Abstract

Objective: to determine the usefulness of reagent strips in the evaluation of pleocytosis, cerebrospinal fluid glucose and protein levels for early and rapid diagnosis of meningitis in children.

Methods: we included cerebrospinal fluid samples of 164 children admitted to the outpatient clinic of Communicable Diseases of the General Pediatric Center (Fundação Hospitalar do Estado de Minas Gerais, CGP-FHEMIG) during the daytime hours from May of 1997 to May 1999, and who presented with clinical suspicion of meningitis. Patients ranged in age from one month to 12 years (median 12 months). Results from the cytological and biochemical assay (cellularity, cerebrospinal fluid glucose and protein levels) were obtained from 154 patients. These results were subsequently compared with the reaction of cerebrospinal fluid in reagent strips.

Results: the cytological and biochemical assay identified 43 cases of probable bacterial meningitis, 19 of probable viral meningitis, and 83 with no alterations. According to the reagent strips, there were 41 cases of probable bacterial meningitis, 2 of probable viral meningitis, and 71 with no alterations. By comparing the results of reagent strips and those of the cytological and biochemical assay, we obtained values for sensitivity, specificity, positive and negative predictive values, and accuracy (respectively 90.7; 98.1; 95.1; 96.4; and 96.1). Statistical analysis using McNemer test did not indicate significant differences between the two methods in the diagnosis of bacterial meningitis (P=0.68). Kappa statistics indicated a high level of agreement between the tests (P<0.0001).

Conclusions: our results suggest that reagent strips may be a useful additional resource in the diagnosis of bacterial meningitis, especially when it is difficult to collect a sufficient amount of cerebrospinal fluid or to indicate the initial treatment.

Introduction

The diagnosis of bacterial meningitis must be clearly established as early as possible due to the high morbidity and mortality of the disease, which varies according to the causing agent, patient’s age, and time of diagnosis. The prevalence of bacterial meningitis is approximately 3 cases per 100,000 inhabitants. In Brazil, data provided by the Ministry of Health show more than 297,000 cases of
meningitis during the last ten years (1990 through 2000), among which approximately 52% were reported as bacterial meningitis.2

In industrialized countries, after the regular use of the conjugate vaccine against Haemophilus influenza b (HIB), implemented in 1986, Streptococcus pneumoniae (pneumococcal meningitis) has become the major cause of meningitis after the neonatal period, with a mortality rate greater than 20%.1,3 During approximately 10 years of anti-Hib vaccination in the United States, the incidence of the causing agent has dropped approximately 90%.1,4 This, however, is not the situation in developing countries, where massive anti-HIB vaccination was not adopted. Neisseria meningitidis (meningococcal meningitis) alters its epidemic form, with a mortality rate around 13%, presenting higher rates according to the presence of prognostic factors.1,5 Newborns are the most commonly affected individuals, reaching a morbidity and mortality rate of approximately 30%.6

Prompt diagnosis of meningitis is fundamental for early treatment. Unfortunately, meningitis is more frequent during the first two years of life, when signs and symptoms are usually nonspecific and simulate any type of infection. The classic triad - headache, fever, and vomiting - is more frequently identified among preschool and school-age children; however, it affects 2/3 of adults.1,7

Many times, patients are submitted to lumbar puncture so that diagnosis can be elucidated, but technical difficulty in obtaining enough material or a delay in the cytological and biochemical assay usually get in the way of promptly deciding on antibiotic therapy implementation. The use of quick cerebrospinal fluid (CSF) tests by means of urine reagent strips has been described in few studies,8-11 in which sensitivity and specificity reached respectively 97% and 100%. The aim of the present study is to assess the use of these reagent strips for the early and prompt diagnosis of bacterial meningitis.

**Methods**

**Place of study and studied patients**

Patients admitted to the outpatient clinic of Communicable Diseases of the General Pediatric Center (Fundação Hospitalar do Estado de Minas Gerais, CGP-FHEMIG) in Belo Horizonte, were included in the study. These patients sought the clinic in the daylight time and on weekdays, between May 1997 and May 1999. As there was a clinical suspicion of meningitis, they were submitted to lumbar puncture.

**Referral and inclusion criteria**

As this hospital is a reference in terms of communicable diseases in the state of Minas Gerais, it received patients with suspected meningitis, which was confirmed when they showed evidence of an infectious process without any other defined source; when they presented an infectious process with altered mental status; and when their infectious process revealed specific manifestations of the central nervous system (seizures, neurological deficits, signs of meningeal involvement, nuchal stiffness, and signs of intracranial pressure).

**Selection of patients and use of reagent test strips**

After the decision on lumbar puncture by the assistant doctor on duty, one of three residents qualified for test reading, and the staff in charge of data collection were called in. Parents or guardians were informed about the study, and requested to sign an informed consent form allowing the participation of their children in the study. The first lumbar puncture provided CSF samples for the routine cytological and biochemical assay and for the Combur®-10 reagent strip test (Table 1).

**Table 1 - Commercially available reagent strips**

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Manufacturer</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biobrás®</td>
<td>Biobrás</td>
<td>Brazil</td>
</tr>
<tr>
<td>Combur®</td>
<td>Boehringer Mannheim</td>
<td>Germany</td>
</tr>
<tr>
<td>Medtest®</td>
<td>Medtest</td>
<td>Germany</td>
</tr>
</tbody>
</table>

The strips are the same used in urine tests: small strips with specific spots for enzymatic and oxidation-reduction reactions of several substances. The technique was not clearly defined beforehand; as a result, there were some variations as to the reaction time, and amount of CSF used.8,9,11 One or two drops of CSF were dripped onto the reactive part of the strip for determining the amount of leukocytes, protein, and glucose. After that, 60 seconds were allowed (following the manufacturer’s recommendations), and the excessive fluid was then wiped off. The result obtained was compared with the package standards, and the readings were entered on the patient’s medical record. All lab exams were conducted by different professionals who did not know about the results obtained by the other researchers.
Interpretation of the cytological and biochemical assay and reagent strip test results

The parameters for analyzing cytological and biochemical alterations in the CSF were defined according to the available literature3,13,14:

– Cellularity: normal if <10 cells/mm³; slight increase between 11 and 50 cells/mm³; moderate increase between 50 and 200 cells/mm³, major increase if >200 cells/mm³.
– Glucose: normal if >45 mg/dl; reduced if <45 mg/dl (or < 2/3 of serum dosage).
– Proteins: normal if <40 g/l; slight increase between 41 and 100 g/l; major increase if >100 g/l.

The parameters used to determine CSF alterations through reagent strips (according to the values supplied with the product) were:

– Leukocytes: negative in case of “negative” reaction; slight increase if reaction equals 10-25 leukocytes/ul or 75 leukocytes/ul; major increase if reaction equals 500 leukocytes/ul.
– Glucose: reduced in case of “normal” reaction; normal if reaction equals 50, 100, or 300 mg/dl.
– Proteins: normal in case of “negative” reaction or 30 g/l; increased if reaction equals 100 or 500 g/l.

A definite etiological diagnosis of meningitis was obtained only when the causing agent was isolated in culture and/or antigen detection test and/or positive blood culture, combined with altered CSF cytological and biochemical assay.7

The diagnosis of probable bacterial or viral meningitis through the cytological and biochemical assay or through reagent strips was given only when all three parameters implied:

– Bacterial meningitis: remarkable increase in the amount of cells and proteins, and reduction in glucose levels.
– Viral meningitis: slight or moderate increase in the amount of cells, slight increase in protein levels, and normal glucose levels.

Visual color comparison may be subjective, especially in cases with slightly altered CSF.9-11,15-17 The isolated increase in the level of cells or proteins was analyzed separately, and was not considered characteristic of meningitis. The presence of up to 50 cells in the CSF, without any other alterations, may be attributed to previous history of seizure, trauma associated with puncture, or reaction to a systemic process.

Statistical analysis

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of reagent strips were calculated based on the cytological and biochemical assay. The 95% confidence interval was calculated for the values obtained for each of the five parameters.

The concordance rate between the cytological and biochemical assay and reagent strips was assessed by the Kappa statistics. This method showed variations between -1.0 (major discordance) and +1.0 (excellent concordance). Values less than 0.4 mean low concordance, values between 0.4 and 0.75 mean good concordance, and values greater than 0.75 denote excellent concordance.18

Finally, the McNemar test was used to assess the discordance rate among the studied methods. The level of statistical significance was P<0.05.

Ethical aspects

The investigation protocol was approved by the Ethics Committee of the hospital.

Results

At the beginning, 164 patients were included in the study. Among the 164 CSF samples, 10 were lost due to reagent strip misreadings. Therefore, 154 CSF samples were analyzed - 100 males (64.9%). Age ranged between one month and 12 years (median of 12 months).

The cytological and biochemical analysis of CSF revealed 43 cases of suspected bacterial meningitis; 19 cases of suspected viral meningitis; 83 cases without alterations; 8 cases with increased cellularity; and one case with unanalyzed hemorrhagic CSF.

The reagent strip test revealed 41 cases of suspected bacterial meningitis; 2 cases of suspected viral meningitis; 71 cases without alterations; and 39 cases with increased cellularity. An isolated increase in protein level was observed in one case.

Among the 43 suspected cases of bacterial meningitis diagnosed through the cytological and biochemical assay, 39 cases were also diagnosed through the reagent strip test; one case did not show any alteration; two cases presented increased cellularity (10 to 500 cells/mm³); and one case presented increased protein level (500 mg/dl).

Among the 19 samples that were supposedly viral, as shown by the cytological and biochemical assay, 2 presented alterations compatible with meningitis on the reagent strip; 14 had increased cellularity; 1 case had reactions that were compatible with bacterial infection; and 2 cases did not show any alterations on the reagent test strip.

One case in which the routine CSF exam and reagent strip test remained unaltered, there was growth of Neisseria in culture.
Table 2 - Distribution of methods used for the diagnosis of 22 patients with bacterial meningitis with defined etiological agent

<table>
<thead>
<tr>
<th>Diagnostic method</th>
<th>Meningococcal meningitis</th>
<th>Hib</th>
<th>Pneumococcal meningitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture (LCR)</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Latex</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Culture + Latex</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Blood culture+ Latex</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Culture + Blood culture</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Latex+Culture+ Blood culture</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 3 - Diagnosis of bacterial meningitis through routine cytological and biochemical assay and reagent strip test

<table>
<thead>
<tr>
<th>Método</th>
<th>Positive cytological and biochemical assay</th>
<th>Negative cytological and biochemical assay</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive reagent strip</td>
<td>39 90.7%</td>
<td>2 1.2%</td>
<td>41 26.6%</td>
</tr>
<tr>
<td>Negative reagent strip</td>
<td>4 9.3%</td>
<td>109 98.8%</td>
<td>113 73.4%</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>111</td>
<td>154</td>
</tr>
</tbody>
</table>

Sensitivity: 90.7% (CI 95% 77.8%-97.4%)
Specificity: 98.1% (CI 95% 93.6%-99.7%)
PPV: 95.1% (CI 95% 83.4%-99.4%)
NPV: 96.4% (CI 95% 91.1%-99.0%)
Accuracy: 96.1% (CI 95% 91.7%-98.5%)

In three cases of Brazilian purpuric fever, the cytological and biochemical assay of CSF and reagent strip test did no show any alterations.

Of the 43 cases of meningitis regarded as bacterial type by the cytological and biochemical assay, diagnostic certainty was obtained in 22 cases (51.2%), of which 48.8% presented undetermined etiology. Ten cases presented with *Neisseria meningitidis*; seven cases revealed *Haemophilus influenza*, and five cases presented with *Streptococcus pneumoniae* (Table 2).

There was no statistical discordance between the compared tests, since the P value obtained through the McNemar test was 0.68. High statistical concordance was obtained, with Kappa = 0.9 and P < 0.0001 (Table 3).

Discussion

The ideal test for defining the bacterial etiology of meningeal infection must have sensitivity, specificity, and predictive values ideally close to 100%, which means that...
no case of meningitis would go unidentified. In addition, the prompt diagnosis of meningitis would allow faster rehabilitation and evolutionary prognosis. Not even routine CSF cyto logical and biochemical assay (cytometry, cytology, glucose and proteins) are able to identify all cases of meningeal infection; therefore, the clinical and evolutionary status as well as other complementary lab tests must be assessed in conjunction.

The use of easily applicable tests that can determine whether antibiotic therapy will be necessary must be stimulated. The aim of this study was to evaluate the use of an extremely easy method, in which results can be obtained through a reaction within seconds. Although the antigen detection test is widely spread due to its sensitivity and ease of application, it depends on reliable technique and on resources that are not yet available in several places. The use of reagent test strips as a routine procedure may be considered as a useful complementary method for the prompt diagnosis of bacterial meningitis in cases where sufficient CSF is not easily obtained or in cases where quick decision for antibiotic therapy must be taken immediately.8,9,12,17

The variation and subjectivity of strip color assessment may interfere with readings, thus requiring qualified personnel. Previous studies show that it is difficult to define normal and altered values. In addition, they point out that slight alterations might not be detected.9-11,15,16 Therefore, in the present study, it was necessary to follow well-defined criteria for the diagnosis of meningitis. The isolated increase of any parameters did not define the diagnosis, which was based upon cellular, glucose, and protein level alterations.

Moosa et al.8 studied the reactions for leukocytes, protein, and glucose in the CSF from 234 patients using this method, and found a sensitivity of 97%.2 Schwartz et al.9 only assessed the reaction for proteins and glucose in the reagent strips, using the same brands of strips that were employed in the present study. By analyzing the 58 CSF samples collected from 43 patients, they concluded that it was possible to quickly identify cases with well-defined alterations, although it was difficult to determine the threshold for normal values. Fleibloom & Muller10,11 investigated CSF glucose levels of respectively 119 and 54 patients, and reported difficulty in the identification of slight alterations. Salvador et al.12 conducted a study with 68 patients, in which they separately analyzed sensitivity and specificity for each of the items analyzed by the reagent strips, thus validating the test for the diagnosis of bacterial meningitis. These authors pointed out the necessity for the presence of polymorphonuclear leukocytes, which have esterase, an enzyme that reacts with the corresponding spot on the reagent strip.

Therefore, there was no statistically significant discordance as to the diagnosis of bacterial meningitis through the two methods presented in our study (P=0.68). We may infer that reagent strips had good sensitivity in the studied sample (90%). In addition, the significant diagnostic concordance (P<0.0001) indicates that the reagent strip method may be a thriving and useful diagnostic tool for meningeal infections, especially in cases that present technical difficulties, hastening the adoption of due measures as early as possible. It is necessary that studies with large-sized samples be carried out so that routine tests can be recommended.

References