Value of cerebrospinal fluid examination in the diagnosis of meningitis in the newborn

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Abstract
Between 1 October 1988 and 30 September 1991 the results of all 896 cerebrospinal fluid examinations from 736 neonates were correlated with clinical diagnosis, treatment, and outcome. The prevalence of fungal or bacterial meningitis in babies requiring lumbar puncture was only 0.95%. Gram staining had a sensitivity of 68% and a positive predictive value of only 46% for the diagnosis of meningitis. Primary cultures directly onto agar plates had a sensitivity of 81% and a positive predictive value of 46%. Broth enrichment cultures did not improve sensitivity and were frequently found to be false positive. Empirical treatment should not be altered unless more than a few organisms are seen on Gram staining. Primary cultures are adequate for the diagnosis of fungal and bacterial meningitis. Enrichment cultures should be performed only when the Gram stain and/or cell count suggests meningitis is likely. Clinicians should be aware that diagnostic tests performed in populations with a low prevalence of disease are likely to generate many false positive results and have a low positive predictive value.

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When systemic infection is suspected in the newborn it is usual to carry out a lumbar puncture to exclude meningitis. The procedure is not without risk and possible complications include acute respiratory compromise, trauma, introduction of infection, spinal epidermoid implantation tumours, and contamination of cerebrospinal fluid (CSF) with bone marrow cells.1–6 We wished, therefore, to assess the utility of Gram stain, primary culture, and enrichment culture in establishing the diagnosis of bacterial and fungal meningitis in babies admitted to the special care baby unit at the John Radcliffe Maternity Hospital, Oxford.

Patients and methods
All babies admitted to the unit, who underwent a lumbar puncture during a three year period beginning 1 October 1988 were included in the study. We divided infants into two groups based on whether the lumbar puncture was performed for suspected early onset infection (up to 48 hours of age) or after 48 hours when suspected late onset infection was the usual indication. Babies having a lumbar puncture after the neonatal period (>28 days old) were classified separately. The unit has a policy of delaying lumbar puncture in small preterm infants with respiratory distress because of the risk of intraventricular haemorrhage in the first two days after birth.7 During the review period the clinical course, treatment, and outcome of all neonates with suspected infection had been prospectively recorded and cases of meningitis noted.

Using data recorded concurrently in the bacteriology department the results of all CSF examinations performed on neonates during this review period were analysed. Details recorded were adequacy of the sample, white cell count, red cell count, Gram stain, and culture result. Primary culture was performed by inoculating one drop of the centrifuged deposit of CSF onto each of three agar plates: chocolate agar and 5% horse blood agar plates (incubated in air+5% carbon dioxide) and a second 5% horse blood agar plate (incubated anaerobically). All plates were incubated for 48 hours at 37°C. Enrichment culture was performed by adding a drop of the deposit to a tryptose soy broth which was routinely subcultured onto agar plates after three days incubation or before this if growth was visible.

In addition, all cases of neonatal meningitis and their corresponding lumbar puncture results from 1 May 1984 to 30 September 1988 were available for analysis from a prospectively recorded database. It was possible, therefore, to review the medical notes of all infants diagnosed with meningitis during that period. Cases of definite bacterial or fungal meningitis were defined by a suggestive clinical presentation, for example thermal and respiratory instability, feed intolerance and/or seizures, supported by positive primary culture or postmortem evidence if a lumbar puncture had not been carried out. Cases of probable meningitis were defined by suggestive clinical features together with either a positive enrichment culture or, if the culture was negative, a positive Gram stain or high white cell count (>100×10⁹/l). All such cases received appropriate antibiotic treatment for meningitis (>10 days intravenous treatment). A false positive (contaminated) culture was defined by the growth of an organism without CSF pleocytosis or positive Gram stain together with a clinical picture which did not suggest meningitis. Such cases all received less than five days intravenous antibiotics. A traumatic blood stained CSF sample was defined using the criteria of Visser and Hall, namely a red cell count greater than 10×10⁹/l.8

The Epi-Info version 5 was used for statistical analysis (Centers for Disease Control, Atlanta). The χ² test with Yates’s correction
**Table 1** Distribution of lumbar punctures by age and indication

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>No of babies</th>
<th>No of lumbar punctures</th>
<th>Lumbar punctures/patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤28</td>
<td>736</td>
<td>896</td>
<td>1:2</td>
</tr>
<tr>
<td>Early infection (&lt;48 hours)</td>
<td>564*</td>
<td>586</td>
<td>1:0</td>
</tr>
<tr>
<td>Late infection (&gt;48 hours)</td>
<td>225*</td>
<td>310</td>
<td>1:3</td>
</tr>
<tr>
<td>&gt;28</td>
<td>63†</td>
<td>166</td>
<td>2:6</td>
</tr>
<tr>
<td>Total</td>
<td>760</td>
<td>1062</td>
<td>1:4</td>
</tr>
</tbody>
</table>

*Fifty three infants had a lumbar puncture for both suspected early and late onset infection.
†Twenty four infants did not also have a lumbar puncture during neonatal period.

result was employed in comparisons, unless an expected cell value was <5 when Fisher’s exact test (two tailed) was employed.

**Results**

(A) FREQUENCY OF CSF EXAMINATION

Between 1 October 1988 and 30 September 1991, 19491 babies were born at the John Radcliffe Maternity Hospital, and of these 1625 (8.3%) were admitted to the unit. A further 178 babies were referred from other centres. Of the 1803 babies admitted to the unit, 760 (42%) underwent lumbar puncture. Table 1 summarises the distribution of lumbar punctures by age and indication. Table 2 summarises the birth weight and gestational age of the 896 neonates who required the procedure.

(B) ADEQUACY OF SAMPLE

Data recorded on adequacy of sample is presented in table 3. One third of all samples were unsuitable for full cytological analysis because the sample was grossly bloodstained, clotted, or too small.

(C) VALUE OF GRAM STAINING

Between 1 October 1988 and 30 September 1991 five cases of definite bacterial meningitis (three early, two late), one case of probable bacterial meningitis (late), and one case of definite candida meningitis (late) were diagnosed. The prevalence of bacterial and fungal meningitis in neonates undergoing lumbar puncture in our unit was 0.95% (7/736). There was a higher yield of true positives from late lumbar puncture (4/310, 1.3%) compared with early (3/586, 0.5%) but the difference was not statistically significant.

**Table 2** Gestational age and birth weight of neonates undergoing lumbar puncture

<table>
<thead>
<tr>
<th>Birth weight (g)</th>
<th>Early (&lt;48 hours)</th>
<th>Late (&gt;48 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1500</td>
<td>45 (8)</td>
<td>46</td>
</tr>
<tr>
<td>1500–2500</td>
<td>188 (33)</td>
<td>540</td>
</tr>
<tr>
<td>&gt;2500</td>
<td>331 (59)</td>
<td>18 (21)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>332 (59)</td>
<td>176 (216)</td>
</tr>
<tr>
<td>&lt;31</td>
<td>32 (6)</td>
<td>532</td>
</tr>
<tr>
<td>≥31</td>
<td>532 (94)</td>
<td>553</td>
</tr>
<tr>
<td>Total</td>
<td>564</td>
<td>586</td>
</tr>
</tbody>
</table>

**Table 3** Adequacy of lumbar puncture from neonates for cytological analysis

<table>
<thead>
<tr>
<th></th>
<th>No (%)</th>
<th>Inadequate</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td>299 (33)</td>
</tr>
<tr>
<td>Early</td>
<td>586</td>
<td>189 (32)</td>
</tr>
<tr>
<td>Late</td>
<td>110</td>
<td>35 (31)</td>
</tr>
<tr>
<td>&lt;1500 g</td>
<td>222</td>
<td>72 (32)</td>
</tr>
<tr>
<td>≥1500 g</td>
<td>674</td>
<td>171 (25)</td>
</tr>
</tbody>
</table>

Gram staining was carried out on all 896 CSF specimens sent from neonates and was positive in 13, six of which were considered true positives and seven false positives. In all the false positive Gram stains, only scanty organisms were seen. There was only one false negative Gram stain result which was from the child with candida meningitis. Thus, the sensitivity of Gram staining was 86%, specificity 99%, positive predictive value 46%, and the negative predictive value 99-9%.

Between 1 May 1984 and 30 September 1988 10 other cases of bacterial or fungal meningitis were recorded. One case was diagnosed only at postmortem examination. In five of the nine cases diagnosed by lumbar puncture organisms were seen on Gram staining giving a sensitivity for the test for the combined review periods of 68% (5/616).

(D) VALUE OF PRIMARY CULTURE

Primary culture was performed on all 896 specimens. A total of 13 positive results were obtained. Of these 13 results, seven were considered false positive and the organisms isolated are listed in table 4. There was no overlap between the false positive cultures and the false positive Gram stain results. There was only one false negative primary culture result, in a baby with a probable late onset meningitis. Gram negative rods were seen in the stained deposit but the organisms failed to grow on both primary and enrichment culture. The baby had received antibiotics before lumbar puncture. Details of the six true positive cultures are summarised in table 4.

**Table 4** Contaminants isolated on primary or enrichment culture (false positives) from 108 lumbar punctures

<table>
<thead>
<tr>
<th>Primary</th>
<th>No</th>
<th>Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase negative staphylococci</td>
<td>3</td>
<td>80</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Haemolytic streptococcus</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Acinetobacter sp</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Oxidase positive Gram negative rod</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Branhamella catarrhalis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Streptococcus bovis</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Total | 111*
culture results are given in Table 5. The sensitiv-
yty of primary culture was 86% with a
pecificity of 99%, the positive predictive value
6%, and the negative predictive value
9%. Of the nine cases of meningitis diagnosed by
lumbar puncture recorded between 1 May
1984 and 30 September 1991, seven were
positive on primary culture. Thus for the com-
bined review periods the sensitivity of primary
culture was 81% (6/7/16).

(E) VALUE OF ENRICHMENT CULTURE
The value of enrichment culture was also
assessed. Enrichment was performed on all
896 lumbar puncture specimens and these
were positive in 101. All were false positive.
Combining false positive primary and enrich-
ment cultures there were therefore 108 con-
taminated cultures, and these were more often
seen when the lumbar puncture was done for
possible late sepsis (53/310, 17%) than in
those done early (55/586, 9-4%; p=0.001).
There was no evidence that lumbar punctures
done in babies with a low birth weight
(<1500 g) were more likely to be contami-
nated (early 446, 9% and late 28/176, 16%)
than those done in larger babies (early 51/540,
9% and late 25/134, 18-7%). Table 4 sum-
maries the identity of organisms grown from
contaminated cultures. Coagulase negative
staphylococci (CoNS) were by far the most
common isolates. There were 12 neonates
from whom CoNS were isolated from blood
and CSF simultaneously. In each case the
isolate from the CSF was distinguishable from
that in the blood by antibiogram (differing by
three or more sensitivity tests from a panel of
douE). Three of these infants were treated for
CoNS septicaemia for between nine and 13
days; the CSF isolates were regarded as con-
taminants. In the other nine cases the CoNS
in both the blood and the CSF were
regarded as contaminants. CoNS were not iso-
lated from subsequent CSF samples obtained
from these babies and none developed a
clinical picture suggestive of meningitis.
In the light of the apparent lack of value of
enrichment culture, we reviewed the contribu-
tion of this procedure in the diagnosis of cases
of meningitis recorded in the period 1 May
1984 to 30 September 1988. Of the nine cases
diagnosed antemortem by lumbar puncture
two grew organisms only on enrichment. Both
had been classified as probable cases.

Discussion
Neonatal meningitis is uncommon. The inci-
dence of 0.36/1000 live births/year which we
observed in Oxford is comparable with a
national figure of 0.32/1000/year reported for
England and Wales.9 The proportion of babies
admitted to the unit who underwent lumbar
puncture is high (42%). This is a reflection of
the difficulty of making the diagnosis of meni-
genial infection on clinical grounds in sick
neonates. Thus lumbar puncture is performed
as a screening procedure in a population with
known risk factors for meningitis but where the
prevalence of the disease is low (0.95%).
Under these conditions there is a need for a
test with high sensitivity. However, attempts to
increase the sensitivity of a test may lead to a
fall in specificity. One third of samples in our
study were inadequate for cytological assess-
ment either because they were too small or
grossly bloodstained. Others have found that
between 15 and 50% of samples are unsuitable
in this way.8,10 Furthermore up to 15% of
neonates may have bacterial meningitis in the
absence of a CSF pleocytosis.8 Thus greater
emphasis must be placed on the results of
Gram stain and culture.
Gram staining is a less sensitive method than
culture for detecting micro-organisms. The
sensitivity of 68% for the two review periods is
acceptable, given the low prevalence of menin-
gitis in the study population. This compares
well with the 80% sensitivity observed in
studies of childhood meningitis where the
prevalence of the disease is much higher.11,12
The two cases of candida meningitis in the
combined review periods both generated false
negative Gram stain results. This emphasises
the difficulty of diagnosing this condition
rapidly.13 However, a negative Gram stain in a
neonatal lumbar puncture should reassure the
clinician as this has a high negative predictive
value (99-9%). The low positive predictive
value of Gram staining (46%) is a consequence
of the low prevalence of meningitis in this
population. In all false positive Gram stains
very few organisms were seen on the film.
Specificity could be improved if these were not
reported. However, considering the 16 cases of
meningitis diagnosed by lumbar puncture in
the combined review periods, very few
organisms were seen on films in three cases of
true meningitis and therefore sensitivity
would suffer. No particular morphological type
was associated with false positivity or true
positivity. The causes of false positive Gram
stains include contamination of specimen
tubes, glass slides and Gram reagents,14 and
the epithelial cells from the child's skin.15
However, this is the first study to quantify the
size of the problem in a large series of tests.

The interpretative value of primary culture
is similar to Gram staining, though culture is
more sensitive (81%). Though the positive
predictive value is low (46%), interpretation is
made easier by the amount of growth and the
identity of the organism. Cultures growing
group B streptococci were always significant.
Cultures yielding CoNS were, in our series,
always contaminants. The significance of
aerobic Gram negative rods and candida was difficult to assess as these were found as both contaminants and significant cultures. We observed a non-significant trend towards an increased yield of true positives from late lumbar punctures (4/310, 1.3%) compared with early (3/586, 0.5%). This has also been observed by others and may reflect different indications for lumbar puncture in the two groups, maternal reasons being more important for suspected early onset infection.10

We have not evaluated the effect of antibiotic treatment given during labour. A recent review of practice in the USA showed that 8.8% of babies had been exposed to antibiotics in this way.10 This may influence the yield of cultures from early lumbar punctures. However, we found only one baby with partially treated meningitis, and this child's cultures were negative because he had himself received antibiotics. Partially treated meningitis appears to be uncommon in neonatal units.10 This may be because lumbar puncture is usually done before antibiotics are started and/or because the high proportion of inadequate specimens means that if cultures are negative, no further interpretation can be made.

Enrichment culture is designed as an exquisitely sensitive technique to resuscitate organisms damaged by the host response and antibiotic treatment. In the second review period (1988–91) 896 enrichments were performed generating 101 false positives without improving on the sensitivity of primary culture. Combining the two review periods and given an annual lumbar puncture rate of about 300 a year, two cases of probable meningitis were detected by an estimated 2225 enrichments which would have generated an estimated 240 false positives. There are few data available on specificity of CSF culture as most series exclude false positive results,11 12 yet such data are vital to the doctor at the bedside who is attempting to interpret the clinical significance of the result. A recent review of practice in a neonatal unit in the USA where the laboratory used equivalent techniques to our own, showed that of 1104 lumbar punctures performed in 826 babies only 17 cultures were positive and of these 12 were considered false positives.10 The identity of organisms grown on enrichment may help with interpretation as most are derived either from skin (CoNS and diphtheroids) or the environment (aerobic sporining bacilli, Pseudomonas sp). However, most of these organisms have also been described as rare causes of meningitis in neonates and the generation of so many false positives may lead to diagnostic uncertainty, repeat lumbar punctures, and unnecessary antibiotic treatment. The origin of these contaminants is uncertain. They could arise at both the bedside and/or during handling in the laboratory.

In conclusion, because lumbar puncture is performed in neonates as a screening procedure, the bacteriological tests carried out behave quite differently when compared with the same tests performed on CSF from adults or children suspected to have meningitis where the prevalence of the disease being sought is greater. The positive predictive value of Gram staining in the neonatal lumbar puncture is so low that unless more than a few organisms per several high power fields are visualised empirically antibiotic treatment should not be altered on the basis of this test alone. Primary cultures have adequate sensitivity, and though positive predictive value is low, the identity of organisms aids interpretation. To perform enrichment culture on all lumbar punctures from neonates does not seem cost effective. It should be confined to samples from babies who have already received antibiotics, or where organisms are seen on the Gram stain, or where there is a raised white blood cell count in the CSF. Clinicians should be aware that diagnostic tests performed in populations with a low prevalence of disease are likely to generate many false positive results and have a low positive predictive value. Systematic review of the performance of laboratory tests against clinical gold standards leads to more rational use of laboratory resources and improves interpretation at the bedside.

5 Spiedel BD. Adverse effects of routine procedures on preterm infants. Lancet 1978; i: 864–5