Short reports

Blood stained cerebrospinal fluid responsible for false positive reactions of latex particle agglutination tests

P A M Camargos, M S Almeida, G L Filho, K W R Batista, A G Carvalho, C L Pereira

Abstract

The accuracy of the latex particle agglutination test (LPAT) was assessed in blood stained cerebrospinal fluid (CSF) specimens from 166 paediatric patients, aged from three months to 13 years. A commercial LPAT kit was used to detect *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, and *Neisseria meningitidis* A, B, and C soluble antigens. Culture of CSF specimens was used as the standard and all laboratory procedures were performed blind. The mean CSF erythrocyte count was 66 406 cells/mm³ in the cases and 11 560 cells/mm³ in the controls. The sensitivity and the specificity of LPAT were 83.8 and 94.0%, respectively, suggesting that LPAT is a useful diagnostic tool even in blood stained CSF specimens.

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Department of
Pediatrics, School of
Medicine, University
of Minas Gerais
P A M Camargos

School of Medicine
K W R Batista
A G Carvalho
C L Pereira

Division of Medical
Biology, Ezequiel Dias
Foundation
M S Almeida
G L Filho

Correspondence to:
Professor P A M Camargos,
Faculdade de Medicina da
Universidade Federal de
Minas Gerais, Departamento
de Pediatria, Avenida
Alfredo Balena 190,
30.130-100 Belo Horizonte
MG, Brasil

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In the mid-1970s simple, fast, and inexpensive methods for the identification of the causative agents of purulent meningitis became available following the development of immunochemical techniques, such as the latex particle agglutination test (LPAT). The high sensitivity and specificity reported for

this method (ranging from 80 to 100%), ease of execution and interpretation, and that prior use of antibiotics does not alter the results suggest that this test may be a useful diagnostic tool, particularly in developing countries.¹⁻⁴

Almost all previously published studies have evaluated LPAT performance in non-bloody lumbar punctures. Some studies rejected blood stained cerebrospinal fluid (CSF) samples, considering these unsuitable for testing.^{5,6} However, blood stained samples are not uncommon, particularly in paediatric patients.

This study was designed to assess the performance of a commercial LPAT kit (Slidex-meningite, Biomérieux, Marcy l'Etoile, France) in blood stained CSF samples obtained from infants and children aged from three months to 13 years.

Methods

The investigation was conducted between March 1991 and February 1993 in Belo Horizonte City, Minas Gerais State, Brazil, at the General Pediatrics Centre, a reference centre for the diagnosis and treatment of meningitis. On physical examination, all patients included in the study presented with clinical and neurological abnormalities suggestive of meningitis. Lumbar puncture was indicated in all patients and all CSF specimens obtained were blood stained. All of the patients had an erythrocyte CSF count ≥ 30 cells/mm³. Patients whose lumbar puncture yielded a CSF volume below 2.0 ml were excluded from the study.

An erythrocyte count, culture, and the LPAT, carried out according to the manufacturer's instructions to which included heating and centrifuging the specimens, and other traditional laboratory procedures for the diagnosis of meningitis were performed on the CSF samples within 24 hours of receipt. All tests were performed blind.

LPAT was assessed by comparing the proportion of positive and negative results with the results obtained following culture. Patients with a negative CSF culture for *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis* served as controls. Sensitivity, specificity, and overall agreement were estimated by calculating the respective 95% confidence interval (CI). The χ^2 and Student's *t* tests were used to deter-

Table 1 Patient characteristics

Characteristics	Cases n (%)	Controls n (%)	Two tailed p value*
Sex			
male	21 (67.7)	85 (62.9)	>0.05
female	10 (32.3)	50 (37.1)	
total	31 (100.0)	135 (100.0)	
Age group			
three months to five years	25 (80.6)	102 (75.6)	>0.05
five to 14 years	6 (19.4)	33 (24.4)	
total	31 (100.0)	135 (100.0)	
Culture results			
positive			
<i>Haemophilus influenzae</i> b	20 (64.4)	—	—
<i>Neisseria meningitidis</i> C	4 (13.0)	—	
<i>Streptococcus pneumoniae</i>	3 (9.7)	—	
<i>Neisseria meningitidis</i>	3 (9.7)	—	
<i>Neisseria meningitidis</i> B	1 (3.2)	—	
<i>Staphylococcus aureus</i>	—	3 (2.3)	
<i>Bacillus</i> sp	—	2 (1.5)	
<i>Acinetobacter calcoaceticus</i>	—	1 (0.7)	
<i>Staphylococcus epidermidis</i>	—	1 (0.7)	
<i>Pseudomonas</i> sp	—	1 (0.7)	
negative	—	127 (94.1)	
total	31 (100.0)	135 (100.0)	
Erythrocyte count**	66 406 (355 182) (range 30-1 980 000)	11 560 (53 967) (range 32-570 000)	<0.05

* χ^2 (sex/age group) or Student's *t* tests (erythrocyte count). **Expressed as mean (SD).

Table 2 Latex particle agglutination test in blood stained cerebrospinal fluid

LPAT	Cases (positive CSF culture)	Controls (negative CSF culture)	Total
Positive	26	8	34
Negative	5	127	132
Total	31	135	166

Sensitivity: 83.8% (95% CI 65.8–94.8).

Specificity: 94.0% (95% CI 88.0–98.0).

Overall agreement: 92.2% (95% CI 86.2–96.2).

mine the statistical significance of the sex and age distribution, and of the mean erythrocyte count. Significance was set at $p < 0.05$.

The research protocol was approved by an independent Ethical Committee. The decision to perform a lumbar puncture was taken by the attending paediatrician.

Results

Patient characteristics are presented in table 1. The disease and control groups were comparable with regard to age and sex.

The mean erythrocyte count, however, differed substantially when the disease and control groups were compared. Meningitis caused by *H influenzae* type b was more prevalent in the patients (64%) and negative cultures were obtained mainly in the control group (94.1%). The overall performance of LPAT is presented in table 2.

False positive results (eight cases from the control group) were caused by cross reactivity with *H influenzae* type b (four cases), *N meningitidis* C (three cases), and *S pneumoniae* (one case) antisera.

Discussion

Despite the low number of children in the disease group, the sensitivity found in the present study was similar to that in the literature,^{1–5,7,8} implying that LPAT successfully detected bacterial soluble antigens in the blood stained CSF samples. The objective of this study, however, was to analyse the control group which constituted an appropriate sample size. The specificity (94.0%) and 95%

CI (88.0–98.0%) obtained were similar to those obtained in other studies which did not include blood stained samples.^{1–5,7,9} Cross reactivity was observed in only eight (6.0%) of the 135 controls.

The results presented here differ from those obtained in the study by Thirumoorthi and Dajani,⁶ where 12 of 16 patients with blood stained samples had false positive results.

Some authors^{7,9} have speculated that non-specific reactions may occur because of the presence of plasma constituents, such as rheumatoid factor, or cold reactive IgM antibodies, in the CSF specimen. According to these investigators,^{7,9} however, these reactions are eliminated by centrifuging and heating the specimen, a procedure used in this study.

Our results suggest that if blood stained CSF specimens are centrifuged and heated, then LPAT can be used to diagnose bacterial meningitis.

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