

A review of diagnostic tests for congenital syphilis in newborns

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Received: 24 October 2009 / Accepted: 13 February 2010 / Published online: 25 March 2010
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Abstract Congenital syphilis (CS) can occur when a mother is inadequately treated or not treated at all for an active *Treponema pallidum* infection. Symptoms of CS are often subtle and non-specific, and it is estimated that up to 60% of affected infants are asymptomatic at birth, making the diagnosis dependent on laboratory findings. Despite decades of experience with CS, problems still arise in its diagnosis because laboratory test results for children at risk for CS can be inconclusive and no single diagnostic test can be used to diagnose CS. The development of diagnostic tests such as enzyme immunoassays, immunoblotting and polymerase chain reaction (PCR) has increased the sensitivity and specificity of diagnoses, but the detection of specific IgM is currently the most sensitive serological method, and the presence of specific IgM should be considered as evidence of a congenital *T. pallidum* infection. Suspected cases can also be confirmed or excluded by serial post-partum tests of antibody kinetics. The authors note strongly that it is considered unethical not to treat a baby at risk of contracting CS, even without a definitive diagnosis. In this review, we describe the various microbiological methods—and their shortcomings—used in the laboratory diagnosis of CS.

Introduction

Congenital syphilis (CS) is caused by a *Treponema pallidum* infection acquired from a mother with untreated

or inadequately treated syphilis. Most infants at risk for CS can easily be identified by a positive maternal serologic test. However, the World Health Organization (WHO) estimates that maternal syphilis is responsible for 460,000 abortions or stillbirths and 270,000 low birth weight and/or premature babies every year (Table 1).

Transmission to the foetus can occur during any stage of a pregnancy, and while it usually occurs via the placenta, it may also occur during delivery if maternal genital lesions are present. The risk of CS transmission is also recognised to be higher in conjunction with a recent maternal infection and lack of treatment [15, 17, 18]. The estimated rates of vertical transmission in untreated women are 70–100% for primary syphilis, 67% for secondary syphilis and 40–83% for early latent syphilis [19]. Transmission from mothers with late latent syphilis to their infants can also occur, but at an estimated transmission rate of only 10% [20–22]. CS is also more likely to occur when the mother has high non-treponemal test titres (Venereal Diseases Research Laboratory, VDRL) of ≥ 16 at treatment or at delivery, and if short intervals between treatment and delivery (<4 weeks) occur [23, 24].

The diagnosis of CS is problematic. More than half of all infected infants are asymptomatic at birth, and the signs in symptomatic infants may be subtle and non-specific.

Clinical disease

CS can be divided into two clinical syndromes: early and late CS. Early CS is diagnosed during the first two years of life, and includes stillbirths. Late CS refers to cases that present after 2 years of age, and usually manifest near puberty. It mainly affects the bones, teeth and nervous system [25–28].

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Table 1 Overview of the reported sensitivity and specificity of IgM assays in newborns at risk or with clinical or asymptomatic congenital syphilis (CS) and healthy newborns

Test	Sensitivity	Specificity	Reference
FTA-ABS IgM assay			
Clinical CS	88–92%		[1, 2]
Asymptomatic CS	65–83%		[1, 2]
At risk for CS	ND		
Healthy newborns		91–98%	[1, 2]
(19S)FTA-ABS IgM assay			
Clinical CS	72–78%		[3–6]
Asymptomatic CS	25%		[5]
At risk for CS	2%		[3, 6]
Healthy newborns		100%	[5–7]
IgM immunoblot			
Clinical CS	83–100%		[4, 8–13]
Asymptomatic CS	100%		[8]
At risk for CS	4–42%		[4, 8, 10, 11, 14, 16]
Healthy newborns		67–100%	[4, 9–11]
IgM ELISA			
Clinical CS	88–100%		[3, 6, 8, 16]
Asymptomatic CS	86%		[8]
At risk for CS	2–66%		[3, 6, 8, 16]
Healthy newborns		100%	[6]

Early CS can present at any time before 2 years of age, but a review of the literature indicates that it usually presents in the neonatal period, and seldom later than 3–4 months of life. Most affected infants are asymptomatic at birth, with two-thirds developing symptoms by 3–8 weeks.

CS is responsible for a variety of clinical symptoms, the most characteristic of which are prematurity and low birth weight (10–40% of infants), hepatomegaly with or without splenomegaly (33–100%), a blistering skin rash (40%) and bone changes seen on X-ray (75–100%) [29, 30]. Other early signs include pseudoparalysis, respiratory distress, bleeding and fever [31]. Clinical examination is important. However, it is estimated that up to 60% of infants with CS are asymptomatic at birth [32]. Therefore, the diagnosis of CS in a newborn can only be made through a review of the mother's testing and treatment history, and through the infant's clinical and laboratory findings.

No single diagnostic test can diagnose CS, and it is considered unethical not to treat a baby at risk of CS, even without a definitive diagnosis. Diagnostic tests such as enzyme immunoassays, immunoblotting detecting specific IgM and polymerase chain reaction (PCR) have made the diagnosis of foetal syphilis more sensitive and specific, and clinical features such as hepatomegaly, jaundice and bone changes can resolve within three months of birth if symptomatic infants are given the appropriate therapy.

Laboratory diagnosis

The detection of *T. pallidum* during pregnancy and in newborns

The most sensitive and reliable method for detecting *T. pallidum* in clinical specimens has been the rabbit infectivity test (RIT). RIT provides definitive evidence of viable *T. pallidum*, but the use of the RIT as a routine diagnostic procedure is impractical because it requires animal testing and is not widely available. Dark-field microscopy has also been used to study amniotic fluid to confirm foetal infection. This method relies on fresh, good-quality specimens. The findings of spirochetes in amniotic fluid may be a marker for more severe foetal disease [33, 34].

The immunofluorescent staining of *T. pallidum* is an alternative to dark-field microscopy [35], but in both techniques, failure to find the organism by microscopy does not exclude a diagnosis of CS. The detection of *T. pallidum* by PCR in amniotic fluids, neonatal sera and neonatal cerebrospinal fluid (CSF) has proved to be a fairly sensitive alternative method (78–86%), with a reported specificity of 100% for diagnosing CS compared to RIT. This could prove to be an important additional tool to dark-field microscopy and treponemal serology [13, 14, 36, 37]. While one study found that RIT was more sensitive than PCR analysis, this was probably caused by inhibitory factors in the clinical specimens [36].

The use of non-*T. pallidum* tests in newborns

The first antibody test for syphilis, named after the bacteriologist August von Wassermann [38], was described in 1906 and was based on complement-fixation and reactivity to cardiolipin. His assay was the basis of the still widely applied non-treponemal tests, the Venereal Diseases Research Laboratory (VDRL) test and the rapid plasma reagin (RPR) test, both based on this same principle. Between 56 and 66% of infants born to mothers with positive syphilis serologic findings are positive for non-treponemal syphilis tests [39–41]. One of the diagnostic criteria sometimes used to diagnose CS is the detection of non-treponemal antibodies by the VDRL or the RPR test. This diagnostic criterion requires that the infant's VDRL/RPR titre be at least four-fold (two or more dilutions) greater than that of the mother's serum at birth, indicating foetal antibody synthesis. This suggests CS, but should not be the only marker used to make the diagnosis [12, 42]. The estimated sensitivity of this method is only 4–13% [40, 42–44], with a specificity of 99% [40].

Most newborns with a four-fold titre ratio had obvious symptoms, and all had abnormal findings that enabled the diagnosis of CS to be made without the use of this criterion [40]. In addition, no asymptomatic CS infections were detected with this method. It has also been suggested that the height of the titre of non-treponemal assays may be more useful than the ratio of newborn and maternal titres, since all newborns with RPR titres of 1,024 or greater had CS, while all newborns with titres lower than 1,024 did not [40].

For the above reasons—and given their low sensitivity—the clinical application of non-treponemal tests seems to be very limited. However, the diagnosis of CS can be excluded if the VDRL/RPR tests become non-reactive before the age of six months in an infant who has not received treatment [45].

The detection of *T. pallidum*-specific IgG in infants

The *T. pallidum* haemagglutination assay (TPHA), the *T. pallidum* particle agglutination assay (TPPA) and the fluorescent treponemal antibody absorption test (FTA-ABS) are more specific serological tests for syphilis, but serology cannot differentiate between the other treponematoses (yaws, pinta and bejel). While they demonstrate IgG as well as IgA and IgM antibodies to *T. pallidum*, they do not distinguish among them. Since IgG from the mother is passively transferred to the newborn, a reactive serologic test in the neonate does not necessarily indicate that the infant is infected. It can be due to maternally derived IgG. Although fetuses are capable of producing IgG as early as 12.5 weeks of gestation, most immunoglobulin of this class at birth is of maternal origin [46]. Thus, it is not surprising that the IgG reactivity of an infant's sera mirrors that of its

mother in most instances, making IgG detection in newborns of limited value for diagnosing CS. In general, it is not used in routine diagnostics as a method of confirming infection in newborns. However, as in the case of non-treponemal tests, the TPPA/TPHA and FTA-ABS tests can be used to exclude CS if the tests are non-reactive before the age of one year in an infant who has not received treatment, or if these serologic tests are non-reactive in the mother and the infant [45].

The detection of *T. pallidum*-specific IgM in infants

The detection of specific IgM in a newborn is a very reliable serological method for the diagnosis of CS because maternal IgM, in contrast to IgG, does not cross the placenta. The foetus is capable of mounting an immune response by 24 weeks of gestation and can produce specific IgM antibodies [47, 48]. While a negative IgM result cannot exclude the diagnosis of CS, a positive result should be considered as evidence of a congenital infection with *T. pallidum*. IgM tests can be negative, particularly in cases of early infection, and it should be recognised that the diagnosis of CS at birth is not always possible, no matter which serologic test is used. However, IgM testing can be used to identify women with a higher likelihood of active infection and who are, therefore, at a higher risk of infecting their babies. Performing the IgM test on mothers has sometimes been more effective at identifying infected babies than performing the IgM test on the babies themselves [49]. Three different methods are currently used to detect treponemal-specific IgM in neonates for the diagnosis of CS: (1) 19S FTA-ABS tests, (2) IgM immunoblots and (3) IgM ELISAs.

(1) 19S FTA-ABS Because *T. pallidum* antigens are not all unique, and antibody reactivity with some of them may exist in normal serum, an absorption step was needed that used a non-pathogenic treponeme (*T. reiteri*, now called *T. phagedenis*). This gave rise to the more specific FTA-ABS test. A modification of the FTA-ABS test to detect IgM to *T. pallidum* was introduced in 1968 [2]. However, clinical testing in newborns showed a false-positive rate of 10% and a false-negative rate of up to 35% [1]. The protocol was, therefore, modified to improve the performance of the FTA-ABS test for IgM detection, with separation of the IgG fraction (maternal) from the IgM fraction (sedimentation coefficient 19S, foetal) prior to the FTA-ABS test. The 19S (IgM) FTA-ABS test is usually only available in specialised reference laboratories [32]. The reported sensitivity of the 19S FTA-ABS test in clinical CS cases is 72–77% [3–6], with a specificity of 100% [5–7]. One to two percent (1–2%) of asymptomatic children at risk were found to be positive for IgM [3, 5, 6]. Following IgG and rheumatoid factor (RF)

removal, there was an improvement in the specificity of the FTA-ABS IgM test, but this occurred at the expense of a loss of sensitivity, particularly in asymptomatic newborns [5].

(2) *IgM immunoblot* Approximately ten different treponemal proteins are identified with IgM antibodies present in the sera of infected, symptomatic infants with immunoblot techniques. Reactions with 47, 17 and 15.5 kDa were found in most reactive blots, together with a variety of other reactions, including those corresponding to 72, 45, 42, 37, 34.5, 31, 24 and 7 kDa [4, 50]. Not all of the infected infants' sera reacted with all these antigens, but the 47-kDa band is one of the major immunogens in neonatal IgM antibody production in CS [4, 9, 10, 51] and is also detected in the blood of foetuses [34]. The 45- and 42-kDa antigens represent either breakdown products of the 47-kDa antigen or other related molecular weight derivatives [52].

IgM immunoblot results are considered to be reactive in neonates when one or more of the 47-, 45-, 30- and 15/17-kDa reactive bands are present [49]. Lewis et al. used an even stricter interpretation in which the presence of at least five visual bands (including 47, 17 and 15.5 kDa) in serum samples was required to be considered as a reactive blot (the others were 42, 34.5, 31 and 24 kDa). In CSF, any IgM-reactive band in the 47 to 15.5 range was considered to be positive [4].

The IgM immunoblot is very effective in confirming CS in symptomatic infants, with a reported sensitivity of 83–100% [4, 9, 10, 34]. In samples from asymptomatic infants at risk for CS, 4–22% were reported to be reactive in the IgM immunoblot [3, 4, 10, 11, 14]. The patterns of IgM reactivity were similar for both asymptomatic and symptomatic infants [10]. The IgM immunoblot was more sensitive than the FTA-ABS IgM assay when used for the screening of newborns at risk for CS [4]. No reactivity in an IgM immunoblot was detected in the control groups (infants born from adequately treated mothers or healthy controls or children with similar symptoms but with other causes [4, 9–11]), leading to a specificity of 100%.

(3) *IgM ELISAs* Both in-house and commercially available IgM ELISAs have been evaluated for the detection of IgM responses in CS and asymptomatic cases [3, 6, 8]. Most IgM ELISAs are based on sonic extracts of *T. pallidum* or a mix of cloned antigens [53]. The sensitivity (88–100%) and specificity (100%) of the IgM ELISAs are high, and positive IgM results of 3–7% are detected in asymptomatic cases at risk for CS. Not many studies have compared the IgM ELISA to the IgM immunoblot, but the IgM ELISAs seem to be slightly less sensitive compared to the IgM immunoblot assays. A total of 21 out of 25 infants with a positive IgM blot were also positive in the IgM ELISA. Two of the false-negative ELISA results were in patients with single bands on

the immunoblot [8]. However, the IgM ELISA is an acceptable alternative for laboratories that have less experience with immunoblotting, and it is easier to perform.

The detection of *T. pallidum*-specific IgM in CSF

Neurosyphilis is believed to occur in 60% of infants with symptomatic CS, as judged by the presence of CSF abnormalities, such as reactivity in the VDRL test, pleocytosis and elevated protein content [54]. Others report that neurosyphilis is less common in infants with CS [14, 43], especially if the infants are asymptomatic. Guideline recommendations include a CSF examination in infants of untreated or inadequately treated mothers [55, 56]. However, lumbar punctures in newborn infants are invasive and not entirely innocuous procedures.

A specific IgM response to *T. pallidum* antigens—particularly the 47-kDa antigen—was detected in the CSF of infants, showing clinical and laboratory evidence of CS, with fewer additional reactions than in the serum [43]. However, the IgM immunoblotting of serum identified all infants who had a *T. pallidum* infection of the central nervous system [57]. The sensitivity of IgM immunoblotting in CSF was low, suggesting that this test is of limited use [57]. Comparison of IgM detection in CSF by immunoblot (2/7) and the detection of treponema in CSF by RIT (6/7) and PCR (5/7) revealed that the detection of *Treponema* in CSF was more sensitive than IgM detection [14].

None of the CSF samples from children with asymptomatic CS were IgM-positive [4, 11, 43] and no IgM was detected in the CSF of infants with late-onset disease [43]. Therefore, no benefit from CSF examination can be expected in an asymptomatic infant.

Follow-up serology

All seroreactive infants (or infants whose mothers were seroreactive at delivery) should receive careful follow-up examinations and serologic testing at one month and at least every two to three months thereafter until the maternal antibodies have disappeared and the serological tests become non-reactive. In the case of an IgM-positive response at birth, treatment should be started and follow-up tests arranged at one, two, three, six and 12 months in order to assess response to treatment. After treatment in adults, the IgM antibodies usually disappear within one year, but can persist in a minority of patients [58]. In the absence of detectable IgM in the newborn, or if the IgM test is inconclusive, the suspected diagnosis can be confirmed or excluded by serial post-partum tests of antibody kinetics only.

During follow-up, non-treponemal test reactivity should decline by three months of age, and should be non-reactive

by six months of age if the infant was not infected. The serological response after therapy may be slower for infants treated after the neonatal period (>1 month). Treponemal tests are less effective for evaluating treatment responses because the results for an infected child can remain positive, despite effective therapy. IgG antibodies are eliminated in non-infected infants, with a half-life of 20.5 days, so passively transferred maternal treponemal antibodies can be present in an infant until 15 months of age [59].

A reactive treponemal test after 18 months is diagnostic of CS. If the non-treponemal test is non-reactive at this time, no further evaluation or treatment is necessary, as long as there is documentation of adequate therapy in the neonatal period. If the non-treponemal test is still reactive at 18 months of age, the infant should be re-evaluated and treated for CS. Given the appropriate therapy, the serological markers (RPR/VDRL and IgM) in symptomatic infants usually disappear within six months [9].

Cord blood versus serum samples

The Centers for Disease Control and Prevention (CDC) suggests serological testing for syphilis on a newborn's serum sample, rather than on the cord blood. This is because serum samples show fewer false-positive results, whereas cord blood samples can become contaminated by maternal blood [55]. Most cord and newborn serum samples (79/87, 91%) were within two tube dilutions of each other in the RPR test [40]. If newborn serum samples were taken to be the true-positive samples, then the number of false-positive cord blood RPR tests was 17/171 (10%). The number of false-negative cord RPR tests was reported to be 9/171 (5%) [40].

Rheumatoid factors in CS

The interference of RFs in the measurement of IgM antibodies in adults against *T. pallidum* was described as early as 1959 [60]. IgM RF has also been detected in CS, and over 90% of infected infants have circulating RF [61, 62]. Elevated RF levels were found to correlate with liver and renal involvement, as well as the extent of the disease. Following treatment, both RF levels and VDRL titres in the infants declined at a similar rate. These findings indicate a close relationship between the disease process and IgM RF levels [61].

Infant sera that contained RF maintained its reactivity even after purification of the IgM and removal of the maternal IgG. This suggests that rigorous fractionation of serum immunoglobulins may not be necessary for IgM immunoblot analysis in neonates [4, 9, 10]. However, all of the serum sample tests from asymptomatic infants at risk, characterised by very the faint staining of only a few IgM

reactions, yielded non-reactive blots when they were fractionated and the IgM fractions were retested [4].

Conclusion

All infants born to mothers who have reactive non-treponemal and treponemal test results should be examined thoroughly for clinical evidence of congenital syphilis (CS). Furthermore, symptoms of CS are often subtle and non-specific, and up to 60% of affected infants are asymptomatic at birth, so all infants at risk should be evaluated by serological assays.

Because maternal IgM does not cross the placenta, an IgM-positive neonatal serum should be considered as evidence of congenital infection. The IgM immunoblot currently seems to be the best available method to detect *Treponema pallidum*-specific IgM in neonates [51]. The IgM immunoblot is able to identify infants with clinical CS with high sensitivity, and IgM reactivity is sometimes the only evidence of an active infection in asymptomatic infants [4, 14]. Suspected cases can also be confirmed or excluded by serial post-partum tests of antibody kinetics.

Although tremendous progress has been made over the years in the new serologic techniques applicable to CS, there is still no single serologic assay that will reliably diagnose or exclude CS in young infants. Since it is considered unethical not to treat a baby at risk of CS, even without a definitive diagnosis, treatment decisions should not be based on definitive evidence of CS only, but also on the likelihood of infection.

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