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Significance of a Positive *Toxoplasma* Immunoglobulin M Test Result in the United States

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A positive *Toxoplasma* immunoglobulin M (IgM) result is often interpreted as a marker of an acute infection. However, IgM can persist for several years, and *Toxoplasma* commercial IgM diagnostic test kits can yield a number of false-positive results. For these reasons, a chronic *Toxoplasma* infection can be erroneously classified as an acute infection, resulting in serious adverse consequences, especially in pregnant women, leading to emotional distress and unnecessary interventions, including termination of pregnancy. Interpretation of *Toxoplasma* serology at a reference laboratory can help differentiate a recently acquired infection from a chronic infection. Serological test results for 451 patients with positive *Toxoplasma* IgM and IgG test results obtained at nonreference laboratories (NRLs) that were referred to Palo Alto Medical Foundation Toxoplasma Serology Laboratory (PAMF-TSL) to determine whether the patient was acutely or chronically infected were retrospectively reviewed. PAMF-TSL results established that of the 451 patients, 335 (74%) had a chronic infection, 100 (22%) had an acute infection, and 7 (2%) were not infected, and for 9 (2%), results were indeterminate. Positive *Toxoplasma* IgM and IgG test results obtained at NRLs cannot accurately distinguish between acute and chronic infections. To do so, testing at reference laboratories is required, as mandated in 1997 in a letter from the Food and Drug Administration (FDA) to clinicians and laboratories in the United States.

Toxoplasmosis is a parasitic infection caused by the intracellular protozoan *Toxoplasma gondii*. One-third of the world's human population is infected (1). In the United States, each year about one million people acquire this infection, with 30% seroconversion by 70 years of age (2). Acute infection is generally asymptomatic or presents with mild nonspecific symptoms in immunocompetent people. Acute infection acquired during pregnancy can also lead to congenital anomalies and fetal demise (3, 4). In the United States, the incidence of congenital toxoplasmosis is around 2,774 cases per year (0.7 case per 1,000 live births) (5). After an acute episode is over, *Toxoplasma* protozoa encyst and remain latent for the life of the host, mainly in heart, brain, eye, and muscle tissues. This chronic or latent infection generally runs a benign course in the immunocompetent population but can reactivate with end organ involvement in people with weak immune systems, such as patients with advanced HIV disease or those on immunosuppressive therapy (6). In immunocompromised individuals, toxoplasmosis can result in life-threatening disease.

In patients with *Toxoplasma* infection, it is important to establish whether they have an acute or chronic infection. In pregnant women, for instance, a diagnosis of acute infection is an indication of the risk of potential transmission of the parasite to the fetus and should trigger treatment to prevent vertical transmission and testing of amniotic fluid for *Toxoplasma* by the PCR; if fetal infection is confirmed by a positive PCR, treatment is indicated to ameliorate clinical sequelae for infected offspring. In contrast, diagnosis of chronic infection (acquired prior to gestation) in an otherwise immunocompetent woman essentially does not carry any risk for congenital toxoplasmosis (7).

Distinguishing an acute *Toxoplasma* infection from a chronic one is based on serological data (8). Serological testing in nonreference laboratories (NRLs) in the United States relies on detection of immunoglobulin M (IgM) and immunoglobulin G (IgG). A positive *Toxoplasma* IgM test is often considered a marker of an acute infection. However, IgM can persist for several months to years after an acute infection, thus making the distinction between an acute and a chronic infection challenging (9, 10). High-IgG-avidity test results in patients with positive *Toxoplasma* IgM test titers can establish that the patient has been infected for at least 3 to 5 months, but low-avidity test results alone are not necessarily diagnostic of an acute infection. Furthermore, positive IgM test results at NRLs can also represent a false-positive reaction. In a serological study of toxoplasmosis during an outbreak in British Columbia, Canada, 46.4% of 153 specimens positive by the Platelia *Toxoplasma* IgM test (but negative for IgM at the PAMF-TSL) tested negative by the Sabin-Feldman dye test (IgG), implying falsely elevated NRL IgM among patients without serologic evidence of *Toxoplasma* infection (11). These two phenomena (persistence of positive *Toxoplasma* IgM in some patients with chronic infection and false-positive results) are well known by laboratories, but to date, there are no published data on the estimation in the United States of the number of potential misinterpretations that would result from considering IgM positivity the sole marker of an acute infection.

Studies done in the 1990s and early 2000s have shown persis-

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tently elevated or false-positive IgM tests obtained by commercial kits compared to the double-sandwich IgM ELISA developed and performed at Palo Alto Medical Foundation Toxoplasma Reference Laboratory (PAMF-TSL) (11–14). Unlike NRL, the PAMF-TSL performs a complete panel of standardized *Toxoplasma* serologic tests that help distinguish an acute infection from a chronic one. PAMF-TSL is a nonprofit organization that has been solely dedicated to the laboratory diagnosis of *Toxoplasma gondii* infection for more than 50 years and also serves as the reference laboratory for the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA) in the United States.

The purpose of this study was to evaluate the overall positive predictive value (PPV) of positive *Toxoplasma* IgM test results obtained at NRLs in the United States.

MATERIALS AND METHODS

We performed a retrospective study of all serum samples that were reported to be positive for both *Toxoplasma* IgG and IgM at NRLs and were referred to PAMF-TSL from January 2003 through December 2013 to assess whether the positive IgM result represented a true acute infection or a chronic infection. The study was approved by the Institutional Review Board at the Palo Alto Medical Foundation Research Institute. The methods for serological testing at PAMF-TSL have been described elsewhere (15–20). In brief, all referred sera were tested initially with the Sabin-Feldman dye test (IgG) and immunocapture IgM enzyme-linked immunosorbent assay (ELISA). Samples that were positive for both the dye test (IgG) and IgM ELISA were tested further with the IgA ELISA, IgE ELISA, differential agglutination of AC and HS antigens (AC/HS test), and IgG avidity (bioMérieux, Lyon, France) to differentiate acute from chronic infections. PAMF-TSL has worked for decades with this battery of serologic tests to determine the stage of *Toxoplasma* infection. Moreover, an acute serologic profile at PAMF-TSL correlates accurately with an infection acquired within 6 months (21).

Study patients were classified in 4 groups according to their PAMF-TSL serologic profiles: acute infection, chronic infection, no infection, and indeterminate. Serological criteria used to define infection status were as follows. For acute infection (100 patients), criteria were a positive IgG dye test, positive IgM ELISA (≥2), and an acute AC/HS pattern and/or low avidity; in one patient, AC/HS and avidity were equivocal, but at least one follow-up sample confirmed acute infection. For chronic infection with positive IgM (119 patients), criteria were a positive IgG dye test, a positive IgM ELISA (≥2), and a nonacute AC/HS pattern and/or high avidity. In 20 patients, AC/HS results and avidity were equivocal, but at least one follow-up sample confirmed chronic infection. In 10 patients, AC/HS results and/or avidity was equivocal, but both IgG and IgM titers were low and near the cutoff; thus, the patients were classified as having chronic infection. For chronic infection with negative IgM (200 patients), criteria were a positive IgG dye test, a negative IgM ELISA, and a nonacute AC/HS pattern and/or high avidity. In 3 patients AC/HS and/or avidity were equivocal, but at least one follow-up sample confirmed chronic infection. In 26 patients, AC/HS results and/or avidity was equivocal, but the IgG titer was low and near the cutoff; thus, they were classified as having chronic infection. For chronic infection with equivocal IgM (16 patients), criteria were a positive IgG dye test and an equivocal IgM ELISA. In 3 patients, AC/HS results and/or avidity was equivocal but the IgG titer was low and near the cutoff; thus, these patients were classified as having chronic infection. For the classification "no infection" (7 patients), criteria were negative IgG dye test result (5 patients had negative IgG and IgM results, and 1 each had negative IgG and equivocal IgM results and negative IgG and positive IgM results). In the patient with positive IgM, the follow-up sample 4 years later revealed the same test results (IgG negative and IgM positive). For the indeterminate classification (9 patients), criteria were a positive IgG dye test, a negative or equivocal IgM ELISA, and an acute AC/HS pattern and low avidity. None of these patients had follow-up samples. The serological profiles of the patients were independently analyzed by the authors (R.D., K.G., C.P., and J.G.M.) in two separate analyses. If discrepancies were encountered (only 1 out of 451 patients), a consensus was reached by all parties reviewing test results at a subsequent meeting with all the authors present. Samples were excluded if the collection dates of sera for serological testing at the NRL and PAMF-TSL differed by more than 6 months. Patient demographics regarding age, gender, and pregnancy status were also obtained from the PAMF-TSL database. An analysis was performed to calculate the proportion of *Toxoplasma* IgM-positive samples from NRLs that were classified as representing acute infection, chronic infection, or no infection or were indeterminate.

TABLE 1 Demographics of 451 patients whose sera were referred to Palo Alto Medical Foundation Toxoplasma Serology Laboratory between January 2003 and December 2013

Characteristic (*n*)	No. (*n* = 451)	%
Gender (448)		
Male	16	4
Female	432	96
Age (450)		
<18	13	3
≥18	437	97
Pregnancy (432)	402	93

There were a total of 770 patients in our database for whom NRLs had obtained positive results in the *Toxoplasma* IgM and IgG serology and who had serum specimens referred to PAMF-TSL for confirmatory testing of a presumed acute *Toxoplasma* infection. Three hundred eighteen patients were excluded from the study because the date of serum collection for serological tests at the NRL could not be determined (297 patients) or because serum collection dates at the NRL and PAMF-TSL differed by more than 6 months (21 patients).

Of the remaining 452 patients, one was an infant. The infant was excluded from final analysis because of difficulty in interpreting toxoplasma serology and the different IgM assay (immunosorbent agglutination assay [ISAGA]) used for infants ≤6 months of age. Thus, 451 patients were available for the final analysis. All of them had had specimens referred to PAMF-TSL within 6 months of initial testing at NRLs.

RESULTS

Of 448 patients (out of 451) for whom gender was known, 432 (96%) were females (Table 1). Pregnant women comprised 93% (402) of female patients. Ninety-seven percent of patients were adults. The median age was 32 years (range, 4 to 73 years). The median interval between blood collection for serological testing at the NRL and at PAMF-TSL was 15 days (range, 0 to 175 days). Ninety-five percent of the patients had referred serum samples collected within 90 days and 76% had samples collected within 30 days of initial serum collection at NRL.

For only 100 (22%) of the 451 patients, PAMF-TSL serologic profile was consistent with an acute *Toxoplasma* infection (Table 2). PAMF-TSL IgM ELISA was positive for all 100. For 335 (74%), the PAMF-TSL serologic profile was consistent with a chronic infection. Out of these 335 patients, 119 (36%) had a positive PAMF-TSL IgM ELISA. The stage of infection could not be determined for 9 (2%) patients (indeterminate category). Seven (2%) other patients had a negative IgG dye test, implying the absence of *Toxoplasma* infection (false-positive test), and only one of these patients had a positive PAMF-TSL IgM ELISA. Overall, PAMF-

TABLE 2 Final interpretation of Palo Alto Medical Foundation Toxoplasma Serology Laboratory test results for 451 patients found to have positive toxoplasma IgM at nonreference laboratories

Interpretation (*n*)	No.	%
Acute infection (all IgM positive) (451)	100	22
Chronic infection (451)	335	74
IgM positive (335)	119	36
IgM negative (335)	200	60
IgM equivocal (335)	16	5
No infection (451)	7	2
IgM positive (7)	1	14
IgM negative (7)	5	71
IgM equivocal (7)	1	14
Indeterminate (451) (IgM negative or equivocal)	9	2

TABLE 3 Demographics and interpretation of toxoplasma serology of 297 patients whose time between blood collection for serological testing at nonreference laboratories and Palo Alto Medical Foundation Toxoplasma Serology Laboratory could not be determined[a]

Characteristic (*n*)	No.	%
Male (290)	22	8
Female (290)	268	92
Age <18 yr (292)[b]	12	4
Age ≥18 yr (292)	280	96
Pregnancy(268)	224	84
Acute infection (all IgM positive) (297)	67	23
Chronic infection (297)	215	72
IgM positive (215)	72	33
IgM negative (215)	133	62
IgM equivocal (215)	10	5
No Infection (297)	8	3
IgM positive (8)	1	13
IgM negative (8)	7	88
Indeterminate (297) (IgM negative or equivocal)	7	2
Specificity of NRL IgM		66
PPV of NRL IgM		23
PPV of PAMF-TSL IgM		48

[a] NRL, nonreference lab; PPV, positive predictive value; PAMF-TSL, Palo Alto Medical Foundation Toxoplasma Serology Laboratory.
[b] All patients were more than 1 year of age.

TSL IgM ELISA was found to be positive in 220 (49%) patients. Of these 220 positive patients, 100 (45%) were classified as having an acute infection. Thus, the sensitivity, specificity, and PPV of IgM ELISA at PAMF-TSL for an acute infection were 100%, 63%, and 45%, respectively. (Indeterminate interpretations and equivocal IgM ELISA results were excluded from the specificity calculation.) In contrast, the PPV of NRL IgM was only 22%.

To determine whether the significance of a positive *Toxoplasma*-specific IgM test result changed over time, we analyzed two periods: (i) from January 2003 to December 2008 (276 patients) and (ii) from January 2009 to December 2013 (175 patients). In the first group, 70 patients (25%) were diagnosed with an acute infection following serological testing at PAMF-TSL. In the second group, 30 patients (17%) were diagnosed with an acute infection ($P = 0.05$).

We did a subanalysis of 402 pregnant women and found no major difference in the proportion of patients having an acute serologic profile. For 79 out of 402 (20%) pregnant women, PAMF-TSL testing resulted in an acute infection (versus 22% overall). We also performed a subanalysis of patients whose sera were collected for serological testing at PAMF-TSL within 15 days of the date of initial serum collection at NRLs to minimize changes in serological profile over time. There were 240 patients in this category, and 27% (versus 22% of patients overall; $P = 0.16$) had a serological profile consistent with an acute infection.

A separate analysis was conducted for 297 patients whose time interval between blood collection for serological testing at the NRL and PAMF-TSL could not be determined. The demographics (age, gender, and pregnancy status), proportion of patients with acute versus chronic infection based on PAMF-TSL criteria, specificity, and PPV of positive IgM for an acute infection were almost identical to the previous results (Table 3).

DISCUSSION

We conducted a large retrospective review on the role of the *Toxoplasma* serological profile at PAMF-TSL applied to referred sera of 451 patients who had positive *Toxoplasma* IgM and IgG test results at NRLs. Using well-established PAMF-TSL criteria for an acute infection, only 22% of 451 patients had a serologic profile consistent with an acute infection, whereas 335 (74%) patients had PAMF-TSL results consistent with a chronic infection. Although the decrease in significance of the positive IgM test results over 10 years was marginally significant, it appears that there is a trend toward lower performance in the IgM tests carried out in NRLs over time.

Another 2% of patients were determined not to have *Toxoplasma* infection at all (based on negative IgG dye test at PAMF-TSL). These findings confirm that a positive current *Toxoplasma* IgM result at an NRL is not necessarily diagnostic of a recent infection, since titers can remain positive for several months after an acute episode of *Toxoplasma* infection and false-positive IgM test results can occur. This is in overall agreement with previous reports of persistently positive IgM in chronic toxoplasmosis (9–11, 13, 22).

Furthermore, the number of patients with positive IgM decreased by nearly half (49%) when samples were tested at PAMF-TSL, implying that the NRL IgM test is less specific and/or remains positive longer than the PAMF-TSL IgM ELISA. However, even the PAMF-TSL IgM ELISA was positive in 36% of patients with chronic toxoplasmosis (PPV, 45% for acute infection) suggesting that IgM positive test results in chronically infected patients can also be a result of persistence of the *Toxoplasma* IgM, apparently without clinical consequence. Contrary to the IgG dye test, which is considered the reference test for IgG detection against *T. gondii*, there is no reference method for IgM detection. Even reference laboratories cannot rely on this single test for the diagnosis of an acute infection. In case of positivity of IgM, it is thus important to perform additional tests in order to conclude whether an infection is acute or chronic. This is of utmost importance for pregnant women, in order to make an appropriate therapeutic decision.

The PPV of NRL IgM of 22% in our study is lower than the 40% in a large study involving 811 pregnant women by Liesenfeld et al.,

even though the confirmatory serology was performed at PAMF-TSL in both studies (12). In the latter study, the gestational age of pregnant women was taken into account when *Toxoplasma* serology was interpreted. Thus, if *Toxoplasma* serology was performed in the third trimester and if acquisition of infection was determined to be during pregnancy (even if more than 6 months prior to the test date), it was labeled as a recent infection. Also, the IgG avidity test was not done or not available in the study done by Liesenfeld et al. (12). A high-avidity test can help confirm chronic infections when the other serologic markers, including AC/HS, are ambiguous. A lack of avidity test in the study by Liesenfeld may also have resulted in a higher proportion of acute infection diagnoses. In a study on the clinical utility of positive IgM test results in pregnant women obtained at their NRLs, Garry et al. found the IgM test to have a very low PPV (6%) for an acute infection when their serum underwent confirmatory testing at PAMF-TSL (13). Furthermore, the sensitivity, specificity, PPV, and negative predictive value (NPV) of PAMF-TSL IgM ELISA in their study were 100%, 83%, 25%, and 100%, respectively. The lower PPV of NRL and PAMF-TSL IgM ELISA in their study could be explained by the differences in the patient population and the inclusion of patients without serological evidence of toxoplasmosis (i.e., negative IgG but positive IgM). In contrast, all our patients had a positive IgG and IgM at the NRL. PPV also depends on the epidemiological data (e.g., prevalence) of toxoplasmosis in the population under study. However, adjusting the PPV in our study for epidemiological data is not possible, since prevalence and incidence data are not available for the various regions where the specimens were sent from.

Laboratory testing for toxoplasmosis, including IgG, IgM, and IgG avidity, varies greatly by method and kit (23–25). Furthermore, the specificity of IgM has been found to vary from lot to lot even from the same manufacturer (14). It is critical that the method and kit used be reported so that appropriate comparisons and conclusions can be made. In this study, PAMF-TSL IgM was compared against various methods and kits (used at different NRLs), but we were limited by the lack of knowledge on the specifics of each of the methods and kits used by these labs. Of the serum samples with positive IgM tests at NRLs, only 49% were found to be positive at PAMF-TSL and only 22% were found to indicate an acute infection. The higher specificity at PAMF-TSL may be related to the fact that a double-sandwich ELISA method is used, which is known to confer higher specificity without sacrificing sensitivity (19). In addition, commercial kits in Europe are routinely evaluated in labs where timing of the infection is accurately available, since they have access to samples with known date of seroconversion (because systematic serological screening is routinely performed during pregnancy). In contrast, in the United States, as required by the FDA, commercial kits need to be evaluated in the United States. Thus, their performance is evaluated in laboratories such as PAMF-TSL, where timing of infection can only be estimated by confirmatory testing in a single serum sample.

A significant limitation of this study is the extended time period (up to 6 months) between the collection dates of blood specimens for serologic testing at the NRL and PAMF-TSL. However, it generally takes about 6 months for the serologic profile of an acute *Toxoplasma* infection to evolve into a chronic pattern, and hence, this should not affect our interpretation. Moreover, the median time between the outside serologic testing and PAMF-TSL was only 15 days, and more than 90% of patients had blood specimens sent to PAMF-TSL within 90 days of initial testing, suggesting that the acute serological profile was unlikely to have changed to a chronic pattern between the 2 tests. When the analysis was restricted to patients whose date of serum collection for serological testing at PAMF-TSL was within 15 days from the initial date of serum collection at NRLs, the proportion of patients with an acute serological profile was not statistically significantly different from that of the overall patients (27 versus 22%).

It is important to emphasize that even a positive IgM test result at PAMF-TSL should not be used as a stand-alone test in determining the stage of *Toxoplasma* infection but rather should be used in conjunction with other elements of the serologic tests, including IgG, IgA, IgE, AC/HS, and avidity tests, available at PAMF-TSL. This is important for an accurate interpretation of the *Toxoplasma* serologic profile, especially in pregnant women, for whom a false-positive IgM result can result in emotional distress, unnecessary amniocentesis with potential complications, and even abortion. Moreover, correctly classifying a positive IgM as indicating a true acute versus persistently positive chronic infection or as a false-positive test and relaying the information to the treatment team has been shown to decrease unnecessary abortions by 50% (12). In fact, in 1997 the FDA issued a public health advisory cautioning the health care professionals against misinterpreting a positive IgM test in pregnant women (http://www.fda.gov/MedicalDevices/Safety/AlertsandNotices/PublicHealthNotifications/ucm062411.htm; accessed 2 June 2015). Unfortunately, according to a recent survey, only 8% of obstetricians and gynecologists in the United States are aware of false-positive results associated with IgM (26).

In conclusion, *Toxoplasma* IgM (as tested by both NRLs and reference laboratories) can remain positive for several months even with contemporary assays and by itself cannot distinguish a recently acquired infection from a chronic one. A complete serologic panel should be performed at the reference laboratory as mandated by the FDA for an accurate interpretation for all *Toxoplasma* IgM specimens identified as positive by NRLs.

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We have no conflicts of interest to declare.

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