

Reagent Strips as an Aid to Diagnosis of Neonatal Meningitis in a Resource-limited Setting

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ABSTRACT

Background: Without early recognition and treatment, neonatal meningitis (NM) has a high mortality and morbidity. Although some neonates have features of NM, many do not. In many low-resource settings, the laboratory support to diagnose NM is not available, and bedside diagnostics are needed.

Methods: This retrospective study was conducted in a neonatal unit in Uganda. Clear cerebrospinal fluid samples were routinely screened for glucose, protein and leukocytes on a Combur[®]-10 urinalysis reagent strip. A definitive diagnosis was made using laboratory analysis. The results of the screening and definitive tests were compared.

Results: The reagent strip showed moderate sensitivity and high specificity for leukocytes $\geq 10 \times 10^6$ cells/l, high sensitivity for protein ≥ 100 mg/dl and high specificity for glucose < 50 mg/dl.

Conclusion: The use of reagent strips has the potential to improve and hasten the diagnosis of probable NM in settings where adequate or timely laboratory support is not available.

KEYWORDS: child health, paediatrics; neonatal sepsis; meningitis; Africa

BACKGROUND

Worldwide, over 6 million children aged < 5 years die annually; nearly all of these deaths are in the developing world and almost half of these deaths (44%) occur within the neonatal period [1]. Neonatal sepsis is one of the leading causes of neonatal mortality and is estimated to account for 23% of neonatal deaths worldwide and therefore at least 520 000 neonatal deaths globally each year [1]. Neonatal meningitis (NM) is an infection of the meninges, the membranes that surround the brain and spinal cord. It is a devastating illness, and in low-

income countries (LICs), it is associated with both high mortality and morbidity [2]. Although some cases of NM have specific features, many neonates with meningitis have no localizing signs [3]. It has been shown that up to 18% of infants with clinical neonatal sepsis can also have a positive cerebrospinal fluid (CSF) [4, 5]. It is therefore gold standard to perform a lumbar puncture (LP) on any neonate with signs of sepsis or meningitis.

In LICs, NM frequently remains a clinical diagnosis, as healthcare facilities often have inadequate laboratory support. Therefore, many cases of NM are

missed or inadequately treated [6]. Early recognition and diagnosis of NM can expedite the initiation of antibiotics and reduce the high mortality and neurological sequelae associated with NM [7]. In high-income countries, identification of the causative organism in the CSF remains the gold standard for the diagnosis of NM; however, culture positivity is low, and abnormal CSF parameters are often used to predict and support a diagnosis of NM. In LICs, culture methods are either not available or are limited; therefore, the laboratory examination of CSF for protein, glucose and leukocyte levels still remains the investigation of choice. Yet, in many LICs, the trained personnel, supplies and laboratory equipment are not always available to perform CSF analysis. Even when they are available, substantial delays in receiving results can occur, and in turn, this can delay the initiation of appropriate treatment. Point-of-care tests are therefore needed to ensure that all cases of NM are effectively diagnosed and treatment is both promptly and appropriately given.

It is possible that urinalysis reagent strips can improve diagnostic detection of NM in settings where robust and reliable laboratory support is not available. Other studies have investigated the utility of urinary reagent strips for semi-quantitative analysis of CSF in older children and adults finding it to be a rapid and relatively reliable method for diagnosing bacterial meningitis [8–11]. Although one of these studies included neonates, as of yet, no study has focused solely on the neonatal population.

METHODS

This was a retrospective study of routinely collected data, conducted in the Neonatal Unit in Mbale Regional Referral Hospital (NNU-MRRH) in eastern Uganda. In NNU-MRRH, it is routine to perform a LP on any neonate with clinical sepsis or suspected meningitis. Timing of the LP depends on the availability of a trained clinician; it is always performed within 24 h of admission and ideally before administering antibiotics. One of three trained clinicians, two non-specialist junior doctors and one clinical officer, performed all the LPs. The CSF samples were routinely sent for standard laboratory analysis to make a definitive diagnosis of NM. The CSF samples were analysed in the laboratory for glucose,

protein and leukocyte levels. The cell count was performed by microscopy using a Neubauer counting chamber and protein, and glucose analyses were done using an automated analyser (Cobas C111). Since July 2016, in addition to sending the CSF for laboratory analysis, it has been standard practice in NNU-MRRH to use Combur[®]-10 (Boehringer Mannheim) urinary reagent strips as a bedside screening test for meningitis whilst the laboratory results are pending.

Haemorrhagic samples cannot be interpreted using the reagent strips and were therefore not included in this analysis. For all macroscopically non-haemorrhagic samples, immediately following collection of the laboratory CSF sample, the Combur[®]-10 urinary reagent strip was used to screen the CSF for protein, leukocytes and glucose. Using a sterile 2 ml syringe, two drops of CSF were placed on the leukocyte, protein and glucose patches and the colour change compared with the reference chart on the reagent strip container. To allow a preliminary diagnosis of meningitis to be made rapidly, all colour changes were read immediately after the LP by clinician performing the procedure. If any of the three parameters were abnormal on the reagent strip, the treatment was adjusted to cover for NM. The treatment was later amended if needed when the final laboratory results became available.

The CSF white cell count is higher at birth than later in infancy. A normal CSF white cell count is $<20 \times 10^6$ cells/l in a term neonate. The Combur[®]-10 reagent strip is designed to detect leukocytes by the esterase activity, an enzyme primarily found in neutrophils. Depending on the colour change of the patch, the level of leukocytes can be graded as no colour change for $<10 \times 10^6$ cells/l, 1+ for $10-75 \times 10^6$ cells/l, 2+ for $75-500 \times 10^6$ cells/l and 3+ for $>500 \times 10^6$ cells/l. The reagent strip was considered positive if there was any colour change $\geq 1+$.

The CSF protein level is also higher at birth. Normal CSF protein level in a term neonate is up to 100 mg/dl. The reagent strip is able to detect protein from 30 mg/dl up to 500 mg/dl. Depending on the colour change of the patch, the level of protein can be graded as no colour change <30 mg/dl, 1+ for 30–100 mg/dl, 2+ for 100–500 mg/dl and

3+ for >500 mg/dl. The reagent strip was considered positive if the protein patch was $\geq 2+$.

A normal term neonate has CSF glucose of ≥ 45 mg/dl or a CSF: blood glucose ratio ≥ 0.6 . Measurement of blood glucose was not routinely possible, so only absolute values of CSF glucose were considered. For the glucose patch on the Combur[®]-10 strip, no colour change is glucose <50 mg/dl, 1+ for 50–100 mg/dl, 2+ for 100–300 mg/dl, 3+ for 300–1000 mg/dl and 4+ for >1000 mg/dl. If there was no colour change, then the reagent strip was considered positive for meningitis.

Convenience sampling was used for this retrospective study. The patient records between September 2016 and May 2017 were reviewed. Neonates were included when CSF samples had been taken, and both the reagent strip and definitive laboratory results were recorded. No record of the screening test result was recorded on the laboratory request, and therefore, the laboratory technicians were not aware of the preliminary results. The diagnostic accuracy of the three reagent strip patches was compared with the laboratory gold standards for glucose, leucocytes and proteins. A 2×2 cross-tabulation of reagent strip diagnosis vs. laboratory diagnosis was created for each parameter. Sensitivity, specificity, positive predictive value and negative predictive value were calculated for each individual parameter. The precision of the estimates was expressed by calculating the 95% confidence intervals (CIs).

Ethical clearance for this study was granted by the Mbale Regional Referral Hospital Research and Ethics Committee.

RESULTS

The records of neonates with clinical neonatal sepsis who underwent a LP to investigate the possibility of meningitis were identified. Between September 2016 and May 2017, there were 73 cases identified where both a reagent strip result and definitive laboratory result were available. The age ranged from 1 to 27 days. All cases were term neonates. For all cases, treatment was given according to the screening result from the reagent strip. Treatment was later adjusted based on the final laboratory result. The macroscopic

appearance of the CSF was not available. All 73 cases had a corresponding laboratory protein and leucocyte level available. Corresponding laboratory glucose levels were only available for 66 cases. The accuracy of the reagent strip is shown in Table 1.

Leucocytes

The reagent strip showed moderate sensitivity (63.6%, 95% CI 30.8–89.1%) and high specificity (81.8%, 95% CI 70.4–90.2%) for an overall leucocyte count $\geq 10 \times 10^6$ cells/l. Leucocyte differential counts were not reported.

Protein

The strip showed a high sensitivity (94.1%, 95% CI 71.3–99.9%) for protein ≥ 100 mg/dl, although it was less specific (79.0, 95% CI 66.1–88.6%).

Glucose

For glucose <50 mg/dl, the strip was highly specific (100.0%); however, the sensitivity was low (6%).

DISCUSSION

NM carries a high mortality and a high risk of complications such as postinfectious hydrocephalus, neurodevelopmental delay and cerebral palsy. All these risks are substantially higher in LICs where the delay to diagnosis or inability to diagnose NM increases them considerably. If the diagnosis and therefore treatment of NM are to be improved in such settings, then access to simple, affordable and sustainable bedside diagnostic tests is needed.

In this study, Combur-10 reagent strips were used to provide a rapid analysis of the CSF. The results suggest that CSF protein >100 mg/dl, leucocytes ≥ 10 cell/mm³ and glucose <50 mg/dl can be determined with reasonable accuracy using the reagent strip. The strip had a high sensitivity and specificity for proteins ≥ 100 mg/dl. Although the specificity for leucocytes was acceptable, the strip had only a moderate sensitivity for leucocytes $\geq 10 \times 10^6$ cells/l. For glucose, the strip was highly specific, although the sensitivity was low. The major difference between CSF and urine, for which the strips were designed, is the lower pH of the urine. It is possible that this variation in pH and the slight differences in the biochemistry of these fluids may have affected the

Table 1. Diagnostic accuracy of the reagent strip

Screening test	Reference standard	True positive	False positive	False negative	True negative	Sensitivity	Specificity	PPV	NPV
Positive leucocytes	$\geq 10 \times 10^6$ cells/l	7	12	4	54	63.6 (30.8–89.1)	81.8 (70.4–90.2)	36.8 (22.8–53.5)	93.1 (86.0–96.8)
Positive proteins	≥ 100 mg/dl	16	12	1	45	94.1 (71.3–99.9)	79.0 (66.1–88.6)	57.1 (44.3–69)	97.8 (87.0–99.7)
Negative glucose	< 50 mg/dl	2	0	30	34	6.3 (0.8–20.8)	100.0 (89.7–100.0)	100.0	53.1 (50.9–55.4)

Notes: NPV = negative predictive value; PPV = positive predictive value.

sensitivity and specificity of the strips for CSF. The development of a simple CSF specific strip with three patches for leucocytes, glucose and proteins would be highly beneficial.

Even in high-resource settings with adequate laboratory support, culture positivity of CSF remains low even in the presence of abnormal CSF parameters. This is partly because the majority of neonates get septic with low levels of bacteria. In addition, some bacterial pathogens are difficult to culture and require laboratory facilities that are often not available in LICs. Poor sensitivity is also because of antibiotics use in the community before seeking medical care, again particularly in LICs. Finally, the abnormal CSF parameters may be because of alternative pathologies, such as viral, parasitic, protozoal or fungal infections. As the availability of molecular testing for these pathogens increases, our ability to identify causative pathogens will improve. In the meantime, until a better test is available for the diagnosis of NM, many settings will continue to rely on CSF analysis. In settings where timely laboratory support is not available, the reagent strip has the potential to improve the detection of possible cases of NM. In these settings, where the risk of mortality and complications from undiagnosed NM is substantially higher such an approach has considerable benefits.

The results from our study for protein levels were comparable with that of Chikkannaiah *et al.* [8] in India who used the same reagent strips in a population aged 2 days to 75 years. For proteins ≥ 100 mg/dl, they observed a similarly high sensitivity (96.0%) and specificity (87.1%). The strip interpretation for glucose < 50 mg/dl was also similar showing a 100.0% specificity and only a 14.2% sensitivity. Their study did however show a higher sensitivity and specificity for leucocytes than reported in our study, 97 and 95%, respectively. Joshi *et al.* included patients from 1 day to 75 years. They demonstrated higher leucocyte sensitivity of 85.2% and specificity of 89.6%. For protein levels > 100 mg/dl, a sensitivity of 92.6% and specificity of 87.5% were comparable with our neonatal data. Similarly, they showed that a negative glucose < 50 mg/dl had a low sensitivity of 46.2% and high specificity of 98.0%. Visual colour comparison of the reagent strips is subjective and may account for much of the variation seen between these studies.

Although not reported in any of these studies, it is possible that the presence of microscopic red blood cells or bilirubin may have affected the interpretation.

Other similar studies that have used the Combur[®]-10 have not included neonates. Kumar *et al.* [9] used the Combur-10 reagent strip in children but did not include patients aged <3 months. They observed a positive correlation between the reagent strip and the laboratory tests for leucocytes, proteins and glucose of kappa 0.94, 0.82 and 0.82, respectively. Parmar *et al.* [10] also used the same reagent strip in children mainly aged 1–5 years and found a similar but slightly lower correlation between the strip and the laboratory tests of kappa 0.78, 0.78 and 0.75. In Brazil, Romanelli *et al.* [11] studied patients aged between 1 month and 12 years. They reported an overall sensitivity of 90.7% and specificity of 98.1% for the reagent strips.

Our study has several strengths. The CSF analysis was carried out blinded to the results of the reagent strip. It is the first study to focus exclusively on neonates, whose CSF parameters are different. Limitations of our study were the retrospective nature of the design and the small sample size. Although we were able to correlate the findings on reagent strip to the laboratory analysis, the microbiology results are not routinely carried out and were not available for comparison. As described above, it is possible that some of the cases treated for NM had elevated parameters because of other aetiologies such as viral meningitis.

This study has demonstrated that reagent strips can be used effectively as a screening test and that they correlate with laboratory CSF analysis. Whilst we continue to improve our understanding of the aetiology of NM in such settings and develop better rapid diagnostic tests for NM, reagent strips have the potential to improve our detection of probable NM.

CONCLUSION

Many hospitals in LICs lack the equipment and staff needed to perform CSF analysis and culture. Reliance on clinical detection of NM means that a significant proportion of sepsis cases are misdiagnosed and inappropriately treated. This study demonstrates that the use of urine reagent strips for

rapid CSF analysis in neonates is valuable. This technique is of benefit not only when laboratory facilities are not available but also to hasten the diagnosis of NM even when facilities are available. The development of a simple CSF specific strip with three patches for leucocytes, glucose and proteins with values that correspond more closely to the cut-offs that are used for the diagnosis of NM would be beneficial.

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