Lumbar Cerebrospinal Fluid Biomarkers of Posthemorrhagic Hydrocephalus of Prematurity: Amyloid Precursor Protein, Soluble Amyloid Precursor Protein α, and L1 Cell Adhesion Molecule

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BACKGROUND: Intraventricular hemorrhage (IVH) is the most frequent, severe neurological complication of prematurity and is associated with posthemorrhagic hydrocephalus (PHH) in up to half of cases. PHH requires lifelong neurosurgical care and is associated with significant cognitive and psychomotor disability. Cerebrospinal fluid (CSF) biomarkers may provide both diagnostic information for PHH and novel insights into its pathophysiology.

OBJECTIVE: To explore the diagnostic ability of candidate CSF biomarkers for PHH.

METHODS: Concentrations of amyloid precursor protein (APP), soluble APPα (sAPPα), soluble APPβ, neural cell adhesion molecule-1 (NCAM-1), L1 cell adhesion molecule (L1CAM), tau, phosphorylated tau, and total protein (TP) were measured in lumbar CSF from neonates in 6 groups: (1) no known neurological disease (n = 33); (2) IVH grades I to II (n = 13); (3) IVH grades III to IV (n = 12); (4) PHH (n = 12); (5) ventricular enlargement without hydrocephalus (n = 10); and (6) hypoxic ischemic encephalopathy (n = 13). CSF protein levels were compared using analysis of variance, and logistic regression was performed to examine the predictive ability of each marker for PHH.

RESULTS: Lumbar CSF levels of APP, sAPPα, L1CAM, and TP were selectively increased in PHH compared with all other conditions (all $P < .001$). The sensitivity, specificity, and odds ratios of candidate CSF biomarkers for PHH were determined for APP, sAPPα, and L1CAM; cut points of 699, 514, and 113 ng/mL yielded odds ratios for PHH of 80.0, 200.0, and 68.75, respectively.

CONCLUSION: Lumbar CSF APP, sAPPα, L1CAM, and TP were selectively increased in PHH. These proteins, and sAPPα, in particular, hold promise as biomarkers of PHH and provide novel insight into PHH-associated neural injury and repair.

KEY WORDS: Amyloid precursor protein, Biomarker, Cerebrospinal fluid, Intraventricular hemorrhage, L1CAM, Neurosurgery, Posthemorrhagic hydrocephalus, Preterm, sAPPα

ABBREVIATIONS: APP, amyloid precursor protein; **ELISA,** enzyme-linked immunosorbent assay; **HIE,** hypoxic ischemic encephalopathy; **IVH,** intraventricular hemorrhage; **L1CAM,** L1 cell adhesion molecule; **LP,** lumbar puncture; **NCAM-1,** neural cell adhesion molecule-1; **NICU,** neonatal intensive care unit; **p-tau,** phosphorylated tau; **PHH,** posthemorrhagic hydrocephalus; **PMA,** postmenstrual age; **RCG,** rostrocaudal gradient; **sAPPα,** soluble amyloid precursor protein α; **sAPPβ,** soluble amyloid precursor protein β; **TP,** total protein; **VAD,** ventricular access device; **VEWOH,** ventricular enlargement without hydrocephalus

Intraventricular hemorrhage (IVH) is a common, severe neurological complication of prematurity, occurring in one-third of infants born ≤28 weeks postmenstrual age (PMA).[1,2] Up to one-half of preterm infants with IVH develop posthemorrhagic hydrocephalus (PHH),[3-5] a condition associated with substantial neurological disability and complex, lifelong neurosurgical care.[1,6] Despite the morbidity associated with PHH and its treatment, there is no consensus regarding the diagnosis or treatment of PHH, largely because of a dearth of quantifiable PHH metrics.

At present, clinical assessment of infants with PHH includes physical parameters such as head circumference, tenseness of the anterior fontanel, and splaying of cranial sutures, as well as changes in vital signs, which occur late in the disease course. As a result, clinicians rely in large part on semiquantitative imaging-based measurements of ventricular size for clinical decision making. However, ventricular size is an imperfect metric for the treatment of neurological disease in preterm infants. Neurological injury (eg, IVH, infarct, hypoxia-ischemia), white matter volume loss, and impaired brain development are all common among preterm infants and impact ventricular size, irrespective of PHH. New tools are needed to complement existing measures of ventricular size and inform clinical research aimed at improving the diagnosis, treatment, and outcomes of infants with PHH.

Cerebrospinal fluid (CSF) proteins have been investigated as candidate biomarkers of PHH by our group and others.[7-13] In particular, CSF levels of amyloid precursor protein (APP) isoforms, L1 cell adhesion molecule (L1CAM), and neural cell adhesion molecule-1 (NCAM-1) have been shown to be elevated in PHH and to normalize after initiation of clinical treatment.[7] Furthermore, CSF APP levels have been shown to be associated with ventricular size and possibly intracranial pressure throughout the active PHH treatment interval.[14] Thus, CSF levels of APP and related proteins represent promising candidate biomarkers of PHH. The potential role of CSF biomarkers in PHH should not be limited to diagnosis of the condition, however, nor even monitoring therapeutic efficacy, but may extend to the prediction of long-term neurological outcome and developmental impairment.

Prior investigations into CSF biomarkers of PHH and other types of pediatric hydrocephalus, including those from our group, share the limitation of using CSF acquired via lumbar puncture (LP) (in subjects without hydrocephalus) as the comparator for ventricular CSF obtained from children with hydrocephalus.[7,8] Comparing ventricular with lumbar CSF introduces the potential for sampling artifact, because rostrocaudal protein gradients (RCGs) exist between ventricular and lumbar CSF and may vary both by protein and by neurological disease.[15,16] For hydrocephalus, RCGs may also vary by hydrocephalus etiology.[16]

In the current study, we account for RCGs in PHH by comparing the levels of candidate biomarkers (APP, soluble APP-α, soluble APP-β, Aβ_{1-42}, NCAM-1, L1CAM, tau, and phosphorylated tau [p-tau]) in CSF obtained from human infants exclusively via LP. Using this approach, candidate CSF biomarkers were compared not just between control and PHH but across a number of newborn neurological conditions, including IVH alone, hypoxic ischemic encephalopathy (HIE), and ventricular enlargement without hydrocephalus (VEWOH).

METHODS

Research Subjects

Approval from the Washington University Human Research Protection Office was acquired prior to initiation of this study (WU

HRPO numbers 201101887 and 201203126). As part of routine clinical care, all preterm infants ≤34 weeks estimated PMA admitted to the St. Louis Children's Hospital Neonatal Intensive Care Unit (NICU) underwent routine head ultrasound examinations between 0 and 5 days of age to screen for IVH or other neurological injuries. IVH was graded according to the Papile criteria,[17] and, if present, ultrasounds were repeated weekly to monitor for progressive ventricular dilatation. PHH was diagnosed in infants who demonstrated a frontal-occipital ratio ≥0.55 on ultrasound or magnetic resonance imaging (MRI),[18] progressive increase in occipitofrontal circumference, splaying of the sagittal suture ≥2 mm in the midparietal region, and palpation of the anterior fontanel above the level of the surrounding bone.[19] The current study includes subjects who were diagnosed with PHH and underwent LP before the neurosurgical implantation of a ventricular access device (VAD) for either diagnostic purposes (eg, to rule out infection before VAD implantation) or therapeutic purposes (eg, to enable ventricular decompression while awaiting VAD) (Table 1). None of the 12 infants with PHH in this study had loculated hydrocephalus or a trapped fourth ventricle at the time of acquisition of the CSF sample, and none went on to later develop a trapped fourth ventricle or require treatment for loculated hydrocephalus (shunt system with >1 ventricular catheter or fenestration procedure) at a median follow-up 27.2 ± 15.4 months. Additional subjects with clinical findings and radiological evidence (ultrasound and/or MRI) consistent with HIE or VEWOH were recruited for the study. MRIs of HIE subjects involved injury to the deep gray nuclei, cerebral cortex, and subcortical white matter. Those with VEWOH showed ventriculomegaly from cerebral malformation, "arrested hydrocephalus," or other unidentifiable causes but did not develop macrocephaly (occipitofrontal circumference >97th centile) or require neurosurgical treatment. A cohort of infants diagnosed with IVH who did not go on to develop PHH were also included in the study. Infants without any known neurological insult or injury who underwent LP for routine sepsis evaluation (where all cultures were deemed negative and sepsis was ruled out) were recruited and termed "controls" (Table 1).

Acquisition of Biospecimens

Lumbar CSF samples were collected from February 1, 2012 to March 1, 2015. During this same time interval, the rate of posthemorrhagic ventricular dilatation at our institution was 5 to 7 cases/year (total of 17 cases). Twelve of these patients (65%) were recruited and included in this study. Lumbar punctures were performed by the clinical team in the NICU under sterile conditions and for clinical purposes only. Immediately after LP, the samples were transported directly to the St. Louis Children's Hospital clinical laboratory where they were frozen at −20°C for a maximum of 2 days. They were then transported on ice to the Washington University Neonatal CSF Repository, where they were stored at −80°C. Before experimental analysis, samples were thawed and centrifuged at 2500 rpm for 6 minutes, and the supernatant was analyzed for protein levels.

In all cases, CSF microbiological cultures sent at the time of the LP remained sterile (cultures were monitored for 3.68 ± 0.13 days); per our NICU practice, anaerobic CSF cultures were not routinely performed. Data for CSF cell count and differential analysis were recorded from the electronic medical record. Using Bonadio et al's[20] proposed threshold red blood cell count of >1000/mm^{3}, "traumatic" LPs were noted in 15 of 56 (26.8%) of the CSF samples (IVH and PHH samples excluded), which is consistent with or lower than previously reported data for LPs in neonates.[21,22] For neonatal plasma samples, blood was collected from

TABLE 1. Summary Characteristics of Subjects in Each of the Six Groups[a,b]

	Sample Size (n)	PMA Birth (wk)	Birthweight (g)	PMA Sample (wk)	Age of Sample (wk)	FOR
Control	33	29.7 ± 3.8	1539 ± 809.6	33.7 ± 4.3	4.0 ± 3.5	0.41 ± 0.04
IVH I-II	13	30.9 ± 4.5	1714 ± 795.2	33.0 ± 3.6	2.1 ± 2.4	0.41 ± 0.03
IVH III-IV	12	28.8 ± 5.9	1448 ± 921.1	34.8 ± 6.7	6.1 ± 5.0	0.50 ± 0.04
PHH	12	27.0 ± 3.2	1031 ± 430.5	29.5 ± 2.8	2.6 ± 1.4	0.64 ± 0.03
VEWOH	10	35.3 ± 4.6	2397 ± 1274.7	39.7 ± 5.0	4.4 ± 4.1	0.49 ± 0.08
HIE	13	37.6 ± 2.4	3023 ± 587.7	39.3 ± 2.9	1.8 ± 2.2	0.36 ± 0.03

[a]PMA, postmenstrual age; FOR, frontal-occipital ratio, a measure of ventricular size; IVH, intraventricular hemorrhage; PHH, posthemorrhagic hydrocephalus; VEWOH, ventricular enlargement without hydrocephalus; HIE, hypoxic-ischemic encephalopathy.
[b]Values are shown as mean ± standard deviation.

newborn infants in evacuated tubes with anticoagulant. The plasma was separated within 2 hours, aliquoted, and stored at −80°C until experimental analysis.

Measurement of Protein Concentrations

Commercially available enzyme-linked immunosorbent assays (ELISAs) were used to measure the concentration of APP, soluble APP α and β (sAPPα and sAPPβ), Aβ_{42}, L1CAM, NCAM-1, tau, and p-tau. Sandwich Duoset ELISA development systems (R&D Systems, catalog #DY-850 and DY-2408 respectively; Minneapolis, Minnesota) were used to measure APP and NCAM-1 as previously described.[14] L1CAM levels were measured using a commercially available kit (DRG, catalog #EIA5074; Mountainside, New Jersey) and have also been described previously.[14] sAPPα and sAPPβ were measured using ELISAs (IBL-International, catalog #27734 and 27732, respectively; Toronto, Ontario, Canada). The levels of tau, p-tau, and Aβ_{42} were also determined by ELISAs (Fujirebio, Ghent, Belgium; catalog #80226, 80062, 80177). In each instance, ELISAs were run according to the manufacturer's protocol. In brief, plates were coated with a primary capture antibody and blocked before incubation with CSF or plasma samples. After washing, the secondary antibody was added, followed by streptavidin-horseradish peroxidase and tetramethylbenzidine. The reaction was then stopped with sulfuric acid. The plates were washed between each of the previous steps, with the exception of the final one. All ELISAs were run in duplicates and the 96-well plates were read at 450 nm on a Versamax microplate reader (Molecular Devices; Sunnyvale, California). Protein concentrations were then determined using a 4-parameter logistic standard curve as detailed by the manufacturers.

Measurement of Total CSF Protein

The Pierce Bicinchoninic Acid protein assay kit (Thermo Scientific; Waltham, Massachusetts) was used to estimate the total protein (TP) level in each sample. In brief, samples and bovine serum albumin standards were placed into microplate wells in duplicate. The working reagent was then added and the plate was incubated at 37°C for 30 minutes. The plate was then cooled to room temperature, and the absorbance was measured at 562 nm on a plate reader. TP levels were determined using a 4-parameter logistic standard curve.

Statistical Analysis

One-way analysis of variance was used to test for association of CSF proteins with PHH and alternate outcome categories (IVH, VEWOH, HIE, and control). A Bonferroni corrected threshold (0.05/9 = 0.0055) was calculated for multiple comparisons. Proteins APP, sAPPα, and L1CAM showed association and were used in simple logistic regressions of PHH on candidate biomarker concentration to examine their predictive ability. Sensitivity, specificity, and odds ratios were used to evaluate candidate cut point concentrations. Significance was set at a P value of < .05. SAS 9.4 was used for all analyses.

RESULTS

Clinical Characteristics and Subject Groupings

Six groups of research subjects were identified based on clinical history and events and radiologic findings (ultrasound and/or MRI): (1) control subjects (no known neurological insult or injury, n = 33); (2) IVH grades I to II (n = 13); (3) IVH grades III to IV (n = 12); (4) PHH (n = 12); (5) HIE (n = 13); and (6) VEWOH (n = 10). Please refer to Table 1 for summary characteristics of subject groupings. There was no difference among the first 4 groups for birth weight, PMA at the time of birth, or PMA at the time of acquisition of the CSF sample; however, compared with PHH subjects, subjects with HIE and VEWOH tended to have higher birth weights (P < .001 and P = .007, respectively) and be older at birth (P < .001 and P = .002, respectively).

Candidate CSF Biomarkers of PHH

Lumbar CSF levels of APP, sAPPα, sAPPβ, Aβ_{42}, NCAM-1, L1CAM, tau, p-tau, and TP were measured as detailed in Methods (Table 2). Among APP and its derivatives, CSF APP and sAPPα were increased in PHH compared with all other conditions (P < .001 and P < .001, respectively; Figure 1A, B). CSF sAPPβ was different between PHH and control (P = .044), and Aβ_{42} was not significantly different among any groups. CSF L1CAM was elevated in PHH relative to all other conditions (P < .001; Figure 1C). NCAM-1 was elevated in PHH relative to the other conditions with the exception of IVH III to IV (P =

TABLE 2. Lumbar CSF Levels of APP, sAPPα, sAPPβ, Aβ$_{1-42}$, NCAM-1, L1CAM, Tau, P-tau, and TP Measured Across the Six Primary Groups and Plasma[a,b]

	APP	sAPPα	sAPPβ	Aβ$_{1-42}$	NCAM-1	L1CAM	Tau	P-tau	TP (μg/mL)
Control	406.7 ± 290.8	136.2 ± 81.7	1139 ± 942.7	0.792 ± 0.3	140.5 ± 68.5	74.43 ± 71.7	6.09 ± 4.7	1.14 ± 0.6	1077 ± 435.1
IVH I-II	313.1 ± 237.3	163.1 ± 130.9	848.9 ± 460.5	0.861 ± 0.5	104.9 ± 51.9	63.36 ± 33.1	3.46 ± 3.1	0.99 ± 0.5	1365 ± 1098
IVH III-IV	523.1 ± 278.6	377.9 ± 376.5	1085 ± 698.6	1.274 ± 0.7	258.9 ± 129.3	76.00 ± 51.1	9.55 ± 12.9	1.47 ± 0.8	1963 ± 952.1
PHH	1416 ± 806.3	1667 ± 1227	16 678 ± 36 068	0.953 ± 0.7	356.4 ± 152.4	191.8 ± 93.5	20.28 ± 33.9	1.98 ± 1.2	3748 ± 2403
VEWOH	330.7 ± 267.9	97.4 ± 60.9	659.5 ± 760.1	0.821 ± 0.7	119.4 ± 77.1	28.68 ± 22.3	5.68 ± 6.4	0.71 ± 0.6	772.2 ± 371.3
HIE	371.3 ± 209.5	168.9 ± 171.6	1002 ± 1605	0.335 ± 0.2	184.9 ± 72.9	24.82 ± 22.0	9.20 ± 9.6	0.85 ± 0.4	1060 ± 448.7
Plasma	139.87 ± 21.4	54.02 ± 31.9	21.43 ± 3.2	0.555 ± 0.2	482.33 ± 63.1	15.75 ± 4.1	0.242 ± 0.1	0.109 ± 0.001	49139 ± 6187

[a] APP, amyloid precursor protein; HIE, hypoxic-ischemic encephalopathy; IVH, intraventricular hemorrhage; L1CAM, L1 cell adhesion molecule; NCAM-1, neural cell adhesion molecule-1; PHH, posthemorrhagic hydrocephalus; sAPPα, soluble amyloid precursor protein α; sAPPβ, soluble amyloid precursor protein β; TP, total protein; VEWOH, ventricular enlargement without hydrocephalus.
[b] Protein levels are shown as mean ± standard deviation and all are in ng/mL, except TP, which is in μg/mL.
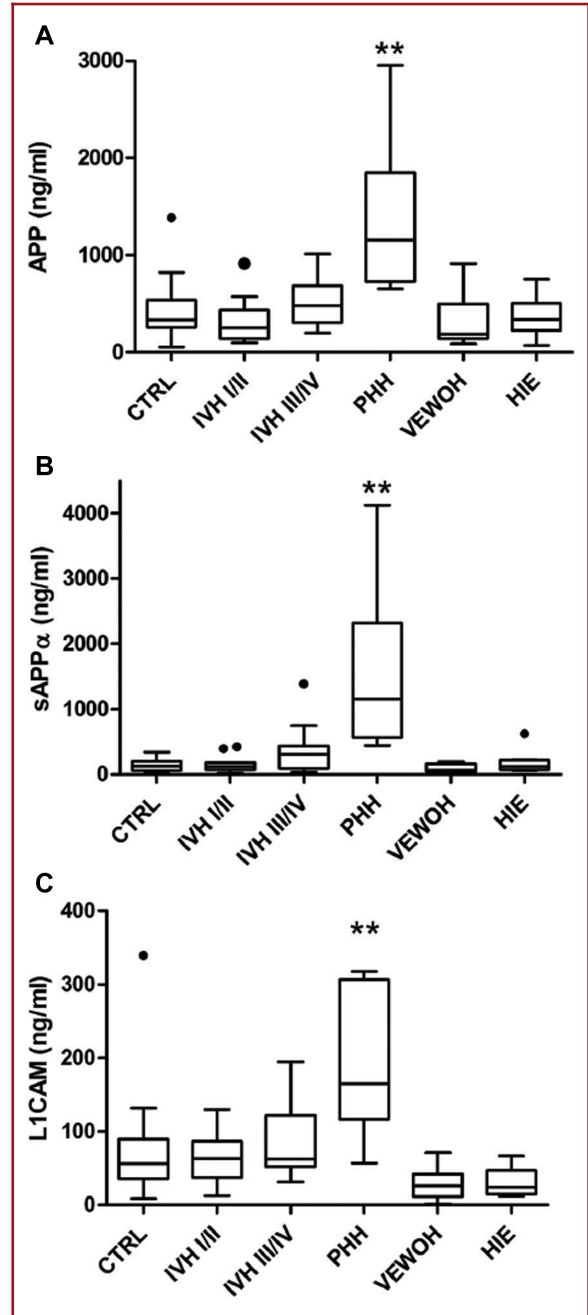
FIGURE 1. *Comparison of lumbar CSF APP **A**, sAPPα **B**, and L1CAM **C** levels across 6 neonatal comparison groups: (1) control subjects (CTRL, no known neurological insult or injury); (2) IVH grades I to II; (3) IVH grades III to IV; (4) PHH; (5) HIE; and (6) VEWOH. Boxes represent the median with 25th and 75th percentiles and the whiskers depict the interquartile range multiplied by 1.5. The PHH levels of all 3 proteins were significantly different than all other groups (P < .050; denoted by **). APP, amyloid precursor protein; HIE, hypoxic ischemic encephalopathy; IVH, intraventricular hemorrhage; L1CAM, L1 cell adhesion molecule; PHH, posthemorrhagic hydrocephalus; sAPPα, soluble amyloid precursor protein α; VEWOH, ventricular enlargement without hydrocephalus.*

.137). Total tau was not different among groups ($P = .095$), but p-tau was variable, greater in PHH than control ($P =.015$), IVH I to II ($P = .022$), HIE ($P < .007$), and VEWOH ($P < .006$). To account for multiple comparisons, the Bonferroni threshold was calculated as 0.05/9 or 0.0055. Three candidate CSF biomarkers, APP, sAPPα, and L1CAM, met the corrected threshold for significance ($P < .001$ for each) and were selective for PHH.

Total CSF protein was also elevated in PHH ($P < .001$; Table 2), consistent with findings from other groups studying various forms of hydrocephalus.[9] Although it is possible that blood or blood breakdown products contributed to this difference, this is unclear because TP was significantly lower in IVH III to IV compared with PHH ($P = .005$). To examine whether blood or blood breakdown products could be contributing to the increases in CSF APP, sAPPα, L1CAM, and NCAM-1, the levels of these proteins were measured in age-matched neonatal plasma samples. With the exception of NCAM-1, which trended toward higher levels in the plasma samples compared with PHH CSF ($P = .139$), APP, sAPPα, and L1CAM were significantly lower in plasma than in PHH CSF ($P = .009$, $P = .003$, and $P = .003$, respectively). These relationships extended to plasma vs control CSF; NCAM-1 was higher in plasma ($P < .001$), while APP and L1CAM trended toward lower levels in plasma ($P = .085$ and $P = .122$, respectively); sAPPα was significantly lower in plasma ($P = .015$). Thus, it is unlikely that blood or blood breakdown products are responsible for the observed PHH-associated increases in CSF APP, sAPPα, and L1CAM.

Logistic regression was used to evaluate the relationship between each of the candidate CSF biomarkers (APP, sAPPα, sAPPβ, Aβ$_{42}$, NCAM-1, L1CAM, tau, and p-tau) and CSF cell count parameters (total cells, nucleated cells, red blood cells, neutrophils, lymphocytes, monocytes, eosinophils, and macrophages). None of the candidate biomarkers demonstrated a significant relationship with any of the cell count parameters, with the exception of CSF L1CAM with CSF neutrophils ($P = .022$) and CSF monocytes ($P = .022$).

CSF APP, sAPPα, and L1CAM as Diagnostic Biomarkers of PHH

The predictive ability of lumbar CSF APP, sAPPα, and L1CAM levels for PHH were examined by first generating a logistic plot for each candidate biomarker using the measurements for CSF samples from infants with PHH and those without PHH (control, IVH grades I-II, IVH grades III-IV, HIE, VEWOH) (Figures 2A-2C). Simple logistic regression was performed on each biomarker to determine the strength of prediction for PHH and cut points were chosen based on combinations of sensitivity and specificity (Tables 3-4). CSF APP (cut point = 699.67 ng/mL, sensitivity = 0.91, specificity = 0.89), sAPPα (cutpoint = 514.43 ng/mL, sensitivity = 0.91, specificity = 0.95), and L1CAM (cut point = 113.33 ng/mL, sensitivity = 0.91,
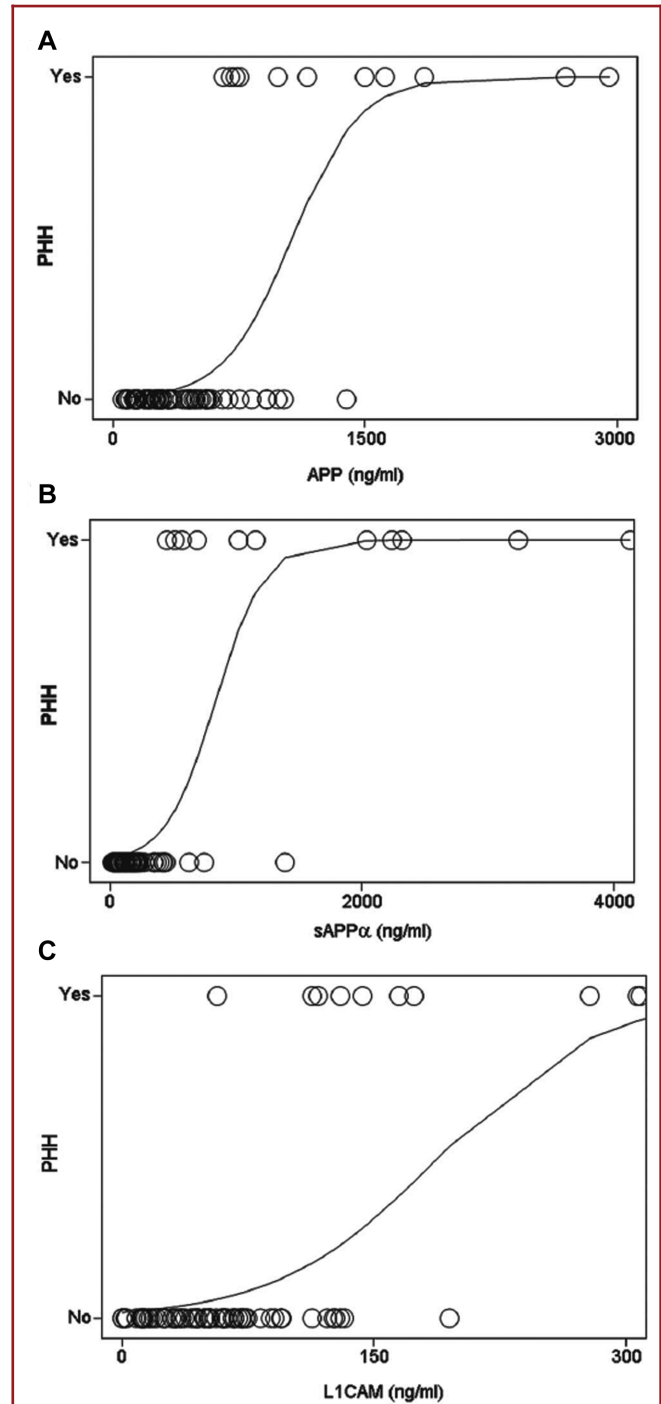
FIGURE 2. *Logistic curve for APP* **A**, *sAPPα* **B**, *and L1CAM* **C** *showing the relationship between protein concentration and the presence or absence of PHH. For the purposes of this analysis, PHH samples were assigned a value of 1.0 and samples from all other conditions were assigned 0.0. APP, amyloid precursor protein; L1CAM, L1 cell adhesion molecule; PHH, posthemorrhagic hydrocephalus; sAPPα, soluble amyloid precursor protein α.*

TABLE 3. Sensitivity and Specificity of Candidate Biomarkers for PHH for Throughout a Range of Concentrations[a]

APP			sAPPα			L1CAM		
Conc.	Sens.	Spec.	Conc.	Sens.	Spec.	Conc.	Sens.	Spec.
653.62	1.0	0.87	441.94	1.0	0.94	56.76	1.0	0.57
699.67	0.91	0.89	514.43	0.91	0.95	113.33	0.91	0.89
823.55	0.64	0.90	688.65	0.73	0.97	125.62	0.73	0.91

[a]APP, amyloid precursor protein; Conc., candidate biomarker concentration (in ng/mL); L1CAM, L1 cell adhesion molecule; sAPPα, soluble amyloid precursor protein α; sens., sensitivity; spec., specificity.

specificity = 0.89), yielded odds ratios for PHH of 80.00, 200.00, and 68.75, respectively.

DISCUSSION

In recent years, biomarkers have been sought to facilitate the diagnosis of PHH and improve clinical management of this devastating condition (reviewed in Mehrar[8]). Previous work from our group has shown that ventricular CSF levels of APP, L1CAM, and other protein mediators of neurodevelopment are increased in PHH and resolve after initiation of neurosurgical treatment.[7] CSF APP, in particular, also appears to be associated with ventricular size, and possibly intracranial pressure, in infants undergoing treatment for PHH.[14] Along with much of the work in this field, these studies share the limitation of using lumbar CSF as the comparator for ventricular CSF obtained from children with PHH. Such a comparison introduces the potential for sampling artifact due to RCGs, which have been observed for specific proteins in a number of diseases.[15,16,23,24] The objective of the current study was to eliminate the potential bias of RCGs and examine the levels of APP, L1CAM, and related proteins using lumbar CSF from infants with PHH and other neurological conditions relevant to the neonate.

Studies into RCGs suggest that the levels of brain-derived proteins (eg, neuron-specific enolase and S100B) remain consistent regardless of access site, whereas blood-derived proteins (eg, albumin and β-trace) tend to be higher in lumbar CSF.[25] These findings are supported by recent proteomic analysis.[26] However, at least 1 study found differential abundance of amyloid isoforms (lumbar levels higher) and tau isoforms (ventricular levels higher) in normal-pressure hydrocephalus, with albumin levels highest in lumbar samples.[16] The etiology of RCGs remains uncertain, although investigators have speculated that the gradients reflect diffusion or transport of serum proteins into the CSF, or CSF proteins into the blood, as a function of distance from the brain (the primary site of brain-derived proteins).[16,23]

The findings presented in this study provide convincing evidence that lumbar CSF levels of APP, sAPPα, and L1CAM are elevated in untreated PHH compared with neonates without neurological injury and those with IVH alone (grades I-II and grades III-IV), HIE, and VEWOH. Related APP derivatives sAPPβ and Aβ$_{42}$, as well as tau and p-tau, did not demonstrate the same degree of selectivity for PHH. NCAM-1 was increased in both PHH and grade III to IV IVH, possibly because of its high concentration in plasma. Thus, in this study of relatively small sample size, CSF APP, sAPPα, and L1CAM appeared the most appropriate CSF biomarkers of PHH. The strong predictive ability of APP, L1CAM, and, in particular, sAPPα, for PHH is an important and entirely novel finding.

The high levels of TP observed in PHH in our lumbar CSF samples will not surprise clinicians who treat PHH regularly. In many centers, serial LPs are used as a temporizing measure for PHH until the condition resolves or the infant undergoes a more definitive neurosurgical procedure, and large increases in TP are expected in this setting. To our knowledge, a comparison of TP in lumbar CSF of infants with IVH alone, PHH (or other neurological conditions), or adults with hydrocephalus, has not been reported previously.

TABLE 4. Logistic Regression Parameters Using Highlighted Concentrations From Table 3 as Cut Points[a,b]

	APP	sAPPα	L1CAM
P	0.0006	0.0019	0.0006
ROC AUC	0.954	0.981	0.921
Cut point (ng/mL)	699.67	514.43	113.33
Odds ratio (95% CI)	80.00 (8.9-722.5)	200.00 (18.9-2118.4)	68.75 (7.7-611.4)
Relative risk (95% CI)	33.5 (4.6-243.6)	46.92 (6.6-335.3)	31.1 (4.3-226.6)

[a]APP, amyloid precursor protein; CI, confidence interval; ROC AUC, receiver operating characteristics area under the curve; CI, confidence interval; L1CAM, L1 cell adhesion molecule; sAPPα soluble amyloid precursor protein α.
[b]Bonferroni corrected threshold (0.0055).

In addition to any potential role in PHH diagnosis or clinical management, CSF biomarkers of PHH give important insight into the pathophysiology of this condition. PHH afflicts preterm infants during the critical developmental period corresponding to 23 weeks PMA to term-equivalent age and beyond. Seminal neurodevelopmental events, including precursor migration and differentiation, synaptogenesis, and the generation of neural networks occur during this interval and are almost certainly affected by PHH. As noted previously, many of the CSF biomarkers involved in PHH are protein mediators of neurodevelopment.[7] For example, L1CAM is involved in neuronal migration, axonal growth, and synaptogenesis.[27-32] Indeed, mutations in L1CAM have been linked to the debilitating conditions of mental retardation, aphasia, spastic paraplegia, adducted thumbs (MASA syndrome), X-linked hydrocephalus, and corpus callosum hypoplasia, retardation, adducted thumbs, spastic paraplegia, and hydrocephalus (CRASH syndrome).[33-37]

APP has long been known to be increased in the setting of axonal injury or stretch,[38] but accumulating evidence also strongly supports that APP is an important trophic factor, with roles in neural stem cell development, neurite outgrowth and synaptogenesis, and neural repair.[39] Posttranslational APP processing is complex and may occur at a number of stages, each of which affects the ultimate fate and biological activity of APP and its derivatives. Perhaps the most critical processing point is the cleavage of cell surface APP by α- or β-secretase. If cleaved by α-secretase (a disintegrin and metalloproteinase), sAPPα is generated; if cleaved by β-secretase (β-site APP-cleaving enzyme 1), sAPPβ results.[40] Although β-site cleavage and sAPPβ are associated with downstream production of amyloid-β_{1-42}, the primary component of neuritic plaques in Alzheimer disease, sAPPα appears to function as a trophic factor for neural precursor cell development, neurite growth and synapse formation, and even neuronal growth, survival, and repair (reviewed in Dawkins and Small[39]). The results from this study clearly demonstrated specific increases in CSF sAPPα in PHH, and, although it is possible that sAPPα may simply result from the neurological injury itself, it is intriguing to consider that sAPPα may be serving a neuronal survival or repair function.

It is worth noting that in adults, sAPPα and sAPPβ, but not tau and p-tau, are also diagnostic biomarkers for distinguishing between idiopathic normal-pressure hydrocephalus and Alzheimer disease.[41] Specifically, the cutoff value for predicting cognitive outcomes in Miyajima et al's[41] study was 198 ng/mL, which is considerably lower than our value of 514 ng/mL for sAPPα. In a related study, Pyykko et al[42] concluded that increases in APP isoforms were independent of neuroinflammation mechanisms in patients with idiopathic normal-pressure hydrocephalus. This finding lends further support to our contention that the trophic effects of APP are important for functional outcome.

The role of neuroinflammation in PHH is an active area of study for our laboratory and others.[43-48] Intraventricular hemorrhage with or without PHH may incite inflammatory processes within the CSF and within the central nervous system more broadly, potentially creating a chemical meningitis. To explore the influence of neuroinflammation in PHH, we analyzed the relationship between our candidate CSF biomarker levels and the CSF cell count and differential analysis results (including total cells, nucleated cells, red blood cells, neutrophils, lymphocytes, monocytes, eosinophils, and macrophages) in the CSF samples tested. In this relatively small series, no significant relationships existed between CSF APP, sAPPα, sAPPβ, Aβ_{42}, NCAM-1, L1CAM, tau, p-tau, and differential cell counts in CSF, with the exception of CSF L1CAM and neutrophils ($P = .022$) and monocytes ($P = .025$). This is not entirely surprising, because the biomarkers investigated in this study are primarily associated with neurodevelopment and neurological injury, rather than inflammation. One relevant exception to this generalization is L1CAM, which has recently been shown to be induced by transforming growth factor-$\beta 1$[49] and have a signaling role in inflammation via nuclear factor-κB.[50]

Limitations

A number of limitations in this study must be acknowledged. In this study of human newborn infant lumbar CSF samples, there were no true "control" infants; even those subjects with no known neurological injury were born prematurely and subject to the influence of myriad clinical factors that could potentially confound the results (eg, clinical status concerning for sepsis, long periods of ventilation). Additionally, in utero or developmental events and pre- and perinatal complications (preterm labor, rupture of membranes, breech presentation, placental abruption) could potentially influence CSF protein levels in these very preterm infants. In the analysis of our specimens, spectrum bias may have attenuated the comparison between conditions, particularly between IVH III to IV and PHH. Similarly, the degree of ventricular enlargement in our VEWOH samples was modest, again introducing the possibility of spectrum bias and limiting the ability to rigorously test the effect of ventricular enlargement alone (no hydrocephalus) on CSF biomarker levels. Of note, VEWOH and HIE infants had greater PMA at birth and higher birth weights than the other groups, limiting stringent comparisons between groups. Finally, although it would be ideal to examine biomarker levels in both ventricular and lumbar CSF simultaneously, clinical and ethical challenges prevent this from being performed routinely in these preterm infants.

Future studies will focus on validating the results of this study in a larger collection of CSF samples and examining the levels of these candidate biomarkers of PHH (APP, sAPPα, and L1CAM) in parallel blood samples. We will also explore the relationship of these biomarkers to neurosurgical (shunt malfunction or infection, need for surgical revision, association with multiloculated hydrocephalus) and neurodevelopmental outcomes. Similarly, we will also investigate the levels of these and related biomarkers in hydrocephalus of other etiologies.

CONCLUSION

In the current study, we report the novel observation that the lumbar CSF levels of APP, sAPPα, and L1CAM are specifically and significantly elevated in untreated PHH. In addition to their potential role as diagnostic biomarkers, these proteins provide novel insights into the pathophysiology of PHH and possible mechanisms of neural injury and neural repair.

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