JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1989, p. 2133–2135
0095-1137/89/092133-03$02.00/0

Vol. 27, No. 9

Significance of Specific Immunoglobulin M in the Chronological Diagnosis of 38 Cases of Toxoplasmic Lymphadenopathy

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Received 21 February 1989/Accepted 17 May 1989

The persistence of immunoglobulin M (IgM) antibody to *Toxoplasma gondii* in sera from 38 patients after toxoplasmic lymphadenopathy was investigated by using an indirect immunofluorescence assay, a double-sandwich enzyme-linked immunosorbent assay, and an immunosorbent agglutination assay. Positive predictive values at 3 and 6 months after lymphadenopathy were, respectively, 45 and 73% for the indirect immunofluorescence assay, 25 and 45% for the double-sandwich enzyme-linked immunosorbent assay, and 22 and 43% for the immunosorbent agglutination assay.

A major goal in the diagnosis of toxoplasmosis is to determine the time of onset of infection, particularly in asymptomatic infections acquired during pregnancy. Thus, sensitive techniques were developed to detect early-phase response immunoglobulin M (IgM) antibodies. However, when more sensitive techniques were used, it became apparent that specific IgM could be detected even several months after the acute phase of infection (2, 5, 8, 12).

Time course studies on this particular issue have suffered from the limited number of patients studied, uncertainty about the onset of infection, and lack of availability of current techniques.

Here we report the follow-up of the serological profiles of 38 patients with acute acquired *Toxoplasma* lymphadenopathy. *Toxoplasma gondii*-specific IgM antibodies were detected by using three different assays: an indirect immunofluorescence assay (IFA), a double-sandwich enzyme-linked immunosorbent assay (DS ELISA), and an immunosorbent agglutination assay (ISAgA).

A total of 19 male and 19 female patients were examined. Their relevant mean age was 26 years (range, 15 to 37 years). They were diagnosed as having acute acquired *Toxoplasma* lymphadenopathy by both clinical and serological criteria. All other known causes of lymphadenopathy were excluded by clinical, hematological, and serological methods (11). All of the patients in this study had a similar clinical course of infection, and they were followed up at intervals for clinical and serological reassessment at our department.

A total of 102 serum samples were available and assayed for *T. gondii*-specific IgM antibodies (i) by an IgM IFA (10), for which rabbit anti-human IgM antiserum was purchased from Dako (Glostrup, Denmark) and used at a dilution of 1:20 in phosphate-buffered saline (titers in serum of ≥1:32 were considered positive [12]); (ii) by an IgM DS ELISA (8), for which commercially available reagents were used (Toxonostika IgM; Organon Teknika, Rome, Italy) (cutoff values were calculated according to the guidelines of the manufacturer); and (iii) by IgM ISAgA (3), for which mouse anti-human IgM monoclonal antibody was purchased from bioMerieux (Marcy l'Etoile, France); samples were recorded as positive by a previously published method (3).

The presence or absence of rheumatoid factor in the serum samples was assessed with a latex agglutination test kit (Behringwerke, Scoppito, Italy).

Statistical analysis was performed to evaluate (i) the rate at which the three IgM assays became negative by actuarial analysis (6) and (ii) the positive predictive value (PPV) of each IgM assay, i.e., the probability that an individual with a positive IgM assay was infected within a given period of time. The adopted statistical methods are described in detail in the Appendix.

Table 1 indicates the times at which serum samples were available from each patient after the clinical onset of *Toxoplasma* lymphadenopathy. Moreover, it indicates the first time at which the serum samples were negative in the IgM assays.

The actuarial presence of *T. gondii*-specific IgM detected by three different techniques in the patients under study is given in Table 2. Whereas IgM was detected by ISAgA in all but one patient within 3 months after toxoplasmic lymphadenopathy (97% positivity), in a considerable proportion (30%) of serum samples from the same period specific IgM antibodies were not found by using IFA. By using DS ELISA, specific IgM antibodies were detected in sera of 85% of patients within 3 months after the clinical onset of infection. Moreover, *T. gondii*-specific IgM lasted up to 9 months in 94% of patients when detected by ISAgA, whereas a marked decrease of IgM positivity by IFA and, to a lesser extent, by DS ELISA was found throughout the study period (Table 2).

T. gondii-specific IgM persistence after 3 and 6 months in the study population computed by actuarial analysis enabled us to estimate the probability that infection occurred within a given time period. Thus, assuming an average incubation period of toxoplasmic lymphadenopathy of 14 days (1), the probability of infection during the previous 3 or 6 months in an individual with a positive IgM titer determined by IFA was calculated to be 45 or 73%, respectively. In the case of a positive IgM titer as determined by ISAgA, the probability of infection during the previous 3 or 6 months was calculated to be 22 or 43%, respectively (Table 3).

These results confirm previous reports that showed that ISAgA and, to a lesser extent, DS ELISA are much more sensitive than IFA in the detection of *T. gondii*-specific IgM in serum (2, 3, 12).

The occurrence of false-negative results in the IgM IFA

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TABLE 1. Time of serum sampling in 38 patients with *Toxoplasma* lymphadenopathy

Patient no.	Age (yr), sex[a]	Serum sampling time (wk from clinical onset)
1	25, M	16,[b] 24, 28
2	22, M	24,[b] 36, 44, 72[c]
3	19, F	8,[b] 12, 20
4	37, M	8,[c] 16[b]
5	18, F	8,[b] 16, 28
6	23, M	8, 12, 16,[b] 24, 36
7	26, F	14, 20, 32,[b] 36
8	24, F	36,[c] 52[b,d]
9	32, F	12,[b] 32
10	28, F	16,[b,c] 24
11	31, M	48,[b] 56, 60, 72
12	17, F	16, 20, 24, 48, 56[b]
13	34, F	40,[b] 48, 56, 72[d]
14	21, F	36,[b] 44[d]
15	15, F	16, 20, 36[b]
16	19, M	28,[b] 40[c,d]
17	19, M	4, 12[b,c,d]
18	28, F	4, 12, 20[b]
19	29, F	40, 72[b,c,d]
20	21, M	12, 16, 20, 48[b]
21	23, M	8, 12[b] 16
22	16, M	20, 28
23	27, M	8,[b] 12, 24, 28
24	20, M	40, 72
25	28, F	28, 36[b]
26	26, F	12
27	32, F	12, 20, 32
28	34, M	4, 12[b]
29	26, M	4, 8, 16,[b] 24
30	21, M	12, 16[b]
31	29, F	12,[b] 16
32	31, F	72[b]
33	30, F	12, 16, 20
34	32, M	8, 16, 24
35	18, M	12, 20
36	35, F	12,[b,c] 20[d]
37	31, M	4
38	36, M	4, 12

[a] M, Male; F, female.
[b] Sample became negative on the basis of IgM IFA.
[c] Sample became negative on the basis of DS ELISA.
[d] Sample became negative on the basis of IgM ISAgA.

has been well documented by Pyndiah et al. (9) and Filice et al. (4), who proposed the use of gel filtration of sera to avoid a possible competitive effect by a high IgG titer for antigenic determinants in common with IgM. However, in our experience this technique fails to demonstrate a significant increase in the sensitivity of the IgM IFA (unpublished data).

TABLE 2. Persistence of *T. gondii*-specific IgM antibodies following *Toxoplasma* lymphadenopathy

No. of mo[a]	No. of patients	% of samples with IgM antibodies determined by[b]:		
		IFA	ISAgA	DS ELISA
0–3	23	70	97	85
4–6	20	43	94	76
7–9	14	24	94	68
10–12	9	12	67	45
13–15	4	4	56	45
16–18	6	1	37	15

[a] From the time of clinical onset.
[b] Percentages computed by actuarial analysis.

TABLE 3. PPV of IgM assays in diagnosis of the acquisition time of acute symptomatic *Toxoplasma* infection

Assay	PPV (%) at[a]:	
	3 mo	6 mo
IFA	45	73
ISAgA	22	43
DS ELISA	25	45

[a] PPV is the probability that an individual with a positive test was infected during the previous 3 or 6 months.

More interestingly, results of this study provide a tool to calculate the PPVs of IgM assays for diagnosis of the acquisition time of *Toxoplasma* infection. Thus, although these predictive values are merely indicative of infection, since they are based on a small sample of patients and their confidence intervals are large, they show that a positive IgM IFA is the most predictive assay to ascertain a recently acquired *Toxoplasma* infection.

Phenotypic and functional immunologic abnormalities, with imbalances of the CD4/CD8 lymphocyte ratio and depression of *T. gondii*-specific T-cell proliferation, have been reported during acute *Toxoplasma* lymphadenopathy (7). Consequently, our data on IgM positivity and persistence cannot be applied to different clinical situations. However, there is no evidence of whether such imbalances also occur in the antibody synthesis and, more importantly, in the isotype shift from immunoglobulin class M to immunoglobulin class G.

Thus, the predictive values given in Table 3 could also be tentatively assessed in different clinical situations, e.g., in patients with acquired asymptomatic infections. With respect to toxoplasmosis diagnosis, our data raise questions as to the suitability of using high-sensitivity tests in which the specificity is affected only by the long persistence of positivity. Instead, these tests should be integrated with a more specific IgM assay (e.g., IFA) to estimate the infection acquisition time.

The combined use of two tests characterized by different sensitivities and specificities would be helpful under different clinical circumstances and particularly when the clinician must face the decision of whether to perform an invasive diagnostic procedure (node biopsy or fetal blood sampling).

APPENDIX

Statistical methods. Statistical analysis was aimed at assessing (i) the rate at which serum samples in the three IgM assays became negative and (ii) the PPV, i.e., the probability that an individual with a positive IgM assay was infected within a given period of time.

(i) Rate at which serum samples became negative. The available data on the rate at which serum samples became negative were not complete, as several patients were lost to follow-up while they were still IgM positive, whereas in other patients the interval between the last positive test and the first negative one was quite long (e.g., >6 months). As a consequence, we adopted a method similar to that used in actuarial analysis (6), which is based on the following two assumptions: (i) individuals who are lost to follow-up while they are still positive by a test have the same probability of turning negative, in any given period, as those who are still in follow-up and (ii) those individuals who have long intervals (>3 months) between a positive and a negative test have the same probability of becoming negative, per unit time, as those who were more closely monitored during follow-up.

Analysis was restricted to the 18 months following the clinical onset of infection; this period was then divided into six quarters.

If we define P_i as the number of patients still in follow-up and IgM positive by the test (during that interval of time or, when not available, by any following test) and N_i as the number of patients with the first negative test during the same interval of time, the probability of remaining positive by a test within each quarter period i (interval-specific, negativization-free survival [NFS_i]) was computed as: $NFS_i = P_i/(N_i + P_i)$. The cumulative probability of still being positive by the test at any time since the infection was obtained as the product of all the interval-specific probabilities NFS_i from time zero through time x.

(ii) PPV. Assuming that the infection rate was constant over time at any given moment, the probabilities that any individual was infected 0 to 3 or 4 to 6, 7 to 9, or 10 to 12 months previously are all identical.

Once the curve of the rate at which serum samples became negative was computed, the probability that an individual with a positive test was infected within a given time span could be estimated as the ratio between the sum of the actuarial probabilities of being positive in that period and the sum of all the interval-specific actuarial probabilities from time 0 to 3 months through 16 to 18 months (for simplicity, all tests were assumed to become negative in 100% of the cases at 18 months since the clinical onset of infection).

In the present analysis, we estimated the probabilities that an individual with a positive IgM assay was infected during the previous 3 or 6 months. For example, the 6-month predictive value of a positive IgM IFA was equal to (70% + 43%)/(70% + 43% + 24% + 12% + 4% + 1%) = 73% (Table 2).

This work was supported by Ministero Pubblica Istruzione and by Ministero della Sanità.

The skillful technical assistance of Franca Miletich and Luigi Corradino is gratefully acknowledged. We are indebted to Elisabetta Bicocchi for cooperation in the copy editing.

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