THE IMPORTANCE OF IgM POSITIVITY IN LABORATORY DIAGNOSIS OF GESTATIONAL AND CONGENITAL SYPHILIS

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From January 1, 2009 through December 31, 2011, from 33,753 blood samples for syphilis screening, *Treponema pallidum* infections were confirmed in 241 pregnant women at the Department of Dermatology, Venerology, and Dermatooncology of Semmelweis University Budapest. In this period, four children born to inadequately or untreated women were confirmed to have congenital syphilis. The height of rapid plasma reagin (RPR) titer was measured to determine the stage of the infection and to examine the success of the antilues therapy. The diagnosis of maternal syphilis infection was confirmed with enzyme linked immunosorbent assay (ELISA), *T. pallidum* particle agglutination (TPPA), and IgG and IgM immunoblots. Maternal IgM immunoblot results identify mothers at risk of delivering babies with congenital syphilis better than the height of maternal RPR titer. The standard serological tests are less useful in newborns because of IgG transfer across the placenta. IgM test which depends on the infant's response has more specificity in diagnosing congenital syphilis.

Keywords: maternal IgM, fetal IgM, immunoblot, RPR, congenital syphilis

Introduction

Although risk of fetal infection is much higher during early maternal syphilis (the first year of infection) than during later stages, *Treponema pallidum* can be transmitted from the bloodstream of the infected woman to her developing fetus at any time during pregnancy [1]. Current Hungarian guidelines suggest that all pregnant women should be tested for *T. pallidum* infection in the first trimester, but some laboratory uses only rapid plasma reagin (RPR), a nontreponemal test for screening. Nontreponemal tests become positive only 6 weeks after exposure [2], in secondary syphilis they can show a false-negative result because of prozone effect [3], and despite that they are reactive in the early latent stage, their reactivity decreases with increasing latency [4]. Treponemal tests ELISA or TPPA can be used for screening, in all stages with or without signs and symptoms of syphilis [2]. Rawstron et al. determined maternal IgM status to be more helpful in identifying babies at high risk of congenital infection than a maternal RPR titer $\geq 1:16$ [5]. Our study was undertaken to evaluate *T. pallidum* IgM status in 241 mothers with syphilis to determine if positive tests indicate a risk of congenital syphilis better than a reactive rapid plasma reagin test.

Because standard serological tests for syphilis detect both immunoglobulin IgG and IgM, including transplacentally acquired maternal IgG, they cannot be used to provide a laboratory diagnosis of congenital syphilis when a single serum sample is tested. In this study, *T. pallidum*

IgM immunoblots were evaluated for the identification of babies with congenital syphilis.

Materials and methods

We used rapid plasma reagin (RPR) (Omega Diagnostics, UK), *T. pallidum* particle agglutination (TPPA) test (Fujirebio Inc., Japan), and enzyme immunoassay (Syphilis II-EIA) test (BioRad, France) to investigate 241 maternal serum samples and 242 serum samples of the babies born to these mothers. IgM immunoblots (MAST, UK) were prepared from all the 483 serum samples, but the IgM–IgG complex and the maternal IgG were evaluated with Mast-sorb (MAST, UK) from the infants' serum. IgG immunoblots (MAST, UK) were prepared from the 241 maternal serum samples. One of the infants had symptoms like neurosyphilis; this diagnosis was confirmed with Venereal Disease Research Laboratory (VDRL) test (Omega Diagnostics, UK) and TPPA in the cerebrospinal fluid of this infant.

Results

Positive syphilis serology was noted in 241 pregnant women of the 33,753 delivering serum samples at the Department of Dermatology, Venerology, and Dermatooncology of Semmelweis University, Budapest, Hungary from January 1, 2009 through December 31, 2011 (Table 1). In

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this three-year period, 230 of the mothers were served with an adequate prenatal care. A total of 217 cared mothers had already been adequately treated for syphilis before pregnancy; they were advised to repeat therapy, according to the Hungarian guidelines [6]. These women had RPR titer of 0–1:16; results of TPPA, EIA, and IgG immunoblot tests were positive, but IgM immunoblots results were negative. Children born to these mothers had RPR titers of 0–1:8, equal or less than those of their mothers. All TPPA and EIA reactions were positive in these children, but only IgM immunoblots were negative. These infants were uninfected, without any symptoms of congenital syphilis at birth. They received careful follow-up examinations and serological testing at 0, 3, 6, 9, and 12 months after birth.

RPR titer was negative in two mothers who acquired syphilis stage I during their pregnancy; the diagnosis was confirmed with positive TPPA, EIA, and IgM immunoblot positive results and with negative IgG immunoblot results. They were immediately treated with intramuscular benzathine penicillin. None of the two children presented clinical or laboratory evidence of infection; no positive serological test, including IgM immunoblot, was found in these two infants. Nine pregnant women received the diagnosis of syphilis latent *recens* during their pregnancy; RPR titers in this group of mothers were 1:16 and 1:32 in five and four cases, respectively. Except for the IgM immunoblot, all the treponemal tests were positive in these mothers. After adequate treatment, the RPR titers decreased fourfold. Their infants were born with the same serological results as the mothers, but they were uninfected and showed no symptoms. Syphilis *latens tarda* was the diagnosis of two pregnant mothers; RPR titers were 0, results of TPPA, EIA, and IgG immunoblot tests were positive, but those of IgM immunoblot test were negative. After adequate treatment during pregnancy, the infants of these mothers had the same serological results as their mothers, but none of them was infected.

Finally, 11 women, having received inadequate prenatal care, classified as latent syphilis patients, were first found to have reactive test results for syphilis at delivery. Seven mothers had the diagnosis of syphilis *latens tarda*, with negative RPR and IgM immunoblot results, but with positive TPPA, EIA, and IgG immunoblot tests. At delivery, the RPR test results of their infants were negative, but TPPA and EIA were positive. None of these children had a positive IgM immunoblot result.

The other four mothers had the syphilis *latens recens* diagnosis at delivery, with an RPR titer at least 1:2 to a maximum of 1:256. TPPA, EIA, and IgG immunoblot tests were all positive. One mother with an RPR titer of 1:64 has already had IgM negative immunoblot result. Her baby had the same serological results and had no clinical symptoms of syphilis, after adequate treatment the follow-up examinations and serological testing at 0, 3, 6, 9, and 12 months of birth confirmed the absence of congenital syphilis infection.

The last three women were found to have positive IgM results together with the other reactive syphilis tests at delivery. The first of them, an intravenous drug user, has had an RPR titer only of 1:2, but one of her twin sons has died immediately after birth. The RPR titer of this 'A' infant was 1:8. RPR titer of 'B' infant was 1:2; his treponemal tests, including IgM immunoblot, were all positive. Clinical symptom of seizures suggested the existence of neurosyphilis in this child. Lumbar puncture was performed to obtain cerebrospinal fluid for VDRL and TPPA tests. VDRL was negative, but TPPA was positive of a 1:80 titer. The second mother–child pair had the same RPR titer of 1:256; all treponemal tests, including IgM immunoblot, were positive in both of them, but the infant had no symptoms of congenital syphilis. The last mother had an RPR titer of 1:64; TPPA, EIA, and IgG and IgM immunoblots were positive. The premature daughter had RPR titer of 1:64 and positive results of TPPA, EIA, and IgM immuno-

Table 1. Date and staging of maternal syphilis diagnosis

Before pregnancy	During pregnancy			At delivery	
Successfully treated	Syphilis I	Syphilis <i>latens recens</i>	Syphilis <i>latens tarda</i>	Syphilis <i>latens recens</i>	Syphilis <i>latens tarda</i>
<i>n</i> =217	<i>n</i> =2	<i>n</i> =9	<i>n</i> =2	<i>n</i> =4	<i>n</i> =7

n = Number of the mothers with the identified diagnosis

Table 2. Serological results of mother–child pairs from cases when maternal syphilis *latens recens* diagnosis was established only at delivery

	Maternal RPR titer	Maternal IgM	RPR titer of newborn	IgM of newborn	Diagnosis of newborn
Mother 1	1:64	Negative	1:64	Negative	Uninfected
Mother 2	1:2	Positive	Twin A: 1:8	Positive	Connatal syphilis
			Twin B: 1:2	Positive	Connatal syphilis
Mother 3	1:256	Positive	1:256	Positive	Connatal syphilis
Mother 4	1:64	Positive	1:64	Positive	Connatal syphilis

blot. Prematurity was the only non-specific clinical manifestation of congenital syphilis in this case (Table 2).

Discussion

Globally, nearly two million pregnant women are infected with syphilis each year. Approximately 50% of women with untreated syphilis have been transmitting the infection to their newborn child, resulting in profound adverse outcomes including an estimated 440,000 perinatal deaths each year [7]. In Hungary, there was no congenital syphilis from 1978 until 1994. Both in 1994 and 1996, three early cases of congenital syphilis were observed, all in foreigners and from pregnancies without prenatal care. Since 1994, however, almost every year a case has been observed in Hungary. In 2007 and 2008, we revealed one and two cases, respectively [8]. In a three-year period from 2009 to 2011, we confirmed four congenital syphilis cases in Hungary. In Switzerland, in contrast to international guidelines, screening for syphilis in pregnancy is not generally recommended, but within the Swiss population, infectious syphilis cases in women of childbearing age increased substantially from 2006 to 2009 [9]. In Hungary, the performance of screening examinations within prenatal care is compulsory in order to prevent the development of syphilis, but only in the first trimester of pregnancy.

RPR test used for screening has the advantage of being inexpensive, widely available, and necessary for determining the efficacy of treatment. Limitations of this non-treponemal test include the lack of sensitivity in primary and late syphilis and the possibility of a prozone reaction or false-positive results [4]. Prozone reactions occur in 1–2% of patients with secondary syphilis [10], when antibody is in excess and blocks the normal antibody-antigen reaction. Dilution of the serum sample exhibiting this prozone reaction is adequate to obtain a readily detectable reaction. Some Hungarian laboratory uses only RPR test from undiluted serum samples, so they may detect a false-negative result in prenatal screening.

Treponemal tests like EIA or TPPA are technically more difficult to perform and more expensive, but they remain reactive for years with or without treatment. Due to the recommendation of Centers for Disease Control and Prevention (CDC), Binnicker et al. suggest to use a reverse syphilis screening algorithm, in which sera are screened using an automated treponemal test (e.g. EIA) [11]. Samples that are reactive by EIA are then tested by RPR to assess disease and treatment status and provide a supplemental marker of infection recommended that sera testing reactive by EIA but nonreactive by RPR be analyzed by the TP-PA assay [12]. Several antigens that elicit high antibody titers during *T. pallidum* infection and are not cross-reactive with serum from patients with other common spirochetal diseases have been identified [13]. TPPA and EIA tests have explored the use of these recombinant

antigens. While false-positive results can occur also with treponemal tests [2], we used these two tests from different manufacturers, as screening and confirming methods.

From the 241 mothers with positive syphilis screening tests, 217 were successfully treated before pregnancy. The next 13 pregnant women diagnosed with syphilis during pregnancy opted for treatment. The rest of 11 mothers were diagnosed with syphilis only at delivery. All our four congenital syphilis cases were from mothers without undergoing prenatal care and syphilis screening. The success rate for mother-to-child transmission intervention (number of successful interventions/number of syphilis positive women who received intervention) was 100% in our cases. In the program supported by the Shenzhen local government, the success rate was lower, 99.1%, because of mothers who refused treatment or had late diagnosis and treatment [14]. Due to the better compliance of the patients, the performance of compulsory screening examinations within prenatal care is a good prevention against the development of congenital syphilis in Hungary.

Rawstron et al. determined maternal IgM status to be better indicator for a risk of congenital syphilis than a maternal RPR titer $\geq 1:16$. However, they described that neither a titer $\geq 1:16$ nor TP IgM reactivity identified all mothers who delivered infected infants; they found babies with congenital syphilis whose mothers had negative TP IgM and titers $\leq 1:8$ [5]. In our study, the mother of the twins with congenital syphilis had only an RPR titer of 1:2. None of the mothers of the infected children was IgM negative in our cases. The infant of the untreated mother, who had an RPR titer of 1:64, but was negative for IgM, was uninfected.

Infected infants can produce IgM *in utero* after 3 months [4]. Previous studies using either ELISA or *T. pallidum* IgM WB have similarly found that IgM antibodies cannot be detected in all babies with congenital syphilis [15, 16]. Serodiagnosis of congenital syphilis is difficult because of the transfer of the IgG antibodies from mother to fetus. Fetus produces IgM antibodies (rheumatoid factor), against maternal IgG [14]. IgG–RF complex reacts in IgM immunoblot, or maternal IgG compete with fetal IgM for the Ag-binding position, resulting in false-positive or false-negative tests, respectively. To eliminate maternal IgG and IgG–RF complexes from the sera of newborns, Mastsorb reagent was used before IgM immunoblot tests. In previous studies with IgM immunoblot test, more reactivity was observed to the 47-kDa antigen than to antigens of 45 kDa or lower [17–19]. All our infants IgM-reactive sera demonstrated also this reactivity. We found reactivity to antigens of 45, 17, and 15 kDa only in one case.

Our observations confirmed that antenatal syphilis screening with the parallel use of treponemal (EIA, TPPA) and nontreponemal (RPR) tests facilitates treatment during pregnancy. Successful treatment offsets vertical transmission. The use of IgM immunoblot examination allowed the identification and treatment of high-risk newborns.

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