ORIGINAL RESEARCH

Adjustment of Cerebrospinal Fluid Protein for Red Blood Cells in Neonates and Young Infants

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OBJECTIVE: To determine the relationship between cerebrospinal fluid (CSF) red blood cell (RBC) count and CSF protein in neonates and young infants undergoing lumbar puncture.

DESIGN: Cross-sectional study.

SETTING: Urban tertiary care children's hospital.

PATIENTS: Infants 56 days of age and younger who had a lumbar puncture in the emergency department between January 1, 2005 and July 31, 2009 were eligible for inclusion. Infants with missing laboratory data, exceedingly high CSF red blood cell counts, or conditions known to elevate CSF protein were excluded.

MEASUREMENTS: Linear regression was used to determine the association between CSF RBC and CSF protein.

RESULTS: Of 1986 infants, 56 days of age or younger, who underwent lumbar puncture in the emergency department during the study period, 1241 (62.5%) met inclusion criteria. The median age was 34 days (interquartile range: 19-46 days); 45% of patients were male. The median CSF RBC count was 40 cells/mm^3 (interquartile range: 2-1080 cells/mm^3); 11.8% of patients had a CSF RBC $>$10,000 cells/mm^3. CSF protein increased by 1.9 mg/dL (95% confidence interval: 1.7-2.1 mg/dL) per 1000 CSF RBCs for all included patients. Restricting analysis to patients without pleocytosis yielded comparable results, as did subanalyses by age and delivery type.

CONCLUSIONS: We found that CSF protein concentrations increased by approximately 2 mg/dL for every 1000 CSF RBCs. These data may assist clinicians in interpreting CSF protein concentrations in infants 56 days of age and younger in the context of traumatic lumbar punctures. *Journal of Hospital Medicine* 2012;7:325–328. © 2012 Society of Hospital Medicine

Traumatic lumbar puncture (LP) occurs when peripheral blood is introduced into the cerebrospinal fluid (CSF) as a result of needle trauma, which causes bleeding into the subarachnoid space. Traumatic LPs occur in up to 30% of LPs performed in children.[1,2] In addition to affecting the CSF white blood cell count, the presence of CSF red blood cells (RBCs) is associated with higher CSF protein concentrations due to the higher protein concentration in plasma compared with CSF and to the release of protein from lysed red blood cells. CSF protein concentration has been used in clinical decision rules for the prediction of bacterial meningitis in children.[3] Elevated protein levels are difficult to interpret in cases of traumatic LP, and a diagnosis of bacterial meningitis may be more difficult to exclude on the basis of CSF test results.[4]

The interpretation of CSF protein levels is further complicated in the youngest infants due to both the changing composition of the CSF as well as the higher rates of traumatic LPs.[5] Therefore, studies establishing a correction factor, adjusting observed CSF protein levels for the presence of CSF RBCs, that included predominantly older children may not be generalizable to neonates and young infants.[6] We sought to determine the relationship between CSF RBC count and CSF protein in infants $\leq$56 days of age who underwent LP in the emergency department (ED).

METHODS

Study Design, Setting, and Participants

This cross-sectional study was performed at The Children's Hospital of Philadelphia (Philadelphia, PA), an urban, tertiary care children's hospital. The Committees for the Protection of Human Subjects approved this study with a waiver of informed consent.

Infants 56 days of age and younger were eligible for inclusion if they had an LP performed as part of their ED evaluation between January 1, 2005 and July 31, 2009. At The Children's Hospital of Philadelphia, infants 56 days and younger routinely receive LPs for evaluation of fever.[7–9] Patients undergoing LP in the ED were identified using computerized order entry records as previously described.[5,10]

We excluded patients with conditions known to elevate CSF protein, including: serious bacterial infection (bacterial meningitis, urinary tract infection, bacteremia, pneumonia, septic arthritis, and bacterial gastroenteritis),[11] presence of a ventricular shunt, aseptic meningitis (positive CSF enteroviral polymerase chain reaction or CSF herpes simplex virus polymerase chain reaction), congenital infections (eg, syphilis), seizure prior to presentation, and elevated bilirubin (if serum bilirubin was obtained). Due to the fact that grossly bloody CSF samples are difficult to interpret, we excluded those with a CSF RBC count >150,000 cells/mm³, a cutoff representing the 99th percentile of CSF RBC values in the cohort after applying other exclusion criteria.

Study Definitions

Bacterial meningitis was defined as either the isolation of a known bacterial pathogen from the CSF or, in patients who received antibiotics prior to evaluation, the combination of CSF pleocytosis and bacteria reported on CSF Gram stain. Bacteremia was defined as the isolation of a known bacterial pathogen from blood cultures excluding commensal skin flora. Urinary tract infection was defined as growth of a single known pathogen meeting 1 of 3 criteria: (1) ≥1000 colony-forming units per mL for urine cultures obtained by suprapubic aspiration, (2) ≥50,000 colony-forming units per mL from a catheterized specimen, or (3) ≥10,000 colony-forming units per mL from a catheterized specimen in association with a positive urinalysis.[12–14]

Statistical Analysis

Data analysis was performed using STATA version 12 (Stata Corp, College Station, TX). Linear regression was used to determine the association between CSF RBC and CSF protein. We analyzed the following groups of children: 1) all eligible patients; 2) children ≤28 days versus children >28 days; 3) vaginal versus cesarean delivery; and 4) patients without CSF pleocytosis. In the primary subanalysis, CSF pleocytosis was defined as CSF white blood cells (WBCs) >19 cells/mm³ for infants ≤28 days of age and CSF WBCs >9 cells/mm³ for infants ≥29 days of age, using reference values established by Kestenbaum et al.[10] Alternate definitions of CSF pleocytosis were also examined using reference values proposed by Byington et al[15] (age ≤28 days, >18 cells/mm³; age >29 days, >8.5 cells/mm³) and Chadwick et al[16] (age 0-7 days, >26 cells/mm³; age 8-28 days, >9 cells/mm³; age 29-49 days, >8 cells/mm³; and age 50-56 days, >7 cells/mm³). We did not correct CSF WBCs for the RBC count because prior studies suggest that such correction factors do not provide any advantage over uncorrected values.[17] Finally, linear regression analysis was repeated while including subjects with >150,000 RBC/mm³ to determine the effect of including those patients on the association of CSF RBC count and protein concentrations. Subjects with grossly bloody CSF specimens, defined a priori as a CSF RBC >1,000,000/mm³, were excluded from this subanalysis.

RESULTS

There were 1986 infants, 56 days of age or younger, who underwent LP in the ED during the study period. Patients were excluded for the following reasons: missing medical record number (n = 16); missing CSF WBC, CSF RBC, or CSF protein values (n = 290); conditions known to elevate CSF protein concentrations (n = 426, as follows: presence of a ventricular shunt device [n = 48], serious bacterial infection [n = 149], congenital infection [n = 2], positive CSF polymerase chain reaction [PCR] test for either enterovirus or herpes simplex virus [n = 97], seizure prior to presentation [n = 98], or elevated serum bilirubin [n = 32]). An additional 13 patients with a CSF RBC count >150,000 cells/mm³ were also excluded.

For the remaining 1241 study infants, the median age was 34 days (interquartile range: 19 days-46 days) and 554 patients (45%) were male. The median CSF RBC count was 40 cells/mm³ (interquartile range: 2-1080 cells/mm³); 11.8% of patients had a CSF RBC count >10,000 cells/mm³.

CSF protein increased linearly with increasing CSF RBCs (Figure 1). The increase in the CSF protein concentration of 1.9 mg/dL per 1000 CSF RBCs for all patients was similar between different age groups and delivery types (Table 1). Restricting analysis to those patients without pleocytosis also yielded comparable results; applying 2 other definitions of pleocytosis did not change the magnitude of the association (Table 1).

In a subanalysis, we then included subjects with a CSF RBC count >150,000/mm³; one extreme outlier with a CSF RBC equal to 3,160,000/mm³ remained excluded. Inclusion of more traumatic samples lessened the overall correction factor. The CSF protein increased by 1.22 mg/dL (95% confidence interval: 1.14-1.29 mg/dL) per 1000 RBC/mm³ increase in the CSF. In the subset without CSF pleocytosis, the CSF protein increased by 1.44 mg/dL (95% confidence interval: 1.33-1.57 mg/dL) per 1000 RBC/mm³.

Three children had high CSF protein values (>500 mg/dL) despite the relative paucity of CSF RBCs. Two of these infants had respiratory syncytial virus bronchiolitis; neither infant had signs or symptoms of neurological illness. While details of the labor and delivery were not available, the CSF sample for one of these infants was reported to have xanthochromia, and the other infant was reported to have had a traumatic LP with a CSF sample that subsequently cleared. The third infant had fever without a specific source identified, but had a birth history of vaginal delivery and prolonged labor. The CSF sample from LP for this patient was reported as grossly bloody by the performing clinicians and by the Clinical Microbiology Laboratory, despite a CSF red blood cell count of only 5500 cells/mm³.
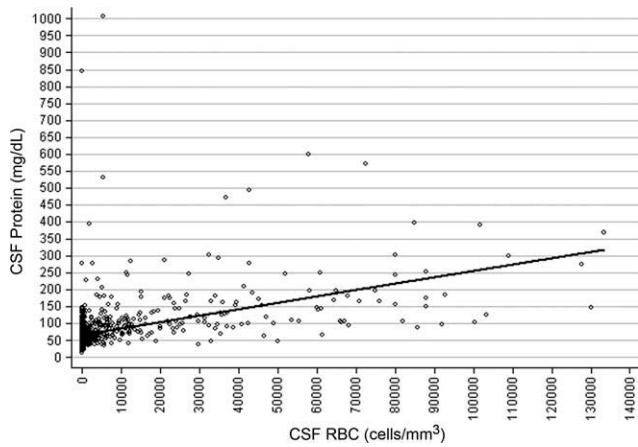
FIG. 1. Scatter plot of cerebrospinal fluid (CSF) red blood cell (RBC) (cells/mm^3) versus CSF protein level (mg/dL; n = 1241).

DISCUSSION

In a large cohort of infants ≤56 days of age, CSF protein increased by approximately 2 mg/dL for every 1000 cell/mm^3 increase in CSF RBCs. This correction factor is higher than previously reported correction factors from studies including older infants and children.[6,18] Some of this difference may be explained by the presence of old blood related to the trauma of labor and delivery. Previous work has demonstrated that the presence of xanthochromia, another RBC breakdown product, in the CSF of young infants was associated with maternal labor and elevated CSF protein.[19] Consistent with this hypothesis, the correction factor was nominally higher in those infants born by vaginal delivery compared with those born by cesarean section.

Several infants in our study had high CSF protein levels despite a paucity of CSF RBCs. By convention at our institution, the protein and glucose values are determined from the second tube, and the WBCs and RBCs are determined from the third tube. However, we could not determine the order in which the specimens for protein and RBCs were collected for individual specimens. Additionally, it is possible that delayed clearance of blood from a traumatic LP would cause the

CSF protein level to be high, as measured in the second tube, but lead to few RBCs in the third tube. These circumstances could explain the discrepancy between CSF protein and CSF RBCs counts for some patients.

The CSF protein adjustment factor for infants ≤56 days of age in our study was almost twice the correction of 1.1 mg/dL for every 1000 RBC increase reported by Nigrovic et al among infants ≤90 days of age.[6] There are differences in the design of the 2 studies. We excluded subjects with exceedingly large numbers of CSF RBCs and restricted inclusion to those 56 days of age or younger. When subjects with >150,000 RBCs/mm^3 were included, the correction decreased to a value comparable to that reported by Nigrovic et al.[6] Therefore, it is possible that inclusion of subjects with grossly bloody specimens in prior studies skewed the association between CSF protein and CSF RBCs. The number of subjects in our cohort with >150,000 CSF RBCs was too small to calculate a relevant correction factor for infants with exceedingly high CSF RBC counts.

The results of this study should be considered in the context of several limitations. Details regarding labor and delivery were not available. We suspect that old blood related to the trauma of birth provides partial explanation for the higher correction factor in neonates and young infants compared with older children. However, differences in CSF blood-brain barrier permeability may also contribute to these differences, independent of the CSF RBC count. Additionally, though the study population included a large number of neonates and young infants, a relatively small proportion of subjects had high CSF RBC counts. Therefore, our results may not be generalizable to those with exceedingly high CSF RBCs. Finally, available clinical prediction rules to identify patients with CSF pleocytosis, who are at very low risk for bacterial meningitis, include CSF protein as a predictor.[3,20,21] Although CSF protein in children with traumatic LPs may need adjustment prior to application of the clinical prediction rule, further study is needed before implementing this approach.

In conclusion, we found that CSF protein concentrations increased by approximately 2 mg/dL for every 1000 CSF RBCs. Correction of CSF protein for those with extremely high CSF RBCs may not be appropriate, as conventional linear models do not apply. These data may assist clinicians in interpreting CSF protein concentrations in infants 56 days of age and younger in the context of traumatic LPs.

TABLE 1. Association Between Cerebrospinal Fluid Protein and Red Blood Cell Count

Patient Group	No. of Patients	Change in CSF protein (mg/dL) per 1000 RBCs (95% CI)
All eligible	1241	1.9 (1.7-2.1)
No CSF pleocytosis*	1085	2.0 (1.7-2.4)
Age		
Age ≤28 days	481	1.9 (1.5-2.3)
Age >28 days	760	1.9 (1.7-2.1)
Mode of delivery†		
Vaginal	741	1.9 (1.7-2.2)
Cesarean	366	1.7 (1.4-2.0)

Abbreviations: CI, confidence interval; CSF, cerebrospinal fluid; RBCs, red blood cells.
* β-Coefficient for the subgroup without pleocytosis as defined by Byington et al[15] was 2.2 (95% CI: 1.9-2.5); β-coefficient for the subgroup without pleocytosis as defined by Chadwick et al[16] was 2.3 (95% CI: 2.0-2.7). † Data addressing mode of delivery was missing for 134 included patients.

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