	Positron emission tomography
PFC	Prefrontal cortex
PMC	Pontine micturition centre
PSP	Progressive supranuclear palsy
RAS	Reticular activation system

RAVLT	Rey auditory verbal learning test
RCG	Rostro-caudal gradient
SAE	Subcortical arteriosclerotic encephalopathy
sAPP	Soluble amyloid precursor protein
SAS	Subarachnoid space
sNPH	Secondary NPH
SSCD	Somnolence-sopor-coma disorder
SSVD	Subcortical small vessel disease
SVD	Subcortical vascular disease
TIMP	Tissue inhibitor of metalloproteinases
ULOQ	Upper limit of quantification
VA	Ventriculo-atrial
VAD	Vascular dementia
VCSF	Ventricular CSF
WMC	White matter changes
VRS	Virchow-Robin spaces
VP	Ventriculo-peritoneal

1 INTRODUCTION

In 2015, it was estimated that 47.5 million people suffered from dementia worldwide, and numbers are thought to double every 20 years [1]. Regardless of numbers, each case of dementia is a burden not only to society, but a psychological and social burden to families, friends and not least to the afflicted person.

For adequate prognosis, planning and exploration of treatment options, an exact diagnostic method of the diseases causing dementia is of essence. Additionally, long term planning in terms of assistance from caregivers and society to the individual suffering from neurodegenerative disorders could improve the daily life of patients and caregivers.

Among the dementias, there are a few that are regarded as "reversible", including normal pressure hydrocephalus (NPH) [2]. NPH is a condition of the elderly with enlarged ventricles despite a normal CSF pressure. Clinically, the characteristic symptoms of NPH consist of gait disturbances, impaired balance, cognitive deterioration and incontinence, sometimes referred to as Hakims triad [2-4].

NPH has been known as a clinical syndrome since the neurosurgeon Salomón Hakim identified it in 1957 at Hospital San Juan de Dios in Bogotá, Colombia [5]. Not surprisingly, the finding was regarded with initial scepticism when Hakim showed that the symptoms of dementia could be reversed in hydrocephalic patients with normal CSF pressure, a phenomenon that was

previously thought restricted to dementia secondary to vitamin deficiencies and to endocrine disorders [2 3].

Hydrocephalus is divided into communicating and non-communicating where in the latter there is a blockage of CSF flow and in general a high pressure in the CSF. Herein I will focus on communicating hydrocephalus with a "normal" pressure (NPH).

NPH is classified as either secondary (sNPH) if there is a known cause, or idiopathic (iNPH). The secondary forms are seen following various kinds of brain trauma, subarachnoid haemorrhage, meningitis or stroke [6]. The idiopathic form is more elusive, with no definite aetiology being found as of today.

The aetiology of iNPH remains an enigma. We know that it is under-diagnosed and under-treated [7] but that the vast majority of cases of iNPH are improved by shunt surgery [8]. The focus of this thesis is to elaborate on how CSF biomarkers can aid in finding and diagnosing the patients that suffer from this disorder and by studying the biomarkers, helping us to understand a bit more of the pathophysiology at work.

2 IDIOPATHIC NORMAL PRESSURE HYDROCEPHALUS

The prevalence of iNPH has been difficult to assess accurately and thus the numbers have varied [9-12], perhaps due to that only a small minority of the patients are thought to be diagnosed and even fewer are being treated by shunt insertion [7]. Population based studies have estimated the prevalence of iNPH to be as high as 5,9 % in the population of 80 years and higher [12]. Probably, only about 20% of patients with the diagnosis are treated, possibly attributed to poor knowledge of the disorder and its treatment options.

The only method for managing the hydrocephalic state being used today is inserting a shunt, usually a ventriculo-peritoneal, or a ventriculo-atrial shunt [13]. Shunt surgery improves around 80 % of the patients [8]. If not treated, the patients condition will deteriorate. They will still improve after surgery, albeit to a lesser degree than if they had been operated early. The delay means loss-of-function that cannot be restored [14 15].

2.1 DIAGNOSIS

"The cardinal early features of normal-pressure hydrocephalus in our patients were a mild impairment of memory, slowness and paucity of thought and action, unsteadiness of gait and unwitting urinary continence. The symptomatology was unobtrusive, having no assignable date of onset, and evolved over a period of weeks or a few months" [3].

To diagnose iNPH, evidence are collected from clinical history, physical examination, and brain imaging. There are two different set of guidelines for diagnosing iNPH and also the procedure to diagnose according to these guidelines varies between centres [16] [17]. In this thesis, the International guidelines will be used.

The *clinical history* should focus on the mode of onset (insidious), its temporal course (progressive) and severity of symptoms. Diagnosing iNPH is further dependant on that no known factor, such as previous head-trauma, meningitis or intra-cerebral haemorrhage is explanatory of the condition (in that case, the term would be secondary NPH, sNPH). A close examination of other medical conditions is also of great importance since there are a number of diseases of the elderly that can easily be misinterpreted for iNPH. The presence of incontinence as well as its type and extent, should be explored.

In the *physical examination*, gait and balance should be tested. To diagnose iNPH, at least gait/balance disturbance should be present, accompanied by either impairment of cognition, or incontinence, or both. Retropulsion is often seen, either spontaneous or provoked. Cognitively, the patients are usually showing a slowing of thought, inattentiveness, apathy, and encoding and recall problems.

Using *brain imaging* (usually Magnetic resonance imaging, MRI), ventricular size can be measured. Evans index (EI) $\geq$ 0,3 is used as a cutting point for an increase in ventricular size as compared to cerebral matter [9]. EI is calculated by the maximum width of the frontal horns divided by the maximum inner width of the skull [9]. Further, imaging is used to secure that the aqueduct is

open (to rule out a non-communicating hydrocephalus) and to estimate the level of cortical atrophy. Other radiological biomarkers have been put forth, such as disproportionately enlarged subarachnoid space hydrocephalus (DESH) but there is no consensus on the application in diagnosis and prediction [18].

The lumbar CSF opening pressure (CSF-OP) should be measured and be within 5-18 mm Hg or 70-245 mm H_2O.

Clinically, there are a number of potentially difficult differential diagnostic challenges. The gait pattern in iNPH can be misinterpreted for, or affected by, Parkinson's disease (PD) (including atypical parkinsonian syndromes), arthritis of the joints, and polyneuropathy of different aetiologies. The affected cognition can sometimes be misinterpreted for other forms of neurodegenerative diseases, such as Alzheimer's disease (AD), subcortical vascular dementia (SVD), Parkinson's disease with dementia (PDD), Lewy body disease (LBD), other dementias or depressive disorders. Urinary incontinence can also be present in other neurological conditions such as post stroke but also as manifestations of primary urological disorders

2.1.1 GAIT

"The mechanism that allows a 6 foot tall human to walk on his two hind legs is imperfect but the nature of the imperfection has yet to be identified" [4].

The gait disturbance is usually the first symptom to become evident and it is often referred to as gait apraxia [4]. The hydrocephalic gait is characterised by

hypokinetic movement which in turn is composed of reduced stride length (albeit with greater variation), reduction of foot-to-floor clearance (due to insufficient extension of the knee) with a tendency to strike the ground flat whilst walking, and a "*disturbance of the dynamic equilibrium"* [19]. This latter component is evident by an enhanced step width and an outward rotation of the feet. The patients also lose balance whilst turning. The interstep variation is diminished, leading to inability to compensate for body sway. Slight reduction of the arm and trunk movement during walking is seen [19 20]. The gait has been described as being "glued to the floor" [3 20] or as "magnetic" [16] since the foot clearance is extremely low. Interestingly, these problems are restricted to the elevated patient. When in bed, normal limb movement is seen [4]. The gait is worsened as the symptoms progress in time, leading to the need of a wheelchair and eventually to immobility as truncal apraxia develops.

The gait disturbances are thought to be partially explained by impaired balance. The inability to compensate for body sways, was in Fisher's view attributed to *"a slowness in correcting a potential instability"* [4]. A possible distortion of visual input (visual axis), leading to a fast movement backwards, as if the body compensates for a fall forward has been suggested [21-23].

2.1.2 COGNITION

The patients show a slowing of the mind and are often seen as lacking initiative and as indifferent, what Fisher termed the *"abulic trait"* [4]. These traits are associated with as well subcortical as frontal types of dementia, suggesting a pattern of "fronto-subcortical dementia" [24]. Some evidence points to that

the fronto-subcortical deficit is manifested early in the process and in time becomes more of a global cognitive impairment as the syndrome progresses, highlighting the importance of an early diagnose [25]. Hellström et al have reported that iNPH patients seem more impaired in the fields of mental speed and executive functioning than actual memory disturbances [26]. The cognitive symptoms are preferably examined using neuropsychological testing [27].

Using organic psychiatry classification [28], iNPH patients initially suffer from astheno-emotional disorder (AED) a condition that is characterised by difficulties regarding concentration and memory, fatigue, irritability and/or emotional lability. As the disease progresses, emotional-motivational blunting disorder (EMD) (with apathy, emotional indifference and a lack of drive) develops, and might lead to, or coexist with, somnolence-sopor-coma disorder (SSCD) with impaired wakefulness, general slowing and dampening of cognitive, emotional, conative and motor processes [29]. Following surgery, the inverse order of symptom recovery is seen, and the latter responding the most favourable to the procedure [30 31]. The symptoms of SSCD have been linked to the ascending reticular activation system (RAS) [31].

Cognitive improvements are seen following surgery, especially in the most severely demented group [25], even though some evidence supports that iNPH patients still do not match healthy individuals of the same age [25 26 30]. Vascular comorbidity has been shown to worsen the cognitive performance [32] but the magnitude of improvement following surgery is not affected by vascular comorbidity [26].

2.1.3 INCONTINENCE

The neurological mechanism for the incontinence in NPH patients is thought of as an "uninhibited neurogenic bladder" [33]. This means that the usual central descending inhibition of the primitive reflex of contraction of the detrusor muscle is inhibited, leading the muscle to contract prematurely, resulting in urgency and frequent voiding.

Sakakibara et al [34], examining in detail the bladder dysfunction in iNPH found that storage symptoms were more prominent than voiding symptoms. More specifically, urinary urgency, nocturnal frequency, urgency incontinence, diurnal frequency, retardation when initiating urination, prolongation/poor flow, sensation of post-void residual, straining, and intermittency was seen. The authors argue that overactive bladder (OAB) is probably the initial manifestation of urinary dysfunction symptom in iNPH.

The incontinence is not always recognised by the patient, especially in advanced stages, which can be an indicator of a frontal executive dysfunction [16].

2.1.4 OTHER SYMPTOMS ASSOCIATED WITH INPH

Other symptoms frequently occur in iNPH patients. Among those are impaired wakefulness and an increased need of sleep (also a part of the symptoms in SSCD) which as previously reported responds well to shunt

treatment [31]. Moreover, paratonic rigidity, retropulsion, cerebellar signs and focal neurological signs are seen [21-23 35 36].

2.2 PREDICTION

Up to 80 % of patients improve by shunt-surgery [8] but there are still patients that do not respond to shunt treatment for reasons unknown. Being able to predict which individual patient that would not benefit from shunt placement would also mean that these patients could be spared the risk of brain surgery. Therefore, finding ways to choose the right patients for shunt-placement for iNPH has been the Holy Grail of iNPH research.

So far, the quest is quite disappointing. We do know that comorbidities, including heavy vascular co-morbidity do not mean that patients would not respond to shunt-placement [37 38]. We also know that patients with a long-standing symptomatology still respond to treatment [15].

To date, the method with the best sensitivity as to predict favourable outcome of surgery is assessing the clinical response to removal of CSF [39-42]. This is performed by the Tap-test (TT) or External lumbar CSF drainage (ELD). However, even if these tests can aid in the inclusion of patients eligible for surgery, a negative test does not exclude the possibility that patients still can benefit from shunt surgery. The sensitivity of the test for successful outcome of shunt surgery is around 75-92 % for the TT and 80-100 % for the ELD. The specificity is however 26-61 % for the TT and 50-100 % for the ELD [43].

Therefore, these supplementary tests can aid in the inclusion of patients for shunt surgery but cannot be used for exclusion.

There is a need for improvement of additional diagnostic tests for iNPH. The problem with the poor specificity in the tests used for prediction is that it will lead to under-diagnostics of patients that suffer from the disorder and that could benefit from shunt surgery.

3 CSF IN HEALTH AND INPH

Traditionally, CSF is thought to be mainly produced in the choroid plexus located in the ventricles, although some amount is thought to be produced in the brain parenchyma [44]. The choroid plexus is comprised of numerous villi protruding into the ventricles, lined with cuboidal epithelium. Beneath the epithelium, there are plentiful of arteries.

The CSF is said to derive from an ultrafiltrate of plasma and is (in healthy individuals) produced at a rate of approximately 0.34 ml/min or approximately 500 ml/day [45]. The formation rate of CSF has been shown to be relatively indifferent of CSF pressure [46]. In iNPH, the rate of CSF production is in the same range or slightly reduced in comparison to HI [47]. The total volume of the CSF in HI is reported at about 250 ml [48] of which about 80 ml is held in the spinal canal [49].

From its production site in the lateral ventricles, the CSF is said to flow through foramen Monroe and enters the third ventricle. From there, it enters the fourth ventricle via the aqueduct. From the fourth ventricle, the CSF enters the subarachnoid space (SAS) via the two lateral foramina of Luschka and the central foramen of Magendie [50] (Fig 1).

Conventionally, CSF is said to have its primary absorption site in the superior sagittal sinus, through the arachnoid villi, but there are also other routes of absorption, such as spinal absorption [51] as well as along blood vessels and cranial nerves [52].

Hakim explained the nature of iNPH by referring to the law of Pascal which states that *"the pressure applied to an enclosed fluid, is transmitted undiminished to every portion of the fluid and to the walls of the containing vessel"*. Applying this law, he argued that the fluid exerts a greater force on the ventricular walls despite the normal pressure in an enlarged ventricular system naming it the *hydraulic press effect*. Therefore, he argued, initially there probably had been a ventricular dilation secondary to an increased pressure but once the ventricles had been dilated, they were being held enlarged by the fact that a greater strain was being applied despite the pressure being normal [2 3]. As the production rate of CSF is within the same range as of HI [47], it is hypothesized that decreased CSF absorption could explain the excess amount of CSF in iNPH [53]. There seems to be a trans-capillary absorption presumably as a response to inadequate outflow [54]. In secondary cases (sNPH) there is a possible explanation for the reduced absorption in terms of previous bleeding to the SAS, and immune activation with inflammation. In iNPH, if the cause is impairment in outflow, the aetiology of the blockage is unknown.

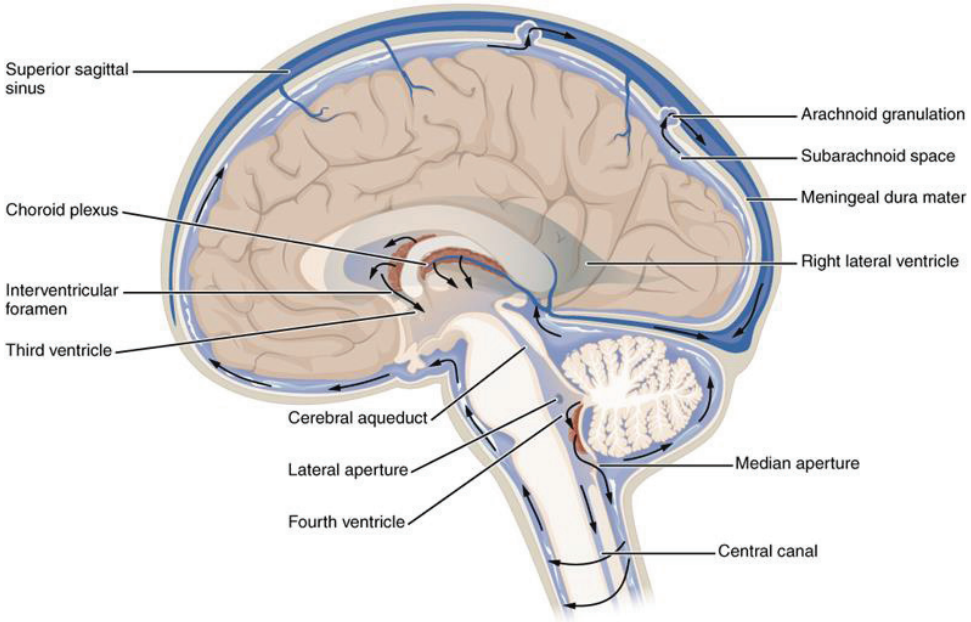

Figure 1. An overview of the traditional view of the CSF circulatory system

The CSF is thought to represent the fluid microenvironment of the extracellular space of the brain (ECS) as CSF is said to lies in direct contact with the ECS [44]. The extracellular /interstitial fluid (ECF/ ISF) is traditionally said to communicate with the CSF via periventricular (Virchow-Robin) spaces and the exchange is mediated through gap-junctions in the Pia and ependyma. The spaces are said to be in dynamic equilibrium [45 46]. By its contact, CSF regulates the composition of the ECF, providing it with nutrients, serves to clear metabolic waste from the interstitium and serves as a medium for chemical signalling within the brain. The CSF's purpose is further to serve as a shock absorbent fluid, protecting the brain floating in CSF from rubbing against the cranium and contributes to the regulation of intracranial pressure.

3.1 NEW VIEWS ON CSF AND ITS CIRCULATION

In recent years, much interest has been given to re-evaluating many of the traditional views on CSF, its production, flow pattern and absorption [55]. These new thoughts have influenced how to think about the CSF biomarkers ability to truly reflect direct parenchymal processes.

The glymphatic system is thought to be the brain's version of a solution to waste clearance, an analogy to the lymphatic system in the rest of the body and its proposed role is to regulate CSF-ISF interchange [56]. Its name derives from *glia* and *lymphatic* to indicate the importance of the astroglia cells for this system.

In this model, subarachnoid CSF recirculates through the brain parenchyma via paravascular spaces. The CSF flows via the Virchow-Robin spaces (VRS) surrounding the penetrating arterioles (extensions of the pial arteries) and is transported by bulk flow through the parenchyma to peri-venous spaces [56]. Newly discovered lymphatic vessels seem to surround the dural sinuses and drain into deep cervical lymph nodes and is a possible missing link in the understanding of the brains immune-surveillance system [57]. These lymphatic vessels have also been visualized in meninges in humans in vivo [58].

The water-transporting capability of the astrocytes is cardinal to the glymphatic system. The astrocytic foot processes cover the microvasculature [59]. Polarized Aquaporin-4 (Aqp4) channels in the astrocyte membrane are thought to facilitate water in- and outflow of the parenchyma and hence the

CSF-ISF interchange [60] [61]. This interchange is thought to decline with advancing age, possibly as a result of loss of Aqp4 polarization surrounding the penetrating arteriole secondary to reactive astrogliosis [62].

Sleep is thought to increase CSF-ISF turnover by expanding the extracellular space, thus allowing more CSF to enter the parenchyma [63]. This sleep-induced change in ECS is believed to be mediated by extracellular ion concentrations [64]. As the ISF/CSF recirculates and mixes in the parenchyma, it is proposed to clear metabolic waste, including Aβ by washing and hence "clean" the ECS [56].

In this view, the interchange of ISF and CSF would be more tightly regulated than previously thought. Also, the directed flow from the choroid plexus and eventually to the arachnoid granulations is challenged and is now thought to involve a more to- and fro pattern directed by arterial pulsatility and respiratory rate which might also effect the direct ISF/CSF exchange in the peri-capillary spaces [55]. Further, ISF production site is coming into question, and is now thought to be, at least to a large extent, a product of capillary secretion [52].

Taken together, the view of concentrations of CSF biomarkers in lumbar CSF representing concentrations in ISF may be challenged and this discussion will continue. Nevertheless, there is a communication between ISF and CSF and as such, it is generally accepted that CSF biomarkers can be used as a way to study pathophysiological dynamics in the brains parenchyma.

4 CSF BIOMARKERS

"A biomarker does not substitute for a brain"
Martin Möckel

A biomarker is by definition *"a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"* [65].

CSF biomarkers are in this thesis viewed as a form of "chemical footprints" of on-going procedures in the parenchyma. They are used to extrapolate theories on cerebral physiology and pathophysiology since the content of fluid is thought to represent the condition of the microenvironment of the brain via the CSF [66].

CSF biomarkers offer a tool to be used in clinical practice in the aid of diagnosing different diseases leading to dementia as well as other neurological disorders. Today, this method is widely used in the diagnosis of AD [67] and is now a part of the diagnostic criteria [68].

For iNPH there are several areas of potential use for CSF biomarkers. Being one of the few disorders causing dementia that is to a certain extent reversible, the need for precise diagnostic methods for iNPH is of essence. Herein lies the need for biomarkers that are able to differentiate between iNPH and other types of dementia in the clinical setting and to provide a more solid foundation when planning for health care and social support for patients suffering from iNPH. There is also a need to understand the underlying

pathophysiologic mechanism, as well as the dynamic, reversible nature of the syndrome and its relation to symptomatology. Furthermore, there is an ongoing search for biochemical markers that could predict outcome of shunting in iNPH. Some progress has been made, but no marker has so far showed enough sensitivity and specificity to be of practical use in selection of candidates eligible for operation [13 69-78].

4.1 AMYLOID PRECURSOR PROTEIN-DERIVED PROTEINS AND THEIR HOMOLOGUES

In these studies, amyloid metabolism is studied using the derivates of Amyloid precursor protein (APP), soluble APP alfa and -beta (sAPPα, sAPPβ) and Amyloid β (Aβ)-fragments of different lengths, (Aβ-38, Aβ-40 and Aβ-42).

APP is a large, transmembrane protein [79 80]. It has a large extracellular domain and a small cytoplasmic tail. Full-length APP is cleaved by α- (ADAM10) or β-secretase (BACE1) [81] generating sAPPα ("the non-amyloidogenic pathway") and sAPPβ ("the amyloidogenic pathway") [82 83] respectively. Following α- and β-cleavage, intramembranous proteinolysis by γ-secretase generates Aβ-fragments of varying lengths from sAPPβ (Aβ-X, with the number X corresponding to the number of amino acids in the fragment) and p3 from sAPPα [83-86].

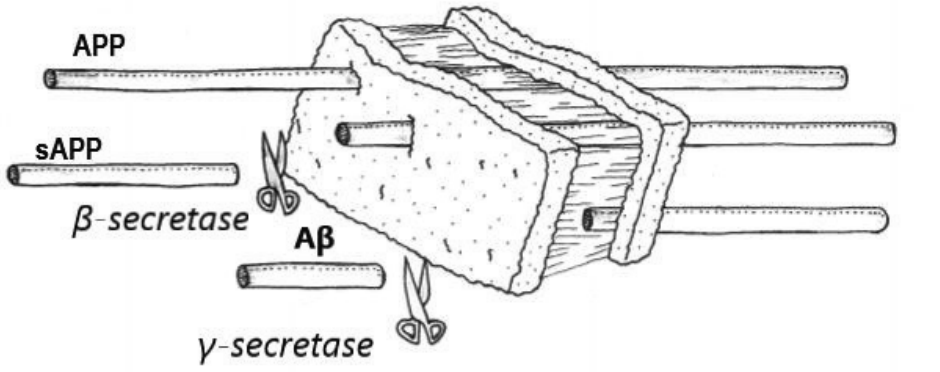

Figure 2. Enzymatic cleavage of APP in the amylogenic pathway is initiated when β-secretase cleaves the ectodomain of the transmembrane protein. Subsequently, γ-secretase generates Aβ of different lengths by intramembranous proteinolysis. Illustration by Jomi Jutlöv.

The amyloid hypothesis of AD states that an early, or initiating, event in AD is the alteration of Aβ metabolism [87]. An absolute or relative increase in the hydrophobic Aβ species Aβ42 and -43 (by increased production or reduced clearance) leads to the formation of amyloid plaques that are being deposited in the brain parenchyma. The main component of the amyloid plaques in AD is Aβ42 [88]. Lowered levels of Aβ42, or a reduced Aβ42/40 ratio in CSF are explained by the plaque deposits [87 89] which is supported from studies of APP-transgenic mice where there was an inverse relationship between plaque-burden and Aβ levels in ISF, not explained by a reduction in APP-production rate [90].

The burden of amyloid deposits increases linearly with age and is primarily located to precuneus, temporal cortex and anterior- and posterior cingulate [91]. Aβ oligomers inhibit Hippocampal long-term potentiation (LTP) in vivo and damage synaptic structures [83 92]. It is thought that it is rather the soluble oligomers (released from the plaques), not the plaques per se, that are synaptotoxic [92]. The cognitive decline in AD is not very well correlated to the amount of plaques but it is hypothesized that amyloid deposits may lead to downstream phenomena (such as activation of the innate immune system and the formation of tangles) leading to neuronal dysfunction in AD [92].

The physiological role of the evolutionary conserved APP-family is not yet fully understood. There are strong indications that processing of APP is important during brain development, synaptic functioning and dendritic formation. Both sAPPα, and –β seem to be important to synapse formation and might act as signalling molecules regulating neuronal growth and interaction [93]. Accumulating evidence suggests that sAPPα has a neuroprotective role and is important for synaptic plasticity, learning and memory (although the main receptor target is not known). One possible mechanism is by regulating NMDA receptor function, and thus LTP [94 95]. α-secretase cleaving, and subsequent increased level of sAPPα is increased by neuronal activity [79].

In rats with kaolin induced hydrocephalus, Aβ has been shown to accumulate possibly as an effect of down regulation of LRP-1, the main efflux transporter of Aβ over the blood-brain-barrier (BBB) [96 97]. The theory has been put forth that NPH and AD share a common pathophysiological aetiology in that

reduced clearance of Aβ would lead to AD-like pathology in iNPH-patients [97 98].

The APP homologue APP- like protein 1 (APLP1) is cleaved by the same enzymatic machinery as APP resulting in the non-amyloidogenic APLP1 derivates APL1β25, -27, and -28. [99-102]. Being processed by the same enzymes although not aggregating in plaques, the ratio of APL1β28/ total APL1β has been suggested as a marker for the relative production of Aβ42 to total Aβ. [100]. The ratio APL1β28/ total APL1β has been shown to increase in patients with AD, lending support to the notion of increased ratio of Aβ42/total AB as an underlying mechanism of AD [103].

4.2 TAU-PROTEINS

Tau binds to (mainly neuronal) microtubule, stabilising it and aiding its assembly of the protein [104]. In AD, tau becomes hyperphosphorylated, leading to microtubule instability and impaired axonal transport. Tangles, the other neuropathological hallmark of AD, has been shown to be made up mainly of fibrils containing aggregated hyperphosphorylated tau [87]. Tau is implicated in a large number of other neurodegenerative diseases [105]. In cortical neurodegenerative processes with axonal death, there is an outflow of tau into the CSF. Thus the level of tau in CSF reflects the extent of the damage to cortical structures [87 106].

4.3 BIOMARKERS OF WHITE MATTER DAMAGE

Neurofilament light (NFL) reflects large-calibre myelinated axonal damage [69]. More specifically, it is said to mirror the loss of intermediate filament protein that leaks through injured cell membranes of large, myelinated axons [70 106]. NFL has been used as a cerebrospinal fluid (CSF) biomarker reflecting neuronal death and axonal degeneration in several neurological diseases [107] but NFL is now regarded as a more general marker of neuronal degeneration [108] . Disorders with mainly cortical engagement do not typically exhibit high concentrations of NFL [109]. Higher concentrations have been associated with disease progression and NFL has been suggested as a disease-intensity marker, rather than a marker of a specific aetiology [110].

Myelin basic protein (MBP) is a membrane protein of oligodendroglia and comprises 30-40 % of the myelin in the CNS. Oligodendroglia cells wrap membrane processes around neural axons in the CNS which highly increases the speed of nerve conduction velocities [111]. Presumably, elevated levels of MBP in the CSF is due to leakage of MBP from the periventricular white matter [112] and elevated MBP in the CSF is an indicator of demyelination [45 70].

4.4 INFLAMMATION AND ACTIVATION

Chemokines and cytokines are regulators of the inflammatory system and are released by activated astro- and microglia cells in response to various

inflammatory threats to the CNS, including misfolded extracellular proteins and damaged synapses [113]. Activated microglia is seen in relation to amyloid deposits [114] but if the macrophage activity is aiding the recovery, or worsening the condition, is a matter of debate [115].

IL-8 is a chemo attractant, acting on neutrophils, but is also thought to act on migrating monocytes, together with MCP-1 contributing to firm adhesion of monocytes to vascular endothelium under flow conditions [116]. IL-8 binds to CXCR1 and CXCR2. Links have been established between IL-8 and the development of atherosclerosis [116].

IL-10 is an anti-inflammatory cytokine. In mice it has been shown that lack of IL-10 leads to more severe atherosclerosis, whereas increased levels of IL-10 show opposite effect, as well as decreased recruitment of monocytes [117].

Monocyte chemoattractant protein I (MCP-1) binds to the CCP2 receptor on migrating monocytes, and is involved in diapedes and migration of monocytes [115 117 118]. MCP-1 acts as a chemoattractant of astroglia [119] and is present in amyloid plaques, probably of microglia origin [119]. It is further a known marker of peripheral tissue macrophages [120] and also released from astro- and microglia in the CSF, facilitating the migration of macrophages [121].

Glial acidic fibrillary acidic protein (GFAP) is a protein synthesized in fibrillary astrocytes and increased concentrations is an indicator of acute damage to astroglial cells or a marker of astrogliosis [122 123]. The protein is the main component of the astroglial filament and the CSF concentrations increase with age [123]. YKL-40 is, in vivo, mostly associated with astrocytes and

is elevated in particular in diseases with CNS inflammatory origin but also in the healthy elderly. It seems as if elevated GFAP and YKL40 are indicative of reactive gliosis but more of acute, than in chronic stages of gliosis [124].

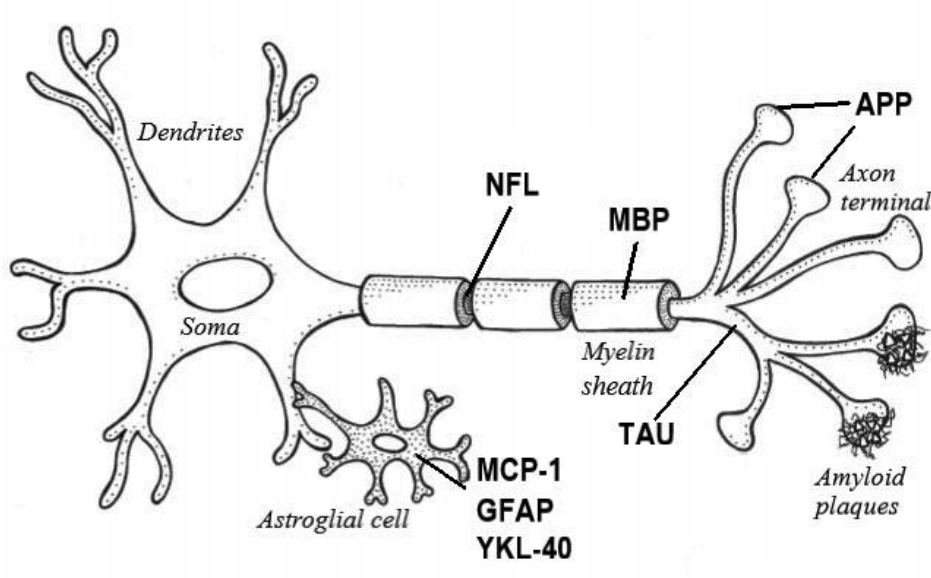

Figure 3. Schematic illustration of the origin of some of proteins measured in this thesis. NFL is located in myelinated axons, MBP in the myelin sheath of oligodendroglia cells, MCP-1, GFAP and YKL-40 are found in astroglial and tau in cortical neurons. APP and amyloid-β are located in axon terminals. Illustration by Jomi Jutlöv.

CSF biomarkers in idiopathic normal pressure hydrocephalus.

5 AIMS

The overall aim of this thesis project is to explore the diagnostic and prognostic potential of CSF biomarkers in iNPH. By measuring markers reflecting different pathophysiological aspects, we aim to elucidate underlying pathophysiologic mechanisms of iNPH.

The specific aims for the different papers were:

I. To explore the pathophysiology of iNPH by examining a broad spectrum of CSF biomarkers and evaluate the diagnostic value of the biomarkers chosen.

II. To examine CSF concentrations of APLP1-derived peptides in iNPH, especially if the APL1β28 form was increased, and to explore the prognostic value of amyloid-related CSF biomarkers.

III. To validate the differential diagnostic significance of CSF biomarkers reflecting amyloid cascade function, AD-related amyloid β (Aβ) production and aggregation, cortical neuronal damage, tau pathology, damage to long myelinated axons and astrocyte activation. All of which hypothetically separates iNPH from other common neurodegenerative disorders.

IV. To specifically expand the knowledge of pathophysiological similarities and differences between iNPH and SSVD, with healthy controls as a reference group, using a broad panel of CSF biomarkers reflecting amyloid pathology, subcortical neuronal degeneration, myelin damage, astrogliosis and markers of extracellular matrix remodeling.

CSF biomarkers in idiopathic normal pressure hydrocephalus.

6 METHODS AND STUDY DESIGN

6.1 INPH PATIENT COHORT

The iNPH-patients were included and diagnosed at the Hydrocephalus Unit at Sahlgrenska University Hospital, the referral unit for Västra Götaland region/western Sweden. The diagnosis was made according to international guidelines [16]. Diagnosis included symptom duration > 2 months, with gait problems gradually developing and mental disturbances probably attributed to iNPH. Incontinence and balance difficulties could be present. The clinical diagnosis was complemented with MRI findings (i.e. EI > 0,3, an open aqueduct and no other known cause of ventriculomegaly). All patients were clinically assessed by a neurologist who reviewed the patients' clinical history and performed a neurological exam. A physiotherapist assessed gait and balance and a neuropsychologist tested the subjects for cognitive deficits. In addition, an MRI was performed and images were evaluated by an experienced neuroradiologist. Severity of the disorder was staged using the iNPH scale, see below [27]. As a part of the evaluation, all patients were subjected to lumbar puncture, where opening pressure was measured and 10 mL of CSF was collected. Samples were collected in the morning with the patient in a recumbent position.

Peri-ventricular changes, deep white matter changes and lacunar infarcts seen on MRI were evaluated. No patients showing signs of acute hydrocephalus (i.e. symptom duration < 2 month), inability to perform the tests needed for the study, restricted life-expectancy due to other causes (e.g. malignancies),

showing other medical contra-indications to surgery or opposing inclusion despite earlier approval were included in the studies.

All patients diagnosed with iNPH and accepting shunt surgery were operated upon and given a shunt with a Rickham reservoir and an anti-siphon device. In most cases, a ventriculo-peritoneal (VP) shunt was placed but there were cases where this was not possible because of technical difficulties (e.g. prior operations in the peritoneal cavity) and in these cases, a ventriculo-atrial shunt (VA) was offered.

At six months after surgery, the patients were subjected to the same clinical examinations and an MRI scan. In patients who did not show significant improvement, shunts were checked for patency and all shunts were functional at the follow-up examination.

All patients and healthy individuals or their next of kin gave their oral and written informed consent to participate in the studies. The Regional Ethical Review Board in Gothenburg/Sweden, Kuopio/Finland and Linköping/Sweden approved ethical permission for the studies.

6.1.1 STAGING OF SEVERITY; THE INPH SCALE

For disease staging and also to quantify severity of symptoms and improvement after surgery, the iNPH scale, developed at the Hydrocephalus Unit, was used in the studies [27]. The scale covers the domains of gait, balance, cognition and continence and uses both ordinal scales and continuous

measures. Gait is given double weight given that it is reported as the major complaint of patients with iNPH, as well as their caregivers [125].

The *gait* domain is measured by letting the patients walk 10 meters in a free manner. Number of steps and time taken were recorded. The test is performed twice, using the most favourable result. Additionally, an ordinal scale for measuring gait was applied. 1 = Normal, 2 = Slight disturbance of tandem walk and turning, 3 = Wide based gait with sway, without foot corrections, 4 = Tendency to fall, with foot corrections, 5 = Walking with cane, 6 = Bi-manual support needed, 7 = Aided, 8 = Wheelchair bound. The three different tests were converted into scores, added and divided by three (or the number of tests performed), thus adding up to a domain score.

Balance was measured using an ordinal scale where; 1 = Able to stand independently for more than 30 sec on either lower extremity alone, 2 = Able to stand independently for less than 30 sec on either lower extremity alone, 3 = Able to stand independently with the feet together for less than 30 seconds, 5 = Able to stand independently with the feet apart (1 foot length) for more than 30 seconds, 6 = Able to stand independently with the feet apart for less than 30 seconds, 7 = Unable to stand without assistance. The rating score was then converted into a domain score.

Neuropsychology was measured by the Grooved pegboard, the Rey Auditory Verbal Learning Test (RAVLT) and the Swedish Stroop test [26]. For measuring manual dexterity, the grooved pegboard was used. The test was performed twice and the fastest time recorded. Verbal learning and recall was measured by the RAVLT. A total of five trials were performed and the sum of the total

trials recorded and later converted into scores. In the Stroop test selective attention, cognitive flexibility and processing speed are measured. Two areas are given points; colour naming and an interference task. Scores are converted and the total four scores are added and divided by four (or by the number of tests performed), thus adding up to a domain score.

Continence was covered by an ordinal scale, where the rating is given by the most reliable source, due to this somewhat delicate nature. 1 = Normal, 2 = Urgency without incontinence, 3 = Infrequent incontinence without napkin, 4 = Frequent incontinence with napkin, 5 = Bladder incontinence, 6 = Bladder and bowel incontinence. The result was then converted into a domain score.

In all, the domain scores were added up (gait given double weight) and divided by 5 (or the number of domains available).

The resulting scale is a measurement of severity of the disease, ranging from 0-100 where 0 is the most severe state. When constructing the scale, it was designed with reference to a group of healthy elderly individuals and 100 can thereby be seen as representing normality. Within the scale, the score is to be seen in relation to other iNPH patients, thus reflecting the severity of the disease in relation to other patients with the same disease. To define improvement following surgery an increase in $\geq$ 5 points on the scale was used.

6.2 BIOCHEMICAL ANALYSIS

All of the chemical analyses were made by methods based on antibody detection.

Immunoassays were used for most of the analyses. Briefly, the immunoassay method is based on quantification of the analyte using specific antibodies. One antibody is coated in excess on a plate (capture antibody) in a well. Then, the sample is administered to the well, leading the analyte in the sample to react with the antibodies. After washing, another analyte-specific antibody (detection antibody) is administered and binds to a different epitope on the molecule, thus creating a complex (or a "sandwich") between the analyte and the two antibodies. The detection antibody will carry a label that allows for detection [126-128], either by an electrochemiluminescent plate-based assay or enzyme-linked immunosorbent assay (ELISA)

For some analysis, the XMap technology was used [129]. The multianalyte assay is developed as to be able to measure several analytes at once, thereby reducing the number of analyses and the amount of CSF needed. Monoclonal capture antibodies (Mab) specific for their epitope are constructed. Spectrally specific carboxylated beads are covalently coupled with the MAbs. A plate filled with several (in this case 96) wells are pre-washed. The beads are placed in wells together with biotinylated detector MAbs. CSF samples are applied and incubated over night. After washing, plates were read by Luminex 100, by flow cytometric separation of the different antibody-coated microspheres [129 130].

6.2.1 CSF SAMPLING

Lumbar CSF was obtained from the iNPH patients prior to surgery. All lumbar punctures were performed in the morning to avoid any influence on the result from possible diurnal fluctuations in biomarker levels. The lumbar puncture was made with the patient in the recumbent position.

Ventricular CSF was sampled through the catheter introduced in the right lateral ventricle at the time of shunt surgery. The first 2 mL of CSF were discarded and the next 8 mL were collected. Postoperative ventricular CSF was sampled at the postoperative re-examination through a puncture of the Rickham reservoir.

The CSF, collected in polypropylene tubes, was centrifuged at 2,000×g at room temperature for 10 min. The ensuing supernatant was aliquoted in screw-cap polypropylene tubes and stored at −80°C pending biochemical analyses.

6.2.2 ANALYTICAL METHODS

For these studies, the following analytical methods were used to determine CSF biomarker concentrations. For each study, all analyses were performed batch-wise in one round of experiments by board-certified laboratory technicians at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden. The laboratory technicians were blinded to clinical data

In *study I, II,* and *IV,* NFL was measured by enzyme-linked immunosorbent assay (ELISA) technology using a commercial kit (UmanDiagnostics NF-light®) with a lower limit of detection of 50 ng/L as described in Norgren et al [109]. In *study III*, NFL concentration was measured using an in house enzyme-linked immunosorbent assay (ELISA) as previously described in Gaetani et al [131]. In this method, monoclonal antibodies NfL21(coating) and NfL23 (detection), targeted at the core domain, are used. Lower limit of quantification (LLOQ) is 78 pg/mL and the upper limit of quantification (ULOQ) is 10,000 pg/mL. Coeficient of variation (CV) was below 13 %. The method from Uman Diagnostics and the in-house ELISA are strongly correlated ($r = 0.9984$, $p < 0.001$) [131]. The initial ELISA method for determining NFL was described by Rosengren et al. where polyclonal antisera were used [132]. The method was later elaborated by Norgren et al with monoclonal antibodies which yielded higher sensitivity and specificity, no cross-reactivity with the NF-intermediate (NF-M) and heavy chain (NF-H) and the advantage of being able to establish a stable method using the monoclonal antibodies MAb 47:3 (coating) and MAb 2:1 (tracer).

The analysis of MBP (*I, IV*) was performed with an ELISA (Active® MBP, Diagnostic Systems Laboratories Inc., Webster, Texas, USA), according to the manufacturer's instructions.

CSF YKL-40 (*II*) concentration was measured by solid phase sandwich ELISA (R&D Systems, Inc., Minneapolis, Minnesota, USA) according to the manufacturer's instructions.

GFAP (*IV*) concentration was measured by an in-house sandwich ELISA method using antisera anti-GFAP IgG polyclonal antibodies from two species, rabbit anti-GFAP IgG and hen anti GFAP IgG. Goat anti-rabbit IgG was used as a detection antibody [123].

Amyloid β isoforms (Aβ38, Aβ40, and Aβ42), the sAPP isoforms (sAPPα and sAPPβ) (*I, II, III* and *IV*) and the inflammatory markers IL-8, IL-10 (*I*) and MCP1 (*I, III*) were analyzed by electrochemiluminescence assays described by the kit manufacturer (Meso Scale Discovery, Gaithersburg, MD, USA) [133]. As for sAPP isoforms, the capture Ab for sAPPα is the Mab 6E10 and for sAPPβ a neoepitope-specific antibody is used.

The APLP1-derived peptides APL1β25, APL1β27, and APL1β28 (*II*) were analyzed using a commercial ELISA (IBL International, Hamburg, Germany). The samples were analyzed according to the kit insert with minor modifications. The CSF samples were diluted 1:20 for APL1β25, 1:10 for APL1β27, and 1:5 for APL1β28 by the dilution buffer contained in the kit. All samples were analyzed in duplicate and CV for standards and samples was < 5 %.

CSF T-tau and P-tau (*I, III*) were measured with flow cytometry by the Luminex® xMAP® technology using the INNO-BIA AlzBio3 kit (Innogenetics, Ghent, Belgium), as previously described in detail in Olsson et al [129]. CV was below 10 %.

The concentrations of matrix metalloproteinase (MMP) -1, -2, -3, -9, -10 and tissue inhibitor of metalloproteinase 1 (TIMP1) (*IV*), were measured using single- or multiplex electrochemiluminescent ELISA (Meso Scale Discovery, Rockville, Maryland, USA), following the manufacturer's instructions with minor modifications. CV was below 15 % for all assays.

6.3 RADIOLOGICAL EVALUATION

In study *II* and *IV,* the extent of radiological white matter lesions in iNPH patients (*II & IV*) and patients with SSVD *(IV*) were staged according to the age-related white matter changes (ARWMC) scale [134].

All patients had undergone radiological examination as a part of the diagnostic routine and the rating was performed on the images available. All iNPH patients had undergone MRI. In the SSVD group, patients had undergone MRI or CT. All radiological staging was made by the same observer (AJ).

The ARWMC scale is constructed to be able to be used for both computed tomography (CT) and MRI images. White matter change is defined as bright lesions ≥ 5 mm on T2, proton density (PD) or fluid attenuated inversion recovery (FLAIR) on MRI or hypodense areas of ≥ 5 mm on CT. Rating is made in five different domains: frontal, parieto-occipital, temporal, basal ganglia (striatum, globus pallidus, thalamus, internal/ external capsule and insula) and infratentorial/ cerebellum. In each region, the left and right

hemisphere is rated separately, giving a total of ten regions. In each region, the ARWMC is rated from 0 to 3. The scale is given in Table 1.

Table 1. The Age related white matter changes (ARWMC) scale.

White matter lesions	
0	No lesions (including symmetrical, well-defined caps or bands)
1	Focal lesions
2	Beginning confluence of lesions
3	Diffuse involvement of the entire region, without involvement of U fibres
Basal ganglia lesions	
0	No lesions
1	1 focal lesion (≥ 5 mm)
2	> 1 focal lesion
3	Confluent lesions

6.4 STATISTICAL ANALYSIS

Due to non-symmetrical distribution of data, non-parametric statistics were used in most of the analysis (*I, II and IV*). Pairwise comparison was performed by the Wilcoxon Mann-Whitney U-test. The Kruskal Wallis test was used for

multiple comparisons. Changes between pre- and postoperative examinations and CSF concentrations were analyzed by the Wilcoxon signed rank test. For comparison of two proportions, the Fisher's exact test was used. For associations between two independent variables, the Spearman rank order correlation was chosen.

In *study III*, parametrical statistics was used to maximize the potential for constructing a combined predictive model. The One-way ANCOVA, corrected for age and sex, with Dunnett's multiple comparisons test was used to compare all groups to iNPH and HI. To construct the predictive model, univariable logistic regression analysis was performed for each individual CSF variable to separate iNPH vs non-iNPH disorders. Stepwise selection of the significant variables was used to select a multivariable logistic model and the chosen model was cross-validated. Area under ROC-curve (AUC-statistics) was calculated for description of goodness of models for iNPH vs HI, non-iNPH, cognitive disorders and movement disorders.

In all studies, significance tests were two-sided and alpha was set to $p < 0.05$. If not otherwise stated, no correction for the mass significance effect was made in order to avoid type II errors. Statistical analyses were made using IBM SPSS Statistics for Windows version 20 (*I*), 21 (*II*) and 25 (*III, IV*) (SPSS, Chicago, IL, USA), SAS Version 9 for Windows (SAS Institute, Cary, NC, USA) and GraphPad Prism© for Windows version 8.0.2. (GraphPad Software, La Jolla California USA, www.graphpad.com).

AJ performed the statistics in study *I, II* and *IV*. In study *III,* statistics were performed by Anders Pehrsson and Nils-Gunnar Pehrsson at Statistiska Konsultgruppen/ Gothenburg.

6.5 STUDY DESIGN AND PATIENT SELECTION

6.5.1 STUDY I

In *study I*, we included 27 patients with iNPH and 20 healthy elderly.

Patients were selected retrospectively, 15 men and 13 women, aged 57 to 79 and diagnosed according to standard protocol. All patients received a ventriculo-peritoneal shunt with a programmable valve with an anti-siphon device and a Rickham reservoir.

Lumbar CSF (LCSF) was obtained prior to surgery, at the time for clinical evaluation. Per-operative ventricular CSF (VCSFper) was sampled through the catheter introduced in the right lateral ventricle at the time of shunt surgery. The first 2 mL of CSF were discarded and the next 8 mL were collected. Postoperative ventricular CSF (VCSFpost) was sampled at the 6-month postoperative re-examination through a puncture of the Rickham reservoir.

Analyses for comparisons were made on previously gathered lumbar CSF samples from elderly healthy individuals, 11 men and 9 women [49]. These individuals were recruited from the population register of the City of Gothenburg and the Swedish retired people's organization, ages ranging from 64 to 76. Criteria of exclusion included neurological-, or psychiatric illnesses (including addiction of alcohol and drugs) or back- or spinal problems. All control subjects underwent neurological testing and blood tests measuring

liver- and kidney function, blood count, ions and blood sugar were performed, assuring that these tests came out within the normal range. None of the subjects chosen were treated with centrally working analgesics, or psychopharmacological drugs.

Table 2. Sex and age at baseline for iNPH and controls. INPH scale score (0-100) staging pre- and postoperatively and outcome for iNPH patients. INPH staging is given as median and interquartile range (IQR).

	HI	iNPH		
		Pre op	Post op	Outcome
	(n = 20)	(n = 27)	(n = 27)	(n = 27)
Female (n (%))	9 (46)	13 (47)		
Age (mean (SD))	70.6 (3.6)	69.6 (6.6)		
Gait		45 (31 to 82)	77 (50 to 90)	13 (3 to 31)***
Neuropsychology		60 (42 to 77)	80 (55 to87)	8 (-2 to 15)**
Balance		67 (67 to 67)	67 (67 to 83)	0 (0 to16) NS
Continence		60 (0 to 100)	80 (60 to 80)	0 (0 to 20) NS
Total		59 (46 to 75)	73 (58 to 84)	13 (3 to 21)***

** $p < 0.01$, *** $p < 0.001$, NS; non-significant. Significance calculated by Man Whitney U test.

6.5.2 STUDY II

For *study II*, we included 20 patients with iNPH and 20 neurologically healthy controls.

We selected 10 patients who improved substantially from surgery and 10 who did not. All patients were diagnosed with iNPH according to standard protocol, underwent surgery with installment of a ventriculo-peritoneal shunt with a programmable valve with an anti-siphon device and a Rickham reservoir.

Patients were selected retrospectively from our local database that at the time included 176 patients that had full pre- and postoperative iNPH scale scores and had sufficient amount of frozen CSF samples available. From that material, we selected the 10 patients that benefitted the most (defined as "total" outcome on the iNPH scale). In patients that did not improve (defined as < 5 p improvement in the iNPH scale at post-operative exam), medical records were scanned in order to establish that the shunts were functional at the time of re-evaluation. None had complications or other conditions that could explain that improvement was not reached. In that group, the 10 patients that benefitted the least were selected. Vascular risk factors were documented for all patients and white matter changes were scored using the age related white matter changes ARWMC-scale [134].

As for controls, we selected twenty persons undergoing knee-surgery and that had given their consent to CSF sampling in conjunction with receiving spinal anesthesia. All controls had a normal mini mental state examination (MMSE) score and had a normal neurological status.

Table 3. INPH scale score pre op, post op and outcome (median and IQR) in the improved and non-improved group.

	Pre op		Post op		Outcome	
	Improved	Non-improved	Improved	Non improved	Improved	Non-improved
	n = 10	n = 10	n = 10	n = 10	n = 10	n = 10
Gait domain	33 (16 to 48)	54 (35 to 69) NS	84 (57 to 100)	50 (34 to 81) NS	49 (26 to 57)	-1 (-6 to 11)***
Cognitive domain	64 (37 to 73)	60 (46 to 80) NS	75 (57 to 85)	69 (48 to 80) NS	10 (6 to 21)	3 (-6 to 11) NS
Continence domain	60 (20 to 80)	80 (60 to 80) NS	90 (75 to 100)	70 (55 to 80) NS	30 (0 to 45)	0 (-20 to 5)**
Balance domain	67 (67 to 71)	67 (67 to 83) NS	75 (67 to 87)	67 (67 to 83) NS	0 (-4 to 20)	0 (-16 to 0) NS
Total iNPH score	50 (36 to 64)	63 (56 to 70) NS	77.3 (71 to 87)	64 (52 to 71)*	26 (21 to 30)	1 (-3 to 3)***

*p ≤ 0.05 ** p ≤ 0.01, *** p ≤ 0.001, NS; non-significant. Significance calculated by Man Whitney U test

Table 4. Comparison of clinical data of improved and non-improved iNPH patients at baseline.

	Improved n = 10	Non-improved n = 10	
Age, mean (SD)	70.3 (3.2)	71.6 (8.0)	NS
Female, n (%)	5 (50)	3 (30)	NS
Sickness duration (months), mean (SD)	42 (21)	34 (28)	NS
Diabetes, n (%)	2 (20)	2 (20)	NS
Hypertension, n (%)	5 (50)	6 (60)	NS
Cardiovascular disease, n (%)	2 (20)	1 (10)	NS
MMSE, median (Q1-Q3)	23 (22 to 28)	26 (24 to 28)	NS
ARWMC, median (Q1-Q3)	6 (4 to 10)	11 (5 to 20)	NS
EI, median (Q1-Q3)	0.43 (0.38 to 0.46)	0.39 (0.36 to 0.41)	NS

NS = non-significant. Significance calculated by Man Whitney U test

6.5.3 STUDY III

In *study III* we included 82 patients with iNPH, 70 with Parkinson's disease (PD), 34 with multiple systems atrophy (MSA), 34 with progressive supranuclear palsy (PSP), 15 with corticobasal degeneration (CBD), 50 with Alzheimer's disease (AD), 19 with frontotemporal dementia (FTD), 75 with vascular dementia (VAD) and 54 neurologically healthy individuals (HI).

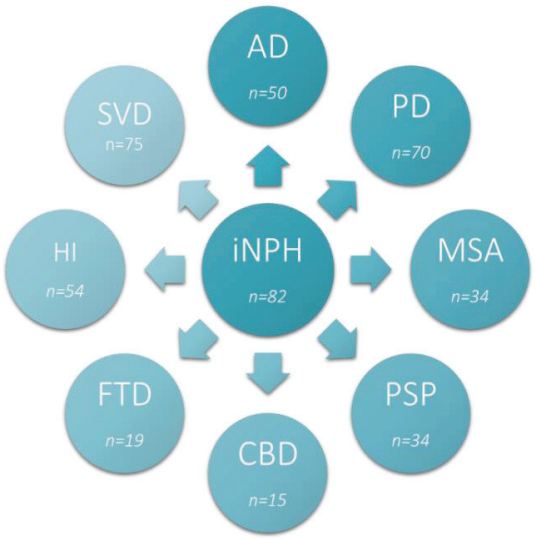

Figure 4. An overview of the different patient cohorts.

The 82 patients with iNPH (diagnosed by standard protocol), were selected retrospectively from our database. Patients that had received the diagnosis of iNPH and had undergone pre- and postoperative examination were

consecutively included. All patients had received a ventriculo-peritoneal shunt with an adjustable valve, anti-siphon device and a Rickham reservoir.

From the Sahlgrenska University Hospitals' movement disorders unit, we included 153 patients with movement disorders. The movement disorders group included 70 patients with definite PD according to the United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria [135]; 34 with probable multiple system atrophy (MSA) according to Gilman's criteria [136] ; 34 with probable or definite progressive supranuclear palsy (PSP) according to the National Institute of Neurological Disorders and Stroke and Society for Progressive Supranuclear Palsy, Inc. clinical criteria [137] ; and 15 with probable corticobasal degeneration (CBD) according to Armstrong et al.[138].

The cognitive disorders group included 144 patients. In this group, 50 patients with AD and 19 patients with frontotemporal lobar degeneration (FTLD) were diagnosed at the Department of Neurology at Kuopio University Hospital, Kuopio, Finland by an experienced neurologist specialized in memory disorders. All patients in the AD group met the NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer´s Disease and Related Disorders Association) criteria for probable AD [139] and FTLD patients were diagnosed according to the Neary criteria [140]. The 75 VAD patients were diagnosed at the Department of Geriatric Medicine at Linköping University Hospital, Sweden using the ICD-10 criteria [141] and sub grouped according to the NINDS-AIREN (Association

Internationale pour la Recherche et l'Enseignement en Neurosciences) criteria [142].

Fifty-four (54) HI were included in the analysis. The group was composed of 20 individuals from Kuopio University Hospital, Finland and 34 from Linköping University Hospital, Sweden. At both centers, neurologically healthy individuals with an MMSE score ≥ 26, undergoing planned surgical orthopedic intervention with spinal anesthesia and provided their informed consent to CSF sampling were included.

Table 5. Sex and age in iNPH, contrast groups and healthy individuals.

	HI	iNPH	AD	FTLD	VAD	PD	MSA	PSP	CBD
	n = 54	n = 82	n = 50	n = 19	n = 75	n = 70	n = 34	n = 34	n = 15
Female, n (%)	32 (59)	29 (35)	29 (58)	14 (74)	45 (60)	23 (33)	20 (59)	20 (59)	10 (67)
Age, mean (SD)	71 (10)	73 (7)***	71 (7)	69 (9)	79 (6)###&&&	60 (12)###&&&	65 (8)###	70 (7)	68 (9)

Age is presented as mean and SD. Sex is presented as number of females and %. Significance testing in comparison with iNPH and healthy controls was done by Kruskal-Wallis one-way analysis of ranks. Pair-wise analysis was made by Wilcoxon-Mann-Whitney U-test and shown as *** $P < 0.001$ (all groups), ### $P < 0.001$ (versus iNPH); &&& $P < 0.001$ (versus HI). No correction for the mass-significance was made.

6.5.4 STUDY IV

For *study IV*, we included 52 patients with iNPH, 17 patients with subcortical small vessel disease (SSVD) and 28 healthy individuals (HI).

The iNPH patients were diagnosed with iNPH according to standard protocol between 2007 and 2012 at the Hydrocephalus research unit and CSF samples were selected retrospectively. All patients had undergone pre- and postoperative evaluation according to protocol and had received a ventriculo-peritoneal shunt with an adjustable valve, anti-siphon device and a Rickham reservoir.

In collaboration with the memory clinic at Sahlgrenska University Hospital, we included patients with SSVD and HI. All were a part of the Gothenburg MCI study [143].

SSVD patients were diagnosed using the Erkinjuntti criteria [142]. More specifically, the patients were required to have mild, moderate or severe white matter changes (WMC) according to Fazekas classification [134] and predominant frontosubcortical symptoms such as mental slowness, executive dysfunction and extrapyramidal motor signs but without pronounced memory loss.

Healthy individuals were primarily recruited through senior citizens organizations, e.g. at information meetings on dementia, and a small proportion were relatives of patients [143]. None of the HI had diseases known to cause cognitive impairment nor did they exhibit any cognitive decline.

Table 6. Age, sex and MMSE in iNPH, SSVD and controls.

	iNPH n = 52	SSVD n = 17	HI n = 28
Age, mean (SD)	72 (7)*,#	71 (7)	68 (4)
Female, n (%)	23 (44)	12 (71)	10 (36)
MMSE	24 (22-27)***,###	27 (25-28)&&&	30 (29-30)

Age is presented as mean and SD. Sex is presented as number of females and %. MMSE is presented as median and interquartile range (IQR). Significance testing in comparing all groups was done by Kruskal-Wallis one-way analysis of ranks. Pair-wise analysis was made by Wilcoxon-Mann-Whitney U-test. * P < 0.05, *** P < 0.001 (all groups), # P < 0.05, ### P < 0.001 (iNPH versus controls); &&& P < 0.001 (SSVD versus controls). No correction for the mass-significance was made.

6.5.5 OVERLAP

Study design and patient selection differed between the different studies. Even so, there is a substantial overlap between them. In Table 7 the overlap is shown. Each column represents one study and each row shows how many patients in that study that are present also in the other studies, i.e. the number of patients common to the specific studies. The bottom line represents the total number of patients in each study.

Table 7. Overlap of patients in study I-IV.

STUDY I	STUDY II	STUDY III	STUDY IV
4			
4	4	4	
4	4	4	4
9		9	
1			1
	3	3	
	8	8	8
	1		1
		24	
		24	24
			8
6		6	6
28	**20**	**82**	**52**

CSF biomarkers in idiopathic normal pressure hydrocephalus.

7 RESULTS

7.1 SEPARATING INPH FROM HEALTHY INDIVIDUALS WITH CSF BIOMARKERS

The potential of separating iNPH patients from healthy individuals (HI) was explored in *study I, II, III and IV*.

In *study I*, we reported that iNPH patients exhibited elevated levels of NFL and MCP1 in combination with a lowering of all APP-derived proteins as well as tau proteins. The interleukins IL-8 and IL-10 were not elevated, nor was MBP in comparison with HI. Moreover, albumin levels (absolute concentrations and CSF/ plasma ratio) were measured and did not significantly differ between iNPH patients and HI. Results from *study I* are presented in Table 8.

Table 8. CSF biomarkers in iNPH and HI (I).

	iNPH (n = 28)	HI (n = 20)
NFL (ng/L)	1260 (840-2290)	825 (653 -1243)*
MBP (ng/L)	1.5 (1.1 -1.9)	1.3 (1.0 1.5) NS
Aβ38 (ng/L)	637 (438-894)	1641 (1231 -2173)***
Aβ40 (ng/L)	5067 (3634-6573)	10083 (7626 -12794)***
Aβ42 (ng/L)	221 (156-325)	498 (391 -669)***
sAPPα (ng/L)	505 (338-739)	1110 (727 -1244)***
sAPPβ (ng/L)	176 (110-258)	414 (250 -545)***
T-tau (ng/L)	39 (34 -50)	84 (64-107)***
P-tau (ng/L)	39 (33 -50)	59 (47 -75)**
IL-8 (ng/L)	34 (26-38)	31 (26-40) NS
IL-10 (ng/L)	0.66 (0 -0.9)	0.67 (0 -0.8) NS
MCP1 (ng/L)	746 (602-874)	628 (564 -686)*
Albumin CSF	287 (188 -408)	232 (203-280) NS
Albumin ratio	6.8 (5.0 -10)	5.6 (4.5 -6.4) NS

Analysis made by Wilcoxon Mann-Whitney U-test and shown as * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$,NS; non-significant. Values are given as median and Q1-Q3 range.

In *study II*, the biomarker focus was placed on amyloid metabolism. Data supported the results from *study I* of a lowering of all APP-derived proteins in iNPH in relation to HI. The metabolic products of the APP homologues APP- like protein 1 (*APLP1*) were also determined. Here, APL1β28 was slightly reduced in iNPH while APL1β25 and -27 were elevated. NFL and YKL40 did not differ between iNPH and HI (Table 9).

Table 9. CSF biomarkers in iNPH and HI (II)

	iNPH n = 20	HI n = 20
NFL (ng/L)	1185 (731-2103)	938 (610-2141) NS
APL1β25 (ng/L)	2591 (2296-2951)	2180 (1898-2386) ***
APL1β27 (ng/L)	1083 (887-1177)	874 (796-964) ***
APL1β28 (ng/L)	1423 (1317-1550)	1621 (1422-1797) **
Aβ38 (ng/L)	502 (266-625)	1114 (819-1445) ***
Aβ40 (ng/L)	3676 (2190-4748)	7682 (6366-9809) ***
Aβ42 (ng/L)	241 (144-405)	754 (493-1058) ***
sAPPα (ng/mL)	207 (157-259)	416 (323-665) ***
sAPPβ (ng/mL)	119 (92-170)	280 (182-389) ***
YKL40 (ng/mL)	122 (90-167)	137 (104-177) NS

Analysis made by Wilcoxon Mann-Whitney U-test and shown as ** $p \leq 0.01$, *** $p \leq 0.001$,NS; non-significant. Values are given as median and Q1-Q3 range.

Results from *study III* are presented in Table 10. Here we used the significant biomarkers from *study I*. The study could replicate the results from *study I*, with a lowering of all APP-derived proteins and tau proteins whereas MCP1 was elevated. NFL was elevated at a trend level but did not reach statistical significance.

Table 10. CSF biomarkers in iNPH and HI (III)

	iNPH n = 82	HI n = 54
NFL (pg/mL)	1155 (821-1676)	754 (540-1771) NS
MCP1 (pg/mL)	477 (421-561)	387 (341-477)*
Aβ38 (pg/mL)	1543 (1089-1824)	2148 (1681-2591)***
Aβ40 (pg/mL)	3727 (2808-4723)	5123 (4313-6494)***
Aβ42 (pg/mL)	345 (274-438)	477 (364-707)***
sAPPα (pg/mL)	429 (312-562)	679 (471-796)***
sAPPβ (pg/mL)	324 (238-387)	482 (363-634)***
T-tau (pg/mL)	206 (157-307)	296 (209-477)NS
P-tau (pg/mL)	30 (24-37)	44 (35-59)***

Analysis made by One-way ANCOVA with Dunnett's multiple comparisons test corrected for age and sex and shown as * $p \leq 0.05$, *** $p \leq 0.001$, NS; non-significant. Values are given as median and Q1-Q3 range

APP-derived proteins and biomarkers of damage to the subcortical zone were the focus of *study IV*. All APP-derived proteins were lower in iNPH than in HI. NFL, MBP and GFAP were all elevated. MMP-10 was slightly elevated but the concentration of markers of extracellular matrix remodeling did not differ between iNPH and HI.

Table 11. CSF biomarkers in iNPH and HI (IV)

	iNPH n = 52	HI n = 28
sAPPα (pg/mL)	384 (303-593)	850 (694-1207)***
sAPPβ (pg/mL)	227 (170-325)	516 (446-664)***
Aβ38 (pg/mL)	1333 (823-1928)	2855 (2266-3261)***
Aβ40 (pg/mL)	3541 (2206-5648)	7009 (5570-7814)***
Aβ42 (pg/mL)	361 (232-496)	693 (510-931)***
NFL (pg/mL)	1592 (1012-2519)	889 (694-1072)***
GFAP (pg/mL)	876 (659-1146)	559 (381-718)***
MBP (pg/mL)	1,997 (1,407-2,503)	1,446 (1,228-1,632)***
MMP-1 (pg/mL)	26 (16-47)	24 (19-33) NS
MMP-2 (pg/mL)	21190 (18965-23600)	21317 (18423-23549) NS
MMP-3 (pg/mL)	221 (162-322)	238 (201-344) NS
MMP-9 (pg/mL)	160 (114-205)	129 (89-160) NS
MMP-10 (pg/mL)	49 (38-67)	42 (31-49)*
TIMP-1 (pg/mL)	99329 (87306-113161)	86094 (78696-107987) NS

Analysis made by Wilcoxon Mann-Whitney U-test and shown as * $p \leq 0.05$, *** $p \leq 0.001$,NS; non-significant. Values are given as median and Q1-Q3 range.

In Table 12, results displaying the difference between iNPH and HI are shown schematically. It can be seen that the results with lower levels of APP-derived proteins and tau proteins were replicated consistently. There was an elevation of MCP1. The significance of an elevated NFL and MBP differed between the studies.

Table 12. An overview of the difference in CSF biomarker concentration between iNPH patients and HI.

	I	II	III	IV
NFL	↑	NS	NS	↑
MBP	NS	-	-	↑
GFAP	-	-	-	↑
YKL40	-	NS	-	-
MCP1	↑	-	↑	-
Albumin CSF	NS	-	-	-
Alb ratio	NS	-	-	-
IL-8	NS	-	-	-
IL-10	NS	-	-	-
MMP-1	-	-	-	NS
MMP-2	-	-	-	NS
MMP-3	-	-	-	NS
MMP-9	-	-	-	NS
MMP-10	-	-	-	↑
TIMP-1	-	-	-	NS
Aβ38	↓	↓	↓	↓
Aβ40	↓	↓	↓	↓
Aβ42	↓	↓	↓	↓
sAPPα	↓	↓	↓	↓
sAPPβ	↓	↓	↓	↓
APL1β25	-	↑	-	-
APL1β27	-	↑	-	-
APL1β28	-	↓	-	-
T-tau	↓	-	↓	-
P-tau	↓	-	↓	-

Direction of arrow indicates level in iNPH as compared to HI.
NS = non-significant

7.2 THE DIFFERENTIAL DIAGNOSTIC CAPACITY OF CSF BIOMARKERS

In *study III*, we tested the CSF biomarkers that differed significantly between iNPH and HI in *study I* on a material consisting of some of the most common iNPH mimics; AD, FTLD, VAD, PD, MSA, PSP and CBD. We could show that concentrations of APP-derived proteins remained low in iNPH. The sAPPs were lower in iNPH than in AD, FTLD, VAD, PD and PSP but did not reach statistical significance in comparison with MSA and CBD. Most of the Aβs were lower in iNPH with Aβ42 equaling the level in AD, VAD and CBD. Tau proteins distinguished iNPH from the cognitive disorders, but did not separate iNPH from the movement disorders. MCP1 remained elevated in iNPH compared to the other disorders but this result was only significant vs PD and MSA when correcting for the effect of age and sex (Table 13).

Similarities and differences between iNPH and SSVD were analyzed in *study IV*. These two disorders share much of the vascular risk factor profile and the clinical (subcortical) picture. Hence, the discrimination between these two disorders remains the most clinically challenging. We could conclude that APP-derived proteins (except Aβ42) were lower in iNPH than in patients with SSVD. There was no statistical difference in biomarkers of white matter damage (NFL or MBP) nor in astroglia activation (GFAP) or extracellular matrix remodeling markers (MMP-1, -2, -3, -9, -10 and TIMP-1) (Table 14).

Table 13. CSF biomarkers concentrations in iNPH and AD, FTLD, VAD, PD, MSA, PSP and CBD (III)

	iNPH n = 82	AD n = 50	FTLD n = 19	VAD n = 75	PD n = 70	MSA n = 34	PSP n = 34	CBD n = 15
T-tau (pg/mL)	245 (131)	980 (333)***	342 (146)	651 (594)***	224 (105)	315 (200)	329 (247)	366 (230)
P-tau (pg/mL)	32 (12)	96 (27)***	45 (13)	63 (41)***	33 (12)	35 (18)	46 (38)*	42 (19)
NFL (pg/mL)	1717(1963)	1977 (3104)	2089 (1401)	2646 (3475)	839 (622)	2322 (987)	2219 (2761)	2137 (1178)
Aβ38 (pg/mL)	1526 (519)	2710 (807)***	2324 (608)***	2136 (672)***	2056 (624)***	1888 (856)*	2125 (1076)***	2091 (684)*
Aβ40 (pg/mL)	3800 (1193)	6541 (1654)***	5801 (1222)***	5477 (1540)***	5067 (1391)***	4650 (1815)*	5099 (1965)***	5197 (1561)**
Aβ42 (pg/mL)	364 (138)	318 (95)	569 (204)***	387 (191)	548 (187)***	489 (203)*	488 (170)*	505 (216)
sAPPα (pg/mL)	446 (178)	865 (310)***	738 (279)***	631 (262)***	715 (262)***	597 (209)	650 (309)**	585 (224)
sAPPβ (pg/mL)	321 (121)	599 (192)***	502 (166)**	484 (188)***	503 (178)***	414 (142)	470 (212)***	452 (154)
MCP1 (pg/mL)	492 (109)	436 (162)	400 (101)	456 (115)	382 (128)**	365 (70)**	410 (121)	448 (272)

CSF biomarker concentrations are shown as mean and SD. Significance testing in comparison with iNPH was done by One-way ANCOVA corrected for age and sex with Dunnett´s multiple comparisons test and shown as * P < 0.05, ** P < 0.01, *** P < 0.001.

Table 14. CSF biomarkers in iNPH and SSVD (IV)

	iNPH n = 52	SSVD n = 17
sAPPα (pg/mL)	384 (303-593)	683 (475-847)**
sAPPβ (pg/mL)	227 (170-325)	417 (232-458)**
Aβ38 (pg/mL)	1333 (823-1928)	2196 (1749-2505)**
Aβ40 (pg/mL)	3541 (2206-5648)	5428 (4678-6838)**
Aβ42 (pg/mL)	361 (232-496)	474 (320-558) NS
NFL (pg/mL)	1592 (1012-2519)	1638 (1150-3149) NS
GFAP (pg/mL)	876 (659-1146)	820 (472-976) NS
MBP (pg/mL)	1,997 (1,407-2,503)	1,691 (1,461-2,351) NS
MMP-1 (pg/mL)	26 (16-47)	34 (20-54) NS
MMP-2 (pg/mL)	21190 (18965-23600)	22244 (21146-25104) NS
MMP-3 (pg/mL)	221 (162-322)	250 (186-372) NS
MMP-9 (pg/mL)	160 (114-205)	163 (107-193) NS
MMP-10 (pg/mL)	49 (38-67)	63 (40-76) NS
TIMP-1 (pg/mL)	99329 (87306-113161)	105464 (87590-142345) NS

CSF biomarker concentrations are shown as medians and interquartile ranges (IQR). Significance testing was made by Wilcoxon-Mann-Whitney U-test and shown as ** P < 0.01, *** P < 0.001, NS; non-significant.

Table 15. Schematic overview of biomarker concentrations in iNPH mimics in comparison with iNPH.

	AD	FTLD	VAD	SSVD	PD	MSA	PSP	CBD
NFL	NS	NS	NS	NS	NS	NS	NS	NS
MBP	-	-	-	NS	-	-	-	-
GFAP	-	-	-	NS	-	-	-	-
MCP-1	NS	NS	NS	-	↓	↓	NS	NS
MMP-1	-	-	-	NS	-	-	-	-
MMP-2	-	-	-	NS	-	-	-	-
MMP-3	-	-	-	NS	-	-	-	-
MMP-9	-	-	-	NS	-	-	-	-
MMP-10	-	-	-	NS	-	-	-	-
TIMP-1	-	-	-	NS	-	-	-	-
Aβ38	↑	↑	↑	↑	↑	↑	↑	↑
Aβ40	↑	↑	↑	↑	↑	↑	↑	↑
Aβ42	NS	↑	NS	NS	↑	↑	↑	NS
sAPPα	↑	↑	↑	↑	↑	NS	↑	NS
sAPPβ	↑	↑	↑	↑	↑	NS	↑	NS
T-tau	↑	NS	↑	-	NS	NS	NS	NS
P-tau	↑	NS	↑	-	NS	NS	↑	NS

Arrows indicate level in comparison with iNPH. NS = non-significant. Except for iNPH vs SSVD, results are corrected for age and sex.

In *study III*, we constructed a predictive model for iNPH. The model consisted of T-tau, Aβ40 and MCP-1 (simplified model 10*MCP1 - Ab40 - 5*T-tau). ROC-curve (AUC-statistics) was calculated and yielded an AUC of 0.87 (iNPH vs HI), AUC 0.86 (iNPH vs non-iNPH disorders), AUC 0.80 (iNPH vs movement disorders) and AUC 0.92 (iNPH vs cognitive disorders). A prediction plot was constructed and is shown in Fig 5.

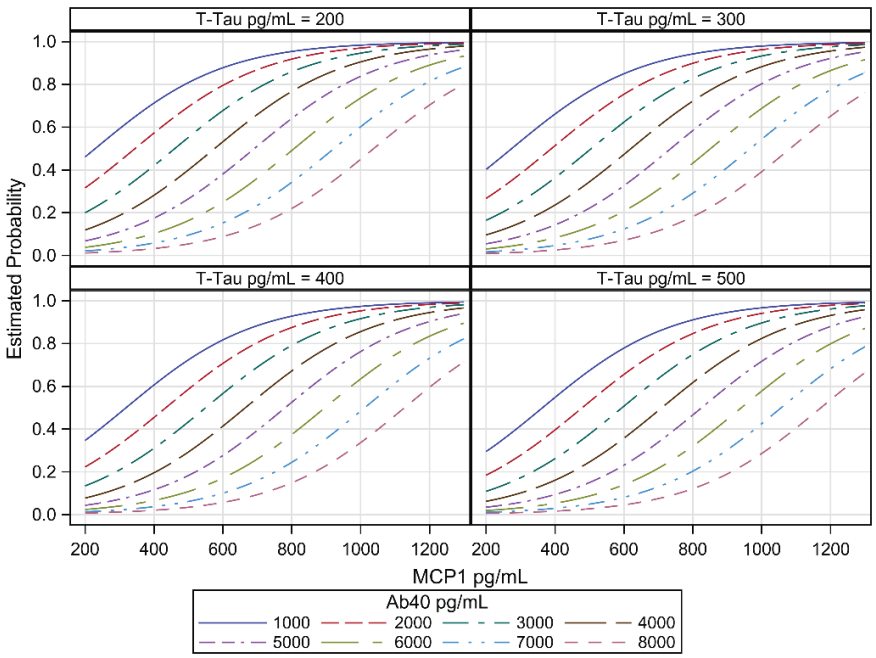

Figure 5. Prediction plot for estimating the probability of a patient suffering from iNPH or a non-iNPH disorder. T-tau is given in 4 concentrations, Aβ40 is given in 8 different intervals whereas MCP-1 is shown as a continuous variable on the X-axes. Estimated probability of iNPH is given on the Y-axes.

7.3 CSF BIOMARKERS IN VENTRICULAR CSF

In *study I*, we compared the concentration of the biomarkers in lumbar with ventricular CSF, the rostro-caudal gradient (RCG). The APP-derived proteins, NFL and IL-8 were higher in lumbar than in ventricular CSF whereas the tau proteins, MBP and MCP-1 were higher in ventricular CSF. Results are shown in Fig 6 and 7.

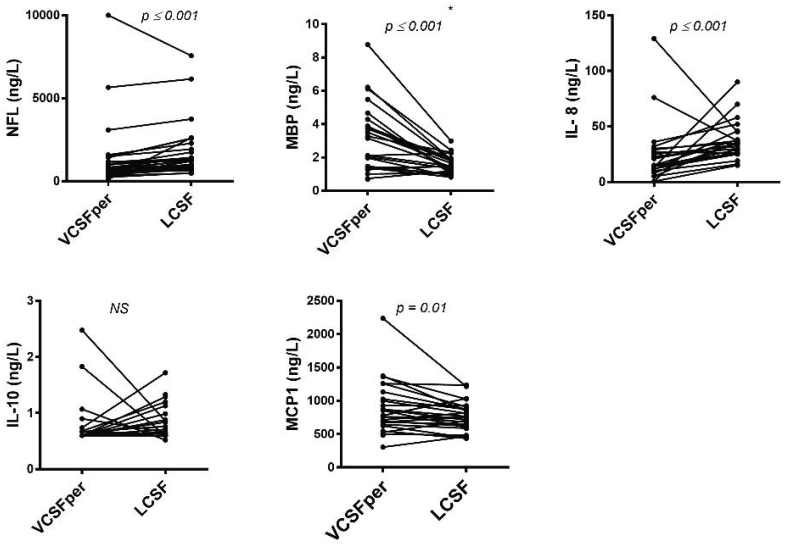

Figure 6. Concentrations of NFL, MBP IL-8, IL-10 and MCP-1 in ventricular CSF per op and lumbar CSF pre op. Significance is calculated by the Wilcoxon Mann-Whitney U-test (I).

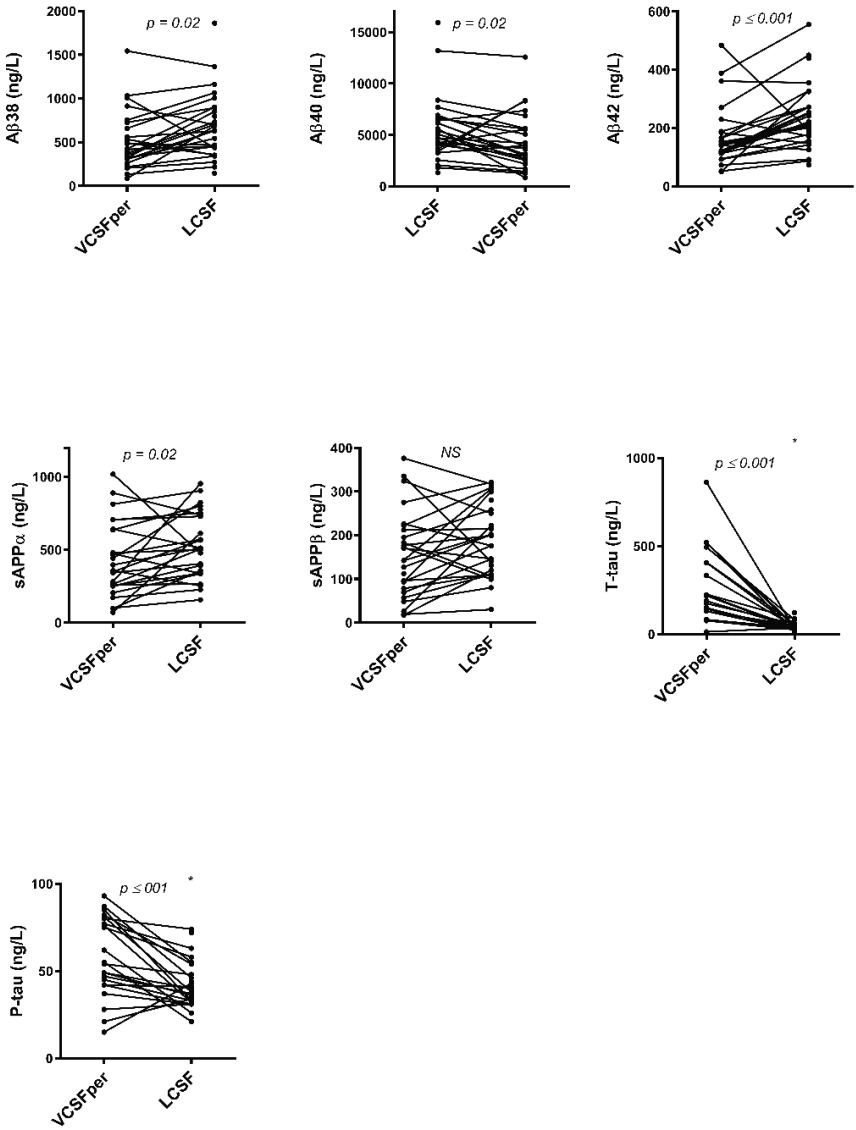

Figure 7. Concentrations of APP-derived proteins and tau proteins in ventricular CSF per op and lumbar CSF pre op. Significance is calculated by the Wilcoxon Mann-Whitney U-test (I).

The biomarkers change in ventricular CSF following surgery (fig 8 and 9). The APP-derived proteins and P-tau did increase. Results on T-tau are more conflicting. At a group level, levels did decrease but looking at individual patients, it rather seems as if most of the patients remained relatively unchanged, with some patients showing a more pronounced reduction. For NFL, the levels slightly increased although many patients remained stable in their levels. MBP did decrease.

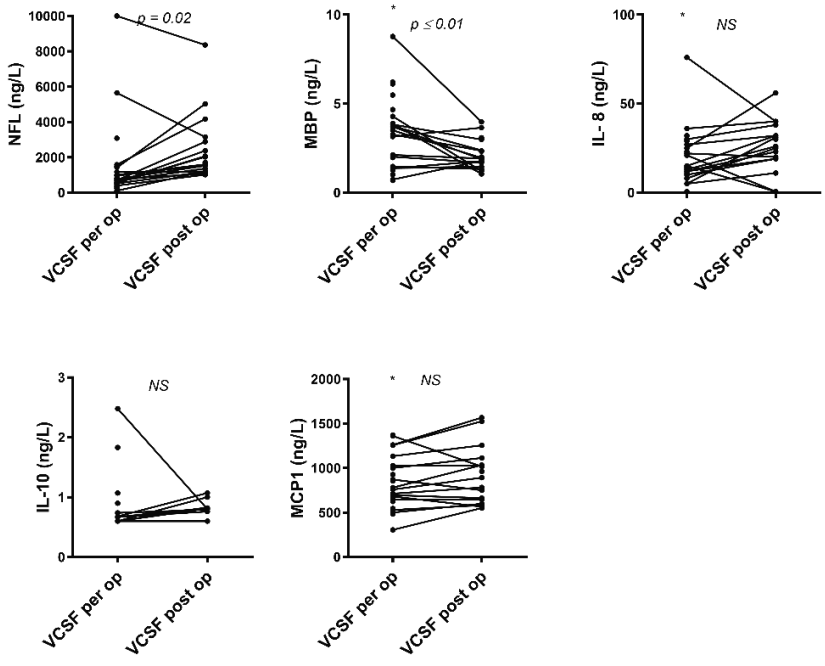

Figure 8. Concentration of NFL, MBP, IL-8, IL-10 and MCP-1 in ventricular CSF per- and post surgery. Significance is calculated by the Wilcoxon signed rank test (I).

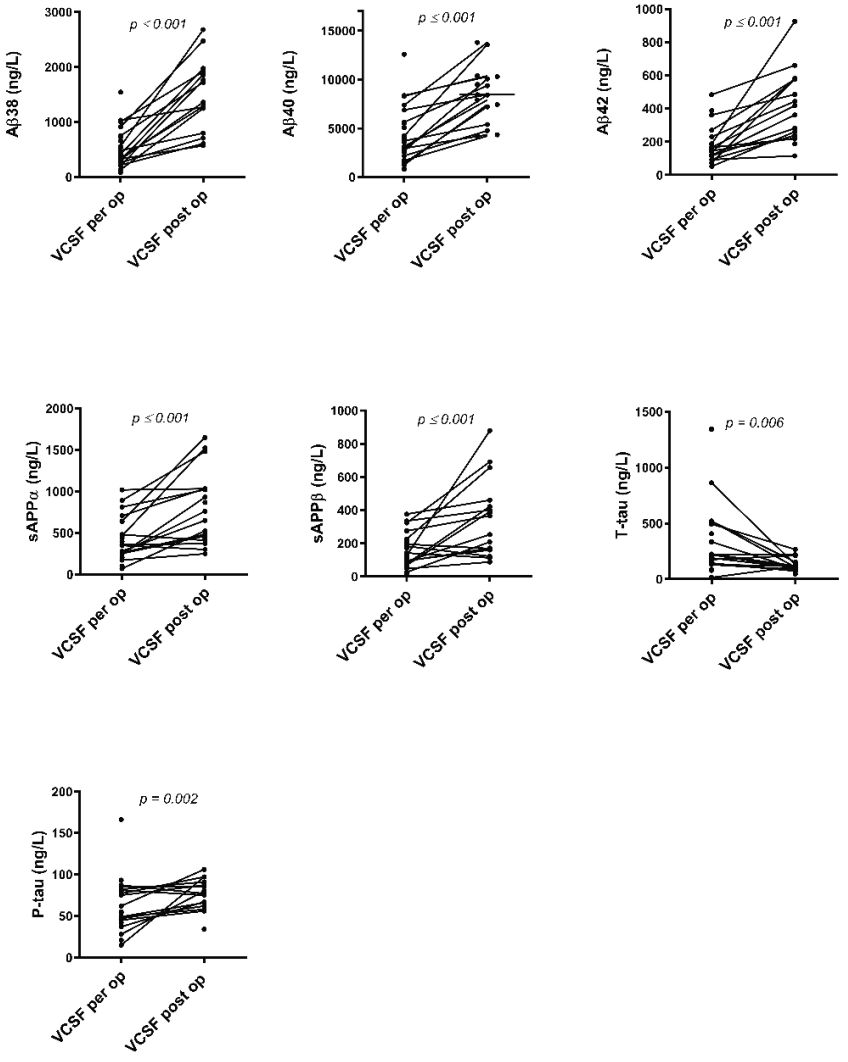

Figure 9. Concentration of APP-derived proteins and tau proteins in ventricular CSF per- and post surgery. Significance is calculated by the Wilcoxon signed rank test (I).

7.4 PREDICTING SHUNT RESPONSE BY CSF BIOMARKERS

In *study II*, we aimed to examine if the biomarker profile differed depending on whether the patient was improved by surgery or not. However, even in these heavily dichotomized groups, none of the CSF biomarker levels at baseline differed between the groups (Table 16).

Table 16. CSF biomarker levels in improved vs non-improved iNPH patients.

	Improved n = 10	Non-improved n = 10
NFL (ng/L)	1186 (869 to 1670)	1085 (699 to 2432) NS
APL1β25 (ng/L)	2532 (2174 to 2958)	2820 (2401 to 2954) NS
APL1β27 (ng/L)	1067 (900 to 1157)	1085 (867 to 1214) NS
APL1β28 (ng/L)	1423 (1264 to 1568)	1458 (1291 to 1562) NS
Aβ38 (ng/L)	500 (308 to 605)	503 (224 to 677) NS
Aβ40 (ng/L)	3731 (2642 to 4740)	3677 (1522 to 4789) NS
Aβ42 (ng/L)	241 (155 to 370)	244 (122 to 438) NS
sAPPα (ng/mL)	205 (175 to 279)	212 (144 to 297) NS
sAPPβ (ng/mL)	114 (95 to 155)	127 (75 to 181) NS
YKL40 (ng/mL)	122 (99 to 153)	134 (84 to 180) NS

Analysis is made by Man Whitney U test., NS; non-significant. Values are given as median and IQ-range.

7.5 RADIOLOGICAL WHITE MATTER CHANGES

White matter changes were assessed in *study II* and *IV*. ARWMC were most abundant in the frontal and parietal-occipital areas. In *study II*, the patients were divided into improved and non-improved. Non-improved patients had slightly more ARWMC (median (IQR) 11, (5-20)) than improved patients (median (IQR) 6, (4-10)) but the difference did not reach statistical significance.

Compared to patients with SSVD, iNPH patients displayed less ARWMC. Ventricle size, though, was slightly larger in iNPH than in SSVD.

In table 17 and 18, ARWMC scores are presented for each sub-region and as a total score. In every domain ARWMC is reported for right (R) and left (L) hemisphere separately.

Table 17. ARWMC in iNPH (II).

Brain region	iNPH n = 20
Frontal	
R	1.5 (1 -3)
L	1.5 (1 - 3)
Parietal-occipital	
R	1 (1 - 2)
L	1 (1 - 2)
Temporal	
R	0 (0 - 1)
L	0 (0 - 1)
Basal ganglia	
R	0 (0 - 1)
L	0 (0 - 1)
Infratentorial	
R	0 (0 - 2)
L	0 (0 - 2)
Total	9 (4 - 13)
Evans' Index	0.4 (0.36 - 0.45)

Values are given as median and Q1-Q3.

Table 18. ARWMC in iNPH and SSVD (IV).

	iNPH	SSVD
Brain region	n = 51	n = 14
Frontal		
R	1 (1-2)	2 (2-3)
L	1 (1-2)	2 (2-3)
Parietal-occipital		
R	1 (1-2)	2.5 (1-3)
L	1 (1-2)	2.5 (1-3)
Temporal		
R	0 (0-1)	0 0-0)
L	0 (0-1)	0 (0-0.25)
Basal ganglia		
R	0 (0-1)	1 (0-2.25)
L	0 (0-0)	1 (0-2.25)
Infratentorial		
R	0 (0-1)	1 (0-2)
L	0 (0-1)	0.5 (0-2)
Total	7 (4-11)	12 (8-22)**
Evans' Index	0.4 (0.37-0.44)	0.31 (0.27-0.38)***

Values are given as median and Q1-Q3. ** P < 0.01, *** P < 0.001.

8 DISCUSSION

The overall aim of this thesis was to explore the diagnostic and prognostic potential of CSF biomarkers in iNPH and hereby elucidate underlying pathophysiologic mechanisms of the disorder. Here, I will start by discussing the pathophysiological implications following the CSF biomarker studies.

8.1 AMYLOIDS IN INPH

In all of our studies, iNPH patients presented with lower levels of all APP-derived proteins in comparison with HI. Being the surrogate marker for AD plaque pathology, Aβ42 is the marker studied the most, together with the other component of plaques: Aβ40. Most studies have found lower Aβ42 [69 144-152] and Aβ40 [145 149 152] in NPH/ iNPH in comparison to HI even if some groups presented compatible levels between iNPH and HI of Aβ42 [153-156] and Aβ40 [146 155]. However, it is noteworthy that nearly all studies that showed compatible levels are from the same research group, indicating a possible overlap in these studies. All studies on CSF biomarkers in iNPH so far have showed lower levels of sAPPα and –β in iNPH [146 149 152 153 155 156] as well as Aβ38 [149 152 155].

APL1 is a homologue to APP [99]. In *study II* we could show that the cleavage products of APL1, APLP1β, were not affected to the same extent, nor in

the same manner as APP. These findings are supported by the only other study on the subject [155]. As such, we would like to argue that reduced levels of APP-derived proteins are specific and characterize the CSF biomarker profile of patients with iNPH in comparison with HI.

There have been theories proposing a common pathological state in NPH and AD. According to these theories, NPH and AD share a common pathophysiological aetiology in that reduced clearance of Aβ would lead to AD-like pathology in iNPH-patients [97 98], a theory in part based on observations of rats with Kaolin-induced hydrocephalus (i.e. rather sNPH than iNPH).

We believe that the results from studies expanding the APP-derived biomarkers studied here, clearly speaks against this hypothesis of a common pathophysiology. In AD, there is an isolated reduction of Aβ42 in CSF whereas the other Aβ and sAPP fragments remain unaltered or even elevated. In iNPH there is a more general reduction of APP-derived proteins in CSF. We believe that this indicates disturbances in different parts of the amyloid cascade, where AD is a downstream phenomenon in the cascade. iNPH on the other hand, seems to suffer from an upstream disturbance or/ together with a reduced clearance affecting all fragments. In CSF, when measuring Aβ42 levels between iNPH and AD, our results (*III*) as well as results from several other studies will indicate that levels of Aβ42 do not differ between the groups [78 144 150 151 153 157]. Some studies have also shown an increase [145 155 156 158] or a decrease of Aβ42 [147 148] in iNPH in comparison with AD. Aβ38 is lower in iNPH than in AD [155] and in contrary to our study, Aβ40 has found to be in the same level as in AD [145 155]. As for

the sAPPs, our study (*III*) and all other studies show that patients with iNPH have lower levels than patients with AD [153 155 156].

Around 30 % of patients with iNPH do exhibit AD-like pathology in cortical biopsies [159]. As AD is a common comorbidity or differential diagnosis, we argue that it is of importance not to base the evaluation on Aβ42 solely, but to simultaneously look at different parts of the cascade to aid in diagnostic queries.

There have been previous attempts in treating patients with AD with a low flow ventriculoperitoneal shunt with the underlying assumption that this would facilitate CSF turnover and hence drain the CSF of accumulated amyloid. However, these attempts, although initially promising, have not been successful [46 160 161].

If APP clearance is hindered by the elevated centrifugal pressure in iNPH and if this would explain the lower concentrations, it might be that APPs can be cleared from a stagnant pool in the ECS once the force applied is being removed. This has led to some objections for the usage of CSF biomarkers in iNPH and that this reduction of ECS and thus clearance of amyloids into the CSF might hinder the interpretation of differences in biomarker concentrations analysis between iNPH and AD [162].

There are few studies that have addressed the relation between ISF and CSF levels of CSF biomarkers. Herruka et al reported the level of Ab1-42 to be quite similar between ISF and ventricular CSF (VCSF) levels [163]. Tau proteins were higher in ISF than VCSF. Aβ42 and P-tau remained quite

stable in ISF in a 21 h period during microdialysis, whereas T-tau was increased initially and falling to a stable plateau within hours. This could indicate that the levels in CSF are actually mirroring the actual amyloid levels, arguing against an isolated mechanistic drainage dysfunction. We believe that such a dysfunction would also hinder the drainage of APL1 derived proteins which does not seem to be the case [149 155]. Larger studies analysing the relation between ISF/CSF concentrations are needed.

A common objection to the suggested CSF biomarker findings in iNPH is that of a possible dilution mechanism due to increased CSF amount. However, there seems to be no correlation between ventricle volume and CSF biomarkers [164]. Also, the finding that not all proteins behave in the same way would argue against a dilution mechanism that accounts for the low APP-derived proteins.

The slight increase of APL1β 25 and 27 seen in *study II* could indicate increased cleavage of APLP-1 by γ-secretase as a response to a reduction of its primary substrate APP. In iNPH an increase in γ-secretase activity in cortical biopsies in patients with iNPH with Aβ-pathology has been shown [165] whereas in Downs syndrome (where APP is overly-expressed) there is a decreased level of all APL1β which could be in accordance with a less available γ-secretase [166]. These findings support the notion of less available APP proteins in iNPH.

Something happens with APP-derived proteins after shunt surgery. In *study I*, concentrations in ventricular CSF increased post op [152]. Tarnaris et al, studying biomarker changes in iNPH during ELD, showed that Aβ42

increased during lumbar drainage [72]. The same increase of Aβ post op has been reported earlier [155]. However, post op values are not so easily interpreted. The shunt would probably change the CSF flow dynamics as a new route of low resistance is being introduced. This would also presumably affect the manner in which amyloids are drained if the studies indicating the importance of the glymphatic system for amyloid drainage are proven right [56].

More studies of biomarkers pre- and post shunt surgery are warranted to bring the dynamic changes caused by shunt surgery into clarification.

8.2 CORTICAL PATHOLOGY IN INPH?

T-tau indicates cortical neural damage whereas P-tau is indicative of mis-phosforylation of tau which is seen in neurofibrillary tangles in AD [67 106]. In *study I* and *III* we measured both T-tau and P-tau in iNPH and they were found to be lower in iNPH than in HI. A number of studies have shown compatible [144-148 150 153-155 167] or lower [69] levels of T-tau and compatible [78 146 147 150 151 153 156] or lower [69 148 154 155] levels of P-tau. There are two studies with contradictory results. Kudo et al and Kapaki et al have showed elevated levels of T-tau [73 78]. In the first study, the cohort was mixed between idiopathic and secondary cases with aetiologies known to increase tau levels. In the second study, T-tau was significantly increased but looking closer at the results, there were two patients with iNPH that had very high

levels of T-tau whereas the rest of the patients seemed to have values well in line with the control sample which might have distorted the statistics.

In *study III* we could show that the movement disorders (PD, PSP, MSA and CBD) exhibited levels of T- and P-tau in the same range as iNPH. The same has been shown previously [144 148 150 154] and tau-proteins do not seem to separate iNPH from movement disorders. The cognitive disorders did however have elevated tau proteins. AD patients exhibit higher levels of T- [78 144 145 147 148 150 153-155 157 158] and P-tau [78 147 148 150 151 153 155-158] than iNPH. The same can be said when comparing to FTLD [145 150]. Dementias of vascular origin show more mixed results. Previous results on VAD showed levels similar to iNPH [150] whereas the VAD-patients in our study had higher levels. SAE have shown higher levels [69]. We know that there are individuals with VAD in our study that have cortical infarctions which might explain higher tau levels.

Patients with mild cognitive impairment (MCI) have shown higher levels of tau proteins than patients with iNPH [147]. A large proportion of MCI patients go on to develop AD and thus it is likely that presence of AD would obscure the image.

Given these results, we believe that iNPH does not involve any extensive damage to cortical structures and that elevated tau levels in patients with iNPH would indicate presence of cortical pathology (e.g. AD) that needs to be taken into consideration by the clinician.

8.3 DAMAGE TO WHITE MATTER AND GLIA ACTIVATION

MBP, a marker of oligodendroglia, is in hydrocephalus presumed to reflect myelin destruction in the periventricular zone [112]. There is an increase of MBP in CSF in iNPH (*I, IV*) (even if not reaching significance in *study I).* Previously, only one study has measured MBP in hydrocephalic (i.e. not NPH) patients and 80 % exhibited increased levels, even if this mixed cohort of high- and low pressure hydrocephalus might not represent an iNPH cohort [112]. Following surgery, MBP concentrations are lowered which we interpret as a reduction of myelin destruction.

As previously stated, there are many clinical similarities between iNPH and SSVD, thought to arise from subcortical structures [168]. Studies on SSVD had indicated a CSF biomarker subcortical profile [169] and we wanted to try this panel for our iNPH patients. The markers of extracellular matrix remodelling (MMPs and TIMP1) were not elevated in iNPH in comparison to HI nor to SSVD (*IV*). Thus, in this study, we could not reproduce the suggested profile for subcortical CSF biomarkers [169]. No other study has measured these markers in iNPH.

NFL, an axonal degeneration marker [106] was elevated in *study I* and *IV* but not significantly so in *II* and *III.* NFL has been shown to be increased in iNPH in comparison with HI before [69 167 170 171] and the levels seem compatible to those in SSVD/SAE/BD (*IV)* [69 167 171]. NFL was initially regarded as mainly representing large, periventricular axons but is now believed to be a more general marker of axonal degeneration [108 109].

According to our clinical experience, we often find that iNPH patients present with NFL levels in the higher span of the reference interval but we seldom see very high levels in iNPH. One possible explanation to the high NFL concentrations seen in earlier studies could be that these studies included a mixture of idiopathic and secondary cases of NPH [167 170 171]. These results are in line with the notion that NFL is rather a marker of disease intensity than marker of a specific aetiology [110].

Astrocyte activation is in these studies mirrored by YKL40 (*II*) and GFAP (*IV*). YKL40 was not found elevated (*II*) whereas GFAP was elevated in comparison with HI but not with SSVD (*IV*). Elevated GFAP in iNPH has been shown before [167 170 172] and also the findings of compatible levels in SSVD [167]. It could be speculated that the reason for the discrepancy might be the low neuroinflammatory component in iNPH that does not trigger YKL40 [124] production. Astrocyte activation has also been implicated in iNPH by elevated Leucine-rich α2-glycoprotein (LRG) which is thought to be expressed by peri-capillary astrocytes [154 173-175].

MCP-1 is also a cytokine which acts as a chemoattractant of astroglia, apart from the recruitment of monocytes from the periphery [115]. It seems to be elevated in CSF of iNPH patients as compared to HI as shown in *study I* and *III*. It was also included in the predictive model to capture astroglial activation. Considering that no other group has measured MCP-1, these results needs replication.

Taken together, the CSF biomarker profile in iNPH is supportive of a subcortical profile with gliosis and possibly with astroglial activation. In

addition, the ARWMC staging in *II* and *IV*, are supportive of such a position with WMC being quite extensive even if they radiologically do not match those of SSVD. Postoperatively, MBP levels decreased in ventricular CSF (III) and previous studies have shown that also the WMC tend to improve post surgery [37 167].

8.4 VASCULAR CHANGES IN INPH

Following the results in *study I,* we shaped the hypothesis of a biomarker pattern in iNPH reflecting a decreased periventricular metabolism which is also supported by MRI findings [176 177]. In *study IV*, we looked closer at this periventricular zone by exploring similarities and differencies between iNPH and SSVD, which we know both exhibit periventricular changes. The clinical pictures of iNPH and SSVD can often be very similar. The cognitive features are both fronto-subcortical (as opposed to posterior in e.g. AD) [168] and gait and urinary symptoms are frequent in both disorders. These similarities are not surprising, if we assume that the clinical manifestations in both disorders are partially the results of periventricular changes. In *study IV*, we saw that many patients with SSVD show ventriculomegaly and WMC are extensive in both disorders, even if they are more pronounced in SSVD. There is an increasing body of evidence focusing on the importance of vascular risk factors in patients with iNPH [178-180]. To complicate matters even further, we know that patients with iNPH and extensive white-matter changes (Binswangers disease) can

respond to shunt treatment [37 181]. As for CSF biomarker changes, we can see that the two disorders do share patterns of biomarker alterations even if the changes in the iNPH patients are more pronounced.

Even if incompletely understood, the aetiology of SSVD is thought to arise from arteriolar dysfunction which mainly affects highly vascularized tissues, such as the brain's white matter [168 182-184]. Contemporary research on NPH is separating sNPH cases from iNPH cases to a larger extent than earlier. We can see that this shift has somewhat changed our view on some of the CSF biomarkers. Presumably, focusing on pressure dynamics might have over-stressed the similarities of s- and iNPH in favor of looking at the underlying factors and that the aetiologies might be more different than previously thought. It may well be that the idiopathic form of NPH, as opposed to the secondary form, might arise from vascular changes. The nature of these changes are not completely understood. The new hypothesis on the glymphatic system might provide a piece of the puzzle. One could speculate that iNPH patients have a down regulation of APP proteins in response to a reduced parenchymal clearance of Aβ due to reduced turnover and this is what we see in lumbar CSF. We believe that further research into vascular changes and glymphatics will be an important future line of research in iNPH.

8.5 PREDICTING OUTCOME?

The question of predictability was addressed in *study II*. We could not find any CSF biomarker, nor any clinical marker that could be used to predict which patient that would benefit from shunt surgery. Non-improved patients presented with more ARWMC than the improved. In this small material, the difference between these heavily dichotomized groups was not significant but this might be attributed to a type II error due to small sample size. Regardless, patients with vascular changes improve to the same extent as those without and even extensive white matter changes should not be used as an argument against shunt surgery [37 171].

There have been numerous attempts at finding objective criteria that could be used to select patients that would benefit from surgery, or rather not expose patients to surgery that we know would *not* benefit. As previously discussed, several tests can aid in patient selection, the most widely used being the CSF tap-test or other tests based on clinical response to CSF removal [39]. None of them have yet been able to show a specificity high enough to safely exclude patients from shunt surgery [43]. In an attempt to find markers of shunt responsiveness, Luikku et al used the disease state index (DSI) which consisted of demographical, clinical, radiological, and biochemical data from 284 patients (54 non-responders) to analyze which patients would benefit from shunt surgery. They concluded that the AUC of predicting shunt responsiveness was still 0.58 and thus remains extremely challenging [185]. Judging from the evidence it seems that to *diagnose* and to *predict reversibility* are two different tasks in iNPH. There are many

iNPH researchers that would oppose this view. In many centers world-wide, TT is still being used for diagnosing iNPH and in the Japanese guidelines, a positive TT is required to be diagnosed with probable iNPH [17]. We do believe that the diagnosis of iNPH can still be correct even if the patient is non-responsive to shunt treatment, and that outcome after shunting is mere a measure of reversibility, not diagnosis.

Co-morbidity is, needless to say, an important factor when attempting to predict the disease course for the individual patients. There are some studies indicating that CSF biomarkers could be used to map co-morbid neurodegenerative disorders and thus to predict long-term cognitive outcome [151 186 187]. Neurodegenerative co-morbidity does not seem to impair the short term results [38 186] and it seems that even if co-morbidities are present, the "iNPH part" might still be reversible even if the course of the neurodegenerative disorder progresses over time as it would without shunt surgery. To that extent, CSF biomarkers could offer a good tool to signal the presence of co-morbidities that might influence the outcome.

8.6 CAN WE USE CSF BIOMARKERS TO DIAGNOSE INPH?

Our first research question was whether CSF biomarkers can aid in diagnosing iNPH. There are several answers to this question. We can conclude that the results from these studies lend support to the idea that iNPH has pathophysiological features that are discernible in the CSF

biomarker pattern. But, it is rather the pattern than the individual marker that seems to hold the greatest promise of a diagnostic tool for iNPH. Following the studies in this thesis and of others research, we propose that the biomarker pattern in iNPH is summarized with a lowering of all APP-derived proteins, no elevation of tau proteins and possibly a slight elevation of NFL and MCP1.

As a diagnostic team, we face a pre-selected group of persons that present with complaints that *could* be attributed to iNPH. As such, the clinical importance of CSF biomarkers is rather to distinguish which patients that have a pattern that can be attributed to iNPH from those that do not. In *study I* we were able to show this pattern and in *study III* we were able to repeat the findings and show that the combination of T-tau, Aβ40 and MCP-1 separated iNPH patients from patients with movement- and cognitive disorders of different origin than iNPH with a high sensitivity and specificity. In sum, we do believe that CSF can aid as an additional tool when diagnosing iNPH.

8.7 GENERAL METHODOLOGICAL CONSIDERATIONS

Some general methodological considerations that need to be addressed have in part been mentioned earlier but will be elaborated upon here.

As for building a model to predict if a person can be said to suffer from iNPH, we have to consider how representative our sample is. Even if the CSF biomarkers are analysed using different samples, they are all from the same pre-selected setting of patients referred to the unit with the suspicion of iNPH. This would render the prevalence of iNPH much higher than in the general population (with an estimated prevalence of up to 2 % of persons over 65 years [12]). Going even further, our contrast diagnoses are, even if at a specialized neurological hospital clinic, not under the suspicion of iNPH. In the real clinical setting, boundaries between different disorders are far more blurred. In addition, as stated in the 'methods' section, there is an overlap of patients in the different studies. Taken together it is of great importance that the results reported here are tested on other iNPH cohorts and that we carefully consider which patients the findings might be applicable to. This being said, we do think that the results can be of use. We do not claim to have found a diagnostic marker for iNPH that can be used in a preclinical setting (yet). But, going from the results presented herein, we do believe that we are ready to try the results prospectively at our clinic. This will be one of the upcoming studies.

Moreover, it is possible that our iNPH cohort might in turn represent a sub-population as the exact algorithm for diagnosing iNPH differs across the globe. Therefore, we do believe that it is of great importance to carefully examine CSF biomarkers in different iNPH cohorts to bring this into clarity.

In order to deliver diagnostic CSF biomarkers there are some concrete methodological limitations. Many of the assays are not as of yet in clinical

practice but are rather developed for research purposes. The between run variability is too large to be able to compare absolute levels between different studies, making it, at this stage, impossible to discuss cut-off levels for the biomarkers chosen. We know that it can be done, as in the case with core AD-biomarkers, but we are not there yet.

9 CONCLUSIONS AND FUTURE PERSPECTIVES

This thesis indicates that iNPH exhibits a CSF biomarker pattern that distinguishes patients with iNPH from healthy individuals of the same age but also from many of their mimics. This profile is characterized by a lowering of all APP-derived proteins, no elevation of tau proteins and an elevation of MBP, GFAP and MCP1.

We believe that these CSF changes reflect pathophysiological processes characteristic of iNPH. The lowering of APP-proteins seems specific and might be a consequence of a reduced production or/ in combination with a reduced clearance, specific for APP metabolites. No elevation of tau proteins indicates that there is no substantial cortical pathology in iNPH. The elevation of MBP indicates destruction of white matter and elevated GFAP and MCP1 indicates astrocyte activation.

As we view CSF biomarkers as a tool to study pathophysiological processes in iNPH we want to continue elaborating on these findings and combine them with additional biomarkers reflecting other aspects of pathophysiology. We plan to look more closely at synaptic function (in importance to APP) in iNPH by analyzing novel CSF biomarkers of synaptic function. Also, we want to continue exploring the relation between ISF and CSF. In order to do this, we would need to combine biochemical analysis (presumably with microdialysis and ventricular CSF) with radiological techniques, both MRI and possibly Positron emission tomography (PET). This combination of biomarkers (neuroimaging and biochemical) will also be combined with clinical data in order to explore if there is a close association between our biomarkers and clinical symptoms.

The biochemical changes in CSF of patients with iNPH seem to share common features with patients with SSVD. This could indicate that the underlying pathophysiology in iNPH and SSVD share common features, possibly connected to shared vascular risk factors and burden of vascular disease. These findings need elaborating, preferably by strengthening the collaboration with research milieus studying SSVD with the aim to explore longitudinal development of disease as well as similarities and differences that might be related to the reversibility of iNPH. It might even be that a proportion of the patients today classified as SSVD alone might actually benefit from shunt placement. This would relate to identifying biomarkers of reversibility. Even if we are not there yet, novel knowledge on pathophysiological processes might provide windows to explore this further.

As of today, we have not found any CSF biomarker that can aid in the selection of patients eligible for shunt surgery but we can see that the CSF profile changes after surgery indicating a substantial effect from the shunt. More studies on CSF biomarkers in lumbar CSF pre- and post-surgery are needed as such studies are lacking in the literature. We want to understand how the shunt procedure affects the biomarker concentrations and also to see if alterations in CSF biomarkers are linked to improvement from surgery. We are collecting both lumbar pre – and post surgery CSF samples and also ventricular CSF per- and post surgery in order to investigate these relations further.

In order to combine these pathophysiological findings from the studies, we constructed a CSF biomarker algorithm of T-tau, Aβ40 and MCP-1 that could prove useful for distinguishing iNPH from cognitive and movement disorders at specialized clinics. As for diagnostic purposes, we wish to elaborate on the model that we have shaped here. One step would be to assess the model on a different iNPH cohort. We have initiated a collaboration with another iNPH center for this purpose. We are also in the process of collecting CSF material prospectively on our cohort with no overlaps to the studies herein. We would also encourage other researchers to replicate the results presented in this thesis.

As the biochemical techniques advance quickly, many of the methods are now sensitive enough to measure CNS derived proteins in peripheral blood. We are already working on some of the CSF markers in plasma and/or serum and we hope that this field will expand in the future, opening up for blood tests aiding diagnostics in the not so distant future.

We hope that the results from this thesis and the studies to come will prove useful for our patients and their kin. We owe it to our patients to continue trying to render iNPH less idiopathic.

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CSF biomarkers in idiopathic normal pressure hydrocephalus.

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