Acta Pædiatrica ISSN 0803–5253

SHORT COMMUNICATION

# Neutrophils and red blood cells in the cerebrospinal fluid of newborns

Otávio A Moreno-Carvalho (nascimentocarvalho@hotmail.com)[1], Maria-Regina A Cardoso[2], Cristiana M Nascimento-Carvalho[3], and the Newborn CSF Study Group

1.Cerebrospinal Fluid Laboratory, José Silveira Foundation, Salvador, Brazil
2.Faculty of Public Health, São Paulo University, São Paulo, Brazil
3.Department of Pediatrics, School of Medicine, Federal University of Bahia, Salvador, Brazil

**Correspondence**
Cristiana M. Nascimento-Carvalho, M.D., Ph.D.,
Rua Prof. Aristides Novis N° 105/1201B,
Salvador, Bahia, Brazil – CEP 40210-630.
Tel: +55-71-32357869 |
Fax: +55 71 33320725 |
Email: nascimentocarvalho@hotmail.com

**Received**
26 December 2007; revised 25 February 2008;
accepted 4 March 2008.

DOI:10.1111/j.1651-2227.2008.00769.x

Lumbar puncture (LP) is routinely performed in order to investigate meningitis in the febrile newborn (1). The contamination of cerebrospinal fluid (CSF) with blood during a traumatic LP makes interpretation of the cell count difficult (2). Several authors have used a definition for nontraumatic LP, even in newborns, that includes several hundreds of red blood cells (RBCs) (3–5). The presence of neutrophils in the CSF is regarded as a sign of inflammation after the neonatal period (2); nonetheless, presence of neutrophils has been accepted as a normal characteristic of the CSF profile in newborns (3). This study aims to evaluate the association of the presence of neutrophils with the presence of RBCs in noninfected and nonhaemorrhagic CSF obtained by LP in newborns (age $\leq$ 28 days), with risk factors for central nervous system (CNS) infection.

The CSF charts of all newborns who underwent LP between April 2003 and December 2005 at the CSF Laboratory, José Silveira Foundation, Salvador, Brazil, were reviewed. Whenever a child underwent several LP, only the first one was included in this study. The performance of the laboratory procedures was prospectively standardized before the period of the study. Examination of the CSF included the cell count (cells/mm$^3$) by using the Fuchs–Rosenthal chamber; cytomorphological profile for which accelerated gravitational sedimentation technique and Leishman staining were performed; protein and glucose concentration (mg/dL), by using the trichloride acetic acid turbid and the enzymatic method, according to Trinder, respectively. Gram-stained smear, bacterial and mycological CSF cultures were performed according to routine methods.

**Abbreviations**
CI, confidence interval; CNS, central nervous system; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; HTLV-1, human T-lymphotropic virus; LP, lumbar puncture; RBC, red blood cell; RR, rate ratio.

CNS haemorrhage was defined as the presence of RBCs inside macrophages (6,7). The LP was performed by the request of the assistant physician, at seven neonatal intensive care units in the city of Salvador. All LP and CSF exams were performed by the same physician (O.A.M.C.). Newborns were classified as premature when the gestational age was under 38 weeks. For those patients with gestational risk for or clinical presentation suggesting congenital infection, serology to cytomegalovirus, herpes simplex virus, human immunodeficiency virus (HIV), human T-lymphotropic virus (HTLV-1), rubella, syphilis and toxoplasmosis, image studies (ultrasonography and X-ray) and ophthalmologic testing were performed. Exclusion criteria included any alteration that was attributable to CNS haemorrhage or anomaly, congenital infection or meningitis. The study was approved by the institutional review board of each neonatal intensive care unit and by the Ethics Committee of the Federal University of Bahia.

Out of 213 examinations, 44 were excluded due to absence of clinical data (n = 19), CNS haemorrhage (n = 14), congenital infections (n = 6), CNS congenital anomalies (n = 4) and meningitis (n = 1). Therefore, the study group comprises 169 patients. The median age (days) was the same (4) in the full-term and in the premature group (range: 0–26 and 0–24 days, respectively). Mean gestational age (weeks) was $33 \pm 3$ (median = 33; range: 25–37) in the premature group. None of the patients in the study group had CNS haemorrhage as a possible diagnosis. LP was performed because of obstetric risk for neonatal infection (44.4%), gestational risk for congenital infection (18.9%), fever (16.6%), respiratory distress and apnoea (11.8%), seizures (2.9%), lethargy (2.4%), cyanosis (1.8%) and hepatosplenomegaly (1.2%). Table 1 shows the descriptive analysis of CSF parameters. Overall, RBCs 25th, 50th and 75th percentiles were 1, 40 and 165, respectively. One thousand RBCs corresponded to the 92th percentile. Neutrophils were detected among CSF samples with (58.6%, 75/128) or without (24.4%, 10/41) RBCs

**Table 1** Descriptive analysis of the cerebrospinal fluid parameters of high-risk newborns without central nervous system infection or haemorrhage

| CSF parameter | Gestational age | | |
|---|---|---|---|
| | Full-term (n = 71) | Premature (n = 98) | Total group (n = 169) |
| WBC/mm$^3$* | 7 (0.3–33) | 6 (0.3–43) | 6 (0.3–43) |
| ANC/mm$^3$* | 0 (0–9) | 0 (0–11) | 0 (0–11) |
| ALC/mm$^3$* | 2.7 (0.2–15) | 2 (0.2–16) | 2.4 (0.2–16) |
| AMoC/mm$^3$* | 3 (0.1–20) | 2 (0.08–25) | 3 (0.08–25) |
| AMaC/mm$^3$* | 0.3 (0–3) | 0.2 (0–6) | 0.2 (0–6) |
| APC/mm$^3$* | 0 (0–0.2) | 0 (0–0.06) | 0 (0–0.2) |
| AEC/mm$^3$* | 0 (0–0.4) | 0 (0–0.8) | 0 (0–0.8) |
| ABC/mm$^3$* | 0 (0–0) | 0 (0–0.06) | 0 (0–0.06) |
| RBC/mm$^3$* | 40 (0–12 000) | 42 (0–7800) | 40 (0–12 000) |
| Protein (mg/dL)* | 70 (29–165) | 102 (36–177) | 90 (29–177) |
| Glucose (mg/dL)* | 45 (5–72) | 44 (12–116) | 44 (5–116) |
| Presence of neutrophils† | 42.3 | 56.1 | 50.3 |
| Presence of RBCs† | 71.8 | 78.6 | 75.7 |

*Results in median (range).
†Results in proportions (%).
WBC = white blood cell; ANC = absolute neutrophil count; ALC = absolute lymphocyte count; AMoC = absolute monocyte count; AMaC/mm$^3$ = absolute macrophage count; APC/mm$^3$ = absolute plasma cell count; AEC = absolute eosinophil count; ABC = absolute basophil count; RBC = red blood cell.

**Table 2** Results of the multivariate model for cerebrospinal fluid neutrophil count among high-risk newborns without central nervous system infection or haemorrhage

| Variables | RR | p-value | 95% CI |
|---|---|---|---|
| Red blood cells/mm$^3$ | 1.0002 | 0.000 | 1.00015–1.00029 |
| Protein (mg/dL) | 1.0032 | 0.356 | 0.99638–1.01013 |
| Glucose (mg/dL) | 1.0095 | 0.102 | 0.99813–1.02106 |
| Age (days) | 1.0202 | 0.271 | 0.98453–1.05719 |
| Gestational age (weeks) | 0.9812 | 0.558 | 0.92069–1.04562 |

RR = adjusted prevalence rate ratio; CI = confidence interval.

($p < 0.001$). As the neutrophil count data set was overdispersed, adjusted prevalence rate ratio (RR) with 95% confidence interval (CI) was estimated using generalized linear model, where distribution was chosen to be a negative binomial and link function was log (Table 2). For each RBC in the CSF the risk for the presence of neutrophil is 0.02%.

RBCs were frequently detected in the CSF profile of newborns and its presence was independently associated with the presence of neutrophils. It has been demonstrated in adults that even minimal blood contamination can result in the presence of one to two neuthophils in normal CSF and the number of CSF neutrophils was strongly correlated with the degree of CSF blood contamination (8). By analyzing the results reported herein, RBCs were not detected in one-quarter of our patients and 1000 RBCs was in the 92th percentile. Therefore, a minimally traumatic tap may be detected by the presence of RBCs under the amount of 1000. Moreover, the presence of neutrophils in CSF may be partly secondary to minimal trauma during LP and the presence of neutrophils due to such a trauma must be expected when the risk is 100%, that is, from 50 RBCs. Since there is no clear RBCs cut-off to define traumatic LP in the literature (9), based on the foregoing data the authors recommend the use of up to 49 RBCs for defining a nontraumatic LP in newborns.

The poor difference between the CSF parameters of premature and full-term newborns was noteworthy. Nonetheless, we have previously demonstrated that only the mean percentage neutrophil count and the mean protein concentration were significantly different when CSF parameters from premature and full-term newborns were compared (10). Regarding aging, the total WBC count and the protein concentration are expected to decrease as of 4 weeks of age among full-term newborns (11).

Our results highlight the importance of CSF culture to establish the diagnosis of neonatal meningitis. Garges et al. (12) have presented evidence that no single CSF parameter can reliably exclude the presence of meningitis in newborns. We have presented evidence that one of the alterations (presence of neutrophils) regarded as sign of inflammation can be due to minimal LP trauma. Further studies are needed to clarify why neutrophils can be present in the absence of RBCs in noninfected CSF of newborns.

## ACKNOWLEDGEMENTS

M. R. A. C. and C. M. N.-C. are investigators of the Brazilian Council for Science and Technology Development (CNPq).

## References

1. Ishimine P. Fever without source in children 0 to 36 months of age. *Pediatr Clin North Am* 2006; 53: 167–94.
2. Mazor SS, McNulty JE, Roosevelt GE. Interpretation of traumatic lumbar punctures: who can go home? *Pediatrics* 2003; 111: 525–8.
3. Bonadio WA, Stanco L, Bruce R, Barry D, Smith D. Reference values of normal cerebrospinal fluid composition in infants ages 0 to 8 weeks. *Pediatr Infect Dis J* 1992; 11: 589–91.
4. Ahmed A, Hickey SM, Ehrett S, Trujillo M, Brito F, Goto C, et al. Cerebrospinal fluid values in the term neonate. *Pediatr Infect Dis J* 1996; 15: 298–303.
5. Wong M, Schlaggar B, Buller RS, Storch GA, Landt M. Cerebrospinal fluid protein concentration in pediatric patients: defining clinically relevant reference values. *Arch Pediatr Adolesc Med* 2000; 154: 827–31.
6. Reis-Filho JB, Andrade LA. Semiologic value of erythrocyte macrophages in the cerebrospinal fluid. *Arq Neuropsiquiatr* 1980; 38: 231–6.
7. Rascol MM. Cytologic survey of CSF in the meningeal hemorrhages. *Sem Hop* 1980; 56: 449–52.
8. Hayward RA, Oye RK. Are polymorphonuclear leukocytes an abnormal finding in cerebrospinal fluid? Results from 225 normal cerebrospinal fluid specimens. *Arch Intern Med* 1988; 148: 1623–4.
9. Shah KH, Richard KM, Nicholas S, Edlow JA. Incidence of traumatic lumbar puncture. *Acad Emerg Med* 2003; 10: 151–4.
10. Nascimento-Carvalho CM, Moreno-Carvalho OA. Normal cerebrospinal fluid values in full-term gestation and premature neonates. *Arq Neuropsiquiatr* 1998; 56: 375–80.

11. Bonadio WA, Stanco L, Bruce R, Barry D, Smith D. Reference values of normal cerebrospinal fluid composition in infants ages 0 to 8 weeks. *Pediatr Infect Dis J* 1992; 11: 589–91.
12. Garges HP, Moody MA, Cotton CM, Smith PB, Tiffany KF, Lenfestey R, et al. Neonatal meningitis: what is the correlation among cerebrospinal fluid cultures, blood cultures, and cerebrospinal fluid parameters? *Pediatrics* 2006; 117: 1094–1100.

## APPENDIX

The Newborn CSF Study Group members (in alphabetical order):

Francisca S Ribeiro[1], Katiaci Araújo[2], Lícia MO Moreira[3], Maria de Lourdes SC Leite[4], Nair C Chaves[5], Rosana Pelegrini[6]

1. Jorge Valente Hospital and Espanhol Hospital, Salvador, Brazil
2. Aliança Hospital, Salvador, Brazil
3. Department of Pediatrics, School of Medicine, Federal University of Bahia, and Santo Amaro Hospital, Salvador, Brazil
4. Santa Isabel Hospital, Salvador, Brazil
5. Sagrada Família Hospital, Salvador, Brazil
6. Salvador Hospital, Salvador, Brazil

 