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DIAGNOSTIC INFORMATION FROM SEROLOGICAL TESTS IN HUMAN TOXOPLASMOSIS

II — Evolutive study of antibodies and serological patterns in acquired toxoplasmosis, as detected by hemagglutination, complement fixation, IgG- and IgM-immunofluorescence tests

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SUMMARY

A serological study was performed in 136 patients presenting evidences of a recently acquired toxoplasma infection. Serum samples were submitted to 4 different tests, the hemagglutination (HA), complement fixation (CF), anti-IgG (IF-IgG) and anti-IgM (IF-IgM) immunofluorescence tests. In samples collected early in the infection, IF-IgG titers of 1:4,000 or more were seen in 93.5% of patients and IF-IgM was positive in 98.5% with titers in most cases of 1:1,024 or higher. CF showed titers of 1:160 or higher for 94% of patients. However, HA titers were in general low, of 1:4,000 or less in 90% of the cases. Such findings characterize a serologic pattern which can be clearly distinguished from the one observed for most cases with toxoplasma-reacting sera in a population and corresponding to old infections, as we have indicated in a previous study [2]. Successive serum samples could be obtained from 117 patients for varying periods, which permitted to follow antitoxoplasma antibodies detected by different tests. A few serologic transition marks could be distinguished and dated between both patterns of recent and old infections and a transition serologic pattern defined. Thus, pattern I, of recent infection, gived way to transition pattern II by negativation of the IF-IgM test and equalization of IF-IgG and HA titers, usually 2 to 8 months after the onset of clinical disease. Pattern III resulted from a fall of IF-IgG, HA and CF titers to low, old infection values, which was observed in only 1/3 of our cases and sometimes as late as 1 or 2 years.

INTRODUCTION

In a previous paper [2], two different serologic patterns were defined which corresponded, respectively, to recent and ancient toxoplasma infections. In this paper tests were serially performed in cases presenting clinical and serological evidences of acute toxoplasma infection, so that transition between such patterns could be studied.

MATERIAL AND METHODS

Patients

One hundred and thirty six patients were studied, with clinical and serological evidences of recent toxoplasma infection, in most cases presenting the glandular form of the disease. A diagnosis of acute miocarditis was made in 1 case, and in another of intersticial pneumonitis. A third patient, a

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2 years old child, presented clinical evidences of acute encephalitis. From 117 patients it was possible to obtain successive serum samples for varying periods of 2 to 27 months. A total of 531 samples were examined.

Serum samples

Venous blood sera were kept at 4°C until tested, which was done in general within 24 to 48 hours after collecting, or frozen at −20°C when kept for longer periods. Sera were heat-inactivated at 56°C for 30 minutes before testing.

Tests

Every sample was subjected to immunofluorescence tests for IgG (IF-IgG) and for IgM (IF-IgM) antibodies, hemagglutination (HA) and complement fixation (CF) tests. Tests were performed and positive reference sera included as previously described [2]. In fluorescent tests, commercial specific anti-IgG and anti-IgM conjugates (Hyland, Travenol Laboratories, USA) were used. However, for a few tests in the beginning of the study, an anti-globulin conjugate was employed instead of the fluorescent anti-IgG reagent. Serum titrations with both conjugates showed no significant differences when such reagents were diluted for use so as to give a maximal reactivity.

RESULTS

A) *Serological characteristics of samples collected early in the infection*

Sera from 136 patients, collected during the first month of clinical disease, were studied and results displayed in Table I and Fig. 1. Positive IF-IgG tests were seen for 99.3% of the samples, with titers from 1:16 to 1:256,000. In most cases high titers were found, of 1:8,000 or more in about 70% of patients, with values lower than 1:4,000 in only 6.5%. Positive IF-IgM tests occurred in 98.5% of sera, with titers of 1:16 to 1:16,000. Most frequent titers were 1:1,024 or higher, observed in more than 80% of cases. HA test was positive in every case, with titers from 1:64 to 1:16,000. However, in more than 90% of the samples, hemagglutination titers did not exceed 1:4,000.

CF test was also positive in every case, with titers from 1:40 to 1:2,560. In 94% of sera, titers of 1:160 or more were found.

In a previous study of 2,055 reactive sera [2], it was possible to establish equivalences of HA and CF titers to IF-IgG titers observed for same sera. Such equivalences seemed to prevail for old infections, but were not observed for samples with a positive IF-IgM test. The same equivalences were looked for in the present series of acute infection sera through the respective percentages of agreement or disagreement between expected values.

As indicated in Table II, "equivalent" hemagglutination titers were more frequent only when IF-IgG titers were low, of 1:4,000 or less. However, in the majority of cases, which presented high fluorescence titers, lower HA titers than "equivalent" ones were seen. Large titer differences between both tests occurred in about 50% of acute disease serum samples, while observed in only about 10% of reactive samples taken at random (Table III).

For the CF-test, titers observed for acute disease sera were higher than respective "equivalent" values for most samples (Table IV).

B) *Serologic curves*

Variations in test titers during infection were studied through serologic follow-up, which could be obtained for 117 patients.

IF-IgG test

Increasing titers were frequently observed during the first weeks or months of disease. In 1 case a IF-IgG titer of 1:16 was found for the first serum sample obtained, which already presented a IF-IgM titer of 1:8,000, a CF titer of 1:640 and a HA titer of 1:2,000. Two months later a titer of 1:16,000 was found for the IF-IgG test as well as for the HA test, the IF-IgM test was negative, and the CF titer presented no significant modification. In another case, a negative IF-IgG

TABLE I

Titer distribution and 95% confidence intervals for IF-IgG, IF-IgM, HA and CF-tests in serum samples collected from 136 patients during the 1st month of clinical evidences of infection

Titers	IF-IgM	IF-IgG	HA *	Titers	CF
< 16	1.5% (0.2% |—| 5.3%) **	0.7% (0.0% |—| 4.0%)	2.2% *** (0.5% |—| 6.3%)	< 20	0.0% (0.0% |—| 2.7%)
16	0.7% (0.0% |—| 4.0%)	0.7% (0.0% |—| 4.0%)	n.d.	20	0.0% (0.0% |—| 2.7%)
64	5.1% (2.0% |—| 10.2%)	n.d.	8.1% (4.1% |—| 14.0%)	40	2.2% (0.5% |—| 6.3%)
256	9.6% (5.3% |—| 16.0%)	2.9% (0.8% |—| 7.4%)	19.9% (13.7% |—| 27.7%)	80	3.7% (1.2% |—| 8.1%)
1,024	14.7% (9.4% |—| 22.0%)	2.2% (0.5% |—| 6.3%)	48.5% (39.9% |—| 57.3%)	160	14.7% (9.4% |—| 22.0%)
4,000	58.0% (49.1% |—| 66.3%)	22.8% (16.1% |—| 31.0%)	14.7% (9.4% |—| 22.0%)	320	32.4% (24.7% |—| 41.0%)
8,000	8.1% (4.1% |—| 14.0%)	33.8% (25.9% |—| 42.4%)	4.4% (1.6% |—| 9.4%)	≥ 640	47.7% (39.1% |—| 56.4%)
≥ 16,000	2.2% (0.5% |—| 6.3%)	36.9% (29.0% |—| 45.6%)	2.2% (0.5% |—| 6.3%)		

* HA titers accumulated for similar values as in immunofluorescence tests
** 95% confidence intervals
*** < 1:64
n.d. not done

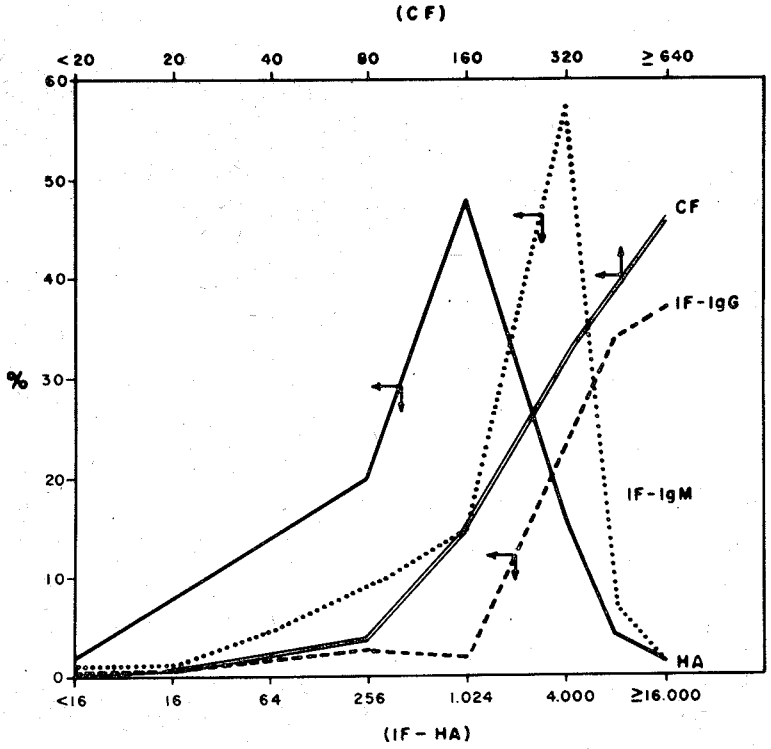

Fig. 1 — Titer distributions for 136 initial cases of acquired toxoplasmosis.

TABLE II

Percentages of agreement or disagreement (and respective 95% confidence intervals) between IF-IgG and "equivalent" HA titers in serum samples from 136 cases of acute toxoplasmosis, collected in the first month of apparent disease

IF-IgG titers	"Equivalent" HA titers	No. of cases	Percent of		
			Agreement	Disagreement due to titers	
				HA < IF-IgG	HA > IF-IgG
≤ 1,024	< 1,024	9	66.7% (29.9% |—| 92.5%)	0.0% (0.0% |—| 33.6%)	33.3% (7.5% |—| 70.1%)
1,024	≤ 1,024	3	100.0% (29.2% |—| 100.0%)	0.0% (0.0% |—| 70.8%)	0.0% (0.0% |—| 70.8%)
4,000	1,024 or 4,000	31	71.0% (52.0% |—| 85.8%)	25.8% (11.9% |—| 44.6%)	3.2% (0.1% |—| 16.7%)
8,000	4,000 or 8,000	45	24.4% (12.9% |—| 39.5%)	75.6% (60.5% |—| 87.1%)	0.0% (0.0% |—| 7.9%)
≥ 16,000	8,000 or ≥ 16,000	51	9.8% (3.3% |—| 21.4%)	90.2% (78.6% |—| 96.7%)	0.0% (0.0% |—| 7.0%)

TABLE III

Comparative study of IF-IgG and HA test titers in: A — sera from 136 cases of acute toxoplasmosis; B — 2,055 reactive sera, taken at random [2]

Serum samples	IF-IgG < HA	IF-IgG = HA	IF-IgG > HA		
			4 ×	16 ×	64 × or more
A	4.5% (1.7% |—| 9.6%) *	17.6% (11.7% |—| 25.2%)	28.7% (21.3% |—| 37.6%)	27.2% (19.9% |—| 35.4%)	22.0% (15.4% |—| 30.0%)
B	3.0% (2.3% |—| 3.9%)	45.7% (43.5% |—| 47.9%)	39.7% (36.9% |—| 41.9%)	8.0% (6.9% |—| 9.3%)	3.6% (2.8% |—| 4.6%)

* 95% confidence intervals

TABLE IV

Percentages of agreement or disagreement (and respective 95% confidence intervals) between IF-IgG and "equivalent" CF titers, in serum samples from 136 cases of acute toxoplasmosis, collected in the first month of apparent disease

IF-IgG titers	"Equivalent" CF titers	No. of cases	Percent of		
			Agreement	Disagreement due to titers	
				CF < IF-IgG	CF > IF-IgG
≤ 1,000	< 20 or 20	9	0.0% (0.0% |—| 33.6%)	0.0% (0.0% |—| 33.6%)	100.0% (66.4% |—| 100.0%)
4,000	20 or 40	31	6.5% (0.8% |—| 21.4%)	0.0% (0.0% |—| 11.2%)	93.5% (78.6% |—| 99.2%)
8,000	80 or 160	45	22.2% (11.2% |—| 37.1%)	0.0% (0.0% |—| 7.9%)	77.8% (62.9% |—| 88.8%)
≥ 16,000	≥ 160	51	100.0% (93.0% |—| 100.0%)	0.0% (0.0% |—| 7.0%)	0.0% (0.0% |—| 7.0%)

test was found for the first serum sample, which presented however a positive IF-IgM test of 1:1,024, CF titer of 1:640 and HA titer of 1:512. After 12 days the patient had a positive IF-IgG test with a titer of 1:1,024 and a 1:8,000 IF-IgM test. One month later both immunofluorescence tests showed the same 1:8,000 titer. In another case, not included in this series, a negative IF-IgG test was seen for a initial serum sample, which presented positive CF (1:640) and HA (1:2,048) tests and a IF-IgM titer of 1:8,000.

In general, maximal IF-IgG titers were already observed at the 1st or 2nd months of clinical disease but only after 3 to 5 months, in a few cases. High titers usually lasted for many months. Only in about 1/3 of the patients IF-IgG titers fell to values as low as 1:4,000 during periods patients were observed. This titer fall occurred between large limits of 2 to 18 months (Table V), but in a few cases higher titers still could be found after 2 years.

IF-IgM test

Maximal titers were seen in general in the first weeks of disease, so that no further significant increases in titers could be detected in most cases. Negativation of the IF-IgM test was observed for 98.3% of the patients. In 2 cases IgM antibodies could never be detected, perhaps due to a very early negativation of the IF-IgM test. Such negativations, when detected, occurred in periods as variable as 1 or 18 months, but for 50% of the patients a previous positive IF-IgM test was found negative after 5 months and for 80%, after 8 months (Table V).

HA test

Hemagglutination titers were in general low in the first weeks or months of infection, which resulted in clear-cut differences between IF-IgG and HA titers. Later, a sudden rise to the high titer values already shown by the IF-IgG test usually followed. In a few cases no such rise occurred, but

equivalent titers in both tests could be seen due to an early decrease in IF-IgG titers to the low titers displayed by the HA test. Large differences, of 16 times or more, between fluorescence and hemagglutination titers were seen in 78% of patients when a first serum sample was tested, but in a few cases such differences became apparent only later, after a few weeks. This was due to rising IF-IgG titers, or paradoxically, to a sudden decrease in HA titers (Fig. 4), to be followed several weeks later by the usual increase to the high IF-IgG titers. In this way the described differences between IF-IgG and HA titers could be found in 89% of our cases.

Equivalence between initially divergent HA and IF-IgG titers came to occur in about 80% of patients within periods as variable as 1 or 18 months, more frequently between 5 and 8 months of infection (Table V).

CF test

Maximal titers were seen early in the infection, in most cases already in the first sample tested. Such high titers lasted for many months and a decrease to lower values, of 1:80 or less, was seen in only 30% of the cases (Table V).

TABLE V

Occurrence of a few transitional marks between recent and old infection serological patterns, in 117 cases of acute toxoplasmosis

Periods	Negativation of the IF-IgM test	Titer equalization between HA and IF-IgG tests	Fall of CF titers to 1:80 or less	Fall of IF-IgG titers to 1:4,000 or less
less than 1 mo.	1.7%	11.1%	0.0%	1.7%
1 — 2 mo.	15.4%	2.6%	2.6%	1.7%
2 — 5 mo.	41.0%	28.2%	8.5%	10.3%
5 — 8 mo.	21.4%	30.8%	7.7%	3.4%
8 — 12 mo.	8.5%	5.1%	4.3%	7.6%
12 — 18 mo.	10.3%	1.7%	4.3%	6.0%
18 — 23 mo.	—	—	2.6%	0.9%
not observed	1.7%	20.5%	70.0%	68.4%

mo. = months

DISCUSSION

Through serologic study of patients with evidences of a recently acquired toxoplasma infection, it was possible to investigate anti-toxoplasma antibodies detected by different tests during the acute phase of the disease. Their variations could be followed for periods of weeks or months which permitted to disclose correlations between results of the several tests and to recognize successive serologic patterns of the infection. It was observed that anti-toxoplasma antibodies usually appear in serum only a few days after the beginning of clinical manifestations of the infection. There were cases showing initially only IgM antibodies, soon followed by IgG antibodies. In other cases it was as if both kinds of antibodies had came together, as in a patient presenting titers of 1:20 for

CF, 1:512 for HA, and 1:256 for both immunofluorescence tests, but one month later such titers had risen to 1:640 for CF, 1:16,000 for IF-IgG and 1:4,000 for IF-IgM, while HA titer remained the same.

In tests including as antigen less soluble components from parasitic walls, as the immunofluorescence and complement fixation tests, titers rapidly rose to high levels, usually within periods of a few days. However, low titers for many weeks or even months was the rule for the hemagglutination test, which employs as antigen very soluble constituents, especially of cytoplasmic origin.

Thus, in initial infections, a very constant and even monotonous serological pattern **could be found**, which displayed as characteristics a positive IF-IgM test, in general with titers of 1:1,024 or higher, IF-IgG titers of at least 1:8,000, CF titers of 1:160 or higher and HA titers not exceeding 1:4,000 and staying several dilutions lower than IF-IgG titers. In a previous study of reactive serum samples taken at random [2], such a pattern had already been suggested as related to recent infections, which could thus be confirmed.

The transition from this pattern to that observed for the majority of reactive samples in a population and recognized as indicating an old infection was investigated and 4 different marks of such a change could be established. These are negativation of the IF-IgM test, equalization of HA and IF-IgG titers, a decrease of IF-IgG titers to values of 1:4,000 or less and a decrease of CF titers to 1:80 or less.

Periods when each one of these changes occurred were investigated. It was verified that IgM antibodies could no more be detected in serum usually between 2 and 8 months after the first clinical manifestations of the infection. Longer periods could be found as well as shorter ones, and even IF-IgM tests that were negative from the first examined sera. Equalization of HA and IF-

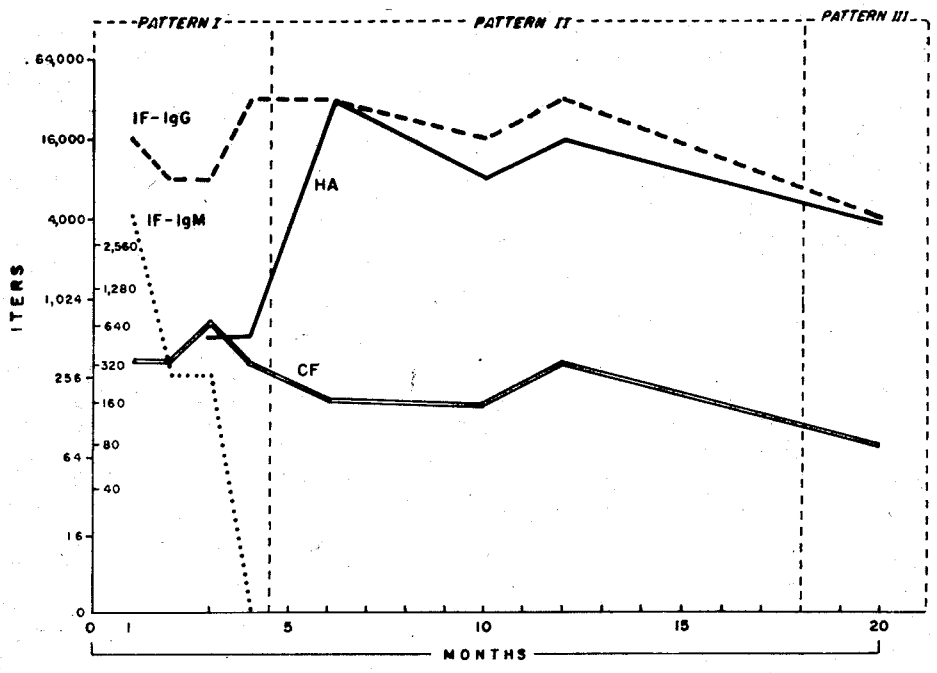

Fig. 2 — Succession of three serologic patterns in patient B.G.V.

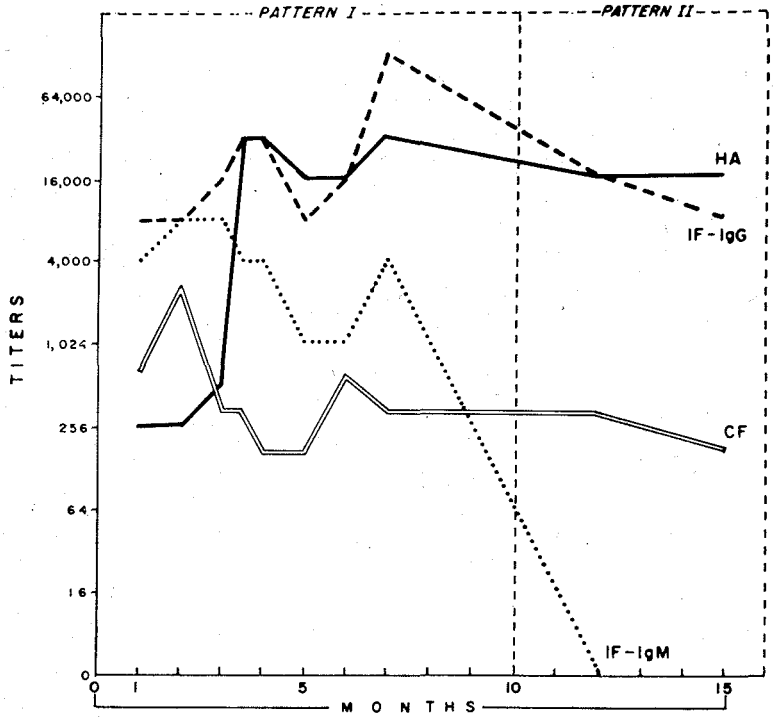

Fig. 3 — Serologic patterns I and II, observed for patient A.R. Jr.

IgG titers was also seen mostly between the 2nd and the 8th months, and generally due to a sudden rise of HA-titers to the already high IF-IgG titer values. Falling of IF-IgG and CF titers to low "old infection" limits usually took a much longer time and in several cases it could not be seen even after more than 2 years.

In this way, 3 serologic patterns could be distinguished, the first corresponding to early infections, the second to a transition stage and the third, to old infections. Pattern III, as previously indicated [2] presents positive IF-IgG and HA tests of low similar titers (1:4,000 or less), a negative or low-titered (1:80 or less) CF test and a negative IF-IgM test. Intermediate pattern II shows a negative IF-IgM test, high and very similar HA and IF-IgG titers, and a positive, high-titered CF test. Succession of the three patterns could be seen in several cases (Fig. 2). Others patients are still presenting pattern II (Fig. 3). There were cases pattern III substituted almost directly pattern I (Figs. 4 e 5).

The possibility of dating the beginning of a toxoplasma infection with the help of serum antibody patterns can be of importance not only for the differential diagnosis of clinical manifestations suggestive of toxoplasmosis, but also for evaluating risks of congenital infection and opportunity for therapeutic measures, especially during pregnancy.

Present data indicate that for clinical purposes serology of toxoplasmosis cannot base itself on isolated tests, even as trustful or sensitive as the Sabin-Feldman dye-test, immunofluorescence or hemagglutination tests, but a battery of tests is frequently necessary to yield a clear diagnostic picture, including not only titers but also correlations between different kinds of antibodies.

It is to be remembered that care must be taken against false positive IF-IgM tests which result from rheumatoid factors or IgM

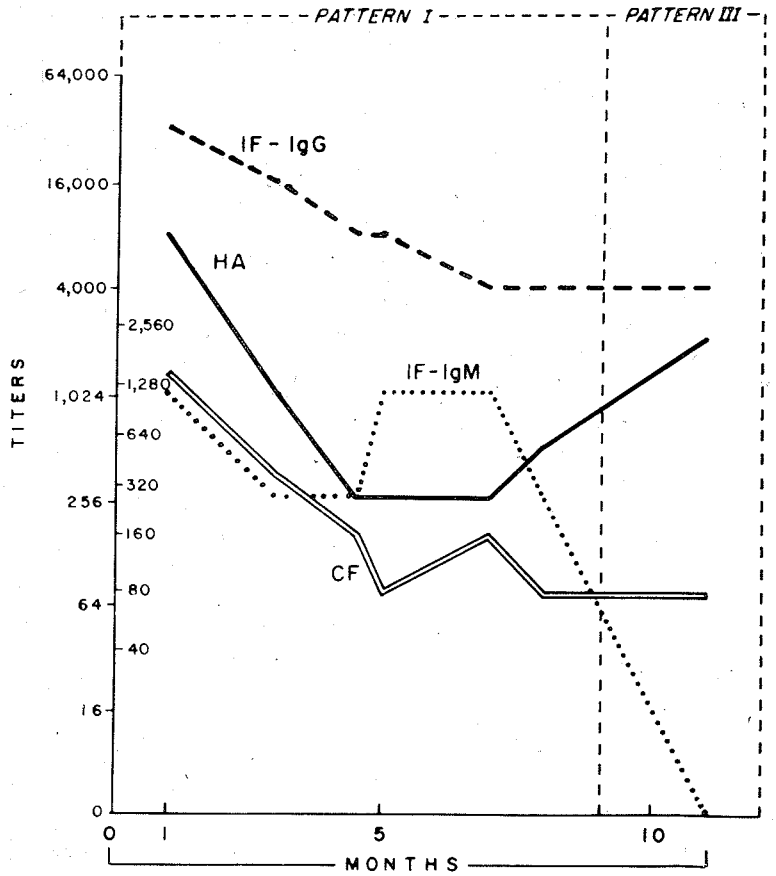

Fig. 4 — Pattern III succeded pattern I, for patient W.J.

anti-IgG antibodies in serum, as we have previously referred [1]. To obtain specific results, we have also described in the referred publication a very simple technique of treating sera with heat-aggregated globulins. Such technique was recently found satisfactory by HYDE et al. [3].

RESUMO

Significado diagnóstico de testes sorológicos na toxoplasmose humana. II — Estudo evolutivo de anticorpos e perfis sorológicos na toxoplasmose adquirida, através de testes de hemaglutinação, fixação do complemento, imunofluorescência anti-IgG e imunofluorescência anti-IgM

Soros de 136 pacientes de toxoplasmose recentemente adquirida foram submetidos a quatro testes para a toxoplasmose, de hemaglutinação, fixação do complemento, imunofluorescência anti-IgG e imunofluorescência anti-IgM.

Em amostras colhidas logo no início, os títulos IF-IgG excederam 1:4.000 em 93,5% dos casos e o teste IF-IgM foi positivo em 98,5%, com títulos, na maioria, de 1:1.024 ou mais. Em 94% dos pacientes o teste CF forneceu títulos altos, de 1:160 ou mais. Em contraste, para o teste HA os títulos foram baixos, não excedendo 1:4.000 em 90% dos casos.

Caracterizou-se, assim, um perfil sorológico bem diverso daquele perfil observado para a maioria das pessoas que em uma

população apresentam anticorpos anti-toxoplasma e que corresponde a infecções antigas, como indicamos em estudo anterior.

O estudo de amostras de soros de 117 dos casos de toxoplasmose recente, colhidas em períodos variados, permitiu acompanhar a evolução sorológica. Foi possível identificar marcos de transição entre esses perfis e a época em que ocorriam, e definir mais um perfil sorológico, de transição.

A passagem do perfil I, de infecção recente, para o perfil II, de transição, foi caracterizada por negativação do teste IF-IgM e equalização dos títulos IF-IgG e HA, em valores já definidos anteriormente como "equivalentes". Ocorreu, em média, de 2 a 8 meses do início das manifestações clínicas.

A queda de títulos dos testes de IF-IgG, HA e CF para os valores habituais de infecções antigas levou ao perfil III, o que observamos, porém, em apenas 1/3 dos casos e por vezes só após 1 ou 2 anos.

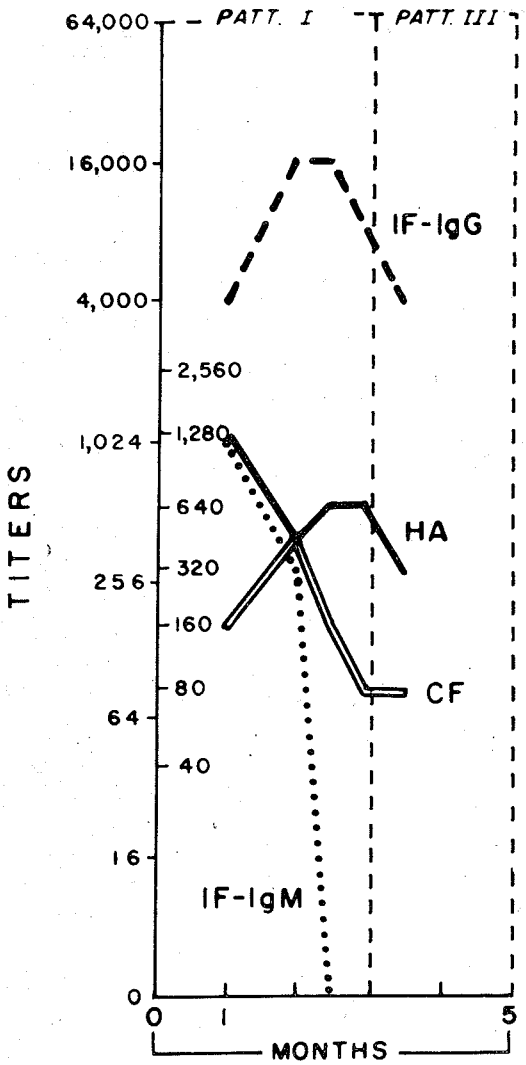

Fig. 5 — Patient W.I.S. Observe rapid evolution from pattern I to III.

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REFERENCES

1. 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.

2. CAMARGO, M.E.; LESER, P.G. & LESER, W.S.P. — Diagnostic information from serological tests in human toxoplasmosis. I — A comparative study of hemagglutination, complement fixation, IgG- and IgM-immunofluorescence tests in 3,752 serum samples. *Rev. Inst. Med. trop. São Paulo* **18**:215-226, 1976.

3. HYDE, B.; BARNETT, E.V. & REMINGTON, J.S. — Method for differentiation of nonspecific from specific toxoplasma IgM fluorescent antibodies in patients with rheumatoid factors. *Proc. Soc. Exp. Biol. Med.* **148**: 1184-1188, 1975.

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