Rev. Inst. Med. trop. São Paulo
14(5):310-313, setembro-outubro, 1972

UDC 616.993.1

RHEUMATOID FACTORS AS A CAUSE FOR FALSE POSITIVE IgM ANTI-TOXOPLASMA FLUORESCENT TESTS. A TECHNIQUE FOR SPECIFIC RESULTS

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SUMMARY

An unusually high percentage of positive fluorescence toxoplasma IgM antibody tests (91.7%) was observed in sera presenting a positive latex test. To verify the hypothesis that rheumatoid factors in sera caused such positive results, different experiments were performed. The addition of rheumatoid factors to sera with IgG toxoplasma antibodies made IgM tests positive. Treating positive latex-test sera with heat-aggregated human "gamma-globulin" resulted in negativation of the IgM toxoplasma tests, with no modification in the titers of IgG toxoplasma antibodies. However, IgM positive tests in sera from cases of acquired toxoplasmosis were not influenced by such a treatment.

Avoiding false positive results through a simple technique as this one could be of help, in view of the diagnostic value of IgM antibody tests and the relatively high frequency of rheumatoid factors in populations living in tropical areas.

INTRODUCTION

The presence of IgM antibodies for *Toxoplasma gondii* in serum is suggestive of a recent toxoplasmic infection [2, 8, 9]. Toxoplasma antibodies as detected by the Sabin-Feldman dye-test or the antiglobulin immunofluorescente test, frequently show high titers for large periods after eventual clinical manifestations have disappeared [1]. In this way, the demonstration of IgM antibodies can be of help, as as guide to therapeutic measures or to the evalution of risks for congenital transmission of the disease. The indirect immunofluorescence test with antiglobulin conjugates specific for the heavy chain of IgM constitutes a very practical technique for the detection and titration of such antibodies in toxoplasma infections [9]. However, as recently suggested by ROWE [10], false positive IgM tests could result from rheumatoid factors present in the serum. Reacting with IgG antibodies fixed *"in vitro"* to the parasitic antigens, these macroglobulinic factors would constitute an IgM layer and determine the staining by the anti IgM conjugate.

When working with serum from cases of rheumatoid arthritis, we have observed an unusually high frequency of positive anti toxoplasma IgM fluorescent tests, which led us to investigate the hypothesis suggested by ROWE.

MATERIAL AND METHODS

Immunofluorescence tests for toxoplasma antibodies were performed as referred by CAMARGO [6], with specific anti IgG or anti

Presented to the VIII Brazilian Congress of Tropical Medicine, February 1972, Belo Horizonte, Brazil

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IgM conjugates. These were prepared from rabbit or sheep immunesera obtained through the immunization of animal either with human IgG or with the heavy chain serum globulin fraction from a case of Waldenström's macroglobulinemia. Immunesera were rendered specific through absorption with polycondensed immuneglobulins obtained as described by AVRAMEAS & THERNYNCK [3]. Specificity of conjugates was determined by immunoelectrophoretic analysis. Also, treatment of sera with 2-mercaptoethanol [11] rendered the IgM tests negative.

Tests for rheumatoid factors were performed according to the usual techniques [5], with latex particles sensitized with human heat-aggregated commercial "gamma-globulin". Qualitative tests were done on slides (RA test) and quantitative tests in tubes.

Serum samples — Were obtained from patients with rheumatoid arthritis, from patients with the lymphoglandular form of acquired toxoplasmosis and from apparently healthy persons showing serum antibodies to *Toxoplasma gondii.*

RESULTS

1) *Fluorescent IgG and IgM tests for toxoplasma antibodies in sera with rheumatoid factors*

Tests for toxoplasma antibodies were performed in 34 serum samples with positive latex tests of titers ranging from 1/80 to 1/10,240. In 24 sera, fluorescent IgG toxoplasma tests were positive, and in 91.7% of these (22 sera) IgM toxoplasma tests were also positive. In 10 sera no toxoplasma antibodies were detected (Table I).

TABLE I

Latex tests and toxoplasma immunofluorescence tests in 34 serum samples with rheumatoid factors

Sera	Latex tests	Toxoplasma immunofluorescence tests	
		Anti IgG	Anti IgM
1	1280(*)	NR(**)	NR
2	1280	4000	1000
3	640	8000	4000
4	1280	1000	256
5	2560	1000	256
6	1280	4000	1000
7	1280	4000	256
8	>160	NR	NR
9	160	4000	1000
10	640	NR	NR
11	80	1000	256
12	320	NR	NR
13	1280	NR	NR
14	5120	256	256
15	80	256	64
16	5120	4000	256
17	>160	1000	1000
18	640	1000	256
19	5120	4000	1000
20	2560	NR	NR
21	>160	4000	64
22	320	256	256
23	640	NR	NR
24	160	4000	256
25	1280	NR	NR
26	80	16	NR
27	640	1000	256
28	2560	>16000	8000
29	>640	4000	64
30	10240	1000	256
31	320	NR	NR
32	640	1000	NR
33	160	NR	NR
34	320	4000	256

(*) End-point dilutions
(**) Non reactive at 1/16

2) *Fluorescence tests for toxoplasma antixoplasma antibodies in sera with rheufactor was added*

Serum mixtures were prepared by adding to a serum with IgG anti toxoplasma antibodies, with a titer of 1/8,000, a positive latex-test serum (titer = 1/2,560) showing negative tests for toxoplasma antibodies.

For this, to 0.5 ml volumes of a 1/16 dilution of the toxoplasma serum, equal volumes of doubling dilutions of the rheumatoid factor serum were added. Toxoplasma immunofluorescence tests were performed with each mixture. IgM tests were positive to a 1/64 dilution of the rheumatoid factor serum, while IgG tests were positive for all mixtures.

The rheumatoid factor serum was then added to 48 other serum samples presenting

a positive IgG – and a negative IgM – toxoplasma test. A 1/8 dilution of sera were used, one volume of the rheumatoid factor serum being added to one volume of each toxoplasma serum. In 45 samples positive IgM toxoplasma tests were then seen.

3) *Fluorescence tests in rheumatoid-factor sera the addition of heat aggregated "gamma globulin"*

To one volume of a 1/16 dilution on 16 serum samples presenting a positive latex test (titers ranging from 1/160 to 1/5,120) and a positive IgM toxoplasma test, one volume of heat-agregated "gamma-globulin" was added. This was a saline solution of commercial human gamma-globulin, with 5 mg of protein per milliliter, heat aggregated for 10 minutes in a water-bath at 63°C.

The serum mixtures were left at room temperature for about 1 hour and then IgG and IgM toxoplasma tests were performed. In every case the IgM test became negative, the IgG test showing the same titers as before. The latex test remained positive in all, with the same or one doubling dilution lower titers.

Heat-aggregated "gamma-globulin" was added in the same way to serum samples from 36 cases of acquired toxoplasmosis showing a positive IgM test, with titers ranging from 1/64 to 1/4,000.

No modification of the results was observed.

DISCUSSION

The frequency of 91.7% of positive immunofluorescence test for IgM toxoplasma antibodies for sera with rheumatoid factors is much higher than the observed percentage in sera collected at random from populational samples. In these, REMINGTON et al. [9], have found only 5.7% of positive IgM tests, in sera with positive IgG toxoplasma antibody tests.

Even in recently acquired cases of lymphoglandular toxoplasmosis we have found only 57.5% of positive IgM tests in 40 patients [2].

Through the addition of rheumatoid factors to sera containing toxoplasma IgG antibodies positive IgM tests resulted.

On the other side, the addition of heat-aggregated "gamma-globulin" to serum samples with rheumatoid factors changed to negative previously positive IgM toxoplasma tests, with no observed modification on the results of the IgG test. This was seen even for sera with positive latex test of very high titers. It is to be remarked that such titers were not substantially changed through the addition of aggregated globulin. Negativation of the latex tests could be obtained only by absorbing sera with polycondensed human IgG, prepared as described by AVRAMEAS & THERNINCK [3]. IgM tests became negative but IgG toxoplasma antibody titers did also fall to very low levels.

Negativation of IgM toxoplasma tests through the addition of aggregated globulin was not observed for sera from cases of the acquired form of toxoplasmosis.

A similar observation on the role of rheumatoid factors in causing false positive tests for IgM antibodies was recently referred to by GREENWOOD et al. [7], in relation to the immunofluorescence test for malaria. Plasmodia could be stained by anti IgM conjugates after incubating infected blood slides with IgG malarial antibodies, followed by a rheumatoid factor serum.

The practical importance of this fact is not to be dismissed. A higher prevalence of rheumatoid factors is found in populations living in tropical areas [4, 7], just where parasitic diseases do occur more frequently and diagnosis problems are more prone to be found.

The addition of heat-aggregated human "gamma-globulin" to serum samples is thus a simple way of avoiding false positive results in the IgM toxoplasma fluorescent antibody test and perhaps in similar tests which can give valuable informations in different parasitic infections.

RESUMO

O fator reumatóide como causa de falsos resultados positivos na pesquisa fluorescente de

CAMARGO, M. E.; LESER, P. G. & ROCCA, A. — Rheumatoid factors as a cause for false positive IgM anti-toxoplasma fluorescent tests. A technique for specific results. *Rev. Inst. Med. trop. São Paulo* **14**:310-313, 1972.

anticorpos IgM anti-toxoplasma. Técnica para a obtenção de reações específicas

Observou-se elevada percentagem (91,7%) de reações positivas de imunofluorescência para anticorpos anti-toxoplasma de tipo IgM, em soros com teste do latex positivo.

Para verificar a hipótese de que se tratassem de falsas reações positivas conseqüentes à presença de fatores reumatóides nos soros, procederam-se a algumas experiências.

A adição de sôro com fator reumatóide a soros com anticorpos anti toxoplasma de tipo IgG, determinou a positivação da reação IgM anti toxoplasma. Misturando-se "gamma-globulina" humana, agregada pelo calor, a soros de casos de artrite reumatóide que apresentavam reação IgM anti toxoplasma positiva, esta se tornou negativa, sem que se alterassem os títulos dos anticorpos de tipo IgG.

Tratamento semelhante de soros de casos de toxoplasmose adquirida, com reação IgM positiva, não determinou qualquer modificação nas reações para toxoplasma, tanto de tipo IgG como IgM.

O afastamento de falsos resultados positivos por meio de processo simples, como aqui descrito, poderá ter utilidade prática, dado o valor da pesquisa de anticorpos IgM em diferentes infecções parasitárias, levando-se em conta que, justamente nas populações de regiões tropicais, costuma ser relativamente alta a ocorrência de fatôres reumatóides.

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Recebido para publicação em 2/3/1972.