

Time Trends of Cerebrospinal Fluid Biomarkers of Neurodegeneration in Idiopathic Normal Pressure Hydrocephalus

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Abstract.

Background: Longitudinal changes in cerebrospinal fluid (CSF) biomarkers are seldom studied. Furthermore, data on biomarker gradient between lumbar (L-) and ventricular (V-) compartments seems to be discordant.

Objective: To examine alteration of CSF biomarkers reflecting Alzheimer's disease (AD)-related amyloid- β (A β) aggregation, tau pathology, neurodegeneration, and early synaptic degeneration by CSF shunt surgery in idiopathic normal pressure hydrocephalus (iNPH) in relation to AD-related changes in brain biopsy. In addition, biomarker levels in L- and V-CSF were compared.

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Methods: L-CSF was collected prior to shunt placement and, together with V-CSF, 3–73 months after surgery. Thereafter, additional CSF sampling took place at 3, 6, and 18 months after the baseline sample from 26 iNPH patients with confirmed A β plaques in frontal cortical brain biopsy and 13 iNPH patients without A β pathology. CSF Amyloid- β_{42} (A β_{42}), total tau (T-tau), phosphorylated tau (P-tau₁₈₁), neurofilament light (NFL), and neurogranin (NRGN) were analyzed with customized ELISAs.

Results: All biomarkers but A β_{42} increased notably by 140–810% in L-CSF after CSF diversion and then stabilized. A β_{42} instead showed divergent longitudinal decrease between A β -positive and -negative patients in L-CSF, and thereafter increase in A β -negative iNPH patients in both L- and V-CSF. All five biomarkers correlated highly between V-CSF and L-CSF (A β_{42} R = 0.87, T-tau R = 0.83, P-tau R = 0.92, NFL R = 0.94, NRGN R = 0.9; all $p < 0.0001$) but were systematically lower in V-CSF (A β_{42} 14 %, T-tau 22%, P-tau 20%, NFL 32%, NRGN 19%). With *APOE* genotype-grouping, only A β_{42} showed higher concentration in non-carriers of allele $\epsilon 4$.

Conclusion: Longitudinal follow up shows that after an initial post-surgery increase, T-tau, P-tau, and NRGN are stable in iNPH patients regardless of brain biopsy A β pathology, while NFL normalized toward its pre-shunt levels. A β_{42} as biomarker seems to be the least affected by the surgical procedure or shunt and may be the best predictor of AD risk in iNPH patients. All biomarker concentrations were lower in V- than L-CSF yet showing strong correlations.

Keywords: A β_{42} , biomarkers, idiopathic normal pressure hydrocephalus, neurofilament light, neurogranin, P-tau, T-tau

INTRODUCTION

The biochemical composition of cerebrospinal fluid (CSF) is commonly used as a surrogate measure to reflect changes in brain metabolism. CSF is assumed to undergo alterations by the time it arrives in the lumbar region (reviewed in [1]). Idiopathic normal pressure hydrocephalus (iNPH) is a geriatric disorder characterized by impaired gait and balance, urinary incontinence, and cognitive decline with evidence of ventriculomegaly [2, 3]. In iNPH patients, CSF shunting is an effective treatment [4]. Shunt-valve puncture in iNPH patients, often used to test the performance of the shunt, allows for the collection of CSF from the brain ventricles, which is easy, painless, and considered safe. In addition, a right frontal cortical tissue biopsy, collected during shunt placement, allows for analysis of brain pathology. Nearly half of iNPH patients show Alzheimer's disease (AD)-related amyloid- β (A β) pathology in biopsies while 10% show concomitant A β and tau pathology [5]. A β_{42} is an amyloid-derived protein that has proven its value to detect A β pathology in AD, as well as concomitant A β pathology in assisting diagnosis iNPH [6–11].

Total tau (T-tau) and tau phosphorylated at amino acid threonine 181 (P-tau) levels in CSF are key diagnostic biomarkers for AD, but their interpretation as markers of neurodegeneration in the brain is less clear (reviewed in [12]). Secretion of T-tau and P-tau could be induced by A β pathology [13, 14], and thus the levels in CSF may at least in part reflect accumulating A β pathology in the AD brain, but CSF T-tau also increases in disorders without plaques, e.g., with

severe neurodegeneration in Creutzfeldt-Jakob disease [15] and acute brain injury such as stroke [16]. However, there is also a step-wise increase with more severe tau pathology, as determined by positron emission tomography [17].

Neurogranin (NRGN) is a post-synaptic protein that is upregulated in the CSF of AD patients [12, 18–24]. Higher CSF NRGN levels correlate with the rate of cognitive decline [25, 26]. Together these data suggest that NRGN may be a marker of monitor synaptic damage in the brain. High CSF NRGN levels associate with A β plaques but not with tau, α -synuclein, or TDP-43 pathology [24], suggesting that, similar to tau, its upregulation in CSF may at least in part reflect a response to accumulating A β pathology in the brain.

Neurofilament light chain (NFL) is a scaffolding protein of the neuronal cytoskeleton that is highly expressed in large caliber myelinated axons with a function in axonal structural support, growth, and regulation. CSF NFL is increased in several neurodegenerative conditions and is hypothesized to leak into CSF upon axonal injury and to be a general biomarker of neurodegeneration [27]. NFL associates with disease progression in AD independent of A β pathology [28].

Prospective studies on CSF biomarkers are sparse and little is known on the changes after CSF shunt. In this study, we compared A β_{42} , T-tau, P-tau, NRGN, and NFL, potential biomarkers of neurodegeneration, in a population of iNPH patients with and without A β pathology in their brain biopsy. The objectives of this study were: 1) to determine whether ventricular CSF (V-CSF) would be superior to lumbar CSF (L-CSF)

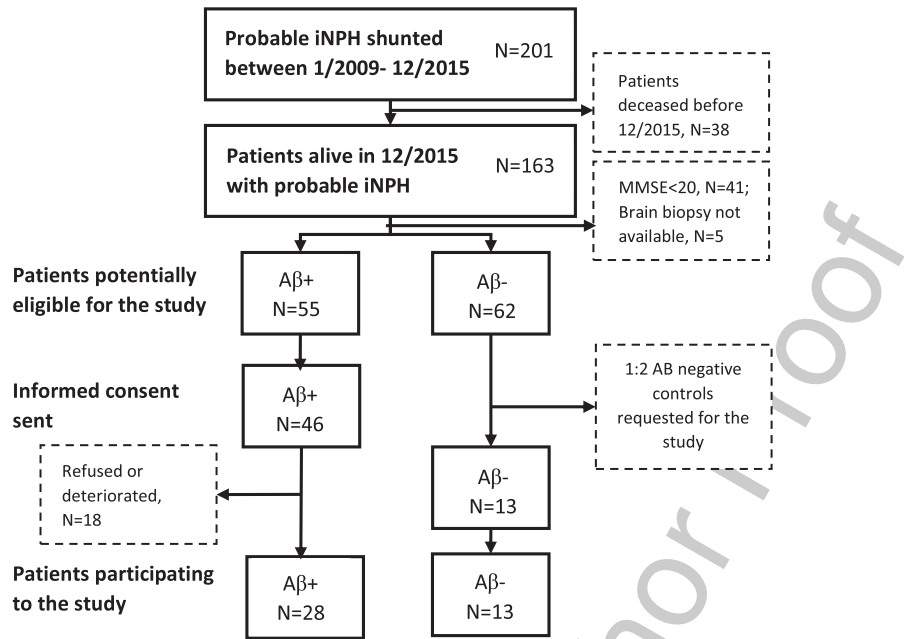


Fig. 1. Selection of shunted iNPH patients presented as a flow-chart. Eligible patients were sorted to groups based on the histopathological examination of A β in frontal cortical brain biopsy. Based on participating A β -positive patients, controls were requested with the ratio of 2 : 1, leading eventually to the group sizes of 28 A β -positive individuals and 13 A β -negative individuals. iNPH, idiopathic normal pressure hydrocephalus; A β , amyloid- β ; MMSE, Mini-Mental State Examination.

for the analysis of these biomarkers; and 2) to analyze and compare the longitudinal change in A β ₄₂, T-tau, P-tau, NRG1, and NFL concentrations in L-CSF and V-CSF samples collected repeatedly over 18 months.

METHODS

Study population and sample collection

Altogether, 201 patients with probable iNPH according to Relkin criteria [3] were shunted by right frontal puncture and ventriculoperitoneal CSF shunt (PS Medical Strata II valve) between January 2009 and December 2015 at the Kuopio University Hospital following a previously described protocol [29] (Supplementary Table 1). Brain biopsies of 41 patients were taken during shunt surgery and analyzed according to the established protocol [30]. The biopsy was taken from the right frontal cortex, 3 cm from the midline and anterior to the coronal suture, and the size of the cylinder-shaped sample was 2–5 mm in diameter and 3–7 mm in length. Biopsies were obtained using either biopsy forceps or since 2010 by disposable Temno EvolutionR TT146 biopsy needle (Merit Medical Systems Inc., South Jordan, UT, USA). Out of them, 28 patients with confirmed

A β plaques in their frontal cortical brain biopsy (5 with concomitant tau pathology) and 13 patients without A β pathology (control group) were included in the study (Fig. 1). Two A β -positive iNPH patients withdrew in early stage (Fig. 2). All participants had Clinical Dementia Rating (CDR) \leq 1 and Mini-Mental State Examination (MMSE) \geq 20. Exclusion criteria were contraindications for lumbar puncture, compromised well-being and serology positive hepatitis B or C, or human immunodeficiency virus.

Pre-shunt (B1) L-CSF ($n = 39$) was obtained during diagnostic CSF diversion, centrifuged and stored at a temperature controlled -80°C freezer. Post-shunting lumbar (L-) and ventricular (V-) CSF were simultaneously collected 3 to 73 months after the shunt placement (median 18 [mean 24] months post-surgery), to allow for putative normalization of the biomarker levels after injury caused by the surgery and thereafter at 3, 6, and 18 months (B0, 3 M, 6 M, and 18 M) sampling points (Fig. 2). All shunt valve punctures ($n = 142$) to obtain V-CSF were successful without blood contamination, any infections or other procedure-related harm. Lumbar puncture after CSF shunt was successful only in 111 out of 142 attempts (78%) and furthermore 4 samples (3.6%) had blood contamination. In addition,

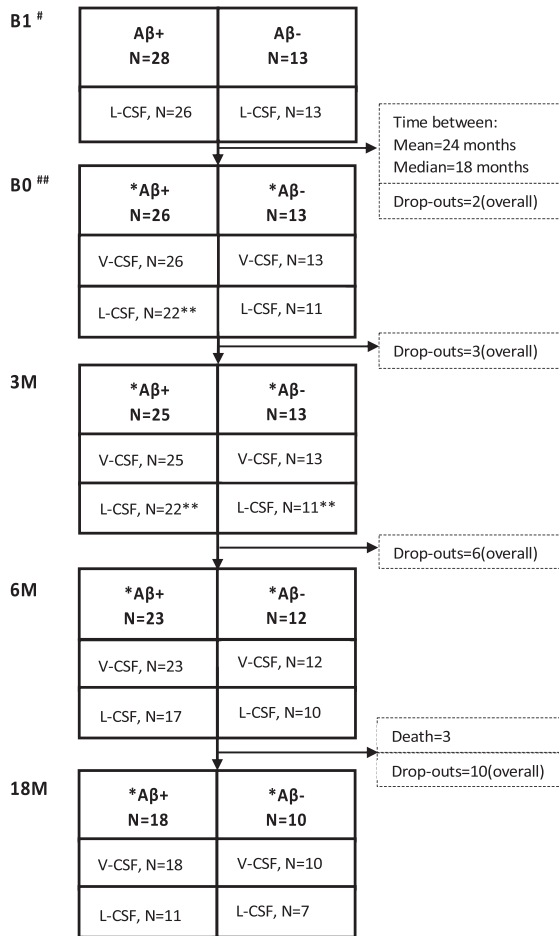


Fig. 2. Sample collection over time with the number of samples collected for V- and L-CSF in the Aβ-positive and -negative groups. Pre-shunt L-CSF was collected prior to the surgery. The 0M sample collection point was at least 3 but up to 73 months (mean 24 months; median, 18 months) after the shunt placement, followed by 3 M, 6 M, and 18 M sample collection points. L-CSF and V-CSF from the same patient were collected on the same day. Drop-outs and deaths between the time points presented as numbers from study population. Cognition was tested at 0 M, 6 M, and 18 M. #7 revision before B1; ##2 revision before B0; *B0 partial samples: 6 in Aβ+ and 3 in Aβ-, 3 M partial samples: 5 in Aβ+ and 5 in Aβ-, 6 M partial samples: 4 in Aβ+ and 4 in Aβ-, 18 M partial samples: 3 in Aβ+ and 5 in Aβ-; **B0: bloody CSF in 2 Aβ+ samples, 3 M: bloody CSF in 1 Aβ+ and 1 Aβ- sample; B1, pre-surgery sample collection time point; B0, baseline visit of the follow-up; 3 M, three-month study visit; 6 M, six-month study visit; 18 M, 18-month study visit; L-CSF, cerebrospinal fluid collected with lumbar puncture; V-CSF, cerebrospinal fluid collected with shunt valve puncture.

effects, centrifuged, aliquoted, frozen and stored at a temperature-controlled -80°C freezer immediately after collection. All samples analyzed in this study had at most one freeze-thaw cycle.

Tissue biopsy results on Aβ and tau pathology were used to divide patients into a control (no AD-type pathology; here referred to as biopsy-negative) group and a group with concomitant AD pathology (Aβ and in five cases also tau; here referred to as biopsy-positive). Patients were APOE genotyped by standard PCR method [31]. DNA was extracted from venous blood using a commercial kit according to the manufacturer's protocol (Illustra Blood GenomicPrep Mini Spin Kit, GE Healthcare, Little Chalfont, UK). Figure 2 shows the number of L- and V-CSF samples collected at the different sampling points per group indicating dropouts at each time. This study was approved by the Ethics Committee, Hospital District of Northern Savo. All participants gave written, informed consent prior to participation into the study.

Biomarker analysis

All CSF and plasma samples were analyzed at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden. CSF concentrations of Aβ₄₂, T-tau, and P-tau were measured with INNOTEST[®] ELISA kits (Fujirebio, Ghent, Belgium). CSF neurofilament light (NFL) concentration was measured with the NF-Light kit (UmanDiagnostics, Umeå, Sweden) [32]. CSF neurogranin (NRGN) concentration was measured using a sandwich ELISA developed at the Clinical Neurochemistry Laboratory [24]. In addition, we measured CSF concentrations of Aβ isoforms Aβ₃₈, Aβ₄₀, and Aβ₄₂ using a multiplexed electrochemiluminescence assay, as described by the kit manufacturer (Meso Scale Discovery, Rockville, MD, USA) [33]. All lumbar and ventricular samples of all sampling time points for each patient were analyzed on the same plate. All biomarker measurements were performed using one batch of reagents by board-certified laboratory technicians blinded to the clinical information.

Statistics

All statistical analysis was performed with IBM SPSS Statistics version 25.00 for IOS. For numerical data, group comparisons and changes over time were analyzed by mixed model multivariate analysis of variance (ANOVA). Pearson correlation coefficients were calculated to evaluate the strength of association

one lumbar puncture led to persisting radicular pain over two months (normal lumbar MRI). Samples were collected in single 10 mL polypropylene tubes to avoid adsorption of proteins to tube walls. CSF samples were mixed to avoid possible gradient

Table 1
Demographic data and biomarker values of the study population with the number of patients

Group		A β +, <i>n</i> = 26	A β -, <i>n</i> = 13	Pooled, <i>n</i> = 39		
Female (%)		11 (42)	5 (38)	16 (41)		
Age; mean (min-max)						
	B1	76 (63–88)	72 (64–80)	75 (63–88)		
	B0	78 (64–89)	73 (65–81)	76 (64–89)		
MMSE; mean						
	B1	24	23	24		
	B0	23	23	23		
	6M	24	24	24		
	18M	23	23	23		
APOE genotype (%)						
	34	10 (38)	2 (15)	12 (31)		
	33	11 (42)	10 (77)	21 (54)		
	24	1 (4)	0 (0)	1 (3)		
	23	4 (15)	1 (8)	5 (13)		
A β +, Brain biopsy amyloid-A β positive, <i>n</i> = 26						
Timescale		B1	B0	3 M	6 M	18 M
Biomarkers (Mean)	Location					
A β ₄₂ (ng/l)	V-CSF		481 (440)	482 (467)	462 (400)	587 (519)
	L-CSF	704 (724)	554 (530)	562 (527)	538 (522)	583 (581)
T-Tau (ng/l)	V-CSF		854 (741)	805 (737)	824 (757)	773 (832)
	L-CSF	248 (220)	1,057 (923)	1,174 (951)	1,039 (923)	1,110 (1,054)
P-Tau (ng/l)	V-CSF		99 (100)	109 (102)	106 (102)	114 (123)
	L-CSF	41 (42)	125 (114)	137 (127)	130 (125)	147 (152)
NFL (ng/l)	V-CSF		2,398 (1,404)	2,215 (1,832)	1,633 (1,382)	2,629 (1,987)
	L-CSF	1,864 (1,179)	2,692 (1,884)	2,669 (2,065)	2,586 (2,077)	3,288 (2,135)
NRGN (ng/l)	V-CSF		592 (529)	651 (532)	631 (549)	530 (519)
	L-CSF	161(42)	704 (494)	825 (619)	622 (546)	902 (653)
A β -, Brain biopsy amyloid- β negative, <i>n</i> = 13						
Biomarkers	Location					
A β ₄₂ (ng/l)	V-CSF		664 (721)	705 (711)	721 (729)	1,048 (1,057)
	L-CSF	786 (771)	767 (795)	760 (777)	825 (810)	1,052 (1,012)
T-Tau (ng/l)	V-CSF		577 (478)	574 (492)	643 (637)	632 (621)
	L-CSF	186 (152)	617 (506)	636 (550)	755 (523)	845 (751)
P-Tau (ng/l)	V-CSF		74 (69)	79 (77)	86 (85)	110 (115)
	L-CSF	34 (27)	78 (70)	86 (86)	98 (85)	146 (145)
NFL (ng/l)	V-CSF		2,860 (1,796)	1,629 (1,432)	1,533 (1,257)	1,757 (1,046)
	L-CSF	1,841 (1,060)	5,136 (4077)	2,974 (2,651)	2,198 (1,647)	3,250 (2,398)
NRGN (ng/l)	V-CSF		462 (325)	497 (468)	500 (389)	498 (432)
	L-CSF	70 (40)	357 (260)	471 (541)	405 (303)	736 (799)

Patients grouping of A β + and A β - is based on the brain biopsy A β histopathological examination result. Age of iNPH patients are presented with mean, minimum and maximum, sex as number and percent, MMSE as a mean and APOE ϵ 4 carriers as numbers and percent in the timescale of B1, B0, 3 M, 6 M, and 18 M. Biomarker concentration values of A β ₄₂, T-tau, P-tau, NFL, and NRGN presented as a means and medians with the timescale and location of sample collection. A β , AD-related amyloid- β MMSE, Mini-Mental State Examination; APOE ϵ 4, apolipoprotein ϵ 4 allele; B1, pre-surgery sample collection time point; B0, baseline visit of the follow-up; 3 M, three-month study visit; 6 M, six-month study visit; 18 M, 18-month study visit; A β ₄₂, Amyloid- β 42 protein; T-tau, total tau; P-tau, tau phosphorylated at threonine 181; NFL, neurofilament light; NRGN, neurogranin; L-CSF, cerebrospinal fluid collected with lumbar puncture; V-CSF, cerebrospinal fluid collected with shunt valve puncture.

208 between biomarkers in lumbar and ventricular CSF.
209 All significances calculated were two-sided with 5%
210 significance level used.

211 Data availability statement

212 All data related but not published within the arti-
213 cle is available and will be shared anonymized upon
reasonable request to any qualified investigator.

RESULTS

214
215 Table 1 summarizes patient demographics, clinical
216 characteristics, and mean biomarker values. Brain
217 biopsy A β -positive patients were weighted in 2 : 1
218 ratio. Brain biopsy A β -positive patients were on the
219 average 3.5 years older than biopsy A β -negative
220 patients (*p* = 0.039). The longitudinal progress of gait
velocity is presented in Supplementary Figure 1F.

Preoperative lumbar CSF A β ₄₂, T-tau, P-tau, NFL, and NRGN concentrations were similar in brain biopsy A β -positive and -negative iNPH patients (Table 1, Fig. 3). After CSF shunt surgery, lumbar CSF A β ₄₂ decreased ($p=0.043$, Fig. 3A), especially in *APOE* $\epsilon 4$ carriers ($p=0.006$). Notable increases were seen in T-tau ($p<0.001$), P-tau ($p<0.001$), and NRGN ($p=0.001$), which remained elevated during the later follow-up (Fig. 3C, E, I). The increase was more prominent in A β -positive patients regarding P-tau ($p=0.025$, Fig. 3E) and tended to be more obvious also in T-tau ($p=0.054$, Fig. 3C), but was not significant for NRGN ($p=0.287$, Fig. 3I). In NFL based on our modelling, the increase after shunt ($p=0.004$) was only temporary and estimated to normalize in 9 months after the surgery (Supplementary Figure 1D). The increase was more obvious in A β -negative patients ($p=0.047$, Fig. 3G) but was rather related with time delay from surgery to the first follow-up which was significantly shorter in A β -negative individuals (average 0.7 versus 2.2 years, $p=0.001$). The temporal dynamics of the measured biomarkers in relation to pre-operative values are presented in Supplementary Figure 1A-E.

Despite the initial decrease after shunt, A β ₄₂ concentration from L- and V-CSF showed increase during the entire follow-up ($p<0.0001$, Fig. 3). Increase was mostly present in brain biopsy-negative iNPH patients as the positive group remained rather stable after post-surgery decrease. The difference of A β ₄₂ was significant between the groups throughout the follow-up ($p=0.009$). In addition, A β ₄₂ was lower in *APOE* $\epsilon 4$ carriers and later increased in non-carriers (Fig. 4A, B) ($p<0.0001$). With NFL, there was no clear longitudinal change between the groups after the post-surgery fluctuation. Although the increase in T-tau, P-tau, NRGN, and NFL after shunting was somewhat more pronounced in the biopsy-positive iNPH patients through the follow-up, it was not significantly different from the biopsy-negative group (Fig. 3).

To circumvent inter-individual variation in absolute levels of the biomarkers, we normalized the values of all sampling points as % change towards pre-shunt L-CSF per patient. Again, we observed a significant increase in T-tau, P-tau, and NRGN after shunting in both L- and V-CSF. This increase was 2.5- to 3-fold for T-tau, 2- to 2.5-fold for P-tau, and 6.5- to 8-fold for NRGN and was sustained over time in both L- and V-CSF. A β ₄₂ showed mild increase of 35% in biopsy A β -negative patients both in L- and V-CSF, as the biopsy A β -positive patients remained

stable. In contrast to A β ₄₂, T-tau, P-tau, and NRGN, there was no clear increase in NFL in the study population as a whole (Fig. 3G, H), besides the transient increase associated with shunt placement described earlier.

The absolute levels of A β ₄₂ (14%), T-tau (22%), P-tau (20%), NFL (32%), and NRGN (19%) measured lower in V-CSF compared to L-CSF (Fig. 5). The absolute levels of each biomarker in L- and V-CSF per patient per time point showed a very strong correlation (A β ₄₂: $R=0.87$, $p<0.0001$; T-tau: $R=0.83$, $p<0.0001$; P-tau: $R=0.92$, $p<0.0001$; NFL: $R=0.94$, $p<0.0001$; NRGN: $R=0.90$, $p<0.0001$) (Table 2). No effect of *APOE* $\epsilon 4$ genotype on T-tau, P-tau, NFL, and NRGN levels was observed. The 18M correlation of L- with V-CSF A β _{42/40} ratio was very strong ($R=0.97$, $p<0.0001$; Fig. 5F).

As expected, T-tau showed a very strong correlation with P-tau both in L- and V-CSF (Table 2, Fig. 6). T-tau and P-tau correlated more weakly with NRGN, a correlation of which was somewhat higher in L- versus V-CSF (Table 2, Fig. 6). A β ₄₂ and NFL, on the other hand, did not correlate well or at all with each other or with T-tau, P-tau, and NRGN (Table 2). Because of a transient increase in NFL in a subset of patients having the 0M sampling point up to 9 months post-surgery, we correlated the NFL values of the pre-shunt, 0M and 3M sampling points of this group with their corresponding T-tau, P-tau, and NRGN values (data not shown). We found a medium to strong correlation between NFL and T-tau (L-CSF $R=0.76$, $p<0.0001$; V-CSF $R=0.63$, $p=0.009$), as well as NFL and P-tau (L-CSF $R=0.64$, $p<0.0001$; V-CSF $R=0.30$, $p=0.263$) in both V- and L-CSF. NRGN showed only medium strength correlation in L-CSF ($R=0.56$, $p=0.001$). These correlations were completely lost in the 6M and 18M sampling point values. They were also absent in those iNPH patients that had their 0M sampling point collected over 9 months post-surgery.

DISCUSSION

In this study, we analyzed longitudinal changes in the concentrations of five potential biomarkers of neurodegeneration (A β ₄₂, T-tau, P-tau, NRGN, and NFL) in V- and L-CSF of iNPH patients. This study provides the first longitudinal analysis of these biomarkers in simultaneously collected L- and V-CSF. It provides also the first longitudinal comparison of these biomarkers towards pre-operatively obtained L-CSF in iNPH patients who had recovered from

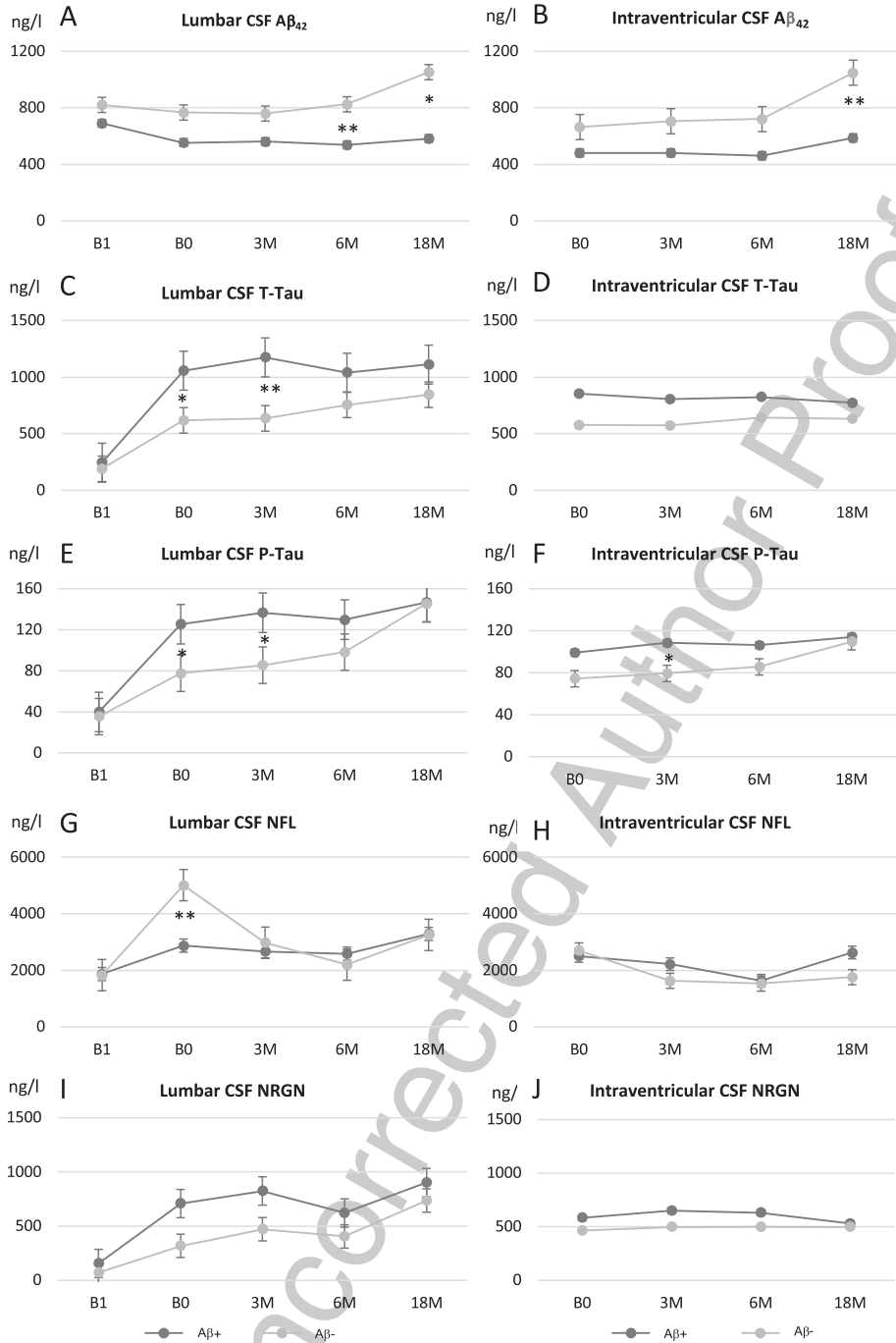


Fig. 3. Longitudinal analysis of biomarkers of neurodegeneration in lumbar (A, C, E, G, and I) and ventricular (B, D, F, H, and J) CSF for amyloid- β_{42} (A β_{42} ; A, B), total tau (T-tau; C, D), tau phosphorylated at threonine 181 (P-tau; E, F), neurofilament light (NFL; G, H), and neurogranin (NRGN; I, J). iNPH patients were grouped into to biopsy positive (dark gray) and biopsy negative (light gray) patients based on the presence or absence of A β pathology in their corresponding frontal biopsy. Values expressed as means \pm standard error. * $p < 0.05$; ** $p < 0.01$ between biopsy-positive and -negative patients in specific time point. B1, pre-surgery sample collection time point; B0, baseline visit of the follow-up; 3M, three-month study visit; 6M, six-month study visit; 18M, 18-month study visit; L-CSF, cerebrospinal fluid collected with lumbar puncture; V-CSF, cerebrospinal fluid collected with shunt valve puncture.

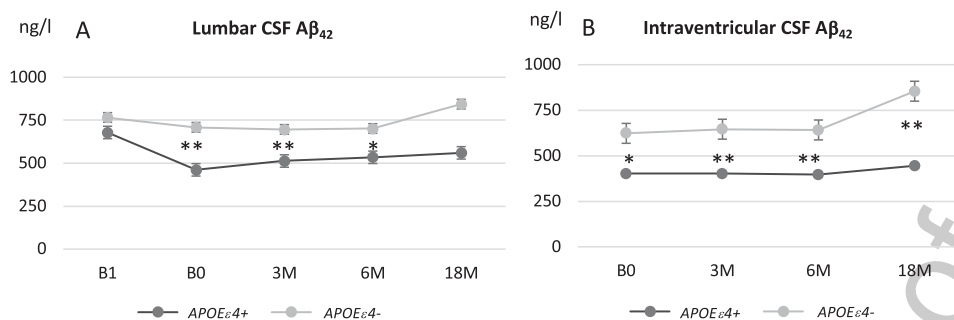


Fig. 4. Longitudinal analysis of A β ₄₂ in lumbar (A) and ventricular (B) CSF in iNPH patients grouped according to APOE ϵ 4 genotype carriers (dark gray) and non-carriers (light gray). Values expressed as means \pm standard error. * $p < 0.05$; ** $p < 0.01$ iNPH, idiopathic normal pressure hydrocephalus; APOE ϵ 4, apolipoprotein E ϵ 4 allele; B1, pre-surgery sample collection timepoint; B0, baseline visit of the follow-up; 3M, three-month study visit; 6M, six-month study visit; 18M, 18-month study visit; L-CSF, cerebrospinal fluid collected with lumbar puncture; V-CSF, cerebrospinal fluid collected with shunt valve puncture.

the shunt surgery for a minimum of 3 months, after which biomarker levels were assumed to have normalized from acute upregulation resulting from the surgery. In lumbar A β ₄₂, we found an interesting decrease after shunt surgery, which was most pronounced in brain biopsy-positive patients. However, during the follow-up, the concentrations stabilized in both groups and eventually increased in the brain biopsy-negative group. To our surprise, we observed a sustained longitudinal increase in T-tau, P-tau, and NRGN levels after surgery. Based on our modelling, NFL showed only a transient increase with levels returning to the pre-shunt levels 6 to 9 months post-surgery (Fig. 7). There was also a trend towards somewhat higher biomarker levels in brain biopsy A β -positive iNPH patients, apart from A β ₄₂, and it would be interesting to investigate whether these differences would become significant in larger groups.

The reason for the sustained increase in T-tau, P-tau, and NRGN after surgery and the NFL decrease toward baseline levels over time remains unclear but may represent the disease process of iNPH or change in CSF flow due to shunt. For AD patients, T-tau and P-tau has been shown to increase over time for 2% per year [34], which may indicate the disease process. In traumatic brain injury (TBI), T-tau levels were shown to already decrease toward baseline levels 20–43 days after the injury [35, 36], while a study in amateur boxers showed that both T-tau and P-tau levels normalized 3 months after brain injury [37]. With NFL, the study from amateur boxers showed that after acute upregulation during the first days after TBI, NFL levels had normalized towards baseline levels in 80% of boxers after 2 weeks [36]. In 20% of boxers, however, NFL levels remained significantly upregulated or were even increased after two weeks compared to

the control group and this was postulated to reflect continued sports-related mild TBI [36]. When taking into account the correlation with time delay from surgery to the follow-up CSF sampling, the increase in NFL was probably rather related to timing than brain biopsy A β profile. If splitting up the group depending on the time delay prior to the first follow-up sample (early: from 3 to 9 M and late: over 9 M), NFL seems to reflect effects of the shunt surgery, i.e., that the temporary increase after CSF shunt, lasting up to 9 months, may at least partly represent the minor injury related with penetration of the brain in CSF shunt surgery.

The interesting correlation of the early NFL concentrations to T-tau, P-tau, and NRGN evokes question whether the T-tau, P-tau, and NRGN upregulation imply the longer lasting neuronal damage due to or despite of shunt surgery. We found no correlation between MMSE and the measured biomarkers, thus, the informative value of biomarkers about the state of cognitive functions with iNPH patients remains unclear. Whether this is explained by a sustained injury or a change of CSF clearance or flow dynamics because of the shunting, remains to be shown. Since iNPH seems to be somewhat progressive despite shunt treatment in a number of patients [2], biomarkers predicting long-term outcome would be valuable.

CSF T-tau is suggested by the NIA-AA research framework to be a biomarker of neurodegeneration or neuronal injury [38]. We show here that this may need to be combined with NFL or other biomarkers of neurodegeneration to assess treatment effects of disease-modifying therapy as levels of T-tau, P-tau, and NRGN may not decrease over time.

The traditional hypothesis of CSF flow has been challenged [39] and there are evidence that CSF

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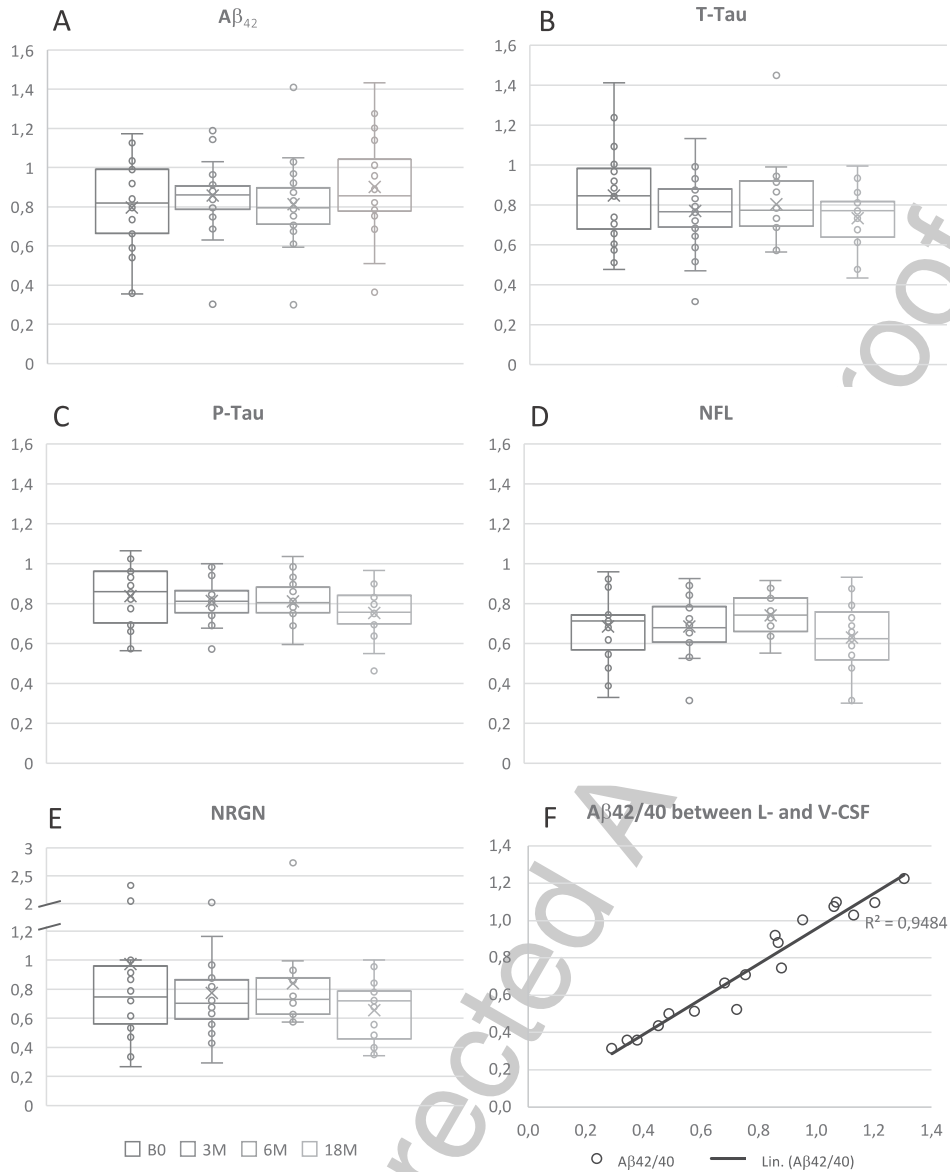


Fig. 5. Ratios of A β_{42} (A), T-tau (B), P-tau (C), NFL (D), NRGN (E), and A $\beta_{42/40}$ ratio (F) in V- and L-CSF. Ratios are presented as box and whiskers plot that portrays the median (center line), mean (cross), Q1 (lower edge of box), Q3 (upper edge of box), minimum and maximum (lines) values. Each boxplot presents all results of one sample collection point of the CSF and single dots demonstrate results of a single iNPH patient. The A $\beta_{42/40}$ result is from 18M time point and presented as correlation matrix. The A $\beta_{42/40}$ ratios presented showed strong correlation between lumbar and ventricular CSF, expressed as Pearson R². Linear trend-line adjusted for values to enhance the visibility of correlation. A β , amyloid- β ; B1, pre-surgery sample collection timepoint; B0, baseline visit of the follow-up; 3M, three-month study visit; 6M, six-month study visit; 18M, 18-month study visit; A β_{42} , Amyloid- β 42 protein; T-tau, total tau; P-tau, tau phosphorylated at threonine 181; NFL, neurofilament light; NRGN, neurogranin; L-CSF, cerebrospinal fluid collected with lumbar puncture; V-CSF, cerebrospinal fluid collected with shunt valve puncture; Q1, quartile 1 holding values up to 25 percentile; Q3, quartile 3 holding values up to 75 percentile.

395 movement is a local mixing and diffusion rather
 396 than unidirectional flow of production and absorp-
 397 tion. Shunt treatment and the iNPH disease itself can
 398 change the CSF flow [40–43] and the composition of
 399 the CSF collected in this study.

400 Simultaneously collected repeated L- and V-CSF
 401 samples indicated that in all 5 biomarkers tested, the
 402 levels were around 14–32% lower in V- compared to
 403 L-CSF. The reason for this remains specula-
 404 tive but the result is in line with a previous report

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405 [6] showing lower levels of T-tau, A β_{40} and A β_{42} in
 406 V-CSF of iNPH patients. All samples from one indi-
 407 vidual were run on the same plate, and the absolute
 408 values of one individual patient for each particular

Table 2

Correlations of A β_{42} , T-tau, P-tau, NFL, and NRGN in lumbar- and intraventricular-CSF

	A β_{42}	T-tau	P-tau	NFL	NRGN
Lumbar CSF					
A β_{42}	1	*-0.23	-0.10	*-0.21	*-0.19
T-tau	*-0.23	1	***0.88	**0.31	***0.60
P-tau	-0.10	***0.88	1	*0.25	***0.55
NFL	*-0.21	**0.31	*0.25	1	*0.17
NRGN	*-0.19	***0.60	***0.55	*0.17	1
Intraventricular					
A β_{42}	1	0.01	*0.19	-0.05	0.07
T-tau	0.01	1	***0.78	*0.24	***0.43
P-tau	*0.19	***0.78	1	0.16	***0.44
NFL	-0.05	*0.24	0.16	1	0.06
NRGN	0.07	***0.43	***0.44	0.06	1
L-CSF & V-CSF					
	***0.87	***0.83	***0.92	***0.94	***0.90

Significances of Pearson-r values presented as * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$. A β_{42} , Amyloid- β 42 protein; T-tau, total tau; P-tau, tau phosphorylated at threonine 181; NFL, neurofilament light; NRGN, neurogranin; L-CSF, cerebrospinal fluid collected with lumbar puncture; V-CSF, cerebrospinal fluid collected with shunt valve puncture.

time point correlated very highly. Thus, we think this cannot be attributed to a technical error in the measurement. A potential reason for the higher concentrations in L-CSF is the dominant diffusion to

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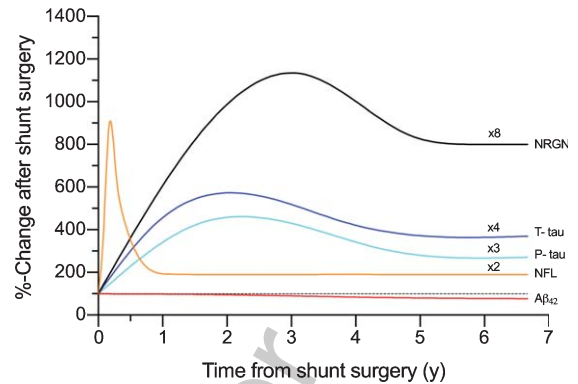


Fig. 7. The schematic presentation of the temporal dynamics in biomarkers of neurodegeneration plotted with the time in years (y) from shunt surgery and percentual change from the pre-surgery values (100%). The plots are formed with local polynomial regression and based on the data shown in Supplementary Figure 1A-E. Multipliers added to figure, are highlighting the longitudinal elevation found for biomarkers. A β_{42} , Amyloid- β 42; T-tau, total tau; P-tau, tau phosphorylated at threonine 181; NFL, neurofilament light; NRGN, neurogranin.

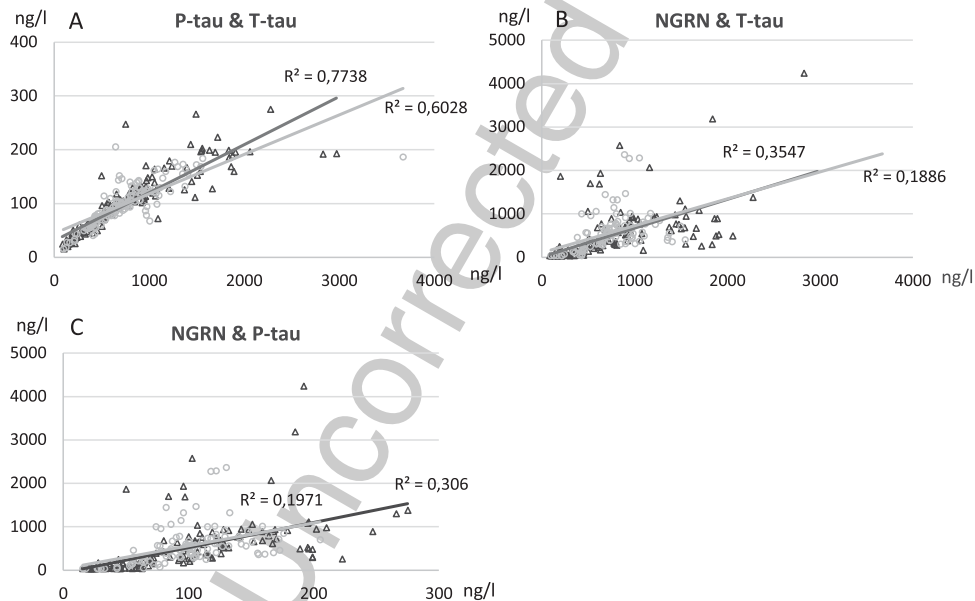


Fig. 6. Correlation analysis of T-tau levels versus levels of P-tau (A), T-tau versus NRGN (B), P-tau versus NRGN (C) in lumbar (L-CSF, black triangle) and ventricular (V-CSF, light gray circle) samples. Pearson R^2 values and significance level were calculated, and linear graphs adjusted according to the values. All time points of B1, B0, 3 M, 6 M, and 18 M are included in correlation analysis. T-tau, total tau; P-tau, tau phosphorylated at threonine 181; NRGN, neurogranin; B1, pre-surgery sample collection timepoint; B0, baseline visit of the follow-up; 3 M, three-month study visit; 6 M, six-month study visit; 18 M, 18-month study visit; L-CSF, cerebrospinal fluid collected with lumbar puncture; V-CSF, cerebrospinal fluid collected with shunt valve puncture.

413 lumbar CSF due to the gravitation and this gradient
414 effect might be amplified by high molecular weight.

415 In previous studies [44–46], V-CSF was shown to
416 contain higher levels of T-tau or P-tau compared with
417 L-CSF in iNPH patients, supporting the postulated
418 theory of a concentration gradient of brain-derived
419 proteins with higher levels in V-CSF. However, in
420 these studies the levels were biased by the surgi-
421 cal procedure in V-CSF sampling that seems to have
422 rather long-lasting effect on CSF biomarkers of brain
423 injury [47]. In other studies presenting T-tau or P-tau
424 levels higher in rostral compared with lumbar CSF
425 [46, 48], a rostro-caudal gradient has been suggested.
426 Consequently, the validity of tap test-collected large
427 volume lumbar CSF that may be “contaminated” by
428 V-CSF remains unclear. In addition, a study [49] pre-
429 senting higher T-tau levels in cisternal CSF compared
430 with L-CSF, had a patient population with trigemi-
431 nal neuralgia or tension-type headache. In this study,
432 with samples collected up to 3 months post-surgery
433 using the shunt valve puncture, we provide a dis-
434 tinct approach for the gradient comparison but cannot
435 determine the effect of CSF shunt on the gradient.

436 Current study confirm that shunt valve puncture is
437 considered to be a safe and feasible option to obtain
438 CSF samples from shunted iNPH patients. How-
439 ever, V-CSF requires specific reference limits for
440 diagnostic purpose since the biomarker values are
441 systematically 14–32% lower than in L-CSF. The
442 lumbar puncture success rate (78%) was notably
443 low, which possibly could be explained by potential
444 shrinkage of spinal dura sac [50] due to contin-
445 uous CSF diversion [51]. The high correlation of
446 biomarker concentrations in V- and L-CSF can be
447 utilized to produce correction factors for specific
448 biomarkers from intraventricular samples. Since the
449 success rate of shunt valve puncture is good and the
450 sampling procedure is easier to repeat, V-CSF analy-
451 sis of shunted iNPH is a promising tool for biomarker
452 diagnostics in the future.

453 We are aware that the total number of brain biopsy
454 A β -negative iNPH patients is half of the biopsy-
455 positive patients, which may have influence on our
456 results. The other issue to consider is the finite num-
457 ber of iNPH patients in addition to the alternating
458 participation to study visits and the limited success
459 rate of lumbar sample collection. Furthermore, A β ₄₀
460 was analyzed only in the first and last time point. We
461 also came by the challenge of variable delay from
462 shunt surgery to the first follow-up sample collection.
463 Especially with the biomarkers related to TBI, e.g.,
464 T-tau, P-tau, and NFL, we had to consider all possible

465 explanations for the fluctuation. In addition, the tis-
466 sue biopsy is rather small, only few cubic mm, and
467 taken from the frontal cortex, thus AD-type pathology
468 present in other areas of the brain could be missed.
469 However, biopsy A β correlates well with autopsy
470 [52] and amyloid PET [53].

471 The examined biomarkers correlated mostly as
472 expected, both between the V-CSF and L-CSF and
473 between other biomarkers. The understanding of the
474 longitudinal behavior of biomarkers of neurodegen-
475 eration, including their diffusion between different
476 compartments is important for the correct assessment
477 of advantages and limitations of these biomarkers as
478 biomarkers of disease progression.

479 In *APOE* ϵ 4 carriers, lumbar A β ₄₂ was lower
480 and showed a steep decrease after shunt insertion
481 and thereafter a minor tendency to decrease while
482 non-carriers showed milder decrease after shunt and
483 thereafter a significant increase. This result is sim-
484 ilar to longitudinal changes previously reported in
485 AD patients [34] and may indicate activation of
486 *APOE*-related clearance of A β by CSF shunt in iNPH
487 patients. Surprisingly, no such *APOE*-related effect
488 was seen in the increase of CSF P-tau. These intrigu-
489 ing preliminary findings motivate further study.

490 CONCLUSIONS

491 Longitudinal follow up shows that after initial
492 upregulation post-surgery, T-tau, P-tau, and NRG1
493 are stable in iNPH patients with or without A β
494 pathology in brain biopsy, while NFL normalized
495 towards its pre-shunt levels. A β ₄₂ instead showed
496 divergent longitudinal decrease between brain biopsy
497 A β -positive and -negative patients in L-CSF, and
498 thereafter increase in biopsy-negative iNPH patients
499 in L- and V-CSF. Thus, A β ₄₂ seems to be the
500 biomarker that is the least affected by the surgical pro-
501 cedure or the presence of shunt and may be the best
502 predictor of AD risk in iNPH patients. The concentra-
503 tion of all biomarkers measured 14–32% lower in V-
504 than L-CSF yet showing strong correlations between
505 the two sample types.

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SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: <https://dx.doi.org/10.3233/JAD-201361>.

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