CSF Biomarkers Predict Gait Outcomes in Idiopathic Normal Pressure Hydrocephalus

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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 (Aβ42), β-amyloid 40 (Aβ40), 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 Aβ40 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 Aβ40 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.

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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. However, differentiating iNPH from other age-related neurologic disorders is complex. Moreover, shunts are associated with a high adverse event rate of approximately 11%, including infection, malfunction, additional surgery, and subdural hematomas. 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.

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. Another comprehensive review by Manniche et al. concluded that tTau and pTau181 might differentiate iNPH from Alzheimer disease (AD), whereas Aβ1–40 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 (Aβ1–42, 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. 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 score ≥4 was used to define improvement in those on whom a TUG could not be obtained.

**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 2,000g 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 Aβ1–42, Aβ1–40, 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: Aβ1–42 3.4%, Aβ1–40 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 LRG1 was 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 characteristics: 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 non-parametric Kruskal-Wallis tests were used for continuous variables depending on whether the variable was normally distributed.
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 Aβ1–40 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 Aβ1–40 (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 Aβ1–40 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 Aβ1–40 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 0.52, 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 in 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) | p Value | p Value |
|----------------------------------|-----------------------|----------------------------------|---------|---------|
|                                  | No improvement (n = 281) | Improved (n = 121) |         |         |
| 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)*   | 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)*  | 42.2 (82.2)           | 107.1 (128.0)        | 0.039   | 296 (163-319) | 746 (496-1000) | <0.001 |
| Interval between baseline and last follow-up in months, mean (SD) | 19.99 (18.36) | 22.2 (19.3) | 0.612 |

Abbreviations: Aβ1-42 = β-amyloid 42; Aβ1-40 = β-amyloid 40; 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. 

* 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.

** 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.

Normal pTau defined as <33.8 pg/mL. Normal NFL defined as <2,417 pg/mL.

after the TT procedure, tTau, pTau181, Evans index, Aβ1-40 and Aβ1-42 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)

| Predictors | Unadjusted models |  | Adjusted models |  |
|------------|-------------------|---|------------------|---|
|            | 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 |
| Transseptal 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 | 0.99 | | | |
| ≥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 |
| Aβ42 | 0.74 | 0.56–0.94 | 0.011 | 0.92 | 0.68–1.23 | 0.576 |

Abbreviations: Aβ42 = β-amyloid 42; Aβ40 = β-amyloid 40; 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.

Discussion

Because of comorbidities and overlapping characteristics between iNPH and other neurodegenerative illnesses, identification of relevant CSF biomarkers could improve diagnosis and treatment outcomes. 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. Surgical insertion of a shunt as a method of permanent CSF diversion is currently the standard method of treatment. 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. 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. 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. Hence, current practice guidelines have not changed.
Evans index is used in most studies of iNPH as one of the prerequisites for making a diagnosis. However, Evans index alone is insufficient to select surgical candidates as it cannot differentiate iNPH from other forms of neurologic diseases. 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 Aβ1–40 levels were not significant in determining long-term responsiveness, but, surprisingly, Aβ1–40 was found to be a significant predictor for treatment outcomes. Aβ1–40 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 Aβ1–40.20 The inability of Aβ1–42 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 Aβ1–42, even in the iNPH group. A recent study was also not able to validate the usefulness of Aβ1–42 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

| Predictors | Unadjusted models | Adjusted model |
|------------|-------------------|---------------|
|            | 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   |
| Aβ1–40     | 0.51      | 0.30-0.80  | 0.005   |           |             |         |
| Aβ1–42     | 0.69      | 0.45-1.15  | 0.127   |           |             |         |

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

The multivariate model uses LRG, pTau, Aβ1–40, Evans index, and transependymal flow as predictors and has the greatest AUC of 0.64 (95% CI 0.58–0.70). Aβ1–40 = β-amyloid 40; Aβ1–42 = β-amyloid 42; CI = confidence interval; LRG = leucine-rich alpha-2-glycoprotein; pTau = phosphorylated tau; 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.
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. 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. Aβ1–40 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, Aβ1–40, LRG, and Evans index and transependymal flow were most predictive. Unlike

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

Bootstrap AUC
Mean (95% CI)
Lasao: 0.76 (0.50–0.96)
pTau: 0.73 (0.48–0.95)
NfL: 0.70 (0.44–0.94)
Aβ1–40: 0.66 (0.39–0.92)
tTau: 0.66 (0.38–0.90)
LRG: 0.55 (0.27–0.81)

In a recent study, 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 Aβ1–40, Aβ1–42, 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. Any alteration in this process might cause Aβ deposition. Because iNPH causes a reduction in CSF outflow absorption, subsequent neurodegeneration may also occur. Because Aβ1–40 and Aβ1–42 are part of the core CSF biomarkers for neurodegeneration, these peptides have been extensively reported in iNPH biomarker studies.

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. In a review by Pfanner et al, Aβ1–42 showed prognostic value for iNPH, whereas Aβ1–40 was not found to be a significant predictor. As posited by Graff-Radford, use of CSF AD biomarkers can be misleading in the investigation of iNPH, potentially due to either
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. 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. The presence of NfL in CSF is associated with a 3.1-fold increased risk of mild cognitive impairment.

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)         |
| Aβ1-42             | 700 (458–1,202)                  | ≤                                      | 0.57 (0.36–0.68)        | 0.41 (0.13–0.85)          | 0.64 (0.16–0.88)         |
| Aβ1-40             | 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)         |
| Aβ1-42             | 1,460 (474–1,617)                | ≤                                      | 0.62 (0.32–0.85)        | 0.72 (0.19–1)             | 0.34 (0–0.85)            |
| Aβ1-40             | 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: Aβ1-40 = β-amyloid 40; Aβ1-42 = 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.
Accordingly, several studies have shown that patients with iNPH exhibit higher CSF NfL levels than controls.\textsuperscript{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.\textsuperscript{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,\textsuperscript{42} is an astrocytic protein displaying perivascular expression in brain that increases with age and nonspecific inflammatory changes.\textsuperscript{43,44} Preliminary work examining LRG1 levels in CSF has shown promising results for differentiating neuroinflammatory diseases with high sensitivity and specificity.\textsuperscript{45} A study by Jingami et al.\textsuperscript{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.\textsuperscript{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.\textsuperscript{47}

This was a single-center study at a tertiary referral center, which limits generalization. A multicenter trial would be necessary for external validation.\textsuperscript{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 cut-off 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.\textsuperscript{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.\textsuperscript{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.

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 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 (occulted sulci at the high convexity) or extraventricular hydrocephalus (CSF collection outside the ventricles not due to atrophy) suggestive of NPH.\textsuperscript{55} 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.\textsuperscript{5} 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.

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.
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 in 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.

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