Toxoplasmonic lymphadenitis—clinical and serologic profile
R. A. Durlach, F. Kaufer, L. Carral and J. Hirt

German Hospital, Buenos Aires, Argentina

Objective To study the serologic profile of several types of test for toxoplasmosis, in order to contribute to the interpretation of antibody kinetics.

Methods The clinical and serologic features of 120 cases of lymphadenopathy with known time of clinical onset were studied during 18 months postinfection. Antibody kinetics was determined by Sabin–Feldman dye test, complement fixation with light antigen, IgM immunofluorescent antibody test, and IgM immunosorben agglutination assay (IgM-ISAGA). Cell-mediated immunity was evaluated by the toxoplasmin skin test.

Results Seventy-five female patients aged 11–54 years (median 27 years) and 45 male patients aged 3–59 years (median 17 years) were studied, 85% of whom were under 30 years of age. Cervical lymph nodes were involved throughout, generally on both sides, with more than one affected ganglion group in 88%. The predominant symptom was asthenia (69%), which persisted in some cases for several months. A negative Sabin–Feldman dye test in a lymphadenopathy with more than three weeks’ evolution excludes a toxoplasma etiology. A positive Sabin–Feldman dye test with negative IgM-ISAGA almost invariably excludes recent infection. The Sabin–Feldman dye test was positive in 94% of patients with titers higher than 1 : 16 000 within the first three months. The IgM-ISAGA yielded 98% of positive results, of which 94% were high titers. Titors ≥ 1 : 160 in the IgM immunofluorescent antibody test and complement fixation were found to be highly indicative of recent infection, since 87% and 91%, respectively, were found within the first three months. A negative skin test plus positive serology values indicates recent infection.

Conclusion Our results indicate that estimation of time of infection on the basis of serologic results is improved by the simultaneous application of several tests, and correlates closely with the presence of clinical lymphadenitis.

Keywords Acute toxoplasmosis, lymphadenitis, Toxoplasma gondii

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INTRODUCTION

Infection by Toxoplasma gondii is widespread throughout the world, its prevalence varying with age and geographic region from 0% to 90% [1,2].

In an immunocompetent host, the primary infection is generally oligosymptomatic and self-limiting, has a favorable prognosis, and requires no specific treatment. Fewer than 10% of infected subjects are symptomatic, with lymphadenopathy as the most frequent clinical form, but even asymptomatic primary infection occurring during pregnancy can cause severe damage to the fetus [3].

The aim of this work was to characterize the clinical and serologic profiles of primary toxoplasma infection of known onset time on the basis of acute toxoplastic lymphadenitis findings.

Humoral immunity detects G and M immunoglobulins, targeting membrane and cytoplasmic antigens. Each immunoglobulin was then evaluated by means of several tests as a marker of the
acute phase for the diagnosis of the parasitosis. Cell-mediated immunity to the parasite was assayed by the toxoplasmin skin test (ST).

The sensitivity of tests for serologic diagnosis of an acute infection is routinely based on raised titers during the acute phase of infection [4]. In order to interpret the results of these tests in the asymptomatic patient or in the pregnant woman, it is necessary to determine antibody kinetics [5,6]. Although quite often in the pregnant woman the probable date of conception is available, antibody titers and time of infection are unknown. An estimate of the latter is essential to establish the prognosis and to decide on the correct therapeutic management.

MATERIALS AND METHODS

One hundred and twenty patients examined at the Toxoplasmosis Department of the German Hospital from 1986 to 1995, who were followed up clinically and serologically, were included in this study. Criteria for inclusion were the presence of single or multiple adenomegalies with a known clinical onset date, and an immunologic response compatible with acute toxoplasma infection: Sabin-Feldman (SF) dye test titer ≥ 1 : 1024, or positive serology with a negative ST dye test. Patients with HIV-positive serology were excluded.

Fifteen patients were referred to us with a histopathologic diagnosis suggesting acute toxoplasmosis based on material obtained by surgical biopsy.

A serologic follow-up was run every three months (trimester) for 18 months with the SF dye test, complement fixation (CF), IgM fluorescent antibody test (IgM-IFA), and immunosorbent agglutination assay (IgM-ISAGA). Cell-mediated immunity was evaluated with the ST. Serologic tests were controlled with standard sera provided by the Institute of Medical Parasitology, University of Bonn. All sera were inactivated for 30 min at 56 °C and processed within 48 h.

Sabin-Feldman dye test

This assay is considered to be the standard method for the diagnosis of toxoplasmosis [4,7]. The technique used was described by the Bundesgesundheitsamt of Germany, with live Toxoplasma of the BK strain, kept by passaging in NMRI mice, using a dilution factor of 4 [8].

In order to facilitate evaluation of results, titers were grouped as negative, low (1 : 4 to 1 : 256), medium (1 : 1024 to 1 : 4000), and high (1 : 16 000 to 1 : 64 000).

Complement-fixation test

Light antigen, prepared and used according to the technique described by the Bundesgesundheitsamt, was provided by the Institute of Medical Parasitology, University of Bonn, Germany [8]. CF titers were grouped as negative, low (1 : 5 to 1 : 20), medium (1 : 40 to 1 : 80), and high (≥ 1 : 160).

IgM immunofluorescent antibody test

The technique used was that described using strain BK toxoplasmas, formulated and fixed by dehydration [8]. The anti-μ-chain-specific antibody marked with fluorescein isothiocyanate was supplied by Bio-Mérieux (Code 75692).

Sera were diluted by a factor of 2, beginning with 1 : 20. Titers were grouped as negative, low (1 : 20), medium (1 : 40 to 1 : 80), and high (≥ 160).

All positive sera were assayed for the rheumatoid factor and the antinuclear antibody, in order to rule out false-positive results [9–11].

IgM immunosorbent agglutination assay

The technique used was that described by Desmonts et al. [12]. The microtiter plates (Nunc Microsorb U96) were coated with anti-μ-chain-specific antibody (Dako A/S Denmark, Code 10425). The specific IgM adsorbed was supplied by adding formalin-fixed Toxoplasma (Toxo AD, BioMérieux, Code 75422). Sera were diluted by a factor of 4.

For all the tests, variation of antibody concentration was considered significant when the increase or decrease in the titer was more than one dilution.

Toxoplasmin skin test

The technique described by Thalhammer was used for preparation of the Frenkel toxoplasmin antigen, which was sterilized in an autoclave for 30 min at an average temperature of 125.5 °C, followed by a sterility control [13–15]. The antigen was injected into the dermis of the left forearm and read 48 h later, the criteria for a positive reaction
being an erythematous area and/or infiltration equal to or greater than 100 mm².

All negative results of the ST with positive serology were repeated in order to rule out errors inherent in the application technique. These patients were tested with the ST every trimester until development of sensitivity to *Toxoplasma* was observed.

**RESULTS**

**Population and clinical data**

The study included 120 patients, 75 female patients aged from 11 to 54 years (median age 27 years), and 45 male patients aged from three to 54 years (median age 17 years).

The male/female ratio was 1:1.6 and was not homogeneous for all ages. Up to approximately 25 years of age, it was 1:1, while above that age females predominated, with a ratio up to 4:6:1. Of the male patients, 85% (38/45) were under 30, the group between 16 and 20 years of age predominating (29%, 14/48). Age distribution was more homogeneous in the female group (Figure 1). Six patients were pregnant, and three presented acute retinochoroiditis; these nine cases were the only ones receiving specific treatment [16,17].

Analysis of the time elapsed between onset of symptoms and the patient’s first medical consultation at a specialized center allowed us to establish that 29 patients (23%) consulted within one month, 19 (16%) in the second month, and 23 (18%) in the third month, with a total of 57% of initial visits within the first trimester. By the end of the second trimester, 82% of the patients had been included in the study.

Cervical lymph node involvement was found in 111 of 120 (92.5%), and occipital localization was found in 88 cases (73.3%), with more than one affected ganglion group present in 88%. Lymphadenopathy was present in regions beyond the neck, such as the axillary (37.5%), inguinal (11.6%) or chin (10%) regions (Table 1).

A relatively low number of patients (12%) presented only one group of affected lymph nodes, invariably located in the head and neck region; the remaining patients had more than one involved group of lymph nodes, which was generally bilateral and pain-free.

The most constant clinical manifestations were asthenia (69%) and fever (45%). Asthenia was not only the most frequent symptom but also the one that lasted longest (Table 2). Duration peaked at over 8 months in one patient and 12 months in another, taking into account the time they needed

![Figure 1 Age distribution.](image-url)
to resume their habitual physical activity. Fever was present in less than half of the cases, lasting from three to seven days. Cephalgia (41%), myalgia (35%), arthralgia (26.6%) and odynophagy unaccompanied by pharyngitis (16.6%) presented variable durations.

The only exanodular locus detected in the 64 patients evaluated ophthalmologically was an acute and symptomatic chorioretinitis in three patients (4.7%) who responded to treatment. Anisocoria was observed in two cases, both resolving spontaneously.

**Humoral immune response**

Patients were followed up serologically, with 323 samples collected during 18 months after the onset of infection; in total, 249 samples were studied with four tests (SF dye test, CF test, IgM-IFA test, and IgM-ISAGA), while 74 were evaluated with three tests (SF dye test, IgM-IFA test, and IgM-ISAGA) or two tests (SF dye test and IgM-ISAGA).

**Sabin–Feldman dye test**

During the first six months, 94% of samples showed high titers, and the remaining 6% medium values. Titers decreased significantly in the third trimester. Thereafter, no significant variation was observed until the fifth trimester, after which an evident decline was recorded (Figure 2).

Two samples taken from 20 patients during the first trimester were assayed, five of which showed a significant increase in titers, which was found only during the first two months after clinical onset.

**Complement-fixation test**

This assay was 100% sensitive with titers ≥ 1 : 10. Titers ≥ 1 : 160 were observed only during the first and second trimesters (30% and 19%, respectively). Only two patients showed high titers in the third trimester, and one in the fifth trimester. Although a significant decrease was recorded in titers from one trimester to the next, no case became negative (Figure 3).

**IgM immunofluorescent antibody test**

For this test, 95% of patients showed positive results in the first trimester, 74% in the second, and 63% in the third.

In the first trimester, 48% of cases presented high titers, which decreased to 17% in the second. Only one patient had high titers up to the fifth trimester. Titers decreased significantly from one trimester to the next, and in the sixth trimester, 74% of the patients were IgM negative (Figure 4).

Of the patients with positive IgM-IFA results, two presented weak positive antinuclear antibody
(titer 1: 20) and two a positive rheumatoid factor. IgM-IFA specificity was confirmed by previously absorbing sera with a specific anti-IgG. In these four patients, IgM-ISAGA based on immunocapture was positive.

**IgM immunosorbent agglutination assay**

We observed 98% (62/63) positive results in the first trimester, while 94% of cases (60/63) had high titers. Analyzed every trimester, high titers decreased significantly after the first trimester (Figure 5).

**Toxoplasmin skin test: cell-mediated immune response**

During the first trimester, 43% (23/54) negative dermal reactions with positive serology were observed, and in the second 34% (16/47), while only two patients still had a negative ST in the third trimester, one of whom remained negative in the fourth trimester.

**DISCUSSION**

Epidemiologic studies of toxoplasmosis in Argentina and Europe have failed to find any significant differences in prevalence between female and male populations [1,18,19]. The low incidence of lymphadenopathy in the male population of our cohort is striking, especially above 30 years of age. Similar findings have been reported by Beverley et al and Montoya and Remington [19,20]. There is no satisfactory explanation for this observation, since differences between male and female immune systems are hardly feasible.

McCabe et al. studied 107 patients by lymph node biopsy whose mean age was 26 years, similar to that found in ours, which was 24 years [21].
We agree with Schneider et al. that lymph node biopsy is unnecessary in cases of acute lymphadenopathy with a recognizable clinical and serologic profile [22–24].

Asthenia was the most frequent symptom, occurring in 69% of our cases, whereas Jones et al. reported 40% [25]. It may persist for months, or even for up to 1 year. Fever, a symptom to be expected in infectious diseases, was found in 45% of our patients, tallying with the findings of Jones et al. (37%), but twice the incidence reported by McCabe et al. [18,25]. Other symptoms in our cohort were non-specific (cephalea, myalgia, and arthralgia, among others), and difficult to distinguish from the fever syndrome.

Acute lymphadenopathy was present in the head and neck region; in some cases, it was also observed in other areas such as the supraventricular (34.1%) and inguinal (11.6%) regions. Lymph nodes were painless, had mild inflammation, were in no case attached to one another or to adjacent planes, and did not suppurate. Other authors report painful nodes in the acute phase [26,27]. In 13% of the cases, we observed involvement of a single ganglion group, in 64% involvement of two to four groups, and in 26% involvement of over five groups.

Reversible anisocoria was a sign added to the protocol, since Morgenfeld and Hirt, as well as Somoza et al., found it in 40% and 37%, respectively, of their cases [28,29]. In our population, we recorded two cases, which were confirmed by three different observers, one of whom was not on our team.

The SF dye test showed high titers in 97% of the cases during the first trimester. A significant increase in SF levels was observed in only 25% of our matched samples, taken within the first two months postinfection. In our study, only two patients presented 1 : 16 titers in the first sample, taken a few days after showing signs of lymphadenopathy, and these increased to 1 : 4000 a week later. Therefore, given the rapid increase in antibody concentration, the likelihood of finding a low titer in a recent toxoplasma lymphadenopathy is minimal; indeed, the same may be said of detecting any significant antibody variation in samples taken at two- or three-week intervals.

A negative SF dye test result in a lymphadenopathy with more than three weeks’ evolution excludes a toxoplasma etiology, while low and stable titers rule out acute infection in immunocompetent patients. Since high titers persist in many cases for over a year, they enable us to suspect, though not to confirm, the acute stage of the disorder.

The drop in SF titers that we recorded at 15-months follow-up contrasts with similar observations by Montoya and Remington at six months and by Brooks et al. at one year [20,30]. Likewise, our mean values proved considerably lower (1 : 16000 versus 1 : 3000 and 1 : 6000, respectively), although they agreed with those found by Welch et al., Saathoff and Seitz, and Del Bono et al. [4,10,31].

Within the first trimester, IgM-ISAGA yielded 98% positive results, 94% of which were high titers. With use of this test, only 2% (1/63) of patients had both acute infection and undetectable IgM, a lower rate than the 10–15% reported in the literature [30,31]. Although titers diminished significantly, most remained positive for 12 months. Therefore, high SF titers with negative IgM-ISAGA seem to be quite infrequent in the first year [32].

Titers exceeding 1 : 160 in the IgM-IFA and CF tests were found to be highly indicative of recent infection, since 87% and 91%, respectively, of such values were recorded within the first two trimesters, the IgM assay proving more sensitive during the first trimester (48% versus 30%).

The ST for cell-mediated immunity, which appears later than humoral assays, is highly useful. A negative ST plus positive serologic values indicates recent infection, since this association rarely occurs after the second trimester.

Antibody kinetics in cases of lymphadenitis was correlated with the time elapsed from onset of infection, in order to determine titers reached in the course of acute toxoplasma infection. Useful information was thus gleaned to estimate the time of infection on the basis of analytic findings [33].

Comparison of the qualitative results of the SF test, CF test, IgM-IFA test, IgM-ISAGA and ST enables us to establish a grid of possibilities whose accuracy depends on the consideration of the quantitative values of each test.

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REFERENCES