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Cerebrospinal fluid biomarkers for prognosis of long-term cognitive treatment outcomes in patients with idiopathic normal pressure hydrocephalus

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ABSTRACT

The prognosis of cognitive improvement after cerebrospinal fluid (CSF) shunting in idiopathic normal pressure hydrocephalus (iNPH) remains uncertain, with no reports on CSF biomarkers related to long-term cognitive prognosis. We performed a preliminary study of CSF biomarker protein levels for cognitive outcome prognostication of two-year outcomes after shunt treated iNPH in 36 patients (13 women) with a median age of 75 years (IQR 69–78). CSF biomarkers included soluble amyloid precursor proteins (sAPP, sAPPα, sAPPβ), amyloid β ($Aβ)_{1–38}$, $Aβ_{1–42}$ and phosphorylated tau (*p*-tau), lipocalin-type prostaglandin D synthase (L-PGDS)/β-trace, and cystatin C. The results clearly showed that *p*-tau levels (sensitivity of 71.4%, specificity of 77.8%, cut-off value of 22.0 pg/mL), $Aβ_{1–38}/Aβ_{1–42}$ ratio (77.8%, 81%, 3.58), and the $Aβ_{1–42}$/*p*-tau ratio (76%, 72.7%, 14.6) in preoperative CSF have the potential to determine postoperative prognosis. Improved cognition may be associated with the improvement in CSF circulation after LPS, which likely induces cystatin C and L-PGDS and switches synthesis from $Aβ_{1–42}$ to $Aβ_{1–38}$.

1. Introduction

Idiopathic normal pressure hydrocephalus (iNPH) is increasingly being regarded as important because of its disease specificity, leading to cognitive disorder, gait impairment, dysuria, falls, and bedridden status in the elderly [5]. Diagnosis of iNPH has progressed with the release of new guidelines [15,20] and introduction of a cerebrospinal fluid (CSF) shunting procedure. However, the mechanism by which shunt surgery improves the symptoms of iNPH is unclear. In the healthy brain, a balance is maintained between the production and absorption of the CSF, with four or five CSF turnovers occurring each day. With increasing age, however, people show increased resistance to CSF absorption. In patients with iNPH, the turnover of CSF appears to have declined more than average due to a reduced ability to absorb CSF [8]. A conspicuous decline in CSF circulation appears to affect the metabolism of a variety of proteins that are produced intracerebrally. In the shunting procedure, the CSF is drained into the abdominal cavity through the shunt system, compensating for the loss of CSF absorption capabilities caused by iNPH. It appears that CSF shunting not only corrects intracranial pressure but also effectively promotes CSF turnover [33].

The prognosis after CSF shunting in iNPH remains uncertain even after the establishment of the latest diagnostic criteria, and only a few reports exist on biomarkers related to prognosis [30]. Most reports on the clinical outcomes of CSF shunting for iNPH are the result of short-term follow-up studies (about 1 year) [7,11], with no reports on CSF biomarkers that predict long-term prognosis.

In this study, we explored the levels of CSF biomarkers for their association with prognosis of cognitive functional outcome in shunt-treated iNPH. We examined and compared patients whose activities of daily living (ADL) improved after CSF shunting and those whose degree of improvement was poor. We analyzed the changes in various biomarkers, focusing on amyloid-related proteins in the CSF, and explored those that may predict the cognitive functional prognosis of

Abbreviations: Aβ, amyloid-beta; iNPH, idiopathic normal pressure hydrocephalus; LPS, lumboperitoneal shunting; iNPHGS, iNPH Grading Scale; MMSE, Mini Mental State Examination; FAB, frontal assessment battery; TMT-A, trail making test part A; mRS, modified Rankin Scale; NC, normal control; LP, lumbar puncture; sAPP, soluble amyloid precursor protein; sAPPα, sAPP alpha; sAPPβ, sAPP beta; APP, amyloid precursor protein; AUC, area under the curve; ROC, receiver operating characteristic; IQR, interquartile range; L-PGDS, lipocalin-type prostaglandin D synthase.

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patients with iNPH. The biomarkers chosen comprised those belonging to or associated with Aβ pathways [6,22].

2. Material & methods

2.1. Patients

Sixty patients (15 women), mean age of 75 years [25% and 75% interquartile range: interquartile range (IQR) 69–78] with iNPH diagnosis at the Department of Neurosurgery [16], Juntendo University in Tokyo, Japan, based on existing guidelines [20], underwent lumboperitoneal shunting (LPS) [23] between September 2008 and May 2012. Inclusion criteria were symptoms and signs, and magnetic resonance imaging (MRI) findings compatible with iNPH [26]. Of these, 13 patients did not have CSF collected. Eleven patients were not followed up, including 4 deaths. Thirty-six patients (13 women) were re-examined 2 years after LPS, prior to this study (Fig. 1). Postoperative course was analyzed using the modified Rankin Scale (mRS) [37], Japanese iNPH grading scale (JNPHGS) [21], the Mini-Mental State Examination (MMSE) [4], Frontal Assessment Battery (FAB) [3], and Trail Making Test A (TMT-A) [27,28]. Performance was compared before and 2 years after LPS.

In the first classification method, we compared two groups: the "*Improved Cognitive group*", who either maintained a favorable cognitive function of 25 points or higher in MMSE score or improved by 3 points in 2 years after LPS surgery; and the "*Poor Cognitive group*", whose MMSE scores were less than 24 points without improvement of at least 3 points after LPS surgery. CSF biomarkers were compared between groups.

In the second classification method, we divided the subjects according to age at surgery (60s, 70s, or 80s) and studied the degree of symptom improvement.

2.2. CSF analysis

The study was approved by the Ethics Committee of Juntendo University. All patients included in the study, or their relatives, gave informed consent to their participation. Written informed consent was also obtained from patients and families prior to LPS placement for all patients who were positive for the tap test. LPS was performed using adjustable valves in all patients, a non-siphon control (NSC) valve with a small lumen catheter (Medtronic Neurosurgery, Goleta, CA) [23].

Lumbar puncture (LP) was performed in the L3–L4 or L4–L5 interspace before LPS. The CSF before LPS was sampled through an 18G spinal needle. Two years after LPS, CSF was sampled again through a puncture of the reservoir using a 27-gauge needle to confirm that the shunt system was operating effectively. No infections were reported following the tap test or shunt valve puncture. We obtained lumbar CSF before and after LPS. All CSF samples were centrifuged to remove cells and debris, aliquoted, and stored in polypropylene tubes at −80 °C until biochemical analysis [18].

Shunt reservoir and lumbar CSF biomarkers were also compared. CSF biomarkers included total soluble amyloid precursor proteins (sAPP) α and β, amyloid β ($Aβ)_{1-38}$, $Aβ_{1-42}$ [32], and phosphorylated tau (*p*-tau) [29]. We also measured lipocalin-type prostaglandin D synthase (L-PGDS)/β-trace and cystatin C, a known chaperone of Aβ protein. The bicinchoninic acid (BCA) method was used for total

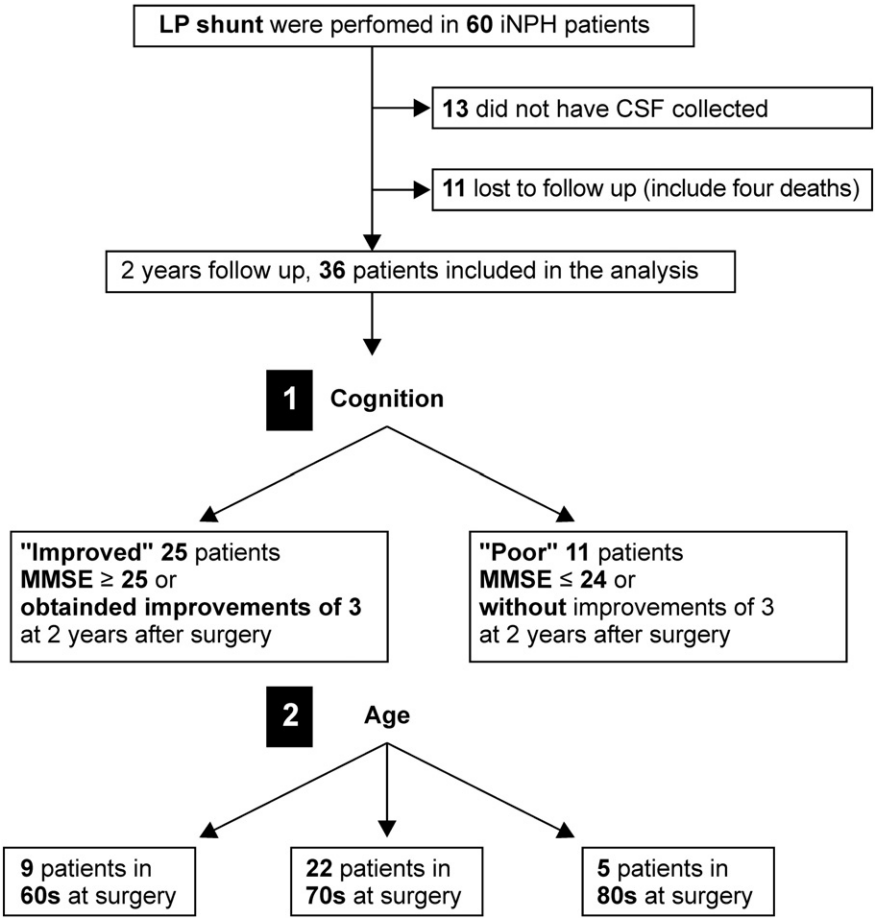

Fig. 1. Overview of patient selection. Four deaths: Four patients died during the course due to myocardial infarction, malignant lymphoma, suffocation due to foreign body aspiration, and complications of liver cancer.

Table 1
Kits used for ELISA and immunoprecipitation.

Target protein	Name of reagent kit	Supplier
sAPP	Human sAPP, Total (highly sensitive) Assay Kit	No27731 IBL, Takasaki, Japan
sAPPα	Human sAPPα (highly sensitive) Assay Kit	No27719 IBL, Takasaki, Japan
sAPPβ	Human sAPPβ (highly sensitive) Assay Kit	No27732 IBL, Takasaki, Japan
$Aβ_{1-38}$	Human Amyloid$β_{1-38}$(FL) Assay Kit	No27717 IBL, Takasaki, Japan
$Aβ_{1-42}$	Innotest beta-amyloid 1–42	Innogenetics, Belgium
L-PGDS	Human prostaglandin D synthase (lipocalin-type) ELISA	RD191113100R, BioVendor, Brno, Czech Republic
Cystatin C	Human cystatin C ELISA	RD191009100, BioVendor, Brno, Czech Republic
P-tau	Innotest phospho tau-181p	Innogenetics, Belgium

sAPP; total soluble amyloid precursor protein, Aβ; amyloid beta, L-PGDS; lipocalin-type prostaglandin D/β-trace, *p*-tau; phosphorylated tau.

protein measurement. Enzyme-linked immunosorbent assays (ELISAs) were used for other biomarker measurements (Table 1) [17].

2.3. Data analyses and statistics

Non-parametric statistical methods were used for all data analysis. The Wilcoxon signed-rank test was used for within-group comparisons of mRS, JNPHGS, MMSE, FAB, and TMT-A scores before and after LPS, and the Mann–Whitney *U* test for comparisons between the "*Improved Cognitive group*" and "*Poor Cognitive group*". Dunnett's T3 test was used to compare age groups (60s, 70s, and 80s). These data are presented as medians (95% credible interval). Statistical analyses were performed with IBM Statistical Package of the Social Sciences Version 18.0 (SPSS, Cary, NC) for Windows. $p < 0.05$ determined with a *t*-test was considered significant.

3. Results

Table 2 shows mRS, JNPHGS, MMSE, FAB, and TMT-A scores before and 2 years after LPS treatment in patients with iNPH (n = 36). Improvements were observed after shunt surgery, with statistically significant differences in all mRS and JNPHGS (gait, cognition, urinary function) scores. LPS surgery related mortality or morbidity or significant postoperative complications were not observed. The *p*-tau, $Aβ_{1-38}/Aβ_{1-42}$, and $Aβ_{1-42}$/*p*-tau ratios predicted prognosis of cognitive function. A group-specific analysis is shown below.

3.1. Comparison between the "Improved Cognitive" and "Poor Cognitive" groups

The CSF biomarkers were compared between "*Improved Cognitive group*" and "*Poor Cognitive group*" (Fig. 2). Receiver operating characteristic (ROC) analysis was performed. Simple ROC analyses were performed for each biomarker to distinguish "*improved*" cognition (Table 3, Fig. 3). Statistically significant differences were seen in the following CSF biomarkers prior to surgery in the "*Improved Cognitive group*": *p*-tau, area under the curve (AUC) = 0.733, p = 0.046 (Fig. 4A); $Aß_{1-38}/Aß_{1-42,}$ ratio, AUC = 0.804, p = 0.009 (Fig. 4B); and $Aß_{1-42}$/*p*-tau ratio AUC = 0.753, p = 0.017 (Fig. 4C). The cut-off values, sensitivity, and degree of specificity were 22.0 pg/mL, 77.8%, and 71.4% for *p*-tau; 3.58 pg/mL, 77.8%, and 81% for $Aß_{1-38}/Aß_{1-42}$ ratio; and 14.6, 76%, and 72.7% for $Aß_{1-42}$/*p*-tau ratio.

Analysis of the $Aβ_{1-38}/Aβ_{1-42}$ ratio revealed that, while the "*Improved Cognitive group*" showed a tendency to shift from $Aβ_{1-42}$ to $Aβ_{1-38}$ ($y = 1.9253x + 526.81$ before shunt surgery versus $y = 3.9725x - 270.11$ two years after surgery), the "*Poor Cognitive group*" showed almost no change ($y = 2.509x + 1047.4$ before surgery and $y = 2.1632x + 2255.7$ two years after surgery) (Fig. 5).

3.2. Comparison of age groups

Table 4 shows age-specific analyses, with 64.0 (IQR 61.0–66.5) years (n = 9) for subjects in their 60s; 75.5 (IQR 73.0–78.0) years (n = 22) for

Table 2
Neurological symptoms and cognitive evaluations before and 2 years after LPS surgery (n = 36).

		All iNPH patients (n = 36)	Improved Cogn. (n = 25)	Poor Cogn. (n = 11)	p-Value (Improved vs Poor)
Age median (IQR)		75 (69–78)	74 (68–77)	78 (73–79)	0.075
MMSE median (IQR)	Before	22 (19–26)	23 (18.5–26)	22 (20–24)	0.512
	After	25 (20–28)	27 (25–29)	20 (19–21)	*0.000
mRS median (IQR)	Before	3 (2–3)	3 (2.5–3.5)	3 (3–4)	0.154
	After	2 (1–2.5)	2 (1–2.5)	3 (2–3)	*0.038
JNPHGS gait median (IQR)	Before	2 (1–3)	2 (1–3)	3 (2–3)	0.616
	After	1 (0–2)	0 (0–1)	2 (1–3)	0.197
Cogn. median (IQR)	Before	2 (1–3)	2 (1–3)	2 (2–3)	0.172
	After	1 (0–2)	0 (0–1)	2 (1–3)	*0.001
Urin. median (IQR)	Before	2 (1–3)	2 (1–2.5)	3 (2–3)	*0.037
	After	1 (0–1)	1 (0–1)	1 (1–2)	*0.028
Total median (IQR)	Before	7 (4–8)	6 (4–7)	8 (7–9)	*0.03
	After	3 (2–5)	2 (1–4)	5 (3–8)	*0.003
FAB median (IQR)	Before	13 (11–14)	12 (10.25–14)	12 (8.5–12)	*0.029
	After	13 (11–14)	13 (11–14)	12 (8.5–14.25)	0.0514
TMT-A median (IQR)	Before	91 (58–150)	90 (56–157)	102 (83.5–187.5)	0.414
	After	77 (49–107)	69 (49–107)	85 (41–112)	0.982

IQR 25% and 75%.
Abbreviations: IQR; interquartile range, JNPHGS; Japan NPH grading scale, Cogn; cognition, Urin; urinary function, mRS; modified Rankin Scale, MMSE; mini-mental scale examination, FAB; frontal assessment battery, TMT-A; trail making scale-A, LPS; lumboperitoneal shunt.
After dividing the subjects into groups according to the degree of MMSE improvement, the only significant differences between groups were seen in the iNPHGS gait and total score. No significant differences were seen in cognitive function (MMSE, FAB, TMT-A). All items, except FAB, improved significantly after LPS. Statistical analysis was performed by the Wilcoxon signed-rank test.
* $p < 0.05$.

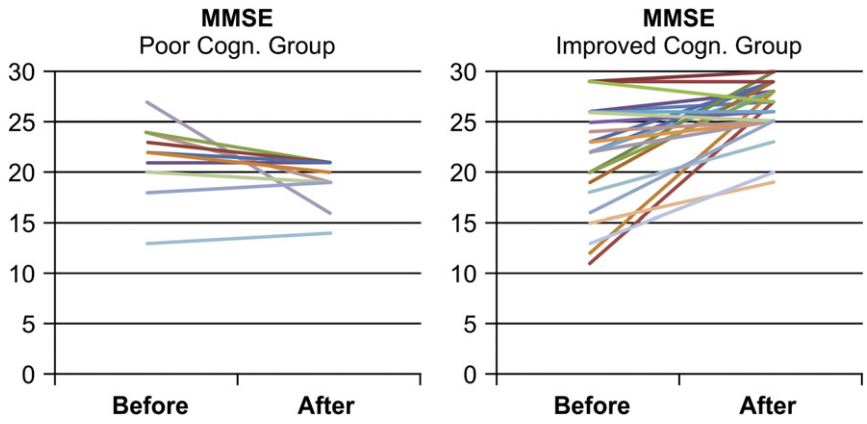

Fig. 2. Comparison of MMSE between the improved cognitive group and poor cognitive group. MMSE comparison between the improved cognitive (n = 25) and poor cognitive groups (n = 11) 2 years after surgery (Before vs. After LP shunt).

subjects in their 70s; and 82.0 (IQR 80.0–83.0) years (n = 5) for subjects in their 80s. Comparisons of these groups revealed no significant differences in preoperative mRS scores between the 60s and 70s groups, but a significant difference of p = 0.032 was seen between the 60s and 80s groups. Preoperative MMSE scores showed no statistically significant differences among the different age groups. Although mRS scores had improved in all age groups 2 years after LPS, a significant difference (p = 0.031) was seen in the 60s and 80s groups (Fig. 6). An evaluation 2 years later revealed that the group of patients who had undergone shunt treatment in their 80s showed only slight improvements in cognitive function (Table 4).

4. Discussion

We previously reported that measurement of sAPPα in the CSF is useful for differential diagnosis of iNPH [19]. CSF amyloid precursor protein (sAPP, sAPPαand sAPPβ) levels in iNPH patients are significantly lower than in normal control subjects of all age groups. In this study, the levels of these APP CSF biomarkers did not change within 2 years after the shunt.

An analysis of iNPH shunt treatment and cognitive function showed that the group whose MMSE scores did not improve 2 years after surgery tended to have had low MMSE scores before surgery. CSF biomarkers *p*-tau, $A\beta_{1-38}/A\beta_{1-42}$ ratio, and $A\beta_{1-42}$/*p*-tau ratio [10,13] were prognostic predictors of cognitive function (Table 3). Moreover, analysis of the $A\beta_{1-38}/A\beta_{1-42}$ ratio revealed that, while the "*Improved Cognitive group*" showed a tendency to shift from $A\beta_{1-42}$ to $A\beta_{1-38}$ 2 years after surgery, the "*Poor Cognitive group*" showed similar results before and 2 years after the same. Concentrations of $A\beta_{1-38}$ and $A\beta_{1-42}$ increased after shunt treatment, perhaps due to a change in γ-secretase activity [12].

In age-specific analyses of the 60s, 70s, and 80s groups, following the iNPH diagnostic guidelines and performing shunting treatment, all age groups attained improved mRS scores; however, younger age at the

Table 3
CSF biomarkers before and after shunt treatment by cognitive function.

		Improved Cognition group (n = 25)	Poor Cognition group (n = 11)	p-Value	Improved Cognition group (n = 25)	Poor Cognition group (n = 11)	p-Value
		Before			After (2 years)		
sAPP (ng/mL)	Median	457.0	581.0	0.229	573.0	553.5	0.243
	IQR	(335.5–650.0)	(390.0–896.0)		(3502.0–1109.0)	(295.0–939.3)	
sAPPα (ng/mL)	Median	124.0	143.0	0.505	165.0	159.0	0.505
	IQR	(71.5–171.5)	(98.8–166.0)		(116.0–210.0)	(129.0–259.0)	
sAPPβ (ng/mL)	Median	141.0	138.0	0.868	177.0	168.0	0.346
	IQR	(99.0–177.0)	(88.0–191.0)		(132.0–228.0)	(129.5–189.0)	
$Aß_{1-38}$ (pg/mL)	Median	1339. 0	1822.0	0.08	2821.0	3732.0	0.099
	IQR	(767.0–2045.0)	(975.0–3029)		(1924.0–3599.0)	(2332.0–5474.0)	
$Aß_{1-42}$ (pg/mL)	Median	486.0	263.0	0.133	825.0	859.5	0.981
	IQR	(290.0–587.0)	(220.5–446.0)		(749.0–911.0)	(382.3–994.8)	
$Aß_{1-38}/Aß_{1-42}$	Median	2.0	6.0	*0.009	3.0	5.0	*0.019
	IQR	(2–3.5)	(3.5–6.5)		(3.0–4.0)	(3.5–6.0)	
P-tau (pg/mL)	Median	20.0	29.0	*0.008	45.0	68.0	0.245
	IQR	(16–23.5)	(23.0–39.0)		(28.0–69.5)	(28.8–102.5)	
$Aß_{1-42}$/*p*-tau	Median	23.0	10.0	*0.017	18.0	10.0	0.067
	IQR	(14.5–29.0)	(8.0–17.0)		(11.5–29.0)	(8.0–16.5)	
L-PGDS (μg/mL)	Median	10.5	15.0	0.139	18.0	17.0	0.33
	IQR	(8.0–12.8)	(9.0–17.0)		(9.5–19.0)	(11.0–27.0)	
Cystatin C (μg/mL)	Median	1.58	1.8	0.223	2.41	3.78	0.554
	IQR	(1.32–2.41)	(1.62–3.01)		(2.05–3.83)	(1.59–4.62)	
Protein (mg/dL)	Median	36.5	35.0	0.831	40.0	42.5	0.541
	IQR	(32.5–44.8)	(32.0–44.0)		(36.0–43.8)	(32.3–50.8)	

IQR 25% and 75%.
Statistical analysis was performed by Mann–Whitney *U* test.
Group with "*Improved Cognition*": MMSE ≥25 or improvement of 3 points, and "*Poor Cognition*": MMSE ≤24 without improvement of 3 points. Preoperative *p*-tau values, $Aß_{1-38}/Aß_{1-42}$ ratio and $Aß_{1-42}$/*p*-tau ratio prior to surgery and 2 years after LPS, predicted improvement of MMSE.
* p < 0.05.

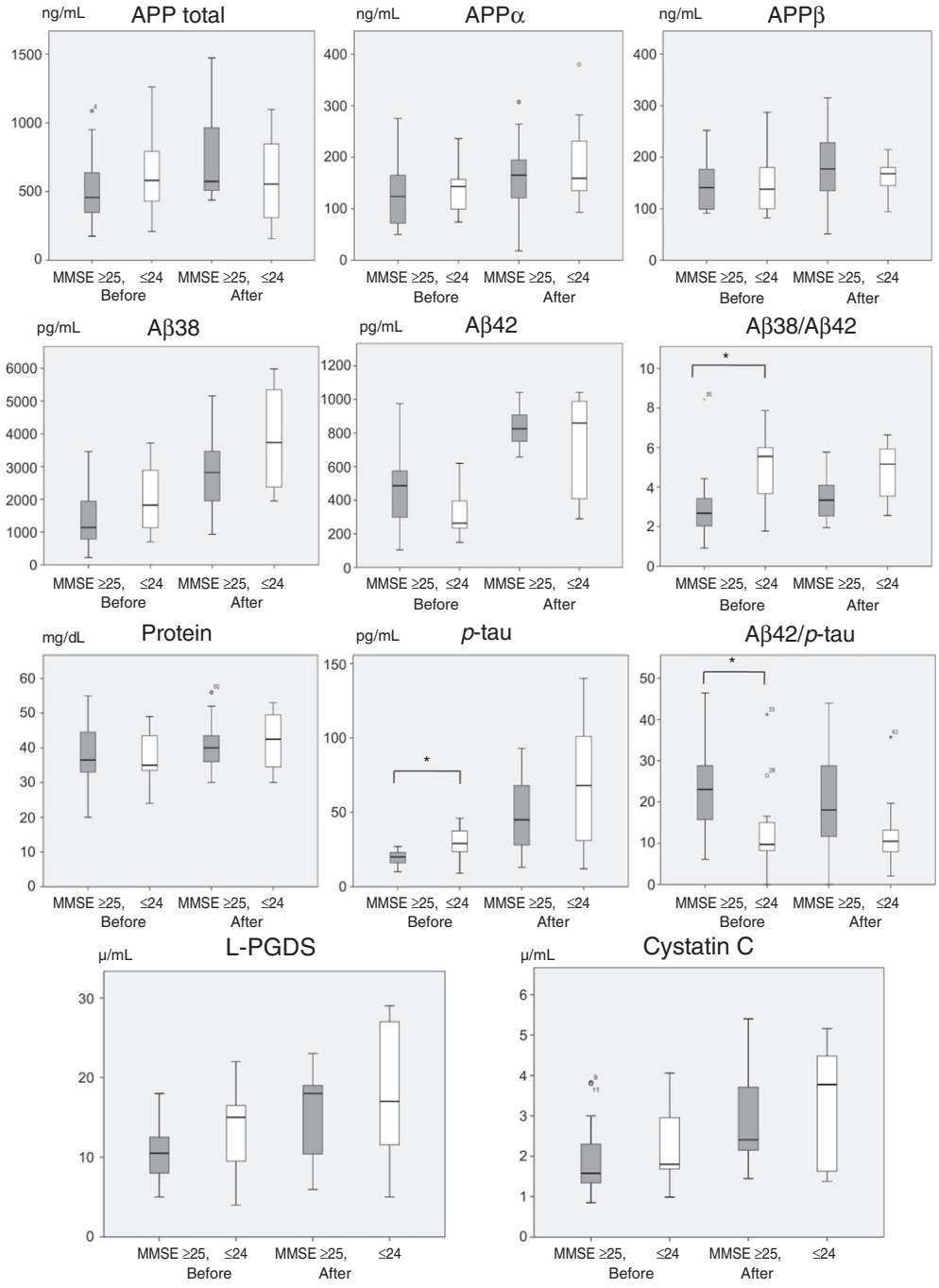

Fig. 3. Changes in CSF biomarkers in the improved and poor cognitive function groups. CSF biomarker changes in the improved (n = 25) and poor cognitive group (n = 11) 2 years after surgery (before vs. after LP shunt); $p < 0.05$.

time of treatment was associated with better mRS scores 2 years later. The group that underwent treatment in their 80s saw slight improvements; however, their MMSE values 2 years later were low and their *p*-tau was high despite a high $A\beta_{1-42}$/*p*-tau, indicating the severity of neuronal damage and loss, suggestive of advanced pathological changes associated with Alzheimer's disease (AD) impacting their cognitive skills [1].

It should be noted, however, that these study findings are the result of single-center experiences targeting an extremely small number of subjects. In addition to our small sample size, this study, similar to previous clinical studies on iNPH, has several further limitations related to the patients' comorbidities [14]. In these studies, AD comorbidity could not be excluded before shunting. Large-scale clinical studies are needed to determine whether early initiation of shunt treatment can contribute to improved cognitive function.

Both improved and poor cognitive groups showed a rise in the concentration of L-PGDS and cystatin C in the CSF after the shunt treatment. L-PGDS, which is one of the most abundant CSF proteins and acts as a prostaglandin D2-producing enzyme and a lipophilic ligand-binding protein, is produced in the arachnoid membrane of the brain, spinal cord, and oligodendrocytes. Its lipophilic nature allows it to function as a chaperone, preventing the formation of neurotoxic agents such as Aβ fibrils [36]. It is thought to be secreted into the CSF as a β-trace protein, and combines with different Aβ fragments with a high level of affinity to constantly suppress Aβ aggregation [9]. Image data of the disproportionately enlarged subarachnoid-space hydrocephalus

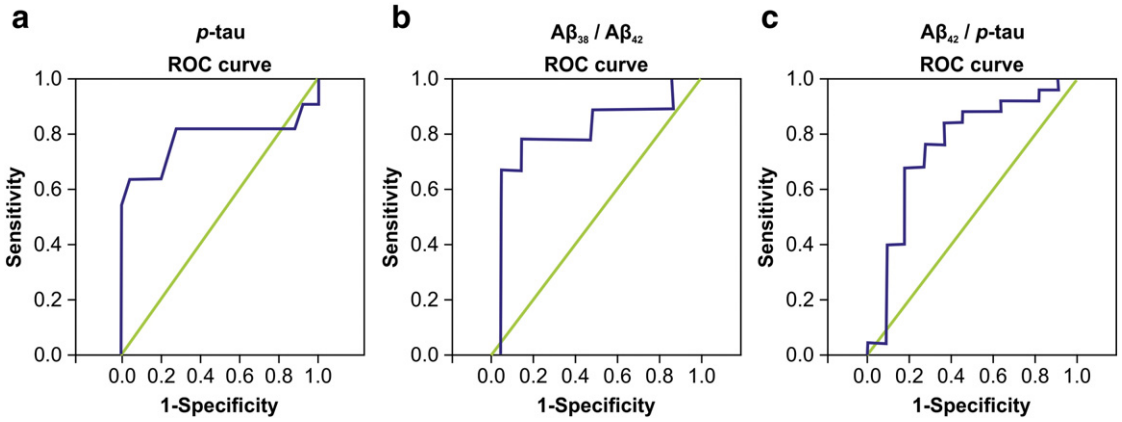

Fig. 4. Receiver operating characteristic (ROC) curves of ELISA data from the 'validation' cohort. ROC analyses of the 'validation' cohort ELISA data were performed for each biomarker to distinguish good cognition from poor cognition. A step-wise logistic regression model was applied to identify a complementary combination of biomarkers that would optimize accuracy (maximize area under the curve (AUC) without including additional non-contributory biomarkers, accepted). Preoperative (A) CSF *p*-tau, AUC = 0.733, p = 0.046; (B) $Aß_{1-38}/Aß_{1-42}$, AUC = 0.804, p = 0.009, and (C) $Aß_{1-42}$/*p*-tau's AUC = 0.753, p = 0.017.

(DESH), which is characteristic of iNPH, show that L-PGDS levels are reduced in iNPH [24]. Our study showed a trend of increasing L-PGDS levels in the "*Improved Cognitive group*," but no increase in the "*Poor Cognitive group*" (Table 4).

Cystatin C is also involved in the metabolism of Aβ protein and is chiefly secreted from the choroid plexus [35]. In iNPH pathology, where the circulation of CSF is chronically impaired, fluid secretion from the choroid plexus into the CSF is also reduced [25,34], including cystatin C. In patients with iNPH, cystatin C levels were low, but increased after LPS treatment.

Choi et al. reported that Aβ polymerization (formation of Aβ oligomers) was promoted by delaying the flow of interstitial fluids in a culture experiment [2]. The results of animal experiments suggest that 10–15% of Aβ is metabolized by interstitial fluids [31]. The extent to which the pathology of iNPH accounts for the reduced turnover of CSF and the metabolism of Aβ is unclear. It is possible that the reduction in enzymes related to Aβ metabolism such as L-PGDS and cystatin C in iNPH creates an environment where Aβ aggregation is increased, forming highly neurotoxic Aβ oligomers. After CSF shunt treatment, turnover of CSF appears to increase, thereby increasing the production of CSF, inducing cystatin C secretion from the CSF choroid plexus, and L-PDGS from the arachnoid membrane of the brain, spinal cord, and oligodendrocytes, thus promoting the metabolism of Aβ.

5. Conclusions

P-tau levels and the $Aβ_{1-38}/Aβ_{1-42}$ and $Aβ_{1-42}$/*p*-tau ratios in preoperative CSF may serve as biomarkers for the prognosis of cognitive function after shunt surgery in iNPH. However, in the age ≥80 operated group, functional prognosis of cognition remains confined, especially on MMSE values. We suggest improved CSF circulation in iNPH after LPS increases the amount of cystatin C and L-PGDS, possibly contributing to Aβ elimination and improvement of a range of symptoms. The switch in Aβ-variant synthesis from $Aβ_{1-42}$ to $Aβ_{1-38}$ also resulted in the improvement of functional prognosis.

Conflicts of interest

The authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

Acknowledgments

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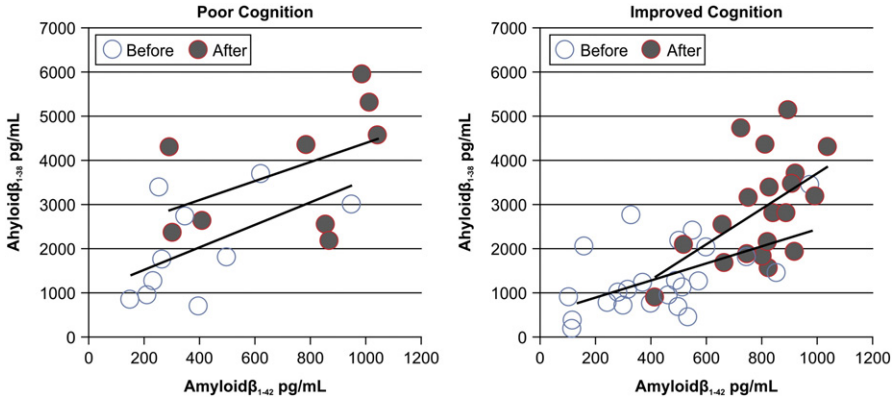

Fig. 5. $Aβ_{1-38}/Aβ_{1-42}$ ratio improvements in the improved (n = 25) and poor (n = 11) cognitive groups. Before: white circle; after: black circle. Improved cognitive group showed a tendency to shift from y = 2.0895x + 419.3 before surgery to y = 3.7455x − 129.2 two years after surgery, and from $Aβ_{1-42}$ to $Aβ_{1-38}$. The poor cognitive group, in contrast, showed almost no changes two years after surgery, with y = 2.132x + 1075.5 before and y = 1.96905x + 2491.1 two years after.

Table 4
Age-specific changes (60s, 70s, and 80s) of mRS, MMSE scores, and various CSF biomarkers in idiopathic normal pressure hydrocephalus.

		60s (n = 9)	70s (n = 22)	80s (n = 5)	60s (n = 8)	70s (n = 19)	80s (n = 5)
AGE	Median	64	75.5	82			
	IQR	61.0–66.5	73–78	80–83			
		Before			After		
mRS	Median	3.0	3.0	4.0	1.0	2.0	3.0
	IQR	2–3.5	3.0–4.0	3.0–4.0	0–2.0	1.0–3.0	2.5–4.0
MMSE	Median	22.0	22.5	18.0	25.0	26.0	19.0
	IQR	20.0–25.5	18.25–26.25	16.5–25.0	21.5–28	20.5–28.0	18.0–25.0
sAPP total (ng/mL)	Median	348.00	534.00	665.0	894.5	546.0	573.0
	IQR	250.0–542.0	385.0–731.0	358.5–819.5	662.5–1124.0	461–1097	1340.0–639.0
sAPP α (ng/mL)	Median	124.0	135.0	150.0	156.5	167.00	129.0
	IQR	73–195	91.25–194.25	85.5–161.0	104.5–292.5	132.75–210.00	105.0–169.5
sAPP β (ng/mL)	Median	134.0	146.0	252.0	159.5	171.0	228.0
	IQR	97.0–183.5	100.0–173.0	92.0–420.5	101.5–210.0	129.0–215.0	145.0–247.0
$Aß_{1-38}$ (pg/mL)	Median	930.00	1762.0	1293.0	3245.0	3171.0	2480.0
	IQR	721.25–2448.75	909.0–2316.5	952.0–2022.5	2388.0–4356.5	2122.0–4747.0	2016.5–2727.5
$Aß_{1-42}$ (pg/mL)	Median	329.00	491.5	457.00	905.0	823.0	659.0
	IQR	184.00–549.50	258.0–603.5	274.75–559.0	827.5–1007.0	724–901	409.0–747.0
$Aß_{1-38}/Aß_{1-42}$	Median	2.30	3.55	2.24	3.41	4.09	3.93
	IQR	1.83–8.77	3.28–5.18	1.99–4.73	2.49–4.44	2.62–5.93	2.54–5.81
P-tau (pg/mL)	Median	20.0	22.0	24.0	38.50	53	38.0
	IQR	16.0–22.5	16.0–27.0	17.5–30.0	15.00–67.25	37–84	20.5–65.25
$Aß_{1-42}$/*p*-tau	Median	16.45	19.60	23.22	25.33	12.02	13.19
	IQR	10.11–27.70	10.23–29.19	12.23–28.19	13.96–36.99	9.79–22.24	9.13–43.94
L-PGDS (μg/mL)	Median	12.00	11.50	10.00	10.05	19	13.0
	IQR	7.57–17.00	8.78–15.0	8.02–11.00	8.56–17.75	16–25	5.48–16.75
Cystatin C (μg/mL)	Median	1.58	1.81	1.40	1.90	2.61	3.23
	IQR	1.23–2.08	1.52–2.93	0.92–1.69	1.52–3.90	2.22–4.17	1.84–3.51
Protein (mg/dL)	Median	36.50	39.00	32.0	40.0	41.0	40.5
	IQR	28.00–47.75	34.00–45.25	25.0–35.5	335.5–42.5	34.0–51.5	34.5–50.25

When multiple comparisons were made by age, statistically significant differences were seen prior to surgery in the APP total (60s vs. 70s, p = 0.044) and L-PGDS (70s vs. 80s, p = 0.044) in the CSF. Two years after shunt surgery, $Aß_{1-42}$ (60s vs. 70s, p = 0.046) and L-PDGS (60s vs. 70s, p = 0.034) showed statistically significant differences.
Although mRS scores improved in all age groups (60s p = 0.016, 70s p = 0.001, 80s p = 0.157), the 80s group showed almost no improvements in cognitive function 2 years after LPS (p = 0.683).

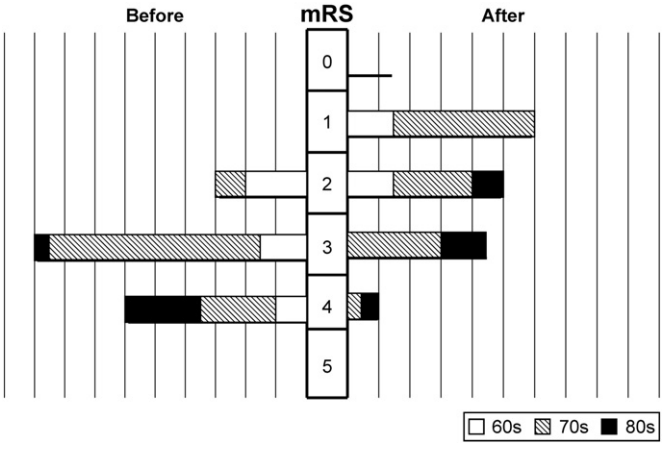

Fig. 6. Comparison of mRS scores in iNPH (60s, 70s, and 80s); before and after LP shunt. Analyses by age group (60s, 70s, and 80s) revealed a significant difference in preoperative mRS between the 60s and 80s groups (p = 0.031).

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