RESEARCH NOTE

Antenatal syphilis serology in pregnant women and follow-up of their infants in northern Italy

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ABSTRACT

Positive syphilis serology was noted in 119 (0.49%) of the 24 053 pregnant women delivering at St Orsola Hospital in Bologna, Italy, from November 2000 through July 2007. Six presumptive cases of congenital syphilis with IgM western blot positive results were found. Two infants had a positive cerebrospinal fluid (CSF) Venereal Disease Research Laboratory test result (one also had a positive CSF PCR result), another presented long-bone lesions, and the remaining three were preterm. These observations confirmed that antenatal syphilis screening facilitates treatment during pregnancy and offsets vertical transmission; moreover, the use of IgM western blot and careful CSF examination allowed the identification and treatment of high-risk newborns.

Keywords congenital syphilis, CSF, PolA PCR, serology, *Treponema pallidum*

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In 1998, the WHO estimated that one million pregnancies each year worldwide were adversely affected by syphilis due to maternal infection [1]. Congenital syphilis (CS) is mainly a consequence of the lack of antenatal care and control of sexually transmitted infections [2]. The foundation of the prevention of CS is the diagnosis of syphilis by serological screening during pregnancy [3]. Current Italian guidelines suggest that all pregnant women should be tested in the first trimester [4]. Several factors contribute to the different manifestations of CS, and even asymptomatic newborns may have early or late postnatal manifestations [5]. Owing to the frequent absence of specific signs of infection at birth, serological tests are often the only means of acquiring a correct CS diagnosis. As immunoglobulins G (IgGs) easily cross the placenta during pregnancy, *Treponema pallidum*-specific IgG detection in infants' sera at birth does not allow detection of high-risk newborns.

The aim of this study was to evaluate the usefulness of *T. pallidum* IgM western blot (WB) and cerebrospinal fluid (CSF) PCR as an aid in the diagnosis of CS during a prospective surveillance study carried out at St Orsola Hospital in Bologna, Italy from November 2000 through July 2007.

All women were screened during pregnancy and at delivery for syphilis by one of the following commercial methods: Syphilis Screening Recombinant EIA (Radim, Pomezia, Italy), LIAISON® Treponema Screen (Diasorin, Saluggia, Italy) [6], or ARCHITECT® Syphilis TP (Abbott Japan Co., Tokyo, Japan).

Positive samples were further analysed by *T. pallidum* haemagglutination assay (AlfaWasserman, Milan, Italy) and an in-house WB [7]; if positive results were confirmed, the rapid plasma reagin (RPR) test (Radim, Pomezia, Italy) was also performed.

A maternal case history was obtained in order to identify high-risk pregnancies. Infants born to syphilis-seropositive mothers were enrolled in a prospective follow-up. Tests were performed at birth (including IgM WB). The source was determined, and propagation and extraction of *T. pallidum* subsp. Nichols strain for WB were performed as previously reported [8,9]. Infants with positive RPR test results at birth, born to mothers not adequately treated, also received long-bone radiography as well as CSF analysis, when possible, including Venereal Disease Research Laboratory testing (Dade Behring, Marburg, Germany). Since 1 January 2006, all CSF samples have been also analysed by a PCR method, as follows. DNA extraction was performed with the QIAamp DNA Mini-kit (Qiagen GmbH, Hilden, Germany) in 400 μL of CSF samples. PCR was performed by using a set of

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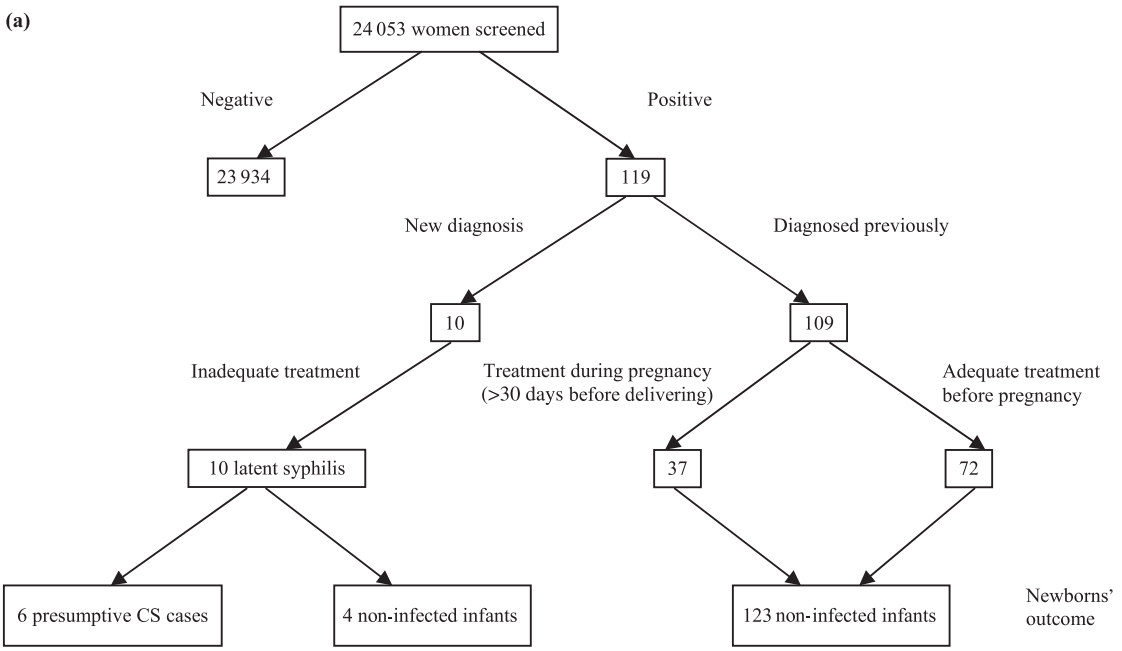
primers [10] amplifying a fragment of the *T. pallidum* DNA polymerase I gene. This primer set generated an amplification product of 377 bp. All amplifications were carried out with Eppendorf Mastercycler Personal (Eppendorf, AG., Hamburg, Germany). The amplicons were electrophoresed on

(a)



(b)

		Mothers' origin			
		Italy (16)	**Eastern Europe (61)**	**South-Central America (18)**	**Others (19)**
Education					
	Primary school		4 (6.6%)	2 (11.1%)	3 (15.8%)
	Intermediate school	6 (37.5%)	24 (39.3%)	9 (50.0%)	9 (47.4%)
	High school	8 (50.0%)	23 (37.7%)	4 (22.2%)	6 (31.6%)
	Degree	2 (12.5%)	5 (8.2%)		1 (5.2)
	Unknown		5 (8.2%)	3 (16.7%)	
Employment					
	Unemployed		7 (11.5%)	4 (22.2%)	
	Housewife	2 (12.5%)	33 (54.1%)	7 (38.9%)	10 (52.6%)
	Employee	12 (75%)	19 (31.1%)	6 (33.3%)	8 (42.1%)
	Self employed	2 (12.5%)			1 (5.3%)
	Unknown		2 (3.3%)	1 (5.6%)	
Marital status					
	Unmarried	8 (50.0%)	31 (50.8%)	7 (39.0%)	3 (15.8%)
	Married	8 (50.0%)	28 (45.9%)	9 (50.0%)	16 (84.2%)
	Unknown		2 (3.3%)	2 (11.0%)	
Father's name on birth certificate					
	Present	14 (87.5%)	53 (87.0%)	15 (83.3%)	19 (100.0%)
	Not present	2 (12.5%)	8 (13.0%)	3 (16.7%)	

Fig. 1. (a) Syphilis serology in pregnant women and the outcome of their newborns. (b) Distribution of maternal characteristics of 114/119 women seropositivefor syphilis. Data regarding the remaining five women (four from eastern Europe and one from South America) are not available, as their babies were put up for adoption. Numbers of women are indicated in boxes.

a 1.5% agarose gel at 100 V for 1 h and visualized after staining with SYBR safe™ (Invitrogen Co., Carlsbad, CA, USA). All seroreactive infants received careful follow-up examinations and serological testing at 0, 3, 6, 9 and 12 months or until the test results became negative.

During the study period, 24 053 women delivered 24 447 infants. Serological evidence of syphilis at delivery was found in 119 women giving birth to 133 children (Fig. 1). The overall syphilis seroprevalence in pregnant women was 0.49%.

Sixty women had already been adequately treated before pregnancy, 12 were advised to repeat therapy during pregnancy because of persistent high-titre RPR which later decreased four-fold, and 37 received the first syphilis diagnosis during pregnancy: the last category of patients were immediately treated with intramuscular benzathine penicillin, and their RPR titres showed a four-fold decrease by the end of the pregnancy. Finally, ten women, classified as latent syphilis patients [11], were first found to have reactive test results for syphilis at delivery, having received inadequate prenatal care. Six of the ten infants born to untreated mothers had positive IgM WB and RPR test results at birth (Table 1). Three infants were preterm (two/three with extremely low birthweight). The remaining three were born at term; two had positive CSF Venereal Disease Research Laboratory test results (one of whom also had a positive CSF PCR result), and the third infant presented long-bone lesions upon X-ray examination (trasversal streaks of radioparency about femoral distal metaphyses), which were no longer evident after therapy. All six infants were hospitalized for treatment with intravenous penicillin G (50 000 U/kg/dose every 8–12 h) for 14 days. The remaining four infants born to mothers who received the first syphilis diagnosis at delivery had negative or weakly positive RPR test results and negative IgM WB test results at birth. The long-bone X-ray examination and CSF analysis results were normal (Table 1). These infants, who were presumptively uninfected, received only a single dose of intramuscular benzathine penicillin (50 000 units/kg).

Finally, 123 asymptomatic infants were born to the 109 mothers adequately treated before or during pregnancy; none of these presented clinical or laboratory evidence of syphilis infection. No positive IgM WB result was found in this group of infants.

The most important risk factor for congenital syphilis is inadequate prenatal care or the absence of care [12–18]. In this study, none of the six infants with presumptive congenital disease had evident clinical signs at birth. Therefore, CS could have been misdiagnosed if serological tests had not been performed at the time of delivery for both mother and newborn. Prematurity was the only non-specific clinical manifestation. Although the aetiology of prematurity among high-risk pregnant women varies, CS is a well-documented cause [16,19].

Table 1. Characteristics of untreated mothers who were seropositive for syphilis and of their infants at delivery and at last follow-up examination

	Maternal profile			Infants' characteristics at birth							Infants' characteristics at last follow-up examination		
	Nationality	Time of residence in Italy before pregnancy (years)	Marital status/husband's nationality	GA (weeks)	BW (g)	IgM WB	RPR	VDRL CSF	PolA PCR	Long-bone X-ray	Age (months)	RPR	TPHA
Case 1	Romania	1	Unmarried	24	695	+	+	ND[a]	Not done	Normal	10	–	+
Case 2	Romania	4	Married/Italian	26	495	+	+	ND[a]	Not done	Normal	12	–	+
Case 3	Romania	1	Unmarried	34	2150	+	+	–	Not done	Normal	4 (lost)?	+	+
Case 4	Russia	2	Married/Italian	40	3660	+	+	+	Not done	Normal	10	–	+
Case 5	Ukraine	1	Unmarried	39	3250	+	+	+	+	Normal	24	–	+
Case 6	Romania	1	Married/Rumanian	38	2800	+	+	–	Not done	Abnormal	12	–	+
Case 7	Cuba	2	Married/Italian	40	3400	–	–	–	Not done	Normal	12	–	–
Case 8	Moldavia	6	Married/Italian	39	3300	–	+	–	–	Normal	9	–	–
Case 9	Romania	1	Unmarried	40	3250	–	+	–	–	Normal	6	–	–
Case 10	Moldavia	1	Unmarried	38	2890	–	+	–	–	Normal	9	–	–

GA, gestational age; BW, bodyweight; WB, western blot; RPR, rapid plasma reagin; VDRL, Venereal Disease Research Laboratory; CSF, cerebrospinal fluid; TPHA, *Treponema pallidum* haemagglutination assay.

The first six infants were presumptive cases of congenital syphilis, whereas the remaining four were uninfected. The ten mothers with inadequate prenatal care were born in foreign countries, but all had been in Italy for at least 1 year (range: 1–6 years) before the pregnancy and four or ten had married Italian citizens. All six infants with congenital syphilis were born to mothers coming from eastern Europe.

[a]CSF was not drawn because it was contraindicated due to the very low birthweight of these infants.

Results obtained by the use of IgM WB, combined with a careful CSF examination including PolA PCR, allowed paediatricians to identify high-risk infants for prompt and adequate treatment, thus avoiding unnecessary therapy and the consequent hospitalization of uninfected infants.

Although, in Italy, prenatal screening for syphilis is scheduled during the first trimester of pregnancy, women are not consistently advised to seek serological syphilis testing during their pregnancies, as already observed [19]. Therefore, the economic rationale in the study area is to test women at delivery to prevent CS; results obtained by an accurate serological examination of mother-infant pair samples allowed the pediatricians to identify high risk infants and to promptly and adequately treat these cases, avoiding unnecessary thereapy and the consequent hospitalization of uninfected infants [20]. Moreover, it is advisable that gynaecologists more closely control foreign women during pregnancy, including the use of serological tests for syphilis, which is a re-emerging problem in the study area [19].

TRANSPARENCY DECLARATION

All authors declare the absence of any dual or conflicting interest.

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