Cerebrospinal fluid biomarkers to differentiate idiopathic normal pressure hydrocephalus from subcortical ischemic vascular disease

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Abstract

Background

Idiopathic normal pressure hydrocephalus (iNPH) remains a challenge to differentiate from subcortical ischemic vascular disease (SIVD). Despite major research efforts, the cerebrospinal fluid (CSF) biomarker profiles of the two diseases are still not known in detail.

Objective

To determine if novel CSF biomarkers neurofilament light (NFL) reflecting axonal damage, the synaptic protein neurogranin (NG), and the astroglial marker chitinase-3-like protein 1 (YKL-40) and the core Alzheimer's disease (AD) biomarkers amyloid-β 42 (Aβ42), total tau (t-tau), phosphorylated tau (p-tau) can differentiate iNPH from SIVD. Patients with AD and healthy controls (HC) were included for comparison purposes.

Methods

Patients with iNPH (n = 28), SIVD (n = 30), AD (n = 57), and HC (n = 33) were retrospectively included from the Danish Dementia Biobank. All patients with iNPH had effect of shunt surgery with a follow-up period of 4 to 69 months. CSF biomarkers were measured using immunoassays.

Results

Lower levels of NFL, NG, Aβ42, and t-tau (p = 0.0037) were found in patients with iNPH versus SIVD, while YKL-40 and p-tau were similar in the two diseases. NFL and Aβ42 were the most reliable biomarkers to differentiate iNPH from SIVD with an area under the curve (AUC) on 0.82 and 0.80, respectively. Combining NFL with Aβ42, t-tau, and p-tau resulted

in an AUC of 0.90, which was equivalent to the diagnostic accuracy of all six biomarkers combined.

Conclusion

An addition of NFL to the CSF panel of Aβ42, t-tau, and p-tau may improve the differentiation of iNPH from SIVD.

Key words: Normal pressure hydrocephalus, vascular dementia, biomarkers, cerebrospinal fluid

Introduction

Idiopathic normal pressure hydrocephalus (iNPH) is one of the few potentially reversible causes of dementia. The characteristic symptom triad of gait disturbance, cognitive decline and urinary incontinence is possibly explained by a disruption of the cerebrospinal fluid (CSF) dynamics. One theory is that impaired CSF absorption leads to a pathological flow of CSF into the periventricular white matter initiating a cascade of pathological processes such as edema, capillary microinfarctions, and potentially reversible neuronal degeneration [1,2]. The ventricular system is enlarged and often surrounded by white matter lesions (WML) on magnetic resonance imaging (MRI) in patients with iNPH. The diagnostic workup can be challenging. Patients may present with symptoms and neuroimaging features that overlap with Alzheimer's disease (AD) and subcortical ischemic vascular disease (SIVD) [3–5]. The supplemental tests, such as the CSF infusion test or tap test, are associated with relatively low sensitivity and/or specificity and complication risks [6]. Consequently, the diagnostic criteria are ambiguous, and only a minority of patients suffering from iNPH receive the diagnosis and are offered a surgical CSF diversion [7]. Treatment is often delayed which may influence the postoperative clinical outcome [8,9]. Therefore, new diagnostic tools are needed to improve diagnostic accuracy, which will possibly also lead to earlier diagnosis.

CSF biomarkers are obvious candidates for such diagnostic tools. Despite major research efforts, a CSF biomarker profile in iNPH has not yet been characterized [10]. A recent systematic review revealed that most research has been focused on the AD biomarkers amyloid-β 42 (Aβ42), total tau (t-tau), and phosphorylated tau (p-tau) [11]. Here, most evidence existed for low levels of t-tau and p-tau to differentiate iNPH from AD and low levels of Aβ42 to differentiate iNPH from healthy controls (HC). Few studies have investigated differences in biomarker levels between patients with iNPH and SIVD. Since iNPH and SIVD may be characterized by several pathologies, a panel of multiple biomarkers

instead of a single biomarker may be more adequate to provide the sufficient diagnostic accuracy.

Neurodegeneration and neuroinflammation are hallmarks of SIVD [12,13]. So far, there is no data to suggest neuroinflammation in iNPH. In addition, degradation of the neuronal structure may only play a minor role in the pathology of the disease, since symptoms can be partly or completely reversed depending on timing of treatment. Neurogranin (NG) and neurofilament light polypeptide (NFL) are promising biomarkers of neurodegeneration [14]. NG is found in the postsynaptic dendrites and is a putative marker of synaptic dysfunction in AD [15]. This pathology correlates with cognitive decline [16] and is believed to precede neuronal degeneration in AD [17,18]. NFL is considered a non-disease-specific biomarker of axonal damage [19]. It is part of the cytoskeleton of mainly subcortical large myelinated axons. This may explain the correlation between NFL and WML in disorders such as multiple sclerosis [20], iNPH [21], and stroke [22].

Chitinase-3-like protein 1 (YKL-40), a putative marker of astrocytic activation, is elevated following stroke [22], in multiple sclerosis [23], and in AD [24]. In addition, it correlates with t-tau and p-tau independently of Aβ [25]. Together, this indicates a possible link between neuroinflammation and neurodegeneration through a non-amyloid-related pathway.

This is the first study to examine differences in the CSF profile of the novel biomarkers of neurodegeneration (NG) and neuroinflammation (YKL-40) in patients with iNPH and SIVD. We aimed to evaluate the potential of NFL, NG, YKL-40, $A\beta_{42}$, t-tau, and p-tau to differentiate iNPH from SIVD and thereby determine which of these CSF biomarkers would be the most promising for use in clinical diagnosis. Patients with AD and HC were included for comparison purposes.

Methods

Study participants

CSF and data on a total of 148 patients including 28 patients with iNPH, 30 with SIVD, 57 with AD and 33 neurologically HC were collected retrospectively from the Danish Dementia Biobank. Between 2009 and 2016, the patients had been referred to cognitive evaluation at Copenhagen Memory Clinic, Department of Neurology, University Hospital of Copenhagen, Rigshospitalet, and Regional Dementia Research Centre, Department of Neurology, Zealand University Hospital on suspicion of a cognitive disorder. Controls were volunteers enrolled only for research purposes. As a minimum, patients and controls underwent diagnostic investigations including medical history, clinical examination, ECG, routine blood analysis, cognitive testing (MMSE, Addenbrooke's Cognitive Examination, supplemented with neuropsychological examination in patients with mild or unclear symptoms), lumbar puncture and structural imaging (head CT/MRI). Also, depending on the patients' symptoms, a PET-FDG functional imaging was performed. All participants gave their written consent to be included in the Danish Dementia Biobank for the purpose of future research. This study was in accord with the Helsinki Declaration of 1975 and approved by the Committee on Health Research Ethics of the Capital Region of Denmark.

Clinical classification

Patients were diagnosed with iNPH if they fulfilled the iNPH international guideline criteria for "probable iNPH" including gait disturbance, cognitive impairment and/or urinary incontinence [4]. Further, enlargement of the cerebral ventricles on diagnostic imaging, which could not be explained by general atrophy, was required. All patients went through a lumbar infusion test, except for one who completed a CSF tap test due to technical issues. If the diagnosis was uncertain after the supplemental testing (n = 9), a tap-test, an ICP monitoring test, and/or a ventricular liquor dynamic test was performed to increase the

diagnostic certainty. All iNPH patients underwent shunt surgery. Patients were not included if they had a known cause of NPH (secondary NPH) or responded less than “fair” according to the “Black Scale for assessment of shunt outcome” on shunt implantation [26,27]. The follow-up period was 23 months in average with a span from 4 to 69 months depending on the need for shunt revisions.

Patients with SIVD were diagnosed according to the VASCOG-criteria [28]. The occurrence of WML and lacunar infarcts were observed on MRI on T2-weighted images and evaluated by two experienced neurologists using the Fazekas scale [29]. A score of minimum 2 was required for patients with SIVD to be included.

Diagnosis of AD were made according to the NIA-AA criteria [30]. There were no indications of hereditary AD among the patients.

The neurological HC did not have subjective or objective signs of cognitive disorder. Control participants with a Fazekas score above 1 or WML above the expected for their age were excluded. Moreover, individuals with any neurological, psychiatric, or physical disease potentially causing cognitive impairment were excluded.

CSF analyses

During the diagnostic examination, a CSF sample was obtained from the participant by puncture in the L3-L4 or L4-L5 intervertebral space. During the diagnostic examination, a CSF sample was obtained from the participant by puncture in the L3-L4 or L4-L5 intervertebral space. YKL-40 concentration was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, Minnesota, USA). NG and NFL concentrations were determined using an in-house ELISA as previously described in detail [31,32].

Commercially available INNOTEST ELISA kits were used to measure the levels of $A\beta_{42}$, t-tau, and p-tau as described by the manufacturer (Fujirebio, Ghent, Belgium). In all four diagnostic groups, there was an unfortunate loss of data regarding values of $A\beta_{42}$, t-tau, and p-tau (iNPH = 4, SIVD = 3, AD = 12, HC = 10).

Statistical analyses

Analysis of demographic, clinical, and neuroimaging data was carried out using Kruskal-Wallis test on interval and ordinal data whereas Chi-Square test or Fisher's exact test were applied on categorical variables. Mann-Whitney U-test was used on the interval and ordinal variables for pairwise comparisons of the diagnostic groups, if significance initially was found. To test the association between biomarker concentrations and the diagnostic groups, age- and sex-adjusted linear models were applied. ROC curves were made to evaluate the diagnostic power of the individual biomarker and in combination by Orthogonal Projections to Latent Discriminant Analysis (OPLS-DA). The Variable of Importance Projection (VIP) score was obtained from the OPLS-DA plot to illustrate the relative contribution of the individual biomarker. The statistical analysis was carried out using IBM SPSS Statistics 25 and R (version 3.6.0, The R Project for Statistical Computing). The R package *ropls* [PMID: 26088811] was used for OPLS-DA analysis.

Results

Demographics, clinical and neuroimaging factors

This study included 28 patients with iNPH, 30 with SIVD, 57 with AD and 33 HC. The demographic, clinical, and neuroimaging data of the study participants are displayed in Table 1. There was no statistical difference in the sex distribution among the diagnostic groups. The HC group was significantly younger than the iNPH, SIVD, and AD group. Patients with

SIVD and AD had significantly lower MMSE scores vs. patients with iNPH and HC. Evans index was the only neuroimaging factor that separated iNPH from the other diagnostic groups. Clinical strokes, lacunar infarcts, hypercholesterolemia and Fazekas score were significantly more common or pronounced in patients with SIVD in comparison with patients with AD, iNPH and HC.

CSF biomarker concentration in the diagnostic groups

Patients with iNPH had lower levels of NFL, NG, $A\beta_{42}$ and t-tau compared with patients with SIVD (Table 2, Fig. 1). No difference in YKL-40 and p-tau were seen between the two disorders.

Low levels of NFL, NG, YKL-40, t-tau, p-tau separated iNPH from AD, while $A\beta_{42}$ were similar in both diseases. In comparison to HC, patients with iNPH had decreased levels of NG, $A\beta_{42}$, t-tau, and p-tau and similar levels of NFL and YKL-40.

ROC analysis revealed that $A\beta_{42}$ and NFL were the most reliable biomarkers to differentiate iNPH from SIVD with an area under the curve (AUC) on 0.80 and 0.82, respectively. In Fig. 2A the diagnostic accuracy of the individual biomarkers is illustrated including the biomarkers combined (OPLS-DA) with an AUC of 0.90. Fig. 2B displays the relative contribution of the individual markers by a Variable Influence on Projection (VIP) score. An OPLS-DA model based on NFL in combination with the three AD core biomarkers, without NG and YKL-40, had the same AUC (0.90) as the model where all six biomarkers were included.

Discussion

In this study, we found that NFL, NG, Aβ42, and t-tau could distinguish iNPH from SIVD by lower levels of all four biomarkers in iNPH vs. SIVD. NFL and $A\beta_{42}$ performed best in the

differentiation of the two diseases. The biomarker levels in patients with AD and HC, who were included for comparison purposes, matched previous published levels [24,31,32].

The higher level of NFL in patients with SIVD compared to patients with iNPH is in accordance with the general belief that axonal loss is a more dominant pathology in SIVD, which may explain the irreversibility of this disease in contrast to iNPH. In contrast, a previous study reported no difference in NFL between iNPH and SIVD [35]. Here, the prevalence of several comorbidities among patients with iNPH was more pronounced in comparison with our patients with iNPH. It is known that comorbid diseases such as cardiovascular disease are associated with WML [34]. Therefore, the lower NFL levels in our patients with iNPH may have been due to a lower Fazekas score. Two other studies reported equal levels of NFL in patients with iNPH and large vessel vascular dementia (VaD) [37,38]. In this subtype of vascular dementia, the subcortical regions may be less affected by axonal loss resulting in less leakage of NFL into the CSF compared to SIVD. Also, one study included both shunt-responders and non-responders in their group of patients with iNPH in contrast to our study which may also have had an influence on the discrepancy in the NFL levels reported [36].

A recent systematic review and meta-analysis illustrated the distribution of CSF NFL in neurological conditions [37]. There was a tendency towards slightly higher levels of NFL in VaD compared with iNPH. However, it should be noticed that these data were based on only two studies including a total of 56 patients with iNPH and two studies including 491 patients with VaD with no distinction between SIVD and VaD. None of the studies included both diagnostic groups.

A high level of NFL is found in several neurodegenerative disorders such as frontotemporal dementia (FTD) and progressive supranuclear palsy [40,41] which indicates that NFL is not a disease-specific marker. Moreover, NFL is already considered a plausible prognostic marker

of disease activity in multiple sclerosis [40] and a risk factor of MCI in a community population [43]. Since serum and CSF NFL correlates positively [44], serum NFL may be applicable as screening tool for neurodegenerative disorders in patients with cognitive impairment in primary care units. With this study, we show that NFL may also be valuable in memory clinics by improving the differential diagnosis of iNPH in combination with a CSF biomarker panel of $A\beta_{42}$, t-tau and p-tau. The highest and second highest level of NFL in our iNPH-group was 2441 ng/L and 1530 ng/L, respectively, with a 95 % confidence interval of 922 ± 160 (data not shown). Based on this we suggest a cut-off value of NFL between 1082 ng/L and 2441 ng/L as clinically useful to distinguish between patients with iNPH and patients with SIVD, who have higher levels of NFL. However, more studies are needed to define a validated cut-off value.

No evidence for an inflammatory component as part of the pathophysiology of iNPH or SIVD was found, since YKL-40 did not differ between iNPH, SIVD, or HC. No other studies have investigated the difference in YKL-40 between patients with iNPH and SIVD, but others did report similar results in patients with iNPH vs. HC and VaD vs. HC, respectively [43,44].

Our findings that NG was low in iNPH in comparison to SIVD, AD, and HC suggest that synaptic degeneration may not contribute to the pathology of iNPH. This is consistent with previous studies reporting a promising potential for NG as an AD-specific marker [32] while it is unaltered or even reduced in several neurodegenerative disorders such as FTD, VaD [46], amyotrophic lateral sclerosis (ALS) [32], Lewy body dementia, and Parkinson's disease [47]. The reduced levels of CSF NG in ALS have been linked to synapse survival [32]. It could be hypothesized that the decreased level of NG is a compensatory neuroprotective response in patients with early stage iNPH. With the possibility of early ongoing neurodegenerative processes among our group of HC (see below), NG may alternatively be

considered a marker of concomitant AD pathology among patients with non-AD dementia disorders.

So far, only one study has investigated $A\beta_{42}$ in iNPH and SIVD and failed to find any difference in $A\beta_{42}$ between iNPH and SIVD [48]. In our study, $A\beta_{42}$ was one of the most reliable biomarkers to differentiate the two entities with a significantly lower level in iNPH vs. SIVD. The discrepancies may be due to more strict diagnostic criteria of SIVD today than was the case in 2004. As we have previously proposed, the low levels of $A\beta_{42}$ may be caused by either a general reduction of brain metabolism in the periventricular areas or an accumulation of Aβ plaques as a cause or a consequence of impaired CSF clearance [11]. A plausible pathophysiological overlap between iNPH and AD is in line with previous reports of high levels of AD pathology in patients with iNPH, even in shunt-responsive patients [47]. As an alternative, the coexistence of AD in iNPH could partly be explained from an epidemiological point of view as an age-related disorder (AD) concomitant with a rare disorder (iNPH). More studies are needed to replicate the findings and clarify the circumstances of reduced $A\beta_{42}$ in iNPH in comparison to SIVD.

T-tau and p-tau, markers of general neurodegeneration and tau tangles were all within normal range in patients with iNPH and SIVD. This is in agreement with two other studies [33,46]. This finding may suggest that neurodegeneration in these patients is mainly restricted to subcortical regions.

Limitations

Surprisingly, similar levels of NFL were observed among patients with iNPH and in HC subjects. Moreover, unexpectedly high levels of t-tau and p-tau were found in HC. We believe that this most likely indicates that healthy individuals who volunteer for dementia research may have subtle cognitive impairment due to early neurodegenerative processes that

are not discovered by standard dementia evaluation.

The sample size for each diagnostic group was relatively small, which is reflected in rather large standard deviations for several of the biomarkers.

There was an unfortunate lack of neuroimaging data on patients with AD due to less frequent use of brain scans at the time of diagnosis.

In this exploratory study, we conducted a two-center retrospective investigation on a highly selected group of patients who fulfilled the international diagnostic guidelines of iNPH, and who had effect of shunt surgery with an average follow-up of 23 months. Hence, our choice of study cohort does not necessarily reflect the target population of patients with iNPH since many patients do not improve from surgery possibly due to prolonged disease duration and irreversible changes or comorbidities. In future studies, where knowledge about the pathophysiology and CSF biomarker characteristics of iNPH is greater, inclusion of a more heterogeneous study population of iNPH would be relevant.

Conclusion

In summary, our findings support the use of a panel of CSF biomarkers to differentiate iNPH from SIVD. Patients with iNPH showed a CSF pattern of lower levels of NFL, NG, Aβ42, and t-tau in comparison to patients with SIVD. NFL performed best in separating the two entities. A combination of NFL, Aβ42, p-tau, and t-tau differentiated iNPH from SIVD as efficiently as all six biomarkers combined. In clinical use an addition of NFL to the already widely used CSF panel of AD core biomarkers may improve the diagnostic accuracy of patients with iNPH and SIVD. Future studies are needed to validate this extended CSF panel in the diagnostic process of dementia disorders and to explore the potential pathophysiological role of subcortical neurodegeneration, amyloid pathology and the compensatory response to synaptic degeneration in iNPH.

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Conflict of Interest

HZ has served at scientific advisory boards of Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by Biogen and Alzecure, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. KB has served as a consultant or at advisory boards for Abcam, Axon, Biogen, CogRx, Lilly, MagQu, Novartis and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg, all unrelated to the work presented in this paper.

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Table 1 - Demographics, clinical and paraclinical factors of study participants

	iNPH	SIVD	AD	HC
N	28	30	57	33
Sex (f/m)	10/18 (35.7%)	6/24 (20.0 %)	27/30 (47.4 %)	14/19 (42.4%)
Age, mean ± SD	72.43 ± 5.6[c]	71.57 ± 7.5[c]	70.28 ± 8.0[c]	64.52 ± 7.6
MMSE, mean ± SD	26.86 ± 3.0[abc]	24.86 ± 3.7[c]	23.44 ± 4.6[c]	28.96 ± 1.3
Hypertension, y/n (%)	17/10 (63.0 %)[c]	22/8 (73.3 %)[c]	24/28 (46.2 %)[ac]	7/24 (22.6 %)
Diabetes Mellitus, y/n (%)	3/24 (11.1 %)	9/21 (30.0 %)[c]	3/51 (5.6 %)[a]	1/31 (3.1 %)
Cardiovascular disease, y/n (%)	8/19 (29.6 %)[c]	12/18 (40.0 %)[c]	17/37 (31.5 %)[c]	2/30 (6.3 %)
Hypercholesterolemia, y/n (%)	4/23 (14.8%)[a]	19/11 (63.3 %)[c]	13/36 (26.5 %)[a]	4/27 (12.9 %)
Clinical stroke or TIA, y/n (%)	2/25 (7.4 %)[a]	12/18 (40.0 %)[c]	10/43 (18.9 %)[ac]	1/31 (3.1 %)
Lacunar Infarcts, y/n (%)	7/15 (31.8%)[ac]	21/5 (80.8%)[c]	3/9 (25.0 %)[a]	0/18 (0.0 %)
Evans Index, mean ± SD	0.41 ± 0.1[abc]	0.33 ± 0.0[c]	0.30 ± 0.1[a]	0.28 ± 0.0
Fazeka score, 0/1/2/3, median (range)	1.5 (0 – 3)[ac]	3.0 (2 – 3)[c]	1.0 (0 – 3)[ac]	1.0 (0 – 1)

Pairwise comparisons of interval and ordinal variables were assessed by Kruskal Wallis test and Mann-Whitney U-test. Categorical variables were assessed by Chi-Square test or Fisher's exact test.

Abbreviations: f = female; m = male; SD = standard deviation, MMSE = mini-mental state examination; TIA = transient ischemic attack. [a] $p < 0.05$ vs. SIVD; [b] $p < 0.05$ vs. AD; [c] $p < 0.05$ vs. HC

Table 2 – CSF biomarker levels in the diagnostic groups

	iNPH	SIVD	AD	HC	*p*-value iNPH vs. SIVD	*p*-value iNPH vs. AD	*p*-value iNPH vs. HC
NFL	922 (397)	1955 (1195)	1207 (843)	775 (637)	**<0.0001**	**0.0261**	ns.
NG	101 (44)	130 (61)	224 (107)	190 (70)	**0.0093**	**<0.0001**	**< 0.0001**
YKL-40	155 (40)	176 (59)	182 (70)	150 (48)	ns.	**0.0270**	ns.
$A\beta_{42}$	411 (222)	697 (251)	337 (184)	1029 (338)	**0.0002**	ns.	**< 0.0001**
T-tau	155 (63)	250 (124)	453 (211)	319 (134)	**0.0017**	**< 0.0001**	**< 0.0001**
P-tau	28 (12)	36 (16)	72 (27)	52 (19)	ns.	**< 0.0001**	**< 0.0001**

Data is shown as mean (SD). Units are pg/mL. YKL-40 in ng/ml. Mann-Whitney U test was used for analysis of data. Abbreviations: NFL = neurofilament light polypeptide; NG = neurogranin; YKL-40 = chitinase 3-like 1 protein; $A\beta_{42}$ = amyloid-β 42; T-tau = total tau; P-tau = phosphorylated tau; iNPH = idiopathic normal pressure hydrocephalus; SIVD = subcortical ischemic vascular disease; AD = Alzheimer's disease; HC = healthy controls; AUC = area under the curve; ns. = nonsignificant.

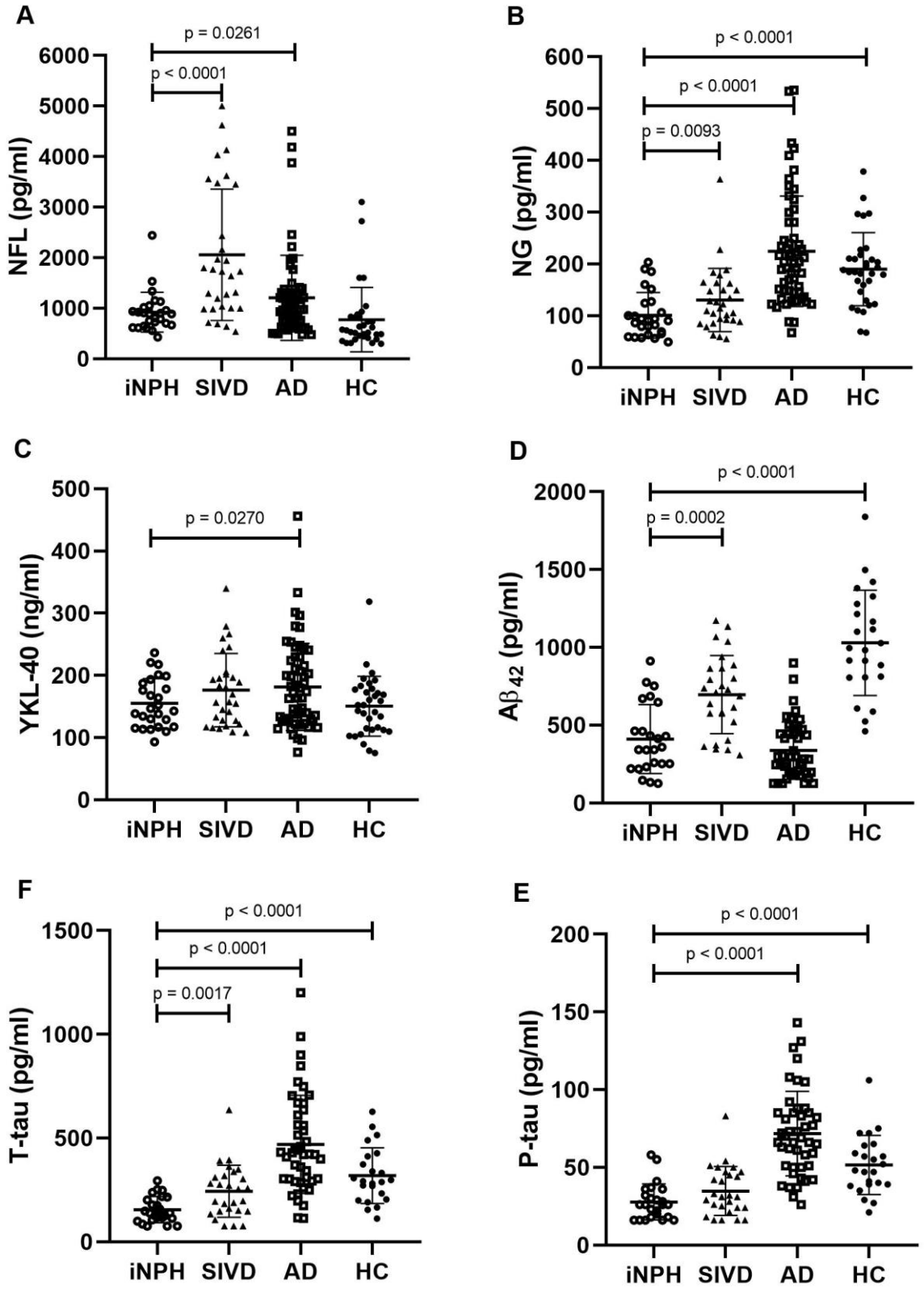
A
p = 0.0261
p < 0.0001
NFL (pg/ml)
6000
5000
4000
3000
2000
1000
0
iNPH SIVD AD HC
B
p < 0.0001
p < 0.0001
p = 0.0093
NG (pg/ml)
600
500
400
300
200
100
0
iNPH SIVD AD HC
C
p = 0.0270
YKL-40 (ng/ml)
500
400
300
200
100
0
iNPH SIVD AD HC
D
p < 0.0001
p = 0.0002
Aβ42 (pg/ml)
2000
1500
1000
500
0
iNPH SIVD AD HC
F
p < 0.0001
p < 0.0001
p = 0.0017
T-tau (pg/ml)
1500
1000
500
0
iNPH SIVD AD HC
E
p < 0.0001
p < 0.0001
P-tau (pg/ml)
200
150
100
50
0
iNPH SIVD AD HC

Fig 1. Scatter plots showing the CSF concentration distribution of all six biomarkers among patients with iNPH, SIVD, AD, and HC. Error bars represent median and interquartile range A) NFL B) NG C) YKL-40 D) $A\beta_{42}$ E) P-tau F) T-tau. Abbreviations: NFL = neurofilament light polypeptide; NG = neurogranin; YKL-40 = chitinase 3-like 1 protein; $A\beta_{42}$ = amyloid-β 42; T-tau = total tau; P-tau = phosphorylated tau; iNPH = idiopathic normal pressure hydrocephalus; SIVD = subcortical ischemic vascular disease; AD = Alzheimer's disease; HC = healthy controls;

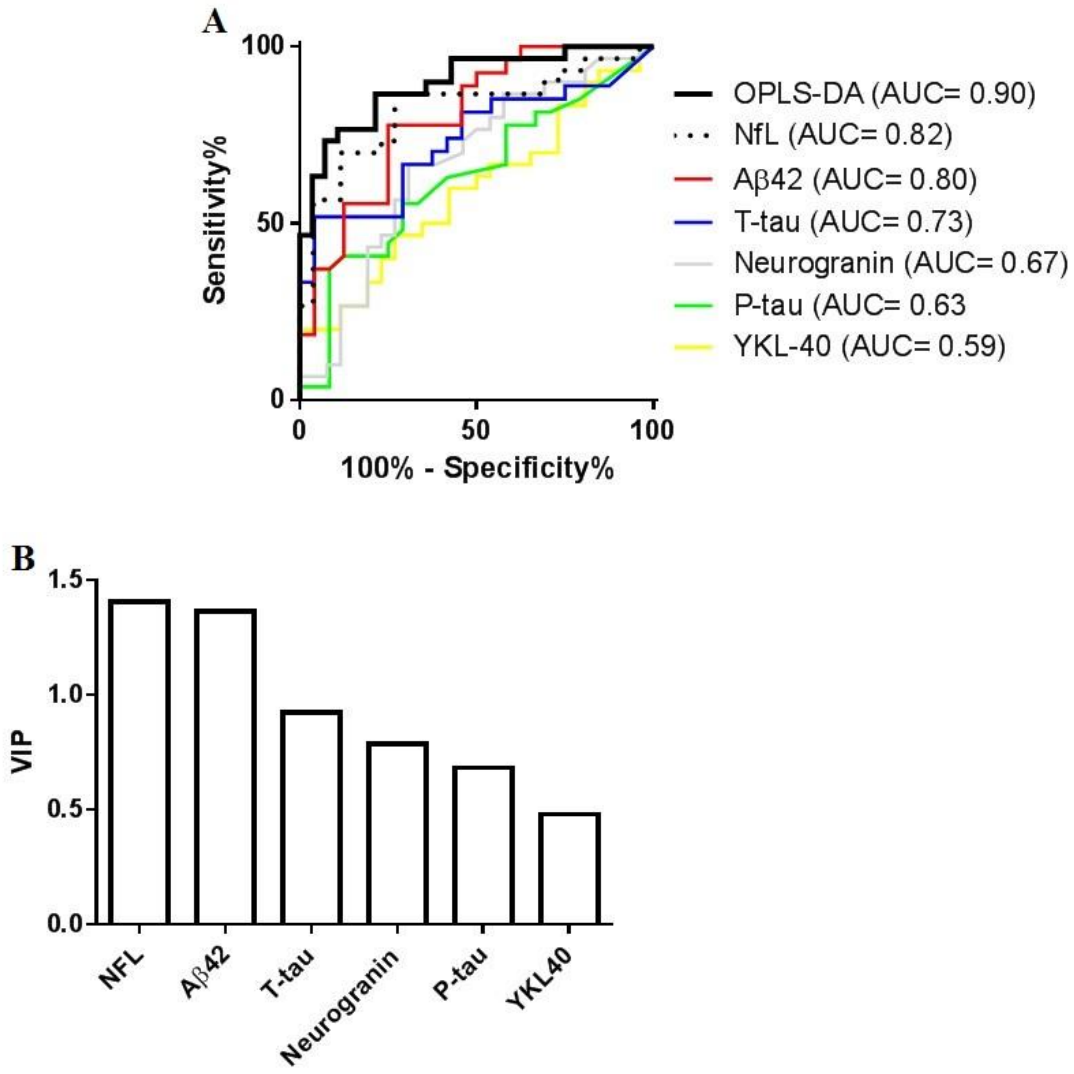

Fig. 2. The diagnostic accuracy of the individual biomarkers and in combination for the differentiation between iNPH and SIVD A) ROC curves of the CSF biomarkers suggest that NFL has most potential in differentiating iNPH from SIVD (AUC = 0.82), while YKL-40 has the least potential (AUC = 0.59). The diagnostic accuracy improves when combining all six biomarkers (OPLS-DA, AUC = 0.90) B) The VIP plot displays the relative contribution of each biomarker to the OPLS-DA model. Especially NFL and $A\beta_{42}$ contribute to the differentiation of iNPH from SIVD.

Abbreviations: NFL = neurofilament light polypeptide; NG = neurogranin; YKL-40 = chitinase

3-like 1 protein; $A\beta_{42}$ = amyloid-β 42; T-tau = total tau; P-tau = phosphorylated tau; iNPH = idiopathic normal pressure hydrocephalus; SIVD = subcortical ischemic vascular disease; AD = Alzheimer's disease; HC = healthy controls; OPLS-DA = Orthogonal Projections to Latent Structures Discriminant Analysis; VIP = Variable Influence on Projection