ORIGINAL ARTICLE

Detection of IgA and low-avidity IgG antibodies for the diagnosis of recent active toxoplasmosis

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Objective: To determine the clinical value of testing IgA and the avidity of IgG (by two commercial systems) for the detection of recent active toxoplasmosis (RAT), and to study the IgG avidity during the course of infection.

Methods: The IgA was tested by a capture ELISA (Pasteur, France) and the avidity of IgG was determined by two modified commercial indirect ELISA methods (Sorin, Italy; Behringwerke, Germany) in 12 patients who were not immunosuppressed (group I) and 57 healthy subjects with a past infection by *Toxoplasma gondii* (group II).

Results: IgA was present in 75% of patients from group I and 21% of subjects from group II. The reliability for diagnosis of RAT was: sensitivity 75%, specificity 84%, positive predictive value 52.9% and negative predictive value 93.3%. In group I, 91.7% of patients had more than 50% low-avidity IgG, by both methods; in group II, 21% of subjects had low-avidity IgG at levels from 40% to 50%, by both methods. The diagnostic reliability of the two methods for the detection of low-avidity IgG in the first samples of RAT was similar when a breakpoint of 50% was used, with values of: sensitivity 91.7%, specificity 100%, positive predictive value 100% and negative predictive value 98%.

Conclusions: The study of IgA is not on its own adequate for diagnosis of RAT. However, testing the avidity of IgG is more reliable for the diagnosis of RAT, in studies of one serum sample or sequential samples.

Key words: *Toxoplasma gondii*, avidity, immunoglobulin G, immunoglobulin A

INTRODUCTION

Until now, the only reliable serologic markers for recent active toxoplasmosis (RAT) have been positive IgM serology and/or a rise in the IgG titers. The interpretation of IgM serology in RAT is hampered by the, sometimes protracted, persistence of specific IgM, the diminished IgM response in newborn babies, and the occurrence of so-called 'natural' antibodies. These last are directed towards multiple antigens of *Toxoplasma gondii* but are not produced by an immune response to toxoplasma infection. In certain Japanese populations that are almost free from toxoplasmosis, up to 30% of healthy adults are IgM responders [1]. This is the reason why immunoblotting is not useful as a reference method for *T. gondii* IgM detection [1,2]. To achieve specificity, manufacturers of IgM-detecting ELISAs tend to minimize the interference of cross-reacting IgM by a simple sensitivity reduction, which also counteracts the IgM persistence mentioned above [3].

The disadvantage of possible false-negative IgM findings early in toxoplasmosis could be eliminated by testing specific IgA in parallel. Many authors have considered parallel testing of specific IgM and IgA valuable in the diagnosis of some recently acquired infections and, with some reservations, in the diagnosis of the infection in the neonate [4,5]. In addition, the problem of IgM persistence can be reduced because specific IgA does not follow the same time course [6]. However, this is not present in all patients and many prefer to rely on serial determinations of IgG in two or three serum samples, with the consequent delay in diagnosis [7–9].

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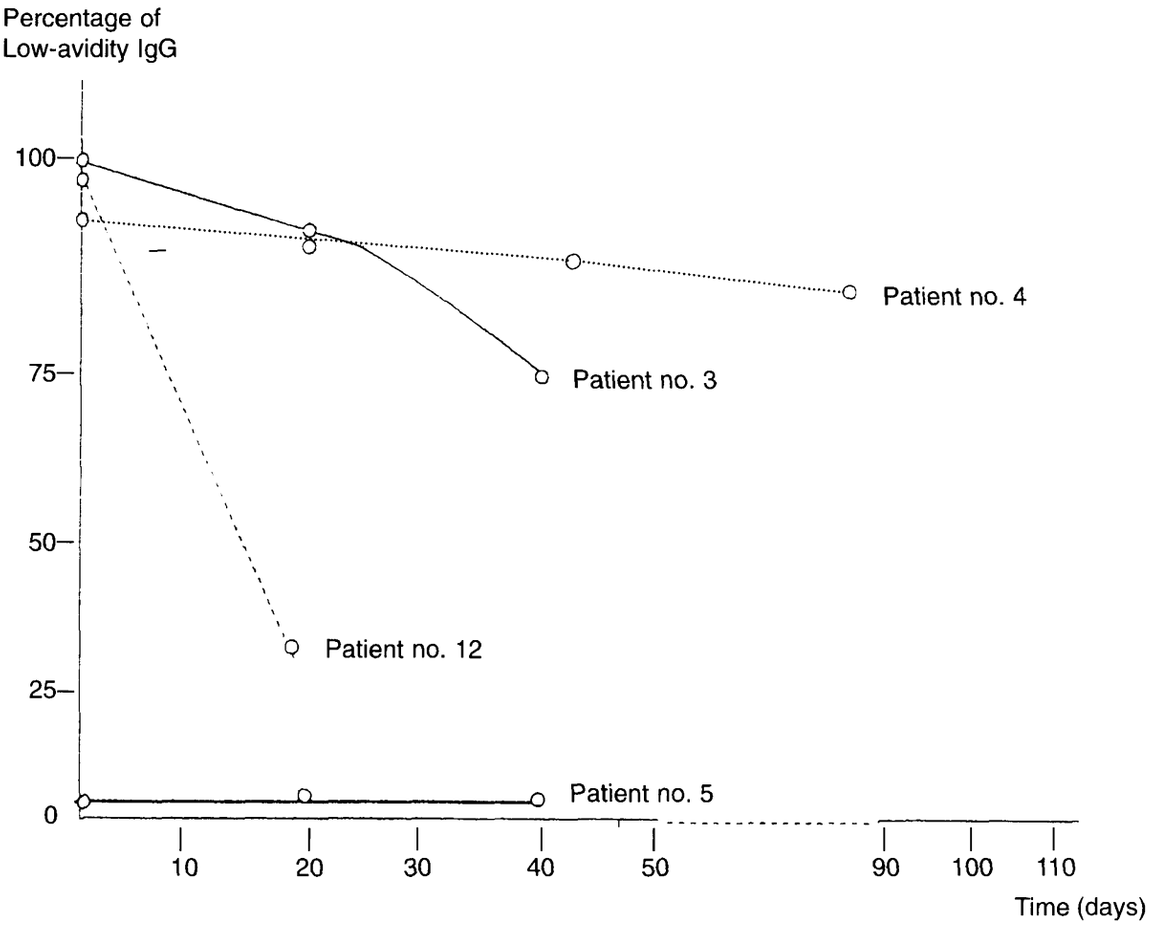

Figure 1 Evolution of percentage low-avidity IgG in patients Nos 3, 4 and 12 (group I) and subject 5 (group IIa).

In this context, the study of IgA and the level of IgG with low avidity for the antigen could reveal additional markers for RAT. Quantification of the low-avidity IgG, using simple techniques, is a new method in serologic diagnosis and follow-up of some infections. It can be used to distinguish between reactivation, reinfection and primary infection [10–13]. Therefore, we set out to determine the clinical value of studying IgA and the avidity of IgG (determined by two methods) in the detection of RAT.

MATERIALS AND METHODS

Subjects and serum samples

Group I consisted of 12 patients, aged 18–30 years, who were not immunosuppressed and who had clinical findings consistent with RAT—an illness including lymphadenopathy and seroconversion in IgM and IgG, detected by ELISA (Sorin Biomedica, Italy). Serum samples were collected 10–15 days after the onset of symptoms. In three patients (nos 3, 4 and 12) we examined further sera at intervals, as indicated in Figure 1.

Group II comprised 57 healthy subjects, aged 20–30 years, with a history of past infection by *T. gondii* defined by the presence of persisting IgG antibodies but with no known immune disorder. This group was subdivided into IIA, comprising seven subjects with IgM and IgG antibodies, and IIB, comprising 50 subjects with IgG but no IgM antibodies. One serum sample was collected from each subject, and in one individual from group IIA (no. 5) further serum samples were obtained at intervals as indicated in Figure 1.

Serologic tests

All initial sera were examined for the presence of specific IgA antibodies and low-avidity IgG antibodies (by two methods). Subsequent specimens were examined for IgA and for low-avidity IgG (by method 1 only).

IgA

A capture ELISA (Pasteur, France) was used. The cut-off, above which a test was considered positive, was obtained from the mean of the positive controls provided by the manufacturer.

Low-avidity IgG, method 1

A commercial test (indirect ELISA, ETI-TOXO-G, Sorin) which uses RH strain *T. gondii* tachyzoites for the antigen, was employed according to the manufacturer's instructions. For quantification of low-avidity IgG, the assay for detection of IgG was carried out in duplicate, simultaneously with and without 8 M urea. The samples were incubated for 1 h in the wells, and then removed. Urea was then applied to the wells for 5 min. For quantification of IgG a standard curve was drawn using the international standards provided with the test. To quantify low-avidity IgG, the difference in the values obtained in the two test runs (without and with urea) was taken and the result expressed as a percentage of the whole specific IgG. The assays were performed twice and the mean value was calculated when the test results differed by 10% or less; when the difference exceeded 10%, the tests were repeated.

Low-avidity IgG, method 2

An automated commercial test (Enzygnost, indirect ELISA, Processor III, Behringwerke, Germany), in which the well is coated with *T. gondii* antigen, was employed according to the manufacturer's instructions. For quantification of low-avidity IgG the assay was carried out in the same way as for method 1, but the urea was applied for 10 min. Exact quantification of the IgG was performed by the a-method calculation system (Behringwerke) [14]. With this it is possible to adjust the test absorbance mathematically to a standard curve, with results in international units. The percentage of low-avidity IgG was derived in the same way as in method 1, with the same requirement of ≤10% difference between duplicate runs.

Low-avidity IgG levels of >50% of total IgG were considered significant, since this value has been associated with RAT [11].

RESULTS

The overall results are presented in Table 1. IgA was present in the first samples of nine patients from group I (75%) and of 12 subjects from group II (21%) and the diagnostic reliability of IgA detection in RAT was: sensitivity 75%, specificity 84%, positive predictive value 52.9% and negative predictive value 93.3%. In the first samples from group I, 11 patients (91.7%) had more than 50% low-avidity IgG, with similar results by both methods. In no subjects from group IIa but in 12 subjects (24%) from group IIb we found low-avidity IgG at levels from 40% to 50%, with similar results by both methods (Table 1). A decrease over time in the low-avidity IgG (method 1) is shown in Figure 1, in the representatives of group I. The diagnostic reliability of the two methods for the detection of low-avidity IgG in the first samples of RAT was similar when a breakpoint of 50% was used, with values of: sensitivity 91.7%, specificity 100%, positive predictive value 100% and negative predictive value 98%.

DISCUSSION

Currently, acute-phase markers are being sought for toxoplasmosis that are more specific than the traditional ones. Two possibilities are the detection of IgA and of a high proportion of low-avidity IgG.

Because of the high sensitivity of ELISA IgA methods, IgA can be detected at early phases of the

Table 1 Subjects with low-avidity IgG and IgA in groups I and II

	Low-avidity IgG (%)											
	0–20		21–40		41–50		51–60		61–80		81–100	
	$IgA^{(+)}$	$IgA^{(-)}$	$IgA^{(+)}$	$IgA^{(-)}$	$IgA^{(+)}$	$IgA^{(-)}$	$IgA^{(+)}$	$IgA^{(-)}$	$IgA^{(+)}$	$IgA^{(-)}$	$IgA^{(+)}$	$IgA^{(-)}$
Method 1												
Group I	0	0	0	0	1	0	1	0	0	1	7	2
Group IIa	2	3	2	0	0	0	0	0	0	0	0	0
Group IIb	4	14	2	16	0	12	0	0	0	0	0	0
Method 2												
Group I	0	0	0	0	1	0	1	0	1	1	6	2
Group IIa	3	3	1	0	0	0	0	0	0	0	0	0
Group IIb	6	17	2	25	0	0	0	0	0	0	0	0

Group I: Recent active toxoplasmosis.
Group IIa: Past toxoplasmosis with IgM positive.
Group IIb: Past toxoplasmosis with IgM negative.

infection, but also for a long time after the onset of the acute phase [6]. Thus persistence of IgA has been reported for a period of more than 6 months after the acute infection [15], or in healthy subjects with a past infection [16]. In some cases of RAT in which IgM could not be demonstrated, IgA was nevertheless present [17]. For these reasons, the clinical value of the study of IgA is limited.

In our experience, the IgA detection test did not achieve diagnostic reliability for RAT; the low sensitivity and positive predictive value are the reasons. These results contradict those obtained by other authors [16,18], even when using the same methods. On the other hand, some authors have obtained results similar to ours [15], concluding that this test is not a valid marker for the diagnosis of the active disease, or at least that it is no better than the study of IgM.

Another marker which has been analyzed for the diagnosis of primary infection by *T. gondii* is the detection of low-avidity IgG. An extensive amount of published work [7,11,13,19–22] underlines the high value of the study of this type of IgG as a diagnostic aid for acute toxoplasmosis without IgM or to exclude past infections, when specific IgM is present. We agree with all these authors in finding 100% diagnostic reliability in the detection of primary infections less than 3 months old. In individuals with past infection the IgG antibodies detected are of high avidity. Using 8 M urea we obtained results similar to those of other authors who used this or another denaturing substance. The literature reviewed on the use of low-avidity IgG antibodies in the diagnosis of infection in general gives breakpoints varying from 70% to 85% [13,21,23,24] to 50% [25,26]. This depends on whether the approach is used for determining primary infection, reactivation or reinfection, on the denaturing substance used and on the different human pathogens being studied. The previous authors, who use a cut-off at a higher percentage of low-avidity IgG, used 6 M urea or diethylamine as a denaturing agent.

By combining an IgG avidity result with the study of IgM it is possible to resolve conflicting situations. Thus IgM-negative primary infections and IgM-positive past infections can be distinguished with low-avidity IgG. Use of this method would be prevented, of course, by the absence of IgG, a phenomenon unlikely to occur, since the IgG response in toxoplasmosis is usually rapid and strong, and occurs in the newborn baby.

Comparing the two methods of determining low-avidity IgG, we find that they have an identical reliability, although they are colorimetric ELISA techniques with IgG quantification by different procedures. The characteristics of the antigens used by the two manufacturers are not known to us. We tried to utilize the same time of application in the urea test, but found it necessary, for technical reasons, to use 10 min with the method 2 (Processor III). With this time we obtained similar results to method 1. Previously we had established that in Processor III 10 min was enough to discriminate low-avidity IgG [27].

Serial study showed disappearance of the low-avidity IgG in 3–5 months. However, a much larger group should be studied to determine the minimum period needed in all cases, as the evolution may vary among subjects. In our series (Figure 1) we observe a decrease of percentage of low-avidity IgG in the three patients with RAT that was higher in patient no. 12.

In conclusion, the study of IgA is not on its own adequate for diagnosis of RAT. IgG avidity tests, however, when combined with IgM study in a single sample, make it possible to establish a recent infection.

References

1. Konishi E. Naturally occurring immunoglobulin M antibodies to *Toxoplasma gondii* in Japanese population. Parasitology 1991; 102: 157–62.
2. Verhofstede C, Van Renterghem L, Plum J. Evaluation of immunoglobulin for the detection of *Toxoplasma gondii* immunoglobulin M antibodies. Eur J Clin Microbiol Infect Dis 1990; 9: 835–7.
3. Verhofstede C, Van Renterghem L, Plum J. Comparison of six commercial enzyme linked immunosorbent assays for detecting IgM antibodies against *Toxoplasma gondii*. J Clin Pathol 1989; 42: 1285–90.
4. Decoster A, Darcy F, Caron A, et al. Anti-P30 IgA antibodies as prenatal markers of congenital toxoplasma infection. Clin Exp Immunol 1992; 87: 310–15.
5. Fourdrinier F, Marx-Chemla C, Aubert D, Bonhomme A, Pinon JM. Value of specific immunoglobulin A detection by two immuno-capture assays in the diagnosis of toxoplasmosis. Eur J Clin Microbiol Infect Dis 1995; 14: 585–90.
6. Francis JM, Joynson DHM. Duration of specific immunoglobulin A antibody following acute toxoplasmosis as determined by enzyme immunoassay and immunoassay and immunoabsorbent agglutination assay. Eur J Clin Microbiol Infect Dis 1993; 12: 556–9.
7. Ades AE. Evaluating the sensivity and predictive value of test of recent infection: toxoplasmosis in pregnancy. Epidemiol Infect 1991; 107: 527–35.
8. Gutiérrez J, Roldán C, Maroto C. Seroprevalence of human toxoplasmosis. Microbios 1996; 85: 73–5.
9. Gutiérrez J, Rodríguez M, Maroto C. A study of IgM antibodies in diagnosis of acute infection by *Toxoplasma gondii* in Spain. Serodiagn Immunother Infect Dis 1996; 8: 85–8.
10. Gutiérrez J, Piédrola G, Maroto C. Reliability of four methods for the diagnosis of acute infection by Epstein–Barr virus. J Clin Lab Anal 1997; 11: 78–81.
11. De Ory F, Casa I, Domingo CJ, Echevarría JM. Application of fluoroimmunoassay to identification of low avidity

specific IgG against pathogenic human viruses and *Toxoplasma gondii*. Clin Diagn Virol 1995; 3: 323–35.
12. Hedman K, Lappalainen M, Söderlund M, Hedman L. Avidity of IgG in serodiagnosis of infectious diseases. Rev Med Microbiol 1993; 4: 123–9.
13. Sensini A, Pascoli A, Marchetti D, et al. IgG avidity in the serodiagnosis of acute *Toxoplasma gondii* infection: a multicenter study. Clin Microbiol Infect 1996; 2: 25–9.
14. Gutiérrez J, Maroto, C, Piédrola G. Evaluation of a new reagent for anti-cytomegalovirus and anti-Epstein–Barr virus IgG. J Clin Microbiol 1994; 32: 2603–5.
15. Huskinson J, Thulliez P, Remington JS. Toxoplasma antigens recognized by human immunoglobulin A antibodies. J Clin Microbiol 1990; 28: 2632–6.
16. Bessières MH, Roques C, Berrebi A, Barre V, Cazaux M, S'eguéla JP. IgA antibody response during acquired and congenital toxoplasmosis. J Clin Pathol 1992; 45: 605–8.
17. Gutiérrez J, De la Higuera A, Maroto MC. Diagnostic value of immunoglobulin A and M in acute toxoplasmosis. Rev Invest Clin 1993; 45: 585–8.
18. Stepick-Bick P, Thulliez P, Araujo FG, Remington JS. IgA antibodies for diagnosis of acute congenital and acquired toxoplasmosis. J Infect Dis 1990; 162: 270–3.
19. Camargo ME, da Silva SM, Leser PG, Granato CH. Avidez de anticorpos IgG especificos como marcadores de infeccao primaria recente pelo *Toxoplasma gondii*. Rev Inst Med Trop Sao Paulo 1991; 33: 213–18.
20. Holliman RE, Raymond R, Renton N, Johnson JD. The diagnosis of toxoplasmosis using IgG avidity. Epidemiol Infect 1994; 112: 399–408.
21. Lappalainen M, Koskela P, Koskiniemi M et al. Toxoplasmosis acquired during pregnancy: improved serodiagnosis based on avidity of IgG. J Infect Dis 1993; 167: 691–7.
22. Vinhall FA, Pena JD, Katina JH, et al. Analysis of aqueous humor in ocular toxoplasmosis: detection of low avidity IgG specific to *Toxoplasma gondii*. Appl Parasitol 1994; 35: 1–7.
23. Thomas HI, Morgan-Capner P, Connor NS. Adaptation of a commercial rubella-specific IgG to assess specific IgG avidity. Serodiagn Immunother Infect Dis 1993; 1: 13–16.
24. Thomas HI, Morgan-Capner P, Cradeck-Watson JE, Enders G, Best JM, O'Shea S. Slow maturation of IgG1 avidity and persistence of specific IgM in congenital rubella: implications for diagnosis and immunopathology. J Med Virol 1993; 41: 196–200.
25. Kangro HO, Manzoor S, Harper DR. Antibody avidity following varicella-zoster virus infections. J Med Virol 1991; 33: 100–5.
26. Schoub BD, Blackburn NK, Johnson S, McAnerney JM, Miller B. Low antibody avidity in elderly chickenpox patients. J Med Virol 1992; 37: 113–15.
27. Gutiérrez J, Rodríguez M, Piédrola, Maroto MC. Behaviour of IgG antibody avidity for the antigen and of IgA in active cytomegalovirus, Epstein–Barr virus, herpes simplex virus and human herpes virus-6 infections. Adaptation of a commercial test. J Infect 1997 (in press).