Failure of the urinary group B streptococcal antigen test as a screen for neonatal sepsis

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Abstract

The accuracy of the urinary group B streptococcal antigen latex agglutination (LA) test for screening infants at risk of group B streptococcal (GBS) sepsis in the first 24 hours of life was prospectively studied in 236 infants for six months. Infection with GBS was defined by a positive blood culture while colonisation was defined by GBS cultured from any other site. The combination of infection and colonisation was used as the gold standard for the LA test. Although the LA test had a sensitivity of 90%, the specificity was only 70%, the positive predictive value 12% and the false positive rate 30%. The overall accuracy was only 71%. The LA test was unable to predict GBS sepsis in infants at risk of the disease. The false positive rate was unacceptably high and could not be potentially accounted for in 11 infants. However, a negative test was useful in excluding GBS disease.

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Systemic group B streptococcal (GBS) infection remains the leading cause of neonatal sepsis in the first 24 hours of life¹ and has a significant mortality rate.² Early recognition and treatment of this disease is therefore paramount in any neonatal care facility.² Rapid confirmation of GBS infection has been reported using GBS antigen detection in body fluids including urine.³⁻⁶ Techniques for urinary GBS antigen detection include latex agglutination (LA), counterimmune electrophoresis (CIE), radioimmunoassay (RIA), and monoclonal sandwich assay (MSA).³⁻⁶ CIE is less sensitive and technically inferior to LA.³⁻⁷ RIA methods for GBS antigen detection have shown poor sensitivity, low specificity, and low positive predictive values.⁷ MSA is a highly sensitive technique, but is unfortunately too time consuming and technically complex to be used as a routine test.⁸ The theoretical advantages of the LA test include the detection of antigen at low concentrations, detection despite antibiotics, technical ease of use and commercial availability.^{3 4 8}

Initial trials assessing the accuracy of LA detection of GBS antigen showed sensitivities and specificities in excess of 95%. These trials were largely performed on patients with positive cultures of blood or cerebrospinal fluid and clinical evidence of sepsis, and used controls not at risk of GBS sepsis.³⁻⁶ More

recently, however, the accuracy of the LA test has been questioned because of reports of high false positive rates for the test.^{6 9}

The variance in sensitivity and specificity of the LA test may in part hinge on the definition of the gold standard of systemic GBS infection. The use of a positive blood culture only as the gold standard may underestimate the incidence of systemic GBS infection as only 46% of infants with early onset GBS pneumonia have a positive blood culture, the remainder having GBS cultured from surface swabs only.¹⁰ A better gold standard for systemic GBS infection includes a positive blood culture or surface colonisation in an unwell infant,¹¹ but in defining the false positivity of the LA test as a screening device, the appropriate gold standard should be the culture of GBS from important screening sites, such as blood, gastric aspirate, urine, cerebrospinal fluid, or swabs from the nose, groin, or umbilicus.

The clinical importance of a positive LA result in the total absence of bacteriological confirmation of GBS has been questioned.⁹ Possible explanations include insufficient blood for culture, maternal antibiotics,⁶ non-specific cross reactivity, colonisation of mucosal surfaces with GBS and systemic absorption of GBS antigen or direct contamination of urine by perineal flora.⁹ Perineal GBS colonisation is unlikely to cause false LA positivity.⁶ In an animal model GBS antigen absorbed through the gastric mucosa could be excreted in the urine and therefore result in a false positive result, although antigenuria was transient.¹²

We undertook to assess the accuracy of the LA test as a screening tool for infants at risk of GBS sepsis in the first 24 hours of life, to assess factors which may contribute to false positivity and to determine the validity of the test in a large tertiary perinatal hospital.

Methods

Infants who were at risk of systemic GBS infection in the first 24 hours of life were prospectively enrolled. Maternal risk factors included antenatal detection of GBS from a high vaginal swab, fever, offensive discharge, prolonged rupture of the membranes and suspicion of chorioamnionitis. Neonatal risk factors included unexplained birth asphyxia, respiratory distress, unexplained prematurity, fever, lethargy, anorexia, poor peripheral circulation or cyanotic episode.

Enrolled infants had blood collected aseptically via a peripheral arterial or venous puncture for a full blood count and blood culture. An external auditory meatal swab and gastric

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Diagnostic accuracy of LA test

	Group B streptococci		
	Present*	Absent	Total
LA positive	9	67	76
LA negative	1	159	160
Total	10	226	236
Sensitivity			90%
Specificity			70%
False positive rate			31%
Positive predictive value			12%
Negative predictive value			99%
Overall accuracy			71%

*Group B streptococcus cultured from any site.

aspirate to detect surface colonisation with GBS, a bag specimen of urine to detect GBS antigen by LA, and a perineal swab at the time of urine collection to exclude contamination of the bag specimen with perineal GBS were also collected.

The full blood count specimen was placed into an EDTA microtube and analysed on a Coulter S Plus II counter for platelet and total leucocyte count. White cell counts were corrected for nucleated red blood cells and differential blood counts were performed manually on Leishman stained blood smears by examining at least 200 cells. Each full blood count was ascribed a haematological sepsis score, as described by Rodwell.¹³ A score of 3 or more signifies an increased likelihood of sepsis.

At least 1 millilitre of blood was collected aseptically for culture and split between two Bactec culture media (PEDS and NR7A). The bottles were processed routinely on a Bactec 660 using a seven day protocol. The gastric aspirate was collected into a sterile container and processed for Gram stain, differential white cell count, and then cultured on to horse blood, chocolate and McConkey agar and cooked meat broth. Culture plates were examined at 24 and 48 hours for growth. Ear and groin swabs were obtained using sterile calcium alginate swabs. The specimens were cultured on to blood and McConkey agar and Trypticase soy broth and examined at 24 hours for bacterial growth. Urine for GBS antigen was collected by bag specimen after cleansing the perineum with sterile water. An aliquot of 0.1 ml of urine was cultured on to blood and McConkey agar and examined at 24 hours for bacterial growth to control for urine overgrowth with GBS as a possible explanation for a false positive LA result. All GBS were identified using colonial morphology and serotyping by agglutination. LA for GBS antigen was performed using the Wellcogen commercial LA kit. Tests were performed according to the manufacturer's instructions. The urine was heated to 100°C for five minutes and concentrated five-fold using Minicom B-5 concentrations before testing. Latex coated with rabbit antibody to GBS antigen was added to an aliquot of the specimen for testing. After mixing, agglutination indicated the presence of the GBS antigen in the specimen. Control latex coated with non-immune rabbit globulins was provided to exclude non-specific agglutination with the specimen. A polyclonal positive

control was used for within test quality control. Urine not processed within two hours of collection was refrigerated to prevent bacterial overgrowth.

Infants were excluded from further analysis if they did not have both blood culture and urine collected for antigen testing, if the LA result was equivocal and not repeated, or if it was positive but the groin not swabbed at the time of urine collection.

An extensive maternal and neonatal clinical evaluation was compiled, including maternal high vaginal swab for GBS (if collected) and the administration of maternal or neonatal antibiotics. An infant was defined as 'unwell' if it had any clinical signs of sepsis and raised haematological sepsis score. Possible trans-placental fetal absorption of maternal antigen to GBS was analysed where maternal HVS swab results were available. Neonatal ingestion of maternal bacteria acquired intrapartum was monitored by microscopy and culture of the gastric aspirate. Cross reaction of the latex with other bacteria was monitored using culture results of the urine when the LA test was positive. The possibility of cross reactivity of the latex with another non-bacterial antigen was not investigated in this study.

In this study a positive LA test was defined as a false positive if all cultures for GBS were negative while a true positive was recorded if any of the cultures were positive for GBS. A negative LA test was defined as a false negative if the LA test was negative with GBS being cultured from the infant's blood or if the infant was colonised with GBS and was unwell. A true negative result meant that the LA test was negative and there was no evidence of invasive GBS disease.

Results

During the second six months of 1992, 266 infants were prospectively enrolled: 30 were subsequently excluded. Nineteen had no urine collected, four did not have blood cultured (one had neither urine nor blood collected), five had equivocal LA results and three infants had a positive LA test but no groin swab.

The remaining 236 infants had a mean birthweight of 3090 g and a mean gestational age of 37.5 weeks; 62 were preterm (<37 completed weeks of gestation). With respect to the LA result, 159 infants had true negative results. Of the nine infants with a true positive LA result, two had a positive blood culture, four were unwell with GBS surface colonisation, and three infants had surface colonisation only. There were 67 infants with a false positive LA test. Only one infant qualified as a false negative result. He was colonised with GBS and was unwell but had both a negative blood culture and LA (table).

The LA test therefore had a sensitivity of 90%, a specificity of 70%, a positive predictive value of 12%, a false positive rate of 31%, with an overall accuracy of 71% (table).

Ten of the 67 infants with a false positive LA test had no gastric aspirate collected. Of the remaining 57 infants, in 22 cases neither the

mother (prenatally or postnatally if breast feeding) nor the infant received antibiotics. Eleven mothers of the remaining 22 infants had vaginal swabs for GBS, none of whom was colonised with the organism. The false positivity could therefore not be discounted in at least 11 infants.

Discussion

This study showed that in our hands the LA test had no value as a screening tool in predicting the presence of GBS in infants at risk of GBS infection. The LA test was defined as a true positive if an infant had confirmed GBS sepsis or was colonised with GBS, as we were assessing the test's efficacy as a screening device, not a predictor of disease. In infants where GBS disease is likely, however, the LA test is indeed accurate.⁴ In our study the LA did indeed have a sensitivity of 90%.

If a positive blood culture only was used as the gold standard, the LA test was insensitive (67%) and non-specific (68%), although it still gave a good negative predictive value (99%). Using a positive blood culture and colonisation in an unwell infant as the gold standard, there was little change in the sensitivity (86%) or specificity (69%). Although the positive predictive value fell to 8%, the false positive rate remained high at 31%. With this same standard, McIntosh and Jeffery¹¹ demonstrated a sensitivity of 88% and a specificity of 98% for the LA test. We are unable to explain the variation in our respective results.

The false positive rate of the LA test (31%) in our institution remains unacceptably high when the test is used as a screening tool. There are several possible explanations for this. Cross reactivity with other bacteria has been described.⁹ However, in no case of false positivity with the LA test did any other organism grow on culture. Contamination of the urine with perineal or rectal flora was postulated by Sanchez⁹ as a potential cause of false positivity. However, culture of the urine and groins of our infants could not explain any of the false positive results. As each urine specimen tested had positive and negative controls run in tandem, non-specific agglutination would not explain the high false positivity.

The suggestion that the antigen may be absorbed from the infant's stomach and excreted in the urine has been demonstrated by Ascher¹² using an animal model. Although the gastric aspirate was not collected in 10 of our infants with false positive LA tests, in the remaining 57 culture and microscopy of the gastric aspirate excluded this as an explanation for the false positivity.

Harris and colleagues have suggested that maternal antimicrobial treatment may be a cause of the apparent false positivity of the LA test.⁶ Even taking this into account, there were

still 22 false positive cases where neither the mother nor infant received antibiotics.

Maternal absorption of the antigen with subsequent transplacental passage might explain some of the false positive results. However, in 11 of the remaining 22 infants the maternal vagina was not colonised with GBS, the remaining mothers not being swabbed because this was not a routine procedure at our institution during the study period.

A final explanation of the false positivity of the LA test may be a cross reaction with another common non-bacterial antigen present in the infant's urine which has not yet been identified.

In conclusion, the LA test in our institution was inaccurate as a screening tool for GBS in infants at risk of early onset GBS disease and therefore its overall usefulness in cases of suspected GBS sepsis must also be questionable. We found the specificity to be poor, with a high false positive rate. Although all but 11 of the 67 false positive cases may be explicable, this is pure supposition. In field conditions the luxury of always having a complete set of investigations from both mother and infant never exists when determining the clinical relevance of a positive LA test. A positive LA test must therefore be interpreted with caution, particularly in a well infant, as this may lead to the unnecessary instigation or prolongation of antimicrobial treatment. The negative predictive value of 99% underscores the test's only value in this study as a reassurance of the absence of GBS sepsis in an at risk infant.

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