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LETTER TO THE EDITOR

# Enrichment culture of CSF is of limited value in the diagnosis of neonatal meningitis

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## Introduction

Neonatal meningitis is difficult to diagnose due to the subtle and nonspecific symptoms in neonates, and confirmation requires cerebrospinal fluid examination (CSF) [1]. Gram stain, culture of CSF directly onto agar plates, and broth enrichment culture are well established methods for diagnosing bacterial meningitis [2–5]. Other methods under evaluation include use of bacterial polymerase chain reaction combined with DNA sequencing [6].

The aim of CSF broth enrichment culture is to facilitate the isolation of damaged organisms and to recover those present in small numbers [7, 8]. The exact origin of enrichment culture is unknown [9]. Beijerinck and Winogradski are believed to be the first to recommend enrichment techniques [10].

We previously reported on the utility of various microbiology tests for the diagnosis of bacterial meningitis in the newborn [7]. We showed that enrichment cultures (inoculation of CSF into a brain-heart infusion broth incubated for 48 hrs) when performed on all lumbar puncture specimens are often falsely positive, because the prevalence of true bacterial meningitis is low in neonatal intensive care units and the test lacks specificity. We suggested that enrichment culture should be confined to settings where the prevalence of bacterial meningitis was higher, such as in samples with raised CSF white cell count (WCC), where organisms are seen on the Gram stain or where pathogens may be difficult to grow such as when babies have already received antibiotics.

The aim of our current study was to assess the performance of enrichment culture when performed on CSF samples selected on the basis of a raised WCC of ≥30 /mm$^3$.

## Methods

We conducted a retrospective study at the Neonatology Department of the John Radcliffe Hospital, Oxford, UK (a tertiary referral centre serving a population of approximately 600,000 people) between January 2006 and December 2008. In the study period 400 lumbar punctures were performed. Of these, 13 CSF samples were excluded from analysis because the patients' notes were unavailable. Thus a total of 387 lumbar punctures from 276 neonates were studied. We defined meningitis as follows:

| | |
|---|---|
| Probable meningitis | Suggestive clinical features together with either a positive enrichment culture or, if the culture was negative, a positive Gram stain or high WCC (≥30/mm$^3$). |
| Definite meningitis | Suggestive clinical presentation supported by positive direct culture from CSF inoculated onto agar plates [1]. |

We did not study the effect of prior antibiotic therapy.

## Results

There were four cases of definite meningitis and 12 cases of probable meningitis during the study period. Gram stain

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**Table 1** Value of Gram stain, direct culture and enrichment culture in the diagnosis of probable and definite meningitis in neonates

| Parameter | Gram stain (%) $n$=385 | | Direct culture (%) $n$=387 | | Enrichment (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | All samples $n$=127[a] | | Normal WCC (< 30 cells/mm$^3$) $n$=31[a] | | Raised WCC (≥ 30 cells/mm$^3$) $n$=45[a] | |
| Sensitivity | 2/16[b] | (12%) | 3/16[b] | (18%) | 3/11[b] | (27%) | 1/1[b] | (100%) | 2/10[b] | (20%) |
| Specificity | 369/369 | (100%) | 370/371 | (99%) | 107/116 | (92%) | 25/30 | (83%) | 32/35 | (91%) |
| Positive predictive value | 2/2 | (100%) | 3/4 | (75%) | 3/12 | (25%) | 1/6 | (16%) | 2/5 | (40%) |
| Negative predictive value | 369/383 | (96%) | 370/383 | (96%) | 107/115 | (93%) | 25/25 | (100%) | 32/40 | (80%) |
| False positive rate | 0/369 | (0%) | 1/371 | (0.2%) | 9/116 | (7%) | 5/30 | (16%) | 3/35 | (8%) |
| False negative rate | 14/16 | (87%) | 13/16 | (81%) | 8/11 | (72%) | 0/1 | (0%) | 8/10 | (80%) |

*n* number of samples in each group, *WCC* white cell count, *CSF* cerebrospinal fluid

[a] Enrichment culture was performed on 127 samples from a total of 387 CSF samples. 51 samples were unsuitable for cell count due to, for example, clotting. From the remaining 76 CSF samples there were 45 samples with raised WCC (≥ 30 cells/mm$^3$) and 31 samples with normal cell count (< 30 cells/mm$^3$)

[b] The denominators differ because enrichment culture could not be performed on 5 samples from the 16. One CSF sample was a definite meningitis (positive direct culture and enrichment culture) with normal WCC (< 30 cells/mm$^3$)

was performed on 385 of 387 samples and direct culture was performed on all 387 samples. Enrichment culture was performed on 127 of 387 CSF samples. Criteria for performance of enrichment culture were a WCC≥30/mm$^3$, organisms seen on Gram staining, or when the specimen appeared blood-stained or was clotted. Of the 127 samples where enrichment culture was performed, 45 samples had raised WCC (Table 1). Table 2 shows the organisms isolated on enrichment culture in all samples stratified by WCC and presence of meningitis.

For the diagnosis of definite and probable meningitis, Gram stain had a sensitivity of 12% (2/16) and a specificity of 100% (369/369) with no false positives. The sensitivity of direct culture (18%, 3/16) and enrichment culture with raised CSF WCC (20%, 2/10) did not differ significantly. The false positive rate of enrichment culture with raised CSF WCC was significantly higher than of direct culture (8% [3/35] versus 0.2% [1/371], $p$=0.002). However, the false positive rate of enrichment cultures (8%) was lower than the 100% found in our previous report (out of 896 samples 101 were positive and all were false positive) where enrichment cultures were performed on all CSF samples.

## Discussion

Our study, to our knowledge, is the first to evaluate the predictive value of enrichment culture in CSF samples selected on the basis of a raised WCC for the diagnosis of neonatal meningitis. We found that even when enrichment culture is done selectively, on samples with a raised WCC, the test does not significantly increase sensitivity and generates many false positives. A previous study by Lessing et al. [8] assessed the value of enrichment culture when performed on all CSF

**Table 2** Results of enrichment culture and disease on CSF samples with no WCC, normal WCC (< 30 cells/mm$^3$) and raised WCC (≥ 30 cells/mm$^3$)

| Organism | No WCC | | Normal WCC (< 30 cells/mm$^3$) | | Raised WCC (≥ 30 cells/mm$^3$) | |
|---|---|---|---|---|---|---|
| | *n* | Meningitis | *n* | Meningitis | *n* | Meningitis |
| Coagulase negative *Staphylococci* | 1 | No meningitis | 1 | No meningitis | 3 | 2 samples no meningitis, 1 probable meningitis |
| *Streptococcus viridans* species | | | 2 | Both no meningitis | 1 | No meningitis |
| *Pseudomonas aeruginosa* | | | 2 | 1 sample no meningitis, 1 definite meningitis | 1 | Definite meningitis |
| *Streptococcus mitis* | | | 1 | No meningitis | | |
| No organism | 50 | All samples no meningitis | 25 | All samples no meningitis | 40 | 32 samples no meningitis, 8 probable meningitis |
| Total | 51 | | 31 | | 45 | |

*CSF* cerebrospinal fluid, *WCC* white cell count

samples in the diagnosis of acute bacterial meningitis in children and found that from 219 enrichment samples 217 were false positive and two were true positive. These two true positive samples either had a raised CSF WCC or abnormal Gram stain. They concluded that CSF enrichment culture should be confined to samples with raised WCC.

Studies performed in adults on the value of broth culture for the diagnosis of meningitis or CNS shunt infection differ on the value of restricting broth culture to samples with raised WCC [11, 12]. This could also be due to the difference in CSF parameters (WCC, protein) associated with meningitis and CNS shunt infections, small study size and retrospective nature of many of the studies.

A possible limitation of our study is the small sample size. The prevalence of meningitis in our sample is low but this does reflect the low prevalence of meningitis in the neonatal population admitted to neonatal intensive care units.

The organisms isolated from enrichment cultures in our study, coagulase negative *Staphylococci*, *Pseudomonas aeruginosa* and *viridans Streptococci* are all organisms commonly associated with contamination. They are very rarely implicated in cases of bacterial meningitis [1].

In conclusion, since the prevalence of bacterial meningitis in neonates is low, selecting CSF samples for enrichment culture on the basis of raised CSF WCC reduces the false positive rate compared to when the test is performed on all CSF samples examined for suspected meningitis. However, it does not improve sensitivity and has a higher false positive rate than direct culture. Therefore, even when applied selectively to those samples with the highest likelihood of culturing a pathogen, enrichment culture has limited value in the neonatal setting.

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