RESEARCH OPEN ACCESS

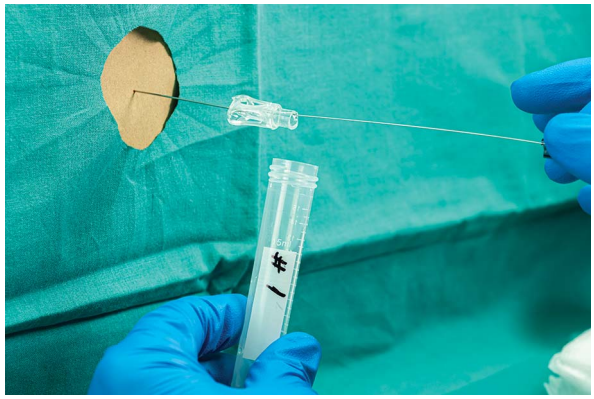
CSF Biomarkers Predict Gait Outcomes in Idiopathic Normal Pressure Hydrocephalus

Jacqueline A. Darrow, MS*, Alexandria Lewis, BS*, Seema Gulyani, PhD, Kristina Khingelova, PA-C, Aruna Rao, MD, Jiangxia Wang, MS, Yifan Zhang, MS, Mark Luciano, MD, Sevil Yasar, MD, and Abhay Moghekar, MBBS

Correspondence
Dr. Moghekar
amogheka@jhmi.edu

Neurology: Clinical Practice April 2022 vol. 12 no. 2 91-101 doi:10.1212/CPJ.0000000000001156

Abstract



Background and Objectives

The assessment of biomarkers in selecting patients with idiopathic normal pressure hydrocephalus (iNPH) for shunt surgery has been limited to small cohort studies and those with limited follow-up. We assessed the potential for CSF biomarkers in predicting immediate response to CSF tap test (TT) and long-term response after shunt surgery.

Methods

CSF was obtained from patients with iNPH referred for CSF TT after baseline assessment of cognition and gait. CSF neurofilament light (NfL), β-amyloid 42 ($A\beta_{1-42}$), β-amyloid 40 ($A\beta_{1-40}$), total tau (tTau), and phosphorylated tau 181 (pTau181) and leucine-rich alpha-2-glycoprotein-1 (LRG1) were measured by ELISA. The ability of these measures to predict immediate improvement following CSF TT and long-term improvement following shunt surgery was compared by univariate and adjusted multivariate regression.

Results

Lower NfL, pTau181, tTau, and $A\beta_{1-40}$ were individually predictive of long-term improvement in gait outcomes after shunt surgery. A multivariate model of these biomarkers and MRI Evans index, adjusted for age, improved prediction (area under the receiver operating curve 0.76, 95% confidence interval 0.66–0.86). tTau, pTau181, and $A\beta_{1-40}$ levels were statistically different in those whose gait improved after CSF TT compared with those who did not. Using a multivariate model, combining these markers with Evans index and transependymal flow did not significantly improve prediction of an immediate response to CSF TT.

Discussion

A combination of CSF biomarkers can predict improvement following shunt surgery for iNPH. However, these measures only modestly discriminate responders from nonresponders following CSF TT. The findings further suggest that abnormal CSF biomarkers in nonresponders may represent comorbid neurodegenerative pathology or a predegenerative phase that presents with an iNPH phenotype.

*These authors contributed equally to this work as co–first authors.

Department of Neurology (JAD, AL, SG, KK, AR, AM), Johns Hopkins University School of Medicine; Department of Biostatistics (JW, YZ), Johns Hopkins University Bloomberg School of Public Health; Department of Neurosurgery (ML), and Department of Medicine (SY), Johns Hopkins University School of Medicine, Baltimore, MD.

Funding information and disclosures are provided at the end of the article. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/cp.

The Article Processing Charge was funded by the authors.

Idiopathic normal pressure hydrocephalus (iNPH) is a putative reversible neurodegenerative disorder that is one of the few treatable causes of cognitive and gait impairment in the elderly.[1] However, differentiating iNPH from other age-related neurologic disorders is complex.[2,3] Moreover, shunts are associated with a high adverse event rate of approximately 11%, including infection, malfunction, additional surgery, and subdural hematomas.[4-6] Hence, specific biomarkers that could differentiate iNPH from other disorders and predict improvement after shunt surgery would be beneficial by ensuring accurate diagnosis and could potentially improve the prediction of shunt response.[7-9]

A recently conducted meta-analysis concluded that β-amyloid protein 42 (Aβ1–42), total tau (tTau), phosphorylated tau 81 (pTau181), neurofilament light (NfL) polypeptide, and the inflammatory biomarker leucine-rich alpha-2-glycoprotein-1 (LRG1) have the most favorable evidence in predicting shunt responsiveness.[5] Another comprehensive review by Manniche et al.[10] concluded that tTau and pTau181 might differentiate iNPH from Alzheimer disease (AD), whereas $A\beta_{1-40}$ might distinguish iNPH from healthy controls. Importantly, this study suggested that a combination of these biomarkers could improve diagnostic accuracy for iNPH. All the studies on which the meta-analyses by Pfanner and Manniche were based share several limitations, including small cohorts, measurement of only a subset of biomarkers, and minimal long-term outcome data, making generalization of findings and drawing definite conclusions difficult. In the present study, we aimed to evaluate the discriminative and predictive role of CSF biomarkers associated with neurodegeneration ($A\beta_{1-42}$, tTau, pTau181, and NfL) and inflammation (LRG1) in a large iNPH patient cohort selected for shunt surgery with long-term gait outcomes.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Eligible patients were those referred to our clinic for a CSF tap test (TT) after exhibiting gait, cognitive, and/or urinary dysfunction. Patients provided informed consent for biospecimen banking from 2012 to 2019 under a Johns Hopkins IRB-approved protocol.

Selection of Patients for Shunt Surgery

Patients underwent large-volume lumbar puncture (CSF TT) according to the guidelines for the assessment of iNPH. Patients were not asked to fast before their procedure.[11] The Timed Up and Go (TUG) test was administered immediately before the large-volume CSF withdrawal to assess gait velocity and dynamic balance. The TUG test was readministered within 1 hour after the CSF withdrawal. Responders were defined as those who showed an improvement of 30% or greater on the TUG test. A global rating of change scale ≥4 was used to define improvement in those on whom a TUG could not be obtained.[12]

Cognitive and MRI Assessments

The Montreal Cognitive Assessment (MoCA) test was used to assess cognitive performance. All patients also underwent a structural MRI scan of the brain; the Evans index was calculated to estimate ventricular enlargement.

CSF Sample Processing Procedures

CSF was collected in 10 mL polypropylene Sarstedt tubes (62.610.018). CSF was transported at room temperature until centrifugation at 2,000*g* for 15 minutes at 5°C ± 3°C. Samples were coded and separated into 500 μL aliquots within 1 hour of collection. Samples were stored in low-binding polypropylene cryovials (Sarstedt; Ref: 101093-760) at −80°C until being thawed once for analysis.

Response to Shunt Surgery

Patients defined as responders (as described above) were scheduled for surgery within 60 days. Patients who underwent shunt surgery were followed at periodic intervals in the clinic according to the standard of care, and the TUG assessment was repeated at every visit. The gait assessments were performed by physical therapists as part of routine clinical care. Improvement following shunt surgery was defined as an improvement in TUG time by 30%, the same criterion used to identify responders to CSF TT and select patients for shunt surgery. Patients who worsened after shunt surgery were also included in the no improvement group.

CSF Assays

CSF $A\beta_{1-42}$, $A\beta_{1-40}$, tTau, and pTau181 were measured using LUMIPULSE G1200 chemiluminescent ELISA (Fujirebio, Malvern, PA) directly from the cryovials without tube transfer. A CSF internal control was run on each day that samples were analyzed. The coefficients of variation were as follows: $A\beta_{1-42}$ 3.4%, $A\beta_{1-40}$ 2.7%, tTau 8%, and pTau181 1.8%. CSF NfL was measured with the Simoa NF-Light Kit using the SRX platform (Quanterix, Billerica, MA). Intra-assay coefficients of variation were 6.1% and 2.3%, and interassay coefficients of variation were <10% for quality control samples with clinically relevant low and high concentrations, respectively. CSF LRG1was measured by a solid-phase sandwich ELISA, Human LRG1 Assay Kit 27769 (IBL America, Minneapolis, MN). The plate was analyzed using a FilterMax F3 microplate reader (Molecular Devices, San Jose, CA). The intra-assay coefficient of variation was <10%, but the interassay coefficient of variation was 30% for the internal native CSF quality control sample due to individual preparation of controls. Hence, results were normalized across plates.

Statistical Methods

Baseline charcateristics: age sex, race, hypertension, Evans index, transependymal flow, the MoCA and TUG test scores, were compared in patients who showed improvement vs. no improvement in gait following a CSF tap test and subsequently shunt surgery. Two-sample *t* tests or nonparametric Kruskal-Wallis tests were used for continuous variables depending on whether the variable was normally

distributed based on the Shapiro-Wilk test for normality. Chi-square tests were used for sex, hypertension, and transependymal flow. Fisher exact tests were used for race and living status. The correlations between the biomarkers were assessed by Spearman correlations and visualized as heat maps. For patients' responses to the TT and shunt surgery, simple univariate logistic regression models and multivariate logistic regression models were used to investigate relationships with biomarkers, demographics, and baseline cognitive measures. The median values and 95% confidence interval (CI) of the regression coefficients from 10,000 runs of bootstrapping are reported. Biomarker concentrations were normalized with their sample means and SDs. Lasso regressions were used to select predictors for the multivariate logistic regression models, with the penalty parameters selected with 10-fold cross-validation. Ten-fold cross-validation was used to evaluate the logistic regression models, and the means of the area under the receiver operating curve (AUC) values were calculated and plotted to compare model performance. The cross-validated AUC R package was used to compute 95% CIs for the cross-validated AUC estimates. One thousand bootstrap samples were used to identify optimal cutoff values of biomarker concentrations (nonnormalized values) at maximum Youden index and accuracy, sensitivity, and specificity at the optimal cutoffs. Nonsupervised random forests were also constructed to summarize the mean decrease in Gini coefficient and mean decrease in accuracy to establish the importance of each variable in predicting treatment outcomes. A sensitivity analysis was performed to compare the baseline characteristics between the patients with and without follow-up after shunt surgery and examine whether patients were lost to follow-up randomly. The analyses were performed using R Studio Version 1.3.1073 (R version 4.0.2) and Stata 16.0. p Values of less than or equal to 0.05 were considered statistically significant.

Data Availability

Anonymized study data pertaining to this report are available on request from any qualified investigator for purposes of replicating the results.

Results

eFigure 1 (links.lww.com/CPJ/A327) provides a graphic representation of the total number of patients referred for iNPH assessment and the reasons for inclusion and exclusion from the study. Of the 420 patients referred for iNPH assessment, 18 had secondary etiologies, including hemorrhage, radiation, or infection, and were excluded (eFigure 1). Of the 402 patients with iNPH who underwent the TT, 121 were judged to be responders and were selected for shunt surgery. In 18 of these 121 patients, postshunt TUG could not be measured for logistical reasons, so the global rating of change scale was administered instead.[13] Response to shunt placement was seen in 90 of the 121 patients. The characteristics of the participants are summarized in Table 1. Most of the patients were followed for at least 12 months following shunt surgery; the mean duration of follow-up for the responders and nonresponders to shunt surgery was 19 and 23 months, respectively, which did not differ between the groups.

Patients with improvement after TT had higher Evans index scores and lower tTau, pTau181, and $A\beta_{1-40}$ concentrations than nonresponders (Table 1). Patients who underwent shunt surgery and improved were younger and had lower TUG scores and lower levels of NfL, pTau181, tTau, and $A\beta_{1-40}$ (Table 1). Higher levels of pTau181 were associated with higher levels of tTau; higher levels of Aβ1–40 were associated with higher levels of Aβ1–42, and higher levels of pTau181 were also associated with higher levels of Aβ1–40 (correlation coefficients of 0.77, 0.77 and 0.73, respectively, eFigure 2, links.lww.com/CPJ/A327). The distribution of these biomarkers across different groups is displayed in eFigure 3.

The univariate logistic regression models indicated that Evans index scores, transependymal flow, pTau181, tTau, and $A\beta_{1-40}$ were associated with improvement after TT. In the multivariate logistic regression model with all 4 predictors, Evans index and transependymal flow were significantly associated with improvement (odds ratio [OR] 1.09, 95% CI 1.04–1.16, $p < 0.001$; OR 1.70, 95% CI 1.07–2.73, $p = 0.029$) (Table 2). However, the multivariate model AUC was 0.64 (95% CI 0.58–0.70) and that for Evans index was 0.61 (95% CI 0.56–0.67) (Figure 1).

For models of improvement after shunt surgery, age, NfL, pTau181, tTau, normalized LRG1, and $A\beta_{1-40}$ showed significant associations with improvement in the univariate models. In the multivariate logistic regression model, improvement after shunt surgery was significantly associated only with pTau181 (OR 0.32, 95% CI 0.11–0.63, $p = 0.003$) (Table 3). The multivariate model had the highest AUC (0.76, 95% CI 0.66–0.86) (Figure 2).

The variable importance plots showed the performance in classifying the patients with respect to their outcomes from the nonsupervised random forest algorithm based on the 2 types of measurements of performances in prediction, the mean decrease accuracy and the mean decrease Gini. The more the accuracy of the random forest decreases due to the exclusion (or permutation) of a single variable, the more important that variable is deemed, and therefore, variables with a large mean decrease in accuracy are more important for classification of the outcome. The Gini coefficient is a measure of homogeneity at each split of the patients from 0 (homogeneous) to 1 (heterogeneous). When building a decision tree, the variable with the lowest Gini coefficient is preferred as the root node. Variables that result in splits with higher homogeneity among the resulting subgroups have a higher decrease in Gini coefficient. A higher mean decrease in Gini coefficient suggests higher variable importance. The nonsupervised random forest algorithm showed consistent results with the logistic regression models (Figure 3). For improvement

Table 1 Patient Baseline Characteristics Compared by Responses to TT and Shunt Surgery

Characteristics	TT response (n = 402)			Shunt surgery response (n = 103)		
	No improvement (n = 281)	Improved (n = 121)	*p* Value	No improvement (n = 28)	Improved (n = 75)	*p* Value
Age, mean (SD)	78.0 (7.6)	77.2 (6.4)	0.330	79.8 (6.5)	76.4 (6.0)	0.014
Male sex, n (%)	179 (63.7)	80 (66.1)	0.643	21 (75.0)	47 (62.7)	0.240
Race, n (%)			0.470			0.679
Caucasian	261 (92.9)	116 (95.9)		28 (100)	71 (94.7)	
African American	15 (5.3)	3 (2.5)		0	3 (4.0)	
Other	5 (1.8)	2 (1.70)		0	1 (1.3)	
Living status, deceased, n (%)	14 (5.0)	5 (4.1)	0.803	3 (10.7)	1 (1.3)	0.060
Hypertension, n (%)	226 (80.4)	100 (82.6)	0.702	26 (92.9)	59 (78.7)	0.143
MoCA score, mean (SD)	21.2 (5.9)	21.1 (5.6)	0.844	19.4 (7.0)	21.7 (5.2)	0.071
MRI Evans index (0–1), mean (SD)	0.36 (0.05)	0.38 (0.04)	0.001	0.37 (0.04)	0.38 (0.04)	0.211
Transependymal flow, n (%)	77 (27.4)	48 (39.7)	0.020	9 (32.1)	31 (41.3)	0.532
TUG score baseline, mean (SD)[a]	24.5 (41.4)	34.7 (49.7)	0.036	35.0 (54.7)	36.3 (53.2)	0.912
TUG score postshunt, mean (SD)[b]		42.2 (82.2)		107.1 (128.0)	18.0 (33.9)	<0.001
Interval between baseline and last follow-up in months, mean (SD)		19.99 (18.36)		22.2 (19.3)	20.0 (18.8)	0.612
NfL, median (IQR)	1,741 (1,077–3,052)	1,512 (944–2,404)	0.073	2,504 (1,337–4,716)	1,298 (933–1,940)	0.002
LRG normalized, median (IQR)	259 (160–457)	269 (182–451)	0.411	269 (204–706)	280 (182–470)	0.402
pTau181, median (IQR)	27.3 (21–41.3)	23.4 (17.8–34)	0.002	33.9 (25.1–62.7)	20.9 (17.6–31.0)	<0.001
tTau, median (IQR)	250 (159–386)	219 (137–309)	0.039	296 (163–531)	211 (132–293)	0.011
$A\beta_{1–42}$, median (IQR)	809 (604–1,070)	757 (510–1,014)	0.103	755 (579–1,079)	746 (496–1,000)	0.370
$A\beta_{1–40}$, median (IQR)	7,395 (5,600–9,456)	6,560 (4,857–8,522)	0.004	8,390 (5,189–10,481)	5,988 (4,581–8,168)	0.010
$A\beta_{1–42}/A\beta_{1–40}$, median (IQR)	0.13 (0.09–0.14)	0.13 (0.09–0.14)	0.253	0.10 (0.08–0.14)	0.13 (0.10–0.14)	0.074
tTau/amyloid ratio, median (IQR)	1,999 (1,223–3,793)	1,934 (1,064–2,921)	0.086	3,285 (1,588–5,520)	1,854 (994–2,471)	0.005
pTau181/amyloid ratio, median (IQR)	223 (155–450)	185 (134–330)	0.021	356 (169–593)	180 (127–276)	0.001
$A\beta_{1–42}$/tTau, median (IQR)	3.49 (1.98–5.60)	3.49 (2.34–5.52)	0.728	2.93 (1.59–4.25)	3.62 (2.40–5.52)	0.078
$A\beta_{1–42}$/pTau181, median (IQR)	32.0 (17.4–44.3)	35.7 (19.3–43.6)	0.398	23.2 (12.7–38.8)	35.9 (20.7–44.5)	0.012
Normal pTau181 and normal NfL, n (%)[c]	160 (56.9)	87 (71.9)	0.007	10 (35.7)	63 (84.0)	<0.001

Abbreviations: $A\beta_{1–40}$ = β-amyloid 40; $A\beta_{1–42}$ = β-amyloid 42; IQR = interquartile range; LRG = leucine-rich alpha-2 glycoprotein; MoCA = Montreal Cognitive Assessment; NfL = neurofilament light; pTau181 = phosphorylated tau 181; TT = tap test (large-volume lumbar puncture); tTau = total tau; TUG = Timed Up and Go.
[a] Patients who were unable to walk were assigned a baseline TUG score of 300. Nine patients had baseline TUG score greater than 200. If the 9 patients are excluded, the mean baseline TUG is 19.5 (SD: 17.8, n = 271) for patients without improvement after TT and 26.4 (SD: 20.3, n = 117) for patients with improvement.
[b] Patients who were unable to walk were assigned a postshunt TUG score of 300. If the 9 patients are excluded, the mean postshunt TUG is 29.9 (SD: 36.5, n = 20) for patients without improvement and 14.2 (SD: 7.6, n = 74) for patients with improvement.
[c] Normal pTau defined as <53.8 pg/mL. Normal NfL defined as <2,417 pg/mL.

after the TT procedure, tTau, pTau181, Evans index, $A\beta_{1–40}$, and $A\beta_{1–42}$ showed the best predictive accuracy. For improvement after shunt surgery, NfL and pTau181 showed superior performance in classifying the outcome (Figure 4).

The sensitivity analysis indicated that aside for higher Evans index, the 18 patients who did not have TUG measures had similar age and CSF biomarker profiles to the 103 who had TUG measures, suggesting that these 18 patients were not significantly different than the full cohort who underwent shunt surgery (eTable 1, links.lww.com/CPJ/A327). Additional analysis including all 121 patients showed that in addition to ptau181, predictors that change in statistical significance include the unadjusted LRG normalized model

Table 2 Logistic Regression Models for Relationships Between Patient Responses to TT and Baseline Characteristics and Biomarkers (n = 402)

	Unadjusted models			Adjusted models		
Predictors	Odds ratio	95% CI	*p* Value	Odds ratio	95% CI	*p* Value
Age	0.99	0.96–1.01	0.296			
Female sex	1.11	0.71–1.78	0.647			
MRI Evans index, per 0.01 pts	1.09	1.03–1.15	<0.001	1.09	1.04–1.16	<0.001
Transependymal flow	1.75	1.11–2.74	0.016	1.70	1.07–2.73	0.029
MoCA (<22)						
22–25	1.00	0.62–1.61	0.999			
≥26	0.80	0.44–1.41	0.435			
NfL	0.97	0.55–1.21	0.806			
pTau181	0.70	0.47–0.91	0.006	0.75	0.46–1.05	0.107
tTau	0.77	0.58–0.97	0.023			
LRG normalized	1.12	0.87–1.37	0.314	1.14	0.88–1.38	0.266
$A\beta_{1-40}$	0.74	0.56–0.94	0.011	0.92	0.68–1.23	0.576

Abbreviations: $A\beta_{1-40}$ = β-amyloid 40; $A\beta_{1-42}$ = β-amyloid 42; CI = confidence interval; LRG = leucine-rich alpha-2 glycoprotein; MoCA = Montreal Cognitive Assessment; NfL = neurofilament light; pTau181 = phosphorylated tau 181; TT = tap test; tTau = total tau; TUG = Timed Up and Go.
The sample size for models with MRI and MoCA is 401. The sample size for the multivariate model is 400. The predictors for the adjusted model were selected by Lasso regression. Median odds ratios from bootstrap samples are reported. *p* Value was obtained by determining the proportions of bootstrapped coefficients smaller and larger than zero and multiplying the minimum proportion by 2.

(changes from being borderline significant $p = 0.066$ to significant $p = 0.039$) and NfL in the adjusted model (changes from $p = 0.091$ to $p = 0.026$) (eTable 2).

The CSF biomarkers individually did not offer much value for predicting improvement after TT as the accuracy of the predictions was not high (Table 4). However, there was stronger evidence of the predictive values of the CSF biomarkers in predicting shunt responders vs nonresponders. We observed higher sensitivity of some of the biomarker tests such as with NfL and pTau181 (0.78, 95% CI 0.52–0.92 and 0.70, 95% CI 0.48–0.96, respectively), although these tests were not as specific individually (0.58, 95% CI 0.25–0.88 and 0.62, 95% CI 0.20–0.91, respectively). This means although we would be able to correctly identify a large proportion of patients who would respond positively to shunt surgery, we would also wrongly identify some patients who would be true nonresponders as responders if the biomarkers are assessed in isolation. In terms of sensitivity, tTau and $A\beta_{1-40}$ were also promising, with comparable sensitivities to NfL and pTau181, but showed weaker performance in terms of specificity.

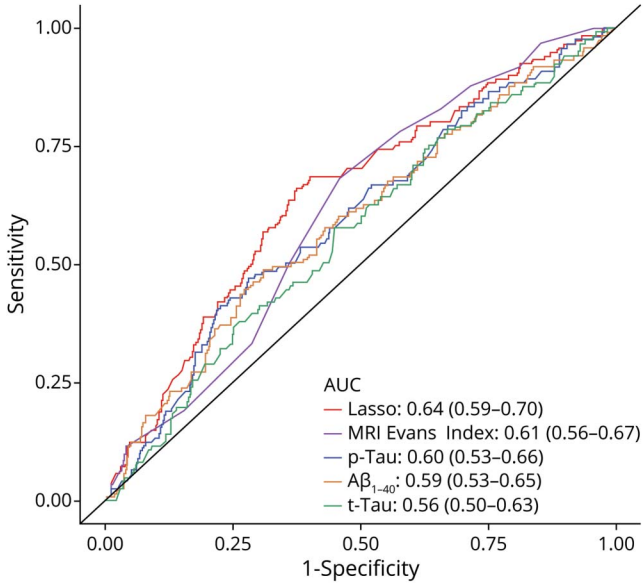
Cutoffs for NfL (1,978.61 ± 655.70 pg/mL) and pTau181 (36.87 ± 12.01 pg/mL) were established from an independent cohort of 50 cognitively normal individuals followed at Johns Hopkins. We found that 63 (84%) patients who improved after shunt surgery had normal pTau181 and NfL values, as did 87 (71.9%) patients who improved after TT (Table 1).

Discussion

Because of comorbidities and overlapping characteristics between iNPH and other neurodegenerative illnesses, identification of relevant CSF biomarkers could improve diagnosis and treatment outcomes.[5] This large cohort study with follow-up gait assessments, over approximately 20 months, shows that a combination of CSF biomarkers involved in neurodegeneration has the potential to identify the subset of patients with iNPH who are likely to have a sustained response from shunt surgery.

Clinically, iNPH is characterized by the triad of gait impairment, cognitive disturbances, and urinary incontinence.[14] On imaging, enlargement of the ventricles with relatively little atrophy may be seen.[10] Surgical insertion of a shunt as a method of permanent CSF diversion is currently the standard method of treatment.[5] Several retrospective studies and smaller prospective studies have demonstrated that shunt treatment can alleviate symptoms in 80% of patients with iNPH if it is distinguished adequately from other neurodegenerative conditions.[6,15] A formal assessment of the efficacy of shunt treatment in a double-blind, randomized trial has yet to be performed due to ethical concerns and the prior lack of valves to turn off a shunt.[16] While 2 small-scale randomized clinical trials, involving 93 and 14 patients each have been conducted about efficacy of shunt surgery in iNPH, a definitive large double-blind randomized trial is still lacking.[17,18] Hence, current practice guidelines have not changed.

Figure 1 The Plot Presents the ROC Curves Generated From the Univariate and the Multivariate Regression (Red) Models Listed in Table 2



The multivariate model uses LRG, pTau, $A\beta_{1-40}$, Evans index, and transependymal flow as predictors and has the greatest AUC of 0.64 (95% CI 0.58–0.70). $A\beta_{1-40}$ = β-amyloid 40; AUC = area under the receiver operating curve; CI = confidence interval; LRG = leucine-rich alpha-2-glycoprotein; pTau = phosphorylated tau.

Evans index is used in most studies of iNPH as one of the prerequisites for making a diagnosis.[19] However, Evans index alone is insufficient to select surgical candidates as it cannot differentiate iNPH from other forms of neurologic diseases.[13] Our study showed that Evans index was significant in predicting immediate improvement from TT but lacked significance in predicting long-term outcomes from shunt surgery.

Our study demonstrated that $A\beta_{1-42}$ levels were not significant in determining long-term responsiveness, but, surprisingly, $A\beta_{1-40}$ was found to be a significant predictor for treatment outcomes. $A\beta_{1-40}$ levels were lower in those who improved after TT than in those who did not and were associated with long-term gait improvement after shunt surgery (Table 1). This finding supports the dilution effect for $A\beta_{1-40}$.[20] The inability of $A\beta_{1-42}$ levels to predict immediate or long-term improvement may reflect the unique older population cohort seen at our center among whom amyloid pathology is more prevalent, resulting in a low $A\beta_{1-42}$, even in the iNPH group. A recent study was also not able to validate the usefulness of $A\beta_{1-42}$ to differentiate iNPH from AD.[21]

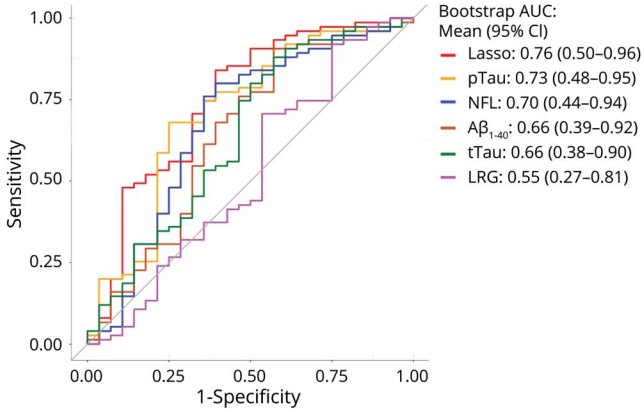
Using conservative improvement criteria, our study extends these findings in a large cohort by showing that elevated baseline levels of pTau181 were associated with poor long-term improvement after shunt surgery. Conversely, pTau181 only modestly predicted immediate improvement after TT. Thus, although

Table 3 Logistic Regression Models for Relationships Between Improvement After Shunt Surgery and Baseline Characteristics and Biomarkers (n = 103)

	Unadjusted models			Adjusted model		
Predictors	**Odds ratio**	**95% CI**	***p* Value**	**Odds ratio**	**95% CI**	***p* Value**
Age	0.91	0.83–0.98	0.016	0.92	0.80–1.03	0.133
Female sex	0.55	0.16–1.42	0.224			
MRI Evans index, per 0.01 pts	1.09	0.95–1.29	0.212			
TUG baseline, per 10 pts	1.01	0.92–1.26	0.859			
MoCA (<22)						
22–25	2.12	0.77–6.58	0.145			
≥26	1.74	0.54–8.64	0.363			
NfL	0.44	0.06–0.76	0.008	0.53	0.08–1.11	0.091
pTau181	0.31	0.11–0.57	<0.001	0.32	0.11–0.63	0.003
tTau	0.43	0.21–0.74	0.003			
LRG normalized	0.68	0.48–1.05	0.066	0.71	0.49–1.52	0.154
$A\beta_{1-40}$	0.51	0.30–0.80	0.005			
$A\beta_{1-42}$	0.69	0.45–1.15	0.127			

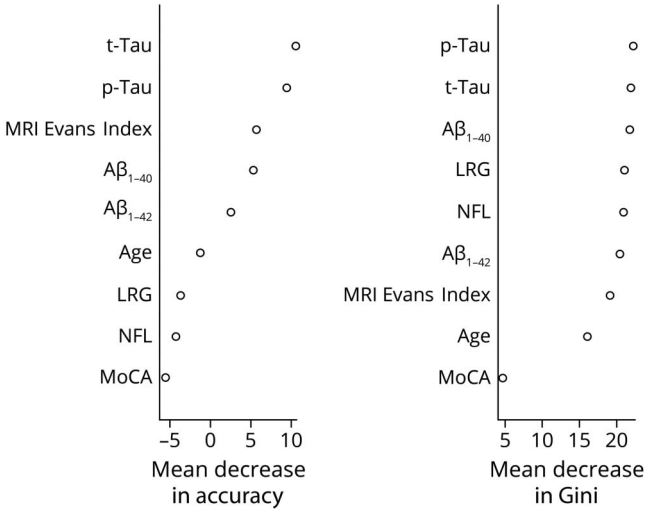
Abbreviations: $A\beta_{1-40}$ = β-amyloid 40; $A\beta_{1-42}$ = β-amyloid 42; CI = confidence interval; LRG = leucine-rich alpha-2 glycoprotein; MoCA = Montreal Cognitive Assessment; NfL = neurofilament light; pTau181 = phosphorylated tau 181; TT = tap test; tTau = total tau; TUG = Timed Up and Go.
The predictors for the adjusted model were selected by Lasso regression. Median odds ratios from bootstrap samples are reported. *p* Value was obtained by determining the proportions of bootstrapped coefficients smaller and larger than zero and multiplying the minimum proportion by 2.

Figure 2 The Plot Presents the ROC Curves Generated From the Univariate and Multivariate (Red) Regression Models Listed in Table 3



The multivariate model uses age, NfL, pTau, and LRG as predictors and has the greatest AUC of 0.76 (95% CI 0.66–0.86). $A\beta_{1-40}$ = β-amyloid 40; AUC = area under the receiver operating curve; CI = confidence interval; LRG = leucine-rich alpha-2-glycoprotein; NfL = neurofilament light; pTau = phosphorylated tau; tTau = total tau.

Figure 3 Variable Importance Plot From Nonsupervised Random Forest Algorithm for Improvement After the TT Procedure



The mean decrease in accuracy attributed to a variable is determined during the classification error calculation phase. The more the accuracy of the random forest decreases due to the exclusion (or permutation) of a single variable, the more important that variable is deemed, and therefore, variables with a large mean decrease in accuracy are more important for classification of the outcome. The mean decrease in Gini coefficient is a measure of how each variable contributes to the homogeneity (purity) of the nodes and leaves in the resulting random forest. The Gini coefficient is a measure of homogeneity from 0 (homogeneous) to 1 (heterogeneous). The changes in Gini are summed for each variable and normalized at the end of the calculation. Variables that result in nodes with higher homogeneity have a higher decrease in Gini coefficient. $A\beta_{1-40}$ = β-amyloid 40; $A\beta_{1-42}$ = β-amyloid 42; LRG = leucine-rich alpha-2-glycoprotein; MoCA = Montreal Cognitive Assessment; NfL = neurofilament light; pTau = phosphorylated tau; TT = tap test; tTau = total tau.

pTau181 may not be a good discriminatory marker, it could play an important role as a prognostic marker when combined with other CSF biomarkers. NfL, a major structural protein of myelinated axons, is an established marker of neuroaxonal integrity.[22]

In addition, in our study, NfL showed significance in the univariate regression for determining shunt responsiveness but not in the multivariate analysis. Elevated NfL levels in CSF at baseline indicated poor shunt responsiveness, suggesting that these patients likely had comorbidities or that their iNPH was sufficiently advanced to cause neuroaxonal injury that shunting could not reverse. Irrespective of the mechanism, an elevated CSF NfL concentration is a poor prognostic marker in iNPH.

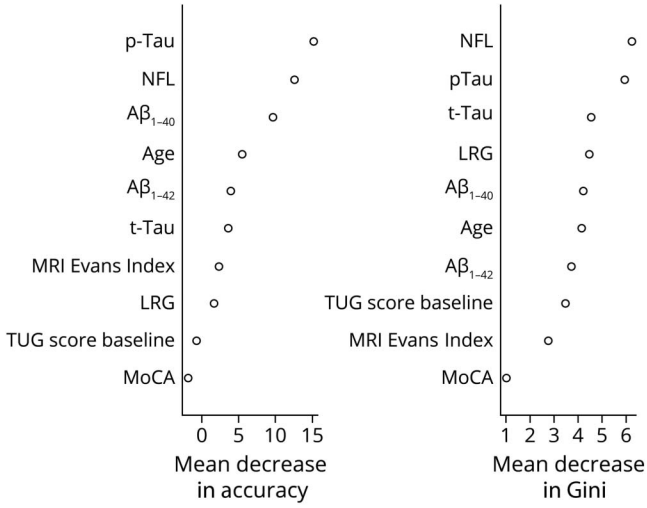
In contrast to previous reports, in our study baseline LRG1 did not discriminate between those who did and did not respond to a TT. However, elevated baseline levels of LRG1 in CSF were associated with poor outcomes following shunt surgery. This finding again suggests that patients had either comorbid neurodegenerative disorders or advanced injury from iNPH.

In our current study, NfL was the best single predictor for patient response after shunt surgery, with pTau181 also having significant predictive ability. However, the most significant predictive potential lay in combining multiple biomarkers. When NfL, pTau181, and normalized LRG1 were combined with age and Evans index in a multivariate model, the predictive value improved. $A\beta_{1-40}$ and tTau, though useful individually in prediction, were not selected into the multivariate model, likely because pTau181 is highly correlated with both, and they do not impart additional information. In predicting immediate improvement from TT, the combined model of pTau181, $A\beta_{1-40}$, LRG, and Evans index and transependymal flow were most predictive. Unlike in a recent study,[21] we were able to show differences in biomarkers between patients with probable iNPH (those who improved after TT) compared with those who did not improve, likely reflecting the larger sample size and the stricter improvement criterion.

Many studies examining CSF biomarker in iNPH have explored the role of CSF biomarkers for AD, in particular $A\beta_{1-40}$, $A\beta_{1-42}$, tTau, and pTau181. Aβs are physiologic peptides present in the normal brain and are thought to be cleared from the brain's interstitial space via the CSF and across the blood-brain barrier.[23] Any alteration in this process might cause Aβ deposition.[24] Because iNPH causes a reduction in CSF outflow absorption,[25] Aβ deposition and subsequent neurodegeneration may also occur.[24] Because $A\beta_{1-40}$ and $A\beta_{1-42}$ are part of the core CSF biomarkers for neurodegeneration, these peptides have been extensively reported in iNPH biomarker studies.[5,26]

Although these 2 kinds of Aβ isoforms differ only in 2 amino acid residues, they vary significantly in their metabolism, physiologic functions, toxicities, and aggregation mechanisms.[27] In a review by Pfanner et al.,[5] $A\beta_{1-42}$ showed prognostic value for iNPH, whereas $A\beta_{1-40}$ was not found to be a significant predictor. As posited by Graff-Radford,[28] use of CSF AD biomarkers can be misleading in the investigation of iNPH, potentially due to either

Figure 4 Variable Importance Plot From Nonsupervised Random Forest Algorithm for Improvement After Shunt Surgery



$A\beta_{1–40}$ = β-amyloid 40; $A\beta_{1–42}$ = β-amyloid 42; LRG = leucine-rich alpha-2-glycoprotein; MoCA = Montreal Cognitive Assessment; NfL = neurofilament light; pTau = phosphorylated tau; TT = tap test; tTau = total tau; TUG = Timed Up and Go.

decreased movement of these molecules from the interstitial compartment or dilution effects, where the excess CSF in iNPH dilutes physiologic CSF components.

Tau, a protein that stabilizes microtubules and is abundant in the neurons, is a known marker of neuronal injury.[29-31] High levels of tTau are found in patients with several neurodegenerative diseases.[5,32,33] Previous studies have not supported tTau or pTau181 as reliable predictors of long-term shunt responsiveness in patients with iNPH when used individually.[5] However, Akiba et al.[34] studied a small cohort of 35 patients and found that low pTau181 levels predicted favorable long-term (3-year) prognosis after receiving a shunt, 3 years after surgery. However, there were no objective gait measures performed in the study to quantify the improvement in this critical feature of iNPH. Another recent study looked at a composite of several AD markers in 50 iNPH shunt recipients and reported that both tTau and pTau181 could predict patients' outcomes after shunt surgery.[35] Nevertheless, their criteria for improvement in any gait parameter were low, at 5% in any gait measure and 1 point on the Mini-Mental Status Examination.

Elevation of NfL in the brain is proportional to the degree of axonal damage in many neurologic disorders, including inflammatory, neurodegenerative, traumatic, and cerebrovascular diseases.[36] Levels are lowest in controls, with intermediate levels in people with mild cognitive impairment, higher levels in those with AD, with the highest levels seen in frontotemporal dementia, amyotrophic lateral sclerosis, and atypical parkinsonian disorders.[26,32] The presence of NfL in CSF is associated with a 3.1-fold increased risk of mild cognitive impairment.[37]

Table 4 Optimal Cutoff Points for Biomarkers for TT and Shunt Surgery

Biomarker	Optimal cutoff, median (95% CI)	Direction corresponding to improvement	Accuracy, mean (95% CI)	Sensitivity, mean (95% CI)	Specificity, mean (95% CI)
TT					
NfL	1,575 (858–2,730)	≤	0.53 (0.40–0.68)	0.49 (0.13–0.79)	0.55 (0.27–0.89)
LRG normalized	170 (139–1,162)	≥	0.46 (0.36–0.68)	0.64 (0.05–0.89)	0.38 (0.16–0.95)
pTau181	22.1 (19.9–36.7)	≤	0.61 (0.42–0.70)	0.47 (0.29–0.83)	0.67 (0.25–0.82)
tTau	234 (140–365)	≤	0.53 (0.41–0.66)	0.53 (0.20–0.82)	0.53 (0.26–0.82)
$A\beta_{1–42}$	700 (458–1,202)	≤	0.57 (0.36–0.68)	0.41 (0.13–0.85)	0.64 (0.16–0.88)
$A\beta_{1–40}$	6,033 (5,200–8,781)	≤	0.60 (0.42–0.68)	0.46 (0.24–0.75)	0.65 (0.29–0.82)
Shunt surgery					
NfL	1,985 (1,424–2,630)	≤	0.72 (0.56–0.84)	0.78 (0.52–0.92)	0.58 (0.25–0.88)
LRG normalized	257 (50–∞)	≥	0.53 (0.26–0.77)	0.61 (0–1)	0.31 (0–1)
pTau181	26.1 (23.5–42.1)	≤	0.68 (0.55–0.81)	0.70 (0.48–0.96)	0.62 (0.20–0.91)
tTau	307 (133–486)	≤	0.67 (0.39–0.82)	0.77 (0.22–0.97)	0.43 (0.10–0.91)
$A\beta_{1–42}$	1,460 (474–1,617)	≤	0.62 (0.32–0.85)	0.72 (0.19–1)	0.34 (0–0.85)
$A\beta_{1–40}$	9,570 (6,389–9,967)	≤	0.69 (0.48–0.84)	0.79 (0.42–0.97)	0.43 (0.13–0.75)

Abbreviations: $A\beta_{1–40}$ = β-amyloid 40; $A\beta_{1–42}$ = β-amyloid 42; CI = confidence interval; LRG = leucine-rich alpha-2 glycoprotein; MoCA = Montreal Cognitive Assessment; NfL = neurofilament light; pTau181 = phosphorylated tau 181; TT = tap test; tTau = total tau; TUG = Timed Up and Go.
Optimal cutoff values were evaluated using 1,000 bootstrap samples and identified based on maximum Youden index. Mean estimates along with 5% and 95% percentile estimates for out-of-bag accuracy, sensitivity, and specificity are reported.

Measuring CSF NfL greatly improves the distinction between many forms of neurodegenerative disease from each other and control participants.[32] Accordingly, several studies have shown that patients with iNPH exhibit higher CSF NfL levels than controls.[9,38-40] These findings could indicate that NfL is a marker for iNPH, but the issue of comorbid neurodegenerative disorders presenting with a predegenerative iNPH phenotype is an alternate hypothesis.[41] In previous studies, it was unclear how NfL concentration correlated with the degree of shunt surgery responsiveness.

LRG1, a novel biomarker for inflammatory diseases,[42] is an astrocytic protein displaying perivascular expression in brain that increases with age and nonspecific inflammatory changes.[43,44] Preliminary work examining LRG1 levels in CSF has shown promising results for differentiating neuroinflammatory diseases with high sensitivity and specificity.[45] A study by Jingami et al.[46] showed no clear evidence that LRG1 was a prospective biomarker for distinguishing between noninflammatory neurologic disorders, as there was no difference in levels between patients with iNPH and AD or responders and nonresponders. Increased concentrations of LRG1 in CSF have been shown in patients with iNPH compared with controls, suggesting a potential role as a disease biomarker or predictor of a positive outcome after shunt placement.[44] Together with NfL, LRG1 potentially allows tracking of the integrity of subcortical structures, offering some discriminatory properties in comparative analyses between iNPH and other neurodegenerative conditions.[47]

This was a single-center study at a tertiary referral center, which limits generalization. A multicenter trial would be necessary for external validation.[48] Because a convenience sampling method was used, there was no set schedule for testing after shunt surgery, although testing around the TT was performed within 1 hour before and after. Furthermore, we used outcome data only from the TUG test to measure speed and dynamic balance. The cutoff of >30% improvement in TUG as a criterion for selection of patients for shunt surgery is arbitrary and does not fully capture the spectrum of improvement after CSF drainage. We did not examine the change in cognitive measures, static balance, or endurance measures, as those are not obtained as the standard of care in our clinic and are performed only if clinical concerns arise. A recent analysis in our cohort demonstrated strong correlations between TUG and measures of balance and endurance.[49] Examining all these measures would potentially improve prediction of shunt outcomes. We also did not assess *APOE* genotype, which can affect clinical outcome from neurologic injuries.[50] The patients without objective TUG measures at follow-up had slightly higher Evans index scores, which might be the reason why this marker was not significant for improvement after shunt surgery in the multivariate regression model for improvement assessment after shunt surgery. Nonetheless, the sensitivity analysis suggests that CSF biomarker profiles were not different between patients with and without TUG data. We did not have volumetric data from MRI to normalize CSF biomarker values for the increased volume of distribution of CSF in patients with ventriculomegaly to account for dilution effects. Finally, we did not further characterize the 28 shunt nonresponders clinically to ascertain their underlying neurologic diagnoses, e.g., Parkinson-plus syndromes and AD.

TAKE-HOME POINTS

- Several reports have suggested that iNPH represents a predegenerative phase of multiple age-related neurodegenerative disorders presenting with a phenotype involving cognition, gait, and bladder control. CSF biomarkers have been suggested as a means of identifying such confounds and potentially select patients for shunt surgery.
- We show that patients, who do not demonstrate long-term improvement in gait following shunt surgery, have elevations in multiple biomarkers that suggest either comorbid neurodegenerative pathology or advanced brain injury from iNPH itself. Irrespective of the cause of this elevation, analyzing biomarkers in combination can identify who is likely to have a sustained gait response to shunt surgery.
- If replicated in independent cohorts and with longer follow-up, the combination of imaging and CSF biomarkers could potentially refine the selection of patients with iNPH for surgery while also facilitating randomized trials of shunt efficacy for this vexing diagnosis.

The strengths of our study include the large cohort size, long duration of follow-up, a strong improvement threshold, and the use of biomarkers that reflect multiple pathologies common in aging. The study included both sensitive (NfL) and specific (pTau181) biomarkers of neurodegenerative disorders that often confound the diagnosis and selection of patients with iNPH for shunt surgery.

In the population-based Mayo Clinic Study of Aging, among 1,494 persons older than 70 years, 20% had ventriculomegaly (Evans index of 0.3 or greater), and 5% had ventriculomegaly and either a tight high convexity (occluded sulci at the high convexity) or extraventricular hydrocephalus (CSF collection outside the ventricles not due to atrophy) suggestive of NPH.[45] In a large population-based study from Sweden, the prevalence of iNPH was estimated at 0.2% between 70 and 79 years and 5.9% for those 80 years and older.[8] Therefore, there is potentially a large population that could benefit from shunt surgery if patients with potential good long-term outcomes can be identified before shunt surgery.

The role of CSF biomarkers for iNPH that would allow clinicians to distinguish it from other neurodegenerative disorders has been assessed in multiple studies. In contrast, the role of CSF biomarkers in predicting long-term outcomes is less well studied but is of increasing interest. Our study extends the current literature

by evaluating not just individual biomarkers but also composites of the most studied CSF biomarkers to predict long-term outcomes after shunt surgery. At the same time, their role in predicting immediate improvement from a TT is limited. Conceptually, these findings would support the hypothesis that, in the subset of iNPH patients who do improve after a TT, elevated biomarkers suggest the coexistence of neurodegenerative disorders like AD, atypical Parkinsonian syndromes and vascular dementia, or there exists irreversible axonal injury from iNPH. These preliminary findings will need to be replicated in other cohorts and, more importantly, looked at in a prospective clinical trial before changes to current clinical practice can be recommended.

Acknowledgment

The authors thank Dr. Marilyn Albert, who provided expertise and guidance regarding drafting the manuscript and substantive editing help. The authors also thank the Lantry Family Foundation and the Myers Family for their philanthropic contributions toward this research project.

Study Funding

This study was supported by the Lantry Family Foundation and the Myers Family.

Disclosure

J.A. Darrow, A. Lewis, S. Gulyani, K. Khingelova, A. Rao, J. Wang, Y. Zhang, M. Luciano, and S. Yasar report no disclosures relevant to the manuscript. A. Moghekar reports research support from Fujirebio Diagnostics Ltd. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/cp.

Publication History

Received by *Neurology: Clinical Practice* August 24, 2021. Accepted in final form January 10, 2022.

Appendix Authors

Name	Location	Contribution
Jacqueline A. Darrow, MS	Johns Hopkins University School of Medicine, Baltimore, MD	Study concept or design, interpretation of the data, drafted and revised the manuscript for content, study supervision, and coordination
Alexandria Lewis, BS	Johns Hopkins University School of Medicine, Baltimore, MD	Study concept or design, interpretation of the data, drafted and revised the manuscript for content, study supervision, and coordination
Seema Gulyani, PhD	Johns Hopkins University School of Medicine, Baltimore, MD	Study supervision and coordination
Kristina Khingelova, PA-C	Johns Hopkins University School of Medicine, Baltimore, MD	Study supervision and coordination
Aruna Rao, MD	Johns Hopkins University School of Medicine, Baltimore, MD	Study supervision and coordination
Jiangxia Wang, MS	Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD	Performed the statistical analyses, interpretation of the data, and revised the manuscript for content
Yifan Zhang, MS	Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD	Performed the statistical analyses, interpretation of the data, and revised the manuscript for content
Mark Luciano, MD	Johns Hopkins University School of Medicine, Baltimore, MD	Study supervision and coordination
Sevil Yasar, MD	Johns Hopkins University School of Medicine, Baltimore, MD	Study supervision and coordination
Abhay Moghekar, MBBS	Johns Hopkins University School of Medicine, Baltimore, MD	Supervised the analysis of CSF, study concept or design, interpretation of the data, drafted and revised the manuscript for content, study supervision, and coordination

References

1. Hakim S, Adams RD. The special clinical problem of symptomatic hydrocephalus with normal cerebrospinal fluid pressure observations on cerebrospinal fluid hydrodynamics. *J Neurol Sci*. 1965;2(4):307-327.
2. Kiefer M, Unterberg A. Differenzialdiagnose und therapie des normaldruckhydrozephalus. *Dtsch Arztebl Int*. 2012;109:15-26.
3. Hebb AO, Cusimano MD. Idiopathic normal pressure hydrocephalus: a systematic review of diagnosis and outcome. *Neurosurgery*. 2001;49(5):1166-1184.
4. Halperin JJ, Kurlan R, Schwalb JM, Cusimano MD, Gronseth G, Gloss D. Practice guideline: idiopathic normal pressure hydrocephalus: response to shunting and predictors of response. *Neurology*. 2015;85(23):2063-2071.
5. Pfanner T, Henri-Bhargava A, Borchert S. Cerebrospinal fluid biomarkers as predictors of shunt response in idiopathic normal pressure hydrocephalus: a systematic review. *Can J Neurol Sci*. 2018;45(1):3-10.
6. Feletti A, D'Avella D, Wikkelsø C, et al. Ventriculoperitoneal shunt complications in the european idiopathic normal pressure hydrocephalus multicenter study. *Oper Neurosurg (Hagerstown)*. 2019;17(1):97-101.
7. Jeppsson A, Wikkelsö C, Blennow K, et al. CSF biomarkers distinguish idiopathic normal pressure hydrocephalus from its mimics. *J Neurol Neurosurg Psychiatry*. 2019;90(10):1117-1123.
8. Jaraj D, Rabiei K, Marlow T, Jensen C, Skoog I, Wikkelsø C. Prevalence of idiopathic normal-pressure hydrocephalus. *Neurology*. 2014;82(16):1449-1454.
9. Tullberg M, Blennow K, Månsson JE, Fredman P, Tisell M, Wikkelsö C. Cerebrospinal fluid markers before and after shunting in patients with secondary and idiopathic normal pressure hydrocephalus. *Cerebrospinal Fluid Res*. 2008;5:9.
10. Manniche C, Hejl AM, Hasselbalch SG, Simonsen AH. Cerebrospinal fluid biomarkers in idiopathic normal pressure hydrocephalus versus Alzheimer's disease and subcortical ischemic vascular disease: a systematic review. *J Alzheimers Dis*. 2019;68(1):267-279.
11. Darrow JA, Calabro A, Gannon S, et al. Effect of patient-specific preanalytic variables on CSF Aβ1–42 concentrations measured on an automated chemiluminescent platform. *J Appl Lab Med*. 2021;6(2):397-408.
12. Gallagher R, Marquez J, Osmotherly P. Clinimetric properties and minimal clinically important differences for a battery of gait, balance, and cognitive examinations for the tap test in idiopathic normal pressure hydrocephalus. *Clin Neurosurg*. 2019;84(6):E378-E384.
13. Tarnaris A, Toma AK, Chapman MD, Keir G, Kitchen ND, Watkins LD. Use of cerebrospinal fluid amyloid-β and total tau protein to predict favorable surgical outcomes in patients with idiopathic normal pressure hydrocephalus: clinical article. *J Neurosurg*. 2011;115(1):145-150.
14. Nassar BR, Lippa CF. Idiopathic normal pressure hydrocephalus. *Gerontol Geriatr Med*. 2016;2:233372141664370.
15. Ghosh S, Lippa C. Diagnosis and prognosis in idiopathic normal pressure hydrocephalus. *Am J Alzheimers Dis Other Demen*. 2014;29(7):583-589.
16. McGirr A, Mohammed S, Kurlan R, Cusimano MD. Clinical equipoise in idiopathic normal pressure hydrocephalus: a survey of physicians on the need for randomized controlled trials assessing the efficacy of cerebrospinal fluid diversion. *J Neurol Sci*. 2013;333(1-2):13-18.

17. Tisell M, Tullberg M, Hellström P, Edsbagge M, Högfeldt M, Wikkelsö C. Shunt surgery in patients with hydrocephalus and white matter changes: clinical article. *J Neurosurg*. 2011;114(5):1432-1438.
18. Kazui H, Miyajima M, Mori E, et al. Lumboperitoneal shunt surgery for idiopathic normal pressure hydrocephalus (SINPHONI-2): an open-label randomised trial. *Lancet Neurol*. 2015;14(6):585-594.
19. Tarnaris A, Kitchen ND, Watkins LD. Noninvasive biomarkers in normal pressure hydrocephalus: evidence for the role of neuroimaging—a review. *J Neurosurg*. 2009; 110(5):837-851.
20. Santangelo R, Cecchetti G, Bernasconi MP, et al. Cerebrospinal fluid amyloid-β 42, total tau and phosphorylated tau are low in patients with normal pressure hydrocephalus: analogies and differences with Alzheimer's disease. *J Alzheimers Dis*. 2017;60(1):183-200.
21. Taghdiri F, Gumus M, Algarni M, Fasano A, Tang-Wai D, Tartaglia MC. Association between cerebrospinal fluid biomarkers and age-related brain changes in patients with normal pressure hydrocephalus. *Sci Rep*. 2020;10(1):9106.
22. Magdalinou NK, Paterson RW, Schott JM, et al. A panel of nine cerebrospinal fluid biomarkers may identify patients with atypical parkinsonian syndromes. *J Neurol Neurosurg Psychiatry*. 2015;86(11):1240-1247.
23. Motter R, Vigo-Pelfrey C, Kholodenko D, et al. Reduction of beta-amyloid peptide42 in the reduction of p-amyloid cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol*. 1995;38(4):643-648.
24. Ray B, Reyes PF, Lahiri DK. Biochemical studies in normal pressure hydrocephalus (NPH) patients: change in CSF levels of amyloid precursor protein (APP), amyloid-beta (Aβ) peptide and phospho-tau. *J Psychiatr Res*. 2011;45(4):539-547.
25. Hamlat A, Adn M, Sid-ahmed S, Askar B, Pasqualini E. Theoretical considerations on the pathophysiology of normal pressure hydrocephalus (NPH) and NPH-related dementia. *Med Hypotheses*. 2006;67(1):115-123.
26. Zetterberg H, Skillbäck T, Mattsson N, et al. Association of cerebrospinal fluid neurofilament light concentration with Alzheimer disease progression. *JAMA Neurol*. 2016;73(1):60-67.
27. Qiu T, Liu Q, Chen YX, Zhao YF, Li YM. Aβ42 and Aβ40: similarities and differences. *J Pept Sci*. 2015;21(7):522-529.
28. Graff-Radford NR. Alzheimer CSF biomarkers may be misleading in normal-pressure hydrocephalus. *Neurology*. 2014;83(17):1573-1575.
29. Johnson GVW, Seubert P, Cox TM, Motter R, Brown P. The tau protein in human cerebrospinal fluid in Alzheimer's disease consists of proteolytically derived fragments. *J Neurochem*. 1997;68(1):430-433.
30. Kapaki EN, Paraskevas GP, Tzerakis NG, et al. Cerebrospinal fluid tau, phospho-tau181 and β-amyloid 1-42 in idiopathic normal pressure hydrocephalus: a discrimination from Alzheimer's disease. *Eur J Neurol*. 2007;14(2):168-173.
31. Zetterberg H, Smith DH, Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. *Nat Rev Neurol*. 2013;9(4):201-210.
32. Olsson B, Portelius E, Cullen NC, et al. Association of cerebrospinal fluid neurofilament light protein levels with cognition in patients with dementia, motor neuron disease, and movement disorders. *JAMA Neurol*. 2019;76(3):318-325.
33. Van Harten AC, Kester MI, Visser PJ, et al. Tau and p-tau as CSF biomarkers in dementia: a meta-analysis. *Clin Chem Lab Med*. 2011;49(3):353-366.
34. Akiba C, Nakajima M, Miyajima M, et al. Change of amyloid-β 1-42 toxic conformer ratio after cerebrospinal fluid diversion predicts long-term cognitive outcome in patients with idiopathic normal pressure hydrocephalus. *J Alzheimers Dis*. 2018;63(3):989-1002.
35. Migliorati K, Panciani PP, Pertichetti M, et al. P-Tau as prognostic marker in long term follow up for patients with shunted iNPH. *Neurol Res*. 2021;43(1):78-88.
36. Gaetani L, Höglund K, Parnetti L, et al. A new enzyme-linked immunosorbent assay for neurofilament light in cerebrospinal fluid: analytical validation and clinical evaluation. *Alzheimers Res Ther*. 2018;10(1):8.
37. Kern S, Syrjanen JA, Blennow K, et al. Association of cerebrospinal fluid neurofilament light protein with risk of mild cognitive impairment among individuals without cognitive impairment. *JAMA Neurol*. 2019;76(2):187-193.
38. Tullberg M, Rosengren L, Blomsterwall E, Karlsson J, Wikkelso C. CSF neurofilament and glial fibrillary acidic protein in normal pressure hydrocephalus. *Neurology*. 1998; 50(4):1122-1127.
39. Tullberg M, Blennow K, Månsson JE, Fredman P, Tisell M, Wikkelsö C. Ventricular cerebrospinal fluid neurofilament protein levels decrease in parallel with white matter pathology after shunt surgery in normal pressure hydrocephalus. *Eur J Neurol*. 2007; 14(3):248-254.
40. Jeppsson A, Höltta M, Zetterberg H, Blennow K, Wikkelsø C, Tullberg M. Amyloid mis-metabolism in idiopathic normal pressure hydrocephalus. *Fluids Barriers CNS*. 2016;13(1):13.
41. Espay AJ, Da Prat GA, Dwivedi AK, et al. Deconstructing normal pressure hydrocephalus: ventriculomegaly as early sign of neurodegeneration. *Ann Neurol*. 2017; 82(4):503-513.
42. Chong PF, Sakai Y, Torisu H, et al. Leucine-rich alpha-2 glycoprotein in the cerebrospinal fluid is a potential inflammatory biomarker for meningitis. *J Neurol Sci*. 2018; 392:51-55.
43. Nakajima M, Miyajima M, Ogino I, et al. Leucine-rich α-2-glycoprotein is a marker for idiopathic normal pressure hydrocephalus. *Acta Neurochir (Wien)*. 2011;153(6):1339-1346.
44. Miyajima M, Nakajima M, Motoi Y, et al. Leucine-rich α2-glycoprotein is a novel biomarker of neurodegenerative disease in human cerebrospinal fluid and causes neurodegeneration in mouse cerebral cortex. *PLoS One*. 2013;8(9):e74453.
45. Graff-Radford N, Gunter J, Thomas C, et al. Ventriculomegaly is a biomarker of gait and cognitive decline. *Alzheimers Dement*. 2017;13(7):P1092.
46. Jingami N, Asada-Utsugi M, Uemura K, et al. Idiopathic normal pressure hydrocephalus has a different cerebrospinal fluid biomarker profile from Alzheimer's disease. *J Alzheimers Dis*. 2015;45(1):109-115.
47. Schirinzi T, Sancesario GM, Di Lazzaro G, et al. Cerebrospinal fluid biomarkers profile of idiopathic normal pressure hydrocephalus. *J Neural Transm (Vienna)*. 2018; 125(4):673-679.
48. Bellomo R, Warrillow SJ, Reade MC. Why we should be wary of single-center trials. *Crit Care Med*. 2009;37(12):3114-3119.
49. Davis A, Yasar S, Emerman I, et al. Standardized regression-based clinical change score cutoffs for normal pressure hydrocephalus. *BMC Neurol*. 2020;20(1):140.
50. Gudmundsson G, Kristjansdottir G, Cook E, Olafsson I. Association of ApoE genotype with clinical features and outcome in idiopathic normal pressure hydrocephalus (iNPH): a preliminary report. *Acta Neurochir (Wien)*. 2009;151(11):1511-1512.