

NEONATAL CONGENITAL SYPHILIS. DIAGNOSIS BY THE ANTI IgM TREPONEMAL FLUORESCENCE TEST

Jacob KIPNIS ⁽¹⁾, Mário E. CAMARGO ⁽²⁾, Celeste FAVA NETTO ⁽³⁾,
A. Walter FERREIRA ⁽²⁾ and Dante B. GUARNIERI ⁽³⁾

SUMMARY

In 20 cases of congenital syphilis, IgM treponemal antibodies were searched for, in infant serums, through the immunofluorescence absorption test (FTA-ABS) using anti IgM conjugates.

The scope was to evaluate the test as a possible guide to the diagnosis of active infection and to the prescription of antisyphilitic therapy. Positive results were found in 13 cases, doubtful in 4 and negative in 3. Cardiolipin tests were positive in all, as well as anti IgG treponemal fluorescence tests. In 4 cases which showed positive IgM tests negative or doubtful results were seen in samples obtained a few weeks later, after treatment.

Possible causes of the failure of the anti IgM treponemal fluorescence test in 35 per cent of our cases are discussed.

INTRODUCTION

The possibility of distinguishing treponemal antibodies produced by the child and indicative of active infection, from those passively transferred from the mother, should give a more solid basis to the prescription of treatment in congenital syphilis. This is especially important for the clinically normal, serologically positive infant of an untreated mother.

Owing to placental impermeability to IgM macroglobulins, a raised IgM level in the blood of infants can be taken as an index of perinatal infections. However, the antibody nature of such immunoglobulins should be determined by serological tests employing antigens from the suspected causative agent.

It has already been demonstrated (SCOTTI & LOGAN ¹⁰; SCOTTI et al. ¹¹) that in congenital syphilis the immunofluorescence test with *Treponema pallidum* is a practical

technique for the identification of treponemal antibody when fluorescein conjugates specific for IgM are used.

The possibility of false negative results in treponemal anti IgM fluorescent tests is however not to be dismissed, since already observed in similar tests (COHEN et al. ⁶; REMINGTON ³).

In the present work we report the results of the anti IgM, fluorescent absorption (FTA-ABS) test in serum specimens from 20 infants showing evidences of congenital syphilis.

MATERIAL AND METHODS

Serum specimens — Twenty five serum samples were collected from 20 infants of both sex and with ages from 13 to 121 days with clinical, radiological and serolo-

(1) Departamento de Ortopedia da Faculdade de Medicina da U.S.P.

(2) Laboratório de Imunologia do Instituto de Medicina Tropical de São Paulo, Brasil

(3) Departamento de Microbiologia e Imunologia da Faculdade de Medicina da U.S.P., São Paulo, Brasil

gical evidences of congenital syphilis. In most cases, only one serum specimen was collected. In five cases a second sample was collected a few weeks to a few months after treatment.

Cardiolipin tests — V.D.R.L. test was performed according to the "Serologic tests for Syphilis Manual, 1964, from the U.S. Department of Health, Education and Welfare"¹². For the complement fixation test the quantitative technique of WADSWORTH et al.¹³ was used and approximated titers determined, as referred by ALMEIDA².

Immunofluorescence tests — Serum specimens were tested in the FTA-ABS test (HUNTER et al.⁷), employing Nichol's strain of *Treponema pallidum* (*) and Reiter's treponeme absorption antigen prepared as described by STOUT et al., 1967. Anti-human globulin conjugates specific for IgG and for IgM were used. Rabbits were immunized with IgG obtained by fractionation of normal human serum. Anti IgG immunoserum was rendered specific through absorptions with IgA and IgM, polycondensed as described by AVRAMEAS & TERNYNCK³. Anti IgM serums, obtained in rabbits and in a sheep immunized with a serum macroglobulin fraction isolated from a patient with Waldenström's macroglobulinemia, were rendered specific through absorptions with cord serum in which IgM is absent or present in only very scanty amounts. Specificity of antisera was ascertained by immunoelectrophoretic techniques. Also, any fluorescence determined by anti IgM conjugates could not be detected after previous treatment of reactive human serums by 2-mercaptoethanol, both in syphilis and toxoplasmosis fluorescence tests.

Conjugates were prepared by labelling anti IgG and anti IgM serums with fluorescein isothiocyanate (**) to F/P weight ratios of 8 to 10. Dilutions for use contained at least one precipitating unit, as defined by BEUTNER et al.⁴.

RESULTS

In the 20 cases studied (Table I) anti IgM treponemal fluorescence tests were positive in 13, doubtful in 4 and negative in 3, in the first serum specimens collected. Cardiolipin tests were positive in all such serums and in most cases high titers were found in the quantitative complement fixation test. Since only small amounts of serum were available, end points could not be determined for a few specimens. Anti IgG fluorescence tests were positive in all serums.

In the five cases from which serum samples were collected after treatment a few weeks or months later, complement fixation titers fell to much lower values or became negative, although V.D.R.L. tests and anti IgG fluorescence tests remained still positive. The IgM fluorescence test, of four previously positive cases, became negative in three and doubtful in one. In a fifth case from which two successive serum samples were available, a first negative IgM test was followed by a doubtful one, in the second specimen.

No relationship could be found between cardiolipin titers and results shown by the IgM fluorescence test.

DISCUSSION

As the results here reported indicate, treponemal IgM antibodies could be undoubtedly demonstrated in most but not in every case of congenital active syphilitic infection, in serum samples collected in the first days of extrauterine life. The test failed in 7 cases (35%), which showed negative or only doubtful results.

Since IgM antibodies in infants serum can be taken as produced by the child and not passively transferred through the placenta, except in cases of placental lesions, the prescription of treatment for syphilis in suspected cases of congenital infection could find a reliable guide on the recognition of treponemal IgM antibodies.

(*) Suspensions gently furnished by Dr. G. NIEL, Hôpital St. Louis, Paris, France.

(**) Crystalline, chromatographically pure, isomer I, B.B.L., U.S.A.

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TABLE I

Serological tests in 20 infants with congenital syphilis

Cases	Sex	Age in days	Serum samples	Results in serological tests			
				Cardiolipin tests		Immunofluorescence tests	
				V.D.R.L.	Complement fixation	IgM	IgG
A.M.A.L.	m	61	3/6/1969	++++	905	+	+
			6/9/1969	+++	2	-	+
M.S.L.	f	59	3/21/1969	++++	> 320	+	+
			8/14/1969	+++	2	-	+
W.S.	m	60	5/20/1969	++++	1130	+	+
			7/3/1969	+++	33	-	+
A.M.S.	m	46	8/13/1969	++++	> 370	-	+
			12/5/1969	++++	-	±	+
M.A.O.	m	121	11/14/1969	++++	> 285	+	+
			1/30/1970	++++	64	±	+
P.O.	f	34	2/14/1969	++++	146	+	+
M.M.B.	f	63	5/31/1969	++++	261	-	+
C.R.S.	f	26	6/19/1969	++++	> 363	±	+
E.A.N.	f	62	7/22/1969	++++	> 284	-	+
R.C.T.N.	m	54	10/1/1969	++++	80	+	+
R.C.T.N.	f	54	10/1/1969	++++	85	+	+
J.C.A.	f	61	10/23/1969	++++	> 320	-	+
W.V.S.	m	47	11/11/1969	++++	64	±	+
S.A.S.	f	55	11/17/1969	++++	> 310	+	+
J.T.M.L.	m	90	11/18/1969	++++	12	±	+
E.A.A.	f	13	12/16/1969	++++	168	+	+
S.A.S.	f	62	2/3/1970	++++	> 800	+	+
A.M.M.	m	46	2/16/1970	++++	142	±	+
P.D.F.	f	66	3/10/1970	++++	> 900	+	+
R.P.L.	m	43	4/10/1970	++++	> 1500	+	+

SCOTTI et al.¹¹, have reported that in a group of 15 infants with a clinical picture compatible with congenital syphilis treponemal IgM antibodies could be found in every case, as well as in three cases which had no clinical signs of syphilis but presented a positive serology.

In our series, the observed failure of the test in a few cases could perhaps derive from an eventual absence of IgM antibodies, in spite of actual infection. Recently, one case of congenital syphilis was reported with no detectable IgM by the immunoradial diffusion technique (ACKERMAN¹). More proba-

bly however, technical reasons are to be searched for. We do not believe a low sensitivity of the anti IgM test could be blamed for such negative results. Although titrations in our positive cases were not performed, a strong fluorescence was observed for most such cases. The satisfactory sensitivity of the test was reflected in a good activity of the same *T. pallidum* antigen when employed in a large number of routine fluorescence tests, and when tested against a positive standard syphilis serum, gently furnished also by Dr. G. Niel (*). The anti IgM conjugate showed also characteristics such as to ensure a high sensitivity for the test. It had a high antiglobulin level and was labelled to the highest possible degree so as to give a maximal reactivity (BEUTNER et al.⁵). In antitoxoplasma IgM antibody tests, in cases of recently acquired *Toxoplasma* infections, the same conjugate was sufficiently active to disclose eventually titers as high as 1:4,000.

Other possible explanation for our negative results could derive from a competition that could occur between IgG and IgM antibodies, with the exclusion of the latter when very high levels of IgG were present, as suggested by the observations of COHEN et al.⁶.

On the other hand, REMINGTON⁹, has observed that not every anti IgM conjugate is sufficiently reliable to demonstrated IgM antibodies in infant serums. PEETOM & MULDER⁸, when analysing such anti IgM conjugates, have observed that a few of them failed to react with many children IgM antibodies, although they reacted with every adult IgM tested. As they suggest, it is possible that absorption of anti IgM immunoserums with cord blood could be responsible for such nonreactivity with certain infant serums. Also, it is possible to assume that our anti IgM serum, prepared by immunizing rabbits with the macroglobulin from one case of Waldeström macroglobulinemia, had a reactivity limited to antigenic sites not found in every infant-produced IgM.

The scanty amounts of serums available, in our cases, did not permit investigations to

verify such hypothesis so as to explain the eventual failures of the test.

RESUMO

Sífilis congênita. Diagnóstico pela pesquisa de anticorpos IgM anti-treponêmicos, por imunofluorescência

Anticorpos IgM anti-treponêmicos foram pesquisados no soro de 20 crianças portadoras de lues congênita, através da prova de imunofluorescência com absorção (FTA-ABS) usando-se conjugados anti-IgM.

A principal finalidade de tal prova é a comprovação de infecção luética em atividade para a instituição imediata da terapêutica específica. Os resultados foram positivos em 13, duvidosos em quatro e negativos em três casos.

As provas sorológicas clássicas com antígeno de cardioplipina e a pesquisa de anticorpos IgG anti-treponêmicos pela imunofluorescência (FTA-ABS teste) foram positivas em todos os casos. Quatro casos em que a pesquisa de anticorpos IgM era positiva, quando examinados pela mesma prova algumas semanas após o tratamento revelaram resultados negativos ou duvidosos.

São discutidas as possíveis interpretações para os 35% de resultados negativos, na pesquisa de anticorpos IgM anti-treponêmicos, em casos comprovados de lues congênita.

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(*) Laboratoires Spécia, Paris, France.

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