Biomarkers in patients with idiopathic normal pressure hydrocephalus

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Thesis submitted for the degree of Doctor of Medicine (MD Res) at the University of London

Declaration

I hereby declare that the work presented in this thesis is my own.

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Abstract

Idiopathic normal pressure hydrocephalus (iNPH) is a condition affecting a small percentage of the elderly population; however it is the only known treatable cause of dementia. Surgical cerebrospinal fluid (CSF) diversion is the only known treatment for the condition today. However, such a procedure is not to be offered lightly and any expected benefit has to balance the associated surgical risks. The prognosis of a favourable surgical outcome has been problematic since the conception of the syndrome. None of current prognostic tests reaches 100% sensitivity or specificity and it is felt that there might be a need for a combination of tests, rather than a single one to maximize the chances of selecting the right patients to offer a surgical CSF diversion procedure. Biomarkers are biological substances that may act as surrogate markers of response to a treatment or to characterise a disease's progression over time.

The aim of this study was to identify CSF markers of favourable surgical outcome in patients with iNPH undergoing the insertion of a ventriculoperitoneal shunt (VPS). We first describe the effects of external lumbar drainage (ELD) on the CSF biochemistry of these patients. Correlations are made with imaging data obtained from volumetric analysis and neuropsychological tests in order to obtain a complete profile of these patients. The rostrocaudal gradients of the CSF markers examined are reported showcasing the need to understand that commonly reported values from lumbar CSF do not necessarily reflect pathological changes occurring at cerebral level. Finally, we report on the individual as well as combined prognostic value of 7 CSF markers on

surgical outcomes at 6 months. The pathophysiological significance of these markers is discussed individually.

It is concluded that the combined power of total tau and Aβ 1-42 may be useful in predicting favourable surgical outcomes at 6 months; further studies applying the findings in a larger cohort and correlating findings with longer outcomes are warranted to enhance the clinical application. The biochemical profile of patients with iNPH appears unique and different than patients with Alzheimer's dementia or control subjects.

Acknowledgements

I would like to thank primarily Mr Neil D. Kitchen for giving me the opportunity to work in the Victor Horsley Department of Neurosurgery and considering me for my position as a Research Fellow. Equally, I would like to thank Mr Laurence D. Watkins for accepting me as his student, acting as my primary supervisor, and being supportive through a long period not just with regards to the thesis, but also acting as a mentor in my professional career. The guidance, mentorship and understanding of both my supervisors have been most invaluable, and the skills (both surgical and research) I learnt during my position will help me in the years to come. Thirdly, I would like to thank Mr Ahmed Toma, my successor to the department who assisted tremendously in every possible way, but has also become a friend along the way. He has helped in making the process to submission tolerable. I wish him good luck with his own important research on NPH.

This work would not have been possible without the tremendous assistance of Dr. Miles Chapman for assisting with the experiments, and Dr. Andrew Church for ensuring the samples were collected appropriately. The hours I spent pipetting were fun, even though I made many times a mess of the lab bench! Equally, Dr. Geoff Keir and Dr. Axel Petzold provided a great insight into experimental design, as well as assistance in understanding all the weird staff about CSF biochemistry. Dr. Louis Lemieux has been invaluable in providing his machines and software for the volumetric analysis performed. The staff at the department of Neuropsychology and in particular Emily Pullen and Lisa Cipolotti, were extremely helpful for carrying out all the required tests mostly in very short notice. I would like to thank Janet Bousquet, Bina Shah, Ida White, and Corinne Docher for all their administrative help while in the department. Also, many thanks to Ms. Joan Grieve and Mr. Michael Powell for assisting in patient recruitment. Finally, I would like to acknowledge Dr Kostas Kallis on statistical advice, and the nursing staff in the

Victor Horsley department of Neurosurgery for being patient with all our lumbar drains! This work was made possible with the kind fellowship of B Braun/ Aesculap Academia who I wholeheartedly thank for supporting research.

I will not forget the patients and their families who placed their trust in the team hoping for an improvement in their quality of life; hopefully our interventions have fulfilled their expectations in most cases and we have helped a little in making their lives better. This work was done with a sincere hope that any findings will add a small stone to the pyramid of knowledge. We should all be Architects !

Lastly, a big thank you to Angharad for all the insight, help and support throughout all these difficult years.

This work is dedicated to my father Vasileios Tarnaris (1940-2012) who passed away suddenly awaiting the award of the degree. Thank you for believing in me.

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Financial disclosure

My salary supporting my research has been provided by a grant from B Braun/ Aesculap.

Publications arising from this work

- Tarnaris, A., L. D. Watkins, et al. (2006). "Biomarkers in chronic adult hydrocephalus." Cerebrospinal Fluid Res **3**(1): 11.
- Tarnaris, A., R. F. Stephenson, et al. (2007). "Diagnosis, Treatment, and Analysis of Long-term Outcomes in Idiopathic Normal-Pressure Hydrocephalus." Neurosurgery **60**(1): E208.
- Tarnaris, A., N. D. Kitchen, et al. (2008). "Noninvasive biomarkers in normal pressure hydrocephalus: evidence for the role of neuroimaging." Journal of Neurosurgery: 1-15.
- Tarnaris, A., A. K. Toma, et al. (2009). "The longitudinal profile of CSF markers during external lumbar drainage." Journal of Neurology, Neurosurgery & Psychiatry **80**(10): 1130-1133.
- Tarnaris, A., A. K. Toma, et al. (2009). "Ongoing search for diagnostic biomarkers in idiopathic normal pressure hydrocephalus." Biomarkers **3**(6): 787-805.

Papers submitted for publication

- Cerebrospinal fluid amyloid-beta and total tau may be able to predict favourable surgical outcomes in patients with idiopathic normal pressure hydrocephalus
- Rostrocaudal dynamics of cerebrospinal fluid markers in idiopathic normal pressure hydrocephalus
- Cognitive and biochemical profile of patients suffering from idiopathic normal pressure hydrocephalus

Conference Presentations

1. **Rostrocaudal dynamics of cerebrospinal fluid markers in idiopathic normal pressure hydrocephalus. A. Tarnaris**, A. Toma, MD Chapman, A. Petzold, ND Kitchen, G. Keir, LD Watkins. **Oral presentation**. Hydrocephalus 2009, Baltimore, USA- September 2009.

2. **Prognostic biomarkers in idiopathic normal pressure hydrocephalus, A. Tarnaris**, A. Toma, MD Chapman, A. Petzold, ND Kitchen, G. Keir, LD Watkins. **Oral presentation.** Hydrocephalus 2009, Baltimore, USA- September 2009.

3. **Prevalence of ventriculomegaly in "falls" clinics. Initial results.** A. Toma, **A. Tarnaris,** S. Das, B. Glickstein, M. Cohen, R. Gray, C. Pulford, L. Watkins. **Oral presentation.** Hydrocephalus 2009, Baltimore, USA- September 2009.

4. **Outcome assessment in normal pressure hydrocephalus: what is the most important? Patient and carers perspective.** A. Toma, **A. Tarnaris,** ND Kitchen, LD Watkins. **Oral presentation.** Hydrocephalus 2009, Baltimore, USA- September 2009.

5. **Investigating shunt function using Continuous Intracranial Pressure Monitoring in adults: single centre experience.** Ahmed Toma, **Andrew Tarnaris**, Neil Kitchen, Laurence Watkins.
Poster presentation Society of British Neurological Surgeons, Birmingham, UK- April 2009**.**
Poster presentation WFNS- Boston, USA- September 2009.

6. **Cognitive and biochemical profile of patients suffering from idiopathic normal pressure hydrocephalus. A. Tarnaris**, A. Toma, E. Pullen, MD Chapman, A. Petzold, ND Kitchen, L. Cipolotti, L. Lemieux, G. Keir, LD Watkins **Oral Presentation** Hydrocephalus 2008, Hannover, Germany- September 2008.

7. **Adjustable shunt valve induced MRI artefact: A comparative study.** Toma A, **Tarnaris A,** Grieve J, Watkins L, Kitchen ND.
Oral Presentation Hydrocephalus 2008, Hannover, Germany- September 2008.
Poster Presentation Society of British Neurological Surgeons, Notthingham, UK- September 2008.

8. **Temporal changes of CSF markers during external lumbar drainage in patients with idiopathic normal pressure hydrocephalus A. Tarnaris,** A. Toma, MD Chapman, A. Petzold, ND Kitchen, G. Keir, LD Watkins **Oral Presentation** Hydrocephalus 2008, Hannover, Germany- September 2008.

9. **Induction of angiogenesis in idiopathic normal pressure hydrocephalus.**
A. Tarnaris, M. Chapman, N. Kitchen, G. Keir, L. Watkins. **Oral Presentation** 4th International Workshop in Hydrocephalus, Rhodes, Greece- May 2007.

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Description of thesis

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Abbreviations used

(2DE)	two-dimensional electrophoresis
(5-HIAA)	5-hydroxyindoleacetic acid
(ABP)	Arterial blood pressure
(ACE)	Angiotensin-converting enzyme
(AChE)	Acetylcholinestarase
(ACT)	Alpha1-antichymotrypsin
(AD)	Alzheimer's disease
(ADC)	Apparent diffusion coefficient
(ADDTC)	Alzheimer's Disease Diagnosis and Treatment Centre
(ApoE)	Apolipoprotein E
(BD)	Binswanger's disease
(BP)	Blood pressure
(BrdU)	Bromodeoxyuridine
(BuChE)	Butyryl cholinesterase
(C)	Cerebral compliance
(CAH)	Chronic adult hydrocephalus
(CBF)	Cerebral blood flow
(Cho)	Choline
(CNS)	Central nervous system
(Cout)	Conductance to outflow
(CRF)	Corticotropin releasing factor
(CSF)	Cerebrospinal fluid
(CT)	Computed tomography
(CV)	Coefficient of variation
(CVD)	Cerebrovascular disease
(DAT)	Dementia of Alzheimer's type
(DSIP)	Delta-sleep-inducing peptide
(DSM)	Diagnostic and Statistical Manual of Mental Disorders
(DWI)	Diffusion-weighted imaging
(DWMH)	Deep white-matter hyperintensities
(DWMLs)	Deep white-matter lesions
(ECF)	Extracellular fluid
(ELD)	External lumbar drainage
(E.L.I.S.A)	Enzyme Linked Immuno-Sorbent Assay
(FDG)	[18F] fluorodeoxyglucose
(GABA)	4-gamma aminobutyric acid
(GFAP)	Glial Fibrillary acidic protein
(GP)	General Practitioner
(HVA)	Homovanillic acid
(H-Tx)	Hydrocephalus Texas
(ICD)	International Statistical Classification of Diseases and Related Health Problems
(ICP)	Intracranial pressure
(iNPH)	Idiopathic form of NPH
(LIT)	Lumbar infusion test
(LP)	Lumboperitoneal
(LP)	Lumbar puncture
(MBP)	Myelin Basic Protein
(MHPG)	3-methoxy-4-hydroxy-phenylglycol
(MID)	Multi-infarct dementia

(MMSE) Mini-Mental State Examination
(MRI) Magnetic resonance imaging
(MRS) MR spectroscopy
(MS) Multiple sclerosis
(NAA) N-acetyl aspartate
(NFL) Neurofilament triplet protein
(NINDS-AIREN) National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherché et l' Enseignement en Neurosciences
(NMR) Nuclear magnetic resonance
(NPY) Neuropeptide-Y
(NPV) Negative predictive value
(NSE) Neuron specific enolase
(NT-3) Neurotrophin-3
(OD) Optical density
(PAI-1) Plasminogen activator inhibitor-1
(PC) Phase-contrast
(Pcsf) CSF pressure
(PD) Parkinson's disease
(PET) Positron emission tomography
(PGDS) Prostaglandin D synthase
(PI) Pulsatility Index
(PPV) Positive predictive value
(PP) Intraparenchymal pressure
(P_{ss}) Pressure at the sagittal sinus
(PVI) Pressure volume index
(PVH) Periventricular hyperintensities
(PS-1) Presenilin-1
(rCBF) Regional cerebral blood flow
(ROC) Receiver operating characteristic
(Rout) CSF Resistance to outflow
(SAE) Subcortical arteriosclerotic encephalopathy
(SH) Protein sulfydryl
(SOM) Somatostatin
(SPECT) Single photon emission computed tomography
(TBAR) Thiobarbituric acid-reactive material
(TNF-α) Tumour-necrosis factor United States
(TTIR) Total tau immunoreactivity
(VP) Ventriculoperitoneal
(VIP) Vasoactive intestinal peptide
(VR) Virchow-Robin spaces
(Xe-CT) Xenon contrast computed tomography

Personal Statement

I have been intimately involved in all aspects of the work described in this thesis. I devised the experimental protocols and drafted the ethics committee applications. I analysed the experimental data, carried out the statistical analysis and have presented the findings at several international conferences.

Nevertheless the analytical techniques I employed were developed from previous studies carried out by the staff of the Department of Neuroimmunology, the department of Neuropsychology and the Department of Clinical and Experimental Epilepsy, Institute of Neurology, London, UK and I am indebted to them for laying the foundations for the work I present in this thesis.

1. Introduction

1.1. Definition and terminology

The term “normal pressure hydrocephalus” (NPH) was introduced by Adams and Hakim in 1965 in a publication reporting three patients with a clinical triad of cognitive decline, gait difficulties and sphincter disturbances associated with ventriculomegaly in air studies and normal pressure of cerebrospinal fluid (CSF) during lumbar puncture (Adams, Fisher et al. 1965). Since then the contradictory terms “normal pressure” and “hydrocephalus” have puzzled the non experts in the field. Hydrocephalus by definition implies high intracranial pressures due to the accumulation of CSF within the ventricular system. The problem has arisen due to the popularity of the bulk-flow theory of CSF flow (Dandy WE and Blackfan 1914) as the only explanatory theory causing hydrocephalus. However, the bulk-flow of CSF cannot explain peculiarities of CSF circulation, such as the idiopathic form of NPH (iNPH).

Due to this contradiction as well as to the fact that the intracranial pressures are not always normal in patients with iNPH, the term has been strongly disputed. It was viewed as an over simplification to classify hydrocephalus only according to the intracranial pressure (ICP) dynamics, therefore as high-pressure or normal-pressure. The classification of hydrocephalus becomes important due to the fact that it is closely linked with the proposed treatment and outcome. Based on pathophysiological considerations, hydrocephalus has been divided into communicating and non-communicating by Dandy, depending on whether or not communication between the ventricular system and the lumbar subarachnoid space is free (Dandy 1919). In this sense NPH represents a form

of communicating hydrocephalus. If we take in mind the classification introduced by Russell (Russell 1949) then NPH represents a form of non-obstructive hydrocephalus. However, NPH (especially its secondary form) might be an obstructed form if we consider that the block in the CSF flow exists extraventricularly and particularly in the level of the Pachionian bodies. In this sense the Russell classification confuses rather than helps in the classification of NPH. The previous two classifications take in account the CSF flow in the major pathways as it is the traditional understanding. A recent classification has been proposed classifying hydrocephalus to “minor pathway hydrocephalus”, differentiating from the conventional classification by Dandy (communicating and non-communicating) or Russell (non-obstructive and obstructive) as “major pathway hydrocephalus (Oi and Di Rocco 2006). In the former term it is understood that CSF absorption does not only happen in the arachnoid granulations, but also with the drainage route via perineural space into the lymphatic system (Brierley and Field 1948; Dohrmann 1972; Boulton, Flessner et al. 1998; Ludemann, Berens von Rautenfeld et al. 2005), and via transependymal–interstitial to the perivascular/ subpial space both in the brain and spinal cord (Becker, Wilson et al. 1972; Hochwald, Boal et al. 1975).

Measurements of CSF dynamics have provided further classification of the syndrome according to the ICP values and the percentage of B waves. Therefore normal pressure hydrocephalus has been classified as i) active, ii) compensated unstable, and iii) compensated stable (Sahuquillo, Rubio et al. 1991).

The duration of hydrocephalus has been taken into account as reflected in the nomenclature used. One of the terms used to describe the same condition is *chronic adult hydrocephalus* (Bret, Chazal et al. 1990). This term proposed by Bret divides hydrocephalus into acute and chronic forms, referring to the chronicity of the CSF disorder implying this long-lasting CSF disorder as the cause of the symptoms of this syndrome. Thus, idiopathic NPH represents a form of chronic communicating hydrocephalus. Based on symptomatology, hydrocephalus has been classified as *symptomatic congenital*, *asymptomatic* and *arrested/compensated* (Larsson, Stephensen et al. 1999). “Arrested hydrocephalus” refers to the stabilization of the patient’s symptoms during the previous years and “symptomatic congenital” means that the condition has been present from birth but symptomatic only as the patient ages. It has been recently suggested that NPH may represent a two-hit disease originating from infancy (Bradley, Bahl et al. 2006), therefore the symptomatology of NPH patients may vary between the arrested and symptomatic congenital forms.

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Figure 1.1.1 Historical trend of research on CSF physiology and treatment for hydrocephalus

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Figure 1.1.2. Proposal of evolution theory in CSF dynamics. Greitz performed an experiment of radionuclide cisternography in ten patients suffering from venous vasculitis. He observed that there was a pool of tracer dye at the convexity in normal subjects that is concomitant with another maximum concentration in the lumbosacral area and not consistent with a local dilution of the tracer at the level of the foramen magnum. He therefore proposed that the CSF circulation is not so much to the bulk flow

as traditionally thought nut mainly due to the pulsatility within the CNS. The diagram above is showing the commonly accepted bulk flow model (Left diagram) and the two types of cerebrospinal fluid circulation related to the proposed concept of the circulation (Middle and right-hand diagram. The dominant pulsatile flow shown in the middle diagram is responsible for the rapid spread of tracers within the extraventricular cerebrospinal fluid spaces, and the comparatively small, almost minute, bulk flow on the right-sided diagram explains the appearance of the cisternogram in normal cases causing washout of tracer in the ventricular system and the basal cisterns (Greitz and Hannerz 1996).

1.2. Historical background

Hydrocephalus was considered a condition of high morbidity before the design and manufacturing of the first successful diaphragm valves in the mid-fifties (Gjerris and Snorrason 1992). Nevertheless this was referring to cases of obstructive hydrocephalus and increased CSF pressure. However as early as 1935 it was noted by Penfield that “it should be pointed out that an occasional exceptional case is encountered in which the CSF spaces are closed and the ventricles progressively enlarge without the measured intraventricular pressure rising above 150-200 mm of water” (Penfield. 1935). Prior to 1964 a few case reports have been published describing patients with the paradox of normal CSF pressure on lumbar puncture and a combination of gait ataxia or apraxia, dementia and urinary incontinence (Riddoch 1936; J Lhermitte and J 1942; Lhermitte J and Mouzon 1942; Yakovlev 1947; Wertheimer and Dechaume 1950; Foltz and Ward 1956; Kibler, Couch et al. 1961; Shulman, Martin et al. 1963; McHugh 1964; Messert and Baker 1966). However, it seemed that the publication of Adams and Hakim (Hakim and Adams 1965) highlighted the syndrome and from then onwards the terminology *Hakim* or *Hakim-Adams syndrome* was used by many authors, even though in one paper this latter syndrome was distinguished as a different entity from the traditional NPH syndrome (Huckman 1981). *Symptomatic occult hydrocephalus with "normal cerebrospinal fluid pressure”* (Miller 1970) and *hydrocephalic dementia* (McHugh 1966; Avant and Toole 1972; Vivenza, Bricolo et al. 1980) were two other descriptive terms used for the syndrome .

In their paper of 1965 Hakim and Adams describe the cases of 3 patients. The first patient a young male aged 16 years had first sustained a head injury complicated by a small right subdural haematoma that was surgically removed. A few weeks later since his clinical condition was not progressing and his level of consciousness was described as “semi-comatose” by the authors, it was decided that a lumbar puncture (LP) is performed; this was done duly and revealed an opening pressure of 150 mm Hg. The patient clinically improved following the LP and a lumbar pneumoencephalogram revealed communicating hydrocephalus. He finally had a ventriculo-atrial shunt 6 weeks following his original head injury. The second patient was a male aged 52 years-old who presented with “gradual failure of memory, mental dullness and apathy, disinterest in personal appearance, unsteadiness of balance and stiffness of legs”. His cognitive symptoms were the first to appear. His wife also noted “an incontinence of urine, which began to occur from time to time”. An LP revealed an opening pressure of 18 mm Hg. He likewise improved following the removal of the spinal fluid but gradual deterioration forced the authors to insert a ventriculoperitoneal shunt. The patient showed a definitive clinical improvement following shunting. In the 3rd case the authors described a 43 years-old male who following a head injury which resulted in hemiparesis and evidence of intracerebral contusion he had “wide fluctuations in alertness, motility, and sphincteric control”. Following the demonstration of communicating hydrocephalus a ventriculo-atrial shunt was performed which resulted in the patient being able to work and lead an independent life.

The authors have clearly described one case of idiopathic (second case), and two cases of secondary NPH. The authors in their discussion state that a pressure of 180 mm water in enlarged ventricles is pathological and may be responsible for the maintenance of hydrocephalic symptoms. They also use the law of Pascal to explain the peculiar phenomenon of normal pressure and increased ventricular size which state that the force exerted on the ventricular wall represents a product of pressure times the surface area. Therefore a given pressure exerts a greater force in a large ventricular area, as the one we have in a hydrocephalic state. In order to account for the symptoms they suggested that the dilatation of the ventricles subjects the major long tracts in the cerebral white matter and corpus callosum to compression and stretching. They also think that the symptoms are reversible once the pressure is not exerted any more along the anatomical structures.

1.3. Epidemiology

NPH is mostly encountered in the over-60's (Trenkwalder, Schwarz et al. 1995; Krauss, Droste et al. 1996). It represents a treatable form of dementia and if left untreated may lead to lethargy, unconsciousness and death (Hakim, Hakim et al. 2001). Exact epidemiological figures for normal pressure hydrocephalus have been scarce however. That is the result of the recent description of the syndrome, the complicated pathologoanatomical and pathophysiological background leading to confusion among clinicians on which patient has NPH and which on doesn't, as well as the commodity of the symptoms of the clinical triad among the elderly population. The prevalence of NPH in the general population has not been quantified. Two European studies in small populations have roughly estimated that 1% to 6% of all dementias are due to NPH and 0.41% of persons in the general population 65 years or older have the disease (Casmiro, Benassi et al. 1989; Trenkwalder, Schwarz et al. 1995). However, both groups felt that NPH is significantly underestimated because many cases go unreported and untreated.

NPH is generally estimated to account for 0.4% to 10% of cases of dementia (Vanneste and van Acker 1990; Vanneste 1994; Larsson, Stephensen et al. 1999; Srikanth and Nagaraja 2005). However, lower prevalence has been reported (Clarfield 2003; Jha and Patel 2004). In a recent questionnaire based study among 53 German Neurosurgical centres the annual incidence of NPH in Germany was estimated to be 1.8 per 100,000 inhabitants (Krauss and Halve 2004). In a national survey in Sweden the incidence of

surgery for all types of hydrocephalus was estimated 3.36 per 1000000 of the population with 47% of them being performed for the idiopathic form of NPH. Both these figures point to an average figure of an incidence of 1.68 per 100000 of population.

In an economic United States (US) study comparing the Medicare expenditure of shunted versus not shunted patients these were found to be $25,477 less in patients receiving a shunt, a cost difference which was statistically significant (Williams, Sharkey et al. 2007). They examined the national hospital discharge survey for the year 2000 showing that only 6000 out of 25000 (i.e. 24%) patients with the diagnosis of hydrocephalus (ICD-9-CM Codes 331.3 and 331.4) had a first shunt (i.e. not a revision) procedure. In that important study they also revealed that age >80 years, and an African-American race are two factors associated with a reduced likehood of receiving a shunt. They have suggested in their analysis that older age appears to be a disproportionate reason not to proceed for surgery and they clearly advocate against such a “habit” suggesting that one should take in account the health status and potential benefit for treatment. One of the limitations of the above study is that the codes do not provide the health status of individuals at their time of assessment. Early diagnosis, referral to a Neurosurgeon and treatment becomes important since a long symptomatic disease become more difficult to treat successfully. Meier has shown that advanced stage NPH has only a 50% chance of improvement versus those who underwent earlier surgery that had a 65% chance of improvement (Meier and Bartels 2002).

1.4. Genetics

No genetic association has been discovered so far for the syndrome of NPH. In 1984 the first case of two siblings suffering from NPH has been reported (Portenoy, Berger et al. 1984). Another case of a family containing two members (a 76 year old man and the 47-year old daughter of his twin sister) representing with adult-onset gait disturbances and in the case of the man with additional cognitive decline and urinary incontinence. It was proposed by the authors that the mode of inheritance in this case would be autosomal dominant with variable penetration without though excluding an X-linked mode of inheritance (Chalmers, Andreae et al. 1999). Another case of monozygotic twins with NPH has been recently reported (Forman, Vesey et al. 2006). A fourth case of a cluster of NPH in a family composed of 3 brothers and the grandson of one of the brothers affected has also been reported. The mode of inheritance from the proband to his daughter's grandson reveals an X-linked mode of inheritance. It was also noted by the authors that two of the affected subjects and the female carrier suffered from ovarian and colon carcinoma. They suggested that the mutation that caused the carcinoma might be related to the same mutation that caused the hydrocephalus (Katsuragi, Teraoka et al. 2000). Nacmias and colleagues in a study of 13 patients investigated the distribution of the apolipoprotein E (ApoE) epsilon4 allele, as well as that of the alpha1-antichymotrypsin (ACT) gene and of allele 1 of the presenilin-1 (PS-1) gene. They observed an increased ApoE epsilon4 allele frequency among NPH patients when compared with controls, thus suggesting that epsilon4 allele may also be involved in the pathogenesis of the disease (Nacmias, Tedde et al. 1997). It is known that the

presence of ApoE epsilon4 allele is considered as a risk factor for the development of Alzheimer's type dementia (AD), and other dementias and therefore their presence might be related to the comorbidity of AD-type dementia in patients suffering from NPH (Corder, Saunders et al. 1993; Frisoni, Calabresi et al. 1994; Savolainen, Paljarvi et al. 1999; Bech-Azeddine, Hogh et al. 2007). In a prospective study of 112 patients with NPH studying the polymorphism of the angiotensin-converting enzyme (ACE) and its relations to the cognitive decline del Mar Matarin and colleagues found that there was no difference in allele distribution between patients and healthy controls, however patients with possession of at least one D allele received less benefit with regards to their cognitive outcome (del Mar Matarin, Poca et al. 2005).

1.5 Theories for development

The pathophysiology of iNPH is still an enigma. Today two principally different mechanisms are considered: A) altered hydrodynamics of the CSF system; and 2) a parenchymal, possibly ischemic, process. The former is demonstrated by modestly raised ICP, increased CSF Resistance to outflow (Rout) and the presence of abnormal B-wave patterns; the latter is indicated by cortical and subcortical decreased blood flow and metabolism as well as periventricular white matter lesions. Research so far has noted impaired autoregulation (Chang, Kuwana et al. 2000; Czosnyka, Czosnyka et al. 2002), a relationship to vascular disease (Bradley, Whittemore et al. 1991; Krauss, Droste et al. 1996; Boon, Tans et al. 1999), decreased cerebral blood flow (Vorstrup, Christensen et al. 1987; Kristensen, Malm et al. 1996; Momjian, Owler et al. 2004), and ischemia (Bradley, Whittemore et al. 1991; Krauss, Regel et al. 1997; Corkill, Garnett et al. 2003). In addition, epidemiological studies have shown that hypertension might be a risk factor (Krauss, Droste et al. 1996).

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Figure 1.5.1.1. Historical account of chronic hydrocephalus. Ruffer in his 1890 “digest” of chronic hydrocephalus gives an insight into the pathogenesis of chronic hydrocephalus. This was recently described as the “two-hit” theory for the development of chronic hydrocephalus (Ruffer 1890).

Other proposed theories included direct vascular compression by the enlarged ventricles (Akai, Uchigasaki et al. 1987; Graff-Radford and Godersky 1987; Vanneste 2000; Meier, Konig et al. 2004), increase in the interstitial fluid pressure (Tamaki, Nagashima et al. 1990; Pena, Harris et al. 2002), and the so-called metabolic theory claiming relative CSF stasis with decreased clearance of various macromolecules being responsible for the pathogenenesis (Marmarou, Takagi et al. 1980; Tullberg, Hultin et al. 2002; Klinge, Samii et al. 2003; Silverberg, Mayo et al. 2003; Kondziella, Sonnewald et al. 2008),

Any theory that will try to explain the pathogenesis and pathophysiology of his syndrome has to explain not only the factors implicated in the initiation of ventriculomegaly, but more over those factors that are responsible for the maintenance of the syndrome and its regression once shunting has occurred. It is only thus that the puzzle might be completed and we might get a full understanding of the syndrome.

Normal pressure hydrocephalus is classically divided into two groups: a) *the secondary form* where there is a background of subarachnoid haemorrhage (E.L. Foltz and Ward 1956), CNS infection, trauma (King 1938), tumour, or aqueduct stenosis (Vanneste and Hyman 1986), and b) *the idiopathic form*, where the cause is unknown. In the case of subarachnoid haemorrhage the blood degradation products produce inflammatory fibrosis in the arachnoid granulations, therefore increasing the resistance to CSF absorption. Initially there is an incidence of acute rise of the intracranial pressure which leads to ventriculomegaly. Following that the CSF is slowly reabsorbed, however the ventricles remain dilated. It is important to note that the pathophysiology of the idiopathic and secondary forms differ and the idiopathic form of NPH tends to present in the elderly (Krauss, Regel et al. 1997), whereas patients with chronic communicating hydrocephalus from prior subarachnoid haemorrhage, meningitis, neurosurgical intervention, or head trauma present in relation to the causing event and generally in a younger age. We will therefore analyze and discuss the pathophysiology of only the idiopathic form.

1.5.1 Altered CSF dynamics in NPH

A disturbance in the CSF dynamics in chronic hydrocephalus has been one of the first theoretical attempts to explain the pathogenesis of the syndrome (Stein and Langfitt 1974; Borgesen and Gjerris 1982). As realized by Dandy, an obstructed bulk flow of CSF at the pacchionian granulations cannot cause communicating hydrocephalus (Dandy 1920). Recent work by Boulton (Boulton, Young et al. 1996; Boulton, Flessner et al. 1998; Boulton, Flessner et al. 1999), Cserr (Knopf, Cserr et al. 1995) and others has challenged the traditional understanding of CSF absorption, and flow MRI studies of Greitz (Greitz and Hannerz 1996), Bateman (Bateman 2004), and others suggest that pulsatility plays a central role in the pathogenesis of hydrocephalus. According to them, the cumulative effect of many pulse waves slowly remoulding the brain is the cause of the ventricular enlargement in chronic hydrocephalus. Intracranial hydrodynamics is thus dependent on the compliance of the thecal sac and the compressible outlets of the bridging veins. Compliance is the capacity of a buffer system to accommodate a volume change and is defined as volume change divided by pressure change (dV/ dP). The expression of decreased compliance in communicating hydrocephalus is increased intracranial pulse pressure and/or decreased intracranial stroke volume. It was suggested that the insertion of a shunt at least initially introduces a new transmantle pressure gradient (Sorteberg, Eide et al. 2004).

Most biological tissues are characterized by plasticity and therefore respond to local forces by local displacement, deformation and remoulding. Small pressure gradients may deform the brain, since it has a high plasticity. The deformation of

the brain and the CSF spaces defines hydrocephalus. The definition of hydrocephalus is enlarged ventricles at the expense of a narrowed subarachnoid space. The brain is displaced towards the skull and the cortical gyri in hydrocephalus are often compressed or flattened. The transmantle pressure gradient i.e. an increased regional force directed from the ventricles towards the subarachnoid space is the only possible force, which could be responsible for such deformation (Hoff and Barber 1974). Hydrocephalus differs from processes lacking a transmantle pressure gradient such as cerebral atrophy, where both the ventricles and subarachnoid space enlarge. The normalization of the CSF spaces following shunting indicates that the transmantle pressure gradient can be narrowed or reversed, which further support that it really exists; recent work though has not supported this argument (Eide and Saehle).

The cranium may be seen as a frequency-sensitive notch filter that suppresses the arterial pulse in the brain. This redistributes the kinetic energy of pulsatility at the heart rate (i.e. cardiac frequency) to smooth, pulseless arterial flow (i.e. zero frequency), which is the cerebral blood flow. This represents the normal spectral distribution of the transfer function between the arterial pulse and the ICP pulse, and is a manifestation of the normal cerebral *windkessel* mechanism.

The loss of cerebral blood flow and augmentation of pulsatility is the main manifestation of an impaired *windkessel* mechanism, and leads to venous stasis, venous hypertension, and reduction in cerebral blood flow. It is suggested that the consequences of impairment of the cerebral *windkessel* mechanism, the mechanism by which the cerebral vasculature renders vascular perfusion of the

microvasculature nearly smooth are ventriculomegaly and CSF malabsorption (Egnor, Wagshul et al.).

Hakim's proposal was that of altered equilibrium of the intraparenchymal and CSF pressure. In brief Hakim suggested that in the normal state the difference between the intraparenchymal pressure (Pp) and CSF pressure (Pcsf) is zero; that is the two pressures are equal applying opposite forces to the ventricular wall. *CSF pressure* is equal to the rate of *CSF formation X CSF outflow resistance + Intracranial venous pressure*. In the case of increased resistance to CSF outflow or increased CSF production, the CSF pressure increases and there is a net positive pressure exerted on the ventricular wall (Pcsf>Pp). When again these forces come into a new equilibrium (Pp=Pcsf) the net difference is again zero, however the ventricles do not return to their previous state (Hakim 1985). When there is a disequilibrium of these two forces (usually when Pcsf> Pp) the brain suffers distortion because of the non hydrostatic loading of its parenchyma.

As a result of the non-hydrostatic distribution of pressure changes the fluid within the cerebral parenchyma is squeezed out into the extracranial venous system, reducing the volume occupied by the parenchyma, thus enabling ventricular enlargement and net CSF volume increase. The brain is a live structure composed of glial cells thus behaving as a viscoelastic material, and cannot absorb for long periods of time the expansive force created by the CSF pressure on the ventricular walls (Hakim, Hakim et al. 2001). The above theories explain the initiation and maintenance of ventriculomegaly but not the clinical picture of the syndrome itself.

1.5.2. Ischaemic process in NPH

1.5.2.1. Autoregulation in NPH

It is well known that the cerebral blood flow (CBF) remains constant over a wide range of intracranial pressure, usually ranging between 0 and 50 mm Hg because of CBF-CSF autoregulation. It was found that there is an enhanced response to acetazolamide administration in patients who do not improve with a shunt (Tanaka, Kimura et al. 1997). The explanation for that maybe that in patients who do not improve there might be an irreversible damage in the brain structure as a result of the pathophysiologic processes which are responsible for the syndrome.

1.5.2.2. Cerebral blood flow

The arterial supply in the white matter mainly consists of long medullar branches from the brain surface and, to a lesser extent, of perforating striate arteries from middle cerebral artery. These consist mainly of end-arteries, which explain why this tissue is sensitive to ischemia. Decreased cerebral blood flow in this area is frequently reported in iNPH, and a connection to the enlarged ventricles and increased Rout has been proposed (Vorstrup, Christensen et al. 1987; Kristensen, Malm et al. 1996; Momjian, Owler et al. 2004; Owler, Pena et al. 2004; Tullberg, Hellstrom et al. 2004). The main methods to assess cerebral blood flow in white matter are based on labeled tracers as in single photon emission computed tomography (SPECT), positron emission tomography (PET), and xenon contrast computed tomography (Xe CT). In recent years, perfusion magnetic resonance

imaging (MRI) has been developed for this purpose (Hertel, Walter et al. 2003). The findings of these studies will be presented in chapter ∫ 1.15.

1.5.2.3. Altered venous hydrodynamics

The importance of the venous system compliance in iNPH is still under investigation. Since the theory of increased resistance to CSF outflow is well established it makes sense to examine the venous system as one of the main pathways of CSF absorption. Occasionally it is easier to study the venous outflow at the level of the internal jugular vein. A study identified that in patients with iNPH there is a retrograde jugular venous flow probably due to valve defect at the level of the internal jugular vein; the sensitivity & specificity of using ultrasonography to detect patients with probable iNPH was 95% and 77% respectively (Kuriyama, Tokuda et al. 2008). Such a defect in the venous outflow might be related with the breakdown of the windkessel mechanism known to exist in communicating hydrocephalus (Egnor, Zheng et al. 2002). During the normal windkessel mechanism intracranial arterial pulsations are dampened by the shift of CSF and venous blood outside the cranial cavity in order to preserve the continuous nonpulsatile blood flow as required at the level of the capillaries (Greitz 1993). Bateman suggested that the central role in pathogenesis in INPH is in fact the elevation in superficial venous system pressure and a reduction in the blood flow returning via the sagittal sinus suggesting also that the deep white-matter hyperintensities (DWMI) is an epiphenomenon rather than the cause of iNPH (Bateman 2008). This elevation in cortical vein pressure appears to be reversible following shunting (Bateman 2003).

1.5.2.4. The role of capillary hydrodynamics

Study of the contribution of the capillary hydrodynamics to the development of the syndrome may only be studied realistically by using a mathematical model (Penar, Lakin et al. 1995). Within the limited studies carried out it has been postulated that increased flow resistance at the level of the capillaries may be a contributing factor to the maintenance of ventriculomegaly. That resistance was suggested to be the result of arterial hypertension known to be prevalent in patients with iNPH (Krauss, Droste et al. 1996). An adaptive process of the brain to chronic hydrocephalus is increase in capillary density and diameter (Luciano, Skarupa et al. 2001).

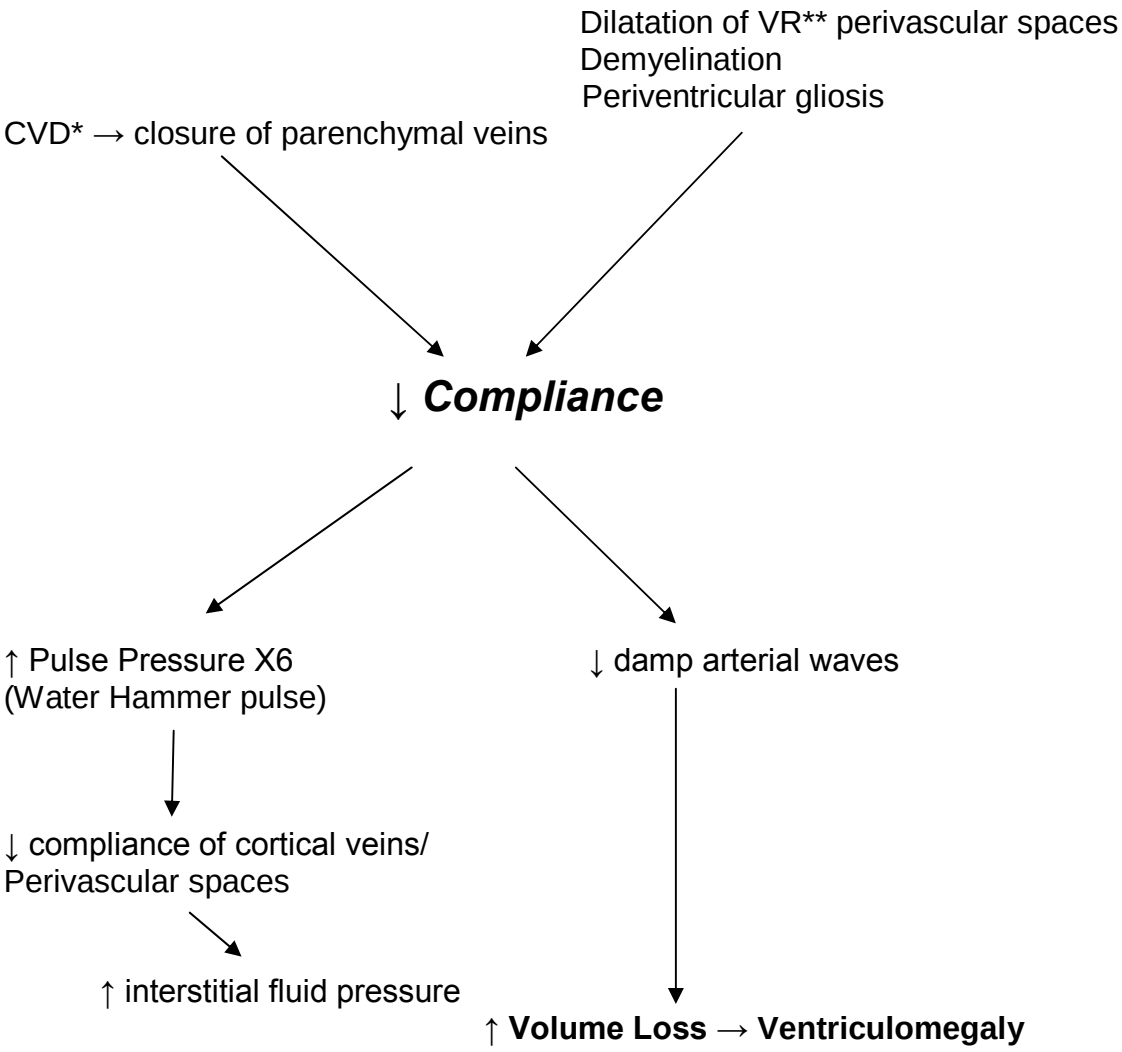

Figure 1.5.2.4.1. Dynamic changes in physiology and pathology that take place during development of the syndrome. Reduced compliance has a central role in it. (* Cerebrovascular disease, ** Virchow-Robin spaces)

1.6. CSF dynamics in chronic hydrocephalus

Idiopathic NPH may cause changes in fluid dynamics in very many ways: CSF resorption routes, as well as CSF production may be altered, the diversion of CSF between fluid compartments might shift, compliance and elastance of brain parenchyma and the ventricular walls might change. Even the input signal to the intracranial space, namely arterial inflow, as well as arteriolar resistance might be changed. Likewise, the venous outflow from the brain might differ from a normal pattern. The answer to such complex pathophysiological processes is more likely embedded in, for example, the profile of ICP measurements, rather than in rough mean ICP values. This necessitates the use of somewhat complex diagnostic tests in order to calculate the intracranial dynamics. The measurement of intracranial dynamics is based on the principle of Kellie-Monroe and the fact that the CSF/blood/brain remains constant at all stages according to the following equation:

$\mathbf{V_{tissue} + V_{blood} + V_{csf} = V_{total} = V_{eq} + V_e}$, where V_{eq} is the equilibrium volume when the ICP is normal and V_e is the elastic volume.

It is also important to appreciate that **CSF pressure= rate of CSF formation X CSF outflow resistance + Intracranial venous pressure**

It therefore follows that for any change in volume the pressure within the cranial cavity will be altered and vice versa (Marmarou, Shulman et al. 1978). This relationship of the intracranial pressure versus intracranial volume may be assessed by CSF volume-pressure studies and is described in terms of elastance

(i.e. elasticity and stiffness) and compliance. The aim of any CSF volume-pressure study is to measure the Rout along with other compensatory mechanism and consider whether the CSF circulation is disturbed and therefore assess the suitability of the patient for surgical cerebrospinal fluid diversion (shunting).

1.6.1. Parameters of CSF dynamics

1.6.1.1. Intracranial pressure

There have been glimpses of hope of a non-invasive way of calculating the ICP by using a mathematical model which by calculation the arterial blood pressure (ABP) waveform linearly transforms the relationship between ABP and cerebral blood flow velocity (Schmidt, Czosnyka et al. 2000). However, the standard method of calculating the ICP remains invasive by inserting a pressure monitor probe either in the subdural space, intraparenchymally, or even intraventricularly. The probe is left in situ for at least 24 hours and then the data is analysed either visually by the clinician, or ideally via a computerised system. Data such as the mean ICP, the waveform and its waves may be then extracted for the whole time. Intracranial pressure oscillation consists of a cardiac-induced component, a respiration-induced component and fluctuation of the base level of ICP. Its waveform is composed of the so called alpha, beta and c waves (Lundberg 1960). Ventricular CSF pressure measurement is considered the gold standard, however a recent paper has suggested that lumbar CSF measurement agrees well with intracranial ICP measurements (Lenfeldt, Koskinen et al. 2007). In normal volunteers, the CSF opening pressure averages 8.8 +/- 0.9 mm Hg when measured by lumbar puncture

in the left lateral recumbent position (Bono, Lupo et al. 2002). ICP as measure of CSF dynamics does not depend on age (Konig, Heissler et al. 2005).

The B-waves are slow and rhythmic oscillations in ICP. They probably originate from oscillations in cerebral blood volume (Magnaes 1976), and may be present in healthy individuals as well (Mautner-Huppert, Haberl et al. 1989). Although they seem to be vasogenic in nature the ICP peaks a few seconds before the blood pressure (BP) peak (Droste and Krauss 1999). It has previously been shown in NPH cases that despite the presence of a normal baseline ICP, an increased frequency of short-lasting ICP elevations (B waves) may be present in NPH. These waves were originally defined by Lundberg as pressure elevations up to 50 mmHg with a frequency of 0.5 – 2/min with an increase in pressure amplitude up to 50 mm Hg (Lundberg 1960). They have been further subdivided into great symmetrical and intermediate waves on the basis of their morphology (Raftopoulos, Chaskis et al. 1992).

Sahuquillo and colleagues have characterized NPH as *compensated unstable* and *compensated stable* according to the percentage of B waves recorded (if they were present for less or more than 25% of the total recording time). In one of their studies they found no statistically significant correlation between the percentage of B waves and the pressure volume index (PVI), compliance or Rout (Sahuquillo, Rubio et al. 1991).

However, a careful interpretation of the ICP pattern is needed due to physiological variations. It has been suggested that ICP recordings in suspected NPH should be

accompanied by polysomnography to avoid misleading results due to variability of B wave appearance dependent on sleep pattern (Droste, Krauss et al. 1994). This is because it was found that the frequency as well as the amplitude of B waves correlate with the REM and other phases of the sleep (Krauss, Droste et al. 1995). The percentage of B waves was used as a diagnostic tool to select patients for shunting; in that sense if a patient exhibited B waves more than 80% of the recording time he was considered a candidate for shunting (Borgesen and Gjerris 1982). The usefulness of ICP monitoring to predict outcomes, although still used today by many clinicians, has been disputed (Williams, Razumovsky et al. 1998; Stephensen, Andersson et al. 2005).

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Figure 1.6.1.1.1. Example of pressure monitoring in a patient with normal pressure hydrocephalus (Czosnyka and Pickard 2004).

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Figure 1.6.1.1.2. Example of B waves

1.6.1.2. Conductance

The main driving force for CSF absorption is the pressure difference between the dural venous sinuses and the CSF, a gradient which is in the range of 20-40 mm of water with the CSF pressure greater than the venous pressure in the superior sagittal sinus in both adults and children (Shulman and Ransohoff 1965). In the normal situation CSF absorption begins after the CSF pressure reaches 68mm CSF pressure following which the rate of absorption and pressure are linearly related (Lorenzo, Bresnan et al. 1974). CSF conductance is a measure of this CSF flow and absorption (units in ml/min/units of CSF pressure). The normal conductance reported in man is in the range of 0.1- 0.15 ml/min/mmHg.

1.6.1.3. Resistance to CSF outflow (R_{out})

Resistance to CSF outflow (Rout) is a measure of the resistance to CSF absorption and is the reciprocal of conductance. The Rout does not correlate with ventriculomegaly (Kosteljanetz and Ingstrup 1985) which explains why ventriculomegaly alone cannot be a reliable feature to distinguish between patients

with iNPH and cerebral atrophy. Rout varies widely in patients examined for suspected disturbances in CSF dynamics, ranging from 0-90 mm Hg/mL/minute (Børgesen SE 1989), whereas in normal individuals it was found to be 9.1+/- 0.8 mm Hg/ml/minute (Albeck, Borgesen et al. 1991). Rout increases linearly with age (Czosnyka, Czosnyka et al. 2001).

The R_{out} can be calculated from formula (3) derived from an infusion test:

$$\mathbf{ICP_{baseline} = R_{out} \times Formation\ rate\ of\ CSF + P_{ss}} \quad \mathbf{(1)}$$

$$\mathbf{ICP_{end\text{-}equilibrium} = R_{out} \times (Formation\ rate + Infusion\ rate) + P_{ss}} \quad \mathbf{(2)}$$

$$\mathbf{ICP_{end\text{-}equilibrium} - ICP_{baseline} = R_{out} \times Infusion\ rate} \quad \mathbf{(3),}$$

where P_{ss} is the pressure at the sagittal sinus, $ICP_{baseline}$ is the ICP recorded at the beginning of the test and $ICP_{end\text{-}equilibrium}$ the ICP reached at the end equilibrium of the test. However, some authors suggested that the plateau pressure, and not the R_{out} calculation, is enough to facilitate patient selection for surgery (Kahlon, Sundbarg et al. 2005).

Several CSF hydrodynamic tests are used to assess Rout. The basic concept is to study the pressure-infusion curve through either bolus injection, constant rate infusion or constant pressure infusion. When compared the first two methods correlate well with the bolus infusion technique giving lower values (Kosteljanetz 1985). A Rout of 18 mm Hg/ml/minute is considered a strong predictor of good outcome following shunting (Boon, Tans et al. 1997; Boon, Tans et al. 1998). Other authors found the Rout not correlating with clinical outcome in early (<1 symptoms) hydrocephalus, but is relevant to outcome in patients with long standing symptoms and associated cerebral atrophy (Meier and Miethke 2003), whereas others

disagreed about its predictive role altogether (Malm, Kristensen et al. 1995; Hebb and Cusimano 2001; Savolainen, Hurskainen et al. 2002; Meier, Konig et al. 2004).

Although infusion testing may be done both via lumbar and ventricular access there is no difference between the two methods favoring therefore for obvious reasons the lumbar infusion test (LIT) (Kahlon, Sundbarg et al. 2005).

The Rout was found to be higher in a study of 35 patients with NPH when compared with patients with cortical atrophy. The outflow resistance was significantly correlated with mean blood flow velocity and cerebral perfusion pressure (both were indirect measures of the capacity for autoregulation). The significance of this finding is that patients with higher resistance to CSF outflow retained their autoregulation, thus excluding largely the presence of concomitant cerebrovascular disease (CVD) (Czosnyka, Czosnyka et al. 2002).

1.6.1.4. Compliance

Tissue compliance is the reciprocal of elastance. It may be calculated as DV/ DP (units in mL/ mmHg). Cerebral compliance (C) is the ability of the brain to adapt to changes in volume (DV) inside the cranium in order to reduce changes in pressure (DP), i.e. C = DV/DP. A brain is described as compliant, (i.e. it has good compliance) if a large change in ventricular volume results in a small change in ICP. In hydrocephalus the compliance of the brain is modified due to the structural changes taking place in the parenchyma as the condition evolves, and therefore will not remain constant. When input volume is plotted in relation to intracranial

pressure the result is not a straight line but rather an exponential curve that first rises slightly, and when the reserves of the system have been exhausted it rises sharply. With a concomitant increase in ICP, there is a decrease of the intracranial compliance, regardless of the source of the pressure increase.

There have been many theories of the structural causes of cerebral compliance. Brain parenchyma is deformable but not compressible and therefore unlikely to contribute to changes in compliance. The same occurs with CSF which acts as a non-compressible fluid medium and since it has a very slow rate of formation and resorption it is unlikely to contribute to changes in compliance. That leaves the intracranial blood volume as the most likely determining factor of cerebral compliance (Chopp, Portnoy et al. 1983).

In fact, the clinical signs and symptoms in NPH as well as ventricular dilation, periventricular oedema, reduced cerebral blood flow, malabsorption of CSF, intracranial pressure waves, increase of mean CSF pressure, increased CSF pulse pressure, increased vascular resistance, hyperdynamic intraventricular CSF flow, increased Pulsatility Index (PI) and decreased intracranial stroke volumes may all be explained by decreased intracranial compliance. There have been attempts of non-invasive measurement of the intracranial compliance by calculating an index , defined as the ratio of the peak-to-peak intracranial volume change (ICVC(p-p)) to the peak-to-peak cerebrospinal fluid (CSF) pressure gradient (PG(p-p)) during the cardiac cycle, measured with phase-contrast (PC) cine MRI (Miyati, Mase et al. 2007).

1.6.1.5. Elastance

In practice elastance indicates the stiffness in brain and is increased linearly with age (Czosnyka, Czosnyka et al. 2001). It is the reverse of compliance. Elastance is determined by using bolus injections to measure the PVI of the CSF pressure–volume curve, where PVI is the volume needed to increase the pressure 10-fold (Marmarou, Shulman et al. 1975).

E= DP/DV (mmHg/ mL)

Volume pressure response (VRP, units in mm Hg/mL) is a direct measure of the elastance.

1.6.1.6. Pressure Volume Index (PVI)

The nonlinear relationship between the pressure and volume elements of the intracranial cavity makes the measurement of compliance a cumbersome procedure. This has been solved by Marmarou and colleagues who changed the graph of the P/V relationship into a semi-logarithmic curve. As a result of this logarithmic transformation the exponential function was found to be linear, and they termed the increased angle a pressure-volume index (PVI). The PVI (units in ml) is the amount of volume necessary to raise the resting pressure by a factor of 10. During an infusion test it can be calculated from the following equation:

$PVI = DV_i / 0.4343 \ln P_p/P_b$,

where DVi is the volume injected, P_p is the peak pressure achieved during the test and P_b is the baseline pressure at the beginning of the test. The PVI in adults is 25.9 +/- 3.7 mL (K Shapiro, A Marmarou et al. 1980).

1.6.1.7. Pulsatility Index (PI)

The elevation of the pulsatility index (PI) at transcranial ultrasound Doppler indicates increased pulsatility in major intracranial arteries. The increased pulsatility is a consequence of decreased intracranial compliance and breakdown of the windkessel mechanism, decreasing the diastolic flow in the arteries. Decreased intracranial compliance also increases the vascular impedance, i.e. increased resistance to pulsating flow, causing a decreased mean blood flow

1.6.2. Methods to calculate the CSF pressure-volume curve

There are five methods of performing tests to assess the intracranial CSF dynamics. These methods utilize a subarachnoid infusion system in which either flow or pressure can be held constant. These tests are quantitative and can often separate a loss of brain substance secondary to a degenerative process from normotensive and hypertensive hydrocephalus. However, calculations of outflow resistance or conductance do not take into account the possibility that the patient's own production of CSF may vary at different intracranial pressure levels, and that the initial pressure level (before the start of the infusion) may vary for reasons unrelated to CSF hydrodynamics.

1.6.2.1. Lumbar infusion test by Katzman

The aim of this test is to quantitatively evaluate the CSF absorptive ability of an individual, which is the conductance to outflow (Cout). During the infusion test

constant steady flow is maintained with an infusion pump. CSF pressures are monitored through a three-way stopcock. In patients with normal CSF absorption mechanisms, infusion at a rate approximately twice the usual rate of CSF formation results in a modestly predictable CSF pressure elevation. In the case of communicating hydrocephalus, the capacity to absorb this additional fluid is reduced; on infusion, CSF pressure rises abruptly, and the procedure is quickly terminated.

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Figure 1.6.2.1.1. An illustration of the infusion test (Katzman and Hussey 1970)

1.6.2.2. Lumbar constant rate infusion test

The lumbar constant rate infusion test was a variant of the infusion test originally described by Katzman (Katzman and Hussey 1970). The test was performed with or without local anaesthesia with the patient in the lateral and horizontal position, in order to obtain an equal level of the ventricular system and the lumbar subarachnoid space. A midline lumbar puncture was made with a 19-gauge needle inserted between the L4 and L5 vertebrae. The needle was then connected to a transducer, which was linked to a computer-based system that allowed pressure sampling each 5th second. After 10 minutes of baseline measurement, the infusion

of normal saline at a rate of 1.5 ml/min or 1 ml/min (if the baseline pressure was higher than 15 mm Hg) is started and continued until a steady state ICP plateau is achieved. If the ICP increases to 40 mm Hg, the infusion is interrupted. Following cessation of the saline infusion, the ICP is recorded until it decreases to steady baseline levels. All compensatory parameters are calculated using computer software based on physiological models of the CSF circulation. Baseline ICP and Rout characterize the static properties of the CSF circulation.

1.6.2.3. Constant pressure infusion test

The test was first described by Eksted (Ekstedt 1977). By infusing artificial CSF at constant pressures and recording the resultant flow, it is possible to obtain information about the hydrodynamic conductance of the CSF outflow pathways. In its original description 2 lumbar needles were inserted via the opening in the back support of a special chair with the patient in the sitting position. The chair was then folded back and the rest of the investigation made with the patient supine. Two pressure transducers were connected to carrier frequency amplifiers. The resting pressure was recorded continuously. The infusion was then started. The first infusion pressure was chosen to be about 0.5 kPa above the resting pressure. The pressure was kept at this level until the rate of flow had levelled off after the initial high rate and the CSF pressure was constant. Then the pressure level was increased by a further 0.5 kPa and the same procedure was repeated. The pressure was kept at a certain level for at least five minutes. In most cases a final pressure of 6 kPa was aimed at, but sometimes, when there was a very good drainage, the content (120 ml) of the bottle with artificial CSF was not sufficient to

attain this level. The next step was the 'post-infusion'. The connection to the bottle was shut off and the pressure was allowed to return spontaneously by elimination of CSF through the physiological pathways. This was continued until a steady plateau value was recorded, which, in most cases, closely corresponded to the initial resting value. The next step in the investigation was then to perform the drainage. The pressure in the infusion bottle circuit was lowered to 0.25 kPa. This caused an outflow of CSF from the subarachnoid space into the bottle. Initially, the rate of outflow was high, but within 10 to 15 minutes the rate levelled off when the intraspinal pressure approached the pressure in the infusion circuit. This part of the investigation was done in order to determine the rate of formation of the CSF. The investigation described a rectilinear relationship between CSF pressure and the flow necessary to maintain each pressure level.

1.6.2.4. Lumbo-ventricular perfusion

The lumbo-ventricular perfusion as described by Borgesen is the most invasive but also the most accurate (Borgesen, Gjerris et al. 1978). This test allows the measurement of the relationship between CSF pressure and CSF absorption; the resorptive capacity of CSF can be measured as Cout. The method is based on a constant infusion rate at different, controlled, constant intracranial pressure levels. Ringer lactate is infused *via* a lumbar cannula at 1.5 to 4.5 ml/min. The pressure level is controlled by the height of the outflow tip of the catheter from the ventricles. The unabsorbed fluid flows out through the catheter from the ventricles, and is measured gravimetrically in two periods of 5 min at 3 to 5 different pressure levels. Cout is then calculated from the resulting rectilinear regression line relating

absorbed volume to pressure level (see equation (2). The compliance of the craniospinal space has no influence on the measurement of C_{out}.

$V_{abs}=V_{inf} + (V_{CSF}-V_{out})$ (1)

$\mathbf{dV_{abs}/dP}$ (mL/ min/ mmHg) (2),

where V_{abs} is the volume of the fluid absorbed, V_{inf} the volume of the infused fluid and V_{out} the volume of the unabsorbed fluid flowing out.

1.6.2.5. Bolus injection method

Bolus injection of fluid into the lumbar thecal sac as described by Marmarou is the least invasive and quickest method (Marmarou 1973). Following placement of a ventricular catheter, a fluid-coupled conventional strain-gauge transducer was connected to the ventricular catheter with a syringe interposed between them for manipulation of CSF. The electrical output of the transducer was recorded continuously on conventional strip charts at 1 mm/second. All pressures were referenced to the right atrium. After a baseline steady-state ICP was established, bolus withdrawal o f 2 to 4 ml o f CSF was performed. The PVI was calculated using the formula:

PVI = AV/log (Po/Pm),

where Po is the initial CSF pressure prior to bolus withdrawal of CSF, Pm is the trough CSF pressure immediately following withdrawal, and AV is the volume of CSF withdrawn. Depending on the PVI and Po, a series of 2 to 10 ml bolus injections were performed allowing 3 to 10 minutes to elapse between manipulations. After each injection, the following parameters were extracted from

the recordings of CSF pressure: the CSF pressure prior to injection (Po); the peak CSF pressure produced by bolus injection (Pp);
and the CSF pressure 1 minute after the bolus injection (Pt). The PVI was calculated for each injection using the formula
PVI = AV/log (Pp/Po). The CSF absorption resistance (Ro) was calculated when Pt was at least 2 mm Hg less than Po, using the formula
Ro = Po/PVI • log [(Pt/Pp) x (Pp - Po/P, - Po)].

1.6.2.6. Computerised constant rate infusion test

This test was carried out as a standard constant rate infusion test. It provides Rout (and other parameters) by an optional lumbar or ventricular constant
rate infusion. The ICP analysis eliminates the influence from unwanted pressure fluctuations and artefacts, and permits calculation of Rout even when a steady pressure level is impossible to obtain. The calculations of Rout, CSF formation rate, and PVI are based upon known relations between the parameters as given by the Davson equation (CSF formation rate=ICP/ (Rout + Pss), where Pss is the pressure of the sagittal sinus), the mono-exponential shape of the pressure/ volume relationship, and the linear relation between the pulse wave amplitude and ICP. Via a lumbar cannula Ringer lactate was infused at constant rate. Excess fluid was collected via an intraventricular catheter, through which also the ICP was measured. The height of the out-flow tube connected to the intraventricular catheter regulates the ICP making it possible to obtain several steady pressure levels at a steady infusion rate. Rout was calculated as the regression coefficient of the ICP/absorbed volume correlation. After re-establishing a steady, normal

pressure infusion with constant infusion rate and monitoring of the intraventricular or lumbar CSF-pressures was performed. The analog pressure signal from the pressure amplifier was converted by an analog-digital converter and stored by the software, installed in a standard personal computer. The ICP-signal was processed by a spectral analysis filtering out noise to make the pattern more salient. From the baseline ICP, the infusion rate, and the end equilibrium-ICP, Rout may be computed.

Technique	*Parameters manipulated*	*Parameter calculated*	*Units*	*Reference*
Lumbar infusion test	Constant flow	Conductance	mL/ min/ mmHg	(Katzman and Hussey 1970)
Lumbar infusion test	Constant pressure	Conductance	ml/ min/ mmHg	(Ekstedt 1977)
Ventriculo-lumbar Infusion	Constant flow	CSF formation/ absorption	mL/ min	(Cutler, Page et al. 1968)
Bolus injection/method	Volume withdrawal/ volume injection	Pressure Volume index	mL	(Marmarou, Shulman et al. 1978)
Computerised infusion test		Resistance to CSF outflow	mmHg/ mL/ min	(Borgesen, Albeck et al. 1992)

Table 1.6.2.6.1. Summary of systems used to study CSF dynamics in man

1.7. Pathology

1.7.1. Experimental studies in hydrocephalus

The contemporary animal model used to study chronic hydrocephalus is that of injection kaolin in the cisterna magna of rats. It is though that the injection of kaolin (aluminium silicate) caused an inflammatory reaction to the meninges obstructing the CSF flow in the subarachnoid space, thus stimulating the conditions of NPH. Experimental studies support the theory of engagement of white matter damage in hydrocephalus. Progressive damage to axons in the periventricular white matter, gradual death of oligodendroglial cells, astroglial hyperplasia, and microglial activation has been seen (Del Bigio and Zhang 1998). It has been shown that ependymal disruption begins as early as 12 hours following CSF flow obstruction. In an experimental study on kaolin-induced hydrocephalus and shunting in kittens, the white matter was found oedematous, with reactive astrocytes and markedly reduced periventricular myelination. Histologically, decreased local cerebral glucose utilization in central white matter was seen in non-shunted hydrocephalic animals (Chumas, Drake et al. 1994).

1.7.2. Neuropathological studies in human subjects

Due to limitation of autopsy cases it is impossible to conclude whether anatomicopathological alterations are present in all cases, or differ depending on the severity or the chronicity of the condition. Neuropathological studies in humans are mainly provided from cortical biopsies. There are only two published pathological autopsy studies; the first of 7 subjects by Akai and colleagues, and a

second on 4 subjects by Brusa and colleagues (Akai, Uchigasaki et al. 1987; Brusa, Piccardo et al. 1991). Cortical biopsies have shown arachnoid fibrosis in 50% and frequent pathological parenchymal changes (Akai, Uchigasaki et al. 1987; Bech, Juhler et al. 1997). Ependymal changes range from normal, stretched, torn or total destroyed epithelium (Akai, Uchigasaki et al. 1987). The ependymal damage depends on the degree of the elevated intracranial pressure (Del Bigio 1993). The gliosis observed was of the astrocytic type ranging from the basement of the ependyma up to a depth varying in the white matter (Brusa, Piccardo et al. 1991). Fibrosis of the choroid plexus has been reported (Jellinger 1976). There is characteristic gliosis in the periventricular area following the onset of CSF obstruction (Jellinger 1976; Weller and Mitchell 1980), accompanied by oedematous subependymal region and demyelination of the periventricular white matter (Akai, Uchigasaki et al. 1987). In brain biopsies of hydrocephalic children large quantities of pinocytic vesicles and enlargement of extracellular spaces has been noted. Histological examination consistently reveals enlarged extracellular spaces in the white matter adjacent to the ventricles of hydrocephalic humans (Foncin, Redondo et al. 1976). This has led many authors to suggest that the enlarged extracellular spaces adjacent to the ventricle act as diffusional pathways for "displaced CSF", as indicated by movement of tracer substances from the ventricle into the parenchyma. Thinning of the corpus callosum and compression of the periventricular white matter is a consistent finding in human hydrocephalus. Axonal degeneration and loss of axons has been reported in long-standing hydrocephalus (Brusa, Piccardo et al. 1991). White matter's demyelination extends to the areas supplied by the anterior and middle cerebral arteries (Akai, Uchigasaki et al. 1987). The loss of myelin appears more prominent than the loss of axons

(Akai, Uchigasaki et al. 1987). It is thought that the axonal and secondary myelin damage occur through a combination of ischemic and mechanical effects. Gross atrophy of the basal ganglia has also been reported in humans and animals (Di Rocco, Di Trapani et al. 1977). Neuronal pyknosis and degeneration have been observed in the cortex of young and adult hydrocephalic humans. Indirect indications of neuronal loss has been reported (Schmidt, Hasselbalch et al. 1996). Vacuolization and degeneration of neurons in the hippocampal formation have been observed in hydrocephalic humans. Yakovlev observed a decreased quantity and reduction in size of the large pyramidal neurons in the paracentral lobules of two chronically hydrocephalic humans (Yakovlev 1947). The vascular changes were those of with multiple microinfarcts, arteriosclerosis, demyelination and loss of axons in white matter; altogether, changes compatible with arteriosclerotic encephalopathy (Akai, Uchigasaki et al. 1987).

Changes typical for Alzheimer's disease (AD) and arteriosclerotic changes have been reported. In two recent retrospective studies, clinical improvement was reported in three of five (Del Bigio, Cardoso et al. 1997) and two of eight (Savolainen, Paljarvi et al. 1999) shunted patients with Alzheimer's disease pathology established by biopsy indicating that comorbidity with this disease does not always mitigate against a beneficial neurosurgical result. There is one comparative study showing significantly more changes of Alzheimer-type in biopsies from hydrocephalus patients than in age-matched autopsy controls (Del Bigio, Cardoso et al. 1997). In a prospective study of 56 patients who underwent ventriculoperitoneal shunting for iNPH and cortical biopsy no specimen biopsy showed inflammation, neoplasm, neurons with Lewy Bodies, Pick bodies, or glial

cells with silver positive inclusions. Amyloid angiopathy and neuropril threads were detected in only a few specimens and sparse accumulations of neurofibrillary tangles were seen in six. Neuritic plaques were found in 23 biopsies whereas 12 patients showed only diffuse plaques. A diagnosis of definite Alzheimer's disease could be made in seven cases (12.5%), probable disease in nine (16%), and possible disease in seven (12.5%) (Golomb, Wisoff et al. 2000). Neurofibrillary tangles (Ball 1976) and granulovacuolar degeneration in hippocampal neurons is prevalent in shunted patients who do not improve (Ball and Vis 1978) and the pathologic changes seen are very similar to those of AD. One of the most important findings of that study is that the patients with positive biopsies for AD had similar improvement in gait, psychometric testing and urinary control when compared with the patients with negative biopsies.

Macroscopical changes observed in patients with hydrocephalus is corpus callosum atrophy as demonstrated with imaging (Jinkins 1991; Thajeb 1993).

1.7.3. Cerebral metabolism

In 1969, one pathophysiological theory suggested that NPH is caused by cerebral perfusion disorders (Greitz 1969). However, most studies show no correlation between the magnitude of changes in the cerebral blood flow due to CSF removal and clinical outcome after therapy (Owler and Pickard 2001).
Previous CBF investigations in which the H2-clearance method and the [14C]iodoantipyrine ([14C]IAP) autoradiography technique were used showed that cortical gray matter blood flow sustained rather mild decreases in adult and

infantile experimental hydrocephalus (Higashi, Asahisa et al. 1986). Only blood flow levels in the white matter were found to be below the ischemic threshold (da Silva, Michowicz et al. 1995).

Additional evidence regarding the disturbance of cerebral metabolism in NPH has been provided by a microdialysis study in ten patients. During the ICP increase artificially induced during lumbar infusion testing reversible changes were observed in energy metabolism in the periventricular white matter without any signs of ischemia. The authors conclude that it is the chronicity of the ICP increases that could cause irreversible axonal injury, later demyelination and therefore the clinical symptomatology of NPH (Agren-Wilsson, Eklund et al. 2005).

CSF may leak into the brain extracellular space under conditions of a permanently disrupted brain-CSF barrier. This kind of abnormal CSF circulation would not cause intracranial hypertension, because the resistance to intraparenchymal CSF flow is probably low. Hypothetically, increased CSF flux in white matter could cause a decrease in CBF in the periventricular zones, provoking specific symptoms (particularly gait disturbances and urinary incontinence).

Compression of the extracellular spaces as described previously in the section of neuropathology may be of functional significance. The movement of neurotransmitters and their metabolites as well as the waste products of energy metabolism is dependent on the volume and tortuosity of extracellular spaces. Del Bigio suggested that if ventriculomegaly causes compression of extracellular spaces, then stagnation of these substances could disturb the homeostasis of the

microenvironment and consequently upset neuronal function. Thus compression of the extracellular space combined with astrogliosis of the white matter could decrease the clearance of waste products from the hydrocephalic brain, a function which is already impaired by reduced outflow of CSF (Del Bigio 1993). The metabolic changes occurring in chronic adult hydrocephalus have been extensively reviewed (Kondziella, Sonnewald et al. 2008). The authors main conclusion was that in NPH from a certain 'point of no return' metabolic impairment becomes decoupled from CSF dynamics and, at least partly, self-sustained. This is probably the reason why despite restored CSF circulation by shunting many patients with chronic hydrocephalus still suffer from severe neurological deficits.

In experimental studies with kaolin-induced hydrocephalus in rats no difference in cerebral glucose utilization rate was found in the periventricular tissue (Richards, Pickard et al. 1985). In human studies, patients with NPH exhibit lower uptakes of glucose and oxygen and a significant release of lactate and pyruvate when compared with normal subjects. Cerebral metabolic rates correlate with an increase in ketone bodies pointing out to a catabolic state in the syndrome of NPH. It has been shown, albeit in a limited number of patients, that these changes reverse post shunting, with an increase in cerebral uptake of glucose and a decrease of the metabolic rate of the ketone bodies suggesting a reversal of the catabolic state in NPH (Lying-Tunell, Lindblad et al. 1981; Kaye, Grady et al. 1990). However, clinical improvement post-shunting does not always correlate with an increase in cerebral uptake of glucose (Tedeschi, Hasselbalch et al. 1995). Impaired oxygen metabolism has been detected by PET studies in the lower areas of the frontal lobe (Ishikawa, Kikuchi et al. 1989). Oxygen extraction is increased in

patients with both idiopathic and secondary forms of NPH when compared with patients with cortical atrophy, or ischemic disease. Furthermore, clinical recovery is correlated with higher preoperative oxygen extraction rates (Mima, Mori et al. 1999). Chemical shift imaging has also been able to point out difference in metabolism in NPH by showcasing an increase in the concentrations in lactate in the ventricular CSF when compared with other forms of dementia and control subjects (Kizu, Yamada et al. 2001). Using proton MRS it was shown that the ratios of non-acetyl aspartate (NAA)/ creatine and non-acetyl aspartate/ choline mean values increased after surgery in a cohort of eleven patients. NAA is present primarily in neurons, axons, and dendrites and is viewed as an indicator of neuronal state and function. NAA is also important for reparative brain processes by enhancing lipid synthesis and repairing injured myelin. These results, therefore, may point out to a reparative process in progress following shunting in NPH (Matarin, Pueyo et al. 2007).

1.7.4. Changes following shunting

Very few studies have studied the pathological changes occurring post-shunting for obvious reasons, so most data we have to date is from experimental animal studies. There is restoration of the brain's dry weight comparable to that of normal animals following shunting (Bannister, Cranley et al. 1994)**.** In shunted kittens, no periventricular oedema was present following shunting (da Silva, Drake et al. 1994). In shunted dogs incomplete restoration of cytoskeletal neuronal damage has been noted (Aoyama, Kinoshita et al. 2006). In another experiment even though there has been clinical improvement following shunting there was no

correlation with white matter atrophy induced due to hydrocephalus. Lack of histopathological changes in white matter points out to another factor implicated in the pathogenesis of hydrocephalus, which as the authors suggested is ischemia (Eskandari, McAllister et al. 2004). Partial restoration of afferent and efferent pathways in the sensorimotor cortex of shunted animals has been reported (Eskandari, McAllister et al. 2004). Re-expansion of the cortical white matter but remnant gliosis suggestive of a protracted phagocytic response to axonal damage that had occurred during the hydrocephalic period was noted in shunted kittens (Hale, McAllister et al. 1992). During the restoration of the cerebral mantle extensive myelin regeneration of residual axons and astroglial proliferation with mesenchymal reaction particularly at capillaries is observed (Yamada, Yokota et al. 1992). Number of capillaries increases post shunting but no effect is observed in larger blood vessels due to hydrocephalus (Del Bigio and Bruni 1988). The reversal of histopathological changes observed has been shown repeatedly to correlate with the time of shunting with milder changes occurring and with more likehood to reverse if shunting is performed earlier rather than later.

In man post shunting changes are more likely to be studied indirectly, that is by the use of imaging features or analysis of CSF markers. A potentially reversible neuronal dysfunction as shown by correlation of the reduction of periventricular hyperintensities on MR imaging with a reduction in CSF markers was suggested post shunting (Tullberg, Blennow et al. 2007). On imaging DWMH might relate to demyelination and periventricular hyperintensities (PVH) to neuronal axonal dysfunction (Tullberg, Hultin et al. 2002). Reversible functional dysfunction of the

corpus callosum in human subjects has been demonstrated (Mataro, Poca et al. 2006).

Table 1.7.4.1. Pathologic changes seen in hydrocephalus. Modified from DelBigio (Del Bigio 1993) and Brusa (Brusa, Piccardo et al. 1991).

Cell type/structure	*Late onset-acute*	*Late onset-chronic*	*Effects of shunting*
Ependyma	Ependymal damage, exfoliation	Periventricular gliosis	Remnant gliosis
White matter	White matter oedema	Axonal stretch +/- loss	No oedema
Capillaries	Capillary compression	? Capillary loss	Capillary restoration
White matter blood supply	Hypoxic ischemic damage	? chronic ischemic damage	Restoration of blood flow
Axons	Gray matter loss, subependymal spongiosis		Remyelination of axons
Neurons	? neuronal pyknosis	? neurofibrillary tangles	Partial restoration of size and synapses
Meninges	Meningoendothelium hypertrophy, fibrosis of Pacchionian bodies		
Subarachnoid space	Obliteration or presence of cystic dilatation		

1.8. Clinical symptoms

The occurrence of the triad of symptoms occurs in 50% of the patients suffering with the syndrome (Pfisterer, Aboul-Enein et al. 2007).

1.8.1. Gait

Even though after the publication of Hakim & Adams dementia was thought to be the prevalent and main symptom of the syndrome, today we know that gait disturbance is usually the first sign and considered the most disabling symptom of the disease This was the result of the work by Fisher who observed in 30 patients who had gait symptoms preceding dementia definitive improvement following spinal drainage or shunting (Fisher 1977; Fisher 1982; Graff-Radford and Godersky 1986). It is assumed that the cause of the symptoms in the syndrome is the result of a compromised axonal function with two main causes: a) a mechanical cause, from compression and axotomy from the distended ventricular walls (Yakovlev 1947), or transependymal CSF diffusion, and b) ischaemia from decreased blood flow in the area (Kristensen, Malm et al. 1996). Thus, in NPH the clinical symptoms result from lesions to sensory and motor tracts travelling through the cerebral white matter. Since the fibers of the corticospinal tract that supply motor function to the legs pass closest to the lateral ventricles in the corona radiata and they are longer, it is not surprising that the gait disturbance is usually the first symptom to appear and the first one to resolve following successful CSF shunting (Graff-Radford and Godersky 1986), however, the fibers to the arms are also likely to be affected. It is now thought that primary frontal damage, lesion of

frontocerebellar pathways, or lesioned corticocortical fibres projecting to the frontal cortex are more likely to cause the condition (Stolze, Kuhtz-Buschbeck et al. 2001). Recent investigations have suggested that the mesencephalic locomotor centre in the dorsolateral midbrain (in the posterior tegmentum just ventral to the inferior colliculus) might be responsible for the gait abnormalities observed in Parkisonism or Parkisonism-like syndromes (Pahapill and Lozano 2000). Indeed the midbrain diametre has been found significantly smaller in iNPH when compared to control subjects (Lee, Yong et al. 2005), and the diametre increases following shunting (Mocco, Tomey et al. 2006). However, the latter study has weaknesses since the observation may well be interpreted as attributed simply due to a shunting procedure and not necessarily correlated to symptom improvement. It would be of interest to determine what changes in mid-brain size occurred in patients who, despite meeting clinical criteria for INPH, did not respond to surgery. This would greatly strengthen the view that increase in mid-brain size may be responsible in part for the improvement seen in these patients.

The gait of NPH is characterised by a triad of: a) reduces stride length, b) reduced step height and c) a disturbance of the dynamic equilibrium. The normal variability of step width and foot angles was decreased, leading to an insufficient compensation of body sway, as it is of particular importance during obstacle avoidance. These results suggest that a disturbed “dynamic equilibrium” during gait is the striking characteristic of the gait pattern in normal pressure hydrocephalus (Stolze, Kuhtz-Buschbeck et al. 2001). The outwards rotated feet and the increased step width observed may be a compensatory mechanism to stabilise locomotion (Stolze, Kuhtz-Buschbeck et al. 2001).

Compared with young adults, neurologically healthy elderly people exhibit 17% to 20% reductions in the velocity of gait and length of stride. The gait disorder in NPH, usually referred to as hypokinetic gait (Sudarsky and Simon 1987), is characterized by being broad-based, with small foot-floor clearance, and low swing-to-stance ratio (Knutsson and Lying-Tunell 1985), reduced gait velocity and a diminished and highly variable stride length. Reduced stride length was proposed to be due to co-contraction of the proximal muscles as revealed by EMG studies (Sudarsky and Simon 1987). In NPH patients lack dorsal extension of the foot and toes, and tend to hit the floor with the whole foot , rather than with the heel first as normal subjects do (Stolze, Kuhtz-Buschbeck et al. 2000). Arm swing does not appear impaired (Sudarsky and Simon 1987). The gait disorder observed in NPH shares these features in common with the gait disorder found in Parkinson's disease (PD) (Stolze, Kuhtz-Buschbeck et al. 2001). Freezing has been observed in between 30% to 56% in 2 studies (Giladi, Kao et al. 1997; Stolze, Kuhtz-Buschbeck et al. 2001). The prevalence of hypo- and hyperkinetic motor deficits, such as akinesia, tremor, dystonia and chorea, in NPH were highest (56 of 65 patients) among the idiopathic forms (Krauss, Regel et al. 1997). In a recent series of 118 NPH cases due to various etiologies, additional hypokinetic motor deficits of the upper extremities usually encountered in PD, such as akinesia, tremor and rigidity, were encountered in 75 percent of affected cases (Krauss, Regel et al. 1997).

Quantitative investigations of motor performance of the hand and arm in NPH are scarce (Soelberg Sorensen, Jansen et al. 1986). However, a clear hypokinetic

deficit of motor performance was found in NPH which is similar in many aspects with that found in PD. It has been proposed that it is not only the gait that is affected in NPH but also posture and balance (Blomsterwall, Bilting et al. 1995).

A characteristic feature of the postural instability in patients with NPH is a tendency to fall backwards, lean backwards and bump down on to the chair (Blomsterwall, Svantesson et al. 2000). External visual or auditory cues improve the walking in NPH very slightly and this comes in contrast with the hypokinetic gait of PD (Stolze, Kuhtz-Buschbeck et al. 2001). Hypokinesia has been suggested to be related to basal ganglia dysfunction (Bugalho and Guimaraes 2007). However, the disequilibrium observed may be related to frontal dysfunction

Only the stride length has been shown to improve following removal of CSF via lumbar tapping. This has a consequence of increase in overall gait velocity (Stolze, Kuhtz-Buschbeck et al. 2000; Stolze, Kuhtz-Buschbeck et al. 2001; Bugalho and Guimaraes 2007; Ravdin, Katzen et al. 2008). Gait velocity may increase in 20-75% of the cases following shunting or tapping (Krauss, Droste et al. 1996; Stolze, Kuhtz-Buschbeck et al. 2000). Following shunting postural functions improve more than the motor ones (Blomsterwall, Svantesson et al. 2000). In a recent study of 33 patients Ravdin and colleagues concluded that the classic features of gait (wide based stride, reduced foot-floor clearance and small steps) cannot predict the responders after a tap test. However, the walking speed, required steps for turning and tendency towards falling were most likely to improve (Ravdin, Katzen et al. 2008); admittedly one would not know the change of gait features following ELD.

1.8.2. Cognitive decline

Patients with iNPH present with dementia which is characteristically of the subcortical type. Subcortical dementia is a clinical syndrome characterized by bradyphrenia, memory impairment, diminished executive function and mood and personality changes. Patients with subcortical dementia are inert, indifferent, and disinterested. The extreme result might be akinetic mutism (Barbizet, Duizabo et al. 1975). It results from dysfunction of subcortical structures, white matter tracts connecting frontal lobe and subcortical nuclei, or frontal lobe regions projecting to specific subcortical targets. The striatum is most closely connected with the frontal lobe, and it is this functional system that is disrupted in subcortical dementia. Projection of the cholinergic fibres from the nucleus basalis to the cortical neurons involved in memory might be stretched and could be involved in depressing CBF metabolism (Iddon, Pickard et al. 1999).

The term “subcortical" refers mostly to physiological rather than anatomical circuits. The finding that in the early stages of NPH the pattern of cognitive impairment is predominantly frontosubcortical, only later becoming more global, contrasts markedly with the pattern in patients with Alzheimer’s disease. The findings of Waldemar (Waldemar, Schmidt et al. 1993) and also Graff-Radford (Graff-Radford, Rezai et al. 1987) give support to this hypothesis as they reported subcortical blood flow abnormalities.

There is another pattern of dementia, namely the cortical dementia of which the main representative is the Alzheimer’s Disease. The spontaneous recall is characteristically impaired in subcortical dementia, but encoding and storage are

largely preserved. Remote memory is generally less impaired when compared with AD but it exhibits no temporal gradient, a characteristic of AD. Subcortical dementia is characterised by the absence of dysphasia, apraxia and agnosia. In contrast in cortical dementia cognitive slowing may occur but is not a necessary symptom and the disorders of higher cortical function, agnosia, apraxia and aphasia, do occur.

With respect to language no aphasia is exhibited although patients in late stage of the syndrome may exhibit difficulties with naming or following auditory commands. It has been suggested that if aphasia is present in a patient then he is less likely to improve following shunting, since this symptom is more suggestive of an Alzheimer's type of dementia (De Mol 1986).

Visuospatial skills are impaired equally in cortical and subcortical dementia. Constructional apraxia has also been observed. Thinning of the corpus callosum has been proposed as the cause of spatial neglect demonstrated in patients with NPH (Jeong, Tsao et al. 2006).

It is now known that the executive functions are predominantly affected in the NPH patients. That is because the prefrontal cortex which is affected in NPH is essential for tasks requiring reasoning, anticipation, goal establishment, strategy formation, shifting mental set, and error monitoring.

Table 1.8.2.1. Diseases in which the syndrome of subcortical dementia has been described
Degenerative disorders
Parkinson's disease
Huntington's disease
Progressive supranuclear palsy
Idiopathic basal ganglia calcification
Spinocerebellar degenerative syndromes
Thalamic degeneration
Vascular disorders
Lacunar state
Thalamic Infarction
Metabolic disorders
Binswanger's disease
Wilson's disease
Hypoparathyroidism
Demyelinating disease
Multiple sclerosis
AIDS encephalopathy
Miscellaneous
Subcortical sarcoidosis
Normal pressure hydrocephalus
Dementia pugistica
Neuro-Behcet's disease

Benson characteristically describes the features of NPH. *'Deranged mental function is the most prominent symptom in NPH. This may range from a mild apathy or mild disturbance of recent memory to severe psychomotor retardation, including akinetic mutism. The dementia characteristically develops at a rapid pace*

but may fluctuate considerably from day to day. The degree of apathy and inattentiveness is marked; in early stages apathy is more striking than depression of cognitive ability'(Benson, LeMay et al. 1970).

The prevalence of dementia attributed to NPH in the general population is essentially unknown. When the cohort of a memory clinic comprised of 196 patients was analysed only 2 were found to have NPH; they estimated NPH as representing only 2% of treatable dementias (Freter, Bergman et al. 1998).

There are few psychometric studies of patients with NPH. A study by Gustafson and Hagberg (Gustafson and Hagberg 1978) reports an overall reduction in cognitive ability, including memory function, although verbal ability is retained. They also comment on the marked difficulties such patients experience in perceptive performance tasks and in inductive reasoning. Gustafson and Hagberg found that their patients did not have aphasic, apraxic or agnostic deficits to the same extent as their control group (patients with AD). Furthermore, such symptoms occurred more frequently in those patients who had a poor result from shunt operation. Those who improved following shunt operation were characterized preoperatively by the symptoms of confabulation, emotional unconcern, gait disturbance and incontinence. These authors point out the similarity between the psychometric profiles of their patients and those described in patients with frontal lobe lesions, in particular the reduced speed of motor and intellectual performance and the deficits in perceptual performance and inductive reasoning (Gustafson and Hagberg 1978).

Traditionally the Mini-Mental State Examination (MMSE) tool has been used to quantify the degree of dementia. However, MMSE may be only used as a screening tool and should not be used as an inclusion or exclusion criterion of considering patients for shunting (Tarnaris, Stephenson et al. 2007). Furthermore MMSE cannot point out to the anatomical part contributing to the dementia. In a multiple regression analyses, education and duration of illness were equally strong predictors of the MMSE score (S. Folstein et al, unpublished data). While failure on cognitive screening tests is associated with failure at work, successful MMSE performance is not a sensitive indicator of the patient's ability to work. Some patients who score in the normal range in the MMSE are unable to function at a job because of their difficulty in initiating and sustaining performance and difficulty with more complex tasks than are covered by the MMSE.

If there are the facilities it is more appropriate that the severity of dementia is assessed with the assistance of a neuropsychological battery of tests and by a qualified neuropsychologist. Neuropsychological battery results may be combined with imaging findings in order to strengthen the evidence and assess the severity of the background pathology (Iddon, Pickard et al. 1999).

With regards to memory there is a loss of immediate and delayed recall (active retrieval of memory), even though there is preservation of storage memory (recognition). The decline in executive functions affects complex information processing (increased reaction time, impaired ability to manipulate acquired knowledge, and decreased cognitive flexibility).Visuospatial perception and visuoconstructive skills may also be impaired (Duinkerke, Williams et al. 2004).

The rate of cognitive improvement following shunting may vary from 26%-66% (Caltagirone, Gainotti et al. 1982; Chang, Agarwal et al. 2006). 66.6% improvement at one year follow-up has been demonstrated by using a neuropsychological battery of tests in one study (Raftopoulos, Deleval et al. 1994). Recovery up to 2 years of the cognitive functions has been described. When the authors used the Wechsler memory Scale it was only after 2 years that all subscales were within normal range; that patient suffered from almost 4 years of progressive cognitive decline (Kaye, Grady et al. 1990). The authors also demonstrated the coupling of anatomical/metabolic (right parietal glucose metabolism) and neuropsychological (visuospatial) alterations that occurred post shunting in that same patient.

A spinal tap when used as a selective diagnostic test cannot predict which patients will have improvement on their cognition post shunting (Tromp, Staal et al. 1989). It has been shown that it is mostly cognitive functions such as fluency, selective attention, motor speed and executive functions that improve postoperatively (Gleichgerrcht, Cervio et al. 2009), but not functions as intelligence (measured by the use of the intelligent quotient in one study) (Tromp, Staal et al. 1989). Preoperative cerebral status such as ventricular-to-brain ratio and cerebral atrophy did not influence cognitive improvement in one study (Stambrook, Cardoso et al. 1988). Younger age and the female genre were found to be predictors of cognitive improvement following shunting (Chang, Agarwal et al. 2006). Patients with iNPH perform worse than healthy individuals on simple and target reaction times, dexterity, memory and learning, working memory, and tests of executive functioning. Patients with vascular risk factors performed worse than those without

(Hellström, Edsbagge et al. 2007). Most of the wide range of neuropsychological functions that are affected by iNPH are markedly improved by shunt treatment, but not completely restored (Hellström, Edsbagge et al. 2008).

1.8.3. Urinary incontinence

Although Adams et al. in their original work did not emphasize the urinary incontinence as a prominent feature in their discussion, 2 of their 3 patients were described as having urinary incontinence (Adams, Fisher et al. 1965). The urinary incontinence demonstrated has often been attributed to the patient not being able to reach appropriate facilities in time due to his gait abnormalities. However, it has been shown that the mechanism is also that of the "uninhibited neurogenic bladder" (Jonas and Brown 1975). The mechanism is loss of the descending signals which normally inhibits the primitive reflex contraction response of the detrusor muscle of the bladder wall during filling. In this latter paper examining 5 patients with NPH, 3 of the cases were aware of the need to urinate and all were distressed by the incontinence. Frequency, nocturnal frequency and enuresis are other urinary disturbances described. In advanced cases, incontinence may be associated with a lack of concern for micturition due to severe frontal lobe dysfunction (Hakim, Hakim et al. 2001). Urinary incontinence usually follows the gait abnormalities and almost always includes urinary urgency (Larsson, Wikkelso et al. 1991; Vanneste, Augustijn et al. 1992; Vanneste 2000). Deformation of periventricular corticospinal tract sacral nerve fibres seems to be the likely reason for incontinence [4]. Ineffective contraction of the detrusor muscle is identified on urodynamic studies (Gleason, Black et al. 1993). Improvement in urodynamic

function has been demonstrated within hours of lumbar tapping. No impairment of sphincter control has been identified (Ahlberg, Norlen et al. 1988). Occasionally, patients do not have frank incontinence but urgency; however the patient not being quick enough to reach the lavoratory in time, he has incontinence. Fecal incontinence may be the late result of this dysfunction, especially if left untreated (Relkin, Marmarou et al. 2005).

The exact anatomical circuit participating in the incontinence mechanism has not been proven. Until today we accept the explanation given by Adams of the deformation of the parasagittal region-connecting long tracts due to the ventriculomegaly (Adams, Fisher et al. 1965). Another suggestion made was that the incontinence may be attributed to the mechanical distortion of the basal ganglia from the ventriculomegaly. This data derives from the study of patients suffering from Parkinsonism who have also neurogenic bladder on customary (Jonas and Brown 1975).

1.8.4. Other symptoms

Motor symptoms in the upper limbs have been described in this group of patients. Akinesia, tremor and rigidity of upper limbs characterised up to 75% of patients with iNPH (Krauss, Regel et al. 1997). A study demonstrated a disturbance in grasping to lift and holding movements which improved (but not normalised) following test CSF drainage. It has been suggested by the same authors that the corticospinal fibres to the upper limbs maybe equally affected as a result of ventriculomegaly (Krauss, Regel et al. 1997). Micrographia is another symptom observed in patients with NPH (Goodman and Meyer 2001).

A psychiatric or behavioural manifestation of NPH has been noted in bibliography. Initially, the symptoms often manifest themselves as depression with marked psychomotor retardation, characterized by symptoms of apathy, inattentiveness, agitation, and poverty of thought which mimic a depressive illness. The inability to recognise the syndrome might result in unnecessary, often prolonged, treatment (Lying-Tunell 1979; Fersten, Glowacki et al. 2005). Rice and colleagues have described five patients in whom psychiatric disturbances, including depression, confusion, delusion, and mental deterioration, were the major feature, while neurological disturbances were relatively less obtrusive (Rice and Gendelman 1973); however, such patients do respond to ventriculoperitoneal shunting and the psychiatric symptoms resolve (Pinner, Johnson et al. 1997). Obsessive compulsive behavior in NPH has been noted (Abbruzzese, Scarone et al. 1994). In one study of 23 NPH cases somnolence-stupor-coma disorder (in 43.5%), astheno-emotional disorder (in all cases), and emotional-motivational blunting disorder (in 22% of the cases) was diagnosed. A variable recovery of these conditions post shunting has been documented (Lindqvist, Andersson et al. 1993).

1.9. Diagnosis

There is not one single diagnostic test that may diagnose the condition with high accuracy. This might be due to the heterogeneous pathologies contributing to the syndrome (Bech-Azeddine, Høgh et al. 2007). However, a consensus from an international group has been reached and diagnostic criteria for the condition have been presented (Relkin, Marmarou et al. 2005). According to these patients may be categorised as “probable”, “possible” and “unlikely”. The assessment of these

three different forms is based in combining relevant history, imaging, clinical and physiological criteria.

A patient with “probable iNPH” would be expected to have onset of symptoms later than age 40, with their symptoms having insidious onset and minimum duration of 3-6 months. The condition would be diagnosed following careful history and exclusion of conditions that lead to the secondary form of iNPH (such as head injury, intracerebral bleeding, central nervous system (CNS) infection, or other known causes of secondary hydrocephalus). Imaging would reveal ventriculomegaly of the communicating type with an Evans index>0.3 and either enlargement of the temporal horns not attributed to hippocampus atrophy, a callosal angle greater than 40 degrees, periventricular lucencies not attributable to vascular causes or demyelination, or an aqueductal or fourth ventricular flow void on MRI. Apart from exhibiting gait disturbance as an essential feature, and either features of cognitive impairment, or urinary incontinence as described in the previous section the patient must demonstrate a CSF opening pressure in the range of 5–18 mm Hg (or 70–245 mm H2O) as determined by a lumbar puncture or a similar procedure.

A large series of diagnostic tests have been suggested. The physicians at the front line of seeing elderly patients such as general practitioners, or elderly care physicians may wish to have a single test that can screen for the syndrome. On the other hand, a neurologist or a neurosurgeon may wish to use a test to aid in diagnosis and predict response to shunting and hence clinical outcome. Commonly held belief of criteria of poor prognosis such as idiopathic type, cortical atrophy,

longstanding symptoms, and presence of dementia in addition to old age should not rule out surgery a priori (Poca, Mataro et al. 2005; Kiefer, Eymann et al. 2006) necessitating therefore further investigations in this group of patients. Therefore early referral to specialist centres result in shorter duration of symptoms and an advantageous clinical outcome (Meier and Miethke 2003).

When evaluating the diagnosis with the use of ancillary tests (i.e. tap test, lumbar infusion studies etc.) it is important to evaluate the patient on more than one occasion an maybe on different days since there might be discrepancies in his performance (Kahlon, Sundbarg et al. 2002).

Modern imaging methods have been employed in a research capacity to measure non-invasively parameters such as ICP or compliance which would previously require invasive methods (Mase, Miyati et al. 2005; Glick, Niebruegge et al. 2006). However, these would require specialized software and processing and are not available widely when compared to the traditionally used lumbar infusion test. Measurement of intracranial hydrodynamic parameters with MR imaging cannot discriminate between responders and non-responders (Bateman and Loiselle 2007). Furthermore, the calculation of stroke volume on cine phase-contrast magnetic resonance imaging might not be useful for prediction of patient selection for shunting (Kahlon, Annertz et al. 2007). Peak CSF flow velocity calculation at the cerebral aqueduct by using the same method may prove more useful (Sharma, Gaikwad et al. 2008). The issue is discussed extensively in a later chapter (∫ 1.15.).

The idiopathic normal pressure hydrocephalus guidelines advocate the use of external lumbar drainage (ELD) as the test with the highest sensitivity (50-100%) and positive predictive value (PPV) (80-100%) when compared with the simple lumbar tap, or the calculation of the CSF outflow resistance following a lumbar infusion test (Marmarou, Bergsneider et al. 2005). The rate of serious complications from external lumbar drainage for iNPH has been reported to be low, with infection being less than 2% (Governale, Fein et al. 2008; Greenberg and Williams 2008). Apart from infections the patient might experience low-pressure headaches due to excessive drainage of CSF; its incidence has been reported as low as 1.7% in a large series (Governale, Fein et al. 2008). The drainage should not be exceeding 20 mLs/ hour as that would increase the chances of low-pressure headaches that might affect their performance following the testing. The testing is usually carried out for 72 hours, although some groups have suggested that 36 hours would be adequate to detect responders from non-responders. Serious complications such as subdural hematoma or subarachnoid haemorrhage have been reported, but appear as low as 1.7% (Governale, Fein et al. 2008). To date there is no data to show how soon the symptoms should be assessed following ELD. Different groups have different approaches; others advocating daily assessment until symptomatic improvement occurs, others assess patients after 72 hours soon after the catheter is removed, and others request the patient to keep a diary documenting symptoms following discharge from the hospital.

The “tap test”, or large volume lumbar puncture is a more appealing method that can be carried out as an outpatient procedure and does not require hospitalization. The method involves the removal of large quantity of CSF, namely about 40-50

mLs and assessment of clinical parametres before and after the testing. According to the guidelines published it is recommended that due to the high PPV (73-100%), but low negative predictive value (NPV) (23-42%) candidates for surgery should not be excluded if the tap test is negative (Marmarou, Bergsneider et al. 2005). Finally, the LIT has been widely used in the past as a method of prognosis and assisting selection of patients for surgery. It offers an insight into the intracranial hydrodynamics of individual patients by infusing CSF via a lumbar catheter in a set pressure or flow rate and then calculating physiological parametres such as the Rout, the Cout, or the PVI and hence the intracerebral compliance. The threshold of Rout which may predict surgical outcomes has varied between 8-18 Hg/ml/min in different studies (Borgesen, Gjerris et al. 1979; Boon, Tans et al. 1997; Kahlon, Sundbarg et al. 2002; Meier and Bartels 2002) with a PPV between 56-96% (Marmarou, Bergsneider et al. 2005). The predictive accuracy of the test increases the more the selection value of Rout increases reaching 92% for an Rout of 18 mm Hg/ml/minute (Boon, Tans et al. 1997). Calculation of the Rout might be useful in cases where the results of the ELD appear equivocal enhancing therefore the prognostic accuracy of the former test. The limitations of this method are that more than one method are presently in use (Ekstedt 1977; Marmarou, Shulman et al. 1978; Borgesen and Gjerris 1982; Czosnyka, Batorski et al. 1990; Borgesen, Albeck et al. 1992) and the estimated Rout vary by method (Eklund, Smielewski et al. 2007). It should also be pointed out that although a patient with increased Rout might be a good candidate for surgery, normal Rout estimations should not exclude one from being offered surgery (Eklund, Smielewski et al. 2007). Instead, supplementary tests should be used. The results of tap test and lumbar infusion

studies has been found to agree only on 45% of cases in one study (Kahlon, Sundbarg et al. 2002).

Other methods used is ICP monitoring which requires a more invasive procedure. In cases of negative CSF removal testing or negative infusion studies it has been suggested that continuous ICP monitoring should be undertaken (Pfisterer, Aboul-Enein et al. 2007). In this latter study 6 out of 11 patients with negative tap test were positive for continuous ICP monitoring and 5 of them improved post shunting. The mean ICP value obtained during monitoring might not be useful for patient selection, so calculation of the frequency or A and B waves of the waveform (Stephensen, Andersson et al. 2005; McGirt, Woodworth et al. 2008), and the pulse pressure amplitude (Eide and Brean 2006) are considered more useful. However, it has been shown that the pulse pressure amplitude obtained during a LIT may be able to predict the intracranial pressure amplitude obtained during ICP monitoring (Eide 2006), hence making the invasive ICP procedure less appealing. However ICP monitoring should not be the only diagnostic tool used for preoperative selection of patients as it has inadequate accuracy (Marmarou, Bergsneider et al. 2005).

Previous studies have found that patients with B-waves in less than 5% of the recording time did not improve (Borgesen 1984), and if there are frequent B-waves, shunting is likely to be successful (Black, Ojemann et al. 1985).

Worth mentioning are also the Japanese guidelines for management and diagnosis of iNPH (Ishikawa, Hashimoto et al. 2008). The authors categorise patients as

“definite”, “probable” and “possible”. They also advocate the use of the tap test as the first diagnostic test, and if positive, the patient should proceed directly to a shunt procedure without requiring additional tests. However in case of a negative tap test they advocate the use of the supplemental tests mentioned already (continuous CSF drainage, continuous ICP monitoring or estimation of Rout) or repeat of the tap test at follow-up.

Neuropsychological assessment before and after temporary drainage may be useful in predicting which patients are less likely to improve cognitively following shunting (Chaudhry, Kharkar et al. 2007). In particular, it was found that absence of improvement on verbal memory after ELD had a high negative predictive value for improvement on memory tests at 3-6 months after surgery (Thomas, McGirt et al. 2005; Chaudhry, Kharkar et al. 2007). Other predictors of cognitive improvement after shunt included young age and female sex (Chang, Agarwal et al. 2006). Worse performance in verbal memory at baseline investigation was associated with a 4 times less chance of improving cognitively post shunting (Thomas, McGirt et al. 2005). The MMSE although it is simple to administer may not be useful in diagnosis of iNPH, or prediction of surgical outcomes (Savolainen, Hurskainen et al. 2002).

Disturbance of gait may closely resemble the features of Parkinson’s disease as stated earlier (Krauss, Regel et al. 1997). Degenerative spinal causes of gait disturbance ought to be assessed with imaging to exclude the concurrence of cervical myelopathy, symptomatic lumbar stenosis or radiculopathy (Rasker, Jansen et al. 1985; Komotar, Zacharia et al. 2008). Common causes of urinary

incontinence such as benign prostatic enlargement in men, pelvic floor disorders in women, or a simple urinary tract infection ought to be considered and investigated accordingly. Also the combination of several different pathologies commonly occurring in older patients should be considered (Vanneste 2000)

1.10. Differential diagnosis

1.10.1. Vascular dementia

The terminology concerning syndromes of diffuse white matter lesions from vascular origin is confusing. The entity dates back to Binswanger's original description from 1894, which designated the neuropathological picture. However, this condition was never expressed in more detail than white matter atrophy and hydrocephalus. Clinical criteria for Binswanger's disease (BD) were later introduced (Caplan and Schoene 1978; Bennett, Wilson et al. 1990). These include cognitive impairment and gait disturbance or incontinence in combination with vascular risk factors and radiological signs of vascular white matter changes. The term subcortical arteriosclerotic encephalopathy (SAE) was introduced by Olszewski in 1962, as a neuropathological term, to describe "a form of cerebral arteriosclerosis in which vessels of the white matter and subcortical gray matter are affected predominantly" (Olszewski 1962).

With the introduction of computed tomography (CT) and MRI where white matter lesions were found more frequently than previously expected, in both symptomatic and asymptomatic subjects, a radiological description was needed. The terms *leukoaraiosis* or *subcortical leukoencephalopathy* were introduced to designate

white matter areas of hypodensity on CT or hyperintensity on T2-weighted MRI. Leukoaraiosis is reported to occur in 41-100% in patients with dementia of presumed vascular origin and in 21-100% in normal control subjects. The clinical significance of leukoaraiosis remains incompletely understood (Pantoni and Garcia 1995).

In the clinical context, the term vascular dementia has become widely used. At least five different systems of diagnostic clinical criteria are being used (Diagnostic and Statistical Manual of Mental Disorders (DSM)-III, International Statistical Classification of Diseases and Related Health Problems (ICD)-10, National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherché et l'Enseignement en Neurosciences (NINDS-AIREN), DSM IV and Alzheimer's Disease Diagnosis and Treatment Center (ADDTC)). These different criteria include different patient cohorts, which render heterogeneity in patient selection in studies (Pohjasvaara, Mantyla et al. 2000). All include focal neurological signs on neurological examination. This may exclude patients with small-vessel subcortical vascular dementia, who frequently do not show clear-cut focal signs. From this reason, a modification of the NINDS-AIREN criteria for subcortical vascular dementia has been proposed (Erkinjuntti 2002). The presence of extensive white matter changes, implying a co-existence of vascular disease and iNPH, has in several studies been reported in patients who benefit from shunt surgery (Bradley, Whittemore et al. 1991; Krauss, Regel et al. 1997; Tullberg, Jensen et al. 2001; Tullberg, Hultin et al. 2002).

Reports of the neuropathological correlates of white matter changes from vascular disease are more numerous than those of chronic adult hydrocephalus. The diffuse ischemic white matter disease is described as reduced number of oligodendroglial cells, reduced myelin content, fewer axons with fiber thinning and fragmentation, and increased number of reactive astrocytes. Degenerative vascular changes and hyaline fibrosis were frequent. No deposition of mature amyloid is noted within white matter, even when the amyloid load is marked elsewhere in the brain (Erkinjuntti, Benavente et al. 1996; Tanoi, Okeda et al. 2000; Englund 2002).

Apart from the above-mentioned neuropathological changes, marked accumulation of axonal transport proteins in the axonal bundles, indicating compromised axonal transport, has been demonstrated (Akiguchi, Tomimoto et al. 1997). In an animal model of global incomplete ischemia, demyelination was found to precede axonal damage. This suggests that the primary event in cerebral hypoperfusion is changes in oligodendrocytes and that changes in neurofilament follow (Kurumatani, Kudo et al. 1998). The neuropathological description of SAE is to great extent similar to that of chronic adult hydrocephalus, which, in combination with similar symptomatology, indicates a final common path of white matter damage in these syndromes.

1.10.2. Alzheimer's disease

The conditions which appear on the top of the list of the differential diagnosis about the cognitive impairment of the patient are dementia of the vascular type (VD, also known as Binswanger's disease) and AD. Patients with iNPH present with

dementia, which is characteristically of the subcortical type. Subcortical dementia is a clinical syndrome characterized by bradyphrenia, memory impairment, diminished executive function and mood and personality changes (Cummings and Benson 1984). Patients with subcortical dementia are inert, indifferent, and disinterested. Subcortical dementia is characterised by the absence of dysphasia, apraxia and agnosia. In contrast in cortical dementia characteristic of Alzheimer's dementia cognitive slowing may occur, however the disorders of higher cortical function, agnosia, apraxia and aphasia, are the predominant symptoms. Although AD and iNPH have different clinical phenotypes, a clinician may end up underdiagnosing iNPH. This might be further complicated by the ex-vacuo ventriculomegaly present in AD patients, but not iNPH (Holodny, Waxman et al. 1998). INPH patients have more severe impairment of attention and psychomotor speed. when compared to AD patients (Ogino, Kazui et al. 2006).

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

Pre-morbid diagnosis can be supported from CSF analysis, where low values of beta-amyloid1-42 (Aβ1-42) and high values of hyperphosphorylated tau (P-tau) is considered typical (Blennow, Wallin et al. 1995; Andreasen, Hesse et al. 1999; Blennow 2004).

In biopsy studies in patients with chronic adult hydrocephalus, changes typical for AD are frequently noted (Bech, Juhler et al. 1997; Del Bigio, Cardoso et al. 1997; Savolainen, Paljarvi et al. 1999; Golomb, Wisoff et al. 2000). The coexistence of AD and chronic adult hydrocephalus is shown not to affect the results from CSF diversion negatively (Golomb, Wisoff et al. 2000) and a study including clinically pure AD patients showed a trend in favor of shunt treatment (Silverberg, Levinthal et al. 2002).

1.10.3. Other conditions

Caplan has described a syndrome of encephalopathy associated with congestive heart failure with neuropsychological symptoms similar to NPH. Patients have apathy and abulia with retained alertness, lacking however the gait disturbance or urinary incontinence characteristic of NPH. Imaging reveals mostly cerebral atrophy with no signs of ventriculomegaly, however the symptoms improve following lumbar puncture resulting also in normalization of the “brain atrophy” (i.e. sulci become smaller and gyri widen (Caplan 2006).

1.10.4. The significance of ventriculomegaly

It can be difficult to differentiate brain atrophy, Parkinson’s disease, vascular encephalopathy, Alzheimer’s disease, Binswanger’s disease, and NPH using morphological criteria alone, such as enlarged ventricles (Savoiardo and Grisoli 2001). The indices used for the measurement of the ventriculomegaly are those of

Evans (Evans 1942), Schiersmann (Schiersmann 1952), and Schaltenbrand and Nfirnberger (Schaltenbrand 1959).

A typical CT scan should demonstrate an Evan's ratio of minimum 0.30 (i.e., the maximal width of the frontal horns divided with the maximal transverse inner diameter of the skull), rounded frontal horns, flattening of the sulci on the convexity, and a low degree of periventricular and white matter lucencies (Wikkelsø et al 1986, Vanneste et al 1993 and 2000, Boon et al 2000). Utilising MRI, an increased velocity of pulsatile CSF in the aqueduct, "the flow voiding sign", has been advocated as a supplementary test (Bradley et al 1986 and 1991b).

Other ways to measure ventriculomegaly is by calculating the ratios used by Poca et al.: Evans index (A/E), third ventricle index (C/E), Cella media index (D/F) and ventricular score (A + B + C + D)/E X100; with A representing the maximum bifrontal ventricular size, B being the distance between the caudate nuclei at the level of the foramen Monroe, C measuring the maximum width of the third ventricle, D representing the minimum width of both cella media, E denotes the maximum inner skull diameter at the level where A and B were taken, and F stands for the maximal outer diameter of the skull at the level where D was measured (Poca, Mataro et al. 2004).

The size of ventriculomegaly does not necessarily correlate with the ICP; indeed small ventricles cannot exclude increase resistance to CSF outflow (Borgesen and Gjerris 1987). High cerebral elasticity and low compliance is a predictor of rapid and marked reduction of the ventricles postoperatively (Tans and Poortvliet 1988; Tans and Poortvliet 1989). Increased ventricular size was not associated with

increased ICP or resistance to CSF outflow, rather the opposite was the case (Sorteberg, Eide et al. 2004).

Most probably, there exists a significant transmantle pressure gradient in the acute phase of hydrocephalus that has dissipated in the chronic phase of the disorder (Stephensen, Tisell et al. 2002). The initial transmantle pressure gradient might thus be responsible for the ventricular dilatation, which once it has occurred, can be maintained even if the pressure gradient is eliminated. Furthermore, as ventricular size increases, ventricular pressure decreases due to physical phenomena explained by Laplace's Law (Portnoy 1971). The present study confirms this relationship by the finding of a negative correlation of ventricle sizes to hydrodynamic parameters, i.e. the larger ventricles our patients had, the lower ICP and Rout were found (Sorteberg, Eide et al. 2004).

1.11. Surgical treatment of idiopathic normal pressure hydrocephalus

A Cochrane review suggests there is no evidence for shunting as treatment for normal pressure hydrocephalus (Esmonde and Cooke), however empirically surgical CSF diversion is known as the only treatment available. Such a question could only be solved by a randomised blinded, placebo-controlled trial of shunting or not all patients with "probable iNPH" eligible to receive treatment; the ethical dilemmas of such a study denying the possibility of treatment in patients who otherwise might be suitable for shunting are obvious. Experimental studies have shown that shunting produces physiological changes in regional blood flow, CSF dynamics and cerebral metabolism (Miyamoto, Tatsuzawa et al. 2007; Klinge,

Brooks et al. 2008; Petrella, Czosnyka et al. 2008), making shunting the only treatment necessarily in existence at present (Boon, Tans et al. 1997). There is an argument that improvement following shunting maybe a placebo effect explaining the deterioration noted after a shunt; such a placebo effect would be very difficult if not impossible to quantify.

A shunt has two modes of action: (1) it diverts CSF from the ventricles, and thereby transports components of the CSF that may play an until now unknown role in the production of symptoms; (2) it modifies intracranial pressure by the present opening pressure and conductance to flow.

The clinical improvement following CSF diversion, using the lumbar CSF tap test or shunt placement, is due to a forced compensatory dilation of the compressed intracranial veins. The forced dilation of the veins is a consequence of the Monroe–Kellie doctrine, since successful shunting is based on a slight over-drainage of CSF, which must be compensated by a matching increase in venous and capillary blood volume. The dilated vessels increase intracranial venous compliance and cerebral blood flow.

The general impression among general practitioners and non-specialists is that the beneficial effect of shunting are short-lasting before the patient deteriorates again (Malm, Kristensen et al. 2000). Hence, physicians might be reluctant to refer patients with significant comorbidities as well as those receiving anticoagulation medication for relevant pathologies. However a recent study has shown that the risk of subdural hematoma or other complications in patients receiving

anticoagulation is not higher than series reporting complications following shunting (Goodwin, Kharkar et al. 2007).

Ventriculoatrial shunting is an alternative method which is nowadays less popular due to its more complex technical nature and its potential for serious complications (Lam and Villemure 1997).

Even though surgical CSF diversion is the established surgical treatment employed other methods have been proposed, such as endoscopic third ventriculostomy. A study carried out in 17 patients with iNPH receiving endoscopic third ventriculostomy instead of ventriculoperitoneal (VP) shunting reported excellent results in 4, good in 7, satisfactory in 3 and poor in 3 patients; the authors used difference in the Kiefer scale to report outcomes (Hailong, Guangfu et al. 2008). Equally good results were presented in another study in a select subgroup of iNPH (Gangemi, Maiuri et al. 2004). However, the results of this method still remain controversial (Longatti, Fiorindi et al. 2004).

Lumboperitoneal (LP) shunting has been tried in the past but has now fallen off favour with surgeons (Selman, Spetzler et al. 1980). The reason is a general concept of LP shunts blocking more often than VP shunts although no evidence of the above exists. It would not be unreasonable to offer LP diversion to a patient who wishes to avoid the possibility of intracranial complications; however, the easy access to CSF sampling and the possibility of programming a valve makes the choice of a VP shunt a more appealing choice among neurosurgeons. A novel method of drainage and testing the suitability for permanent CSF diversion is a

lumbar-subcutaneous shunt which has been recently described by a single group claiming no infection in 46 patients (Ushewokunze, Haja Mydin et al. 2008).

There are numerous valve makes, models, and designs in the market all attempting to restore a “physiological” CSF flow across the valve. In the single randomised trial carried out in patients with INPH its authors found better outcomes when they used a low-pressure than a medium-pressure valve (Boon, Tans et al. 1998); however the rate of overdrainage was higher with low-pressure valves. A retrospective study revealed no difference in outcomes between using flow-regulated valves and differential-pressure valves (Weiner, Constantini et al. 1995).

Even though programmable valves may appear to be the obvious solution to find an optimised pressure setting for any individual it is not easy as one would think. Only one study has presented a formula of calculating setting based on physiological parameters (intracranial, patient’s height and intraabdominal pressure) (Miyake, Kajimoto et al. 2008). Nevertheless, a physician may reprogram the valve setting in the outpatient clinic based on the patient’s symptoms and imaging. There has been growing evidence that a gravitational unit when combined with a programmable valve may have better outcomes since it is able to counteract the siphon effect seen with differential pressure valves (Meier and Lemcke 2006; Meier and Lemcke 2006). Ultimately, the choice depends on the physician’s familiarity with a particular system. The current understanding favours the use of VP shunting with the use of a programmable valve combined with a gravitational unit

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

Many shunt types have been manufactured in the last fifty years since the first implantable shunt valve by Nulsen and Spitz over 50 years ago (Nulsen FE and Spitz 1952). Drake in a review of shunt technology of the last 50 years concluded that in paediatric hydrocephalus it is not the shunt technology but rather factors like the type of hydrocephalus, placement of the ventricular catheter and the ventricular catheter environment that predispose to ventricular failure or not in the paediatric population (Drake, Kestle et al. 2000).

1.12. Complications

Complications in shunting may be thought to be inherent to the procedure (shunting) or attributed to the condition itself (NPH). For example analysis of data from the UK shunt registry that analyses shunting of all types of hydrocephalus

shows that underdrainage can vary between 46-48% of all shunted cases (Richards HK, Seely HM et al. 2000). The 5-year complication rate has been reported to be 50% for shunt procedures (Borgbjerg, Gjerris et al. 1995)

The area was first reviewed systematically by Hebb and Cusimano (Hebb and Cusimano 2001). The authors concluded that the rate of complications is 38%, with 22% of patients requiring additional surgery, whereas there was a 6% combined rate of permanent neurological deficit or death. The authors mentioned that mortality of patients receiving a shunt may be between 2.5-3.3 times when compared to patients with vascular comorbidity; however this analysis did not take into account that mortality is mostly unrelated to the shunt procedure and is due to the co-morbid factors (Malm, Kristensen et al. 2000; Tisell, Hellström et al. 2006).. However, in a later retrospective analysis of the Medicare expenses of elderly patients with hydrocephalus it was shown that propensity to die is significantly greater if a patient does not receive a shunt (Williams, Sharkey et al. 2007).

The incidence of subdural hematomas in a large study reached 19.8% (Zemack and Romner 2002); this might be attributed to overdrainage and may be amenable to surgical evacuation or gradual absorption by adjustment of a programmable valve setting. Again sometimes, it is difficult to distinguish between a hematoma and a hygroma; hence, the actual hematoma rates might be lower. Particularly in NPH, no chronic subdural effusions occurred in one series (Sorteberg, Eide et al. 2004), whereas this complication was reported at frequencies as high as 20% in other series (Bakker, Boon et al. 2002). Williams and colleagues in their economic analysis of Medicare costs in patients with a diagnosis of hydrocephalus (not

exclusively NPH though) receiving a shunt was 12.2% (Williams, Sharkey et al. 2007).

The published guidelines have updated the complication rates. The incidence of subdural haematoma ranges from 2-17%, incidence of infection between 3-6%, postoperative seizures between 3-11% and 21% shunt revision rate (Bergsneider, Black et al.). Mortality due to shunt has been as low as 2% (Bergsneider, Black et al.) In two large prospective studies with well selected patients the rate of revision was 33% (McGirt, Woodworth et al. 2005), subdural hematoma was 3% (Marmarou, Young et al. 2005), subdural hygroma 3% (Marmarou, Young et al. 2005) and shunt infection rate was 3-6.7% (Marmarou, Young et al. 2005; McGirt, Woodworth et al. 2005). Infection of the shunt system will need explantation. Underdrainage might manifest as return of the symptoms or gradual deterioration and requires further investigations.

The complications of underdrainage might be divided into three different types: enlargement of ventricle width after shunt placement together with a persistent or worsening clinical pathology, no change of ventricular width and a clear worsening of clinical pathology, or late worsening of clinical pathology after a clear and early postoperative recovery.

1.13. Outcomes

Improvement after shunt placement in communicating hydrocephalus is dependent on the preshunting CSF circulatory profile, the shunt function after implantation,

and the potential coexistence of hydrocephalus with other disease states, particularly of a cerebrovascular nature.

Early observational work has pointed out that reduction in ventricular size correlated with improved outcomes (Gunasekera and Richardson 1977), however this initial findings have been challenged (Meier and Mutze 2004). Neither periventricular lesions, nor an increased Evans ratio preoperatively was significantly associated with clinical outcome (Pfisterer, Aboul-Enein et al. 2007). If that is the case, then the commonly held view of the stretching of the periventricular fiber cannot fully explain neither the symptomatology nor the improvement post-shunting.

Earlier the diagnosis of NPH was considered when the patient improved following surgery (i.e. it was given post-priori). However, one must confirm that the shunt is functioning before the diagnosis of NPH is rejected. Williams and colleagues found that among the 2/3 of patients with poor outcome following a shunt implantation, 80% had a treatable cause and subsequently clinical recovery occurred in further 70% of this last cohort (Williams, Razumovsky et al. 1998). These results were verified in another study where 61% of the poor outcome patients improved following shunt revision (Kilic, Czorny et al. 2007).

The general impression among general practitioners and non-specialists is that the beneficial effect of shunting are short-lasting before the patient deteriorates again. However, sustained beneficial outcomes have been shown in a mean follow-up duration of almost 6 years. The benefit was shown to 87% of the patients with

regards to the GD, to 86% with regards to cognition and to 80% of patients with regards to incontinence (Pujari, Kharkar et al. 2008). However, in that same study they reported that 25% of patients who reported initial improvement for gait will deteriorate. Worse outcomes than those reported may be related to inappropriate selection of patients, or shunt malfunctions which are not diagnosed appropriately. Unless a normal flow of CSF is demonstrated in a patient with iNPH who deteriorates one should assume that there is a high possibility of a shunt malfunction to account for any clinical deterioration.

One of the most significant hurdles in understanding the clinical course of the intervention is that there is no universally accepted scale in assessing outcomes; this makes the data reported in several outcome studies difficult to extrapolate and synthesize data. Two of the oldest outcome scales are those of Black (Black grading scale) (Black 1980), and the earlier Stein-Langfitt scale (Stein and Langfitt 1974). Whereas the former requires comparison with the preoperative status (i.e. it examines if the patient improved and whether this resulted into a return to previous activities), the latter focuses on functional outcome at the time point of assessment with an emphasis to supervision required for daily activities. A disadvantage of those is that because of the categorical nature a ceiling effect may be observed or the quantification of improvement not appear accurate. There are also other outcome scales such as the Kiefer grading scale (Kiefer, Eymann et al. 2003), the Krauss outcome scale (Krauss, Droste et al. 1996) which are composite scales based on grading the severity of the triad of symptoms. Other authors have used the modified Rankin scale to report outcomes, which is commonly used in vascular/ stroke research and the Barthel index, which is a commonly used daily

activities index. It is hoped that clinicians will proceed to be using one of the afore-mentioned outcome scales rather than the older system of reporting outcomes based on the clinician's impression on clinical improvement (excellent, fair, good etc.). However, more coordinated effort is anticipated and in the recent Hydrocephalus 2008 conference it was stated a task force is required to report a "Consensus on Outcome Scales and Quality of Life measures" (Hazel and Klinge 2008)

1.14. Biomarkers in chronic adult hydrocephalus

Biological markers have traditionally been used in clinical practice in order to support a diagnosis, or monitor the progression of a disease by measuring levels longitudinally. The definition as given by the Biomarkers Definition Working Group was "*A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention*." (Matias, Ferreira et al. 2001). Biomarkers can assist us in this task as they provide an insight to the changes of the cerebral milieu associated with the condition. In order for their use to be established in routine clinical practice, they should demonstrate high sensitivity and specificity. Biomarkers have long been used in the neurosciences for these exact reasons (Feigin 2004; Miller, Glass-Marmor et al. 2004; Rachakonda, Pan et al. 2004; Andreasen and Blennow 2005; Teunissen, Dijkstra et al. 2005; Blasko, Lederer et al. 2006). Some well established biomarkers in the field of neurodegenerative disorders and dementias are the neurofilament light chain polypeptide for multiple sclerosis (MS) (Avasarala 2004), and tau protein in AD (Blennow, Wallin et al.

1995). Extensive research has already been carried out to document the hydrocephalus-induced changes in the composition of CSF (Del Bigio 1989).

Serum biomarkers have played an important role in other neurological conditions, due to the relative accessibility of samples (Raabe, Grolms et al. 1999; Sen, Belli et al. 2005). However, CSF remains the primary fluid of choice to monitor these changes. The concept of the "*sink action"* of the CSF was first introduced by Davson in 1962 to highlight this potential (Davson 1967). The aim of this paper is to review the role that biomarkers play in the diagnosis and monitoring of the clinical progression of chronic adult hydrocephalus (CAH), and to discuss current research and future perspectives.

1.14.1. Serum Biomarkers

Despite serum being an easily accessible biological fluid, only four studies on biomarkers in adult hydrocephalus have been identified, thus demonstrating that there is an open field for the discovery of serum biomarkers in CAH. Vasopressin plasma levels were studied in 11 patients and compared to controls. No significant difference was found between the two groups (Hammer, Sorensen et al. 1982) and similar results were found in another study with 18 patients (Sorensen, Gjerris et al. 1983). Glycoprotein D2 serum levels were measured in 13 patients with NPH and there was no difference compared to control subjects. However, the mean values were significantly lower when compared to those of patients suffering from primary degenerative dementia of Alzheimer's type. Unfortunately, the authors do not comment on the significance of this finding and neither were the findings correlated with imaging or with CSF dynamics studies (Sorensen, Gjerris et al.

1983). Preoperative melatonin levels were studied in six patients with NPH and were found to be lower than controls. Postoperative values did not differ significantly, but shunting restored the preoperative deranged diurnal rhythm of melatonin (Yamada, Iwasa et al. 1991).

1.14.2. Cerebrospinal fluid Biomarkers

Biomarkers in the CSF are potentially more useful because they provide an insight into changes in the brain milieu associated with the condition, and consequently more research has been undertaken on the composition of the CSF. It is assumed that ventricular CSF will reflect the changes happening in the brain parenchyma, and more specifically in the periventricular white matter. Ideally, levels of biomarkers should be measured before and after surgical diversion of the CSF, in order to obtain clinically useful indices for the diagnosis and progression of the disease. Initial attempts took place in the early 80's (Wikkelso and Blomstrand 1982). There are some reasons why good experimental criteria have not always been met: 1) universally accepted outcome scales have not been produced until recently, making comparison between different studies and different groups difficult (Klinge, Marmarou et al. 2005), 2) NPH is a relatively rare disorder and it is only recently that there is increased awareness about its importance, 3) CSF is not always available for repeated sampling, especially in pre-shunt patients with no accessible route into the ventricles (reservoir). However, this latter cohort of patients may provide a better insight into the pathophysiology, because after shunt insertion the CSF dynamics are altered.

1.14.2.1. Neuropeptides

- **Somatostatin**

Somatostatin (SOM) in the CSF has been measured by two groups, in 1991 (Wikkelso, Ekman et al. 1991) and in 2001 (Poca, Mataro et al. 2001). In the study by Wikkelso, CSF SOM levels were significantly lower (p<0.05) in NPH patients when compared to the control group and Poca et al also observed the same. They suggested that the decrease could be the result of damage to the cortical neurons and the nerve terminals of the hypothalamus that normally have high concentrations of SOM. The normal concentrations of SOM in these structures are selectively impaired in experimental hydrocephalus (Ehara, Matsumoto et al. 1982; Rubinow, Davis et al. 1988). Following shunting, SOM concentration significantly increased compared to preoperative values. The authors also detected a significant correlation with visual memory performance (r=0.57; p= 0.032) and with visuomotor speed (r=−0.55; p=0.05), demonstrating that higher concentrations of SOM were associated with better visual memory and increased speed of mental processing, features that are known to be deranged in NPH. These associations did not persist after surgery. After shunting, changes in SOM concentrations correlated significantly (r=0.66; p=0.01) with improved daily life activities (measured with the Rapid Disability Rating Scale-2 (RDRS-2). In another smaller study, levels of SOM were lower in the iNPH group when compared to controls, but were not correlated with either MMSE scores or the Blessed dementia scale (Molins, Catalan et al. 1991).The modulatory role of somatostatin in cognition has already been proposed (Schettini 1991).

- **Vasoactive Intestinal Peptide**

Wikkelsö in 1985 examined the role of the vasoactive intestinal peptide (VIP) in the pathogenesis of NPH and in multi-infarct dementias (Wikkelso, Fahrenkrug et al. 1985). The preoperative concentration of VIP in CSF was significantly lower in NPH when compared to controls, but increased postoperatively. The rationale behind the study was that VIP is a potent vasodilator and therefore may play a role due to the presence of chronic ischemia (Henning and Sawmiller 2001). These results were verified again in a later study (Wikkelso, Ekman et al. 1991) by the same group. Tullberg e*t al*. compared the levels of VIP in 43 patients with NPH and 19 with SAE and found the CSF levels of VIP higher in patients with SAE than with NPH (Tullberg, Mansson et al. 2000). They also noticed that the group of NPH patients suffering from cerebrovascular disease demonstrated higher VIP concentrations than those with other aetiologies. They suggest that higher VIP concentration in patients with SAE could be due to activation of VIP-ergic neurons to accomplish a compensatory vasodilatation. This would not, however, explain why the VIP levels increased following a shunt operation as in the previous study, since cerebral blood flow has been shown to be restored postoperatively (Sutton, Wood et al. 1983). Tisell *et al* compared the levels of VIP in 18 patients with aqueductal stenosis and 19 patients with iNPH (Tisell, Tullberg et al. 2004). The authors correlated the results with outcomes, concluding that levels of VIP correlated weakly with postoperative deterioration in alertness.

- **Delta-sleep-inducing peptide**

Wikkelsö *et al.* investigated the role of delta-sleep-inducing peptide (DSIP) in the CSF of ten patients with NPH and compared the results with that of healthy volunteers and other dementias (Wikkelso, Ekman et al. 1991).The levels of the peptides were again decreased in NPH compared to control levels, but increased significantly in parallel to the clinical improvement following shunting. Since, DSIP is a 9-amino acid peptide with a role in the normal sleep-wakefulness regulation, the results are not surprising, especially since the reduction was more pronounced in subjects with worse psychomotor performance.

- **Neuropeptide Y**

Reduced levels of neuropeptide Y (NPY) in patients with NPH has been found in several studies (Wikkelso, Ekman et al. 1991; Catalan, Sahuquillo et al. 1994; Poca, Mataro et al. 2001). Furthermore, it appears that the levels increase following shunting (Poca, Mataro et al. 2001). Wikkelsö *et al.* investigated the role of this neuropeptide in ten patients with NPH compared to levels in other patients with dementia; he also examined and compared the levels longitudinally three months post shunting. The percent increase in concentration following shunting was strongly correlated with percent change in a functional/activity scale (RDRS-2) (Wikkelso, Ekman et al. 1991). Tisell *et al* in a later study, compared the levels in 18 patients with aqueductal stenosis and 19 patients with iNPH (Tisell, Tullberg et al. 2004). The authors correlated the results with outcomes, concluding that levels of NPY correlated negatively ($r<0.40$) with postoperative improvement in alertness. However, lower CSF levels when compared with controls have been found also in

patients with AD (Alom, Galard et al. 1990), therefore reducing the specificity of this peptide and negating its potential role as a biomarker.

1.14.2.2. Neurotransmitters

Tullberg *et al.* extended the range of biomarkers in a later study by examining the role of 4-gamma aminobutyric acid (GABA) in addition to the two previously mentioned biomarkers (Tullberg, Mansson et al. 2000). Similarly to the previous study, they correlated the biomarkers with surgical results. No correlation was found with the results of shunt surgery and the CSF concentration of GABA. Malm *et al.* considering that a similar biochemical disturbance to that of patients with different forms of dementia, namely disturbance in the cholinergic, serotonergic and noradrenergic system would occur in patients with NPH, measured the levels of 3-methoxy-4-hydroxy-phenylglycol (MHPG), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA), acetylcholinesterase (AChE) and butyryl cholinesterase (BuChE) (Malm, Kristensen et al. 1991). They did not observe any significant differences between patients with NPH, AD, multi-infarct dementia (MID), and controls regarding the concentrations of HVA, 5-HIAA and MHPG, BuChE. They noticed reduce AChE activity both in the NPH and AD group when compared to controls. The levels of these transmitters did not correlate with the degree of ventriculomegaly, dismissing the possibility of a dilutional effect. They observed a positive correlation between outflow conductance in the hydrocephalic group and the concentrations of HVA, and 5-HIAA**.** Acetylcholinesterase activity was positively correlated with MMSE scores. The study highlighted the possibility of a common cholinergic disturbance in NPH and AD.

Homovanillic acid was further studied by Hildebrand *et al* as a reflection of dopamine metabolism, attempting to correlate the concentration with the clinical triad of gait disturbance, dementia and urinary incontinence (Hildebrand, Moussa et al. 1992). No correlation was found in their study and the authors rejected the potential of HVA as a biomarker for this condition. Similarly, levels of HVA did not differ significantly between patients with NPH and controls in another study (Tullberg, Mansson et al. 2000). Another study did not find any difference in the levels of MHPG, HVA and 5-HIAA between cases of obstructive hydrocephalus and iNPH (Spanu, Santagostino et al. 1989).

1.14.2.3. Cerebral metabolites

- **Lactate**

Lactate, an end product of anaerobic glycolysis, possibly represents the element of chronic ischemia implicated in the pathophysiology of normal pressure hydrocephalus Lactate was studied by Malm *et al.* in 15 patients with iNPH and was significantly reduced when compared to controls (n=21) (Malm, Kristensen et al. 1991). Furthermore, they noticed a positive correlation between outflow conductance in the hydrocephalic group and the concentrations of lactic acid. One would expect the levels of lactate to be increased on a background of chronic ischemia. Malm explained this paradox by hypothesising that a) there is an autoregulatory CSF clearance of lactate with a facilitated out transport mechanism in the choroid plexus and cerebral subarachnoid space, b) due to inverse, caudorostral, flow in CSF there is an accumulation of the metabolite in the ventricles and subsequently a transepensymal absorption of it , and c) as a result

of atherosclerosis and the pathological changes induced there is an obstacle to transpendymal diffusion of lactate from extracellular fluid (ECF) to CSF. Nooijen *et al.* however reported higher lactate levels in the NPH group when compared to controls, and significantly higher values when compared with AD (p=0.0005, n=36) and VD (p<=0.01, n=49) concluding that lactate levels might differentiate between adult hydrocephalus and patients with Alzheimer's and vascular dementia (Nooijen, Schoonderwaldt et al. 1997). Although the latter is a large cohort observational study with 57 hydrocephalic patients, the authors did not report correlation with CSF outflow conductance, ventriculomegaly or with surgical outcomes.

- **Free radicals**

Fersten *et al.* studied the role of free-radical peroxidation products in the CSF of 24 patients with NPH, and in particular that of thiobarbituric acid-reactive material (TBAR), and protein sulfydryl (SH) groups (Fersten, Gordon-Krajcer et al. 2004). The rationale behind the study was that free-radical peroxidation alters the structure of biological membranes and may therefore be implicated in the pathogenesis of chronic adult hydrocephalus. The results showed a significant increase in the levels of TBAR, total and soluble SH groups, as well as a decrease in the number of protein thiol groups between the NPH and the control group. The authors imply that peroxidation which damages the cytoplasmic membranes might be one of the factors that affect cognitive functioning.

1.14.2.4. Enzymes

- **Neuron Specific Enolase**

Nooijen et *al.* studied the CSF levels of neuron specific enolase (NSE), a glycolytic enzyme localized in neurons, in 57 patients with NPH. The levels were lower than both the control and the Alzheimer's group. However, this difference was not statistically significant and certainly was not correlated with surgical outcomes (Nooijen, Schoonderwaldt et al. 1997). NSE, was significantly higher in the Alzheimer's and vascular dementia group as compared to the control group, while it did not differ significantly between the two dementia groups (Blennow, Wallin et al. 1994). However, these results were contradicted in another study (Parnetti, Palumbo et al. 1995). These findings are only suggestive that CSF-NSE has potential as a non-disease specific marker for the neuronal degeneration in dementia. NSE as a biomarker in hydrocephalus remains even less well established.

- **Plasminogen Activator Inhibitor-1**

Sutton *et al.* measured the levels of the plasminogen activator inhibitor-1 (PAI-1) in the CSF of patients suffering from various neurological disorders (Sutton, Keohane et al. 1994). In the hydrocephalic patients the levels were not increased compared to the levels of control subjects. Interestingly enough, the levels were increased in the case of AD, cerebral infarction and CNS infection. This finding contradicts results from newborns where high levels have been found in cases of post haemorrhagic hydrocephalus (Hansen, Whitelaw et al. 1997; Hansen, Lapp et al.

2000). PAI-1 is a 52-kDa glycoprotein, which under normal conditions is relatively restricted from entry into the CSF. The study found increased levels particularly in patients with brain tumours, pointing to possible alterations in the blood-brain barrier. The small number of patients in the above study, the negative findings, and the fact that the PAI- is found in a variety of neurological diseases excludes the use of this protein as a potential biomarker for hydrocephalus. However, PAI-1 may play a role in fibrinolysis occurring after a haemorrhage within the CNS. In the absence of adequate fibrinolysis, micro thrombi will obstruct the arachnoid villi and subsequently cause fibrosing arachnoiditis affecting the CSF dynamics.

- **Prostaglandin D synthase**

Mase *et al*. measured the levels of prostaglandin D synthase (PGDS) in 14 patients with normal pressure hydrocephalus and found them significantly lower when compared with a control patient and other patients with dementia patients **(**Lewy body dementia, vascular dementia, Alzheimer's type) (Mase, Yamada et al. 2003). This enzyme is produced in the leptomeninges, and the trabecular cells of the arachnoid membrane and then secreted in the CSF as beta-trace. The authors conclude that the observed decrease is probably due to a degenerative change in the arachnoid membrane and cannot be considered the cause of neurological symptoms in the case of NPH. It is unclear whether these decreased levels were due to decreased CSF production that reflect arachnoid damage, mere dilution, or to the disturbance in fluid dynamics.

1.14.2.5. Neural cell-derived proteins

- **Myelin Basic Protein**

Myelin Basic Protein (MBP) is a known indicator of brain damage and in particular of demyelination (Levin, Hoyle et al. 1985; Miller, Glass-Marmor et al. 2004). It is known that in hydrocephalus there is demyelination of the periventricular white matter and so MBP appears as an attractive marker to study the degree of this pathological process. Sutton *et al.* measured the levels of this protein in the CSF of hydrocephalic patients with different aetiologies and proposed that active hydrocephalus produces significant periventricular demyelination, probably as the result of mechanical stretching (Sutton, Wood et al. 1983)**.** Interestingly, the degree of ventriculomegaly was positively correlated with the levels of MBP. The findings become more interesting since we know from the studies of Whitaker *et al.*, that cerebral atrophy is not associated with elevated MBP values (Whitaker, Lisak et al. 1980). Later, Longatti *et al*. in 1993 examined the levels of MBP pre- and postoperatively. In their study of 17 patients with hydrocephalus who underwent surgical CSF diversion they observed that the levels of MBP decreased following the shunt operation, suggesting that MBP is an index of brain damage and its levels could be used as an indication for shunting (Longatti, Canova et al. 1993). They have not however correlated the levels with shunting outcomes. However, high levels of MBP before shunting may be explained by the pooling of molecules in stagnant CSF, which then decrease after flow is restored by shunting. In another study of 57 patients with NPH, the levels of MBP did not differ significantly between patients with NPH, vascular dementia, AD, and controls (Nooijen, Schoonderwaldt et al. 1997). However, the levels of MBP were higher than controls**.** Although

studies have shown that the MBP levels decrease in humans postoperatively, different results were obtained in rats. In order to examine the possibility that neurons and oligodendrocytes, both of which represent deteriorating cell populations in hydrocephalus, can be regenerated by the proliferating brain cells, rats with kaolin-induced experimental hydrocephalus were later injected with bromodeoxyuridine (BrdU). The BrdU positive cells for MBP were increased from 17% in the hydrocephalic group to 33% at an early stage after the shunt procedure, but were restored to 6% at a later stage after shunting. The differentiation to mature oligodendrocytes appears to be inhibited in hydrocephalus even after the shunt procedure (Fukushima, Yokouchi et al. 2003). Del Bigio *et al.,* who measured the degree of myelination indirectly by measuring the MBP in cerebrum of rats with experimentally induced hydrocephalus, observed that with persistent hydrocephalus, the corpus callosum became thinned, axons were lost, and myelin-related enzyme activities and proteins were decreased. The timing of intervention became important as he showed treatment of hydrocephalus at 1 week largely prevented the damage while shunting at 4 weeks failed to restore the injured white matter concluding that hydrocephalus in the immature rat brain delays myelination, but compensatory myelination is possible if treatment is instituted prior to the development of axonal injury (Del Bigio, Cardoso et al. 1997). This finding correlates with our clinical experience on NPH and the importance of shunting patients as soon as possible in order to achieve the best clinical outcome.

- **S-100b**

The presence of this protein in blood points to the functional and/or morphological disruption of the blood-brain barrier (Sendrowski, Sobaniec et al. 2004). It is a major protein of the cytosol predominantly found in glial cells. Increased levels have already been found in cases of astrogliosis (Migheli, Cordera et al. 1999) and hydrocephalic children (Sendrowski, Sobaniec et al. 2004). S-100b levels in the CSF, were studied by Nooijen e*t al.* who showed that S-100b levels did not differ between patients with NPH (n=44), and controls (Nooijen, Schoonderwaldt et al. 1997), and therefore its role as a marker in chronic adult hydrocephalus is doubtful.

- **Nerve Growth Factor**

Nerve Growth Factor (NGF) is known to promote neuronal recovery from injury and age-related atrophy, being also important in the regeneration in the brain. NGF is not normally detectable in innervated tissues, but ablation of the innervating neurons leads to the production of measurable NGF in the target tissues (Mashayekhi and Salehi 2005). Increased NGF mRNA levels have been detected in the medial septal nucleus, striatum and corpus callosum in experimentally-induced hydrocephalus in rats (Shinoda, Hidaka et al. 2001). Yang *et al.* investigated the role of NGF in the pathogenesis of hydrocephalus. They measured the levels of NGF in nine adult patients with high pressure hydrocephalus (the authors define high pressure as CSF pressure>10 cm H_20) and seven patients with ex-vacuo hydrocephalus (Yang, Chang et al. 1999). The levels were significantly higher postoperatively in the second group, despite the fact that

no significant difference existed perioperatively. The results suggested that the neuronal injury was more severe in the ex-vacuo category, however one may argue that the last measurement was made only four days postoperatively. No correlation was made with surgical results. In a similar study Hochhaus *et al.* measured the levels of NGF and neurotrophin-3 (NT-3) in 42 hydrocephalic children. The levels of both were again elevated in comparison to controls, however the results were only correlated with presenting symptoms and not with outcomes (Hochhaus, Koehne et al. 2001). Increased levels of NGF when compared to controls have been verified in another study of 16 children with communicating hydrocephalus (Mashayekhi and Salehi 2005). These findings suggest the possibility that the elevation of NGF concentration in CSF was caused by increased generation of glial cells that resulted from brain damage.

- **Tau Protein**

Kudo *et al.* studied the role of tau protein by measuring the levels in 20 patients with NPH. Tau concentrations were elevated compared to those of orthopaedic controls (Kudo, Mima et al. 2000). The results of his study were not correlated with surgical outcomes. The levels of the protein were positively correlated, however, with the severity of dementia and with urinary incontinence but not with gait. Tisell *et al* also compared the levels of tau protein in 18 patients with aqueductal stenosis and 19 patients with iNPH, concluding that the levels of tau have no correlation with clinical improvement (Tisell, Tullberg et al. 2004). Tau protein, a microtubule-associated protein has been found to be elevated in the CSF of patients suffering from Alzheimer's disease (Blennow, Wallin et al. 1995), as well as in patients with

Lewy body dementia, corticobasal degeneration (Newman, Rissman et al. 2005), and Creutzfeldt-Jakob disease (Van Everbroeck, Boons et al. 2005) indicating that it is a marker of neuronal degeneration.

Lins *et al.* measured the immunoreactivity of amyloid beta peptide (1–42, Ab42-IR) and tau protein (total tau immunoreactivity (TTIR) in 12 patients with NPH, and compared them with the levels of an equal number of patients suffering from VD, AD, Parkinson's disease (PD) without dementia and 24 controls (Lins, Wichart et al. 2004). TTIR levels in NPH were not significantly changed when compared with the other causes of dementia and controls, whereas Ab42-IR was significantly decreased when compared with Parkinsonian patients and control subjects. The authors combined the results of both markers in a single plot as a method to discriminate between different groups of dementia; all the NPH patients were within the predicted area. Increased levels of TTIR are believed to reflect ongoing neuronal and axonal degeneration or damage, whereas decreased Ab 1-42 may be the result of increased recruitment of Ab1-42 from the CSF and the brain interstitial fluid to deposits in the form of plaques or decreased secretion into the CSF (Samuels, Silverman et al. 1999). A recent experimental study in rats with kaolin-induced hydrocephalus verified the above results showcasing increased accumulation of Ab1- 42 in the periventricular area, around the cortical vessels and the cortical parenchyma; the size of the deposits correlated well with the duration of the condition (Klinge, Samii et al. 2006).

- **Glial Fibrillary Acidic Protein**

Glial Fibrillary acidic protein (GFAP) is a non specific marker indicating gliosis (Bartosik-Psujek and Stelmasiak 2001; Malmestrom, Haghighi et al. 2003). Tullberg *et al*. were the first to find a useful biomarker associated with the progression of normal pressure hydrocephalus. They measured the CSF levels of GFAP in 65 patients with normal pressure hydrocephalus (twenty one of the idiopathic type) and correlated them with preoperative clinical presentation and signs. They observed a two-fold increase in GFAP levels when compared to controls (Tullberg, Rosengren et al. 1998). Similar results regarding GFAP were verified in an earlier study by Albrechtsen *et al.* (Albrechtsen, Sorensen et al. 1985). GFAP did not seem to correlate with severity of symptoms or presentation and had no correlation with the outcome of shunt surgery. In particular, GFAP in CSF suggests an irreversible damage to astrocytes, since GFAP is not secreted by astrocytes.

- **Neurofilament triplet proteins**

Neurofilament proteins are Type III intermediate filament proteins that assemble into neurofilaments, the major cytoskeletal element in nerve axons and dendrites. They consist of three distinct polypeptides, the neurofilament triplet protein (NFL). It has been shown that the metabolism of neurofilaments is disturbed in Alzheimer's disease (Lacoste-Royal, Mathieu et al. 1990). Therefore, NFL may be used as a biochemical marker of neuronal degeneration and particularly of axonal damage (Malmestrom, Haghighi et al. 2003). Tullberg *et al*. measured the levels of

NFL in 65 patients with normal pressure hydrocephalus (21 of the idiopathic type) and correlated them with preoperative clinical presentation and signs. They observed a six-fold increase in NFL levels when compared to controls. NFL as the authors point out is not specific for NPH and therefore cannot differentiate between various types of dementia. This increase in NFL indicates a degeneration of neurons primarily affecting the axonal region with a loss of intermediate filament protein across deranged cell membranes into the interstitial fluid. Although, they report outcome results without using any of the commonly used scales, in the case of NPH they conclude that high preoperative NFL levels are associated with favourable surgical outcomes (r=0.3, p<=0.05), and suggest that NFL can be used as a marker for ongoing axonal damage (Tullberg, Rosengren et al. 1998). However, in another study from the same group, even though the increased levels of NFL in the ventricular CSF of patients with iNPH were verified, the results did not correlate with improvement (Tisell, Tullberg et al. 2004).

- **Sulfatide**

Sulfatide is a glycosphingolipid component of myelin and it has been recently understood by experiments in sulfatide-null mice, to be essential for the maintenance of CNS myelin and axon structure (Marcus, Honigbaum et al. 2006). In an early study, the role of sulfatide was studied in patients with communicating hydrocephalus (Tullberg, Mansson et al. 2000). The preoperative CSF sulfatide levels were found to be higher in the NPH group with cerebrovascular aetiology, when compared with the rest of the NPH patients. The authors postulated the presence of irreversible ischemic white matter lesions in the hydrocephalic group

with cerebrovascular disease. Since the sulfatide levels were normal in most of the NPH patients, they considered that demyelination plays a minor role in the pathogenesis of NPH. In addition, there was no correlation with surgical outcome. The authors state that the results cannot be explained by a difference in CSF dynamics and therefore the concentration of sulfatide can differentiate between NPH and subcortical arteriosclerotic encephalopathy (SAE). The latter condition also known as Binswanger's disease has a similar clinical presentation to NPH (Kovacs, Szirmai et al. 2005), and therefore this contribution is significant in establishing the diagnostic role of sulfatide as a biomarker. Tisell *et al* compared the levels of sulfatide in 37 patients with aqueductal stenosis and iNPH (Tisell, Tullberg et al. 2004). The levels of sulfatide correlated inversely with improvement in psychometric performance; this correlation however was weak.

- **Glycoprotein D2**

Glycoprotein D2 is a glycoprotein enriched in neuronal membranes and probably involved in intercellular adhesion. The levels of this protein were examined in the CSF of 13 hydrocephalic patients and compared with controls and patients suffering from primary degenerative dementia of Alzheimer's type. The levels were significantly lower in the case of NPH. No correlation was made with symptoms, surgical outcomes or outflow conductance. The significance of this study remains unknown in the setting of NPH (Sorensen, Gjerris et al. 1983)

1.14.2.6. Cytokines

Tarkowski *et al.* investigated the role of the tumour-necrosis factor (TNF-α), an inflammatory mediator, and whether NPH triggers its production (Tarkowski, Tullberg et al. 2003). They examined the levels of TNF-α in the CSF of 35 patients with NPH and compared them with controls. In the NPH group the levels were 45-fold higher. The most interesting finding was that TNF- α returned to control levels following shunting in the group that improved following surgery. This factor has a short half-life in CSF, hence accumulation due to CSF stagnation is unlikely, and the increase may be due to increased production preoperatively. The authors also suggest that TNF-α might be a marker for demyelination and suggest TNF-α toxicity is directed to the white matter in patients with NPH.

Recently, the CSF levels of two interleukins, IL-4 and IL-10, have been compared in different neurodegenerative diseases (Stoeck, Bodemer et al. 2005). Levels of both interleukins were significantly higher when compared to controls, but not different when compared to patients with dementia and CJD. The authors suggest that elevated levels of those cytokines might reflect a response to neurodegeneration, and also might trigger neuroregeneration. In another study IL-1 levels in the CSF of patients with AD were
significantly higher when compared with NPH (Cacabelos, Barquero et al. 1991). The results of both studies were not correlated with surgical outcomes. This, together with the small number of patients, means that it is not possible to obtain any solid conclusions.

1.14.2.7. Other biomarkers

We will briefly summarise below studies of biomarkers that have been investigated less, or only in single studies. CSF levels of vasopressin did not differ between NPH patients and controls in two studies of 11 and 18 patients, respectively. Hammer *et al.* suggested that there might be a role of vasopressin affecting the memory, however the results of the study did not substantiate this claim (Hammer, Sorensen et al. 1982)**.** The concentrations of corticotropin releasing factor (CRF) were examined in 14 patients with NPH pre- and postoperatively (Poca, Mataro et al. 2001). The levels of CRF increased significantly post-shunting, nevertheless they remained below normal levels. The authors explain this difference by a possible improvement in cerebral blood flow that is known to occur post shunting. They also noted that the change in CRF correlated negatively with percentage change in postoperative verbal fluency and in the trail-making test B, a test measuring psychomotor speed. No other study has been identified in the literature and unfortunately we cannot gain a better insight into the role of CRF with hydrocephalus. Levels of cholecystokinin, a 33-amino acid polypeptide acting as a neurotransmitter or neuromodulator (Mollereau, Roumy et al. 2005), were found to be significantly lower in 16 patients with NPH when compared with controls and also lower levels were correlated with abnormal ICP values (Galard, Poca et al. 1997). Brettschneider *et al.* studied the CSF levels of leptomeningeal derived β trace protein, beta2 microglobulin and Cystatin C in groups of patients suffering from NPH (n=19), AD (n=30), vascular (n=13) and frontotemporal dementia (n=6). The levels of β-trace protein were lower in the NPH group than the controls and patients with AD suggesting a meningeal dysfunction in the pathogenesis of NPH.

The authors suggest this protein a potential biomarker discriminating between NPH and AD, although no correlation was carried out with surgical outcomes (Brettschneider, Riepe et al. 2004).

1.14.2.8. The use of CSF biomarkers for outcome prognosis

During the recent years, various groups have made efforts to identify CSF markers for neurodegenerative disorders. These efforts have so far been concentrated on other neurodegenerative disorders such as AD (Andreasen, Minthon et al. 2001), PD (Michell, Lewis et al. 2004), Pick's disease, and Lewy Body dementia (Mollenhauer, Cepek et al. 2005). Although improvements of clinical NPH symptomatology have been described after shunting in patients with neuropathologically confirmed concomitant AD (Bech, Waldemar et al. 1999), it has been shown that NPH patients with additional pathology attributed either to vascular dementia or AD generally show worse outcomes after shunting than those with NPH without concomitant pathology (Savolainen, Hurskainen et al. 2002). The pathology of these conditions is so closely interlinked (Del Bigio, Cardoso et al. 1997; Holm, Savolainen et al. 2003; Silverberg, Mayo et al. 2003) that Silverberg *et al.* even suggested a low-flow (up to 140 mL/d) CSF drainage pilot study for patients with Alzheimer's dementia (Silverberg, Levinthal et al. 2002). Therefore, neurochemical parameters that could help to separate NPH from other neurological disorders, which mimic NPH symptomatology, would be of clinical value. Pathological studies have given us an insight into the changes occurring in chronic hydrocephalus. Disruption of the ependymal ventricular lining, interstitial oedema, neuronal degeneration, white matter lesions, gliosis, capillary micro-infarctions and demyelination (Weller, Wisniewski et al. 1971; James, Flor et al.

1980; Miyagami, Murakami et al. 1981; Akai, Uchigasaki et al. 1987; Sutton, Keohane et al. 1994) are consistent findings. Studies from experimentally-induced hydrocephalus in rats have supported these findings and provided us with additional evidence (Klinge, Muhlendyck et al. 2002; Klinge, Samii et al. 2003). Furthermore, we now know that a regenerative process takes place following gradual necrosis in the white matter and axonal injury, and as a response there is the production of new glial cells in the subependymal zone to compensate for the cell loss (Del Bigio and Zhang 1998).

The case for identifying biomarkers in chronic hydrocephalus of adult onset has arisen due to similar developments in other common causes of dementia and the increasing awareness of both the epidemiology of NPH (Trenkwalder, Schwarz et al. 1995; Tisell, Hoglund et al. 2005) and its impact on the quality of life of elderly patients (Gelling, Iddon et al. 2004). Ideally, useful biomarkers should be confirmed with data from multiple disciplines, including neuropsychological testing, blood tests, genetic markers, CSF composition, and brain imaging. Alterations in the neurochemical composition of CSF in hydrocephalus have been widely documented and reviewed (Del Bigio 1989). Newer techniques, which will be discussed in the next section, will provide us with a broad spectrum of biological markers ranging from serum proteins to intracellular mediators that are involved in signal transduction and transcription (Kondziella, Qu et al. 2003).

Definition of a biomarker and applications in patients with chronic adult hydrocephalus: "*The ideal biomarker for Alzheimer's disease (AD) should detect a fundamental feature of neuropathology and be validated in neuropathologically-confirmed cases; it should have a sensitivity of 80% for detecting AD and a*

specificity of 80% for distinguishing other dementias; it should be reliable, reproducible, non-invasive, simple to perform, and inexpensive. Recommended steps to establish a biomarker include confirmation by at least two independent studies conducted by qualified investigators with the results published in peer-reviewed journals." This definition given by the Consensus Report of the Working Group on Molecular and Biochemical Markers of Alzheimer's disease (Ronald and Nancy Reagan Research Institute and the NIA Working Group-1998) adequately highlights the problems that may be faced in attempts to establish biomarkers for adult-onset communicating hydrocephalus. The role of a biomarker is to confirm a diagnosis, serve for epidemiological studies, assess for prediction, monitoring the progression and response to treatment and studying brain-behavior relationships. Any marker will need to be validated against a definite diagnosis. Traditionally the diagnosis of NPH was given only postoperatively on the basis of improvement of the patient. Recently a collaborative attempt was made to categorize patients with NPH as 'possible', 'probable' or 'definite' (Ishikawa 2004). If this categorization were to be used, then biomarkers should only be tested in probable cases to achieve high diagnostic accuracy. The establishment of control groups is another problem that needs a solution. Ideally, the spouses of hydrocephalic patients could be used as controls, as they would be well matched for age and environmental and lifestyle factors. However, medical ethics prohibit us from using invasive procedures (such as serum or CSF sampling) for research purposes in healthy individuals. Since one single biomarker might be inadequate to provide the needed diagnostic accuracy, a combination of more than one biomarker might give a solution. An example of this was the study by Lins *et al.* as analyzed in an earlier

section of this report (Lins, Wichart et al. 2004), and as has already been demonstrated in other neurodegenerative processes (Reiber 1998).

As we have seen, serum biomarkers appear to be no use at the present time because relevant studies have been so few. Serum Glycoprotein D2 was inadequately studied and therefore may not be used in the differential diagnosis with patients suffering from primary progressive dementia**.** Clearly, serum protein concentrations can be influenced by many factors other than brain and CSF composition, therefore a direct reflection of brain metabolism is likely to prove more useful. Studies of biomarkers in the CSF have been much more numerous although, as our review has shown, no biomarker has received enough attention from researchers to emerge with the needed specificity and sensitivity.

In most of the studies so far there is an observed difference in the levels of the biomarker preoperatively and postoperatively, but there are weak correlations with surgical outcomes. In other studies there are observed differences between patients and a control group, but rarely are results correlated with surgical outcomes. TNF-α emerges as a potential biomarker. Although the results were not correlated with surgical outcome, the 45-fold preoperative increase and return to normal post- shunting, shows the potential of this marker. However, only one study of 35 patients, reported results for TNf-α, and therefore the data has yet to be replicated by other research groups. Somatostatin appears to relate to changes and general impairment of cognitive functions, but bigger studies are warranted to highlight better this relationship. A significant correlation was also found between levels of tau protein in patients with NPH and the severity of dementia; again these

results need to be replicated. Sulfatide emerged as a promising biomarker showcasing a sensitivity of 74% and specificity of 94% in distinguishing between NPH and subcortical arteriosclerotic encephalopathy. Also, levels of neurofilament triplet protein >640 mg/L have been identified by Tullberg *et al.* as having a predictive value of 100% for a positive outcome after shunt surgery; the latter remain non-sensitive (17%) but highly specific (100%). Lactate appears a promising distinguishing factor between different forms of dementia and further studies correlating lactate levels with surgical outcomes could reveal its potential as a biomarker.

1.14.2.9. Technical considerations and existing limitations

There are a few problems of technical nature with using establishing and validating the use of biomarkers in this condition. These have been reviewed and acknowledged by Wood (Wood 1980). Firstly, caution should be exercised when interpreting biochemical results as for many potential markers there might be no reference values in the CSF of healthy subjects. There is also a need for age-matched reference values in the evaluation of CNS pathologies (van Engelen, Lamers et al. 1992). Wikkelsö *et al* have noted that in hydrocephalic patients, low neuropeptide values in the CSF can be caused not only by reduced release or increased degradation, but also by an altered distribution volume of the CSF (Wikkelso, Ekman et al. 1991). Indeed this effect was noted with the MBP studies, levels of which decreased after flow was restored by shunting. Furthermore increased CSF levels of certain markers might be explained when one takes in consideration the low CSF conductance (i.e. increased CSF outflow resistance) in

NPH which compromises the clearance of these metabolites resulting in accumulated levels in the CSF. Another controversial aspect of studies on concentrations of neuropeptides using lumbar instead of ventricular samples is whether the effect of the gravity may influence the concentration of the peptides in the samples (Gjerris, Werdelin et al. 1987; Gjerris, Gjerris et al. 1988).

The dynamics of markers in the CSF differ depending on whether the proteins derive directly from the nerve cells, the leptomeninges, or whether they derive from blood and therefore their concentration and dynamics depend on the integrity of the blood-brain barrier (Reiber 2001).

Ideally only ventricular samples would be used in the case of hydrocephalus, even though the spinal absorption pathways have been shown to be involved in the case of communicating hydrocephalus (Luedemann, Kondziella et al. 2002). That is because the ventricular fluid would reflect more accurately the changes occurring in the periventricular white matter. Ideally the technique of microdialysis, which has been developed to quantify regional spatial and temporal changes in brain biochemistry, would be the best tool to monitor such changes. Indeed, there are publicized attempts in applying this in patients with NPH (Agren-Wilsson, Roslin et al. 2003; Agren-Wilsson, Eklund et al. 2005). However, ethical issues and technical limitations for detecting putative markers might be the key issue in pursuing this further. It has also been demonstrated that concentrations of certain markers in the CSF might fluctuate over time, so a sample at one time point might be of limited use (Geracioti, Orth et al. 1992). The sampling during ventricular catheterization seems to influence the concentrations of certain markers as verified by two studies

(Kruse, Cesarini et al. 1991; Woertgen, Albert et al. 2004). This potential pitfall should be kept in mind in the design of relevant future studies. The differences in the pathogenesis of the idiopathic and the secondary form of chronic adult-onset hydrocephalus add more limitations for data collection from appropriate populations.

14.2.10. Future directions and possibilities of the field of CSF biomarkers

Proteomics, since its inception in 1995 (Wilkins, Sanchez et al. 1996) has showed great promise in providing a more detailed insight into the mechanisms of disease in the post-genomic era. The proteome and peptidome maps can provide us with what is called “a bird’s eye view” of the physiological and pathological products and of processes occurring at any one time. The field of clinical proteomics is especially well suited for discovery of biological markers in complex biological fluids, such as plasma, urine, serum and CSF; these in turn reflect the ongoing biological processes in healthy subjects, as well as in several neurodegenerative disorders. The related field of peptidomics is a method for analysing the vast range of the peptides that have been expressed in any cell, tissue or fluid at any given time. These two technologies have already been used successfully to provide us with an insight into the CSF proteome map (Finehout, Franck et al. 2004; Maccarrone, Milfay et al. 2004; Wenner, Lovell et al. 2004; Yuan and Desiderio 2005; Yuan and Desiderio 2005).

Changes in the protein composition of CSF may be indicative of altered CNS protein expression pattern, providing us with a link to the cause or diagnosis of a condition. Other areas of neurology have already benefited from this new area. Mass spectrometry has been used in immunodeficiency virus dementia (Berger, Avison et al. 2005), in CSF from patients with multiple sclerosis (Hammack, Fung et al. 2004), and AD (Puchades, Hansson et al. 2003), or for the identification of protein tumour markers in primary brain tumours (Zheng, Luider et al. 2003).

As with every new diagnostic technology, limitations do exist. At present there is a lack of standardization for the procedures as the techniques are quite new. A low total-protein concentration, a high concentration of albumin and immunoglobins, and a wide range of protein concentrations cause several difficulties in the identification of low-abundance CSF proteins (Yuan and Desiderio 2005). An attempt to apply proteomics has already been carried out in the Hydrocephalus Texas (H-Tx) rat inherited model of hydrocephalus (Li, Miyajima et al. 2005). A recent attempt in one single patient identified 82 proteins of which 25 have not appeared in any previously published two-dimensional electrophoresis (2DE) map of CSF, whereas eleven of them have not been previously reported to exist in CSF (Finehout, Franck et al. 2004). Two new peptides related to neuropeptide FF, a modulator of the opioid system, were detected in the CSF of one patient with NPH (Burlet-Schiltz, Mazarguil et al. 2002). The significance remains unknown at present, but these two studies demonstrate the potential to gain a novel insight into areas that hitherto have not been available.

1.14.2.11. The need for future collaboration in the field of biomarkers

There is a need for continued and collaborative collection of research populations of subjects with chronic communicating hydrocephalus who can contribute to a longitudinal bank of biologic specimens (i.e., imaging data, biological fluid and genetic samples) for identification and verification of novel biomarkers. Similar projects in neurosurgery regarding brain injury (BrainIT) have been recently attempted and are ongoing. However, since NPH is mostly a clinical diagnosis with a radiological verification, such a biomarker might not be forthcoming even by the combined efforts of a controlled multicenter trial**.** Moreover, collaboration is needed between groups, which deal with paediatric and adult-type of hydrocephalus. Although, limitations exist due to the need for data protection, the long-term gain into the insight in the continuum spectrum of this condition will be immense.

Since follow-up and knowledge of the long-term prognosis for the management of this condition is vital, it is necessary to establish common outcome scales which will allow for multiple study comparisons. As we have seen outcomes and end points vary widely between groups dealing with this condition. This effort might also include establishing a national brain bank project for future collaborative research.

Attention should be also given in understanding the epidemiology of this condition. So far, epidemiological data arises mainly from Sweden due its health system

infrastructure. This approach could also include multiple non-medical disciplines that ultimately influence the outcome of geriatric conditions.

1.15. Non-invasive biomarkers: the role of neuroimaging

In patients with normal pressure hydrocephalus (NPH) CT demonstrates ventriculomegaly with ventriculosulcal disproportion and hydrocephalus is of the communicating type. In the periventricular areas of the frontal and occipital lobes hypodense areas represent transependymal passage of CSF. The accuracy of CT in idiopathic and secondary NPH is not known due to the problematic nature of establishing a firm diagnosis in this condition. Ventriculomegaly alone is not a specific feature of NPH as it may be met in ex-vacuo dilatation secondary to cerebral atrophy, PD, MID, AD, and Binswanger's disease (Savoiardo and Grisoli 2001). MRI imaging provides additional physiologic information for NPH when compared with CT scanning by demonstrating a pulsatile flow void across the aqueduct and a hyperdynamic CSF flow on T2-weighted imaging. However, the accuracy of MRI is again not known in diagnosing NPH. Traditionally, isotope cisternography has been used to demonstrate the persistent reflux of the isotope in the ventricular system and the absence from the convexities within 48 hours. However, its use is doubted today (Benzel, Pelletier et al. 1990; Vanneste, Augustijn et al. 1992; Hebb and Cusimano 2001).

Recently, criteria for the diagnosis of NPH have been published (Marmarou, Bergsneider et al. 2005). However, the investigations outlined have an invasive nature (Marmarou, Bergsneider et al. 2005). Neuroimaging due to its non-invasive

nature has the ability and advantage of imaging both structural and functional changes of the brain allowing access to, and a better understanding of the brain disease and the metabolic consequences of hydrocephalus. In that sense it might be able to also provide useful biomarkers that might guide us in the preoperative diagnosis, act as prognostic indicators, or assist us in judging the evolution of the condition. The purpose of this review is to investigate and outline the use of neuroimaging modalities in predicting outcomes for favourable shunting, as well as identifying potential biomarkers.

1.15.1. Structural imaging features

Ventriculomegaly is an essential criterion for the diagnosis and is confirmed in most studies as an Evans index greater than 0.3. This index equates to the ratio of the maximum width of the frontal horns to the maximum width of the inner table of the skull. Other indexes have been used invariably but the Evans index is the one most commonly used having high sensitivity but low specificity for NPH (Waldemar, Schmidt et al. 1993). In an earlier study an Evans ratio less than 0.35 was found of no use in predicting outcomes (Borgesen and Gjerris 1982). Other features of NPH have been identified on imaging. Hippocampal volumes (Golomb, de Leon et al. 1994), small perihipoccampal fissures (Holodny, George et al. 1998), focal impingement of corpus callosum (Qureshi, Williams et al. 1998), distal dilation of the aqueduct (Kurihara, Simonson et al. 1995) and smaller midbrain diameter (Lee, Yong et al. 2005) have been suggested as selecting tools for this group of patients. Although hippocampal atrophy when compared to control subjects has been reported in NPH (Golomb, de Leon et al. 1994; Savolainen,

Laakso et al. 2000), this feature is met also in other conditions (Laakso, Partanen et al. 1996) and therefore its specificity for NPH is limited. The positive predictive value of the size of perihippocampal fissures to distinguish NPH from AD is 86%; however the size of the perihippocampal fissures was assessed subjectively in that study (Holodny, Waxman et al. 1998). Convexity gyral atrophy has a low sensitivity and specificity for predicting surgical outcomes (Benzel, Pelletier et al. 1990). Relating to the previous finding with small cortical sulci having high specificity in predicting surgical outcomes (Borgesen and Gjerris 1982). The absence of sylvian fissure enlargement was another studied feature that has a very low specificity (Benzel, Pelletier et al. 1990). Although the midbrain diameter correlates with gait impairment this features did not correlate with clinical outcomes (Mocco, Tomey et al. 2006).

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Figure 1.15.1.1. Measurement of Evans index (a/b) as a marker of ventriculomegaly from an axial CT brain

1.15.1.1. Volumetric studies

Volumetric studies assessing the distribution of CSF among different intraaxial compartments and the volume of different cerebral components in this cohort of patients might act as a predictor for outcome. A reduction of ventricular volumes may not be apparent to routine interpretation of scans so detailed volumetric studies might be needed for accurate measurements (Anderson, Grant et al. 2002). Ventricular enlargement could predict outcomes with a sensitivity of 82%,

but a smaller specificity (50%) in an early study (Benzel, Pelletier et al. 1990). The ventricular volume was greater than controls, whereas the volumes of brain, grey and white matter components and subarachnoid space were similar to controls (Matsumae, Kikinis et al. 1996). In addition to ventricular volumes, enlarged intracranial CSF volumes when compared to control subjects have been reported (Tsunoda, Mitsuoka et al. 2001; Bradley, Safar et al. 2004). That was confirmed in another study which also used the ventricular/intracranial CSF ratio as a strong suggestion for the diagnosis of NPH (Yoshihara, Tsunoda et al. 1998). The sylvian fissure and basal cisterns' CSF volume were also found larger than patients with AD (Kitagaki, Mori et al. 1998). However, volumetric studies do not seem useful as a means of predicting outcome. Responders and non-responders to shunt surgery (n=26) in a 1-year follow-up did not have any difference in the mean ventricular volume ratio, mean brain volume ratio, mean pericerebral CSF volume ratio, and the mean ratio between ventricular and pericerebral CSF volume (Palm, Walchenbach et al. 2006). Favourable outcomes following shunting in patients with NPH (n=80) did not correlate with decreased ventricular volume 1 year after surgery. In fact, better clinical outcomes were observed in patients with little or no alteration in ventricular size (Meier and Mutze 2004). Holodny and colleagues observed a paradoxical decrease in the size of the dilated fissures and sulci of 5 patients that paralleled the decrease in the size of the lateral ventricles following successful shunting suggesting that the focal fissural and sulcal dilation may represent reservoirs of cerebrospinal fluid analogous to the ventricular system. They concluded that patients should not be denied a shunting procedure solely on the basis of focally dilated fissures of sulci (Holodny, George et al. 1998).

1.15.1.2. Periventricular lucencies

Caution must be exercised to separate periventricular, diffuse, smooth hyperintensities with extension limited to the corona radiata and centrum semiovale attributable to microvascular disease from irregular intensities around the frontal and occipital horns often associated with NPH (Yamada, Fukuda et al. 1978). The pathophysiology and the significance of periventricular lucencies in hydrocephalus are not at all understood and they might represent either oedema or gliosis (James, Flor et al. 1980). The periventricular lucencies seen on MRI prior to shunting represent increased water content in the extracellular space (Aygok, Marmarou et al. 2006), and may be assessed by the use of the T1 and T2 relaxation times. In NPH relaxation times of white matter are longer both in T1 and T2 when compared with controls, and when compared with relaxation times of grey matter (Tamaki, Nagashima et al. 1990). Patients with these lucencies exhibited greater improvement post shunting (Borgesen and Gjerris 1982; Thomsen, Borgesen et al. 1986; Poca, Mataro et al. 2004). This might be explained by the fact that the presence of the lucencies on a patient might indicate that the hydrocephalus is still not fully “compensated” (Bradley 2001) and therefore the pathologic changes are reversible if CSF dynamics are restored. However, their sensitivity and specificity in predicting surgical outcomes remains low (Benzel, Pelletier et al. 1990).

1.15.1.3. Deep white matter lesions

Since an element of chronic hypoperfusion contributes to the development of NPH this is reflected in imaging (Bradley, Whittemore et al. 1991). It is widely accepted that deep white-matter lesions (DWMLs) most frequently are secondary to chronic ischemia caused by hypertensive arteriolosclerosis (Fernando, Simpson et al. 2006). NPH is known to be associated with diffuse white matter damage, even in normal-appearing cerebral white matter (Hahnel, Freund et al. 2000). Elderly patients with idiopathic NPH have more frequent and more severe periventricular and deep white matter lucencies than people in age-matched control groups (Bradley, Whittemore et al. 1991; Krauss, Regel et al. 1997). The lack of white matter signal and more severe periventricular signal on T2W imaging were shown to act as predictors of good response to shunt surgery (Jack, Mokri et al. 1987). The extension of periventricular and deep white matter lesions was found to be inversely correlated with the degree of clinical improvement in 41 patients (Krauss, Droste et al. 1996). However, conventional MRI might not be the best imaging modality to highlight these white matter changes (Hahnel, Freund et al. 2000), and distinguish reliably between patients with NPH and subcortical vascular encephalopathy (Tullberg, Hultin et al. 2002). Since the presence of deep white matter hyperintensities or subcortical lacunar infarctions could not predict poor postoperative outcomes and should not exclude patients from having a surgery the role of MR as a selection tool to exclude patients from having surgery is limited (Tullberg, Jensen et al. 2001).

1.15.1.4. Phase contrast MR studies

MRI studies may provide us with information about the CSF flow void phenomenon across the aqueduct. The degree of the flow void reflects the increased velocity of the pulsatile CSF motion in patients with NPH, which in turn depends on the relative ventricular compliance and surface area (Bradley, Kortman et al. 1986). In addition phase-contrast MR studies can be used to study quantitavely the hyperdynamic flow along the aqueduct of Sylvius, thus calculating the stroke volume or the CSF flow velocity.

In patients with NPH the flow void is usually increased (Bradley, Whittemore et al. 1991). Significantly higher and lower values of the (mean) maximum aqueductal signal intensity were found in the NPH and the ex-vacuo groups respectively, when compared with a control group (Mascalchi, Ciraolo et al. 1990). A significant correlation ($p<0.003$) was initially found between good or excellent response to shunting and an increased CSF flow void in a 5-year follow up (Bradley, Whittemore et al. 1991). However, the same group later disputed its use as a marker of good outcome (Bradley, Scalzo et al. 1996).

The first studies using CSF flow/motion patterns and MRI for the syndrome of NPH took place in 1987 (Jolesz, Patz et al. 1987). Patients with NPH (n=7) showed increased systolic flow rates when compared with patients with ex-vacuo hydrocephalus. In another smaller study (n=18) a stroke volume greater than 42 microL was associated with favorable outcome following shunting (Bradley, Scalzo et al. 1996). It is important to note here that such stroke volume values vary

according to the technique and the scanner used; therefore they cannot be of absolute value unless compared to age-matched controls.

Abnormal CSF flux (as defined by being outside the 2 standard deviations from the mean difference between the maximal rostral and caudal flux) in phase-contrast cine MR in 8 patients was proven to have an accuracy of 88% in predicting outcomes (Egeler-Peerdeman, Barkhof et al. 1998). Luetme et al. concluded that CSF flow greater than 18 ml/min suggests idiopathic NPH and may distinguish from other dementias (Luetmer, Huston et al. 2002). High CSF velocity through the aqueduct was identified as a good predictor of improvement after surgery with a sensitivity of 90% and specificity of 50% (Poca, Sahuquillo et al. 2002). The maximum CSF flow velocity using phase-contrast cine MR in the aqueduct was found to be significantly larger in a group suffering from secondary NPH (9.21 +/- 4.12 cm/sec) when compared to controls (5.27 +/- 1.77, $p < 0.001$) and a group of patients with cortical atrophy (4.06 +/- 1.81, $p < 0.005$) (Mase, Yamada et al. 1998).

However, later studies have not verified the above promising results. No difference was found between the occurrence of aqueductal CSF flow void in 37 patients with idiopathic NPH and an equal number of controls (Krauss, Regel et al. 1997). These negative results were verified by other studies (Parkkola, Komu et al. 2000; Dixon, Friedman et al. 2002). Aqueduct stroke volumes did not differ between patients with NPH (n=16), Alzheimer's dementia and vascular dementia (Bateman, Levi et al. 2005). Dixon and colleagues suggest that MR measurements of aqueductal CSF flow might be useful only if the diagnosis of NPH is ambiguous or

for patients in whom the diagnosis of NPH is suspected but surgery is high risk because of medical co-morbidities (Parkkola, Komu et al. 2000; Dixon, Friedman et al. 2002). However, Bradley suggested that negative results may be explained by the choice of imaging techniques that decrease the sensitivity of the aqueductal flow void sign (Bradley 2001).

1.15.2. Functional imaging features

The role of cerebral blood flow measurements has been systematically reviewed by Owler and Pickard (Owler and Pickard 2001), however it has been noted that none of the measures have succeeded in a better diagnosis and prognosis of NPH, impact due to centre-specific differences in methodology and technique application. It has been shown that areas of frontal lobe, parietal (Larsson, Bergh et al. 1994), thalamic and hippocampus (Tullberg, Hellstrom et al. 2004) are particularly affected. Similar changes in cerebral blood flow and cerebral oxygen utilization have been shown previously both in patients with dementia and chronic hydrocephalus (Grubb, Raichle et al. 1977; Tanaka, Kimura et al. 1997). The role of CBF as a selection tool for shunting has been suggested by Mathew et al. (Mathew, Meyer et al. 1975) and Moretti using tomoscintigraphy (Moretti, Sergent et al. 1988). Ultrasonographic studies using transcranial Doppler (Fritz, Kalbarczyk et al. 1989; Krauss and Droste 1994; Bakker, Boon et al. 2002) have also been used in the past to highlight the role of chronic ischemia in the pathophysiology of chronic adult hydrocephalus. Below, we will investigate in more detail different imaging methods and their role in predicting surgical outcomes.

1.15.2.1. Xenon-enhanced Computed Tomography

Early studies measuring the regional CBF using the method of 133Xe clearance method identified diffusely decreased cerebral blood flow in the frontal area (Baba, Takeyama et al. 1978), whereas increase CBF in the frontal, temporal and basal ganglia areas post shunting correlated with good post surgical outcome (Tamaki, Kusunoki et al. 1984; Meyer, Kitagawa et al. 1985). The method has been also used to measure the difference in CBF following a diagnostic lumbar tap in patients with NPH (Mamo, Meric et al. 1987). Tanaka and colleagues studying 21 patients with idiopathic and secondary NPH concluded that a criterion for postoperative improvement is preoperative hemispheric cerebral blood flow greater than 20 ml/100 g per minute and an impaired vascular response to acetazolamide only in the periventricular white matter (Tanaka, Kimura et al. 1997). When glycerol was administered in 22 patients with secondary NPH, preoperative and postoperative regional cerebral blood flow (rCBF) increased by more than 20% in responders to surgery (Shimoda, Oda et al. 1994). Glycerol acts by increasing cerebral perfusion pressure and improving microcirculation. In this last study no cut-off value for predicting improvement was mentioned. Negative results regarding the use of Xe-CT for predicting outcomes when CBF is measured before and after a diagnostic lumbar puncture have been reported (Kushner, Younkin et al. 1984). Vorstrup and colleagues have found that improvement in CBF correlate strongly with a reduction of ventricular size as measured by Evans ratio (Vorstrup, Christensen et al. 1987).

1.15.2.2. Positron Emission Tomography

The first PET studies were carried out in 1985 and used as a differentiation method between AD and NPH groups. Both groups showed lower cortical rates of [18F] fluorodeoxyglucose (FDG) utilisation than controls. However, AD subjects demonstrated bilateral temporoparietal hypometabolism while the NPH group showed globally diminished glucose use (Jagust, Friedland et al. 1985). Studies by Owler and colleagues have identified the basal ganglia and thalamus as additional areas of hypoperfusion (Owler, Pena et al. 2004). In another study the same group revealed that the cerebral flow is reduced more in the paraventricular region getting progressively normalised towards the subcortical white matter area (Momjian, Owler et al. 2004). Klinge and colleagues using PET estimated the cerebrovascular reserve and global CBF while correlating the results with surgical outcomes. They found that the responder to shunting group (n=31) had lower blood flow values when compared to the non-responder group (n=29) (Klinge, Berding et al. 2002). Following shunting no significant changes in global CBF were observed in both outcome groups. The same group when evaluated 11 patients with 15-O-water PET one-year after surgery found that the responder group had reduced CBF in the frontobasal cortex (Klinge, Berding et al. 2002).

1.15.2.3. Single-photon-emission-computed tomography

The measurement of the regional cerebral blood flow by means of the dynamic single-photon-emission-computed tomography (SPECT) is a method of examination that provides quantitative information regarding the distribution of the

effective cerebral perfusion. SPECT (either alone or in combination with perfusion-weighted MRI) has been used preoperatively as a research tool to assess cortical perfusion before and after a lumbar tap test (Kristensen, Malm et al. 1996; Hertel, Walter et al. 2003). Increase in cerebral perfusion (as assessed by perfusion weighted MRI) when combined with clinical examination helped to predict good outcomes in six out of seven shunted patients (Walter, Hertel et al. 2005). Another study assessing pre and postoperative blood flow changes using SPECT verified CBF improvement in the less disabled NPH group of patients (Piechnik and Hultin 2005). A study of 14 patients identified enlarged subcortical low-flow region, asymmetry of regional CBF (rCBF) in the central white matter, the inferior and mid-temporal cortex when compared to age-matched controls, whilst global CBF was normal (Waldemar, Schmidt et al. 1993). In another study with 23 patients decreased rCBF was identified in the hippocampal regions and in the frontal and parietal white matter as compared to controls, which increased post shunting (Larsson, Bergh et al. 1994). Mataro and colleagues expanded on the specific areas of the frontal and parietal lobes of blood flow improvement post-shunting by using SPECT in 15 patients (Mataro, Poca et al. 2003). Secondary NPH has been also shown to have reduced frontal lobe blood flow when compared with idiopathic cases (Kamiya, Yamashita et al. 1991).

A Dementia Alzheimer's type (DAT) pattern characterised by decreased flow in posterior temporal and parietal areas predicted outcomes with high accuracy in 14 patients with idiopathic NPH (Granado, Diaz et al. 1991). A ratio of 1.05 in the anterior/posterior brain regions acted as a marker for 14 out of 16 clinically improved patients, with the improved group having values below that ratio (Graff-

Radford, Rezai et al. 1987). These results were confirmed in a follow-up study by the same group providing similar accuracy figures (Graff-Radford, Godersky et al. 1989). Enlarged subcortical low flow areas predicted outcome in 10 out of 11 patients (Waldemar, Schmidt et al. 1993). Another study of 22 patients with NPH showed that an increase of more than 80% in CBF after CSF removal was predictive of response to shunt surgery with 77% accuracy (Mori, Maeda et al. 2002). Finally, Chang and colleagues quoted a cut off value for CBF of 35 ml/100 g/ min predicting a favourable outcome (Chang, Kuwana et al. 1999).

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Figure 1.15.2.4.1. An example of perfusion-MRI in patients with NPH. (Walter, Hertel et al. 2005)

1.15.2.4. Diffusion-weighted imaging

Diffusion-weighted imaging (DWI) has been used to demonstrate a lengthened apparent diffusion coefficient (ADC), a measure of the restriction to diffusion of

water in tissues, in periventricular white matter in NPH when compared with age-matched controls (Gideon et al., 1994). This was thought to reflect an increase in intracellular water, demyelination, or myelin-associated water. In a study of 11 patients the poor outcome group had a significantly higher ADC (0.99 versus 1.67 respectively, $p<0.05$) in the periventricular region, whereas the ADC in the good outcome group was similar to that of the controls (Corkill, Garnett et al. 2003). The authors suggested that DWI may act as a selection tool for patients most likely to benefit from surgery.

1.15.2.5. MR spectroscopy

Proton MR Spectroscopy (MRS) may be used to evaluate cerebral metabolism in the clinical setting of hydrocephalus in a longitudinal non-invasive way. Proton Nuclear magnetic resonance (NMR) spectroscopic studies have contributed evidence for the importance of early surgery to relieve the damaging effects of hydrocephalus (Harris, Plant et al. 1996; Harris, Plant et al. 1997). Increased intraventricular lactate concentrations (lactate/ creatinine ratio 0.23) have been found to differentiate successfully between patients with NPH and other types of dementia (Pick's and Alzheimer's disease) (Kizu, Yamada et al. 2001). Significant higher lactate values in the CSF of patients with NPH when compared with a vascular dementia group ($p<0.01$) and patients with Alzheimer's dementia ($p=0.0005$) has also been found by another group supporting the above spectroscopic results (Nooijen, Schoonderwaldt et al. 1997). Another study did not show any metabolic abnormalities, except a definite lactate peak in intraventricular CSF (Braun, Gooskens et al. 2003). This was not attributed to anaerobic

metabolism. The drawback of this last study was that the population was consisted of a mixed population (24 children and adults with a mean age of 28.5 years and various hydrocephalic aetiologies). Shiino and colleagues examined the N-acetyl aspartate (NAA)/ creatinine (Cr) and NAA/ choline (Cho) ratios in the periventricular tissue using MR spectroscopy and correlated those with postoperative outcomes. They found that NAA/Cho ratio had a predictive value of 89.5% for one year outcome as measured by the modified Rankin scale. Patients with an excellent outcome showed a tendency towards higher preoperative NAA/Cr and NAA/Cho ratios in white matter (Shiino, Nishida et al. 2004). NAA concentrations in white matter reflect axonal loss and low values reflect irreversible axonal injury. When Phosphorus^{31} (31P) MR Spectroscopy was used regions of decreased total P31 metabolite signals were observed in two patients with NPH, accompanied by alkalosis in the periventricular area when compared to controls. However, this remains a preliminary study and the role of 31P MRS in adult hydrocephalus is awaited to be further evaluated (Braun, Vandertop et al. 2000).

1.15.2.6. The role of imaging markers in predicting surgical outcomes

Neuroimaging is more attractive as a selection tool for predicting outcomes in this group of patients due to its non-invasive nature. Although for the preoperative diagnosis of NPH imaging is as important as the clinical triad (Hakim and Black 1998), it is currently only an ancillary tool for selection of patients for shunting. As we saw both structural and functional imaging has done little to provide biomarkers fulfilling the definition given above. This is not a reflection of the poor quality of studies, but rather of the problematic nature of establishing a diagnosis for this

condition. In fact, in the past a diagnosis was confirmed only if the patient responded clinically to ventriculoperitoneal shunting, raising the question about the diagnosis of the patients who did not respond. Also many studies are designed as observational studies rather than providing a prediction of favourable surgical outcomes.

The initial search performed revealed 226 articles. However, only 69 studies were relevant to adult hydrocephalus and reviewed further for the purpose of this review. A total of 1581 patients were imaged with 437 patients belonging to the idiopathic form (28% of the total number of patients). 53 studies were excluded. Even though in some of these studies outcomes are provided the authors do not provide analytical data, or do not provide a cut-off value of the marker in order for sensitivity and specificity figures with regards to prognostic value to be calculated. In the excluded group the mean number of patients studied is 22 (total of 1179). Fifteen studies (28%) have imaged the idiopathic form exclusively and 27 studies (51%) use a control group, whereas nineteen studies (36%) use a group with comparative pathology as a control sample.

Sixteen of the studies fulfill the inclusion criteria mentioned in the search strategy section. The mean number of patients of the above sixteen studies is 25 (total of 402 patients). Only three of them (19%) study the idiopathic form of NPH, whereas in 6 of them it is not clear whether the idiopathic or secondary form is studied. Only four studies (25%) use a control group (patients with no pathology), and only one of them uses a control group with a similar pathology (Alzheimer's dementia).

Dynamic and functional rather than structural studies prove more useful. Although structural imaging and volumetric studies have proposed biomarkers of NPH against related neurodegenerative conditions as we saw earlier, their role is rather limited in predicting surgical outcomes. That is a reflection of the nature of the condition and the way the CSF dynamics and regional cerebral blood flow get altered by the intervention procedure (insertion of a ventriculoperitoneal shunt). It is therefore more likely that studies reflecting the metabolic state or quantifying the cerebral blood flow might prove more fruitful in selecting appropriate patients for shunting. The study by Granado and colleagues using a preoperative SPECT scan concluded that a DAT pattern of reduced flow offered high accuracy in predicting six-month outcomes in 14 patients. These last results can be easily explained as a common pathophysiological background has been suggested for both AD and NPH (Silverberg, Mayo et al. 2003; Silverberg 2004). The outcomes were provided using a *GOOD* or *BAD* classification and therefore suffer from lack of standardization with regards to outcome scores. Also their outcomes assessed only the neuropsychological aspect of the clinical triad. Both these studies ought to be replicated by other groups in order to establish useful biomarkers. However, they offer a solid basis and hypothesis from where current studies may be designed trying to validate these results. Bradley's work using the presence of a flow-void as a predictive marker does indeed fulfil the statistical criteria set above. Again no validated outcome scale was used, but the surgical outcomes were stratified as excellent, fair, good or poor. This work's results although very promising due to reflecting the CSF dynamics of the syndrome were negated by a later paper from the same group (Bradley, Scalzo et al. 1996). Since then no other group has produced similar results. The study by Shiino et al. using MR

spectroscopy satisfies the statistical criteria set up previously. However, the results referred to patients with the secondary form of normal pressure hydrocephalus, and therefore might not apply to the idiopathic form. The results point out that reversible axonal loss correlates with good surgical outcome.

There is only Level B evidence in using SPECT in patients with iNPH to assist in the selection for shunting; this method provides high sensitivity, specificity and has an accuracy of 92.8%. The DAT pattern on SPECT in patients with NPH can be predictive of an unfavourable outcome in a 6-months follow-up. There is Level A evidence of MR Spectroscopy to predict surgical outcomes; however the latter study was used in patients with the secondary form of NPH. There is also Level B evidence of using MRI to quantify the CSF flow void in patients with NPH. This latter study suffers from the non-description of patient characteristics, however it provides a long-follow up (mean of 60 months). The results of all these 3 studies have not been replicated by another group.

A problem with choosing a single diagnostic modality to predict outcomes is the heterogeneity in the background pathology of each patient with NPH (Tedeschi, Hasselbalch et al. 1995). Indeed, the pathology of patients with NPH might overlap with other neurodegenerative diseases (Bradley, Whittemore et al. 1991; Krauss, Regel et al. 1997; Savolainen, Paljarvi et al. 1999). Some plausible solutions to override this problem would be to combine neuroimaging biomarkers with biomarkers obtained from CSF analysis or neuropsychological evaluation of these patients. Another solution would be to combine markers of reduced regional CBF with markers of CSF flow. The combination of two markers reflecting the

pathophysiology of this condition will increase the predictive value for selecting patients who are to be benefited by ventriculoperitoneal shunting. The latter practice of course tends to increase the likelihood of a type I error and therefore such studies should be designed with caution.

Imaging may of course provide only surrogate end points, and not true clinical end points as in NPH this means a definitive clinical improvement in long-term follow-up. In NPH this is not always possible and has not been always achieved mainly due to the influence of comorbidity in this cohort of patients. A search in the neurological section of the website of the Massachusetts General Hospital Center for Biomarkers in Imaging (www.biomarkers.org) did not reveal any current attempt in establishing a biomarker for NPH, even though disease such as Alzheimer's dementia has 9 ongoing studies. It is hoped that the current review will act as a platform helping clinicians and researchers alike to identify the problems associated with imaging patients with this syndrome and helping in designing prospective trials.

In clinical practice it seems that at present no single imaging modality may assist clinicians to select patients for shunting. Therefore invasive studies will remain the mainstay of selecting appropriate patients for the CSF diversion procedures for the near future. At present imaging may be only used to verify the extent of ventriculomegaly and exclude cases of gross cerebral atrophy and other pathologies that might explain the symptomatology of the syndrome. However neurosurgeons, neurologists and neuroradiologists ought to collaborate closely in the near future in order to design studies that will aim to discover biomarkers for

favourable surgical outcomes. This also means multicentre prospective studies that should image all patients that are referred to neurosurgeons for the invasive diagnostic tests irrelevant of the result of these tests. Brain SPECT, MR spectroscopy and study of the flow void in patients with NPH have provided Level A and B evidence of being able to predict surgical outcomes and ought to be replicated by other groups. It is of outmost importance that a validated outcome scale is used in all studies with NPH for comparability purposed. The current work should act as a platform to design further studies with larger sample sizes. It is hoped that patients in the future might be able to avoid invasive diagnostic tests if neuroimaging proves its diagnostic accuracy against the current “gold” prognostic standards.

1.16. Overall thesis aims:

The aims of this thesis were:

1) To examine the feasibility of identifying potential biomarkers in lumbar and ventricular CSF of patients with idiopathic normal pressure hydrocephalus by using the ELISA method,

2) To correlate the levels of those biomarkers with imaging and neuropsychological data and postulate of pathophysiological mechanisms and,

3) To correlate the levels of those markers with clinical outcomes in order to identify potential prognostic biomarkers in patients with idiopathic normal pressure hydrocephalus.

Chapter 2 Methods

Patient recruitment and study protocol

The period of the study was February 2005- February 2007. The study was carried out within the National Hospital for Neurology and Neurosurgery (part of the UCLH NHS Foundation Trust).

Consultant Neurologists of the Greater London area referred the patients to our department for clinical evaluation and consideration of surgical CSF diversion. Most patients had already an appropriate physical examination and relevant imaging (i.e. CT/MRI) by the referring team, which would be consistent with the diagnosis of NPH or communicating hydrocephalus.

The inclusion criteria for the subjects were:

a) Gait unsteadiness as well as one of the two symptoms that constitute the classic triad of NPH (i.e. psychomotor retardation, and incontinence of urine), associated with appropriate imaging features of NPH or communicating hydrocephalus on CT/MRI and demonstration of normal CSF pressure (5-18 mm Hg). The eligible patients had the option of proceeding directly to surgery if they so wished, or undergo further testing for suitability of shunt (insertion of ELD). The patients would finally be offered a shunt if they improved in either the walking test or the neuropsychological assessment following ELD. If the patient did not improve in any of the tests they would still have the option to proceed to surgery following a consultation with the patient and family in the OPD.

Exclusion criteria for the subjects were:

A) Medical conditions that would deem the patient unfit for a general anaesthetic.

B) Failure to improve following ELD in either the walking test or neuropsychological assessment.

Recruitment or choosing participants

Informed consent was be taken by the Consultant Neurosurgeon after any further questions that the patient might has have been answered and clarified. Due to some patients with NPH suffering from dementia, the spouse, or a relative of the patient was expected to be present during the informed consent.

Data

The data for the study was collected prospectively by the Research Fellow and entered into a database in a computer. This computer was placed in the Research Office of the NHNN, and is a property of the UCLH Foundation NHS Trust.

The data was collected from carefully reviewing patient's notes and by reviewing imaging films acquired for the purpose of the study.

The time points for collection of the data was before treatment (VP shunt or ELD insertion) and after treatment (if ELD was inserted), and just after the two follow-up appointments (first at 6 weeks, and second at 6 months postoperatively).

For the purposes of data collection and handling Microsoft's Access and Excel programs were used. For the purpose of analysing the data for statistical calculations the latest version of the SPSS package (SPSS Inc, Chicago, Illinois Version 16.0) was used. The SPSS package was used for the production of graphs.

The data was entered in a database and held securely with the help of a password. This password was known to the Primary Investigator and Research Fellow only, or to properly authorised individuals.

The data was pseudo-anonymised for the protection of the subject's anonymity. That means that each subject was given a unique identifiable number specific for this study, which will allow us to track.

Outcome assessment

The surgical outcome was assessed by means of the Black grading scale (Black 1980) at 6 weeks and 6 months. In this scale the patients are categorised depending on the level of activity from excellent to poor in 6 subscales.

Statistical considerations

A Univariate analysis (one factor vs. outcome) was used for the analysis of the continuous factors and used correlation or regression to report this value. For the

categorical factors analysis of variance was used. A multiple regression model with all the factors included was used finally.

Non-parametric tests were used for calculation of all results. The independent samples t-test was used to compare means. The Spearman rank order test was used to assess bivariate correlations. ROC (receiver operating characteristic) curves were used to calculate the sensitivity and specificity of each market in predicting surgical outcomes. The significance level for all tests was set at 0.05. Discriminant functional analysis was used to determine if the combination of any two markers would discriminate successfully between favourable and unfavourable outcomes.

Compliance

Patients could withdraw from the study at any time without giving further explanation. If there were any subjects who would not comply with the follow-up and we did not have notification of their willing withdrawal form the study, we would write initially to the subject's General Practitioner (GP) and enquire on whether there is an awareness of particular problems that prohibited the subject of attending the follow-up. If we did not receive a satisfactory answer form the subject's GP we may have had to contact a telephone interview with the non-compliant subject.

Ethical considerations

The research programme received an approval from the Great Ormond Street Hospital Local Research Ethics Committee (G.S.O.H. L.R.E.C.) on 22nd February 2005 (**REC reference number: 05/Q0508/3**). Furthermore, we received ethical approval from G.S.O.H. L.R.E.C. on 27th July 2006 (**REC reference number: 06/Q0505/59**) with regard to collecting the CSF samples from patients suffering from trigeminal neuralgia.

Finance and Insurance

As this was NHS-sponsored research compensation for harm arising from negligence remained the responsibility of the UCLH Foundation NHS Trust, this being the employer of the Principal Investigator. The Principal Investigator (L. Watkins) had a suitable contract with the Trust that ensures indemnity issues that might arise are covered. This is according to the Health Service Guidelines document, namely HSG (96)48, published on 8 November 1996.

A participant suffering injury as a result of having taken part in research would need to pursue a claim for negligence through litigation, or may be offered an ex gratia payment by the Trust. Each case will be considered on its merits.

As this was an NHS-sponsored research there was no provision to offer advance compensation for non-negligent harm to participants. However, the Trust (UCLH Foundation NHS Trust) is aware of the possibility of a compensation claim should a problem occurs.

Patient recruitment and treatment flowchart

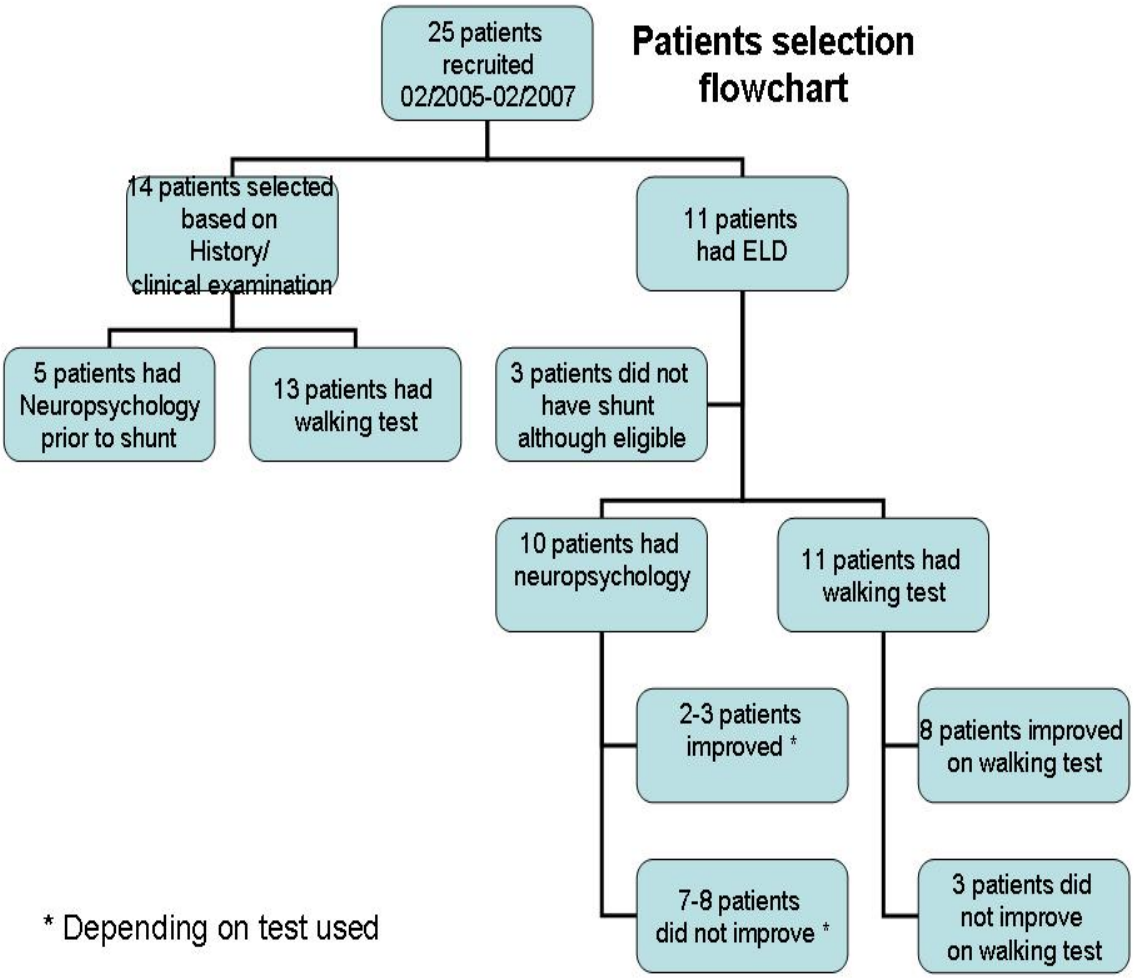


Figure 2.1 Patient selection flowchart

2.1 Cerebrospinal fluid analysis

E.L.I.S.A. (Enzyme Linked Immuno Sorbent Assay) is an immunoassay technique utilizing an antibody labelled with an enzyme marker such as horseradish peroxidase. While either the enzyme or the antibody is bound to an immunosorbent substrate, they both retain their biologic activity; the change in enzyme activity as a result of the enzyme-antibody-antigen reaction is proportional to the concentration of the antigen and can be measured spectrophotometrically or with the naked eye. Many variations of the method have been developed. The advantages of E.L.I.S.A are: 1) its simplicity, 2) rapidity, 3) sensitivity, 4) the possibility of using commercially available reagents or kits, 5) its relatively low cost. Three main methods form the basis of all ELISA's, namely: 1) The direct ELISA, 2) Indirect ELISA and 3) sandwich ELISA. Apart from the calculation of the albumin and lactate ELISA was used for the other 6 markers.

All CSF samples were collected prospectively in polypropylene tubes, centrifuged, and stored at -80^{C} within 2 hours of sampling in 1.5–2-ml Eppendorf tubes until analysis. All tubes were coded, and the CSF was analyzed with the analyst (A.T.) blinded to all other information. The results were independently double checked for accuracy by an author (A.P., G.K. or M.D.C.) who were masked to all other information. Results based on duplicates with a coefficient of variation (CV) more than10% were rejected and repeated unless the manufacturer's instructions suggested otherwise. In order to ensure that the analysts remained masked, the CSF data was entered into a database. Only after this step were the other clinical and paraclinical data linked to each individual sample.

2.1.1. Albumin

2.1.1.1. Description of laboratory method

The method of immunoturbidimetry was used to calculate the CSF albumin concentration. Each of the 96-well plates contained a blank well, a standard well, and a quality control well in addition to the samples tested. We used an antiserum solution (goat anti-human) albumin, and a calibrant solution (human albumin standard 1mg/mL). The reagent was prepared as follows: First a solution of 3% weight/volume PEG60000 in saline was made. Then to each 4 mLs polypropylene tube we added 250 μL of anti-albumin serum mixing well. For each well first 250 μL of reagent were added and then 10 μL of either blank, standard, quality control or tested sample were added. The plate was allowed to stand for 10 minutes and then the optical densities were read at 405 nm.

2.1.1.2. Calculation of results

The tested sample's albumin concentration is derived by the formula Albumin= (Od_{test}-Od_{blank}/ $Od_{standard}$-Od_{blank}) X 1.0 g/L, where OD stands for optical density.

2.1.2. Lactate

CSF lactate was analyzed enzymatically on an YSI 2700 STAT PLUS analyzer (YSI, USA) according to manufacturer's instructions.

2.1.3. Vascular Endothelial Growth Factor (V.E.G.F.)

2.1.3.1. Description of laboratory method

For our experiments we used the Amersham Biotrak Vascular Endothelial Growth Factor [(h) VEGF], Human ELISA system (GE Healthcare, UK Limited). This ELISA was designed for the measurement of natural and recombinant human VEGF165. The isoform VEGF121 cross reacts 100% in the assay. The sensitivity of this assay or lower limit of detection was <8.0 pg/ml. That was the smallest value that was not zero with 95% confidence.

The assay range was 31.3-2000 pg/ml. The standard curve points were 0, 31.3, 62.5, 125. 250, 500, 1000 and 2000 pg/ml.

The steps of the procedure will be summarised below:

1. We added 50 μL of sample diluent to each well.

2. We then added 50 μL of the standard and consecutive CSF samples in duplicate. The plate was covered and was incubated at room temperature (20-25 ^{0}C) for 2 hours. We then decanted and washed the plate 3 times.

3. 100 μL of biotinylated antibody reagent was added to each well.

4. The plate was covered and incubated at room temperature for 1 more hour. At the end the plate was decanted and washed 3 more times.

5. We then added 100 μL of Streptavdin-HRP horseradish peroxidase) reagent to each of the 96 wells.

6. The plate was covered again and incubated once again for 30 minutes. At the end the content was decanted and washed 3 times.

7. 100 μL of premixed Tetramethylbenzidine (TMB) substrate solution reagent was added to each well.

8. The plate was incubated at room temperature again for 30 minutes in the dark. After this final step the optical density was calculated at 450 nm straight away within 30 minutes after stopping the reaction.

2.1.3.2. Calculation of results

The standard curve was used to determine the amount of (h) VEGF. The standard curve was generated by plotting the average absorbance (450-550 nm) obtained for each of the standard concentrations on the vertical (X) axis versus the corresponding (h) VEGF concentration on the horizontal (Y) axis. Readings at 550nm were subtracted from readings obtained at 450 nm. Readings at dual wavelengths corrected for optical imperfections in the microplate.

The aim according to the manufacturer's advice was that all optical density values obtained for duplicates should be within 10% of the mean. Duplicate values that differed from the mean by greater than 10% were considered inaccurate and have been repeated. One of the standard curves obtained is shown below:

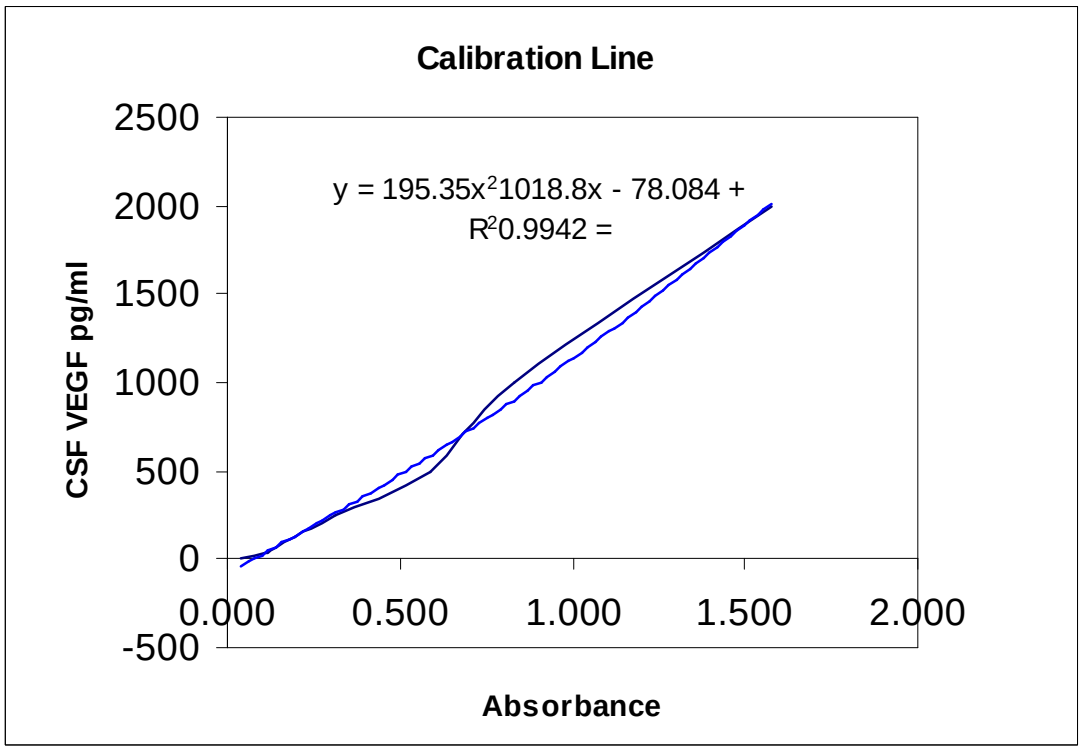


Figure 2.1.3.2.1. Standard curve for VEGF generated by plotting the average absorbance (450-550 nm) obtained for each of the standard concentrations on the vertical (X) axis versus the corresponding (h) VEGF concentration on the horizontal (Y) axis.

2.1.4. 8-Isoprostane

2.1.4.1. Description of laboratory method

The commercial kit 8-Isoprostane EIA Kit (Cayman Chemical Company, Ann Arbor, MI, US) was used for the purpose of our experiments. The assay is based on the competition between 8-isoprostane and an 8-isoprostane – acetylcholinesterase (AchE) conjugate (8-isoprostane tracer) for a limited number 8-isoprostane-specific rabbit antiserum binding sites. The amount of 8-isoprostane tracer that is able to bind to the rabbit antiserum will be inversely proportional to the concentration of 8-isoprostane in the well. This rabbit antiserum-8-isoprostane (either free or tracer) complex binds to the rabbit IgG mouse monoclonal antibody

that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's reagent (which contains the substrate to AchE) is added to the well. The product of the enzymatic reaction has a distinct yellow colour and absorbs strongly at 412 nm. The intensity of the colour, determined spectrophotometrically, is proportional to the amount of 8-isoprostane tracer bound to the well, which is inversely proportional to the amount of free 8-isoporostane present in the well during the incubation. Each of the 96 well plates contained 2 blanks (Blk), two non-specific binding wells (NSB), two maximum binding wells (B_0), and an eight point standard curve in duplicate.

The steps of the procedure will be summarised below:

1 The EIA buffer was prepared (used Vial #4 & 90 mLs H20).

2 The wash solution was prepared (Used Vial #5 & 2 Lt H20 & 1 mL Tween 20 (vial #5a)).

3 The 8-isoprostane standard was prepared and 50 µL was added per well. 50 µL of sample was added per well in duplicate.

4 The tracer solution was prepared (Use vial #2 and 6 mL EIA buffer). 50 µL were added to each well except the Total Activity (TA) and the Blank (Blk) wells.

5 The anti-serum was prepared (Use vial #1and 6 mL EIA buffer). 50 µL were added to each well except the Total Activity (TA), the Non-Specific Binding (NSB), and the Blank (Blk) wells.

6 The plate was incubated for 18 hours overnight.

7 The next day it was washed 5 times.

8 200 µL Ellman's reagent were added to all wells.

9 5 µL of Tracer were added to B1 & B2.

10 The plate was covered and incubated for 60-90 min in dark with gentle shaking.

11 The plate was read at wavelength 405 nm.

2.1.4.2. Calculation of results

According to the manufacturer's instructions a 20% or greater disparity between the apparent concentrations of two different solutions of the same sample would indicate interference and then the sample will be reassessed.

The calculations were made as follows: First the %B/ B_0 value for each sample was calculated. The concentration of each sample was determined by identifying the %B/ B_0 on the standard curve and reading the corresponding values on the x-axis. The specificity of the ELISA for 8-isoprostane is 100%. The detection limit (80% %B/ B_0) is 5 pg/mL.

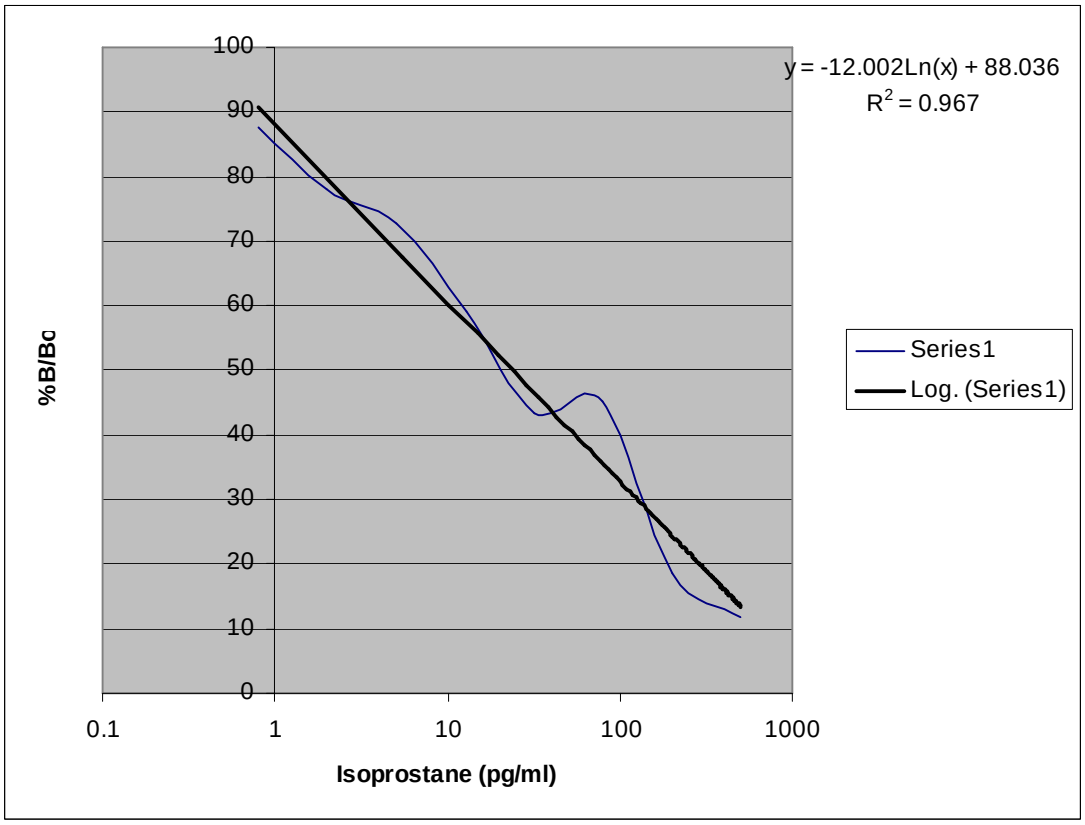


Figure 2.1.4.1. Standard curve for 8-isoprostane

2.1.5. Glial Fibrillary Acidic Protein (GFAP)

2.1.5.1. Description of laboratory method

The in-house method used has been described in detail in the following paper (Petzold, Keir et al. 2004). For the procedure the following were used. A $Barb_2EDTA$ buffer that was made up of 13.1 g sodium barbitone, 2.1. g barbitone and 0.45 g disodium EDTA per litre of solution.

A block solution that was made up of 2% Bovine Serum Albumin (BSA) in working strength $Barb_2EDTA$ buffer (i.e. 2 g BSA in 100 mL buffer solution).

The sample diluent that was made of 0.2% BSA in $Barb_2EDTA$ buffer (i.e. 0.2 g BSA in 100 mL buffer).

The wash solution that was made from 0.2% BSA, 0.05% Tween20 in $Barb_2EDTA$ buffer (i.e. 500 μL Tween20 in 1 Lt Buffer solution).

The 2 antibodies that were used were for capture a monoclonal anti-GFAP (SMI 26, Sternberger) and as a detector HRP (horseradish peroxidase) polyclonal rabbit anti-cow-GFAP (DAKO).

For the calibration procedure we used GFAP (50 μg protein vial) diluted with 500 μL H20 giving a concentration of 0.1 μg/μL (0.1 mg/mL). We then diluted by 1; 1000 to give a stock solution of 100 ng/mL.

The standard curve ranges from 10 ng/mL to 0 ng/mL. We then prepared 2 sets of double dilutions from 10 ng/mL down to 0.078 (7 dilution steps).

The ELISA steps are as follows:

Stage 1: 2 μL anti-GFAP were added to 10.5 mL carbonate buffer (0.05M) and 100 μL added to each well of a 96-well plate. The plate was left overnight at 4 ^{0}C.

Stage 2: the plate was decanted and rinsed with 250 µL wash solution. The wells were blocked with 250 µL block solution for 1 hour.

Stage 3: the plate was rinsed twice with 250 µL wash solution. 50 µL of sample diluent were added. 50 µL of calibration standard, blank, quality control and tested CSF sample were added. The plate was subsequently incubated for 1 hour at room temperature.

Stage 4: The plate was washed 6 times of 5 minutes each with wash solution. 10 µL of the 2nd antibody was added to 10.5 mL of sample diluent. 100 µL of the diluted second antibody were added to each well. The plate was then incubated at room temperature for 1 hour.

Stage 5: the plate was washed again for 30 minutes. 100 µL of DAKO-TMB one step substrate were added to each well. It was then incubated for 15-20 minutes and the reaction was stopped by adding 50 µL of 1M Hcl to the wells. The absorbance was then read at 450 nm.

2.1.5.2. Calculation of results

The sensitivity of the assay is 5 pg/ml, the upper reference limit 9 pg/ml and the standard curve ranges from 0 to 200 pg/ml. One of the standard curves obtained in order to calculate the results per sample is shown below:

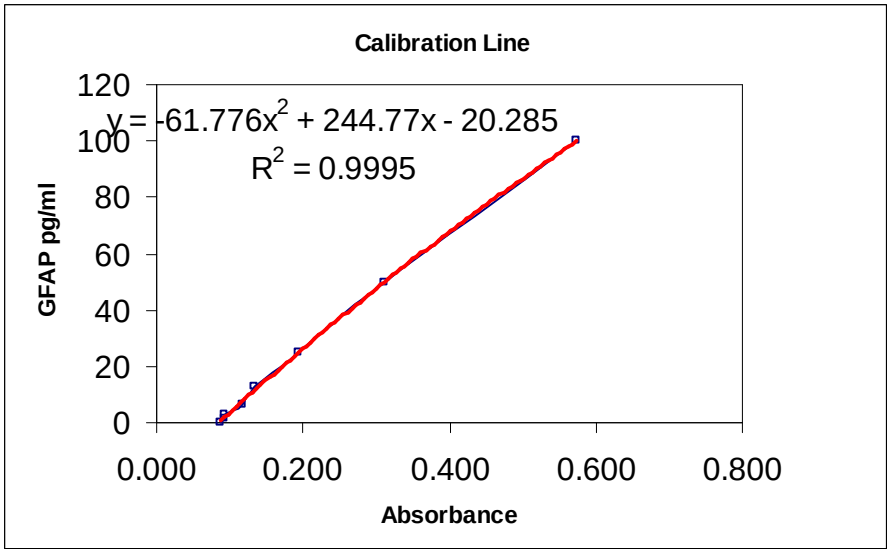


Figure 2.1.5.1. Standard curve for GFAP

2.1.6. Heavy chain of Neurofilament Protein (NfH)

2.1.6.1. Description of laboratory method

The in-house method used has been described in detail in the following paper (Petzold and Shaw 2007). For the procedure the following were used. A $Barb_2EDTA$ buffer that was made up of 13.1 g sodium barbitone, 2.1. g barbitone and 0.25 g disodium EDTA per litre of solution. A carbonate buffer that was made of 13.85 g anhydrous sodium carbonate and 26.1 g sodium hydrogen carbonate. A block solution that was made up of 2% Bovine Serum Albumin (BSA) in working strength $Barb_2EDTA$ buffer (i.e. 2 g BSA in 100 mL buffer solution). The sample diluent that was made of 0.2% BSA in $Barb_2EDTA$ buffer (i.e. 0.2 g BSA in 100 mL buffer). A wash solution that was made from 0.2% BSA, 0.05% Tween20 in $Barb_2EDTA$ buffer (i.e. 500 µL Tween20 (polyoxyethylene 20-sorbitan monolaurate; Fisher Scientific, Pittsburgh, PA) in 1 Lt Buffer solution).

The 3 antibodies that were used were for capture a monoclonal anti-NfH (SMI 35, Sternberger), as a detector a polyclonal anti-NfH (N4142, Sigma) and as a reporter a 3rd HRP swine anti-rabbit (DAKO, Copenhagen, Denmark).

For the calibration procedure we used NfH (50 µg protein vial) diluted with 500 µL H20 giving a concentration of 0.1 µg/µL (0.1 mg/mL). We then diluted by 1:1000 to give a stock solution of 100 ng/mL.

The standard curve ranges from 20 ng/mL to 0 ng/mL. We then prepared 2 sets of double dilutions from 10 ng/mL down to 0.078 (7 dilution steps).

The steps of the ELISA are as follows:

Stage 1: 2 µL anti-NfH (SMI35) were added to 10.5 mL carbonate buffer (0.05M) and 100 µL were added to each of the 96 wells of each plate. The plate was left overnight at 4 ^{0}C.

Stage 2: The plate is then decanted and rinsed with 250 µL NfH wash solution. The wells are then blocked for one hour with 250 µL block solution.

Stage 3: The plate is then decanted and rinsed with 250 µL NfH wash solution. 50 µL of sample diluent are then added. 50 µL of calibration standard, blank, quality control or tested sample is added. The plate is then incubated for 1 hour at room temperature.

Stage 4: The plate is washed 6 times of 5 minutes each with 250 µL wash solution. 10 µL of the second antibody (polyclonal rabbit anti-NfH) are added to 10.5 µL sample diluent. Then 100 µL of diluted second antibody are added to each well. The plate is then incubated for 1 hour at room temperature.

Stage 5: The plate is washed 6 times of 5 minutes each with 250 µL wash solution. 10 µL of the 3rd antibody (HRP swine anti-rabbit) are added to 10.5 µL sample diluent. 100 µL of the diluted third antibody are then added to each well. The plate is then incubated for 1 hour at room temperature.

Stage 6: The plate is washed 6 times of 5 minutes each with 250 µL wash solution. 100 µL of DAKO TMB one-step substrate to each well. The plate is incubated for 15-20 minutes and the reaction is stopped by adding 50 µL 1M Hcl. The absorbance is then read at 450 nm.

2.1.6.2. Calculation of results

The detection limit of the method is 0.1 mg/l with a sensitivity of 0.2 mg/l. The upper reference value for CSF NfHSMI35 levels was 0.73 ng/ml. One of the standard curves obtained using this method is shown below:

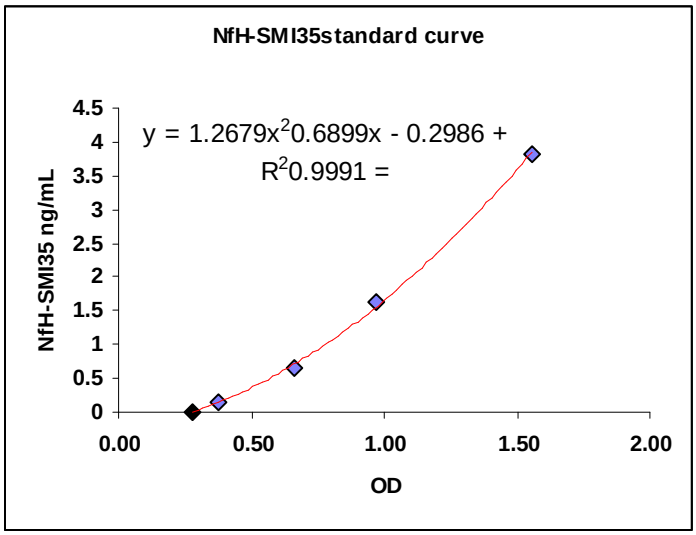


Figure 2.1.6.1. Standard curve for NfH-SM135

2.1.7. Total-tau protein

2.1.7.1. Description of laboratory method

For the experiments the commercial kit INNOTEST hate Ag (Inn genetics, Gent, Belgium) was used. This is a solid-phase enzyme immunoassay in which the human tau protein is captured by a first monoclonal antibody (AT120) bound on the solid phase. CSF samples are added in 25 μL volumes and subsequently incubated with two biotinylated tau-specific monoclonal antibodies (HT7 and BT2). The three monoclonal antibodies recognise different epitopes on the tau protein. These antibodies are then detected by a peroxidase-labelled streptavidin. After addition of substrate solution, positive samples will develop a blue colour.

The conjugate 1 solution is made of 2 monoclonal anti-hTAU antibodies labelled with biotin, in phosphate buffer.

The conjugate 2 solution is made of peroxidase-labelled streptavidin. The conjugate diluent is phosphate buffer with stabilising proteins and 0.03% Proclin 300 as preservative. The substrate is Tetramethyl benzidine (TMB) dissolved in dimethyl sulfoxide. The hTAU standard contains lyophlized recombinant hTAU standard. 500 μL sample diluent are added to it. Starting with this standard (1200 pg/mL) serial half dilutions are carried out in sample diluent to give standards of 600, 300, 150 and 75 pg/mL. The standard blank is the sample diluent alone. The steps of the ELISA are as follows:

Step 1: 75 μL of conjugate working solution are added to each well of the antibody-coated plate.

Step 2: 25 μL of each standard and the tested samples are added in duplicate wells. The plate is left overnight in an incubator at 25 ^{0}C.

Step 3: Every well is then washed 4 times. 100 μL of conjugate solution 2 is added to each well. The plate is covered for 30 minutes in an incubator at 25 ^{0}C.

Step 4: each well is washed 4 times. 100 μL of substrate working solution is added to each well and incubated for 30 minutes at room temperature. Following that, 100 μL of 2N sulfuric acid is added to each well to stop the reaction. The plate is then read at 450 nm absorbance.

2.1.7.2. Calculation of results

According to the manufacturers instructions the test was repeated if individual optical density (OD) values differed by more than 20%. The standard curve was constructed by plotting the mean absorbency value obtained for each of the standard solutions on the horizontal (X) axis versus the corresponding tau concentrations on the vertical (Y) axis. The standard levels range between 75 and 1200 pg/mL. Using the mean absorbency value of each unknown CSF sample determine the corresponding concentration of tau protein in pg/mL from the standard curve.

The lowest detection limit of the assay is +/- 59.3 pg/mL. The mean recovery of tau is 92.16%. Below is an example of a standard curve obtained during the experiments.

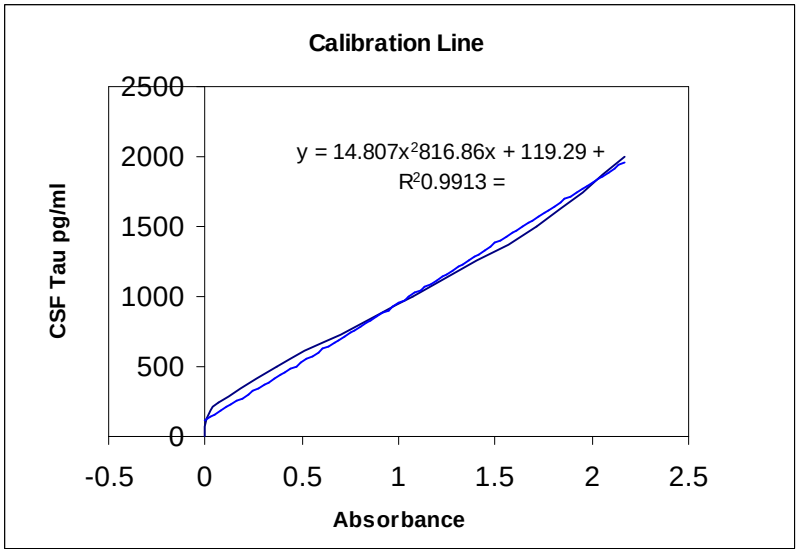


Figure 2.1.7.1. Standard curve for total tau

2.1.8. Amyloid beta peptide 1-42 ($A\beta_{1-42}$)

2.1.8.1. Description of laboratory method

For the experiments the commercial INNOTEST β-AMYLOID $_{(1-42)}$ (INNOGENETICS, Gent, Belgium) was used. The assay is a solid-phase enzyme immunoassay in which the amyloid peptide is first captured by a monoclonal antibody (21F12) bound on the solid phase. CSF samples are added in 25 μL volumes and subsequently incubated with a biotinylated antibody (3D6). This antibody is then detected by am peroxidase-labelled streptavidin. After addition of substrate solution, positive samples developed a blue colour.

The sample diluent is phosphate buffer with stabilising proteins and 0.01% MIT/0.1% CAA as preservative. The conjugate 1 is a mouse anti-β-amyloid$_{(1-42)}$ IgG labelled with biotin> this was diluted 100 X with conjugate diluent 1 prior to use.

The conjugate 2 is a peroxidase-labelled streptavidin containing 0.02% MIT and 0.02% bromonitrodiaxone as preservative. This was diluted 100X with conjugate diluent 2 prior to use.

Conjugate diluent 1 is phosphate buffer with stabilising proteins and 0.01% MIT/0.1% CAA as preservative.

Conjugate diluent 2 is phosphate buffer with stabilising proteins and 0.01% MIT/0.1% CAA as preservative.

Substrate TMB is tetramethyl benzidine (TMB) dissolved in dimethyl sulfoxide (DMSO). Substrate buffer is phosphate-citrate buffer containing 0.02% hydrogen peroxide used to dilute the Substrate TMB. The stop solution is 0.9 N sulfuric acid. The wash solution is phosphate buffer containing 0.01% MIT/ 0.09% CAA as preservative diluted 25X with distilled water.

The standards were prepared by serial dilutions of 1500 μL of the highest standard (2000 pg/mL). The concentrations are as follows: 2000, 1500, 1000, 500, 250, 125 (pg/mL).

The ELISA procedure is as follows:

Step 1: The conjugate solution 1 is prepared and 75 μL are added to each well of the plate.

Step 2: 25 μL of each standard including the blank and the tested samples are added to duplicate wells. The plate is washed 5 times.

Step 3: 100 μL of the conjugate solution 2 is added to each well. The plate is covered and incubated for 30 minutes at room temperature. The plate is then washed 5 times.

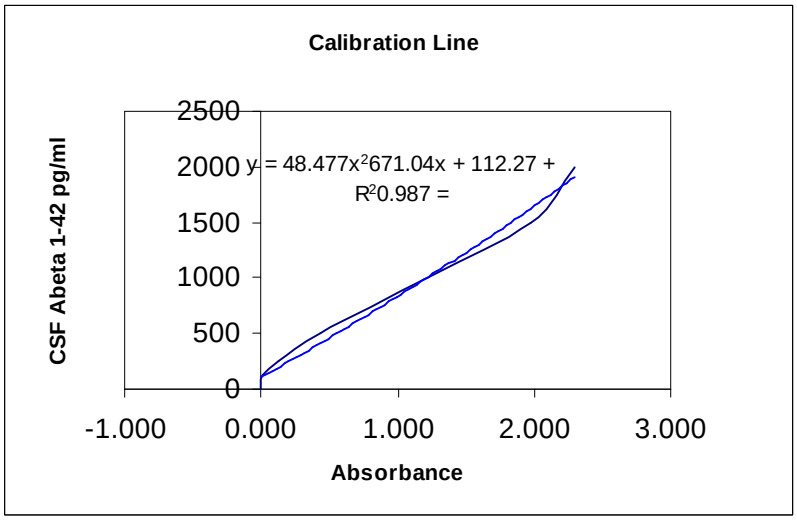
Step 4: 100 μL of substrate is added to each well. The plate is then incubated for 30 minutes at room temperature in the dark.

Step 5: 50 μL stop solution is added to each well. The plate is read at 450 nm absorbance.

2.1.8.2. Calculation of results

The assay range is between 125 and 2000 pg/mL. The lowest detection limit is +/- 50 pg/mL. The mean absorbance for the standard solutions and the tested samples was calculated. According to manufacturer's instructions if the duplicate values differed by more than 20% the samples were repeated. A standard curve was constructed by plotting the mean absorbance values obtained for each of the standard solutions on the horizontal (X) axis versus the corresponding concentrations on the vertical (Y) axis. Using the mean absorbance value of each tested sample the corresponding concentration of in pg/mL is calculated from the standard curve. An example of one of the standard curves obtained is shown below:

Figure 2.1.8.1. Standard curve for Amyloid beta 1-42



2.2. Neuropsychology assessment

A battery of tests was administered in our Institution by the department of Neuropsychology on all patients who were physically able to undergo testing. According to our protocol there were 4 stages in the assessment: the first prior to a lumbar drain insertion, the second as soon as possible after the lumbar drain was removed, the third in their first follow-up after surgery (usually at 6-8 weeks), and the final stage at 6 months postoperatively. The same battery of tests was administered throughout to all patients. Patients who did not speak English fluently were excluded by the neuropsychological assessment. The battery was composed of the following tests:

i) General intelligence was assessed by ***WAIS-R*** (Wechsler Adult Intelligence Scale, Revised). This test has been used previously in research with NPH patients (Iino, Yoshinari et al. 2000; Ogino, Kazui et al. 2006)

ii) Verbal memory was assessed by the ***RMT Words*** and visual memory by the ***RMT faces*** tests. The Recognition Memory Test (RMT; Warrington, 1984) comprises a verbal (words) and a non-verbal (unfamiliar faces) subtest. It is commonly included in neuropsychological batteries, which are used both in routine clinical assessments and in clinically oriented research (Bird, Papadopoulou et al. 2003). The RMT has also been extensively used in research into neurodegenerative disease. Thus, this test has been used in studying the early memory changes associated with familial Alzheimer's Disease (Fox, Warrington et al. 1998); the distinct cognitive profiles associated with Pick's Disease and Alzheimer's Disease (Mummery, Patterson et al. 2000) and with early-onset

autosomal dominant familial Alzheimer's Disease caused by mutation of the presenilin 1 gene (Janssen, Lantos et al. 2001).

iii) The frontal executive functions were assessed by the ***phonemic verbal fluency*** and ***trail making test B*** tests. Verbal fluency is one of the most frequently used measures of executive functioning (Baldo, Shimamura et al. 2001) and is used regularly by approximately 50% of neuropsychologists (Butler, Retzlaff et al. 1991). The two types of verbal fluency tasks are phonemic and semantic. Phonemic fluency tasks require participants to say (or write) as many words as possible beginning with a specific letter. The Trail Making Test (TMT; Army Individual Test Battery, 1944) is one of the most widely used neuropsychological tests (Retzlaff, Butler et al. 1992). It has been widely accepted as one of the most sensitive general indicators of the presence of brain dysfunction (Crawford, Parker et al. 1992); several studies of the effects of aging have used the TMT as an index of frontal lobe dysfunction (Hänninen and Soininen 1997). The test is composed of two parts A and B and it is the added demands of Part B that supposedly elicit executive processes of some type, resulting in the extrapolation to the frontal lobes (Alvarez and Emory 2006). The test has been used in other studies of cognitive assessment in NPH (Akiguchi, Ishii et al. 2008; Solana, Poca et al. 2009)

iv) The subcortical function was assessed by ***cancelling 0's or A's*** *(Willison and Warrington 1992)*.

2.1.2.1. Criteria for banding on WAIS-R

A subject was categorized as Normal where the score on testing was no more than 10 point drop from NART (National Adult Reading Test – test of premorbid

functioning); Mild if there was a10-20 point drop, and impaired when there was more than a 20 point drop.

Criteria for banding on focal tests: A subject was categorized as Normal where the score on testing was 25th%ile and above; Mild when the score was10-25th%ile; and impaired when the score was below the10th%ile.

Improvement criteria on neuropsychological testing: For the RMT words test = 1 to 2 percentile band change (Bird, Papadopoulou et al. 2003). For the RMT faces test = 2 to 4 percentile band change (Bird, Papadopoulou et al. 2003). For the Phonemic Verbal fluency = 1 SD change (Murkin, Baird et al. 1997) based on the idea of single case analysis technique which uses each patient as his/her own control- endorsed 1999 at an international consensus meeting as the preferred method of defining cognitive impairment. Although this technique is susceptible to the potential bias of regression toward the mean. For the cancelling tasks 1 SD change (Murkin, Baird et al. 1997).

2.3. Imaging analysis- Volumetric process

All the MRI images were obtained at the Department of Radiology at our Institution on a 1.5 T system (GE Signa EchoSpeed , General Electric Medical Systems, Milwaukee, WI) using the same protocol. The T2-weighted images wares transferred to a Sun UNIX workstation (Sun Microsystems, Palo Alto, California, USA) and converted to the *Analyze* format using the *Analyze* software (*Analyze* 7.5, Biomedical Imaging Resource, Mayo Foundation, Rochester, MN, USA) for

volumetric analysis using our software, "*MRreg*" (Lemieux, Wieshmann et al. 1998). The ventricular (VV), intracranial (ICV), periventricular (PVL), deep white matter hyperintensities (DWMH) and white matter (WM) volumes were measured using the "seed and region growing" algorithm of *MRreg*. The method has been described in detail elsewhere (Lemieux, Liu et al. 2000). In brief a slice comprising the structure of interest is displayed using *MRreg* and the display intensity level and window settings selected to optimise visualisation in terms of contrast with neighbouring structures and for consistency across datasets; this is helped by visualising data from different patients in a preliminary phase. A threshold level is then selected and a seed is placed in the region of interest by mouse clicking. Automatic 2D region growing automatically connects the seed point to all neighbouring voxels, in the original, unmagnified, image, that have an intensity value equal to or above the threshold value. "Spillage" of the connected region across anatomical boundaries can be corrected manually. The volume of the structure of interest within each individual slice is calculated by multiplying the number of connect voxels by the voxel volume; these are summed to give the total structure volume. The minimum and maximum threshold values for the PVL and DWMH were different at all patients representing the different pathological origin of these imaging features.

For the calculation of VV the lower or first slice used was through the third ventricle at the level of the thalami extending to the highest visible lateral ventricular slice. The same slices were used for the calculation of all parameters. The periventricular lucencies (PVL) were calculated by placing the seeds in the periventricular white matter of the frontal and/or occipital horns and by selecting

the threshold manually. The same was done for the deep white matter hyperintensities (DWMH). In order to account for volumetric differences per subject due to anatomical (related to gender, head size, etc) variations, the ventricular volumes were normalised to the intracranial volume. The following relative volume measures were also calculated: ventricular per PVL ratio (VV/PVL) to express the impact of ventriculomegaly on the PVLs, ventricular per DWMH ratio (VV/DWMH) to represent the degree of ventriculomegaly versus the vascular ischemic load , as well as ventricular per WM ratio (VV/WM) to express the impact of ventriculomegaly on white matter volume. We also calculated the PVL/WM ratio as an index of severity of PVL and the DWMH/WM ratio as an index of gravity of ischemic changes in this cohort. The operator (AT) was blind to the identity and clinical status of the subject during the volumetric process.

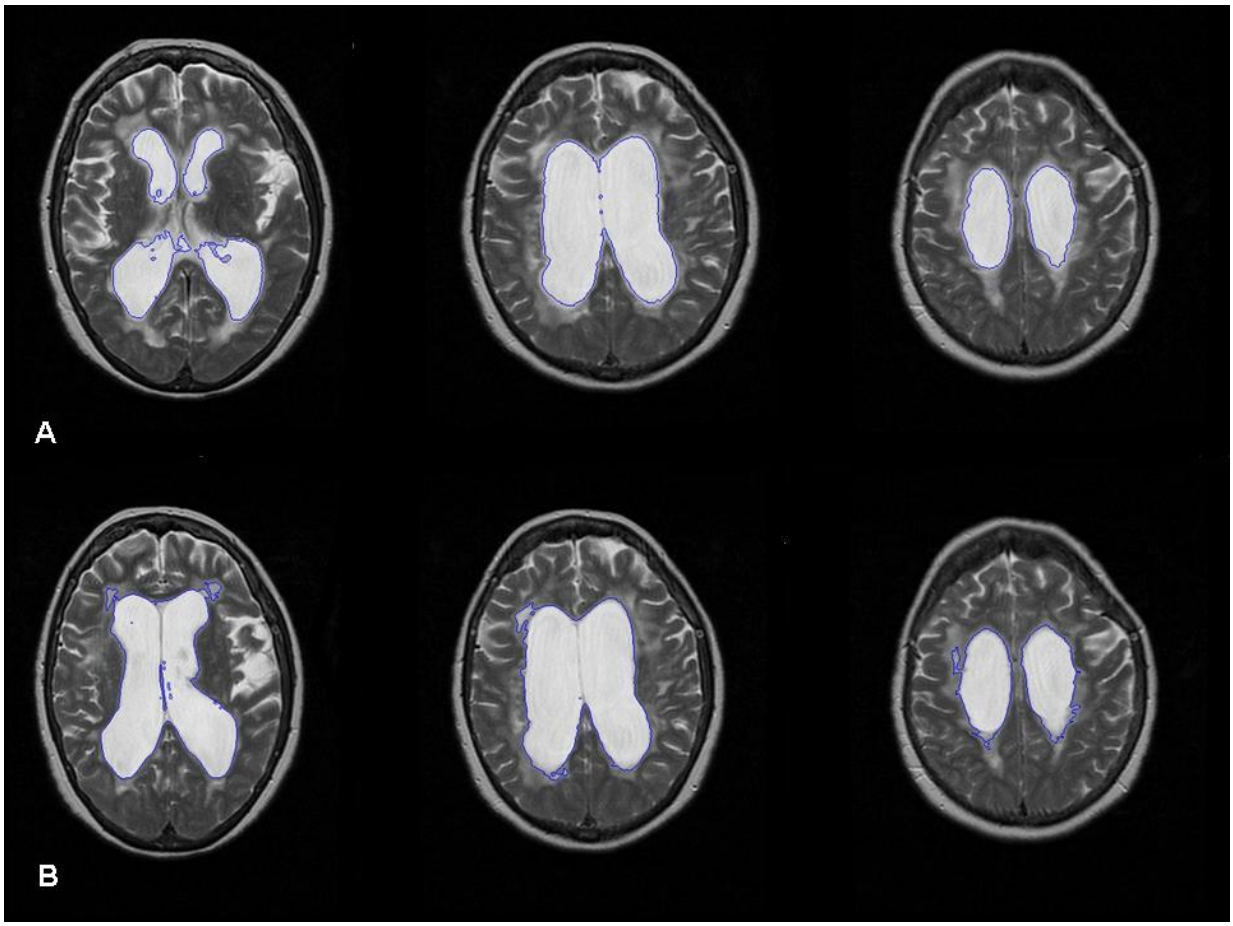


Figure 2.3.1. An example of the volumetric process in a single patient. The top row (A) shows measurement of intraventricular volumes (VV) in the lower slice chosen at the level of thalami (left), with two higher slices presented (middle and right picture). The bottom row (B) shows calculation of periventricular lucencies (PVL) in similar slices (middle and right picture) for comparison.

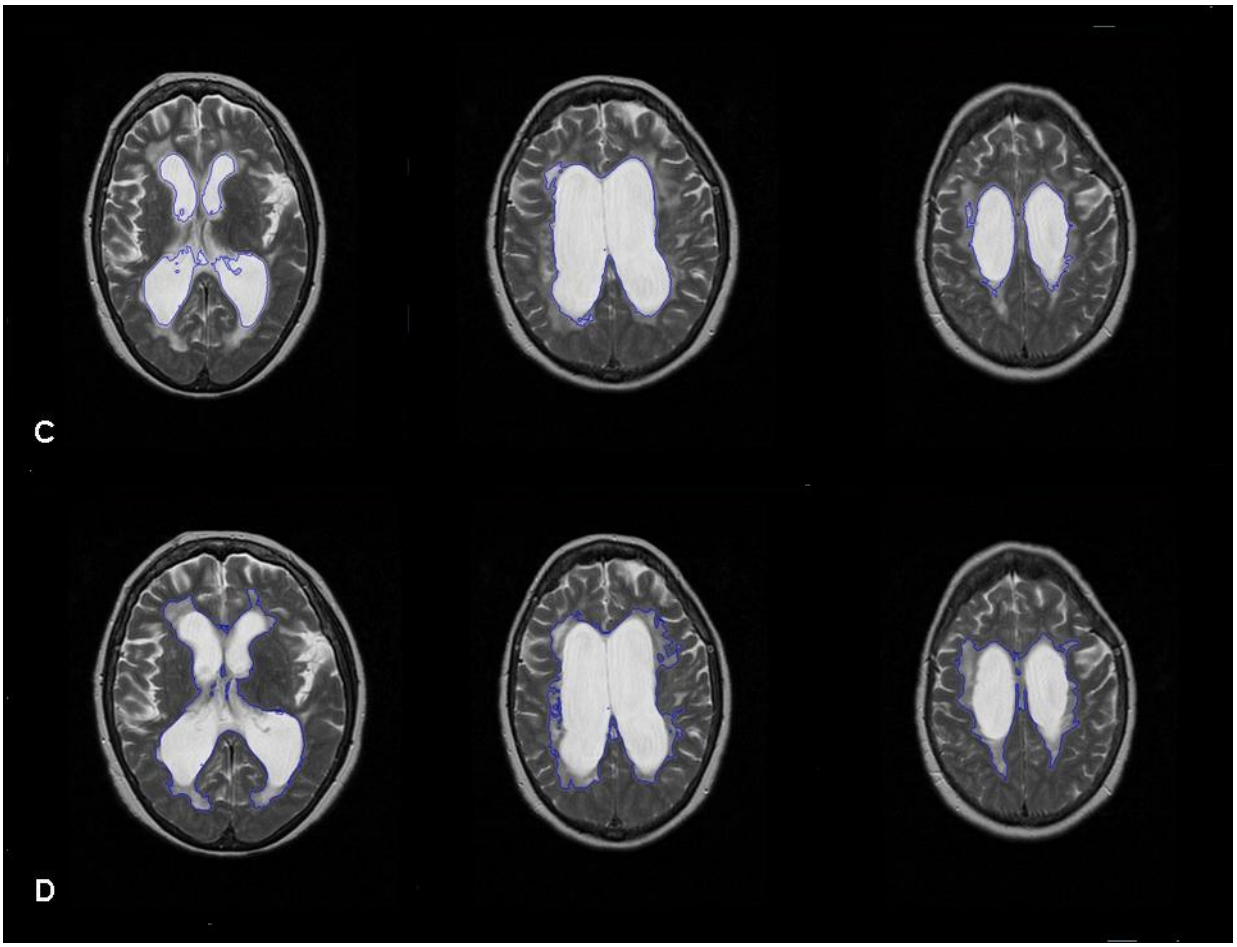


Figure 2.3.2. An example of the volumetric process in the same patient (continued). The top row (C) shows measurement of periventricular lucencies (PVL) in the lower slice chosen at the level of thalami (left), with two higher slices presented (middle and right picture). The bottom row (B) shows calculation of deep white matter hyperintensities (DWMH) in similar slices for comparison. The minimum and maximum threshold values for the PVL and DWMH were different at all patients representing the different pathological origin of these imaging features.

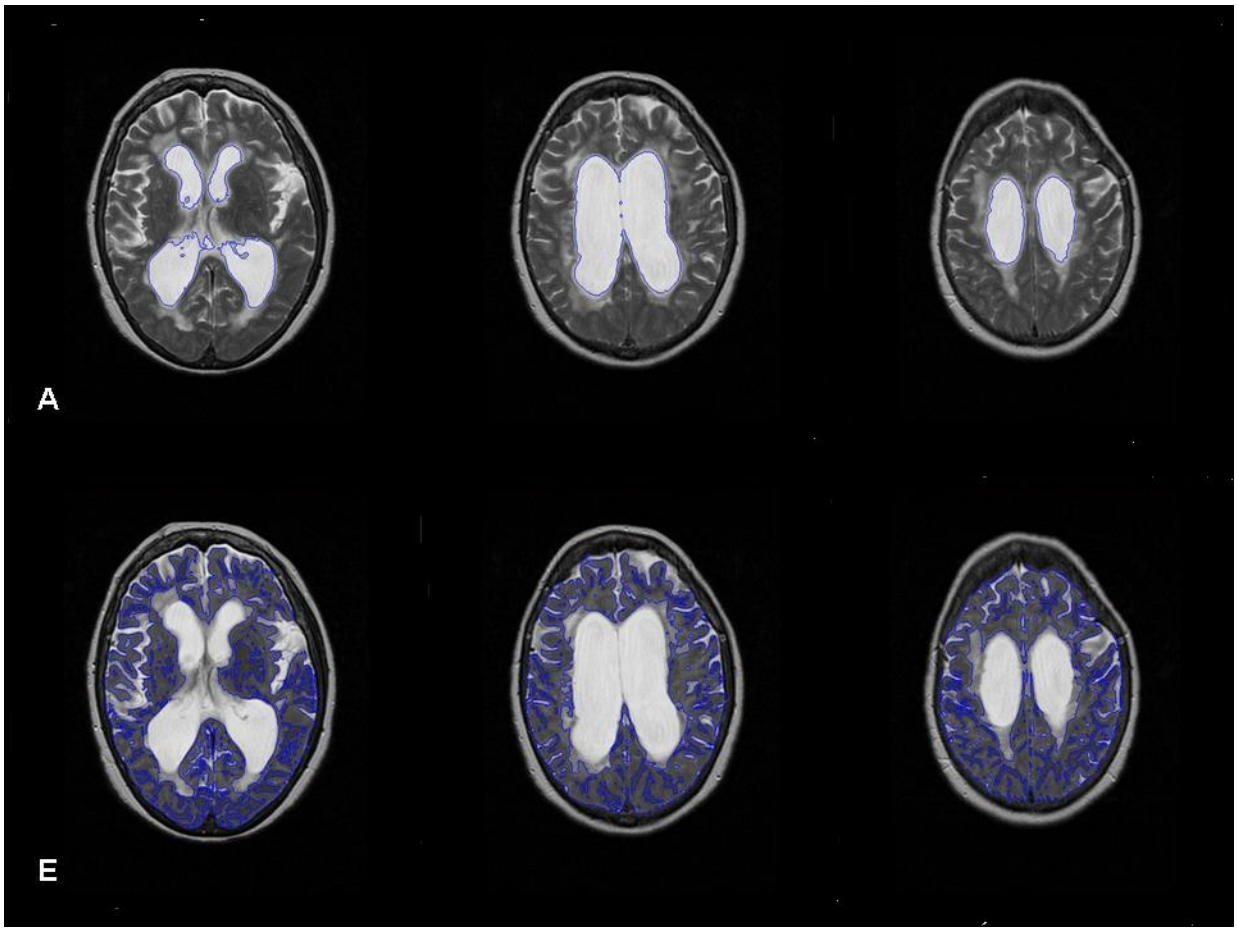


Figure 2.3.3. An example of the volumetric process in the same patient (continued). The top row (A) shows measurement of intraventricular volumes (VV) in the lower slice chosen at the level of thalami (left), with two higher slices presented (middle and right picture). The bottom row (E) shows calculation of white matter volume (WM) in similar slices for comparison.

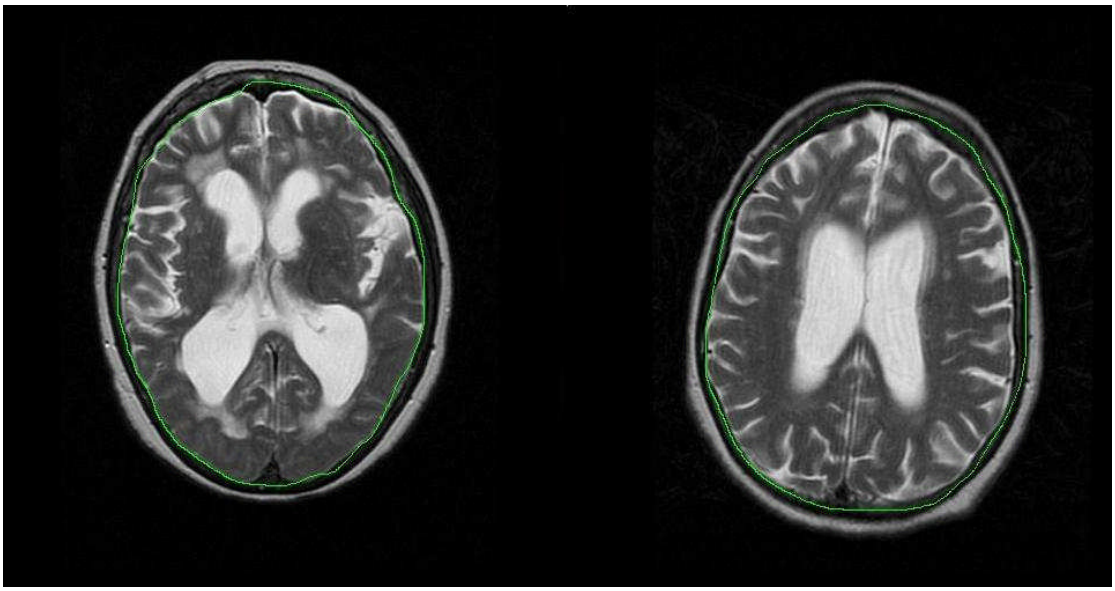


Figure 2.3.4. An example of the volumetric process in two different patients. The green line outlines the total intracranial volume in the respective slices chosen for demonstration. The method of volumetry on this occasion was manual tracing and not region drawing.

2.4. Insertion of external lumbar drain

The procedure was carried out under general anaesthetic. The patient was positioned in a lateral decubitus position as for a lumbar puncture. The skin was prepped and draped in a standard fashion, and the subcutaneous tissue was infiltrated with 1% Lignocaine and Adrenaline 1:200.000. A 14-gauge Tuohy needle was inserted with the bevel facing cranially. Once CSF flow was obtained the pressure of CSF was recorded; following that 5 MLs of CSF were sent for a standard cell count, microbiological analysis, as well as kept for research purposes. Following CSF sampling a lumbar drainage catheter with wire stylet was inserted until resistance was met (between 20-30 cm length). The needle and the stylet were subsequently removed and satisfactory CSF flow was confirmed

visually. The catheter was then attached to the supplied Luer-Lok connector, secured with a 2.0 silk ligature and then connected to a closed CSF drainage reservoir with a three-way stopcock. The site was dressed with a 2 X 2 inch gauze and a transparent Opsite dressing. The drainage was set to 10-15 mLs/ h for 72 hours. For the purposes of the study 2 further CSF samples were taken under strictly aseptic conditions; the first after 48 hours of drainage (day 2) and the second just prior to removal of the LD (day 3).

2.5. Insertion of ventriculoperitoneal shunt

The procedure is carried out under general anaesthesia. Intravenous antibiotics with skin flora coverage were given at induction. The patient was placed in the table supine with the head turned 90^0 to opposite side (if no contraindications) and an ipsilateral shoulder roll in place. The area of the proposed skin incision was shaved using a disposable razor. The cranial, neck, thoracic and abdominal area were prepped initially with betadine detergent scrub for 5 minutes and then betadine paint. A small semilunar scalp flap was marked as the incision in the posterior parietal area. In the abdominal area a horizontal 3-4 cm was marked in the right subcostal area. The patient was then draped over the exposed neck, thorax and abdomen, and the skin is infiltrated with 1% Lignocaine and Adrenaline 1:200.000. Following a hockey-stick scalp incision, haemostasis is achieved with bipolar cautery. A burr hole is fashioned using an air-powered perforator 3 cm from midline and 6 cm up from the inion. A subgaleal pocket for the valve is made in plane above the pericranium and a shunt-passer path is developed with a haemostat. Following an abdominal skin incision, and subcutaneous tissue

dissection the anterior rectus sheath is incised. The rectus muscle is then split in layers in the direction of fibres. Straight forceps are placed initially in the posterior rectus sheath and after its incision the peritoneum is identified. Straight forceps are placed onto the peritoneum and the peritoneal cavity is entered. A bent metal shunt-passer is advanced from the cranial to caudal direction and the shunt tube is passed through from the cranial to the abdominal end. The passer is then removed. The dura is coagulated with bipolar cautery and incised in a cruciate fashion with a no.11 blade. The pia is cauterized and subsequently incised. Following study of each patient's individual imaging a trajectory is planned and a styleted ventricular catheter is passed aiming usually for the ipsilateral medial canthus. When CSF backflow is achieved the stylet is removed and the ventricular catheter is forwarded to a depth according to the surgeon's preference. The first few mLs of CSF are discarded and then 5 mLs of CSF were sent for a standard cell count, microbiological analysis, as well as kept for research purposes. The distal shunt catheter is fed into the peritoneal cavity with non-toothed forceps, and a purse string suture secures the catheter position. The wounds are closed in layers with interrupted Vicryl and clips to skin.

The choice of the valve and valve settings depended on the individual surgeon's preference, taking into account the degree of ventriculomegaly and physiological measurements such as intracranial or CSF pressure. Two patients had a fixed pressure Delta valve (Medtronic, PS Medical) with medium settings. Ten patients were implanted with a Strata programmable valve (Medtronic, PS Medical) with initial settings ranging from 1.0-2.5 performance level. Nine patients were implanted with the proGAV Programmable valve (Aesculap-Miethke) with settings

ranging from 5-10 cm H_20. One patient was implanted with the Polaris programmable valve (Sophysa) pre-programmed at 150 mm H_20.

Chapter 3 Results

3.1. Epidemiology

25 consecutive patients were recruited in total between February 2005 and February 2007. Out of this group one patient died prior to having a ventriculoperitoneal shunt (VPS) insertion, one patient refused to have the procedure even though he underwent ELD and fulfilled the criteria to enter the study, and one patient underwent the procedure after February 2007 hence only partial data (pre and post lumbar drain) exists. The patient who died underwent ELD and also fulfilled the criteria to have a VPS.

22 patients had a VPS inserted in the above period and we report on their outcomes.

There were 15 males and 7 females. The mean age of the subjects was 71.45 (+/- 9.5) years. The mean symptom duration was 45 (+/- 59) months.

3.2. Clinical characteristics

Fourteen patients (64%) had gait ataxia as the first presenting symptom; 6 patients presented firstly with cognitive decline and 2 patients with urinary incontinence. Four patients (18%) have diabetes mellitus, 7 (32%) had hypertension, 1 (4.5%) had suffered a transient ischaemic attack (TIA) previously, 3 (14%) patients had a previous cerebrovascular accident (CVA), 1 had suffered a myocardial infarction (MI) and 1 had concomitant peripheral vascular disease (PVD).

All 22 patients had gait ataxia and cognitive decline at the time of assessment and 17 (77%) had urinary incontinence. Seventeen patients had the entire triad of the symptoms at the time of the assessment.

On clinical examination 5 (23%) patients had documented mild motor weakness of the lower limbs. 2 (10%) patients had concomitant cerebellar symptoms. 8 (36%) patients had extrapyramidal symptoms. 9 (41%) patients had apraxia. The gait independence is displayed in Table 3.2.1.

Gait score	Description of gait (n=22)	Number of patients per category
0	Normal	0
1	Unstable but independent gait	8
2	Walking with one cane	4
3	Walking with two canes or a walking frame	9
4	Walking not possible	1

Table 3.2.1. Description of gait

Clinical examination characteristics (n=22)	Number of patients	Percentage of total (n=22)
Difficulty rising from chair	9	41
Difficulty withstanding a push from front or rear	11	50
Difficulty in maintenance of standing	7	32
Starting hesitation	7	32
Turning hesitation	12	55
Arm swing during gait	14	64
Wide based gait	13	60
Short Stride	18	82
Brakynesia affecting gait	17	77
Improvement of walking upon counting aloud	4	18
Writing disturbance	9	41
Frontal release signs	13	69

Table 3.2.2. Clinical characteristics on examination

A 10-metre walking test was carried out in all patients, apart from one for whom walking was not possible at the time of examination. The average number of steps and the time taken in seconds was measured from two attempts. Where possible we would ask the patient to walk without assistance; if not possible the patient would use his normal walking aid. The average number of steps needed to transverse this distance was 31.6 (range: 14-58) requiring 25.3 (range: 6.5-84) seconds.

The severity of urinary incontinence was categorised according to table 3.2.3. .

Urinary incontinence score	Description of urinary incontinence (n=22)	Number of patients per category
0	Absent	5
1	Absent but with frequency or urgency	4
2	Sometimes only at night	4
3	Sometimes even during the day	6
4	Frequent	3

Table 3.2.3. Description of urinary incontinence

The average M.M.S.E score for 16 patients was 20.2 (+/- 6.6) with a range of 8-29. 16 patients completed the neuropsychological assessments pre-shunt; the remaining 6 either refused to participate, or the assessment was not completed due to tiredness of the participants during the test or the presence of severe cognitive decline.

3.3. Patients undergoing external lumbar drainage

Eleven patients (out of the total 25) underwent assessment before and after the insertion of a lumbar drainage. As mentioned previously only eight of those 11 had insertion of a VPS, since one patient died prior to VPS insertion, one patient refused the procedure and the third patient had the procedure following the end of this study (February 2007), hence only partial (pre-post drainage) data exists.

The group was comprised of 9 males and 2 females. Age was 71.1 (+/- 5.85) years at the time of assessment. The ages of the 2 populations (ELD and VPS as described in § 3.1. do not differ (p=0.38, paired samples t-test). The preoperative symptom duration is 36.9 (+/- 19.4) months; this does not differ from the previous population (p=0.48, paired samples t-test). The clinical characteristics of those 3 additional patients not described previously will be described below.

Two patients (64%) had gait ataxia as the first presenting symptom and the third presented firstly with cognitive decline. Two patients had diabetes mellitus, and all three had hypertension. One patient was suffering from ischemic heart disease. One had suffered a transient ischaemic attack previously.

All 3 patients had gait ataxia and cognitive decline at the time of assessment and 2 had urinary incontinence.

None of the three patients had motor weakness of the lower limbs. None had demonstrated cerebellar or extrapyramidal symptoms or apraxia. The gait independence was categorised according to Table 3.3.1. .

Gait score	Description of gait category	Number of patients per category
0	Normal	0
1	Unstable but independent gait	1
2	Walking with one cane	1
3	Walking with two canes or a walking frame	1
4	Walking not possible	0

Table 3.3.1. Description of gait

Clinical examination characteristics	Number of patients	Percentage of total (n=25)
Difficulty rising from chair	12	48
Difficulty withstanding a push from front or rear	13	54
Difficulty in maintenance of standing	7	28
Starting hesitation	8	32
Turning hesitation	14	56
Arm swing during gait	14	58
Wide based gait	15	60
Short Stride	21	84
Brakynesia affecting gait	19	79
Improvement of walking upon counting aloud	4	18
Writing disturbance	10	43
Frontal release signs	14	61

Table 3.3.2. Clinical characteristics on examination

A 10-metre walking test was carried out in all 3 patients. The average number of steps and the time taken in seconds was measured from two attempts. The average number of steps needed to transverse this distance was 68 (range: 15-152) requiring 76 (range: 10-171) seconds.

The severity of urinary incontinence was categorised according to table 3.3.3. .

Urinary incontinence score	Description of urinary incontinence	Number of patients per category
0	Absent	1
1	Absent but with frequency or urgency	0
2	Sometimes only at night	1
3	Sometimes even during the day	1
4	Frequent	0

Table 3.3.3. Description of urinary incontinence

2 of the three patients had neuropsychological assessment prior to ELD insertion; the third did not participate due to language barrier.

3.4. Walking test before and after external lumbar drainage

In the table below one may see the results of the walking test.

10 metre Walking test (average of 2 attempts) pre ELD insertion

	N	Minimum	Maximum	Mean	Std. Deviation
Steps required	11	15	152	39.5	39.05
Time taken (secs)	11	6.5	171	38.8	48.15

Table 3.4.1. Walking test before external lumbar drain insertion

Eight patients improved in the walking test following a 72 hours external lumbar drainage. These eight patients were selected to have insertion of a VPS at a later time. The results of the walking test 72 hours post ELD insertion can be seen in the table below.

10 metre walking test 9average of 2 attempts) post ELD insertion

	N	Minimum	Maximum	Mean	Std. Deviation
Steps required	11	15	107	34.15	29.3
Time taken (secs)	11	9.5	90	29.9	32.21

Table 3.4.2. Walking test after external lumbar drain removal

Although there is a reduction in the mean number of steps required and time taken the difference pre and post assessment is not statistically significant (paired samples t-test; p=0.26 for steps required and p=0.16, for time taken).

3.5. Neuropsychological assessment before and after external lumbar drainage

The neuropsychological battery was administered to 10 patients. The test was not possible to be administered to one patient due to language barrier. The results of the pre ELD insertion assessment as well as the difference between the 2 assessments may be seen in the tables below.

Neuropsychological assessment prior to ELD insertion (n=10)						
		Memory tests		**Frontal executive (anterior)**		**Speed (subcortical)**
Tests	**General Intelligence (WAIS-R)**	**Verbal Memory RMT Words**	**Visual Memory RMT Faces**	**Phonemic VF**	**Trail Making Test B**	**Speed (Cancelling 0's or A's)**
Normal	4	6	4	4	1	0
Mild	3	2	0	3	1	3
Severe	3	2	6	3	8	7

Table 3.5.1. Neuropsychological assessment prior to ELD insertion

Pre and Post Lumbar Drain changes

		Memory tests		Frontal executive (anterior)		Speed (subcortical)
Tests	**General Intelligence (WAIS-R)**	**Verbal Memory RMT Words**	**Visual Memory RMT Faces**	**Phonemic Verbal Fluency**	**Trail Making Test B**	**Speed (Cancelling 0's or A's)**
No difference		7	6	9	8	3
Significant improvement	N/A as not repeated post drain due to practise effects	2	1	1	1	3
Significant decline		1	3	0	0	3
Not administered					1	1

Table 3.5.2. Neuropsychological assessment after ELD removal

3.6. Surgical procedure

Twenty-two patients had insertion of ventriculoperitoneal shunt in total. The procedure was carried out by 4 different surgeons, although the majority of the patients (sixteen) were operated by the same surgeon (LW). All the 11 patients who had insertion of a lumbar drain were operated by the same surgeon (LW).

3.6.1.1. Complications of external lumbar drainage and ventriculoperitoneal shunting

One patient (8.3% of the total who underwent ELD) who underwent external lumbar drainage returned 5 days following his discharge home feeling unwell and feverish, as well as being disorientated and confused. He was initially admitted to his local hospital where he was given i.v. Cefotaxime empirically, as well as p.o. Nitrofurantoin for a presumed urinary tract infection (the patient had an indwelling

urinary catheter). On admission in our hospital he was feeling better and he had no photophobia or neck stiffness. An MRI spine did not reveal any spinal abscess and a CT brain showed equal size ventriculomegaly. However, the patient had increased CRP but normal WCC A lumbar puncture showed normal pressure with 41 white cells that were predominantly lymphocytes. The Gram stain was negative and there was no growth of organisms on culture. A urethral swab grew Candida species and the patient was treated accordingly and later discharged home.

Five patients (23% of the total) who received a ventriculoperitoneal shunt had postoperative complications. One patient suffered a pontine ischaemic stroke 7 months following the operation and was not followed-up further. However, this is not thought to be a surgical complication.

Three patients (14%) suffered a subdural haematoma 3, 4 and 7 months postoperatively. One patient had a fixed pressure and two had a programmable valve. In particular, they had a medium Delta valve, a proGAV valve preset at 5 cm H_20, and a Polaris valve preset at 150 mm H_20. One patient (fixed-pressure valve) had bilateral subdural effusions/haematomas that were managed conservatively due to minor symptomatology and eventually disappeared on subsequent imaging. The other two patients required surgical evacuation of the haematoma.

One patient had an ischemic infarct immediately postoperatively in his right occipital lobe that manifested as left sided homonymous hemianaopia.

One patient who was implanted with the proGAV valve experienced low-pressure headaches 6 weeks postoperatively in an initial valve setting of +5 cm H_20. However, the valve was reset to a higher setting (+10 H_20) in an outpatient setting and the symptoms disappeared. Notably there were no infections during our study period.

3.7. Imaging and volumetric data

Fifteen patients had MR imaging preoperatively; the remaining 7 had only CT brain. On three patients the MR imaging obtained was of poor quality due to movement artefact and it could not be used further in the volumetric analysis. However, the ventricular and intracranial volume could be extracted from those three patients and was used accordingly. Ventricular, intracranial and periventricular lucencies volumes were extracted by volumetric analysis from CT scans; however it was not technically possible to extract the volumes of the deep white matter hyperintensities, or white-matter volume due to the technical limitations of CT scanning..

Volumetric characteristics of 22 patients with iNPH

	Minimum	Maximum	Mean	Std. Deviation
Ventricular volume	41.59	276.22	126.7437	57.91708
Periventricular lucencies volume	.74	52.91	28.3744	13.96503
Deep white matter hyperintensities volume	2.32	193.83	45.5371	53.67868
White matter volume	120.39	414.17	275.4692	84.96347
Total intracranial volume	445.48	1244.12	681.3395	200.42722

Table 3.7.1. Volumetric characteristics of patients

Volumetric characteristics of 22 patients with iNPH

	Minimum	Maximum	Mean	Std. Deviation
Evans index	.33	.51	.3842	.04623
Ventricular volume ratio	.08	.31	.1850	.05766
Periventricular lucencies volume ratio	.00	.09	.0418	.02341
Deep white matter hyperintensities volume ratio	.00	.26	.0598	.07385
White matter volume ratio	.00	.58	.2629	.21752
Intracranial volume (mm3)	445.48	1244.12	681.3395	200.42722

Table 3.7.2. Relative volumetric ratios of patients

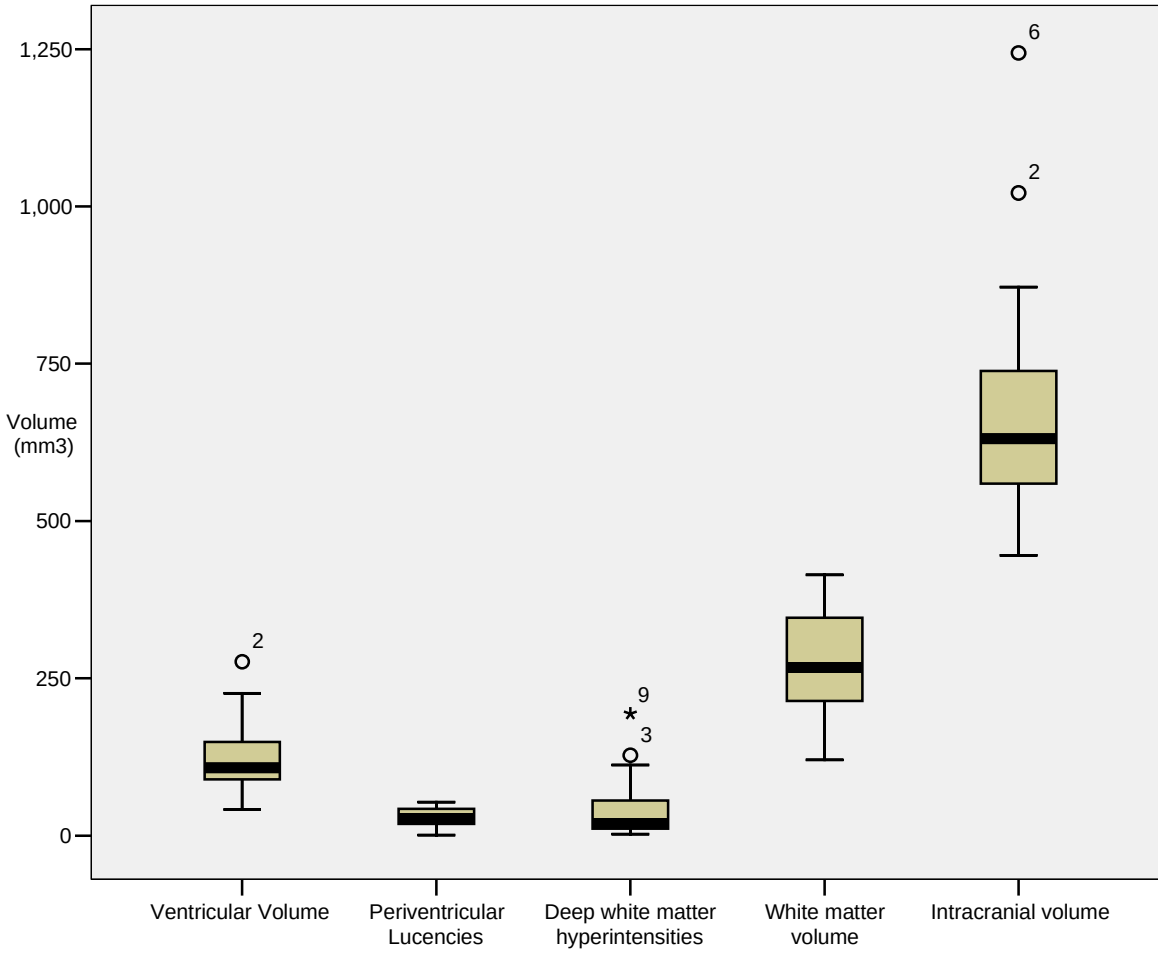


Figure 3.7.1. Boxplot diagram of volumetric characteristics of 22 patients with iNPH

3.8. CSF marker results of patients undergoing external lumbar drainage

There were no differences in red cell count, white cell count or CSF/serum albumin ratio between the samples. There was also no significant difference in the total volumes drained per patient in 72 hours. The results of the assays for the markers can be seen in Table 3.8.1.

CSF markers	Day 0	Difference between Day 0 and Day 2	Day 2	Difference between Day 2 and Day 3	Day 3	Difference between Day 0 and Day 3
CSF/ serum albumin ratio (X 10^3)	8.19 (7.04)	↑, ns	10.62 (10.07)	↓, ns	7.64 (6.47)	↓, ns
Lactate (mmol/L)	2.27 (.26)	↑, ns	2.68 (.23)	↑, ns	2.74 (.26)	**↑, * [0.027-0.033]]**
8-Isoprostane (pg/ml)	38.13 (.29)	↓, ns	37.8953 (.33)	**↑, * [0.027-0.034]**	38.66 (.32)	↑, ns
VEGF (ng/ml)	4.08 (1.14)	**↑, * [0.010-0.014]**	13.61 (2.81)	↑, ns	16.8 (4.09)	**↑, * [0.007-0.011]**
GFAP (ng/ml)	2.01 (1.41)	**↑, * [0.022-0.028]**	6.9750 (.99)	↑, ns	16.3 (6.46)	**↑, ** [0.001-0.003]**
NfH (ng/ml)	0.1618 (.052)	**↓ * [0.07-0.010]**	.0156 (.015)	↑, ns	.0345 (.016)	**↓ * [0.013-0.018]**
Aβ 1-42 (pg/ml)	366.05 (74.71)	↑, ns	464.04 (43.62)	↓, ns	449.02 (74.50)	↑, ns
Total Tau (pg/ml)	151.88 (27.28)	**↑, * [0.006-0.010]**	310.57 (51.68)	**↑, * [0.038-0.046]**	415.55 (69.78)	**↑, * [0.002-0.004]**

Table 3.8.1. Concentrations of markers in all 3 days of collection and the relationship between days 0 and 3. Values represent mean and standard error of mean in brackets. The one-tailed Wilcoxon signed ranks test was used to identify significant differences. Levels of significance: * $p<0.05$, ** $p<0.001$, ns: non significant. In brackets the 95% Confidence Intervals of the levels of significance.

The CSF/serum albumin ratio (Q_{alb}) increases between day 0 and 2 and then decreases in the following 24 hours, in a non-significant fashion. Lactate levels increase as drainage progresses, and this increase reaches statistical significance within 72 hours of drainage. Furthermore, even though lactate levels are within normal limits at time 0 (upper normal limit: 2.40 mmol/L) they become pathological at 48 and 72 hours post drainage. The levels of the 8-isoprostane increased over 72 hours with the difference reaching statistical significance only between 48 and 72 hours. Concentration of VEGF also increased in a statistically significant manner, particularly between day 0 and 2, where the change is most prominent. Similar results occur for GFAP, with the most prominent change happening within

the first 48 hours and the significance reaching p≤0.001 after 72 hours of drainage. The levels of NfH decrease significantly between day 0 and 3. The levels of $A\beta_{1-42}$ increase overall but in a non-significant fashion. A consistent increase is noted in the concentration of total tau as drainage continues.

3.8.1. Correlations between markers

Correlations between the markers were calculated for all three time-points. Table 2 presents the correlations for day 0.

		Lactate	IP	VEGF	GFAP	NfH	$A\beta_{1-42}$	tau
Lactate	Correlation Coefficient	.						
	Sig. (2-tailed)		NS	NS	NS	NS	NS	NS
8-isoprostane	Correlation Coefficient		.					
	Sig. (2-tailed)	NS		NS	NS	NS	NS	NS
VEGF	Correlation Coefficient					**-0.758**		
	Sig. (2-tailed)	NS	NS	.	NS	**0.007**	NS	NS
GFAP	Correlation Coefficient						**-0.676**	**-0.785**
	Sig. (2-tailed)	NS	NS	NS	.	NS	**0.022**	**0.004**
NfH	Correlation Coefficient			**-0.758**				
	Sig. (2-tailed)	NS	NS	**0.007**	NS	.	NS	NS
$A\beta_{1-42}$	Correlation Coefficient				**-0.676**			**0.636**
	Sig. (2-tailed)	NS	NS	NS	**0.022**	NS	.	**0.035**
Total tau	Correlation Coefficient				**-0.785**		**0.636**	
	Sig. (2-tailed)	NS	NS	NS	**0.004**	NS	**0.035**	.

Table 3.8.1.1. Correlations of CSF markers. Table demonstrates only results for the samples collected at day 0. The two-tailed Spearman's test for bivariate correlations was used. Levels of significance: * p<0.05, ** p<0.001, NS: non significant

Lactate has virtually no correlation with either 8-isoprostane or VEGF in day 0, 2 and 3. Equally 8-isoprostane has no correlation with any marker in any of the 3 day samples. VEGF has a negative correlation with NfH (r= -0.758, p=0.007) in the first

sample which does not continue in day 2 and 3. This is because the VEGF levels increase and NfH levels decrease as drainage continues.

GFAP has also a significant negative correlation with total tau level on day 0 (r= -0.785, p=0.004); this becomes non significant as the drainage ensues. $A\beta_{1-42}$ also has a significant negative correlation with GFAP level on day 0 (r= -0.676, p=0.022); this negative relationship is maintained as drainage continues but it becomes non significant.

$A\beta_{1-42}$ and tau have a moderately positive correlation (r= 0.636, p=0.035) which is statistically significant during day 0. This positive trend is maintained in day 2 (non significant) and reverses in day 3 since the concentration of total tau increases as drainage ensues, that of $A\beta_{1-42}$ decreases from day 2 to 3.

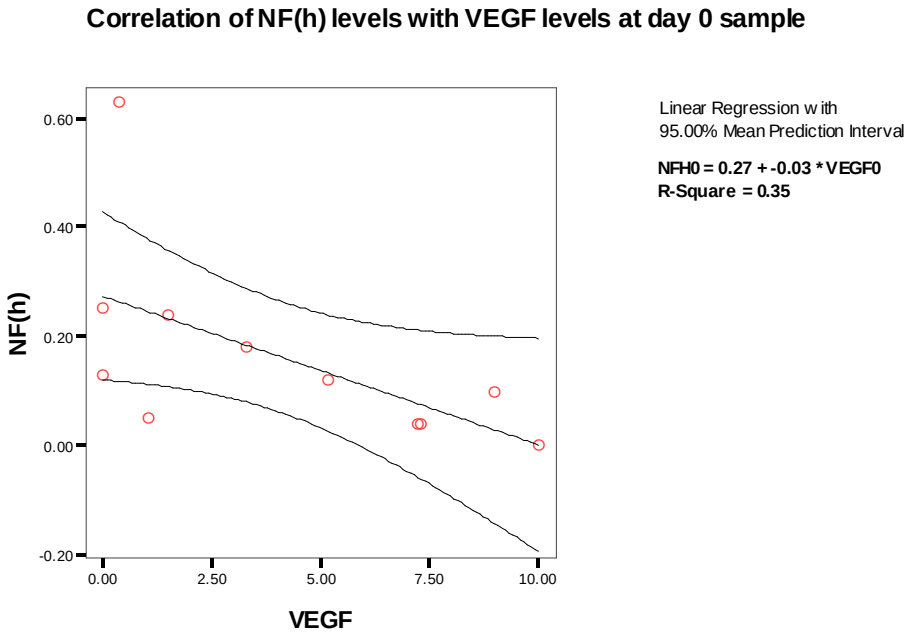


Figure 3.8.1.1. Scatterplot and linear regression analysis of lumbar NfH and VEGF levels. Solid line is best-fit regression line with 95% Confidence Intervals (CIs) (curved lines).

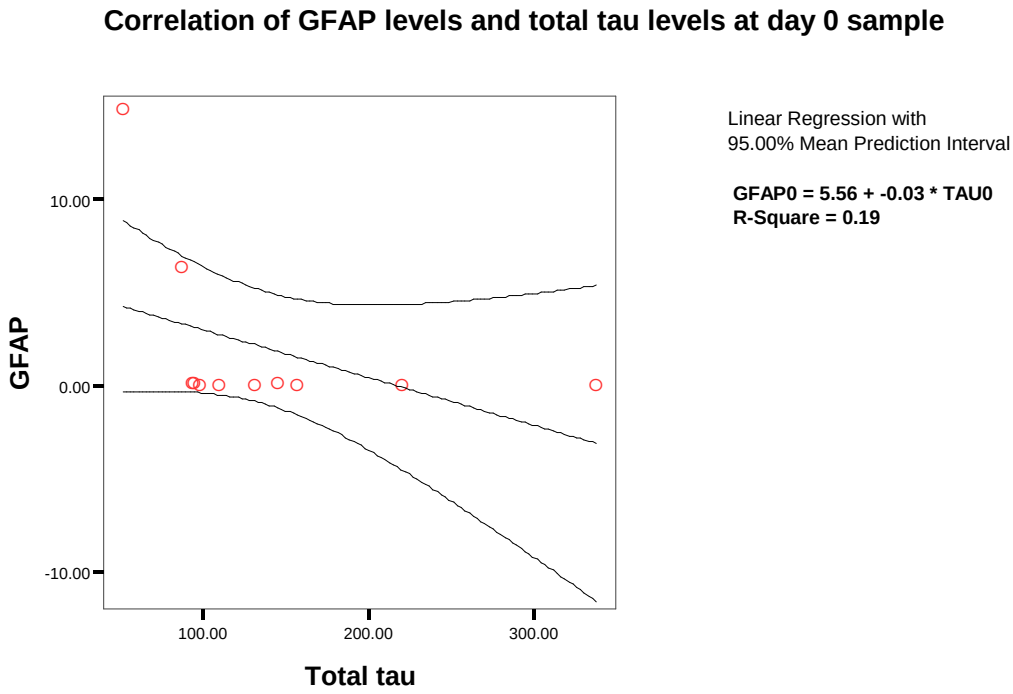


Figure 3.8.1.2. Scatterplot and linear regression analysis of lumbar GFAP and total tau levels. Solid line is best-fit regression line with 95% CIs (curved lines).

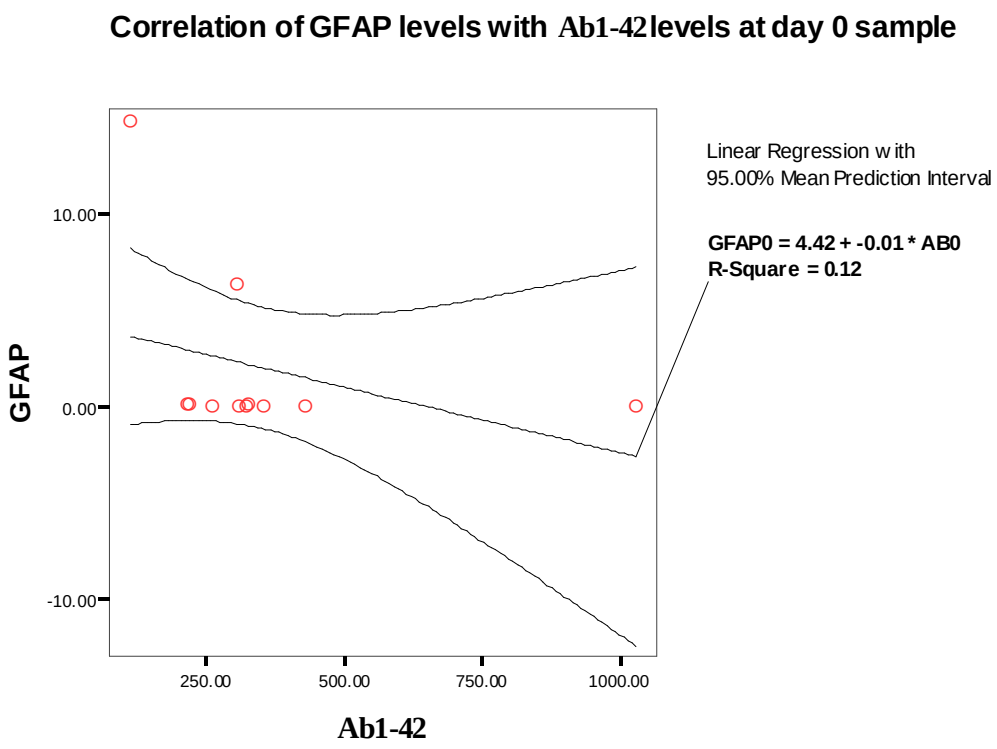


Figure 3.8.1.3. Scatterplot and linear regression analysis of lumbar GFAP and Ab 1-42 levels. Solid line is best-fit regression line with 95% CIs (curved lines).

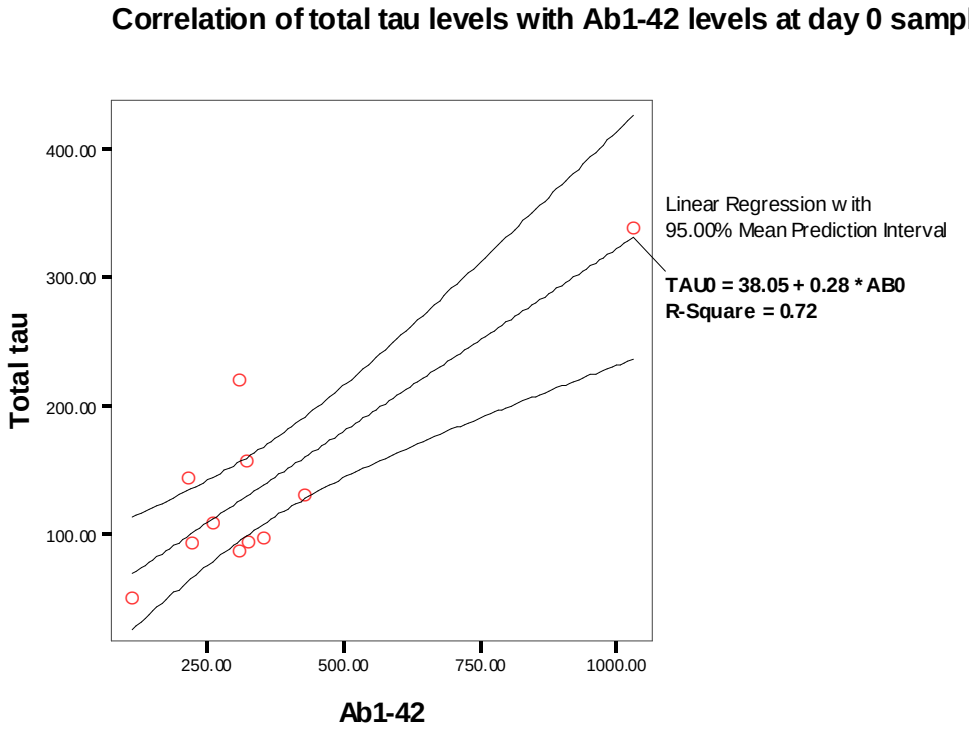


Figure 3.8.1.4. Scatterplot and linear regression analysis of lumbar total tau and Ab 1-42 levels. Solid line is best-fit regression line with 95% CIs (curved lines).

3.8.2. Correlations of CSF markers at day 2 and 3

In the tables below the correlations between the markers as drainage ensues are displayed. It is noted that all correlations noted at the initial CSF sample have become non-significant. There are two trends worth noting. At day 2 there is a positive trend (non-significant) between the levels of 8-isoprostane and total tau (R=0.714, p=0.071). This correlation becomes weaker at day 3 (R=0.583). At day 0 the correlation was negative (R=-0.064, p=0.853).

At day 3 we note a positive trend between the levels of lactate and NfH (R=0.634, p=0.67). This trend was negative at the initial sample (R=-0.237, n.s.) becoming positive at day 2 (R=0.411, n.s.)

Correlations of CSF markers obtained at day 2 of ELD

		Lactate	8-isopro stane	VEGF	GFAP	NfH	Ab1-42	Total tau
Lactate	Correlation Coefficient	1.000	.000	-.059	.311	.411	-.381	.167
	Sig. (2-tailed)	.	1.000	.881	.453	.272	.352	.693
8-isopro stane	Correlation Coefficient	.000	1.000	-.500	-.252	.	.607	.714
	Sig. (2-tailed)	1.000	.	.207	.548	.	.148	.071
VEGF	Correlation Coefficient	-.059	-.500	1.000	.216	.481	-.515	-.252
	Sig. (2-tailed)	.881	.207	.	.608	.190	.192	.548
GFAP	Correlation Coefficient	.311	-.252	.216	1.000	.	-.468	-.505
	Sig. (2-tailed)	.453	.548	.608	.	.	.289	.248
NfH	Correlation Coefficient	.411	.	.481	.	1.0	.082	.412
	Sig. (2-tailed)	.272	.	.190	.	.	.846	.310
Ab1-42	Correlation Coefficient	-.381	.607	-.515	-.468	.082	1.000	.262
	Sig. (2-tailed)	.352	.148	.192	.289	.846	.	.531
Total tau	Correlation Coefficient	.167	.714	-.252	-.505	.412	.262	1.000
	Sig. (2-tailed)	.693	.071	.548	.248	.310	.531	.

Table 3.8.2.1. Correlations of CSF markers obtained at day 2 of ELD

Correlations of CSF markers obtained at day 3 of ELD

		Lactate	8-isopro stane	VEGF	GFAP	NfH	Ab 1-42	Total tau
Lactate	Correlation Coefficient	1.000	-.262	-.008	.183	.634	.000	.024
	Sig. (2-tailed)	.	.531	.983	.637	.067	1.000	.955
8-isopro stane	Correlation Coefficient	-.262	1.000	-.259	-.200	-.525	-.200	.583
	Sig. (2-tailed)	.531	.	.500	.606	.147	.606	.099
VEGF	Correlation Coefficient	-.008	-.259	1.000	.419	.127	.301	-.527
	Sig. (2-tailed)	.983	.500	.	.228	.726	.431	.145
GFAP	Correlation Coefficient	.183	-.200	.419	1.000	-.186	-.367	-.067
	Sig. (2-tailed)	.637	.606	.228	.	.606	.332	.865
NfH	Correlation Coefficient	.634	-.525	.127	-.186	1.000	-.068	-.251
	Sig. (2-tailed)	.067	.147	.726	.606	.	.861	.515
Ab 1-42	Correlation Coefficient	.000	-.200	.301	-.367	-.068	1.000	-.367
	Sig. (2-tailed)	1.000	.606	.431	.332	.861	.	.332
Total tau	Correlation Coefficient	.024	.583	-.527	-.067	-.251	-.367	1.000
	Sig. (2-tailed)	.955	.099	.145	.865	.515	.332	.

Table 3.8.2.2. Correlations of CSF markers obtained at day 3 of ELD

3.9. Cognitive, biochemical and imaging profile of patients suffering from idiopathic normal pressure hydrocephalus

Although the theory of disturbed CSF dynamics in patients suffering from iNPH is well-established, routine CSF dynamic investigations do not always predict favourable surgical outcomes for patients undergoing cerebrospinal fluid diversion. Recently, a review of metabolic disturbance in chronic adult hydrocephalus suggested that during the course of the syndrome there is a “point of no return” of metabolic failure that becomes uncoupled from the CSF dynamics disturbance and therefore self-sustaining (Kondziella, Sonnewald et al. 2008). The triad of symptoms characterising iNPH has been partly attributed to the CSF dynamics failure although it is not clear what the role of the metabolic failure in the progression or maintenance of these symptoms is.

The exact relationship between the cognitive profile of these patients, as well as imaging characteristics and biochemical profile has not been studied extensively. Tullberg et al. have studied the association of neurofilament light chain (NFL) with white matter pathology (Tullberg, Blennow et al. 2007), whilst another group attempted to study the relationship between neuropsychological parameters, CBF and cerebrovascular reserve capacity (CVR) using PET (Klinge, Ruckert et al. 2002; Klinge, Brooks et al. 2008). Iddon et al have studied the associations between white matter hyperintensities and the neuropsychological profile in a small group of patients with iNPH (Iddon, Pickard et al. 1999). Furthermore, there have been recent attempts to understand whether biochemical markers and imaging

play a role in predicting surgical outcomes (Tarnaris, Watkins et al. 2006; Tarnaris, Kitchen et al. 2008).

There has been little research focusing on cognitive dysfunction in NPH and biomarkers, which have used a comprehensive neuropsychological testing battery (Molins, Catalán et al. 1991; Schettini 1991; Mataro, Poca et al. 2003). Research is also limited regarding the degree of ventriculomegaly and cognitive impairment in NPH (Golomb, de Leon et al. 1994; Palm, Walchenbach et al. 2006; DeVito, Salmond et al. 2007).

It seems necessary to attempt to study all these clinical parameters in a single study in order to understand the interrelationships that characterise the syndrome. The aim of this study was to analyse and report the biochemical, cognitive and imaging profile of patients suffering from iNPH. Furthermore, any relationships obtained may provide further insight into the pathophysiology of iNPH.

3.9.1. Patient characteristics

Ten consecutive patients (8 male, 2 female) were recruited prospectively between February 2005 and February 2007 and investigated for possible iNPH as part of a hydrocephalus research programme carried out in our institution. Inclusion criteria for patients were an ataxic gait, combined with cognitive decline and/or urinary incontinence. All patients had pre-operative imaging that demonstrated ventriculomegaly of communicating type and an Evans Index > 0.3. Furthermore, all patients had at least one measurement of CSF pressure during the insertion of an external lumbar drain to confirm a normal CSF pressure (within the limits of 5-18 mm Hg). All 10 patients recruited suffered from the idiopathic form of normal pressure hydrocephalus and fulfilled the criteria of “probable” NPH as laid out in recently published guidelines (Relkin, Marmarou et al. 2005). All participants had signed a consent form with regards to the aims of the study and the study has been approved by the Local Research Ethics Committee.

Mean patient age was 71.4 (range: 62-82) years. Mean pre-operative duration of symptoms was 35.9 (range: 12-60) months. In seven of the ten patients ataxic gait was the first symptom. All patients had ataxic gait as their main symptom at the time of investigation. Nine patients had problems with memory and cognitive decline. Seven patients had urinary incontinence at the time of investigation.

3.9.2. Statistical analysis

All results were calculated and compared for each group using the non-parametric Wilcoxon signed ranks test. The independent samples t-test was used to compare

means. The Spearman rank order test was used to assess bivariate correlations. The significance level was set at 0.05.

3.9.3. Correlation of age and preoperative symptom duration with any of the variables tested

There was no significant correlation with any of the variables except a significant negative correlation of preoperative symptom duration and total tau levels (R=-0.841, p=0.002; Figure 1).

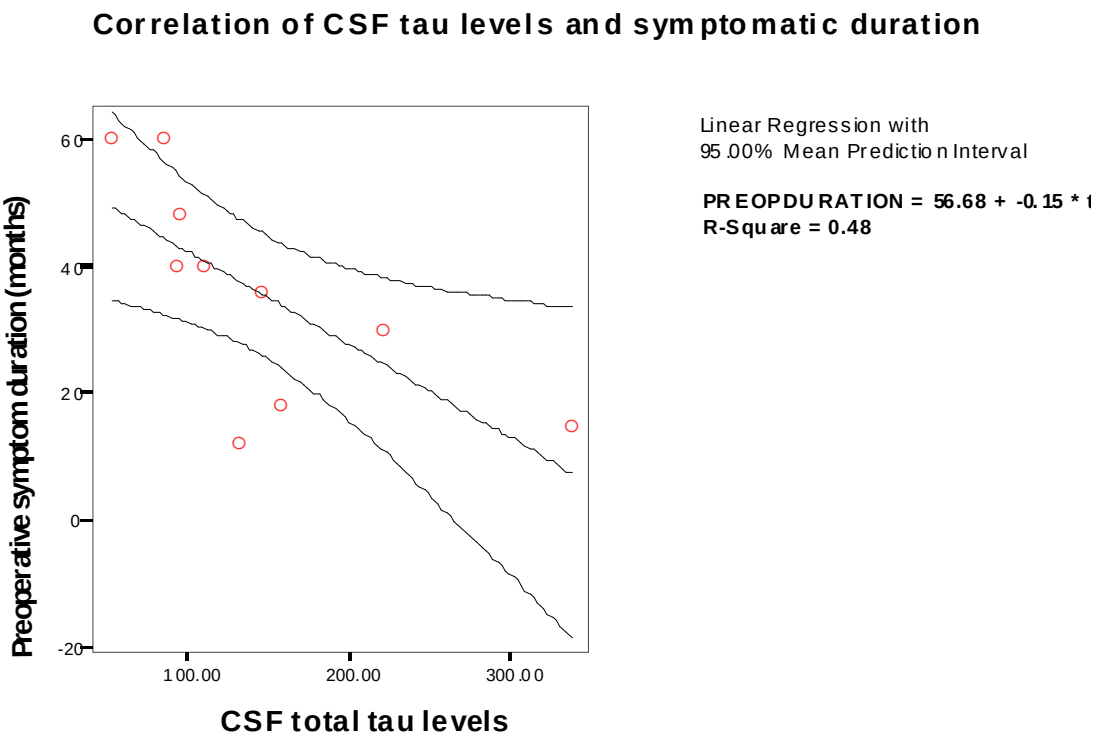


Figure 3.9.3.1. Scatterplot showing linear regression between total tau levels (pg/mL) and preoperative duration of symptoms (in months).

3.9.4. Volumetric analysis

Table 3.9.4.1. shows the mean volumes of the different compartments as well as their respective ratios. The ventricular volume occupies up to 17% of the intracranial volume in the specific area measured. There appears to be in our cohort a larger volume of DWMH (mean: 36.9 mm^3) when compared to the PVL volume (mean 21.5 mm^3). Subsequently PVL occupy 2.99% of the intracranial volume, whereas DWMH occupy 4.13% of the ICV. The volume of white matter had a mean value of 302 mm^3 occupying almost 42% of the intracranial volume.

Volume (mm3)	Mean	SD	% ICV
Ventricular volume (VV)	124	56.11	17.22
Periventricular lucencies (PVL)	21.54	8.37	2.99
Deep white-matter hyperintensities (DWMH)	36.92	58.22	5.13
White matter (WM)	302.04	117.68	41.96
VV/PVL	7.38	5.93	
VV/DWMH	8.43	7.08	
PVL/WM	0.08	0.05	
DWMH/WM	0.05	0.04	
Intracerebral volume (ICV)	719.69	239.74	

Table 3.9.4.1. Volume characteristics in 10 patients with iNPH as well as their ratios to the intracranial volume.

3.9.5. Neuropsychological testing

Table 3.9.5.1 presents the neuropsychological profile of 10 patients. Due to small numbers the patients were grouped as either normal or mild/moderate-severe cognitive impairment. No association was found between the results of the different tests. Also only one performed in the normal range on the Trail Making Tests B, a test of frontal executive functioning. These findings are therefore consistent with the 'fronto-subcortical dementia' profile associated with NPH.

	Test of intelligence	**Recognition Memory tests**		**Executive Functioning**		**Test of subcortical function**
Tests	**WAIS-R**	**RMT words**	**RMT faces**	**Phonemic VF**	**Trails**	**Speed (cancelling)**
Normal	4	6	4	4	1	0
Mild/Moderate - severe cognitive impairment	6	4	6	6	9	10

Table 3.9.5.1. Neuropsychological profile of the 10 patients by using a specific battery of test.

3.9.6. CSF biochemical profile in lumbar CSF

The mean value of lactate in our cohort was 2.24; 3 patients had pathological lactate values (>2.4 which was our laboratory reference value). There are no other studies that present CSF reference values for 8-isoprostanes and VEGF. One of

our patients had no detectable VEGF in the CSF. The mean value of the GFAP levels was 2.19 pg/mL and was within the normal range (upper reference value: 9 pg/mL); one of our patients had pathologically elevated GFAP levels. The mean levels of NfH in our cohort was 0.63 pg/mL which was normal (upper reference value: 0.73 ng/mL); no patient had pathologically raised NfH levels. The mean value of $A\beta_{1-42}$ was 366 pg/mL (median 315 pg/mL) which is lower than the control reference value given by the manufacturers (median 849 pg/mL)(Hulstaert, Blennow et al. 1999); only one patient had pathological high values. Also the total tau levels had a mean of 143 pg/mL (median: 120 pg/mL) which was lower than the control subjects median value for total tau (195 pg/mL) as provided by the manufacturer (Hulstaert, Blennow et al. 1999); in the case of total tau 2 subjects had values above that median value.

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Lactate	**10**	**1.32**	**4.32**	**2.24**	**.90**
8-Isoprostanes	**10**	**36.78**	**39.98**	**38.17**	**1.03**
VEGF	**10**	**.00**	**10.00**	**4.49**	**3.74**
GFAP	**10**	**.05**	**14.93**	**2.19**	**4.89**
NF (h)	**10**	**.00**	**.63**	**.17**	**.18**
Ab 1-42	**10**	**111.92**	**1029.00**	**365.92**	**247.85**
Total tau	**10**	**50.73**	**338.00**	**142.87**	**82.74**

Table 3.9.6.1. Biochemical profile of the CSF obtained during the insertion of a lumbar drain in 10 patients with iNPH. Lactate levels in (mmol/L), VEGF and NfH levels in ng/mL, 8-isoprostane, GFAP, Aβ 1-42 and tau levels in pg/mL.

3.9.7. Correlation of CSF markers with volumetric analysis

Neither lactate nor 8-isoprostane levels had significant correlations with any of the analysed volumes. However, there was a negative trend between the isoprostane levels and the VV/WM ratio (R= -0. 667, p= 0.071). There was a significant positive correlation (R= 0.648, p=0.043) between the levels of VEGF and the VV/ICV ratio; there was also a positive trend between the VEGF and the WM volume (R= 0.667, p= 0.071). There was a significant positive correlation of the levels of GFAP and the VV/ DWMH ratio (R= 0.828, p=0.006). There was a significant negative correlation between the levels of NfH and the VV/ICV ratio (R= -0.657, p=0.039), as well as a negative trend (R= -0.612, p=0.06) with the IVV. In addition, the white matter and NfH levels were inversely correlated (R= -0.778, p=0.023). There was also a significant positive correlation between the PVL/WM ratio and NfH levels (R= 0.738, p= 0.037) and a positive trend with the DWMH/WM ratio (R= 0.667, p= 0.071). $A\beta_{1-42}$ and total tau had no significant correlations with any of the structural volumes, however there was a negative trend between tau and VV/DWMH (R= -0.617, p= 0.077).

3.9.8. Correlations of CSF markers and neuropsychological profile

There was no difference in any of the levels of the examined markers between the patients who had normal and mild/moderate-severe cognitive decline in WAIS-R, RMT Words, and Verbal Fluency test. Patients who were in the normal category, when examined with the RMT Faces test, had lower lactate levels compared to the subjects who performed worse in this particular test (independent samples t-test,

p=0.060). It was also observed that the patient who has pathologically high GFAP levels showed no cognitive decline when tested on the Trail Making Test B.

3.9.10. Correlations of volumetric analysis and neuropsychological profile

Patients who performed normal in the RMT Words had significantly higher ICV than the rest of the patients (879.42 +/- 215 vs. 574.05 +/- 155.6 mm3; p=0.05, independent samples t-test).

3.10.1. Rostrocaudal gradient of CSF markers

The dynamics of CSF markers along the craniospinal axis has not been adequately studied (Grove, Schechter et al. 1982; Menachem, Persson et al. 1989; Sommer, Gaul et al. 2002). Many proteins are produced by the brain parenchyma and then are transported across the ependyma into the CSF spaces. A fraction of some of them is derived from serum, whereas others are produced exclusively by the brain parenchyma. The movement of the molecules across the blood-brain barrier (BBB) and the blood-CSF barrier depends on concentration gradients. The particular dynamics of the brain-derived proteins depend on their sources which are either the
brain cells (neurons, glial cells) or the leptomeningeal cells (Reiber 2003). From then following the bulk flow a portion flows down the spinal axis to the lumbar thecal sac where appropriate reabsroption as well as secretion occurs.

Access to lumbar CSF provides us with diagnostic information about different neurological conditions (Hühmer, Biringer et al. 2006). Levels of markers in the CSF may be studied and conclusions about the pathophysiology mechanisms underlying each condition may be extrapolated (Reiber 1998). However, it is not true that the concentrations of proteins in the lumbar thecal sac reflect accurately the concentrations in higher levels, such as the cisternal or ventricular CSF. Hence obtaining information only from lumbar CSF may leads us to erroneous conclusions. Sampling of higher levels along the craniospinal axis may not be ethically feasible hence limiting the yield of diagnostic information from lumbar CSF only.

Although numerous studies have examined the diagnostic and prognostic significance of markers in lumbar CSF of patients with iNPH, less data exists in the difference between ventricular and lumbar concentration of different markers.

3.10.2. Methods: Ventricular (vNPH) and lumbar CSF (lNPH) was collected from 8 patients fulfilling the criteria of “probable” iNPH. Cisternal CSF was collected from 6 patients suffering from trigeminal neuralgia (TGN) acting as control group (CC). Lumbar CSF was collected from 6 patients investigated for headache and acted as a control group for lumbar samples (LC). The mean age of the 6 TGN patients was 60 (+/-9.3) years (range: 52-76). The mean age of the 6 patients investigated for headache was 52.7 (+/- 10.6) years (range: 37-72). The difference in age of the two control groups was not statistically significant (Mann Whitney U test, p=0.234).

3.10.3. Rostrocaudal gradient of Lactate

There is no correlation of lactate levels and age neither in iNPH nor on control subjects.

Correlation of age and ventricular lactate levels in NPH

			age_nph	vCSF_nph
Spearman's rho	Age of patients	Correlation Coefficient	1.000	-.096
		Sig. (2-tailed)	.	.821
		N	8	8
	Ventricular lactate levels	Correlation Coefficient	-.096	1.000
		Sig. (2-tailed)	.821	.
		N	8	8

Table 3.10.3.1. Correlation of age and ventricular lactate levels in NPH

Correlation of age and lumbar lactate levels in NPH

			age_nph	lCSF_nph
Spearman's rho	Age of patients	Correlation Coefficient	1.000	-.108
		Sig. (2-tailed)	.	.799
		N	8	8
	Lumbar lactate levels	Correlation Coefficient	-.108	1.000
		Sig. (2-tailed)	.799	.
		N	8	8

Table 3.10.3.2. Correlation of age and lumbar lactate levels in NPH

Correlation of age and cisternal lactate levels in TGN

			age_ctrl	cCSF_ctrl
Spearman's rho	Age of patientsl	Correlation Coefficient	1.000	-.029
		Sig. (2-tailed)	.	.957
		N	6	6
	Cisternal lactate levels	Correlation Coefficient	-.029	1.000
		Sig. (2-tailed)	.957	.
		N	6	6

Table 3.10.3.3. Correlation of age and cisternal lactate levels in NPH

Comparison of means between ventricular and lumbar CSF lactate levels in NPH

	N	Mean	Std. Deviation	Minimum	Maximum
ventricular lactate (mmol/L)	8	2.0213	.50879	1.55	2.91
lumbar lactate	8	2.3000	.93274	1.56	4.32

Table 3.10.3.4. Comparison of means between ventricular and lumbar CSF lactate levels in NPH

The rostrocaudal gradient (RCG) for lactate in iNPH is 0.88.

Cisternal lactate levels in patients with TGN (mmol/L)

	N	Mean	Std. Deviation	Std. Error Mean
Cisternal Lactate	6	1.8783	.66490	.27145

Table 3.10.3.5. Cisternal lactate levels in patients with TGN

The RCG of lactate in the control group is 1.17. The values for the control lumbar lactate levels were 1.59 (+/- 0.30) (Wandrup, Tvede et al. 1989). There is no significant difference between the ventricular and lumbar samples in patients with iNPH (Wilcoxon signed ranks test, exact significance (two tailed) p=0.688). There is no statistical difference between the ventricular lactate levels in NPH and the cisternal lactate levels in the control group (Kruskal Wallis test, p=0.519).

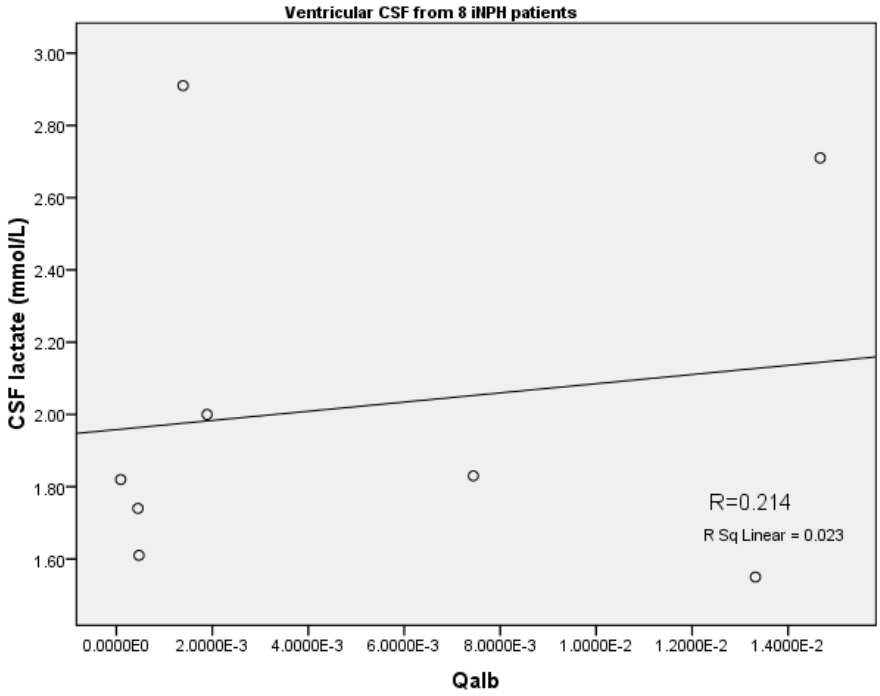


Figure 3.10.3.1. Scatterplot and linear regression between Qalb and lactate levels in CSF

3.10.4. Rostrocaudal gradient of 8-isoprostane

There was a significant negative correlation of the age of iNPH patients with the lumbar levels of 8-isoprostane (table 3.10.4.2.).

Correlation of age with ventricular 8-isoprostane levels in patients with iNPH

			age_nph	vCSF_nph
Spearman's rho	Age of patients	Correlation Coefficient	1.000	.491
		Sig. (2-tailed)	.	.217
		N	8	8
	8-isoprostane levels of ventricular CSF	Correlation Coefficient	.491	1.000
		Sig. (2-tailed)	.217	.
		N	8	8

Table 3.10.4.1. Correlation of age and ventricular 8-isoprostane levels in NPH

Correlation of age with lumbar 8-isoprostane levels in patients with NPH

			age_nph	lCSF_nph
Spearman's rho	Age of patients	Correlation Coefficient	1.000	-.753*
		Sig. (2-tailed)	.	.031
		N	8	8
	8-isoprostane levels of lumbar CSF	Correlation Coefficient	-.753*	1.000
		Sig. (2-tailed)	.031	.
		N	8	8

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.10.4.2. Correlation of age and lumbar 8-isoprostane levels in NPH

Correlation of age with lumbar 8-isoprostane levels in patients with TGN

			age_ctrl	cCSF_ctrl
Spearman's rho	Age of patients	Correlation Coefficient	1.000	.086
		Sig. (2-tailed)	.	.872
		N	6	6
	8-isoprostane levels of cisternal CSF	Correlation Coefficient	.086	1.000
		Sig. (2-tailed)	.872	.
		N	6	6

Table 3.10.4.3. Correlation of age and lumbar 8-isoprostane levels in TGN

Correlation of age and lumbar 8-isoprostane levels in control subjects

			VAR00003	VAR00004
Spearman's rho	Age of patients	Correlation Coefficient	1.000	.800
		Sig. (2-tailed)	.	.200
		N	6	6
	Lumbar 8-isoprostane levels	Correlation Coefficient	.800	1.000
		Sig. (2-tailed)	.200	.
		N	6	6

Table 3.10.4.4. Correlation of age and lumbar 8-isoprostane levels in TGN

The RCG for 8-isoprostane in iNPH is 1. The RCG for 8-isoprostane in control subjects is 1.06. There is no significant difference between the levels of ventricular and lumbar levels in iNPH (Wilcoxon signed ranks test, p=0.641). There is a significant difference between the ventricular and cisternal levels of 8-isoprostane between iNPH patients and TGN controls (Mann-Whitney test, p=0.02). There is not a significant difference between the lumbar levels of 8-isoprostane between iNPH patients and controls (Mann-Whitney test, p=0.74).

Comparison of means between ventricular and lumbar CSF 8-isoprostane levels in NPH

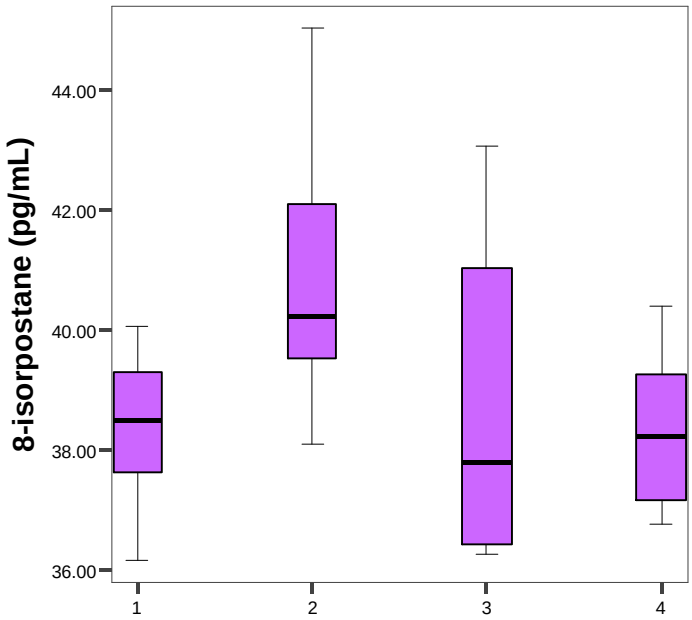
	N	Mean	Std. Deviation	Minimum	Maximum
ventricular 8-isoprostane (pg/mL)	8	38.3938	1.26782	36.16	40.07
lumbar 8-isoprostane	8	38.3288	1.32331	36.78	40.41

Table 3.10.4.5. Comparison of means between ventricular and lumbar CSF 8-isoprostane levels in NPH

Comparison of means between cisternal and lumbar CSF 8-isoprostane levels in control subjects

sample_type	N	Mean	Std. Deviation	Std. Error Mean
cisternal 8-isoprostane (pg/mL0	6	40.8833	2.43341	.99344
lumbar 8-isoprostane	6	38.7450	3.13911	1.56956

Table 3.10.4.6. Comparison of means between cisternal and lumbar CSF 8-isoprostane levels in TGN



1: ventricular NPH, 2: Cisternal control, 3: Lumbar control, 4: Lumbar NPH

Figure 3.10.4.1. Boxplot levels of CSF 8-isoprostane in the 4 groups tested

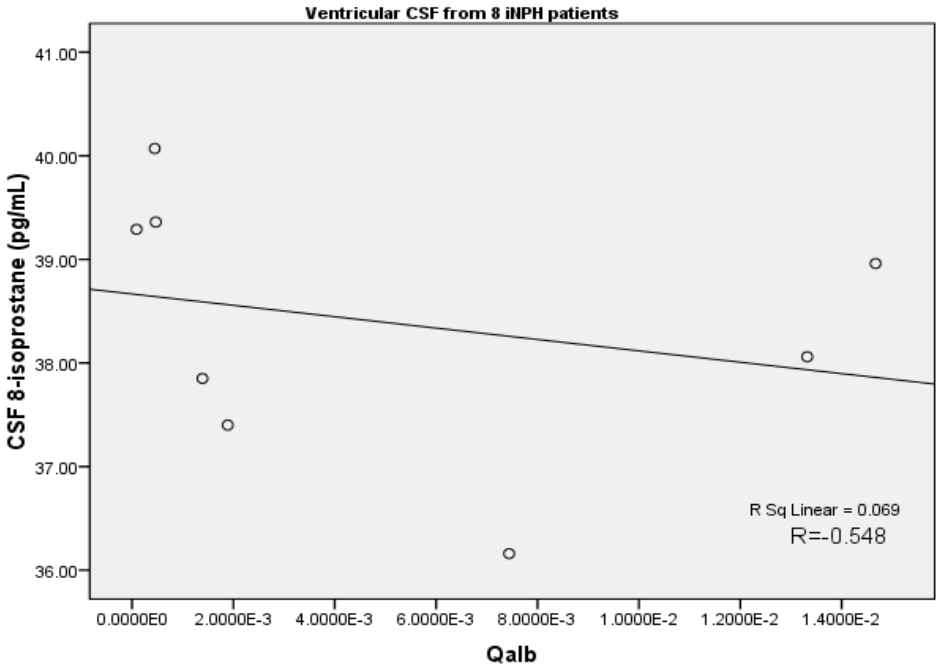


Figure 3.10.4.2. Scatterplot and linear regression between Qalb and 8-isoprostane levels in CSF

3.10.5.1. Rostrocaudal gradient of VEGF

There is no correlation between the age of the subjects and the VEGF levels in either NPH or control subjects. However, there is a positive trend between VEGF cisternal levels and age in patients with TGN.

Correlation of age and ventricular VEGF levels in NPH

			age_nph	vCSF_nph
Spearman's rho	Age of patients	Correlation Coefficient	1.000	-.108
		Sig. (2-tailed)	.	.799
		N	8	8
	Ventricular VEGF levels	Correlation Coefficient	-.108	1.000
		Sig. (2-tailed)	.799	.
		N	8	8

Table 3.10.5.1. Correlation of age and ventricular VEGF levels in NPH

Correlation of age and lumbar VEGF levels in NPH

			age_nph	lCSF_nph
Spearman's rho	Age of patiensts	Correlation Coefficient	1.000	-.024
		Sig. (2-tailed)	.	.955
		N	8	8
	Lumbar VEGF levels	Correlation Coefficient	-.024	1.000
		Sig. (2-tailed)	.955	.
		N	8	8

Table 3.10.5.2. Correlation of age and lumbar VEGF levels in NPH

Correlation of age and cisternal VEGF levels in TGN

			age_ctrl	cCSF_ctrl
Spearman's rho	Age of patients	Correlation Coefficient	1.000	.771
		Sig. (2-tailed)	.	.072
		N	6	6
	Cisternal VEGF levels	Correlation Coefficient	.771	1.000
		Sig. (2-tailed)	.072	.
		N	6	6

Table 3.10.5.3. Correlation of age and cisternal VEGF levels in TGN

Correlation of age and lumbar VEGF levels in control subjects

			VAR00001	VAR00002
Spearman's rho	Age of patients	Correlation Coefficient	1.000	.500
		Sig. (2-tailed)	.	.667
		N	6	6
	Lumbar VEGF levels	Correlation Coefficient	.500	1.000
		Sig. (2-tailed)	.667	.
		N	6	6

Table 3.10.5.4. Correlation of age and lumbar VEGF levels in TGN

The RCG for VEGF in NPH and control subjects is 3.2 and 0.38 respectively. There was a statistical significant difference between the ventricular and lumbar VEGF levels in NPH (Wilcoxon signed ranks test, p=0.036). There was no statistical difference between the ventricular and cisternal VEGF levels in NPH and control subjects respectively (Kruskall-Wallis test, p=0.071). There was a significant difference between the lumbar VEGF levels in NPH and control subjects (Mann-Whitney test, p=0.014).

Comparison of means between ventricular and lumbar CSF VEGF levels in NPH

	N	Mean	Std. Deviation	Minimum	Maximum
ventricular VEGF (ng/ml)	8	18.2375	15.71671	6.10	44.99
lumbar VEGF	8	5.6875	5.38652	.00	15.49

Table 3.10.5.5. Comparison of means between ventricular and lumbar CSF VEGF levels in NPH

Comparison of means between cisternal and lumbar CEF VEGF levels in control subjects

	sample_type	N	Mean	Std. Deviation	Std. Error Mean
ng/mL	cisternal VEGF	6	7.7917	8.84722	3.61186
	lumbar VEGF	6	20.4267	2.75264	1.58924

Table 3.10.5.6. Comparison of means between cisternal and lumbar CSF VEGF levels in controls

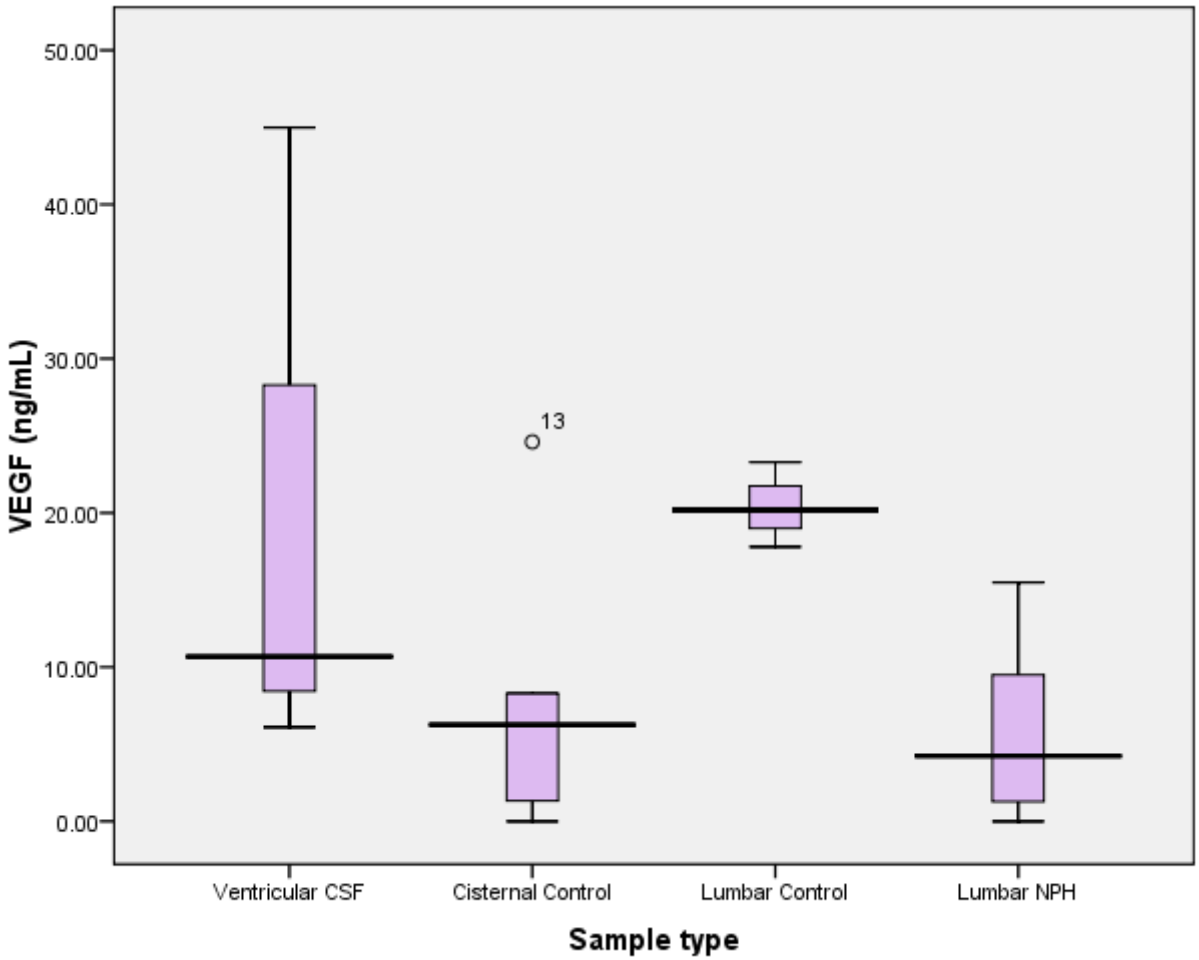


Figure 3.10.5.1. Boxplot levels of CSF VEGF levels in the 4 groups tested

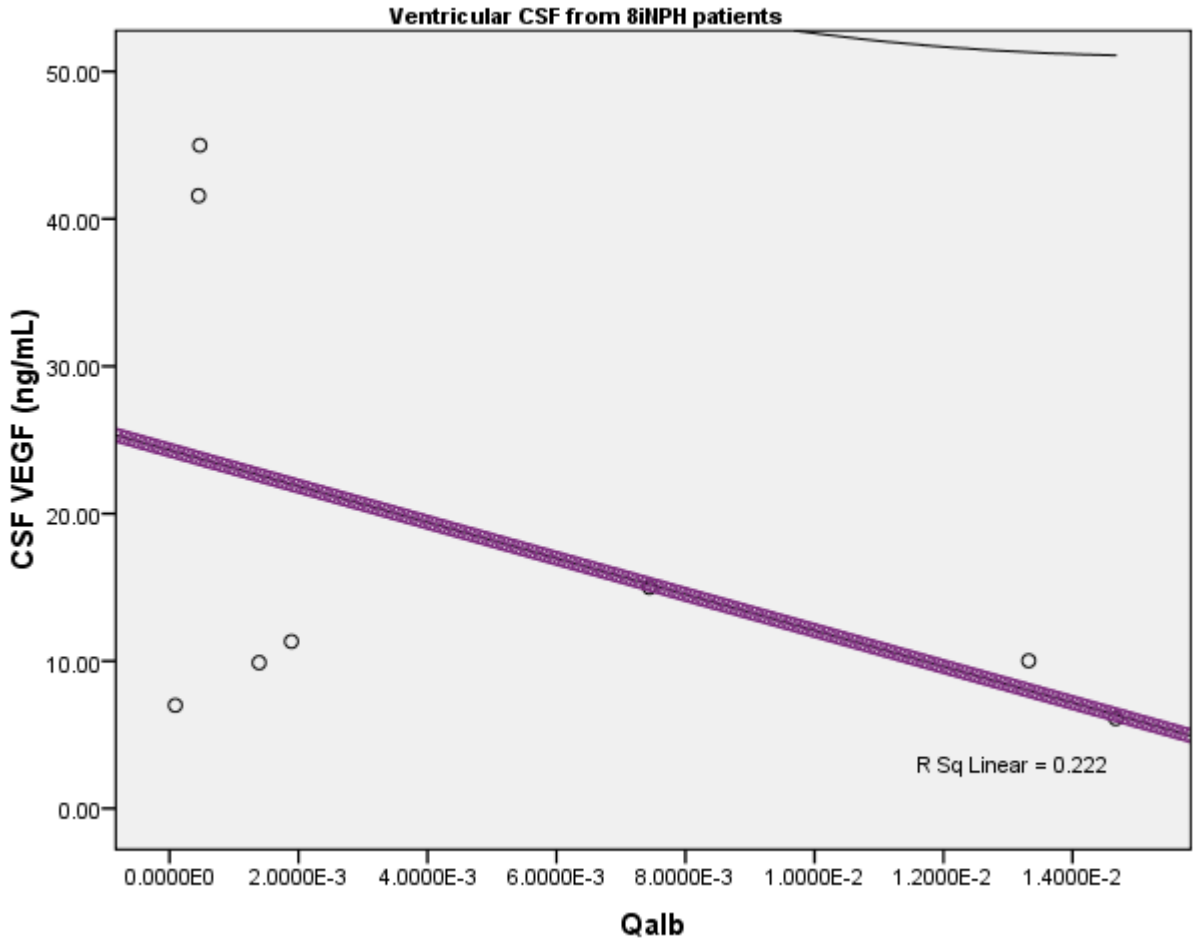


Figure 3.10.5.2. Scatterplot and linear regression between Qalb and VEGF levels in CSF

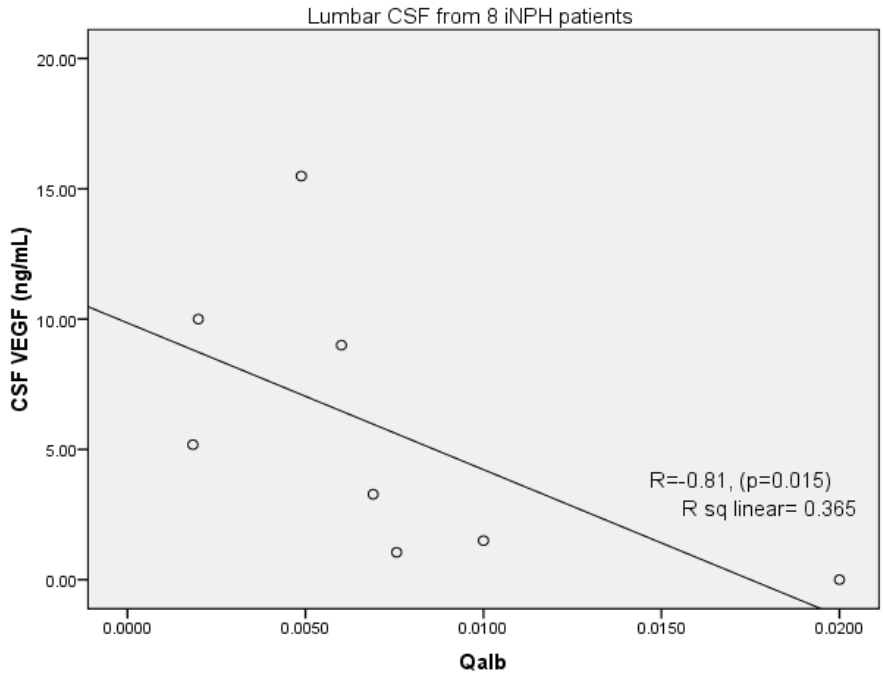


Figure 3.10.5.3. Scatterplot and linear regression between Qalb and VEGF levels in lumbar CSF from 8 iNPH patients

3.10.6. Rostrocaudal gradient of GFAP

There is no correlation of age and ventricular or lumbar GFAP levels in patients with NPH. However, there is a significant negative correlation between the GFAP levels and the age of the control group ($p<0.001$). With regards to the GFAP levels of lumbar CSF from control subjects the reference median value of 0 (range: 0-60) pg/mL were used (Petzold, Brettschneider et al. 2009). The median GFAP in cisternal CSF in patients with TGN is 0.2 pg/mL.

Correlation of age and ventricular GFAP levels in NPH

			age_nph	vCSF_nph
Spearman's rho	Age of patients	Correlation Coefficient	1.000	-.250
		Sig. (2-tailed)	.	.589
		N	8	8
	Ventricular GFAP levels	Correlation Coefficient	-.250	1.000
		Sig. (2-tailed)	.589	.
		N	8	8

Table 3.10.6.1. Correlation of age and ventricular GFAP levels in NPH

Correlation of age and lumbar GFAP levels in NPH

			age_nph	lCSF_nph
Spearman's rho	Age of patients	Correlation Coefficient	1.000	-.398
		Sig. (2-tailed)	.	.329
		N	8	8
	Lumbar GFAP levels	Correlation Coefficient	-.398	1.000
		Sig. (2-tailed)	.329	.
		N	8	8

Table 3.10.6.2. Correlation of age and lumbar GFAP levels in NPH

Correlation of age and cisternal GFAP levels in control subjects

			age_ctrl	cCSF_ctrl
Spearman's rho	Age of patients	Correlation Coefficient	1.000	-1.000**
		Sig. (2-tailed)	.	.000
		N	6	6
	Cisternal GFAP levels	Correlation Coefficient	-1.000**	1.000
		Sig. (2-tailed)	.000	.
		N	6	6

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.10.6.3. Correlation of age and cisternal GFAP levels in controls

The RCG in NPH is 8.85. In normal subjects it cannot be calculated since the median value for a control population is 0 (with a range of 0-60). Although one will assume that the gradient is >1 since the cisternal median GFAP value is 0.2 pg/mL. There is a statistical significant difference between the ventricular and lumbar GFAP levels of NPH patients (Wilcoxon signed ranks test, p=0.034). There

is no statistical difference between the ventricular GFAP levels in NPH and the cisternal GFAP levels in patients with TGN (Mann-Whitney test, p=0.267). The statistics between cisternal and lumbar levels in the control group were not calculated.

Comparison of means between ventricular and lumbar GFAP levels in NPH

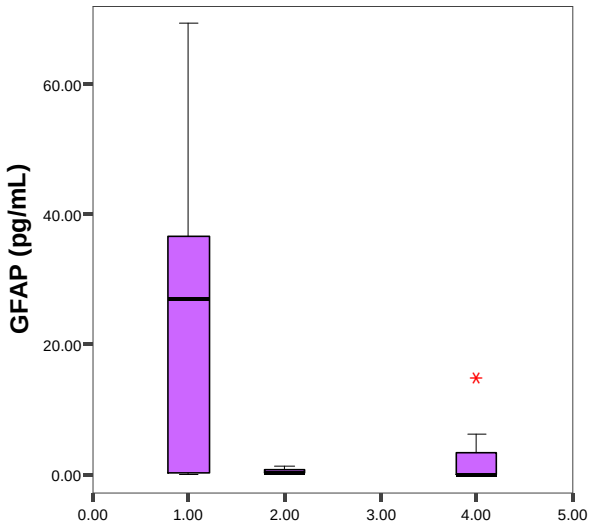
	N	Mean	Std. Deviation	Minimum	Maximum
Ventricular GFAP (pg/mL)	8	24.3529	26.10363	.16	69.32
lumbar GFAP	8	2.7588	5.38169	.05	14.93

Table 3.10.6.4. Comparison of means between ventricular and lumbar CSF GFAP levels in NPH

Cisternal GFAP levels in patients with TGN (pg/mL)

	N	Minimum	Maximum	Mean	Std. Deviation
Cisternal GFAP	6	.13	1.31	.5467	.66199

Table 3.10.6.5. Mean cisternal GFAP levels in TGN



CSF sample 1:Ventricular NPH,2: Cisternal control, 3: lumbar control (not available), 4: Lumbar NPH

Figure 3.10.6.1. Boxplot levels of CSF GFAP levels in the 3 groups tested

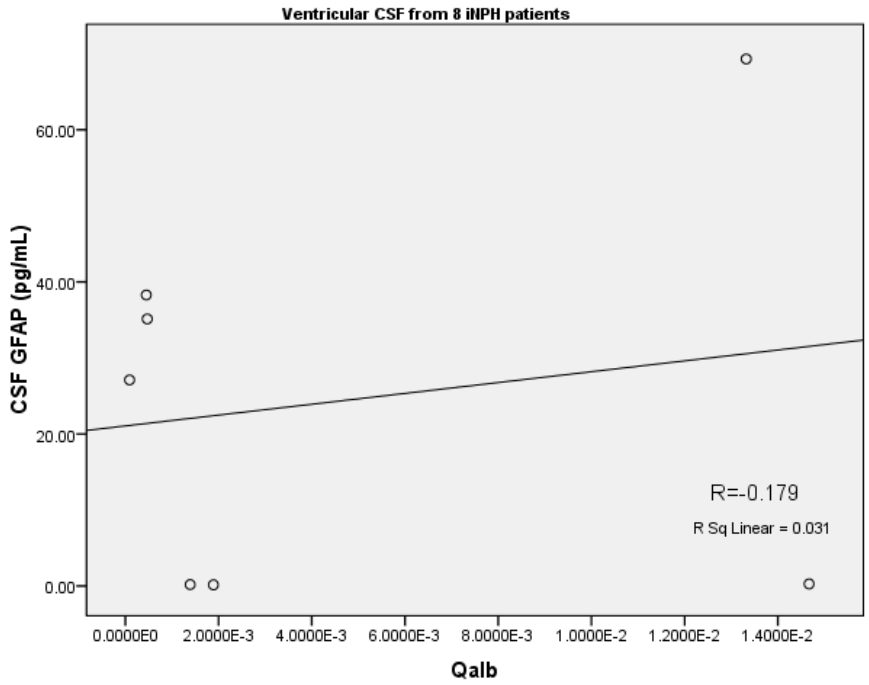


Figure 3.10.6.2. Scatterplot and linear regression between Qalb and GFAP levels in CSF

3.10.7 Rostrocaudal gradient of NfH

There is no correlation of age with NfH levels in any of the groups tested. With regards to the NfH levels of lumbar CSF from control subjects the reference mean value of 0.25 (+/-0.23) ng/mL was used (Petzold, Keir et al. 2003).

Correlation of age and ventricular NfH levels in NPH

			age_nph	vCSF_nph
Spearman's rho	Age of patients	Correlation Coefficient	1.000	.430
		Sig. (2-tailed)	.	.287
		N	8	8
	Ventricular NfH levels	Correlation Coefficient	.430	1.000
		Sig. (2-tailed)	.287	.
		N	8	8

Table 3.10.7.1. Correlation of age and ventricular NfH levels in NPH

Correlation of age with lumbar NfH levels in NPH

			age_nph	lCSF_nph
Spearman's rho	Age of patients	Correlation Coefficient	1.000	-.359
		Sig. (2-tailed)	.	.382
		N	8	8
	Lumbar NfH levels	Correlation Coefficient	-.359	1.000
		Sig. (2-tailed)	.382	.
		N	8	8

Table 3.10.7.2. Correlation of age and lumbar NfH levels in NPH

Correlation of age and cisternal NfH levels in TGN

			age_ctrl	cCSF_ctrl
Spearman's rho	Age of patients	Correlation Coefficient	1.000	.154
		Sig. (2-tailed)	.	.805
		N	6	5
	Cisternal NfH levels	Correlation Coefficient	.154	1.000
		Sig. (2-tailed)	.805	.
		N	5	5

Table 3.10.7.3. Correlation of age and cisternal NfH levels in TGN

The RRG of NfH is therefore 4.84 and 1.08 in NPH and control subjects respectively. There is no significant difference between the ventricular and lumbar levels in NPH (Wilcoxon signed ranks test, p=0.208). There is no difference between the ventricular levels in NPH and cisternal levels in TGN (Mann-Whitney test, p=0.298). There were no statistical calculations between the lumbar levels of NfH in NPH and control subjects.

Comparison of means between ventricular and lumbar CSF NfH levels in NPH

	N	Mean	Std. Deviation	Minimum	Maximum
ventricular NfH (ng/mL)	8	.9225	1.83993	.00	5.45
lumbar NfH	8	.1988	.19475	.00	.63

Table 3.10.7.4. Comparison of means between ventricular and lumbar CSF NfH levels in NPH

Cisternal NfH levels in patients with TGN (ng/mL)

	N	Mean	Std. Deviation	Minimum	Maximum
Cisternal NfH	6	.2740	.59597	.00	1.34

Table 3.10.7.5. Mean cisternal NfH levels in TGN

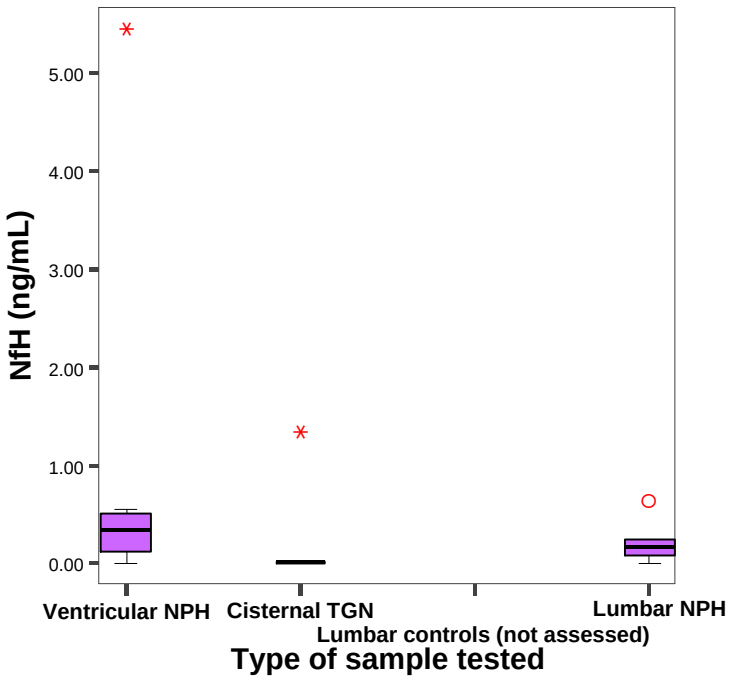


Figure 3.10.7.1. Boxplot levels of CSF NfH levels in the 3 groups tested

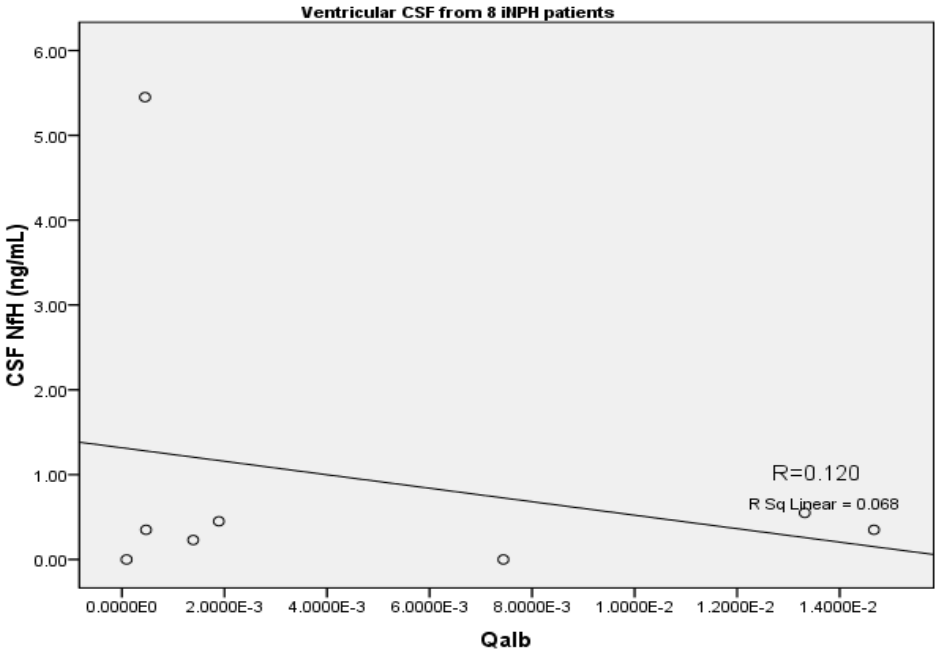


Figure 3.10.7.2. Scatterplot and linear regression between Qalb and NfH levels in CSF

3.10.8 Rostrocaudal gradient of Aβ 1-42

There is no correlation of age with either ventricular of lumbar Aβ 1-42 levels in NPH. There is a trend (but not a significant correlation) between the age of patients with TGN and Aβ 1-42 levels. With regards to the Aβ 1-42 levels of lumbar CSF from control subjects the reference mean value of 849 (682-1063) pg/mL was used (Hulstaert, Blennow et al. 1999). The median cisternal value in patients with TGN is 930.05 (113-1458) pg/mL.

Correlation of age with ventricular Ab levels in NPH

			age_nph	vCSF_nph
Spearman's rho	Age of patients	Correlation Coefficient	1.000	.036
		Sig. (2-tailed)	.	.939
		N	8	8
	Ventricular Ab levels	Correlation Coefficient	.036	1.000
		Sig. (2-tailed)	.939	.
		N	8	8

Table 3.10.8.1. Correlation of age and ventricular Aβ 1-42 levels in NPH

Correlation of age with lumbar Ab levels in NPH

			age_nph	lCSF_nph
Spearman's rho	Age of patients	Correlation Coefficient	1.000	.335
		Sig. (2-tailed)	.	.417
		N	8	8
	Lumbar Ab levels	Correlation Coefficient	.335	1.000
		Sig. (2-tailed)	.417	.
		N	8	8

Table 3.10.8.2. Correlation of age and lumbar Aβ 1-42 levels in NPH

Correlation of age with cisternal Ab levels in TGN

			age_ctrl	cCSF_ctrl
Spearman's rho	Age of patients	Correlation Coefficient	1.000	.771
		Sig. (2-tailed)	.	.072
		N	6	6
	Cisternal Ab levels	Correlation Coefficient	.771	1.000
		Sig. (2-tailed)	.072	.
		N	6	6

Table 3.10.8.3. Correlation of age and cisternal Aβ 1-42 levels in TGN

The RCG is therefore 0.93 and 1.09 in NPH and control subjects respectively. There was no difference between the ventricular and lumbar levels in patients with NPH (Wilcoxon signed ranks test, p=0.398). There was a significant difference between the ventricular levels of Aβ 1-42 in patients with NPH and cisternal levels in TGN (Mann-Whitney test, p=0.035). No statistical calculations were performed for difference between lumbar concentrations in NPH and control subjects.

Comparison of means between ventricular and lumbar Ab levels in NPH

	N	Mean	Std. Deviation	Minimum	Maximum
Ventricular Ab (pg/mL)	8	229.3086	100.27197	108.00	409.92
Lumbar Ab	8	246.7813	86.71816	111.92	325.00

Table 3.10.8.4. Comparison of means between ventricular and lumbar CSF Aβ 1-42 levels in NPH

Cisternal Ab levels in patients with TGN (pg/mL)

	N	Mean	Std. Deviation	Minimum	Maximum
Cisternal Ab	6	841.3250	451.87560	113.00	1458.00

Table 3.10.8.5. Mean cisternal Aβ 1-42 levels in TGN

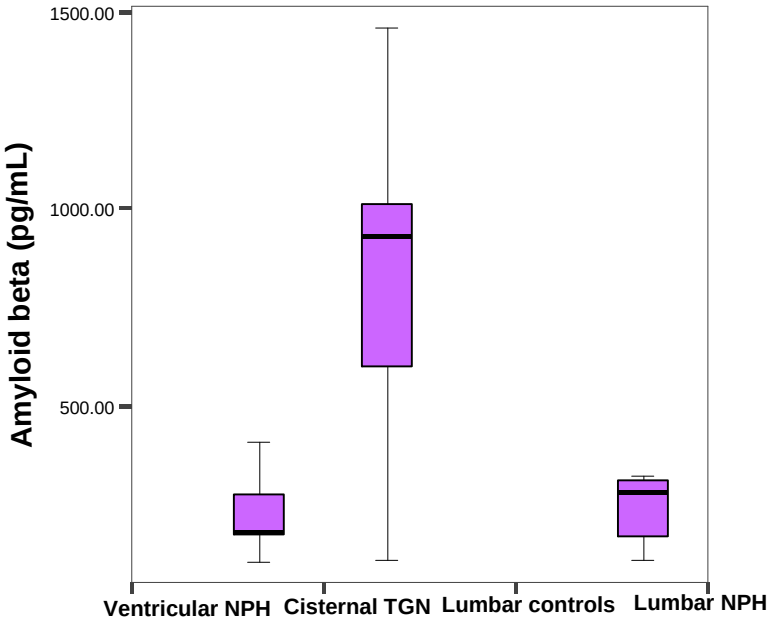


Figure 3.10.8.1. Boxplot levels of CSF Aβ 1-42 levels in the 3 groups tested

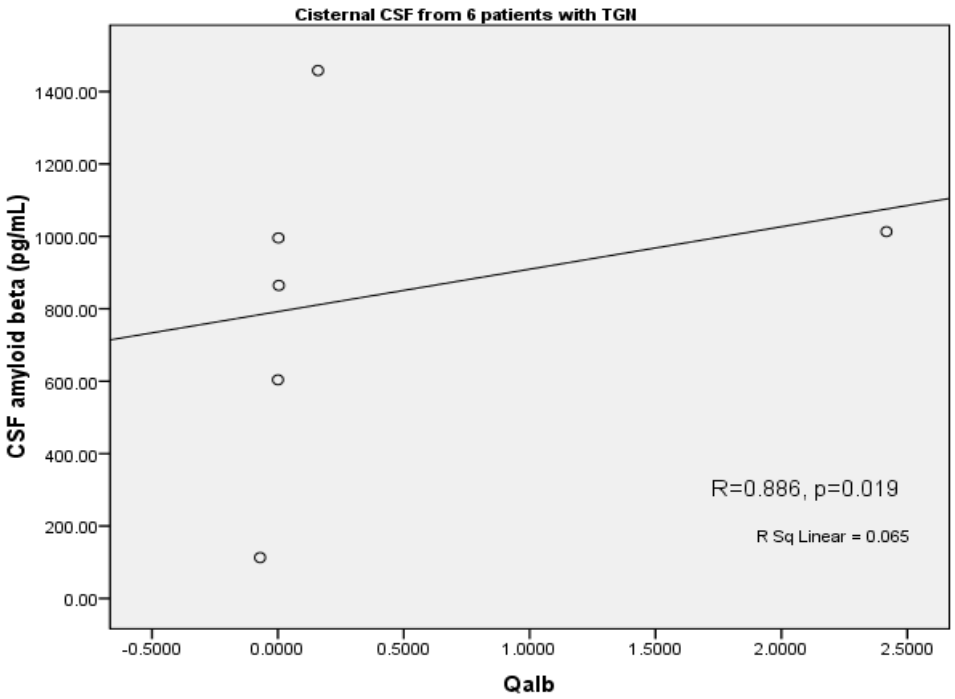


Figure 3.10.8.2. Scatterplot and linear regression between Qalb and Aβ 1-42 levels in CSF

3.10.9. Rostrocaudal gradient of total tau

There is no correlation of total tau levels with age in any of the patient groups. With regards to the total tau levels of lumbar CSF from control subjects the reference mean value of 195 (121-294) pg/mL was used (Hulstaert, Blennow et al. 1999). The median value of cisternal total tau levels in patients with TGN is 285 pg/mL.

Correlation of age with ventricular total tau levels in NPH

			age_nph	vCSF_nph
Spearman's rho	Age of patients	Correlation Coefficient	1.000	.623
		Sig. (2-tailed)	.	.099
		N	8	8
	Ventricular total tau levels	Correlation Coefficient	.623	1.000
		Sig. (2-tailed)	.099	.
		N	8	8

Table 3.10.9.1. Correlation of age and ventricular total tau levels in NPH

Correlation of age with lumbar total tau levels in NPH

			age_nph	lCSF_nph
Spearman's rho	Age of patients	Correlation Coefficient	1.000	.108
		Sig. (2-tailed)	.	.799
		N	8	8
	Lumbar total tau levels	Correlation Coefficient	.108	1.000
		Sig. (2-tailed)	.799	.
		N	8	8

Table 3.10.9.2. Correlation of age and lumbar total tau levels in NPH

Correlation of age with cisternal total tau levels in TGN

			age_ctrl	cCSF_ctrl
Spearman's rho	Age of patients	Correlation Coefficient	1.000	.200
		Sig. (2-tailed)	.	.704
		N	6	6
	Cisternal total tau levels	Correlation Coefficient	.200	1.000
		Sig. (2-tailed)	.704	.
		N	6	6

Table 3.10.9.3. Correlation of age and cisternal total tau levels in TGN

Therefore, the RCG for total tau is 6.01 and 1.46 in NPH and control subjects respectively. There is a significant difference between the ventricular and lumbar levels in NPH (Wilcoxon signed ranks test, p=0.028. The difference between the ventricular total tau levels in NPH and cisternal total tau levels in patients with TGN is statistically significant (Mann Whitney test, p=0.002)

Comparison of means between ventricular and lumbar total tau levels in NPH

	N	Mean	Std. Deviation	Minimum	Maximum
Ventricular total tau (pg/mL)	8	842.2388	665.13976	121.00	1830.00
Lumbar total tau	8	140.1075	70.18270	50.73	258.00

Table 3.10.9.4. Comparison of means between ventricular and lumbar CSF total tau levels in NPH

Cisternal total tau in patients with TGN (pg/mL)

	N	Mean	Std. Deviation	Minimum	Maximum
Cisternal total tau	6	392.3050	386.52071	54.54	1158.00

Table 3.10.9.5. Mean cisternal total tau levels in TGN

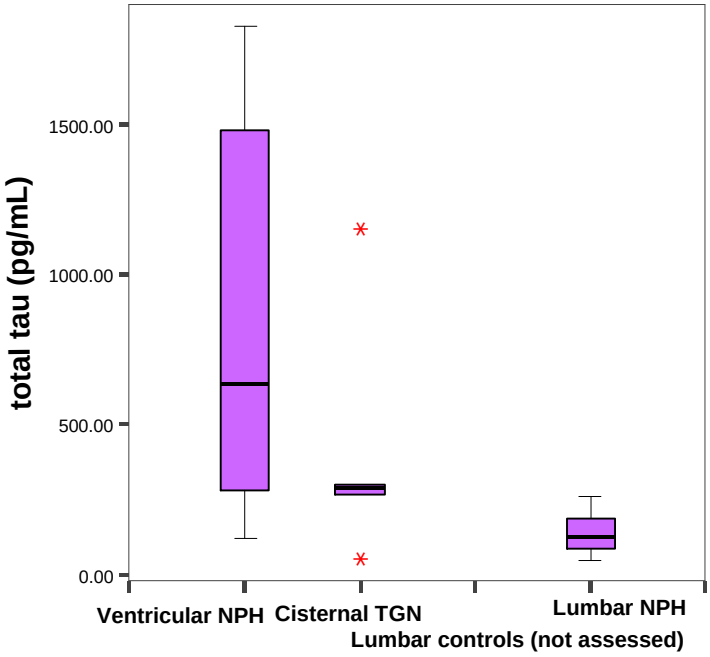


Figure 3.10.9.1. Boxplot levels of CSF total tau levels in the 3 groups tested

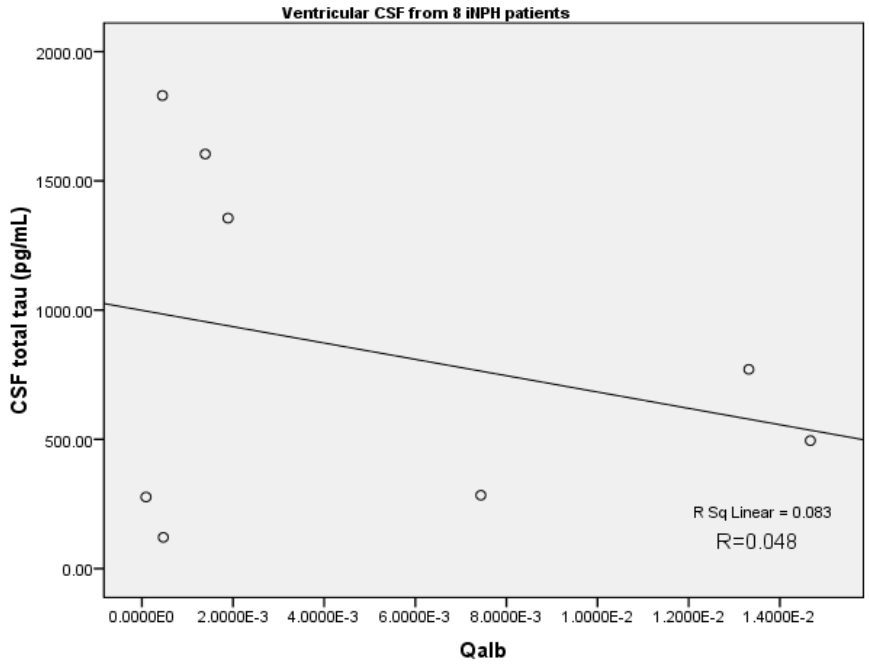


Figure 3.10.9.2. Scatterplot and linear regression between Qalb and total tau levels in CSF

Tabulated table of mean CSF values of markers examined and statistical differences				
	Ventricular CSF in NPH	**Lumbar CSF in NPH**	**Cisternal CSF in TGN**	**Lumbar CSF in controls**
Lactate	**2.02**	**2.3**	**1.87**	**1.59**
8-isoprostane	**38.39**	**38.32**	**40.88 *ƒ***	**38.74**
VEGF	**18.23**	**5.68 ***	**7.79**	**20.42 ‡**
GFAP	**24.35**	**2.75 ***	**0.54**	**0**
NfH	**0.92**	**0.19**	**0.27**	**0.25**
Aβ 1-42	**229.3**	**246.78**	**841.32 (930.05) *ƒ***	**849**
Total tau	**842.23**	**140.10**	**392.3 *ƒ***	**195**

Table 3.10.9.6. Tabulated table of mean CSF values of markers examined and statistical differences

* Significant difference p<0.05 between ventricular and lumbar CSF levels in NPH

ƒ significant difference p<0.05 between ventricular and cisternal levels in NPH and control subjects respectively

‡ significant difference in lumbar CSF levels in NPH and controls.

3.11. Correlation of CSF markers from external lumbar drainage with neuropsychology

There was no difference in any of the levels of the examined markers between the patients who had normal and mild/moderate-severe cognitive decline in WAIS-R, RMT Words, and Verbal Fluency test. Patients who were in the normal category, when examined with the RMT Faces test, had lower lactate levels compared to the subjects who performed worse in this particular test (independent samples t-test, p=0.060). It was also observed that the patient who has pathologically high GFAP levels showed no cognitive decline when tested on the Trail Making Test B.

3.12. Correlation of CSF markers from external lumbar drainage with volumetric data

Neither lactate nor 8-isoprostane levels had significant correlations with any of the analysed volumes. However, there was a negative trend between the isoprostane levels and the VV/WM ratio (R= -0. 667, p= 0.071).

There was a significant positive correlation (R= 0.648, p=0.043) between the levels of VEGF and the VV/ICV ratio; there was also a positive trend between the VEGF and the WM volume (R= 0.667, p= 0.071).

There was a significant positive correlation of the levels of GFAP and the VV/ DWMH ratio (R= 0.828, p=0.006).

There was a significant negative correlation between the levels of NfH and the VV/ICV ratio (R= -0.657, p=0.039), as well as a negative trend (R= -0.612, p=0.06) with the IVV. In addition, the white matter and NfH levels were inversely correlated (R= -0.778, p=0.023). There was also a significant positive correlation between the PVL/WM ratio and NfH levels (R= 0.738, p= 0.037) and a positive trend with the DWMH/WM ratio (R= 0.667, p= 0.071).

$A\beta_{1-42}$ and total tau had no significant correlations with any of the structural volumes, however there was a negative trend between tau and VV/DWMH (R= -0.617, p= 0.077).

3.13. Correlations of neuropsychology and volumetric data in patients undergoing external lumbar drainage

Patients who performed normal in the RMT Words had significantly higher ICV than the rest of the patients (879.42 +/- 215 vs. 574.05 +/- 155.6 mm3; p=0.05, independent samples t-test).

3.14. Surgical outcomes

Black grading scale for shunt assessment			
Excellent	*Pre-illness activity resumed without deficit*	**2**	9.1
Good	*Pre-illness activity resumed with moderate deficit*	**4**	18.2
Fair	*Improvement, but no return to previous work*	**11**	50
Transient	*Temporary major improvement*	**3**	13.6
Poor	*No change or worse*	**2**	9.1
Dead	*Death within 6 weeks of surgery, or a result of surgery*	**0**	0

Table 3.14.1. Surgical outcomes at 6 weeks

3.13.9.1. Surgical outcomes at 6 months

One patient did not return for follow-up despite enquiries made. Neither her or nursing care were available for interview at the 6 months point, hence the outcomes for 1 patient are unavailable. One patient moved abroad following the intervention however the outcome was communicated to us by the daughter of the patient who is a general practitioner and are therefore considered valid.

Black grading scale for shunt assessment			
Excellent	*Pre-illness activity resumed without deficit*	**8**	38.1
Good	*Pre-illness activity resumed with moderate deficit*	**4**	19
Fair	*Improvement, but no return to previous work*	**5**	24
Transient	*Temporary major improvement*	**0**	0
Poor	*No change or worse*	**4**	19
Dead	*Death within 6 weeks of surgery, or a result of surgery*	**0**	0

Table 3.14.2. Surgical outcomes at 6 months

3.15. Prognostic accuracy of CSF markers

For the purpose of providing meaningful statistical calculations due to the small numbers the results are dichotomised as “favourable” which include BGS *excellent, good* and *fair* categories and “unfavourable” which include BGS *transient, poor* and *dead* categories. In this sense in 6 weeks 17 (77.3%) patients had favourable and 5 (22.7%) patients unfavourable outcome. In 6 months follow-up 17 (80.9%) patients had favourable and 4 (19.04%) unfavourable outcome.

For each marker tested we perform a Receiver Operating Characteristic (ROC) curve in order to calculate a cut-off value that will provide a sensitivity and specificity of predicting a favourable outcome. The area under the curve is also calculated and reported with 95% CI. Mean, median, SD and range values for each marker as well as boxplots for the favourable and unfavourable groups are presented. The Youden’s index (equals to Sensitivity + Specificity − 1) is also reported for each marker.

3.15.1. Prognostic accuracy of lactate levels in 6 months

Area Under the Curve

Test Result Variable(s): lactate

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.518	.148	.906	.228	.807

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Table 3.15.1.1. Calculation of the area under curve for ventricular lactate with CI's

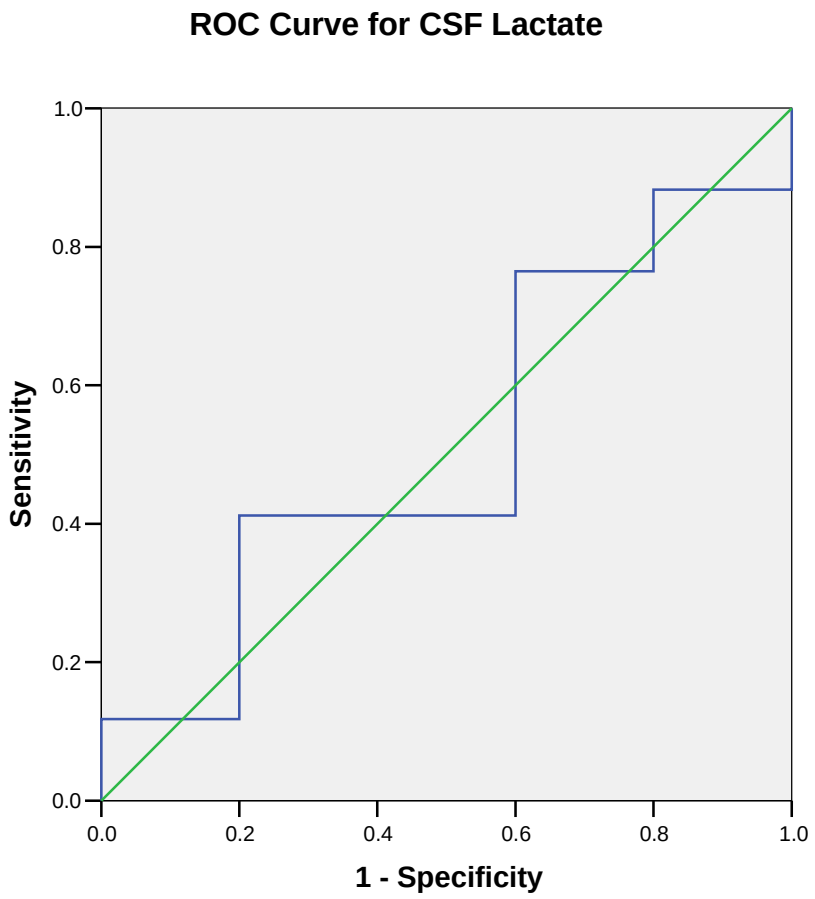


Figure 3.15.1.1. ROC curve for CSF lactate.

A level of CSF lactate=1.58 will have a sensitivity of 88.2% and specificity of 20% of predicting a favourable outcome at 6 months. The Youden's index is 0.08.

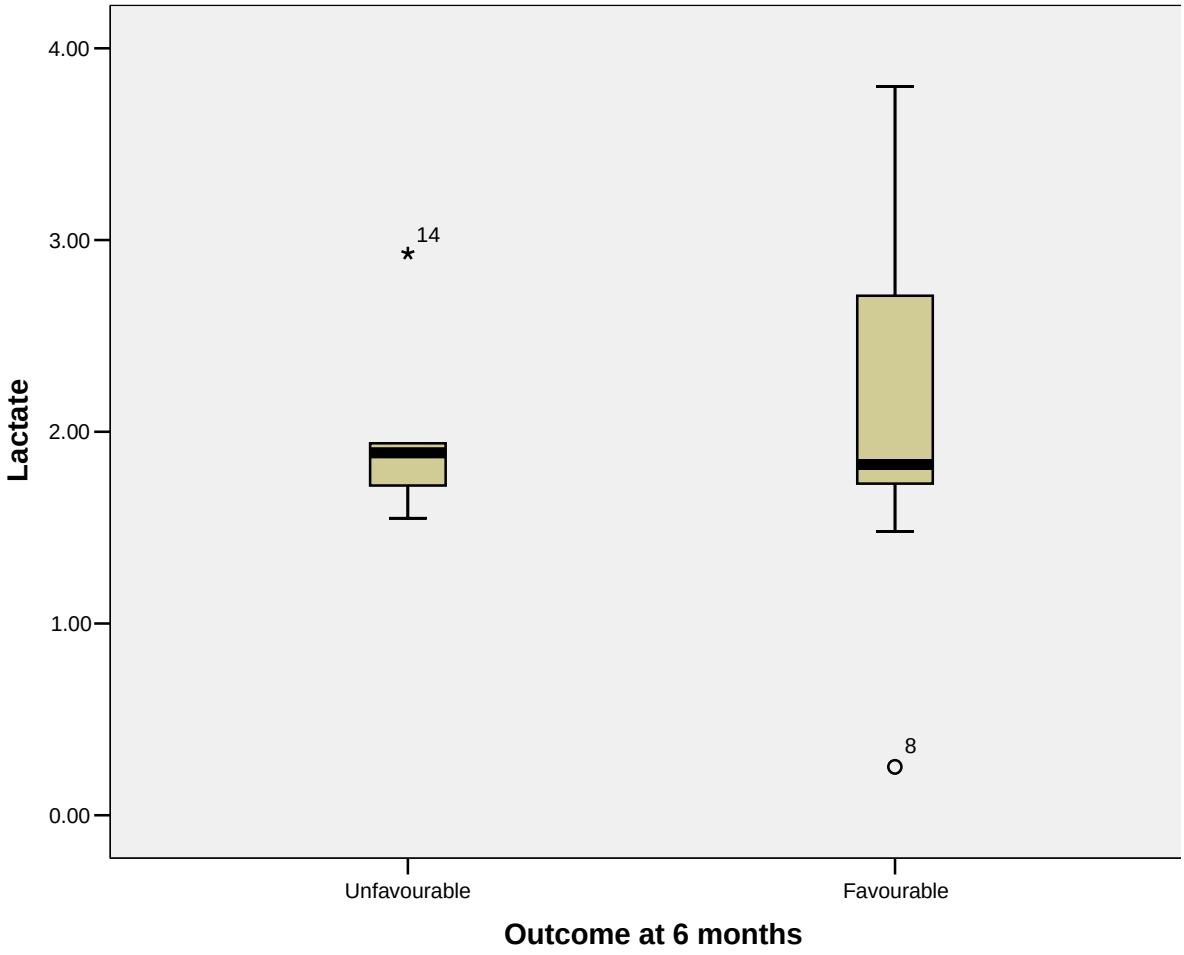


Figure 3.15.1.2. Boxplots of ventricular Lactate levels of favourable and unfavourable groups at 6 months

3.15.2. Prognostic accuracy of 8-isoprostane levels in 6 months

Area Under the Curve

Test Result Variable(s): isoprostane

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.400	.141	.513	.124	.676

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Table 3.15.2.1. Calculation of the area under curve for ventricular 8-isoprostane with CI's

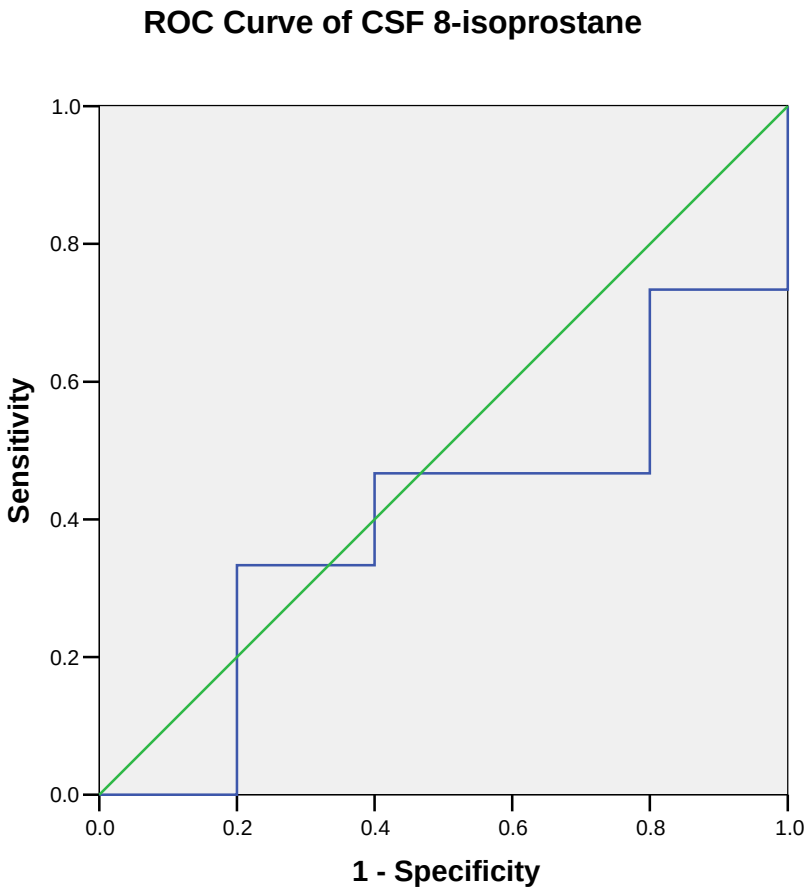


Figure 3.15.2.1. ROC curve for CSF 8-isoprostane.

A level of CSF 8-isoprostane=37.28 will have a sensitivity of 73% and specificity of 20% of predicting a favourable outcome at 6 months. The Youden's index is -0.07.

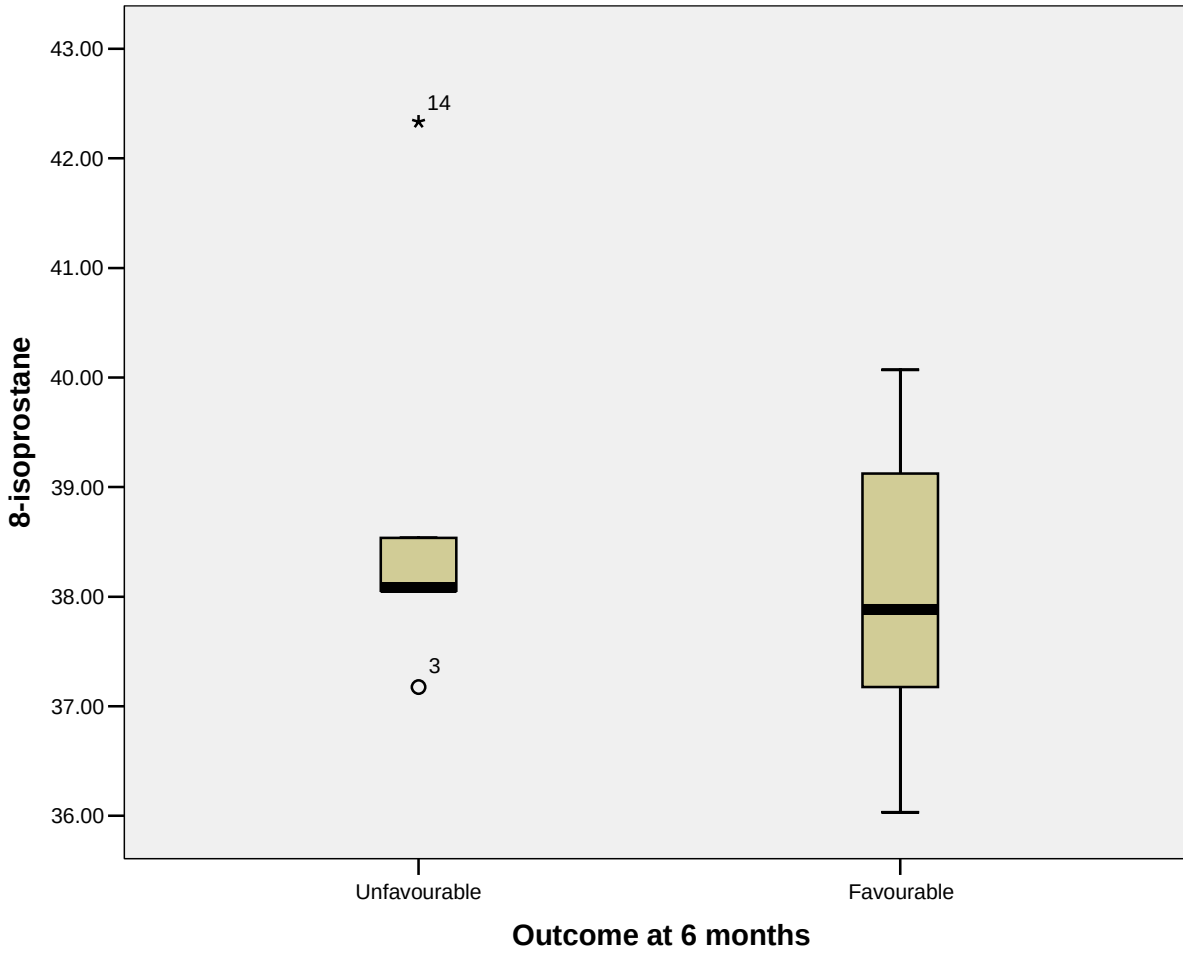


Figure 3.15.1.2. Boxplots of ventricular 8-isoprostane levels of favourable and unfavourable groups at 6 months

3.15.3. Prognostic accuracy of VEGF levels in 6 months

Area Under the Curve

Test Result Variable(s): vegf

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.329	.119	.256	.096	.563

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

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Table 3.15.3.1. Calculation of the area under curve for ventricular VEGF with CI's

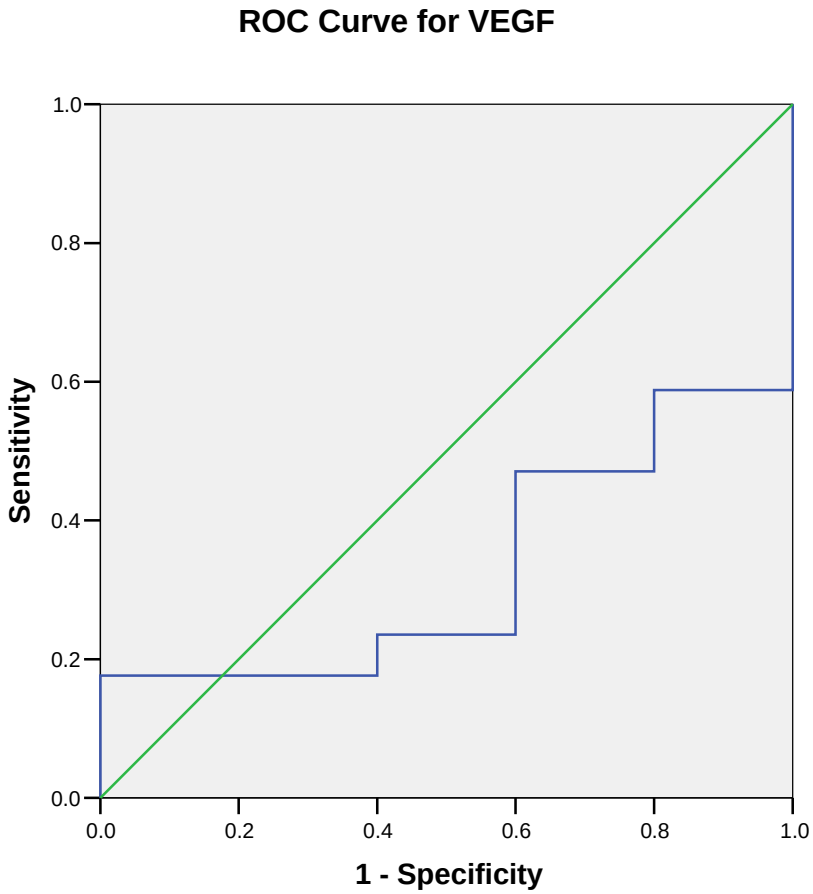


Figure 3.15.3.1. ROC curve for CSF VEGF.

A level of CSF VEGF=9.03 will have a sensitivity of 58.8% and specificity of 20% of predicting a favourable outcome at 6 months. The Youden's index is -0.22

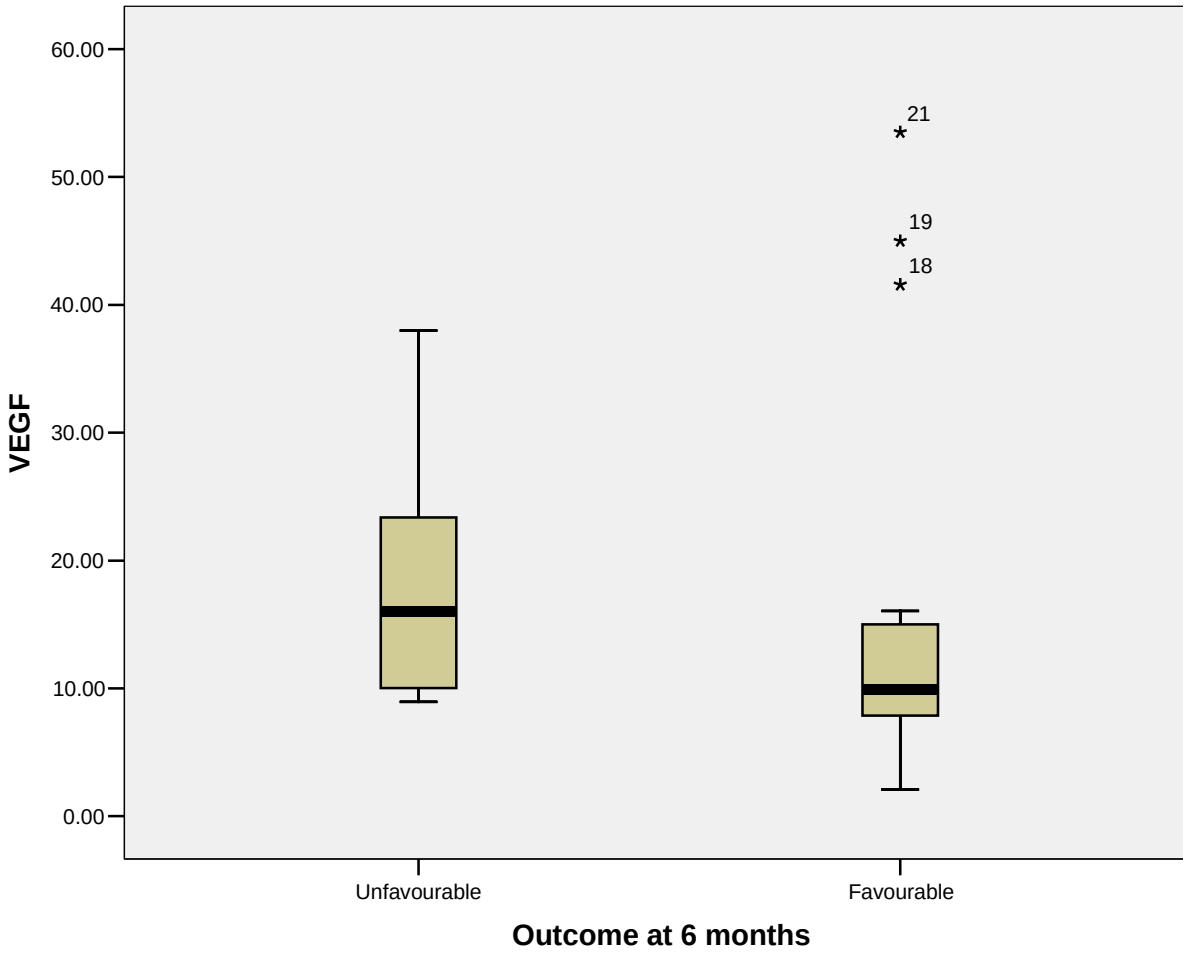


Figure 3.15.3.2. Boxplots of ventricular VEGF levels of favourable and unfavourable groups at 6 months

3.15.4. Prognostic accuracy of GFAP levels in 6 months

Area Under the Curve

Test Result Variable(s):gfap

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.549	.146	.791	.263	.835

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Table 3.15.4.1. Calculation of the area under curve for ventricular GFAP with CI's

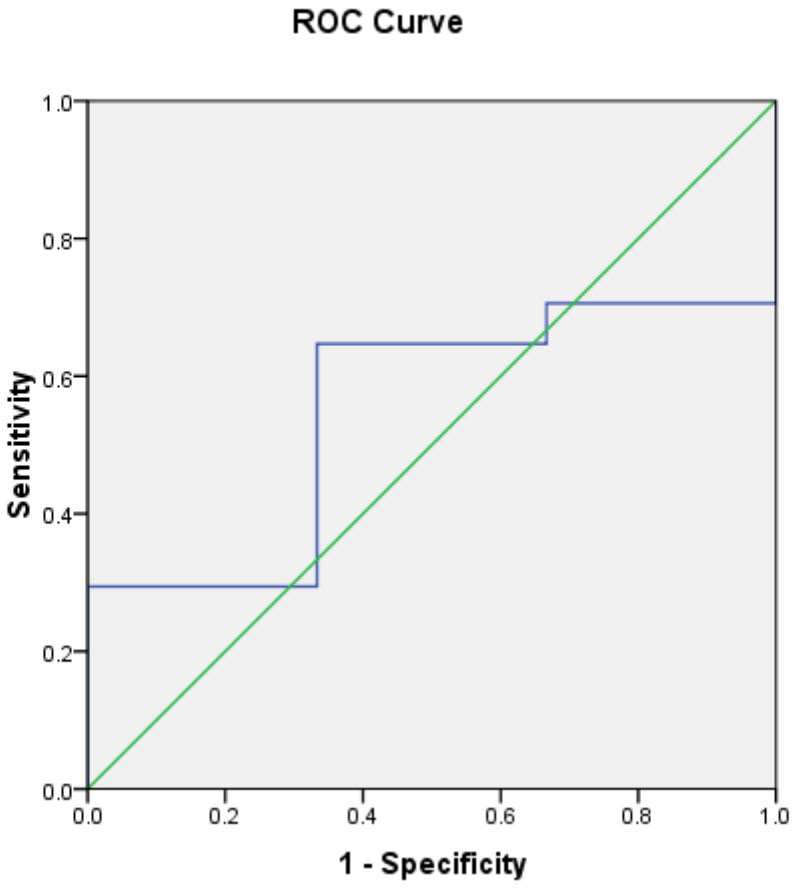


Figure 3.15.4.1. ROC curve for CSF GFAP.

A level of CSF GFAP=0.2 will have a sensitivity of 43.8% and specificity of 75% of predicting a favourable outcome at 6 months. The Youden's index is 0.18.

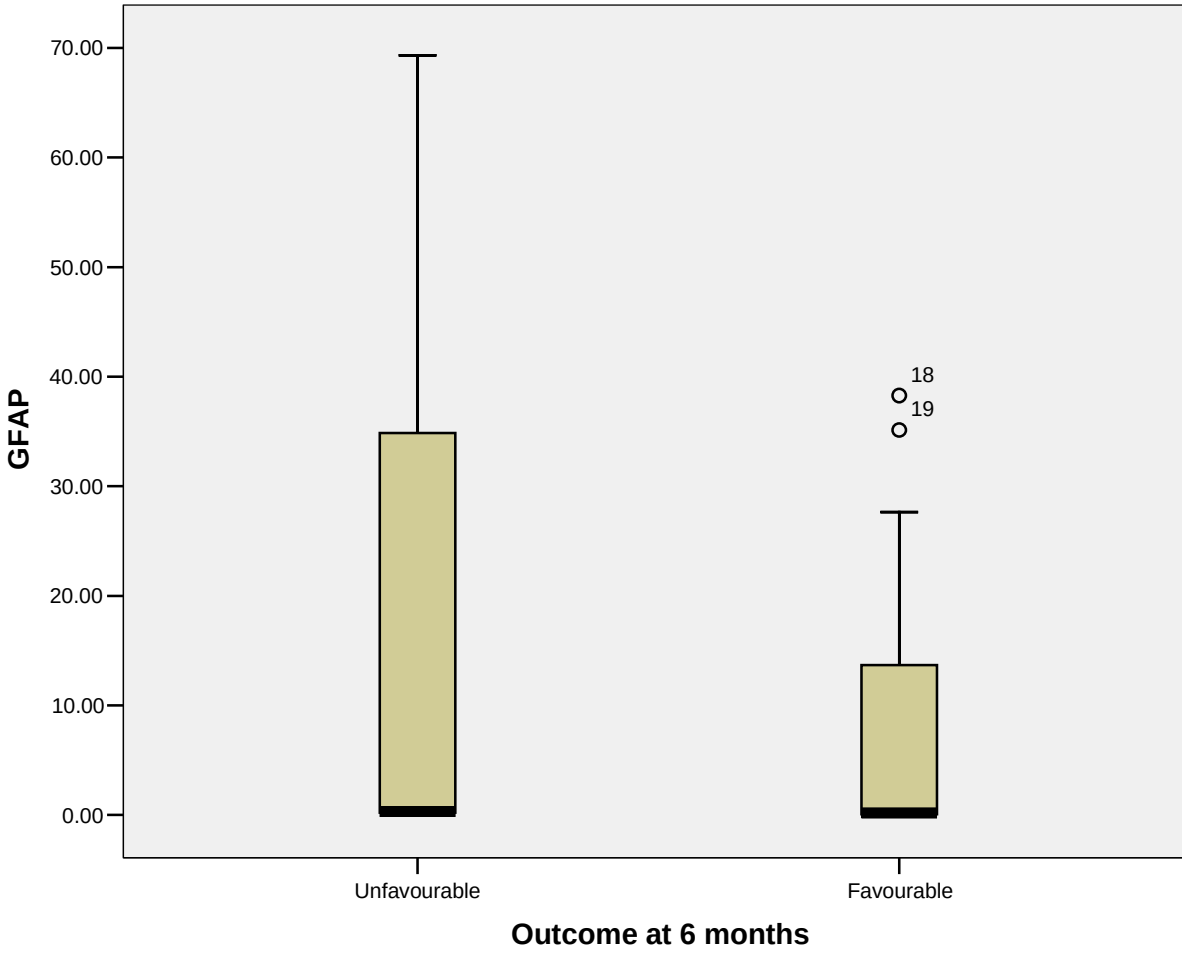


Figure 3.15.4.2. Boxplots of ventricular GFAP levels of favourable and unfavourable groups at 6 months

3.15.5. Prognostic accuracy of CSF NfH at 6 months

Area Under the Curve

Test Result Variable(s): nfl

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.318	.132	.225	.059	.576

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Table 3.15.5.1. Calculation of the area under curve for ventricular NfH with CI's

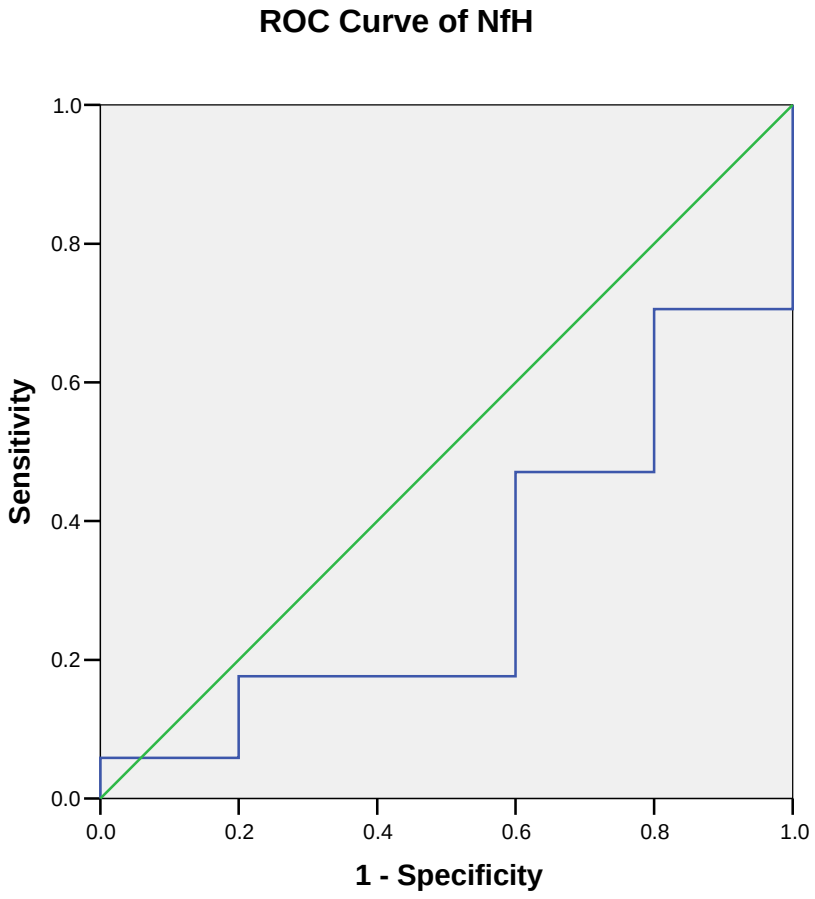


Figure 3.15.5.1. ROC curve for CSF NfH.

A level of CSF NfH=0.15 will have a sensitivity of 70% and specificity of 20% of predicting a favourable outcome at 6 months. The Youden's index is -0.1.

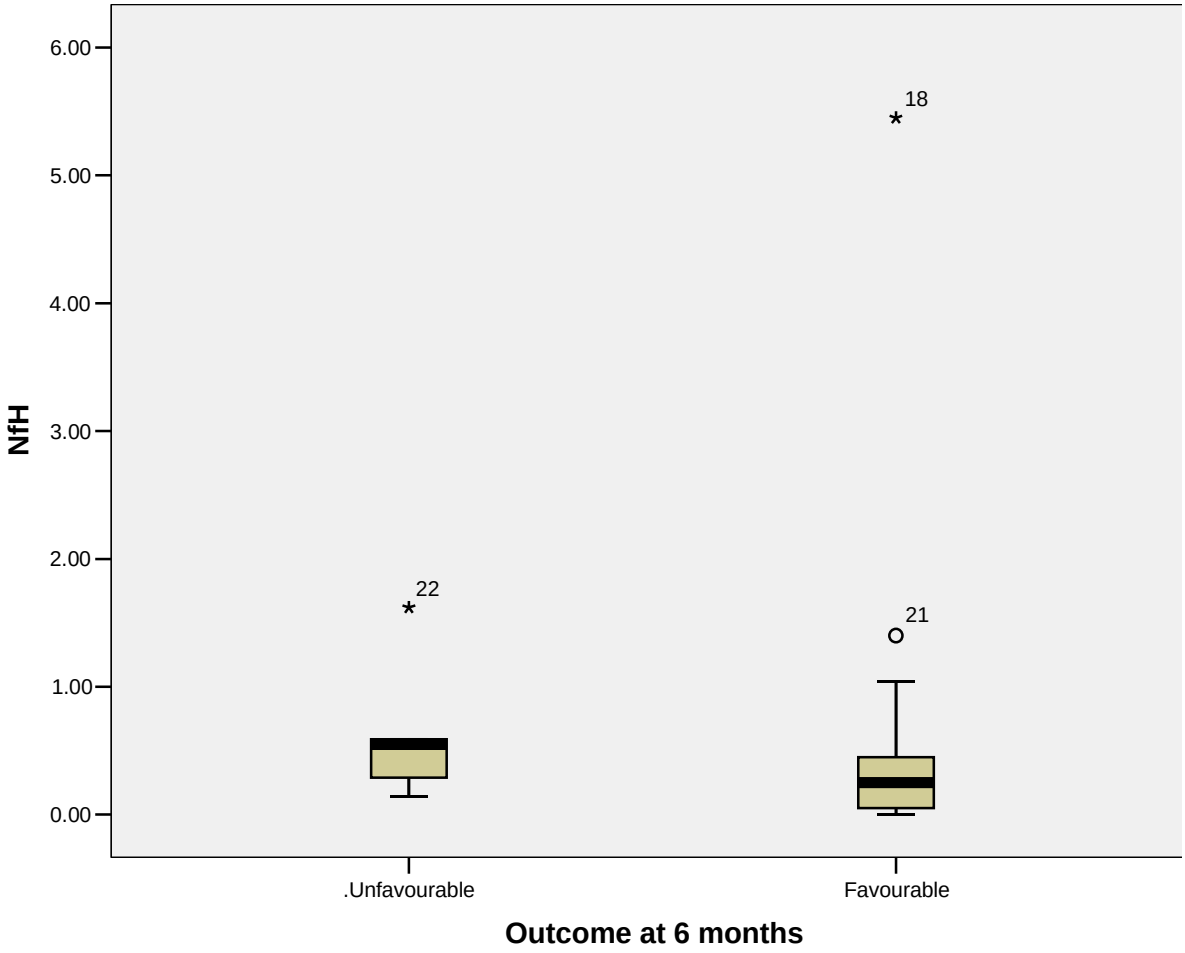


Figure 3.15.5.2. Boxplots of ventricular NfH levels of favourable and unfavourable groups at 6 months

3.15.6. Prognostic accuracy of Aβ $_{1-42}$

Area Under the Curve

Test Result Variable(s): Ab142

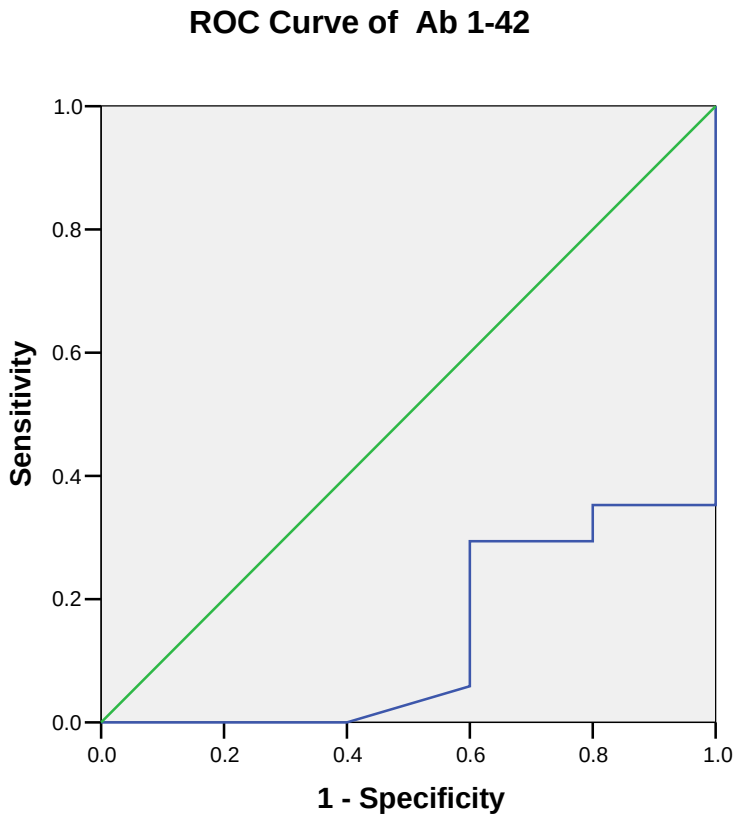
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.135	.087	.015	-.036	.307

The test result variable(s): Ab142 has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Table 3.15.6.1. Calculation of the area under curve for ventricular Aβ $_{1-42}$ with CI's



Diagonal segments are produced by ties.

Figure 3.15.6.1. ROC curve for CSF Aβ $_{1-42}$.

A level of CSF Aβ $_{1-42}$=180 will have a sensitivity of 35% and specificity of 20% of predicting a favourable outcome at 6 months. The Youden's index is -0.45.

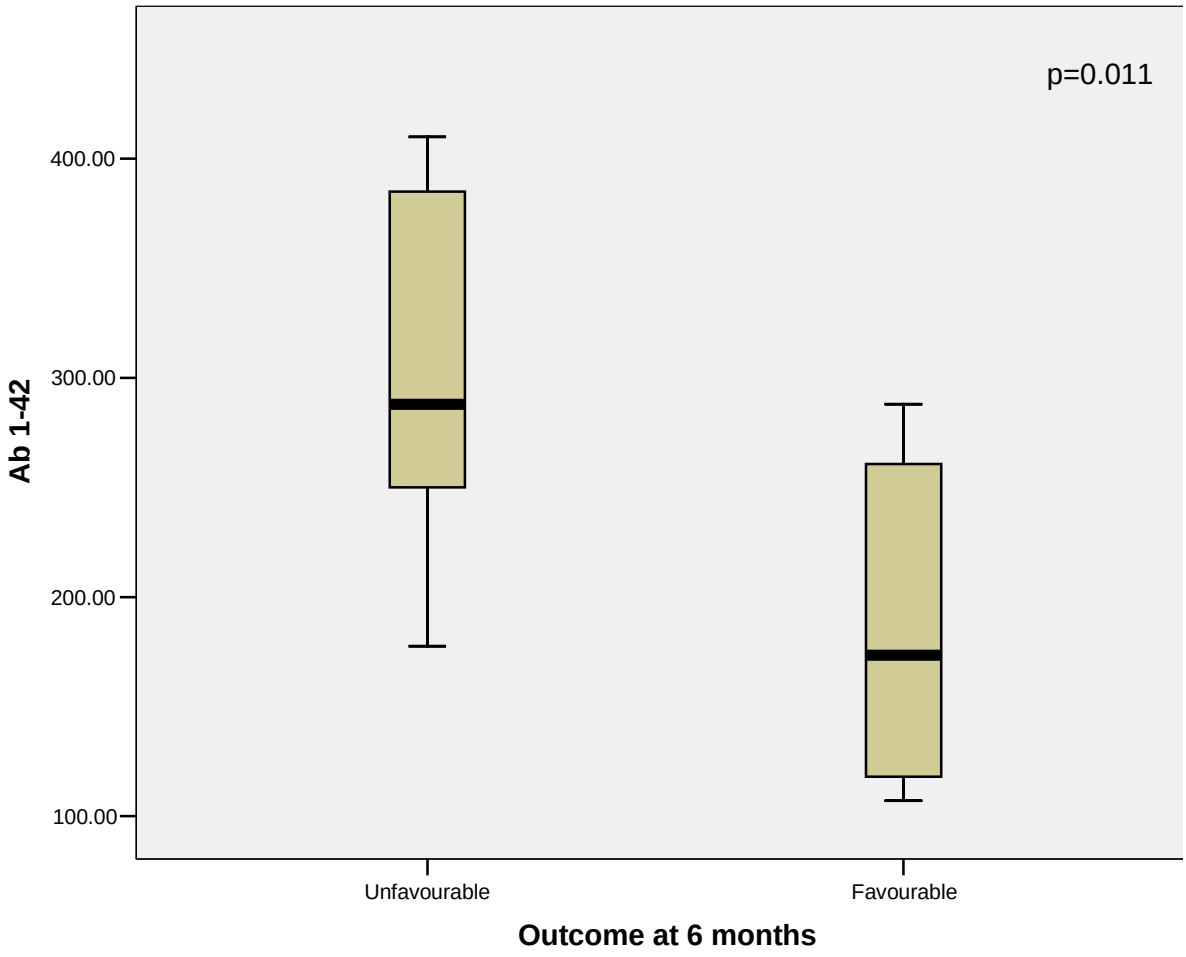


Figure 3.15.6.2. Boxplots of ventricular Aβ $_{1-42}$ levels of favourable and unfavourable groups at 6 months

3.15.7. Prognostic accuracy of CSF total tau levels at 6 months

Area Under the Curve

Test Result Variable(s): tau

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.165	.088	.026	-.008	.337

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Table 3.15.7.1. Calculation of the area under curve for ventricular total tau with CI's

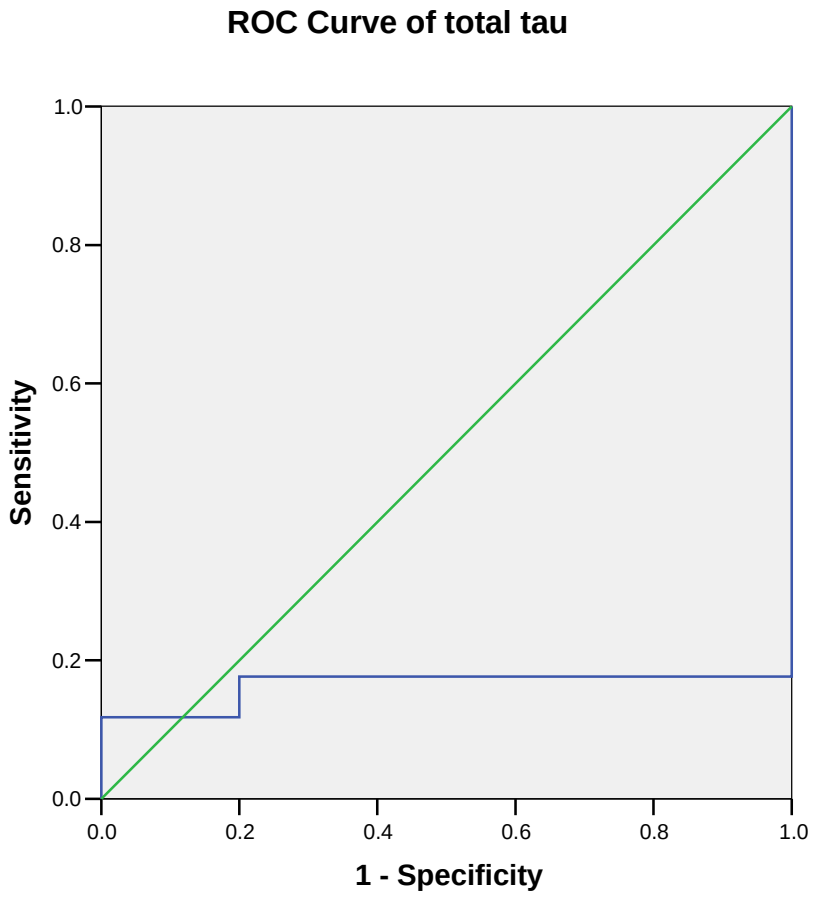


Figure 3.15.7.1. ROC curve for CSF total tau.

A level of CSF total tau=767 will have a sensitivity of 17% and specificity of 20% of predicting a favourable outcome at 6 months. The Youden's index is -0.63.

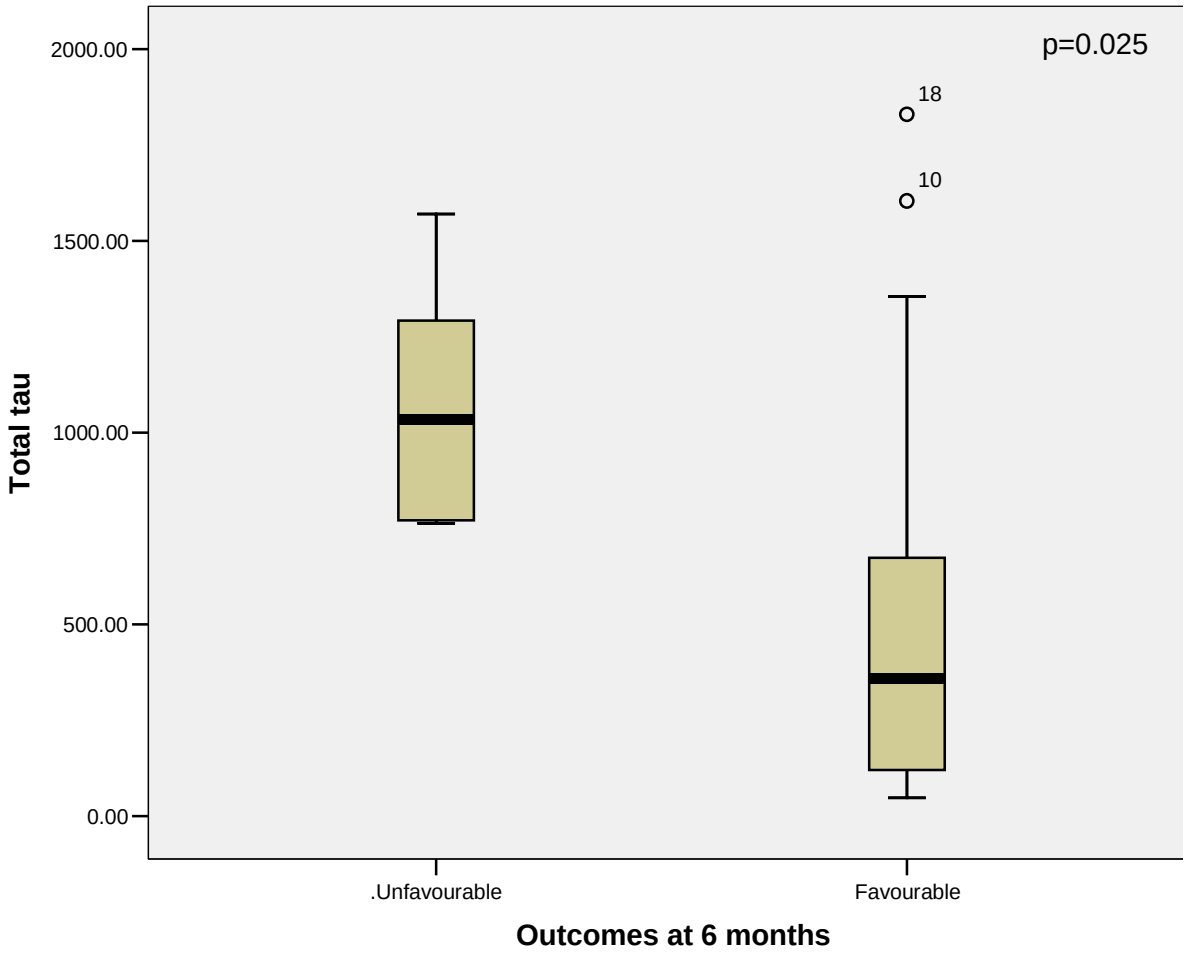


Figure 3.15.7.2. Boxplots of ventricular total tau levels of favourable and unfavourable groups at 6 months

3.15.8. Total tau/ Aβ $_{1-42}$ ratio

Area Under the Curve

Test Result Variable(s): tau_ab142

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.388	.115	.457	.164	.613

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Table 3.15.8.1. Calculation of the area under curve for ventricular Total tau/ Aβ $_{1-42}$ ratio with CI's

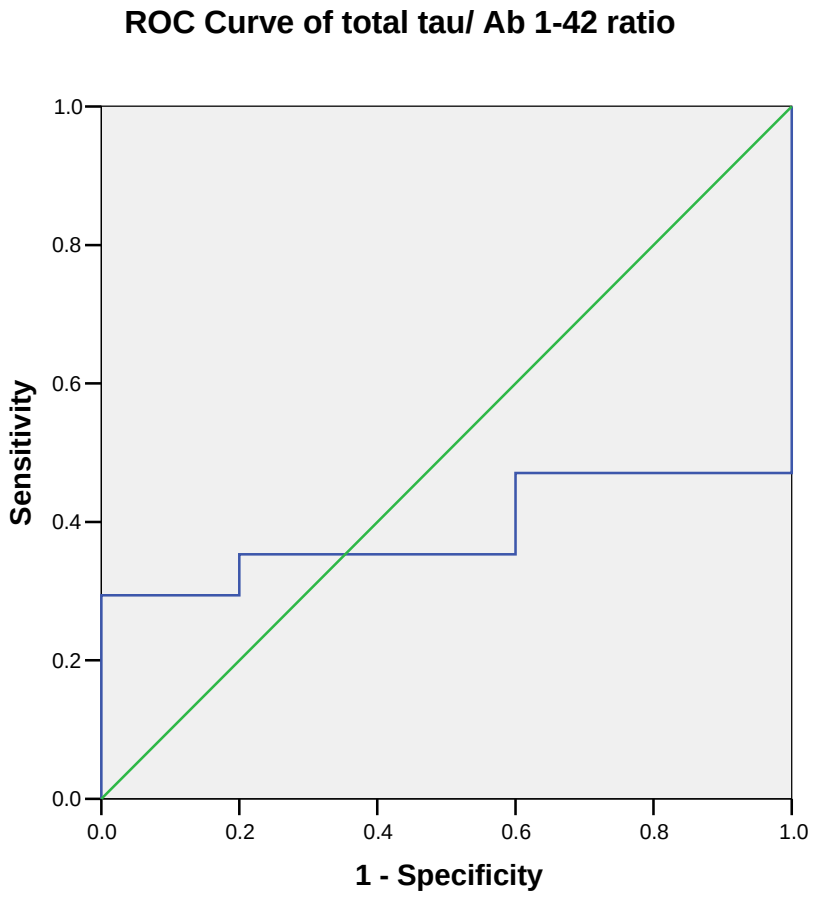


Figure 3.15.8.1. ROC curve for CSF Total tau/ Aβ $_{1-42}$ ratio.

A cut-off level of 2.28 will have a sensitivity of 47% and specificity of 20% of predicting a favourable outcome at 6 months. The Youden's index is -0.33.

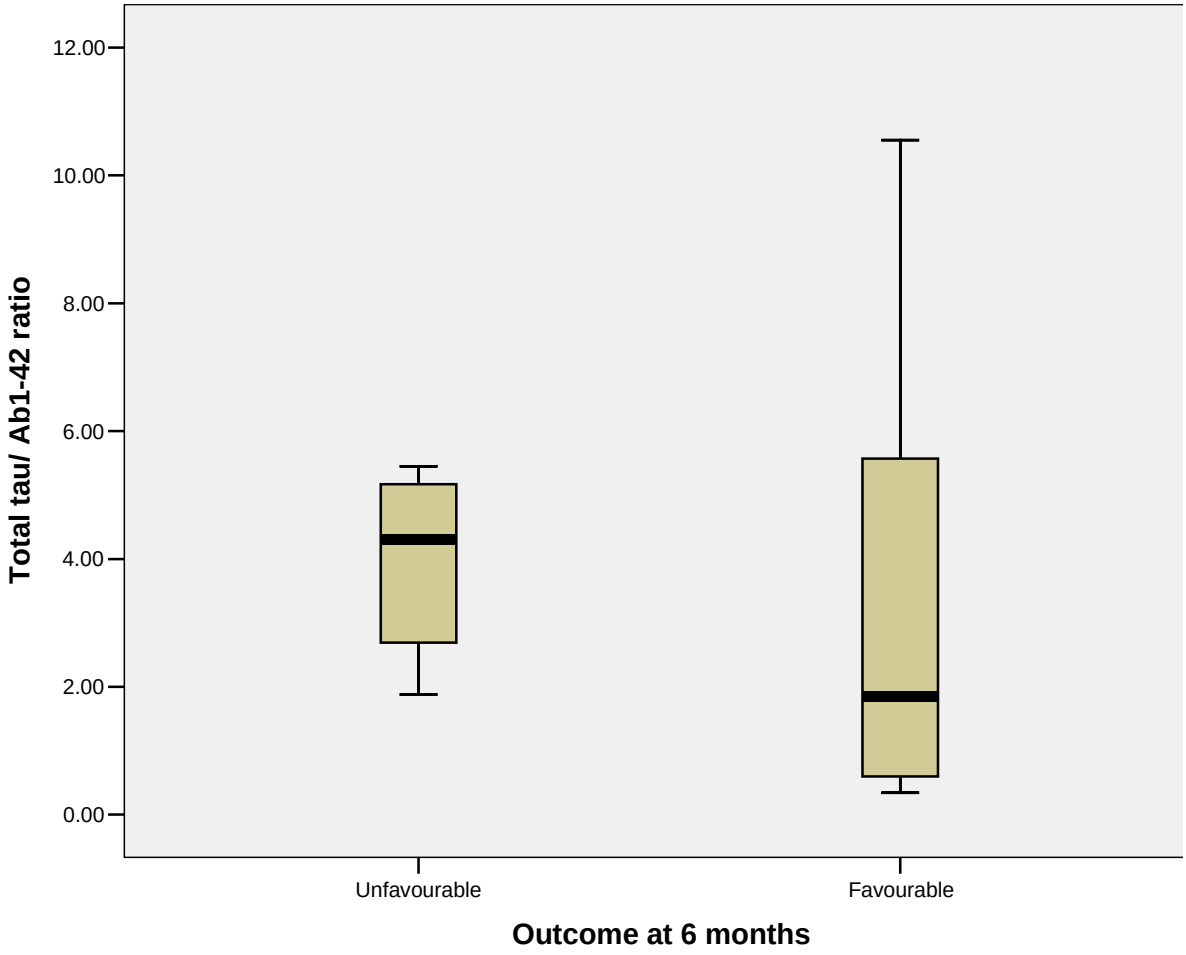


Figure 3.15.8.2. Boxplots of ventricular Total tau/ Aβ $_{1-42}$ ratio of favourable and unfavourable groups at 6 months

A discriminant function analysis of both total tau and Aβ $_{1-42}$ was undertaken in order to calculate the prognostic accuracy of the combination of both markers. The

Wilks' lambda is a multivariate test. Because p <0.05, we can say that the model is a good fit for the data.

The discriminant function coefficients seen below are used to write an equation in order to calculate the discriminant function. Hence:

DF= 0.841 X Aβ + 0.474 X Total Tau

Wilks' Lambda

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1	.590	10.035	2	.007

Standardized Canonical Discriminant Function Coefficients

	Function
	1
ab142	.841
tau	.474

Functions at Group Centroids

6 months Outcome	Function
	1
Unfavourable	1.467
Favourable	-.431

Unstandardized canonical discriminant functions evaluated at group means

Table 3.15.8.2. Discrimination function analysis of the combination of total tau and Aβ $_{1\text{-}42}$

The table demonstrates the group's centroids. The cut score= 1.036 is the average of the two above values. Hence if the:

Score >1.036 by substituting the respective Aβ and total tau in the above equation it is more likely to have an unfavourable outcome and if the

Score < 1.036 then a favourable outcome is more likely

Classification Results[a]

		6 months outcome	Predicted Group Membership		Total
			0	1	
Original	Count	Unfav	4	0	4
		Favour	3	14	17
	%	Unfav	80.0	20.0	100.0
		Favour	17.6	82.4	100.0

a. 81.8% of original grouped cases correctly classified.

Table 3.15.8.3. Classification results of the discriminant function analysis for total tau and Aβ $_{1\text{-}42}$

According to the classification 81.8% of cases were classified correctly with a sensitivity of 80% and specificity of 82.4%

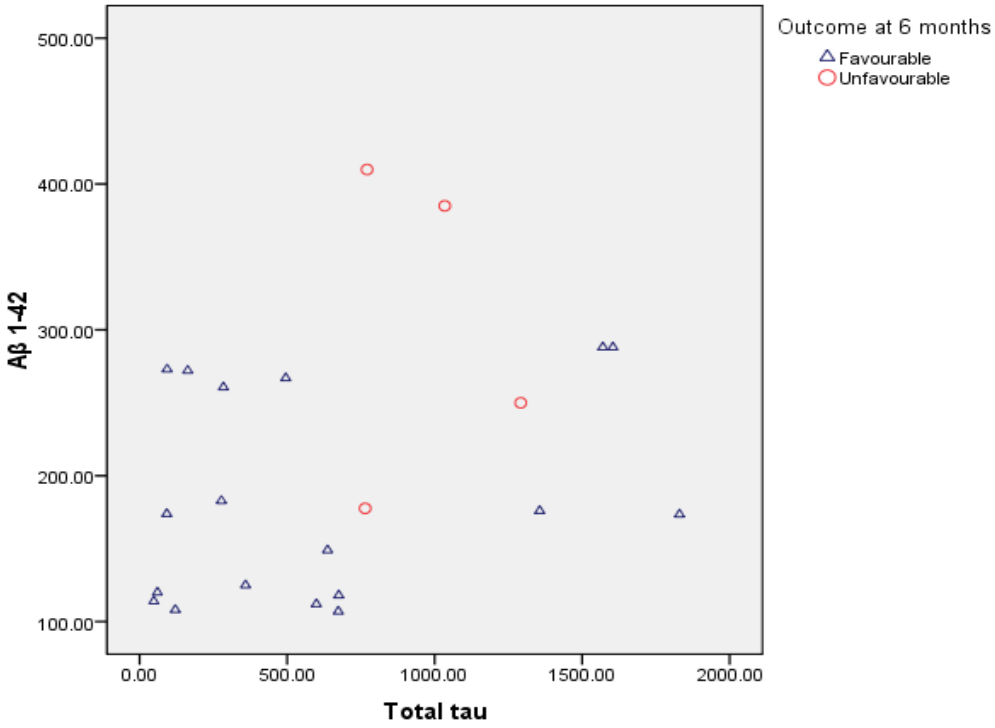


Figure 3.15.8.3. Scatterplot of favourable and unfavourable cases based on the discriminant function analysis for total tau and Aβ $_{1-42}$

3.15.9. Summary of descriptive statistics from all CSF markers

The means and median of the levels of the different markers are presented below. The levels of Aβ 1-42 and total tau are smaller in the favourable group with the difference being statistically significant (Mann-Whitney test, p=0.011 and p=0.025 respectively).

Descriptive statistics of ventricular CSF levels from 22 patients with idiopathic NPH

Outcome		Lactate	8-isopro stane	Ab 1-42	Total tau	Total tau/ Ab 1-42 ratio	NfH	GFAP	VEGF
Unfavourable	Mean	2.0060	38.8366	302.10	1086.2393	3.8980	.6380	17.5350	19.2640
	Std. Deviation	.53882	2.01567	96.060	347.19529	1.55884	.57954	34.52339	11.93046
	Median	1.8900	38.0848	288.00	1034.0000	4.3000	.5500	.3050	16.0000
	Minimum	1.55	37.18	177.60	764.20	1.88	.14	.21	8.96
	Maximum	2.93	42.33	409.92	1570.00	5.45	1.62	69.32	37.98
Favourable	Mean	2.0884	38.0296	177.64	550.9750	3.3118	.6447	8.1100	15.5435
	Std. Deviation	.80601	1.31128	67.902	551.65230	3.08204	1.29287	14.48425	15.44091
	Median	1.8300	37.8855	173.49	359.0000	1.8500	.2500	.1750	9.8900
	Minimum	.25	36.03	107.06	48.00	.34	.00	.01	2.09
	Maximum	3.80	40.07	288.00	1830.00	10.55	5.45	38.28	53.50
Total	Mean	2.0697	38.2314	205.92	672.6260	3.4450	.6432	9.9950	16.3891
	Std. Deviation	.74265	1.50029	90.116	554.55965	2.78629	1.15651	19.20355	14.53661
	Median	1.8300	38.0699	176.80	618.0000	2.7800	.3200	.2200	10.0800
	Minimum	.25	36.03	107.06	48.00	.34	.00	.01	2.09
	Maximum	3.80	42.33	409.92	1830.00	10.55	5.45	69.32	53.50

Table 3.15.9.1. Summary of descriptive statistics from all CSF markers for favourable and unfavourable groups

Comparison of means of CSF markers in favourable and unfavourable outcome groups [b]

	Lactate	8-isoprostane	Ab 1-42	Total tau	Tau_Ab ratio	NfH	GFAP	VEGF
Mann-Whitney U	41.000	30.000	11.500	14.000	33.000	27.000	16.500	28.000
Wilcoxon W	56.000	150.000	164.500	167.000	186.000	180.000	152.500	181.000
Z	-.118	-.655	-2.429	-2.233	-.744	-1.216	-1.466	-1.136
Asymp. Sig. (2-tailed)	.906	.513	.015	.026	.457	.224	.143	.256
Exact Sig. [2*(1-tailed Sig.)]	.940[a]	.553[a]	.011[a]	.025[a]	.493[a]	.249[a]	.148[a]	.283[a]

a. Not corrected for ties.

b. Grouping Variable: good_b

Table 3.15.9.2. Comparison of means of CSF markers in favourable and unfavourable outcome groups

The correlations of the different ventricular markers were also calculated. 8-isoprostane has a significant positive correlation with both NfH and GFAP. Total tau levels correlate positively in a significant fashion with NfH, GFAP and VEGF levels. NfH also has a strong correlation with GFAP levels. Lactate and Aβ 1-42 levels do not correlate with any of the other markers.

Inter-correlations of ventricular CSF markers

			Qalb	Lactate	8-isoprostane	Aβ 1-42	Total tau	NfH	GFAP	VEGF
Spearman's rho	Qalb	Correlation Coefficient		NS	NS	NS	NS	NS	NS	NS
		Sig. (2-tailed)	.							
	Lactate	Correlation Coefficient	NS		NS	NS	NS	NS	NS	NS
		Sig. (2-tailed)								
	8-isoprostane	Correlation Coefficient	NS	NS		NS	NS	.494	.483	NS
		Sig. (2-tailed)						.027*	.042*	
	Aβ 1-42	Correlation Coefficient	NS	NS	NS		NS	NS	NS	NS
		Sig. (2-tailed)								
	Total tau	Correlation Coefficient	NS	NS	NS	NS		.506	.498	.423*
		Sig. (2-tailed)						.016*	.025*	.050*
	NfH	Correlation Coefficient	NS	NS	.494	NS	.506		.703	NS
		Sig. (2-tailed)			.027*		.016*	.	.001**	
	GFAP	Correlation Coefficient	NS	NS	.483	NS	.498	.703		NS
		Sig. (2-tailed)			.042*		.025*	.001**	.	
	VEGF	Correlation Coefficient	NS	NS	NS	NS	.423	NS	NS	
		Sig. (2-tailed)					.050*			.

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.15.9.3. Intercorrelations of all ventricular CSF markers

3.15.10. Logistic regression of markers as predictors of outcome

A logit regression was used as a means of predicting favourable outcome from the ventricular levels of the CSF marker examined. None of the markers proved to be significant predictors of favourable outcome.

Logistic regression as predictor of favourable outcome

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	Lactate	2.451	1.898	1.666	1	.197	11.596
	Ab 1-42	-.032	.030	1.139	1	.286	.969
	Total tau	.000	.008	.001	1	.982	1.000
	Total tau/ Ab 1-42 ratio	-.280	1.770	.025	1	.874	.756
	NfH	.443	1.264	.123	1	.726	1.558
	VEGF	-.026	.107	.059	1	.809	.974
	8- isoprostane	-.939	.914	1.056	1	.304	.391
	GFAP	.031	.077	.164	1	.685	1.032
	Constant	40.629	36.957	1.209	1	.272	4E+017

a. .

Table 3.15.10.1. Logistic regression of all ventricular CSF markers as predictor of surgical outcome at 6 months.

3.16. Influence of epidemiological data on 6-months outcome

A chi-square test was used to find associations between favourable and unfavourable outcomes and epidemiological data. The factors of sex, symptom appearing first, history of diabetes mellitus, hypertension, history of transient ischaemic attacks, history of cerebrovascular accident, history of myocardial infarction, history of ischaemic heart disease and peripheral vascular disease. None of the factors proved to have significant associations with the outcome.

The Mann-Whitney test was used to identify associations between outcomes and age of patient, and symptomatic duration, as well as duration of each individual

symptom. No significant association was identified. In a logistic regression model none of the above factors was a significant predictor of favourable outcome at 6 months.

3.17. Influence of clinical parametres on outcome

Chi-square test was used to test for association between outcome at 6 months and clinical parametres at examination (see table 3.2.2.). None of the parametres had a significant association with outcome.

3.18. Prognostic accuracy of neuropsychological assessment

Cross tabulations were performed in order to test for association between outcome at 6 months and the different test of neuropsychological assessment. None of the associations proved statistically significant. Similarly improvement on the individual tests post drainage cannot predict the 6 months outcome.

	Test of intelligence	Recognition Memory tests		Executive Functioning		Speed (subcortical)
Tests	**WAIS-R**	**RMT words**	**RMT faces**	**Phonemic VF**	**Trails**	**Speed (cancelling)**
Normal	5	7	4	4	1	0
Mild/Moderate -severe cognitive impairment	10	8	11	11	9	15
Post ELD significant improvement	≠	2 (20%)	1 (11%)	1 (11%)	1 (12%)	3 (38%)
Post ELD no difference/ significant decline	≠	8	9	9	7	5
6 weeks significant improvement	0	5 (50%)	5 (55%)	2 (22%)	1 (12%)	3 (38%)
6 weeks no difference/ significant decline	10	5	4	7	7	5
6 months significant improvement	1 (12%)	6 (60%)	4 (40%)	3 (27%)	2 (30%)	3 (38%)
6 months no difference/ significant decline	8	5	6	8	4	5

≠: not repeated post drain due to practise effects

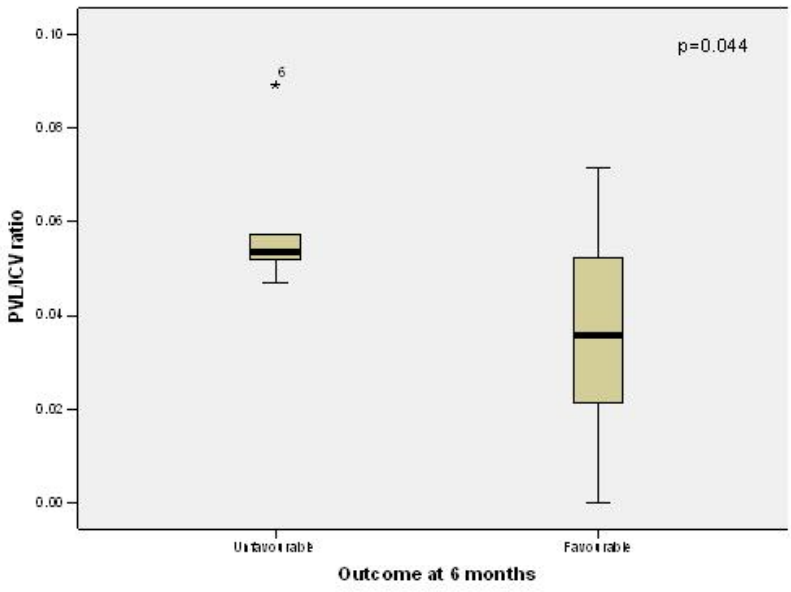
Table 3.18.1. Neuropsychological assessment before and after insertion of ELD, at 6 weeks and 6 months

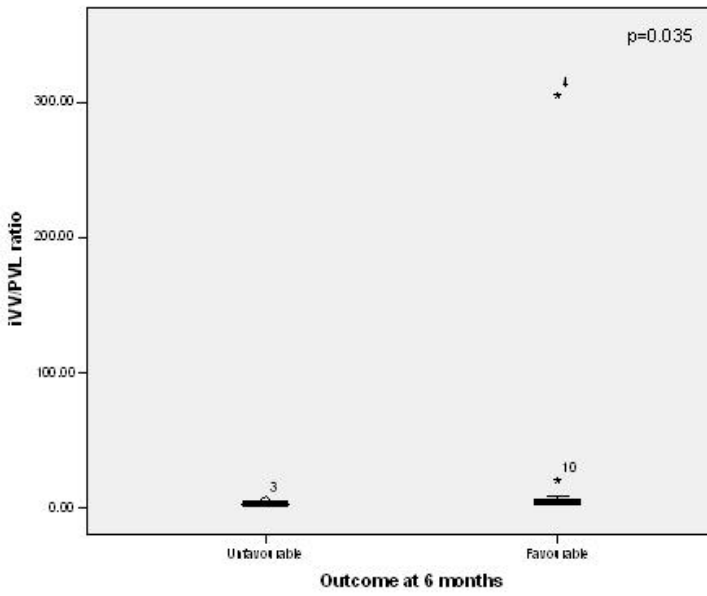
The above table demonstrates improvement in all the tests (apart from the RMT faces test) that continues beyond the first assessment at 6 weeks to the second assessment at 6 months. However no more than 60% of patients that had the assessment demonstrated improvement on individual tests at any time point.

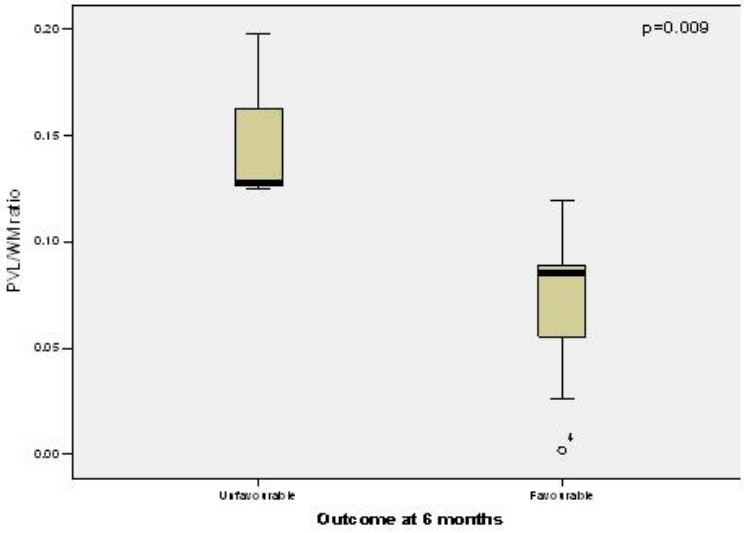
3.19. Prognostic accuracy of volumetric imaging data

The Mann Whitney U test was used to calculate significant differences between the favourable and unfavourable outcome groups. The only significant differences between the 2 groups were in the PVL normalised ratio (0.035 vs. 0.059, p=0.044), the IVV/PVL ratio (28.89 vs. 2.95, p=0.035), and the PVL/WM ratio (0.07 vs. 0.15, p=0.09).

A logistic regression of the volumetric variables did not show any significant predictor of the favourable outcome.







Figures 3.19.1. 3.19.2. and 3.19.3. Boxplots of PVL/ICV ratios, IVV/PVL ratios and PVL/WM ratios and differences between groups with favourable and unfavourable outcome.

3.20 Walking test on follow-up

The walking test at baseline and follow-up is displayed below. 18 patients undertook the follow-up test. The Wilcoxon signed ranks test showed a significant reduction in the number of steps at follow-up (p=0.015; 99 CI=0.012-0.018) and a non significant difference in the time (p=0.125; 99 CI=0.116-0.133).

Walking test at baseline and 6 months follow-up

	N	Mean	Std. Deviation	Minimum	Maximum
Baseline steps	24	38.53	34.6	15.5	152
Steps at 6 months	18	19.56	6.2	14	31
Baseline time (secs)	24	43.8	50.4	6.5	171
Time at 6 months (secs)	18	13.09	7.13	8	31

Table 3.20.1. Walking test at baseline and 6 months follow-up

Chapter 4 Discussion

External lumbar drainage is thought to mimic shunting which could explain its high accuracy in selecting patients for CSF diversion (Marmarou, Bergsneider et al. 2005; Marmarou, Young et al. 2005). No study in the literature to date has recorded the changes in the CSF composition occurring during external drainage. Our study therefore is the first, to our knowledge, which attempts to record such changes. Tullberg et al. on a recent study have recorded increased postoperative levels of lumbar CSF albumin, albumin ratio, neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), ganglioside GD3, and tau, whereas homovanillic acid (HVA), 5-hydroxy-indoleacetic acid (5-HIAA) and 4-hydroxy-3-methoxyphenylglycol (HMPG) and sulphatide levels remained unchanged following shunting in patients with iNPH (Tullberg, Blennow et al. 2008). The increase in tau levels agrees with our current findings. In another earlier study from the same group (albeit in a mixed NPH group of idiopathic and secondary forms) a significant postoperative increase in the same markers as before as well as in gamma amino butyric acid and sulfatide was noted, whereas the lumbar levels in CSF of NFL, HVA, HMPG and 5-HIAA remained unchanged (Tullberg, Blennow et al. 2007)

The role of lactate as a prognostic biomarker

CSF lactate is produced by immigrated leukocytes and by anaerobic glycolysis in the CNS parenchyma. Mean lactate level increased from day 0 to day 2 and then further to day 3. The difference from day 0 to day 3 was statistically significant. One would expect that as the drainage proceeds, lactate level would decrease

since the element of ischaemia is alleviated. Inao et al. have demonstrated a slow seepage of lactate into the CSF following experimental barotrauma in cats hence demonstrating the dynamics of lactate absorption and production into and out of the CSF spaces (Inao, Marmarou et al. 1988).

Two previous studies gave contradictory results with regards to the levels of lactate in NPH (Malm, Kristensen et al. 1991; Nooijen, Schoonderwaldt et al. 1997). Malm et al. found lower lactate levels in iNPH when compared to controls and hypothesised that there is ventricular accumulation and consequent transependymal absorption of lactate due to inverse, caudo-rostral, flow of CSF. However, our results show that the rostrocaudal gradient (RCG) of lactate was 0.93 and 1.13 in iNPH and control subjects, not supporting the above argument. There was no significant differences between the two levels of sampling both in iNPH patients and control subjects verifying the earlier studies of Posner and Plum who found almost equal CSF lactate levels between the cisterns and lumbar thecal sac (Posner and Plum 1967). A ventriculo-lumbar ratio of lactate equal to 0.8 has been found in a study of patients with suspected CNS bacterial infections with higher lumbar than ventricular concentration (Gerber J 1998). In another study comparing lumbar and ventricular lactate levels in patients with CNS infections and cerebral haemorrhage, no significant difference existed between the two (Sommer, Gaul et al. 2002). We postulate that the hydrostatic pressure gradient from the ventricles to the interstitial space is reversed during drainage and therefore the resorption of lactate by the subependyma occurring in normal circumstances (Bateman 2002) might cease. Under normal circumstances lactate clearance from CSF is slow taken up by the brain via diffusion (Prockop 1968), and by the

arachnoid villi by bulk flow (Valenca, Shannon et al. 1971), and partially metabolised to pyruvate. In control subjects it appears that lactate accumulates more in the ventricles, however in subjects with iNPH lactate is in larger concentration in the thecal sac. The higher RCG in control subjects agrees with the suggestion by Bateman. Eide et al. have shown in a microdialysis study of 28 patients with iNPH that in 29% of the participants a reduction was seen in the microdialysate lactate (Eide and Stanisic 2009). This finding corresponds well with our findings and the suggestion by Bateman. This hypothesis is further strengthened by the findings of the other microdialysis study in patients with iNPH where following CSF drainage lactate levels increase (Agren-Wilsson, Roslin et al. 2003). The difference in the two studies is that in the former the probe was placed 20 mm from the cortical surface, whereas in the latter it was placed in the periventricular white matter. During ELD therefore a reduction in the brain lactate occurs due to a reversal of the hydrostatic gradient from the ventricles to interstitial space causing subsequently causing a net flow from the brain parenchyma towards the ventricular space and hence lactate appears in the CSF.

Lactate entrance into the CSF from the brain may occur by diffusion, by bulk flow via extracellular channels, or by direct volume entry by choroid plexus. Furthermore elevated CSF lactate may be derived not only from the brain but also from the circulating blood lactate secondary to impairment of the blood- brain barrier (BBB). However, brain lactate concentration does not depend on serum levels (Posner and Plum 1967). Our data suggests a degree of BBB in the 11 patients undergoing ELD hence suggesting a leakage of serum lactate via the BBB in patients with iNPH

At the initial sample there is no significant correlation with the levels of the other markers or the Q_{alb}. However, as drainage continues a positive trend between NfH and lactate is noticed, that was negative on the initial sample.

Patients who categorized in the normal category, when examined with the RMT Faces test, had lower lactate levels compared to the subjects who performed worse in this particular test. That signified a trend between pathological CSF lactate levels and memory (non-verbal) failure. As visual memory is localised to the right temporal lobe (Ariza, Pueyo et al. 2006), this finding may be considered as being consistent with literature suggesting deficits of some right hemisphere functions in NPH (Iddon, Pickard et al. 1999). This signifies a lateralising effect; whereas lactate levels would be the result of a global ischemic insult. In a study evaluating changes in regional blood flow post shunting, Tamaki et al. did not identify any lateralising effect in blood flow of the temporal lobe of the improved group (Tamaki, Kusunoki et al. 1984). On the other hand a single patient case report correlating neuropsychological and findings from cerebral metabolism (as measured by PET using fluodeoxyglucose F 18) found asymmetric hypometabolism with more extensive involvement of right-hemisphere regions (Kaye, Grady et al. 1990). Hence, although interesting we cannot further elucidate on the significance of this finding.

A cut-off level of CSF lactate=1.58 mmol/L will have a high sensitivity of 88.2% and low specificity of 20% of predicting a favourable outcome at 6 months. With higher lactate levels the sensitivity decreases whereas the specificity increases.

Lactate may be then used for selecting patients for surgery; however patients with higher levels should not be excluded from having surgery on the basis of an expected unfavourable outcome, due to the low specificity of CSF lactate. As proposed from other studies lactate is a marker of cerebral ischemia (Brouns, Sheorajpanday et al. 2008) it makes sense that patients with a lower ischemic load would have better surgical outcomes as previously reported (Boon, Tans et al. 1999). The fact that increased lactate should not exclude patients from having surgery may be explained by the fact that patients with AD or VD may also have increased CSF lactate levels (Parnetti, Reboldi et al. 2000). Increased CSF lactate therefore may be a marker of more than one pathological comorbidity occurring at any one time.

The role of 8-isoprostane as a prognostic biomarker

The isoprostanes are a family of eicosanoids of non-enzymatic origin produced by the random oxidation of tissue phospholipids by oxygen free radicals. F2-isoprostanes are exclusively products of free radical–mediated damage to arachidonic acid which form as esters bound to membrane lipid which are then enzymatically hydrolyzed and released into extracellular fluid. They are known to be elevated by oxidative stress. One of the isoprostanes, 8 –Isoprostane (8-iso PGF2a), has been proposed as a marker of antioxidant deficiency and oxidative stress (Domenico Praticò 2000). Levels have been found increased in ventricular (Montine, Beal et al. 1999) and lumbar CSF (Domenico Praticò 2000) in patients

with AD, and they are thought to enhance its laboratory diagnosis (Montine, Kaye et al. 2001). Lipid peroxidation was increased when compared to controls in NPH (Fersten, Gordon-Krajcer et al. 2004) and similar results suggesting that vascular changes observed in hydrocephalic rats may be due to the high level of lipid peroxidation have been published (Caner, Atasever et al. 1993). Isoprostanes represent the product of free-radical peroxidation of polyunsaturated fatty-acids (Montuschi, Barnes et al. 2004). Oxidative stress and chronic cerebral ischemia are two pathophysiological processes closely linked together (Faraci 2005; Cai, Yan et al. 2008).

Our results have shown that the temporal profile of 8-isoprostane remains mostly unaltered during the 72 hours of drainage, even though there appears to be a significant increase between day 2 and 3. That might mean that; a) the ischaemic process cannot be reversed within such a short time and therefore any changes will not shown in the CSF profile obtained during this study, b) any ventricular changes will not be reflected in the lumbar concentrations, or c) the background ischaemic process is negligible and therefore no temporal difference is shown. Ischaemia is known to be an established pathophysiological process in iNPH as demonstrated by various imaging studies (Bradley, Whittemore et al. 1991; Krauss, Regel et al. 1997; Tanaka, Kimura et al. 1997). Our results of volumetric calculations of the DWMH area in the above set of patients point to an ischemic process which is non-negligible (DWMH represent 5.1% of the total intracerebral volume) hence our third suggestion is not valid. Our second suggestion may be less likely bearing in mind that we found the RCG to be equal to 1. Therefore we propose that the ischemic process may not be able to be reversed within 72 hours

of drainage. In a recent study Lenfeldt et al. showed that recovery of N-acetyl aspartate and choline during 3 days of external lumbar drainage is slow and little different when these metabolites were measured with the aid of proton spectroscopy (Lenfeldt, Hauksson et al. 2008). The results of this latter study might support the hypothesis that 72 hours is not long enough to reverse long-established damage. CSF drainage produced reduction in levels of 8-isoprostane in AD patients undergoing drainage with a low-flow shunt, but significant reduction was produced after 9 months, and not at 3 and 6 months (Praticoa, Yaoa et al. 2004).

CSF F2-isoprostanes levels are elevated early in the course of AD and correlated with disease progression (Montine, Beal et al. 1999). In our study we did not find a correlation with symptom duration. Also, in patients with AD it was found that the levels are correlated in a significant fashion with brain weight and the degree of cortical atrophy (Montine, Markesbery et al. 1999). It is known that in patients with iNPH ventriculomegaly is disproportionate to the degree of cortical atrophy that differentiating their imaging from patients with AD. The volumetric study from 10 patients with iNPH found a negative trend between the isoprostane levels and the VV/WM ratio (R= -0. 667, p= 0.071). The smaller levels of F2 isoprostane in our study (38 pg/mL) when compared with studies of patients with AD (8-128 pg/mL) may be because the ventricular volume versus white matter volume ratio in NPH would be larger. This correlation therefore suggests that in iNPH the main source of 8-isoprostane is the white matter, which would explain the trend observed.

The RCG of 8-isoprostane was 1.0 and 1.06 in iNPH and controls. It appears therefore that 8-isoprostane is secreted and absorbed in an equal rate across the craniospinal axis both in iNPH and control subjects. There are no previous similar studies from patients with AD or other degenerative neurological disorders that have studied the dynamics across the craniospinal axis. A study measuring F2 isoprostane levels from autopsied cerebral samples and ventricular CSF (VF) from AD patients undergoing autopsy, and lumbar CSF *intra vitam* (LF) again from patients with AD showed a brain tissue>VF>LF gradient (Montine, Markesbery et al. 1999). However lumbar levels were measured early in the course of disease, whereas ventricular levels were measured in patients with more advanced AD. In our study the levels were measured a few weeks apart in each case.

Levels of F2 isoprostanes are not influenced by the density of neuritic plaques or neurofibrillary tangles (Montine, Markesbery et al. 1999). That agrees with our findings of no correlation of 8-isoprostane with tau or Aβ 1-42 levels. However there was a significant positive correlation of the ventricular 8-isoprostane both with GFAP (R=0.483, p=0.042) and NfH (R=0.494, p=0.027) levels. Cerebral GFAP has been shown to correlate with markers of oxidative damage in a mouse model of AD pathology (Zhu, Gu et al. 2008). Reactive astrocytes are known to protect neurons from oxidative stress via a glutathione dependent mechanism (Iwata-Ichikawa, Kondo et al. 1999; Chen, Vartiainen et al. 2001; Muntane, Dalfo et al. 2006); this positive correlation therefore signifies a close association between irreversible gliosis and oxidative stress in normal pressure hydrocephalus. It has also been suggested that free radical activity plays an important role in the reorganizations of the neurofilament structure and the cytoplasmic inclusions

observed in several neurodegenerative diseases (Gélinas, Chapados et al. 2000; Bizzozero, Reyes et al. 2007). Our findings therefore suggest a common pathway of oxidative stress affecting both astrocyte function and neurofilament structure as part of a neurodegenerative process.

An interesting finding was that of a significant difference between the ventricular and cisternal levels between patients with iNPH and control subjects suffering from TGN. This finding is unexpected since our initial hypothesis was to use 8-isoprostane to characterise oxidative stress. In our study group 4 patients (18%) had diabetes mellitus, 7 (32%) suffered from hypertension, 1 (4.5%) had suffered a transient ischaemic attack previously, and 3 (14%) patients had a previous cerebrovascular accident. The prevalence of hypertension in both males and female blacks in non-Hispanic whites and blacks in a large US study in the age group 70-79 years old is more than 60% (Burt, Whelton et al. 1995). Our study group therefore appears to have a smaller incidence of hypertension than what expected from an average population. Patients with TGN do not suffer from hypertension more often than a control population; the incidence of hypertension appears equal to that in our group of NPH patients (Teruel, Ram et al. 2009). F2-isoprostanes have been found locally in atherosclerotic plaques (Pratico, Iuliano et al. 1997). The association of TGN and atherosclerosis is established (Lewy and Grant 1938). It might be therefore that the increased cisternal levels in patients with TGN reflect the local disease process. That may also explain the slightly higher cisternal than lumbar CSF levels in our control population.

Although oxidative stress and chronic cerebral ischaemia are two important pathophysiological processes that can influence the surgical outcome in iNPH the prognostic value of 8-isoprostane appears limited at present. The reason for this might be that 8-isoprostanes, as we have discussed, may not be able to characterise the pathological processes influencing surgical outcomes in iNPH. It may be that oxidative damage occurs early in the process of chronic hydrocephalus development and hence at the point of our examination, when most patients had a mean of 4 years of symptoms, the levels have levelled off and hence they do not appear of use in differentiating responders from non-responders. A cut-off level of 37.3 pg/mL or less has a sensitivity of 73% of predicting a favourable outcome; however its specificity appears limited to 20%. Hence with levels less than 37.3 pg/mL we may be able to predict a favourable surgical outcome with greater than 73% sensitivity; however with levels greater than 37.3 pg/mL the specificity is small

The role of VEGF as a prognostic biomarker

There is increasing evidence that blood vessels and angiogenic factors such as VEGF play an important role in the control of neurogenesis via crosstalk pathways (Vagnucci and Li 2003). It has been suggested that neurogenesis continues on the adult brain especially after an ischemic insult and that the role of VEGF in these processes appears central (Lichtenwalner and Parent 2005). We know that chronic vascular insufficiency in the CNS and possibly insufficient VEGF-dependent neuroprotection leads to selective degeneration of motor neurons in mice with a targeted deletion of the hypoxic response element in the promoter of the VEGF

gene (Oosthuyse, Moons et al. 2001). The damage may be the combination of insufficient neuroprotection and impaired neural perfusion. VEGF affects vascular tone by controlling the release of the vasorelaxant nitric oxide (NO) by endothelial cells. Alternatively, VEGF may be required for the normal functioning of perivascular autonomic nerves, which critically regulate vascular tone and, hence, tissue perfusion (Storkebaum and Carmeliet 2004). VEGF's implication in the development of AD has been already suggested (Vagnucci and Li 2003).

VEGF in the CSF has been studied before and has been identified in patients with astrocytomas (Peles, Lidar et al. 2004). In that study the authors measured CSF VEGF level in ten patients with communicating hydrocephalus and identified a median concentration of 8.3 ng/mL, which is not dissimilar from our study. However, the type of communicating hydrocephalus was not further elucidated in that study. The role of VEGF in cerebral ischemia has also been studied in aneurysmal subarachnoid haemorrhage in human subjects (Scheufler, Drevs et al. 2003). Dombrowski et al. have identified increased density of VEGF positivity in neurons, glia and blood vessels in an animal model of hydrocephalus (Dombrowski, Leichliter et al. 2006). Increased levels of VEGF-A were found in children undergoing shunt surgery when compared to a control group; the authors suggested that patients with other conditions of altered CSF flow pathways, which would also be expected to interfere with the
intracranial or intraspinal pulsation absorption mechanisms (as in Chiari malformations, some arachnoid cysts, and the like) also had elevations in CSF VEGF-A (Madsen, Shim et al. 2009).

The level of VEGF increased during the 3 days drainage. This difference was significant between day 0 and day 2 and continued to day 3, even though in the last 24 hours the difference was not significant. Our study supports a degree of angiogenesis in patients with iNPH, even though this angiogenesis does not seem to be induced by ischaemia, since VEGF is not correlated with the other 2 markers we chose to characterise hypoxia. It might be that VEGF is increased as a means to promote neurogenesis/neuroregeneration once the increased transmantle pressure ceases (due to drainage) (Sun, Jin et al. 2003; Storkebaum, Lambrechts et al. 2004).

In the day 0 sample the lumbar levels of NfH are negatively correlated with the levels of VEGF which might signify a reverse established association between neurodegeneration and angiogenesis/ neurogenesis. However, the reversal of this relationship as drainage continues might be explained by the washout of ventricular metabolites. The ventricular levels of VEGF are positively correlated with total tau ($R=0.494$, $p=0.05$), another marker of neurodegeneration. No similar findings have been identified in humans, however in a transgenic model of AD continuous release of VEGF over a 3 month period has led to attenuation of hyperphosphorylated tau and amyloid load suggesting an element of neuroprotection (Jeynes and Provias 2009). Tortuous thickening of the basement membrane, collagen deposition, cerebral amyloid angiopathy, and the presence of plaques/tangles impede diffusion of oxygen (Storkebaum and Carmeliet 2004), thus explaining the positive correlation between total tau and VEGF levels in ventricular CSF. Amyloid plaques characteristic of AD pathology are known to generate reactive oxygen species that damage brain endothelium (Vagnucci and Li

2003). VEGF obtains therefore a central role between a chronic hypoxic/ischemic insult and neurodegeneration in iNPH.

The positive correlation of the VEGF levels with the VV/ICV ratio may mean that VEGF is produced as a response to ventriculomegaly. If this is the case the VEGF secretion might be due to chronic ischemia induced by the ventriculomegaly-produced increased transmantle pressure, which other studies have suggested (Deshpande, Dombrowski et al. 2007). There is, however, no evidence whether the reverse may be true. Experimental studies in rats have shown that infusion of VEGF produces prominent bilateral ventriculomegaly (Harrigan, Ennis et al. 2003); leading to the hypothesis that bilateral ventriculomegaly may be the result of increased VEGF production. The presence of VEGF in choroid plexus is already known (Stopa, Berzin et al. 2001). The positive trend between the white matter volume and the VEGF levels might indicate that the latter is also produced from the white matter (Arai, Deguchi et al. 1998), thus the role of VEGF in ventriculomegaly and in particular in iNPH requires further investigation.

The RCG for VEGF in NPH and control subjects is 3.2 and 0.38 respectively. The ventricular levels were much higher than the lumbar in iNPH patients, whereas the reverse was true in the control population. There was therefore almost a reversal of the RCG in the 2 groups we examined. The dynamics of brain-derived proteins have been elegantly described by Reiber (Reiber 2001; Reiber and Peter 2001; Reiber 2003). He suggested that the CSF flow rate, and not the blood-CSF barrier dysfunction as previously thought, is the most important determinant for understanding the pathological changes if both blood and brain-derived proteins in

CSF. In his work in order to characterise the dynamics he analysed the concentration gradients of CSF /serum albumin ratio (Q_{alb}) and the ventricular/ lumbar CSF concentration gradient. Pathologically decreased CSF flow rate was characterised by increased CSF /serum albumin ratio (Q_{alb}). According to his theory therefore:

1) brain-derived proteins show a decrease of concentration between ventricular and lumbar CSF (i.e. RCG>1) in normal situations; their concentration does not vary with pathologically decreasing CSF flow rate, i.e. in cases of a blood CSF barrier dysfunction, and therefore it is independent of the $Q_{alb.}$

2) In the case of CSF proteins from leptomeningeal cells an increasing concentration between normal ventricular and lumbar CSF is observed (similar to blood-derived proteins) (i.e. RCG<1). In the case of pathologically decreasing CSF flow rate a linearly increasing concentration in CSF is observed, that therefore being dependent on $Q_{alb.}$

3) a group with a non-negligible blood-derived fractions in addition to brain-derived fractions. In this group it is important to decide whether a quotient or an absolute concentration of the brain-derived protein is preferred as the most sensitive evaluation with reference to the albumin quotient.

In the case of VEGF the ventricular levels were independent of Qalb (Spearman's correlation, R=-0.32, p=0.144). VEGF has both a brain and blood derived fraction. A limitation of our study was that we did not have access to serum samples and therefore it is impossible to calculate the blood-derived fraction of the ventricular

VEGF levels. The RCG however is 3.2 in iNPH following the dynamics of brain-derived proteins as described by Reiber. We thus suggest that there is increased release of VEGF in the CSF of patients with iNPH which is mostly derived from the brain or choroid plexus as research has shown. This is positively correlated with the degree of ventriculomegaly, however from the current data we cannot explore whether ventriculomegaly is a cause or a consequence of VEGF release. Furthermore, we are not able to explore the issues of angiogenesis versus neuroregeneration in hydrocephalus.

Despite what seems to be an important role in the pathogenesis and pathophysiology of chronic hydrocephalus VEGF levels have a low sensitivity and specificity in predicting a favourable outcome (VEGF=9.03 ng/mL, sensitivity=58.8% and specificity=20%). The favourable outcome group has smaller VEGF levels however the difference is not statistically significant. If we assume that VEGF is increased because of chronic ischemia we may therefore assume (as before with lactate and 8-isoprostane) that the element of ischemia at the time of assessment of our cohort is not so important in predicting a favourable surgical outcome.

The role of GFAP as a prognostic biomarker

Tullberg (Tullberg, Rosengren et al. 1998) and Albrechtsen (Albrechtsen, Sorensen et al. 1985) have both demonstrated increased GFAP levels in iNPH patients compared to controls. GFAP is particularly abundant in reactive astrocytes and gliotic tissue (Albrechtsen, Sorensen et al. 1985) and raised GFAP in CSF

suggests irreversible damage since GFAP is not actively secreted by the astrocytes.

In our study, the levels increased significantly in relation to time of drainage. This might signify a release of GFAP from a ventricular pool due to previous accumulated GFAP as result of the underlying astrocytic damage. These findings are supported by the high RCG (8.85) we have observed in our patients, and is also supported by other studies (Albrechtsen, Sorensen et al. 1985). It could not possibly be explained by further astrocytic damage since CSF drainage is known to reverse any pathological effects. Whether GFAP levels decrease as one would expect the longer the drainage continues is not possible to conclude from our current findings.

GFAP had a statistically significant negative correlation with both $A\beta_{1-42}$ and total tau in the first sample. This negative relationship with $A\beta_{1-42}$ continued in the next 72 hours but became non-significant. These results signify an inverse relationship between astrogliosis and neuronal degeneration, as is reflected in the CSF profile of these patients. One would expect to see a positive relationship between these two processes, i.e. increased gliosis associated with increased neuronal degeneration as a reflection of ongoing damage. It is for example known that $A\beta_{1-42}$ accumulates in astrocytes as a by-product of their local neuronal debris clearing function (Heneka 2006). Following that lysis of these $A\beta_{1-42}$ burdened astrocytes occurs to form astrocyte-derived amyloid plaques (Nagele, Wegiel et al. 2004). However, even though there have been many studies in heterogeneous populations looking into the above relationship this is not always the case (Joachim, Morris et al. 1989; Rozemuller, Eikelenboom et al. 1989). It has been

reported that astrogliosis in the AD brain, as measured by GFAP levels, increases independently of beta-amyloid accumulation but correlates with the duration of the disease, suggesting that deposition of beta-amyloid reaches an early “ceiling” whereas the gliosis continues throughout the time course of the condition (Ingelsson, Fukumoto et al. 2004). This last finding is supported by our present data. In a previous study that investigated neurofilament light chain and GFAP in patients with NPH (inclusive of secondary cases) this relationship was not examined (Tullberg, Rosengren et al. 1998).

GFAP is a marker of reactive gliosis and it correlates in a positive manner with the VV/DWMH, a ratio that represents the degree of ventriculomegaly versus the vascular ischemic load in this cohort of patients. Positive GFAP immunostaining has been found in areas of gliosis (Fazekas 1993) commonly associated with ventriculomegaly (Rubin, Hochwald et al. 1976), but not in areas related to DWMH (Young, Halliday et al. 2008) which may explain our finding. This diverse and distinct relationship between reactive gliosis and DWMH has been also documented in patients suffering from other types of dementia (Barber, Scheltens et al. 1999).

A significant negative correlation between the GFAP levels and the age of the control group ($p<0.001$). This relationship (Conde and Streit 2006) is established and confirms our findings. The ventricular levels of GFAP were found to have a positive correlation with 8-isoprostane, total tau and particularly NfH. These findings have not previously been confirmed in patients with iNPH. However, neuronal degeneration has been associated with astrogliosis in experimentally

induced excitotoxicity in aged rat brains (Castillo-Ruiz, Campuzano et al. 2007). Increased gliosis has been shown to precede AD pathology in patients with AD, although the correlation was between GFAP and amyloid beta in pathological sections of lateral temporal cortex (Wharton, O'Callaghan et al. 2009)

The RCG in NPH is 8.85. In normal subjects it cannot be calculated since the median value for a control population is 0 (with a range of 0-60). Although one may assume that the gradient is >1 since the cisternal median GFAP value is 0.2 pg/mL. The gradient agrees with the model for brain-derived proteins described by Reiber. Furthermore in iNPH the concentrations are independent of the Qalb again that verifying Reiber's model.

The levels of GFAP are higher in iNPH when compared to cisternal controls, even though the difference is not significant. Similar results have been published by Albrechtsen when compared lumbar GFAP concentrations to patients with AD and controls (Albrechtsen, Sorensen et al. 1985), and Tullberg who compared the lumbar levels of 65 patients with NPH and 40 controls (Tullberg, Rosengren et al. 1998).

A level of CSF GFAP=0.2 pg/mL will have a sensitivity of 43.8% and specificity of 75% of predicting a favourable outcome at 6 months. The Youden's index is 0.18. We therefore conclude that GFAP is the most useful marker among the ones examined with regards to its prognostic use. The levels of GFAP are lower in patients with favourable outcome, although the difference is not statistically significant. It use is particularly demonstrated by its high specificity, meaning that

astrogliosis is a phenomenon specific to patients with iNPH who are likely to improve following shunting. Since raised GFAP has been associated with irreversible brain damage (Taraszewska, Zelman et al. 2002) our findings suggest that good outcomes would be achieved in patients who have not yet sustained irreversible damage due to ongoing ischemia and/ or degenerative processes. It has been demonstrated that shunting can reverse the astrogliosis observed in hydrocephalic H-Tx rats (Miller, McAllister et al. 2007). The irreversible damage maybe a combination of both background processes (i.e. ischemia and neurodegeneration) rather than the product of one only since levels of GFAP did not differ between patients with mixed forms (idiopathic and secondary) NPH and subcortical arteriosclerotic encephalopathy (Tullberg, Mansson et al. 2000).

The role of NfH as a prognostic biomarker

Neurofilament (NF) proteins are major constituents of the neuronal cytoskeleton. Localised in large neurons and axons, they play an important role in neuronal structure. Increased levels of NFs in CSF may reflect neuronal degeneration in neurological disease (Petzold, Keir et al. 2007). The neurofilament light chain has been previously studied in patients with iNPH and its levels were no different between improved and non-improved patients following a ventriculoperitoneal shunt insertion (Tullberg, Blennow et al. 2007). However, the heavy subunit of the neurofilament protein (NfH) has not been investigated so far in iNPH. The drop in level of NfH between the onset of drainage and day 2 was significant. The increase between day 2 and 3 was not significant. NfH levels were reduced significantly within 72 hours. Tullberg et al. did not find a significant difference in the levels of

NfL (light chain of neurofilament protein) as they were measured before and after 3 months in 18 patients with iNPH (Tullberg, Blennow et al. 2008). In their study the levels of NfL increased rather than decreases as in our case with NfH; however the difference may be due to the different protein studied as well as the different time of sampling (3 days versus 3 months).

Our results may indicate the reversal of neuronal degeneration that one would expect to occur with shunting. However, the levels on all three days were within normal limits (upper limit of normal = 0.73 ng/ml (Petzold, Keir et al. 2006)). Such results may reflect either a) a lack of neuronal degeneration in the subjects, or b) an inability of NfH to reflect the neuronal degeneration characteristic of patients with iNPH.

The presence of neurofilaments is related to axonal degeneration (Petzold, Keir et al. 2007) and our findings confirm a negative correlation with white matter volume. This inverse relationship signifies an association between axonal degeneration and white matter volume loss. Indeed it was shown that white matter volume loss may be associated with increased risk of Alzheimer's dementia (Beauregard, Cristinzio et al. 2008). White matter changes being characteristic of demyelination of white matter axons, partial loss of axons and oligodendroglial cells, mild reactive fibrillary gliosis, and fibrohyaline thickening of the small vessels supplying the white matter has been associated with increased levels of the light chain of neurofilament triplet protein (NFL) (Sjogren, Blomberg et al. 2001). Its significant positive correlation with the PVL/WM ratio and positive trend with the DWMH/WM ratio may indicate an origin from the periventricular gliotic areas as well the chronic hypoxic areas of

the deep subcortical white matter. Similar findings about the light chain of the neurofilament protein (NFL) have been reported by other groups (Tullberg, Hultin et al. 2002; Tullberg, Blennow et al. 2007). A significant association of CSF NFL levels with the volume of DWMH in normal subjects has been reported from the authors of the Leukoaraiosis and Disability in the Elderly Study (LADIS) study (Jonsson, Zetterberg et al. 2009). It is worth noting however that the levels of NfH were normal and they might not be able to characterize the pathology observed in iNPH as well as NFL (Tullberg, Blennow et al. 2007). However, the association with the white matter volume warrants further study in a larger sample.

The ventricular levels of NfH are positively correlated with the levels of 8-isoprostane ($R=0.494$, $p=0.027$), total tau ($R=0.506$, $p=0.016$) and GFAP ($R=0.703$, $p<0.001$) in a significant fashion. The relationships with 8-isoprostane and GFAP have been already discussed in the relevant sections. The correlation with the levels of total tau may be explained by the observation that both markers represent a degree of neurodegeneration, and hence that degree is representative in the CSF levels in ventricular sampling. Such positive correlation has not been found in a study measuring markers of neurodegeneration (NfHSMI35, total tau, p-tau, Aβ 1-42 and Aβ 1-40) in various cohorts of dementia (Brettschneider, Petzold et al. 2006); however, the CSF samples were from lumbar fluid and, as our studies show, there are rostrocaudal dynamics that may influence the relationships of different proteins as they move along the craniospinal axis. Another publication which examined the same markers did not examine their relationship (De Jong, Jansen et al. 2007).

The levels of NfH did not correlate with age in any of the groups tested, agreeing with previous reports (Brettschneider, Petzold et al. 2006). The ventricular levels of NfH are almost 5-fold the levels in the lumbar sac. That as well as the fact that the levels of NfH are independent of Q_{alb} verifies again the dynamics for brain-derived proteins as described by Reiber. The ratio in control subjects is slightly over 1.0 again being independent from Q_{alb}. The ventricular levels in iNPH are higher than the cisternal controls albeit in a non-significant fashion. These increased levels are a reflection of the neurodegenerative process in this group of patients

Patients with favourable outcome have lower NfH levels than the unfavourable group, with the difference being non-significant. A cut-off level of CSF NfH=0.15 will have a sensitivity of 70% and specificity of 20% of predicting a favourable outcome at 6 months; the sensitivity increases with levels ≤ 0.15 with the specificity decreasing accordingly. The relatively high sensitivity figures with relatively small levels in predicting favourable outcomes is another indication of neurodegeneration being a burden towards a favourable outcome.

The role of total tau as a prognostic biomarker

$A\beta_{1-42}$ and tau and their neuronal toxicity are known to be associated with neurodegenerative diseases (Taylor, Hardy et al. 2002). The tau protein interacts with other cytoskeleton proteins, such as neurofilament proteins (Hirokawa, Shiomura et al. 1988), which play an important role in the maintenance of the

normal architecture and axonal calibre of the neurons. The over expression of tau causes changes in cell morphology, retards cell growth and dramatically alters the distribution of various organelles transported by microtubule-dependent motor proteins.

Tau has been suggested as a potential biomarker for NPH (Tarnaris, Watkins et al. 2006) and both phosphorylated forms have been found to be increased in NPH patients when compared to controls (Kapaki, Paraskevas et al. 2007); these results were, however, contradicted in a larger study (Agren-Wilsson, Lekman et al. 2007). In both the latter studies $A\beta_{1-42}$ was decreased when compared to controls (Agren-Wilsson, Lekman et al. 2007; Kapaki, Paraskevas et al. 2007). Kapaki et al. identified a cut-off level of the phosphorylated form of tau (P-tau) greater than 47 (pg/mL) which is able to distinguish between AD and idiopathic NPH with a sensitivity of 88.7% and specificity of 88.6%. The authors used a commercial ELISA assay (Innotest, Innogenetics, Gent, Belgium) to determine the total and phosphorylated tau, and β-amyloid 1-42. The authors also found that total tau was increased in both conditions when compared to controls, whereas β-amyloid levels were decreased in both. The diagnosis of iNPH was based on history, examination and relevant imaging but no external lumbar drainage or tap test. A lumbar puncture was performed but the authors do not mention the pressures obtained. Increased tau levels when compared to a control group (orthopaedic subjects) were detected also by Kudo et al. (Kudo, Mima et al. 2000) in a mixed NPH group using the same commercial assay (Innotest, Belgium). However, their results contradict the studies of Agren-Willson (Agren-Wilsson, Lekman et al. 2007), Zemlan (Zemlan, Rosenberg et al. 1999), Lins (Lins, Wichart et al. 2004) and

Gloeckner (Gloeckner, Meyne et al. 2008) where the levels of tau were either lower than the control group or within the normal range. Agren-Willson et al. used an “in house” ELISA published previously (Blennow, Wallin et al. 1995), using patients undergoing orthopaedic surgery as controls. This sample (n=62) of iNPH patients is of value because of its size and its appropriate selection (lumbar infusion test and tap test performed in patients with relevant history and imaging). In the study by Zemlan et al. the levels of the cleaved form of the tau protein were barely detectable with their in house ELISA and Western Blot assays. Lins et al. used a commercial assay (Innotest, Belgium) to measure amyloid as well as total tau immunoreactivities. They constructed a combined evaluation of amyloid b peptide (1–42)-immunoreactivity (Ab42-IR) and total tau protein-immunoreactivity (TTIR) plot described previously (R?sler, Wichart et al. 2001), which discriminated all NPH from the AD samples. They concluded that the combined use of both markers rather than one separately is of diagnostic use. No separate sensitivity or specificity figures were provided though. TTIR was not higher (ns) when compared to controls. Gloeckner et al. in their study using the same commercial assay found lower tau levels than controls (Gloeckner, Meyne et al. 2008).

In patients with NPH the average value of total tau levels of the 4 studies that used the same commercial assay (Innotest, Belgium) was 294.7 pg/mL (Lins, Wichart et al. 2004; Agren-Wilsson, Lekman et al. 2007; Kapaki, Paraskevas et al. 2007; Gloeckner, Meyne et al. 2008). The range of total tau in two studies was from 75 to 1040 pg/mL. We used the same commercial assay having a mean of 140.10 pg/mL. The average level of total tau from control subjects was 188.6 pg/mL and 534 pg/mL in AD subjects, in the same four studies.

Our results from the initial lumbar sample for these two markers agree with those of Agren-Wilsson and colleagues (Agren-Wilsson, Lekman et al. 2007). The increase in total tau levels over the 3 days of drainage was significant, both from day 0 to day 2 and from day 2 to day 3 signifying possibly a release from a ventricular pool. That could coincide with the known “toxic theory” suggested for the pathogenesis of AD (Selkoe 2000) which would mean that either an overproduction or reduced clearance of the tau protein could be responsible for the changes observed in NPH (Silverberg, Mayo et al. 2003).

The lumbar levels of $A\beta_{1-42}$ and tau are positively correlated only in the first 24 hours of ELD meaning that CSF drainage clears an established metabolic pool of these markers, even though this clearance is delayed in the case of tau protein. Our results might mean that there is a predominance of tau rather than $A\beta_{1-42}$ accumulation in NPH, and that is reflected on the increased concentration or that there is a reverse caudo-rostral concentration (RCG=0.93) gradient as we have seen in the case of $A\beta_{1-42}$ protein. The ventricular levels of total tau correlate in a significant positive manner with NfH ($R=0.506$, $p=0.016$), GFAP ($R=0.498$, $p=0.025$) and VEGF ($R=0.429$, $p=0.05$) and the significance of these findings has been discussed earlier.

The ventricular levels of total tau were 6-fold higher than the respective lumbar in patients with iNPH and 1.5 fold higher in the control group. The levels were independent of Qalb verifying Reiber’s theory about the CSF dynamics of brain-derived proteins. The ventricular total tau levels in NPH were significantly higher

than cisternal total tau levels in patients with TGN as would be expected by patients with no clinical signs of a neurodegenerative process. This adds to evidence that total tau characterizes not just the well known neurodegenerative disorders but also patients with iNPH. One study had found contradictory results with total tau levels not being different than the control group (Lins, Wichart et al. 2004); their results (266.9 +/- 29.8 pg/mL) do not differ than ours with reference to the lumbar CSF levels. However, as we have shown the ventricular levels are significantly higher demonstrating the importance of the study of the CSF dynamics along the craniospinal axis for each marker.

Ventricular total tau levels had no associations with any of the structural volumes. An association has been demonstrated between the gray matter loss and neurofibrillary tangles (NFT) pathology (Whitwell, Josephs et al. 2008); however the NFT is a measure of the hyperphosphorylated tau load and not the total tau as was measured in our study. Furthermore the authors studied the NFT load in autopsy and not CSF samples.

Total tau might act as a marker of the progression of the condition. Our findings of a significant negative correlation of preoperative symptom duration and total tau levels ($R=-0.841$, $p=0.002$) in lumbar CSF suggest that a degree of neurodegeneration occurs in early stages of the syndrome. The ventricular levels of the same 10 patients demonstrate the negative correlation exhibited in the lumbar levels, but the relationship is becoming non significant ($R=-0.258$, $p=0.538$). This demonstrates the importance of studying the CSF dynamics for each marker studies since erroneous conclusions may be drawn if one studies only the lumbar

levels of a biomarker. Other proteins such as NfH did not show duration dependence; this needs further exploration and may be due to a specific pathological process in INPH or in the cohort of our patients. Studies in patients with AD have not found a correlation of CSF levels with the disease stage (Kurz, Riemenschneider et al. 1998; Mecocci, Cherubini et al. 1998). Other studies have contradicted these results (Nishimura, Takeda et al. 1998).

A level of CSF total tau=767 pg/mL will have a sensitivity of 17% and specificity of 20% in predicting a favourable outcome at 6 months. The low sensitivity and specificity of the cut-off value may mean that there is more than one pathological process which will determine the final outcome of surgery. Since the cut-off value of total tau is high and comparable to levels seen in patients with AD our results suggest that patients who bare a significant comorbidity of AD are unlikely to have a favourable of surgical outcome. We may also see that the surgical group with the favourable outcome had significantly lower ventricular levels than the unfavourable group (p=0.025).

The role of Aβ 1-42 as a prognostic biomarker

The pathological hallmark of AD are the extracellular amyloid plaques, composed of aggregates of amyloid-beta peptides and the intracellular neurofibrillary tangles, which contain accumulations of hyperphosphorylated forms of the neurofilament-associated protein tau (Poirier 2005). Amyloid peptides (Aβ) are fragments of the amyloid precursor protein (APP), an integral membrane protein. Aβ peptides are continuously generated by neurons and non-neuronal cells via sequential cleavage

of APP by proteases (Selkoe and Kopan 2003). The longer and more hydrophobic Aβ 1-42 fragment is much more prone to fibril formation than Ab 1-40, and even though Aβ 1-42 is a minor form of Aβ, it is the major Aβ species found in cerebral plaques. Damage to neurons may be caused by intracellular and, to a lesser extent, extra-cellular highly reactive and toxic Aβ dimers and oligomers.

The mechanism(s) leading to a reduction in CSF Aβ 1-42 level in patients with AD is still unclear. One possible explanation is that reduction is secondary to the progressive degeneration of neurons. However, after acute ischemic stroke, there is a marked increase in CSF-tau within 1 to 2 days that peaks after 2 to 3 weeks and returns to normal values after 3 to 4 months, whereas
the level of CSF Aβ 1-42 remains unchanged (Innogenetics and Gent 2000)
These data support the hypothesis that the CSF level of tau reflects neuronal damage and degeneration, whereas the CSF level of Aβ 1-42 does not seem to simply be a marker for neurodegeneration. Glial cells participate in the efficient uptake of soluble extra-cellular Ab and in the clearance of this material at localized sites where the Aβ is concentrated (Walsh, Klyubin et al. 2002).

Alternative explanations include reduced production or secretion of b-amyloid in AD brains. Alternatively, a reduction of CSF- Aβ 1-42 level in patients with AD may be secondary to an aggregation in the amyloid deposits, decreasing the amount of Aβ 1-42 that can be secreted to extracellular space and thereby resulting in lower levels remaining in CSF.

Aβ 1-42 is secreted to the extracellular space, which is continuous with CSF. In AD, a portion of Aβ aggregates and is incorporated into highly insoluble fibrils in the plaques. These amyloid deposits consist primarily of Aβ 1-42. It seems that the peptide is cleared out of the CNS to blood mainly via transport through the blood–brain barrier (BBB) (DeMattos, Bales et al. 2002) and/or via the interstitial fluid (ISF) bulk flow into the CSF, and from there into the bloodstream (Silverberg, Mayo et al. 2003).

Amyloid beta aggregates may be as high as 38% of patients with NPH (Holm, Savolainen et al. 2003). Silverberg et al. have proposed a unifying theory that may explain the pathogenesis of both AD and NPH based on CSF circulatory failure leading to the toxic deposition of Aβ in the meninges (Silverberg, Mayo et al. 2003). In a rat model of adult hydrocephalus immunocytochemistry revealed increased expression of Aβ and its transporters (Receptor for Advanced Glycation End products) RAGE as well as an increased of GFAP (Deren, Forsyth et al. 2009). As aging progresses there might be a breakdown in the homeostasis of Aβ as demonstrated by the decrease of the low-density lipoprotein receptor-related protein (LRP-1) receptor in aging rats (Johanson, Flaherty et al. 2006).

With regards to β-amyloid 1-42 levels they were assayed in 3 studies using the same commercial ELISA as in our study (Lins, Wichart et al. 2004; Kapaki, Paraskevas et al. 2007; Gloeckner, Meyne et al. 2008) The average value for patients with NPH was 447.1 pg/mL, whereas that of the control subjects was 718.9 pg/mL and 402.4 pg/mL in AD subjects.

We observed an increase in the levels of $A\beta_{1-42}$ between day 0 and day 2 of ELD, and levels slightly decreased on the third day of drainage, a change which was not significant. Overall there was a non-significant increase in the levels over three days of drainage. We postulate that this is because Aβ 1-42, as said earlier is aggregated in the amyloid plaques and hence only a small fraction that is free is allowed to drain from the ECS to the CSF compartment. It would have been interesting to see whether Aβ 1-42 levels continue to increase or level off if drainage continued further. In a longitudinal study of AD and normal subjects the levels of Aβ 1-42 were shown to decrease over a period of years but no significant difference was recognized in the levels between the initial and last examinations (Kanai, Matsubara et al. 1998). On the other hand another report showed steady levels of Aβ 1-42 levels when they were studied a year apart (Andreasen, Hesse et al. 1999).

The levels of $A\beta_{1-42}$ and tau are positively correlated only in the first 24 hours meaning that CSF drainage clears an established metabolic pool of these markers, even though this clearance is delayed in the case of tau protein. The ventricular levels of $A\beta_{1-42}$ did not correlate with any of the other markers.

$A\beta_{1-42}$ and total tau had no significant correlations with any of the structural volumes. No association between the Aβ burden or CSF Aβ levels and brain atrophy, DWMH or ventriculomegaly were found in other reports (Josephs, Whitwell et al. 2008; Jonsson, Zetterberg et al. 2009).

There is no correlation of age with either ventricular of lumbar Aβ 1-42 levels in NPH. There is a positive trend (but not a significant correlation) between the age of patients with TGN and Aβ 1-42 levels; similar findings with normal groups have been reported (Van Gool, Schenk et al. 1994; Kanai, Matsubara et al. 1998)

The RCG of Aβ 1-42 is 0.93 and 1.09 in NPH and control subjects respectively. The Aβ 1-42 levels are dependent on the Q_{alb} (R=0.886, p=0.019) in the control subjects but not the iNPH group. Increases in Aβ levels due to decreasing CSF clearance and hence dependence in Qalb has been demonstrated in experimental models of chronic hydrocephalus (Silverberg, Caralopoulos et al. 2009).The dynamics of Aβ 1-42 suggest according to Reiber's theory that Aβ 1-42 is secreted by the leptomeningeal cells along the craniospinal axis. It has been indeed shown that there is amyloid accumulation in the cortical leptomeninges of both aged individuals and patients with AD (Hamano, Yoshimura et al. 1997; Shinkai, Yoshimura et al. 1997); the potential of amyloid deposition in the spinal cord has been demonstrated in vivo with MR imaging in cases of amyloidosis and spinal vascular malformations (Hart, Merz et al. 1988; Horowitz, Thomas et al. 1998). The fact that in NPH the RCG is less than 1 may be explained that the ventricular CSF levels are lower than the control levels (229.3 vs. 841.32 pg/mL respectively). Aβ 1-42 would be then secreted along the craniospinal axis resulting in higher lumbar than ventricular levels in iNPH and hence a RCG <1. Authors of a study where the fluctuation of Aβ 1-42 levels were studied assumed that there was no RCG in normal subjects (Bateman, Wen et al. 2007); however they did not sample ventricular CSF but collected 25-35 mLs of lumbar CSF. Our study found a small gradient (1.09). However, as it has been shown the volume of the spinal CSF is a

mean of 81 +/- 13 mL in normal individuals (Edsbagge M, Starck G et al. 2009) rendering the above method of calculating a RCG methodologically incorrect.

There was a significant difference between the ventricular levels of Ab in patients with NPH and cisternal levels in TGN (Mann-Whitney test, p=0.035). The reasons for this have been explained above. It is however of note that the levels are much lower than ones previously reported for patients with iNPH. This may be explained by the <1 RCG that we have demonstrated since the previous studies reported lumbar CSF levels.

The levels of Aβ 1-42 are lower in the favourable group with the difference being statistically significant (Mann-Whitney test, p=0.011). When correction for multiple comparisons is applied however in a logit regression model the difference is not statistically significant (see table 3.15.10.1). A cut-off level of CSF Aβ 1-42=180 pg/ mL will have a sensitivity of 35% and specificity of 20% of predicting a favourable outcome at 6 months. It is worth noting that the cut-off value provided by the ROC curve is much lower than the mean ventricular CSF level. The fact that the Aβ 1-42 levels alone do not prove useful to predict outcomes may be explained by the possibility that there are multiple path anatomical factors that influence the surgical outcome.

The combined role of total tau and Aβ 1-42 as a prognostic biomarker

We decided to examine the ratio of total tau/ Aβ 1-42 in our cohort since in previous publications that used the same commercial ELISA kit as our experiments

this ratio appears different in the groups examined; and in particular it is 0.65 in NPH, 0.26 in control subjects and 1.32 in patients with AD. In our study the unfavourable group had a higher index than the favourable group (3.89 vs. 3.31) but the difference was not significant. A cut-off level of 2.28 will have a sensitivity of 47% and specificity of 20% of predicting a favourable outcome at 6 months.

However, the discriminant analysis undertaken for a combination for total tau and Aβ 1-42 classified more than 80% cases correctly and had a sensitivity and specificity greater than 80%. The above demonstrates the importance of the concomitant AD pathology in predicting surgical outcomes.

The lower values of Aβ 1-42 and total tau in the favourable outcome group raises questions about the pathophysiology of the syndrome. The pathological hallmark of AD are the extracellular amyloid plaques, composed of aggregates of amyloid-beta peptides and the intracellular neurofibrillary tangles, which contain accumulations of hyperphosphorylated forms of the neurofilament-associated protein tau (Poirier 2005). If a reduction of CSF Aβ 1-42 levels in patients with AD is secondary to an aggregation in the amyloid deposits (Tamaoka, Kondo et al. 1994), decreasing the amount of Aβ 1-42 that can be secreted to extracellular space and thereby resulting in lower levels remaining in CSF, why are the levels of total tau lower? Could iNPH represent an earlier stage of a common pathway that may have Alzheimer's dementia as its one end? Or could iNPH as suggested by Silverberg et al. represent one part of a hybrid nosological entity that has its primary origins in CSF circulatory failure? (Silverberg, Mayo et al. 2003). The findings of four studies that have used the same commercial ELISA assays as our studies have suggested

that a combination of lower total tau levels (mean: 294.7 pg/mL) but comparable Aβ 1-42 levels to Alzheimer's dementia (mean: 447.1 pg/mL) may be unique to the biochemical profile of iNPH patients and therefore may be applicable as a diagnostic biomarker (Tarnaris, Toma et al. 2009). Our finding of higher total tau levels in patients with smaller symptomatic duration may represent the earlier stages of a dynamic tauopathy. In patients with longer symptomatic duration total tau levels may be reduced due to abnormal hyperphosphorylated levels of tau being produced. They in turn represent the hallmark of development of an AD-like state; hyperphosphorylated tau behaves as an inhibitory/toxic protein unable to stimulate microtubule assembly, but also sequestering normal tau and contributing to inhibition of assembly and disruption of microtubules. The breakdown of the microtubule network in the affected neurons compromises axonal transport, leading to retrograde degeneration which, in turn, results in dementia (Iqbal 2005). To provide such answers in a future study the levels of hyperphosphorylated tau should be measured in the same cohort.

The role of volumetric analysis as a prognostic tool

Although volumetric analysis was not undertaken as a predictor to surgical outcomes but as a means to find associations with biochemical markers, nevertheless a logistic regression analysis did not reveal any significant predictors for surgical outcome. The favourable group appeared to have a significantly lower PVL normalised ratio and PVL load in the white matter and higher IVV/PVL ratio. In essence the group that had favourable outcome following shunting in our study had relatively larger ventricles and smaller load of periventricular lucencies when

compared to the unfavourable group. However, neither of these parameters were an independent predictor of outcome. It is known that PVLs are due to due to pressure-related changes in the volume of the extracellular fluid (ECF) (James, Flor et al. 1980; Murata, Handa et al. 1981; Page 1985; Takei, Shapiro et al. 1987). Patients with PVLs exhibited greater improvement post shunting (Borgesen and Gjerris 1982; Thomsen, Borgesen et al. 1986; Poca, Mataro et al. 2004). This might be explained by the fact that the presence of the lucencies on a patient might indicate that the hydrocephalus is still not fully "compensated" (Bradley 2001), and therefore the pathologic changes are reversible if CSF dynamics are restored. A study of 26 patients that reviewed the ventricular volume ratio, brain volume ratio, pericerebral CSF volume ratio, and the ratio of ventricular volume to pericerebral CSF volume found no predictive use in the above parameters (Palm, Walchenbach et al. 2006). In our review of imaging as a predictor to surgical response only SPECT and phase-contrast MR imaging were useful in predicting surgical response in patients with iNPH (Tarnaris, Kitchen et al. 2008). Even though one would expect that the ventricles would decrease in size postoperatively and that this change would correlate favourably with clinical outcomes this may not always be the case (Meier and Mutze 2005). This is most likely due to changes of cerebral elastance and an increase in brain "stiffness". Even though shunting restores physiological parameters of intracranial dynamics patients do not always experience clinical improvement accordingly (Petrella, Czosnyka et al. 2008). Another explanation could be that the patients have passed a "stage of no-return" and that the metabolic disturbance might now be the prevalent factor that will determine the improvement or not of the patient (Kondziella, Sonnewald et al. 2008). Nevertheless other authors have argued that computerised measurement of

ventricular size might actually reveal decreased ventricular volume in patients who improve postoperatively (Frucht and Goodman 2002). Ours study despite using computerised-assisted volumetric analysis of intracranial volumes did not confirm the above argument.

The mean ventricular volume in a study of 24 iNPH patients was found to be 109.3 (50.7) mL (Tsunoda, Mitsuoka et al. 2001). These values are very similar to our volumetric finding of 126.7 (57.9) mL. However, our reported ICV of 681.3 (200.4) mL are lower than the values reported by Matsumae et al. (1370 (133) mL in a study of 26 normal subjects) (Matsumae, Kikinis et al. 1996). Very similar ICV to the latter study (1321.7 (118.6) mL) are reported in the over 70's in 215 males (DeCarli, Massaro et al. 2005). Yet, in a recent study of 87 control subjects having imaging while participating in the Alzheimer's Disease Neuroimaging Initiative the intracranial volumes were reported as 997.9 (98.4) mL (Ott, Cohen et al.). Variations in the range of 20-30% for VV have been noted by much earlier reports based on CT and radioisotope ventriculography (Wyper, Pickard et al. 1979); these may be due to methodological differences, since currently no standardization exists with regards to semi-automated intracranial volumetric analysis.

The role of neuropsychology as a prognostic tool

As with volumetric analysis neuropsychological assessment was used primarily as a tool to assess suitability for shunting. Many clinicians use the M.M.S.E instead of a formal neuropsychological assessment (Golomb, de Leon et al. 1994; McGirt,

Woodworth et al. 2005); however, the M.M.S.E. should only be used as a screening tool of dementia due to its limitations of matching all neuropsychological test scores with premorbid intelligence quotient levels and age-matched control data to obtain an accurate perception of any cognitive decline (Savolainen, Hurskainen et al. 2002; Tarnaris, Stephenson et al. 2007). Formal neuropsychological assessment improves the standard diagnostic algorithm used at present in selecting patients for shunt (Farace and Shaffrey 2005).

The baseline cognitive status of our group is not able to predict outcomes at 6 months. Thomas et al. have found that significant improvement in cognitive function occurs when INPH is treated with a shunt, and the likelihood of cognitive recovery is influenced by the extent of baseline cognitive impairment (Thomas, McGirt et al. 2005); however, they only suggested prediction of cognitive outcomes. This disagrees with the findings of Savolainen et al. who found only the recognition of words test (RMT words) as being able to distinguish the patients needing shunt surgery (Savolainen, Hurskainen et al. 2002).

The improvement in cognition in our group continued to 6 months and this has been demonstrated previously (Duinkerke, Williams et al. 2004). However, only half of the patients demonstrate improvement at any time point. Despite the documented improvement patients with iNPH still score less than control healthy population (Hellström, Edsbagge et al. 2008).

The more noticeable improvement in our study occurred in the verbal and visual memory in the 6 weeks assessment, and verbal memory and speed in information

processing in the 6 months follow-up. The repeated administration of the RMT for words and facial recognition has been extensively used in research into neurodegenerative disease (Fox, Warrington et al. 1998; Mummery, Patterson et al. 2000; Chan, Fox et al. 2001). Improvement following shunting may reach up to 67% in the 12 months follow-up period (Raftopoulos, Deleval et al. 1994). In fact it may be that patients with more severe cognitive impairment exhibit more pronounced improvement (Goodman and Meyer 2001). More elderly patients have less chances of cognitive improvement following shunting (Chang, Agarwal et al. 2006); however this is contradicted in a recent large study (Hellström, Edsbagge et al. 2008). The average M.M.S.E. in our cohort was 20 (range 8-29) which is similar to a cohort of NPH with significant CVD comorbidity in a study of 58 iNPH patients; their cohort without CVD comorbidity had a higher M.M.S.E. score on first testing (Hellström, Edsbagge et al. 2007).

The role of epidemiology and comorbidities in influencing surgical outcome

Co morbid factors did not have an influence in surgical outcomes in our study; similar findings have been reported by other studies (Bech-Azeddine, Hogh et al. 2007). In the large European Multicentre study of 146 patients with INPH 25% had DM, 58% had hypertension, 14% had suffered a stroke, 20% had cardiac disease and 10% had peripheral vascular disease (Wikkelso Carsten, Tans Jos et al. 2009). The results of the influence of co morbid factors on surgical outcomes from that study have not been presented yet. In the large Dutch NPH study the incidence of diabetes was 15%, that of hypertension was 28%, 14% had suffered a

stroke, 21% had cardiac disease and 4% had peripheral vascular disease (Boon, Tans et al. 1999). The authors do not discourage the offer of surgery in patients with concomitant CVD, although its role in poor surgical outcomes is acknowledged. In our study the incidence of respective comorbidities was slightly smaller but not dissimilar from the numbers quoted. Hypertension has been strongly associated with iNPH (Casmiro, D'Alessandro et al. 1989; Krauss, Regel et al. 1996). A much higher incidence of DM in patients with NPH has been reported (Jacobs 1977). Patients with iNPH and those with first-ever stroke exhibited very similar survival rates which were reduced when compared to that of a normal elderly population (Malm, Kristensen et al. 2000). Raftopoulos et al. noted that of the 13 patients (56% of the total study group) who died during the first 6 years of follow-up , half of the deaths were caused by brain or heart ischemic related conditions (Raftopoulos, Massager et al. 1996). In our study the outcomes may not be related to the comorbidities due to our short-term (6 months) reported outcomes. It may be that if our cohort is followed-up for a longer period the co morbid factors would have played a more significant role.

Furthermore we found no evidence of age, symptomatic duration of the each individual symptom being able to predict surgical outcomes. This comes in antithesis with many reports of shorter duration of symptoms being a predictor of favourable surgical outcome (Caruso, Cervoni et al. 1997; Meier and Miethke 2003; Kiefer, Eymann et al. 2006; McGirt, Woodworth et al. 2008); on the other hand other studies found symptomatic duration irrelevant to surgical outcomes (Greenberg, Shenkin et al. 1977; Poca, Mataró et al. 2004). In a large study with long follow-up each additional year of iNPH symptom duration was associated with

a 13% lower likelihood of treatment response (McGirt, Woodworth et al. 2008). In our study only 5 patients (23% of total) had symptomatic duration less than 12 months, and only 7 patients (33% of total) had symptomatic duration less than 24 months.

Limitations of the study

Since the drainage was established at 15 mLs/h it is possible that the changes observed in day 2 and day 3 represent the levels of ventricular rather than lumbar CSF. This is because CSF would follow the path of least resistance through the lumbar drain rather than the upstream route via the venous sinuses.

Is the difference in CSF markers during ELD explained by washout from a ventricular pool?

We postulated that the observed change of CSF markers during ELD maybe due to washout from a ventricular pool or actual changes induced by drainage. We have observed significant increases in VEGF, GFAP and total tau levels following ELD which may be explained by a washout of ventricular pooling of these proteins. This is because the above markers demonstrated a RCG>1. However, the changes of lactate, 8-isoprostane, NfH and Aβ 1-42 may not be explained as a washout since the RCG of these markers is less than 1 (equal to 1 in the case of 8-isoprostane). We may then assume that these changes represent physiological changes induced due to CSF drainage.

However, in a recent study it was found that the volume of the spinal CSF compartment was 81 +/- 13 mL (Edsbagge M, Starck G et al. 2009). Similar findings have been confirmed by Gjerris et al. who sampled ventricular and lumbar CSF in patients with NPH (Gjerris, Gjerris et al. 1988). Therefore, using sequential CSF sampling to assess the ventriculo-lumbar gradients of different markers may be methodologically invalid.

It has been shown that variations of body mass index or narrowing of the spinal canal may influence the levels of CSF protein concentration (Seyfert, Kunzmann et al. 2002). In our study the BMI was not recorded and hence not correlated with marker levels. However, all patients were assessed for the possibility of degenerative cervical or lumbar spondylosis either by the referring team or ourselves.

Another limitation was that we have not obtained serum samples for the proteins with a non negligible blood derived fraction in addition to the brain derived fractions assessed; such protein would be the VEGF in our study. Isoprostane dynamics may follow the same rules described by Reiber. In the design of future studies the inclusion of serum samples and appropriate analysis should be included.

One of the limitations of our study was that the markers were measured after 72 hours of external lumbar drainage which might prove insufficient in order to provide any significant alterations in the neuronal environment and the effect this will have on the composition of the CSF. There has so far been one more group which measured the differences postoperatively (Tullberg, Blennow et al. 2007), and although this would be ideal to provide firm conclusions it was not permitted in our

study for ethical reasons. Even though 72 hours is a short time significant alteration did occur in the composition of CSF and the correlations between markers might have been strengthened further if we were able to obtain CSF samples at a later time point.

Our results should be interpreted with caution due to the small sample size, however, they represent a platform to further our understanding of the radiological, biochemical and cognitive manifestations of the syndrome of iNPH. One of the limitations of our study was the volumetric process undertaken. By selecting the slices chosen it is true that not all ventricular volume is calculated as the fourth ventricle is excluded from calculations. In addition, the selection of the PVL and DWMH areas based on the pixel intensities and not on an algorithm may be further improved by a fully-automated method in a future study (Tullberg, Hultin et al. 2002). It has been shown that semi-automated analysis of brain volumes may be comparable to hand-measured volumes (Giesel, Thomann et al. 2008). However, semi-automated or fully-automated volumetry gives continuous values which may be better in assessing correlations with markers than the ordinal values of the Fazekas scale (Wahlund, Barkhof et al. 2001).

The neuropsychological assessment undertaken may be criticized for its complexity which led to many patients not being able to complete the battery at all times, especially preoperatively. At present there is no standardised battery of neuropsychological assessment in patients with iNPH and the problems this creates have been recognised by the international community. This acknowledgement has led to a Neuropsychology Conference for iNPH on

September 16, 2009, where an attempt to reach a consensus opinion on appropriate neuropsychological testing was undertaken.

One of the surgical limitations of our study was that our cohort was not operated by the same surgeon. However, the technique of an insertion of a VPS remains more or less a standard procedure with little variation and hence we do not think that this might have introduced bias in the results. More important than the surgeon remains the choice of valve as well as the setting if a programmable valve is chosen. Drake in a review of shunt technology of the last 50 years concluded that in paediatric hydrocephalus it is not the shunt technology but rather factors like the type of hydrocephalus, placement of the ventricular catheter and the ventricular catheter environment that predispose to ventricular failure or not in the paediatric population (Drake, Kestle et al. 2000). We do not know whether the hydrodynamic properties of each valve may have had a significant role to the surgical outcomes since our cohort is small, however our reported outcomes are in line with those of other studies.

Future directions

One of the most interesting findings of our studies was the dynamics of VEGF and its role. Our current data cannot answer the question of what causes VEGF elevation during drainage i.e. is VEGF a result of the chronic ischemia or does it increase as an attempt to offer neuroprotection. The role of angiogenesis in the pathophysiology of chronic hydrocephalus in both humans and rats has been highlighted recently (Dombrowski, Deshpande et al. 2008; Tarnaris, Toma et al.

2009). The development of chronic hypoxia and ischemia remains central in the evolution of chronic hydrocephalus (Boon, Tans et al. 1999; Bradley 2001). However the element of neurodegeneration has emerged as a parallel pathophysiological pathway and of equal importance to that of chronic ischemia (Silverberg, Mayo et al. 2003; Silverberg 2004). Aberrant angiogenesis appears to be the link of those two processes (Johanson and Jones 2001; Zlokovic 2005). To date though no attempt has been made to investigate the role of angiogenesis and neurogenesis in chronic hydrocephalus.

It has been postulated that NPH may share common pathophysiological mechanism with both vascular dementia and Alzheimer's disease, two conditions that appear commonly in the elderly population (Bateman 2004). Angiogenesis appears as a common pathway of both Alzheimer's and vascular dementia (Tarkowski, Issa et al. 2002; Vagnucci and Li 2003).

Vascular changes noted in an autopsy study were those of multiple microinfarcts, arteriosclerosis, demyelination and loss of axons in white matter; altogether, changes compatible with arteriosclerotic encephalopathy (Akai, Uchigasaki et al. 1987). Changes typical for Alzheimer's disease (AD) and arteriosclerotic changes have been reported. In two recent retrospective studies, clinical improvement was reported in three of five (Del Bigio, Cardoso et al. 1997) and two of eight (Savolainen, Paljarvi et al. 1999) shunted patients with Alzheimer's disease pathology established by biopsy indicating that comorbidity with this disease does not always mitigate against a beneficial neurosurgical result. There is one comparative study showing significantly more changes of Alzheimer-type in biopsies from hydrocephalus patients than in age-matched autopsy controls (Del

Bigio, Cardoso et al. 1997). In a prospective study of 56 patients who underwent ventriculoperitoneal shunting for iNPH and cortical biopsy a diagnosis of definite Alzheimer's disease could be made in seven cases (12.5%), probable disease in nine (16%), and possible disease in seven (12.5%) (Golomb, Wisoff et al. 2000). Neurofibrillary tangles (Ball 1976) and granulovacuolar degeneration in hippocampal neurons is prevalent in shunted patients who do not improve (Ball and Vis 1978) and the pathologic changes seen are very similar to those of AD. One of the most important findings of that study is that the patients with positive biopsies for AD had similar improvement in gait, psychometric testing and urinary control when compared with the patients with negative biopsies.

Deficiency of neurogenesis resulting in abnormal cortical development has been shown in a rat model of obstructive hydrocephalus (Mashayekhi, Draper et al. 2002). Destruction of neurons as a result of chronic hydrocephalus has already been shown (Klinge, Muhlendyck et al. 2002). The role of neurogenesis has not been so far studied in chronic hydrocephalus because models of chronic hydrocephalus have so far focused on the role of ischemia in its development (Del Bigio and Bruni 1988; Klinge, Samii et al. 2003). It is therefore unknown whether the levels of neurotrophic factors alter as a result of the progression of the syndrome. Furthermore we do not know the changes that occur with shunting and whether the functional recovery achieved both in mobility and cognitively is associated with enhanced neurogenesis secondary to shunting.

If the hypothesis of reduced neoangiogenesis and neurogenesis is correct the infusion of growth factors in experimental (animal) models may reduce or reverse

the development of the syndrome. If NPH is an angiogenesis-dependent disorder, then development of antiangiogenic drugs targeting the abnormal brain endothelial cell and neuron might be able to prevent and treat the syndrome. Angiogenesis promoters (such as VEGF) can be studied by immunohistochemistry, and temporally and spatially correlated with markers of neurodegeneration and neuronal death. Furthermore, antiangiogenic agents can be administered to investigate whether pathological features are ameliorated.

In view of potential therapeutic considerations, it would seem worthwhile to delineate, for different brain regions, the time-course of expression of vascular endothelial growth factor (VEGF) and other neurotrophic growth factors induced by the development of chronic hydrocephalus. Such experiments could be carried out in animal models of chronic hydrocephalus, such as by infusing kaolin into the cisterna magna.

Thesis conclusions

We have demonstrated that CSF removal in patients with iNPH produce measurable changes in the composition of CSF which may reflect changes of the ECS milieu in this group. It is not known though whether these changes are sustained or plateau following further CSF removal. Some of these changes may be responsible for the clinical improvement, although we cannot extrapolate this from this data.

We have demonstrated a disturbance in the blood-cerebrospinal fluid barrier which may explain the leakage of proteins across the barrier.

Most of the markers demonstrated gradients across the craniospinal axis. Hence, a careful interpretation of the pathophysiological significance of markers in lumbar CSF is warranted, since pathological associations occurring in the C.N.S. may not be accurately reflected in lumbar CSF.

CSF lactate exhibits the highest sensitivity in selecting patients for shunting, however its low specificity precludes it from being a useful biomarker.

The source of 8-isoprostane appears to be the white matter. Patients with iNPH suffer oxidative stress affecting both astrocyte function and neurofilament structure as part of a neurodegenerative process.

In iNPH there is a degree of angiogenesis as a response to ischemia or as an attempt for neuroregeneration. From the current studies, we are not able to explore the issues of angiogenesis versus neuroregeneration in hydrocephalus. VEGF obtains therefore a central role between a chronic hypoxic/ischemic insult and neurodegeneration in iNPH. We thus suggest that there is increased release of VEGF in the CSF of patients with iNPH which is mostly derived from the brain or choroid plexus. This is positively correlated with the degree of ventriculomegaly, however from the current data we cannot explore whether ventriculomegaly is a cause or a consequence of VEGF release.

The element of ischemia at the time of assessment of our cohort is not so important in predicting a favourable surgical outcome.

An inverse relationship between astrogliosis and neuronal degeneration has been shown in iNPH. However, a larger study population is required to reach a firm conclusion. GFAP appears the most useful single marker to predict outcomes; however it is not as useful as the combination of total tau and Aβ 1-42. The findings suggest that favourable outcomes would be achieved in patients who have not yet sustained irreversible damage due to ongoing ischemia and/ or associated degenerative processes.

The heavy chain of the neurofilament may originate from the periventricular gliotic areas as well the chronic hypoxic areas of the deep subcortical white matter. However, it may not be as useful to characterise the changes occurring in patients with iNPH as the light chain of the same protein. The relatively high sensitivity figures with relatively small levels in predicting favourable outcomes is another indication of neurodegeneration being a burden towards a favourable outcome.

Aβ 1-42, may be aggregated in amyloid-plaque like pathology in iNPH and hence only a small fraction that is free is allowed to drain from the ECS to the CSF compartment. Total tau might act as a marker of the progression of the condition and our results suggest that an element of neurodegeneration occurs in early stages of the syndrome. A combination of Aβ 1-42 and total tau appear to be able to predict with sufficient sensitivity and specificity surgical outcomes.

Patients who have a favourable outcome have lower levels of both total tau and Aβ 1-42 when compared to control subjects; this may be due to a smaller degree of CSF circulatory failure when compared to patients with AD. The biochemical profile of patients with iNPH appears unique and to a degree different than that of AD. On the other hand co-morbid factors like hypertension, previous stroke or cardiovascular disease did not have an influence in surgical outcomes in our study and therefore patients with probable iNPH should not be refused surgery on the basis of the above.

The group that had favourable outcome following shunting in our study had relatively larger ventricles and smaller load of periventricular lucencies when compared to the unfavourable group. However, none of the imaging characteristics as calculated with semi-automated computer software was able to predict surgical outcomes.

Cognitive improvement continues up to 6 months paralleling the overall clinical improvement. Baseline neuropsychological assessment does not proves useful in predicting surgical outcomes, but it should be used to rule out concomitant AD pathology in patients with probable NPH.

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