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Correction of Cerebrospinal Fluid Protein in Infants with Traumatic Lumbar Punctures

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Abstract

In our multi-center cohort of infants < 60 days of age, we identified 2,646 infants with a traumatic lumbar puncture of which 31 (1.2%) had bacterial meningitis. For every 1,000 cerebrospinal fluid (CSF) red blood cells per mm³, cerebrospinal (CSF) protein increased 1.1 mg/dL [95% confidence interval (CI) 1.0–1.2 mg/dL].

Keywords

bacterial meningitis; cerebrospinal fluid; protein; infants

BACKGROUND

Febrile infants < 60 days old who present to the emergency department (ED) are frequently evaluated for bacterial meningitis with a lumbar puncture (LP).¹ Because cerebrospinal fluid (CSF) culture results require at least 24 hours to reliably exclude bacterial growth, clinicians combine immediately available clinical and laboratory factors, including CSF protein, to assess risk of bacterial meningitis.² An elevated CSF protein has been associated with a higher risk of bacterial meningitis in children and may lead to empiric bacterial meningitis coverage.³ However, up to one-third of LPs in infants will be traumatic,^{4,5} increasing both CSF red blood cell (RBC) count and CSF protein, and limiting clinicians' ability to utilize CSF protein values in assessing an infant's risk of bacterial meningitis. Prior single-center studies have shown that the presence of RBCs in the CSF raises the CSF protein between 1.1–1.9 mg/dL for every additional 1,000 CSF RBCs.^{6–10} The ability of CSF protein corrected for CSF RBCs to discriminate between infants with and without bacterial meningitis has not been examined.

We assembled a large, multi-center cohort of infants < 60 days of age with traumatic LPs. We examined the relationship between CSF protein and CSF RBCs and assessed the ability of corrected and uncorrected CSF protein to identify infants with bacterial meningitis.

MATERIALS AND METHODS

We performed a planned secondary analysis of a retrospective cohort study of infants who presented to the ED of one of the 23 participating centers in the Pediatric Emergency Medicine Clinical Research Committee (PEM CRC) herpes simplex virus (HSV) study (details of study protocol previously described).⁵ We limited our analysis to 20 sites which contributed CSF cell counts and protein. The study was approved by each of the participating institutional review boards.

We included infants < 60 days old presenting to a participating ED between January 1, 2005 and December 31, 2013 who had a CSF culture obtained within 24 hours of ED presentation and who had available CSF cell counts and chemistries. We limited our analysis to those with a traumatic LP defined as a CSF RBC > 10,000 cells/mm³, a level of peripheral blood

contamination of the CSF which makes the interpretation of CSF protein challenging.⁴ Infants with CSF RBCs $> 1,000,000$ cells/mm³ were excluded, as we could not confirm the presence of CSF in these samples. Infants could be included more than once if they had more than one eligible ED encounter.

Site investigators abstracted the following data elements from the electronic medical record: patient demographics, laboratory results (complete blood count, CSF cell counts and protein) and microbiologic results [blood, urine and CSF cultures, and viral testing (HSV and enteroviral PCR testing) when performed].

Our primary outcome was bacterial meningitis; defined as the growth of a bacterial pathogen in CSF culture.⁵ We classified the following pathogens as contaminants: coagulase-negative staphylococci, *Viridans streptococci*, *Propionibacterium acnes* and *Corynebacterium* species.

First, we explored the relationship between CSF protein and CSF RBCs using regression with CSF protein as the dependent variable and CSF RBCs as the independent variable. We decided *a priori* to assess the relationship as linear, based on prior evaluations,^{6,7} and to enhance clinical applicability, as non-linear relationships are difficult to apply.

Second, we calculated corrected CSF protein as: Corrected CSF protein = CSF protein – (CSF RBC x Correction Factor), where the correction factor was determined using the beta-coefficient from our regression. A normal CSF protein value was defined as < 115 mg/dL for infants 0–28 days of age and CSF protein < 89 mg/dL for infants 29–60 days of age.²

We calculated the following test characteristics for the corrected and uncorrected CSF protein as a predictor of bacterial meningitis: sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), positive likelihood ratio (+LR), and negative likelihood ratio (-LR). Finally, we calculated the area under the curve (AUC) from the receiver operating characteristic (ROC) curves for both corrected and uncorrected CSF protein.

The analyses and figure for this paper were generated using SAS software version 9.4 (SAS Institute Inc. Cary, NC, USA 2015).

RESULTS

We identified 23,618 LPs, of which 19,819 (83.9%) had both CSF RBC counts and CSF protein available for analyses. Of these, 2,659 (13.4%) were traumatic. We excluded 13 (0.5%) LPs with a CSF RBCs $> 1,000,000$ cells/mm³, yielding a final sample of 2,646 LPs (13.4% of eligible LPs). Median patient age was 25 days [Interquartile Range (IQR) 13–39 days] and 1442 (54.5%) were male. Thirty-one infants (1.2%, 95%CI 0.8–1.6%) had bacterial meningitis.

CSF protein increased 1.1 mg/dL (95% CI 1.0–1.2 mg/dL) for every 1,000 increase in the CSF RBC count (Figure 1A). Using the 1.1 mg/dL correction factor, we found no difference between corrected and uncorrected CSF protein with regard to sensitivity, NPV, PPV, negative likelihood ratio or positive likelihood ratio for detection of bacterial meningitis

(Figure 1B). However, the specificity of corrected CSF protein was higher than the uncorrected CSF protein. While corrected CSF protein had slightly higher AUC for its ROC curve for detection of bacterial meningitis compared to uncorrected CSF protein, discriminatory ability was low for both.

Of the 31 infants with bacterial meningitis, 4 infants (12.9%, 95% CI 5.3–29.0%) had normal corrected CSF protein but an elevated uncorrected CSF protein. All four of these misclassified infants were ≤ 28 days of age and were hospitalized on empiric antibiotics awaiting bacterial culture results. CSF cultures of these infants grew the following pathogenic organisms: *Staphylococcus aureus* (2), *Moraxella* species (1) and *Escherichia coli* (1).

1,006 (38.0%) infants had HSV PCR testing of the CSF performed and 4/1006 (0.4%) were positive. Among these 4 infants, uncorrected CSF protein was elevated in two infants and corrected CSF protein was elevated in only one infant. 473 (17.9%) infants had enteroviral PCR testing performed and 66/473 (14.0%) were positive. Among these 66 infants, uncorrected CSF protein was elevated in 48 infants and corrected CSF protein was elevated in 32 infants.

DISCUSSION

In our large, multi-center cohort of infants with traumatic LPs, CSF protein increased 1.1 mg/dL for every increase of 1000 CSF RBCs per mm³. Corrected CSF protein had a similar sensitivity but higher specificity when compared to uncorrected CSF protein. However, both corrected and uncorrected CSF protein alone had low discriminatory ability to identify infants with bacterial meningitis.

Previous studies have evaluated the relationship between CSF protein and CSF RBCs in CSF.^{6–10} Our results are similar to a previous examination of 1,354 children of all ages, which found a CSF protein to CSF RBC ratio of 1.1 mg/dL per 1,000 CSF RBCs (95% CI 0.9–1.1 mg/dL).⁶ Our results differed from another investigation of 1,241 infants ≤ 56 days of age which found a ratio of 1.9 mg/dL per 1,000 CSF RBCs (95% CI 1.7–2.1 mg/dL).⁷ Both studies examined this relationship in all CSF samples obtained, regardless of CSF RBC counts. In fact, the majority of children in both studies had non-traumatic LPs, where the CSF protein may not require correction.

Our findings add to the current understanding of the optimal interpretation of CSF protein in a young infant with traumatic LPs. First, our large, multi-center population increases the generalizability of our findings. Second, our analysis was limited to infants with traumatic LPs, thus our results are applicable to a very large population for which CSF protein correction may be clinically indicated. Our study compared the predictive ability of corrected vs. uncorrected CSF protein to determine the risk of bacterial meningitis. We found that neither corrected nor uncorrected CSF protein has adequate discriminative ability to be used alone for bacterial meningitis risk stratification. Therefore, clinicians should interpret CSF protein in combination with other clinical and laboratory values when assessing an individual infant's risk of bacterial meningitis. If clinicians incorporate CSF

protein into this evaluation, corrected CSF protein offers higher specificity and slightly higher overall discriminatory ability compared to uncorrected CSF protein. However, this must be weighed against the risk of misclassifying a few additional infants with bacterial meningitis as having a normal corrected CSF protein.

Our study has limitations. First, despite a large sample of infants, bacterial meningitis was rare, and we may be underpowered to detect small difference in sensitivity of CSF protein. Second, we did not have data on clinical appearance, which may limit generalizability of our results. Third, not all infants underwent testing for HSV and enteroviral CSF infections. Therefore, we were unable to test characteristics of either uncorrected or corrected CSF protein for either enteroviral or HSV infection. Finally, as some study infants may have received antibiotic pretreatment before diagnostic lumbar puncture rendering the CSF culture falsely negative, we may have misclassified infants as not having bacterial meningitis. However, antibiotic pretreatment in young infants is exceedingly rare (0.1%).¹¹ Therefore, we would caution against application of our results to infants with antibiotic pretreatment prior to LP.

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Abbreviations

AUC	Area under curve
CSF	cerebrospinal fluid
CI	confidence interval
ED	emergency department
HSV	herpes simplex virus
LP	lumbar puncture
-LR	negative likelihood ratio
NPV	negative predictive value
PEMCRC	Pediatric Emergency Medicine Collaborative Research Committee
+LR	positive likelihood ratio

PPV	positive predictive value
ROC	receiver operating characteristic
RBC	red blood cell

References

1. Baskin MN, O'Rourke EJ, Fleisher GR. Outpatient treatment of febrile infants 28 to 89 days of age with intramuscular administration of ceftriaxone. *J Pediatr*. 1992; 120(1):22–27. DOI: 10.1016/S0196-0644(05)82808-2 [PubMed: 1731019]
2. Shah SS, Ebberson J, Kestenbaum La, Hodinka RL, Zorc JJ. Age-specific reference values for cerebrospinal fluid protein concentration in neonates and young infants. *J Hosp Med*. 2011; 6(1): 22–27. DOI: 10.1002/jhm.711. [PubMed: 20629018]
3. Nigrovic LE, Kuppermann N, Macias CG, et al. Clinical prediction rule for identifying children with cerebrospinal fluid pleocytosis at very low risk of bacterial meningitis. *JAMA*. 2007; 297(1):52–60. DOI: 10.1001/jama.297.1.52 [PubMed: 17200475]
4. Nigrovic LE, Kuppermann N, Neuman MI. Risk Factors for Traumatic or Unsuccessful Lumbar Punctures in Children. *Ann Emerg Med*. 2007; 49(6):762–771. DOI: 10.1016/j.annemergmed.2006.10.018 [PubMed: 17321005]
5. Lyons TW, Cruz AT, Freedman SB, et al. Interpretation of Cerebrospinal Fluid WBC Counts in Young Infants With a Traumatic Lumbar Puncture. *Ann Emerg Med*.
6. Nigrovic LE, Shah SS, Neuman MI. Correction of cerebrospinal fluid protein for the presence of red blood cells in children with a traumatic lumbar puncture. *J Pediatr*. 2011; 159(1):158–159. DOI: 10.1016/j.jpeds.2011.02.038 [PubMed: 21492866]
7. Hines EM, Nigrovic LE, Neuman MI, Shah SS. Adjustment of cerebrospinal fluid protein for red blood cells in neonates and young infants. *J Hosp Med*. 2012; 7(4):325–328. DOI: 10.1002/jhm.1920 [PubMed: 22371377]
8. Mehl AL. Interpretation of traumatic lumbar puncture. A prospective experimental model. *Clin Pediatr (Phila)*. 1986; 25(10):523–526. <http://www.ncbi.nlm.nih.gov/pubmed/3757399>. [PubMed: 3757399]
9. Srinivasan L, Shah SS, Abbasi S, Padula MA, Harris MC. Traumatic lumbar punctures in infants hospitalized in the neonatal intensive care unit. *Pediatr Infect Dis J*. 2013; 32(10):1150–1152. DOI: 10.1097/INF.0b013e31829862b7 [PubMed: 23624433]
10. Nizet, V., Klein, JO. Bacterial Sepsis and Meningitis. In: Remington, JS, Klein, JO, Wilson, CB, Nizet, V., Maldonado, Y., editors. *Infectious Diseases of the Fetus and Newborn*. Vol. 7. Philadelphia, PA: Elsevier Saunders; 2011. p. 223–264.
11. Mahajan P, Kuppermann N, Mejias A, et al. Association of RNA Biosignatures With Bacterial Infections in Febrile Infants Aged 60 Days or Younger. *JAMA*. 316(8):846–857. DOI: 10.1001/jama.2016.9207.

Figure 1.

A. Plot of cerebrospinal fluid protein versus cerebrospinal fluid red blood cells in infants 60 days with a traumatic lumbar puncture, **B.** Test characteristics of uncorrected and corrected cerebrospinal fluid protein for the detection of bacterial meningitis in infants 60 days with a traumatic lumbar puncture

Abbreviations: mg=milligram, dL=deciliter, mm³ = cubic millimeter, PPV=positive predictive value, NPV=negative predictive value, +LR=positive likelihood ratio, -LR=negative likelihood ratio, AUC=area under curve for receiver operating characteristics curve

¹Slope of regression line = 0.0011 (mg/dL)/(cells/mm³)

²Significantly different compared to uncorrected CSF protein at $p < 0.001$

³Significantly different compared to uncorrected CSF protein at $p = 0.00$