Tikrit University

College of Nursing

Basic Nursing Sciences

Second Year - 2023-2024

Microbiology

Parasitology

Toxoplasma gondii

By: assistant lecturer

Ghufran Ayad Ahmed

Toxoplasma gondii

Diagnosis
• Direct microscopic examination.
• Latex agglutination test (LAT).
• Enzyme linked immune sorbent assay (ELISA).
• Polymerase chain reaction (PCR).

Diagnostic Methods

The diagnosis of T. gondii infection or toxoplasmosis may be established by serologic tests, amplification of specific nucleic acid sequences (i.e., polymerase chain reaction [PCR]), histologic demonstration of the parasite and/or its antigens (i.e., immunoperoxidase stain), or by isolation of the organism. Other rarely used methods include demonstration of antigenemia and antigen in serum and body fluids, a toxoplasmin skin test, and antigen-specific lymphocyte transformation

Serologic Tests

The use of serologic tests for demonstration of specific antibody to T. gondii is the initial and primary method of diagnosis. Different serologic tests often measure different antibodies that possess unique patterns of rise and fall with time after infection. A combination of serologic tests is usually required to establish whether an individual has been most likely infected in the distant past or has been recently infected. The clinician and clinical laboratories must be familiar with these problems and consult reference laboratories if the need arises.

A panel of tests (the Toxoplasma Serological Profile [TSP]) consisting of the Sabin-Feldman dye test (DT), double sandwich IgM ELISA ,IgA ELISA, IgE ELISA, and AC/HS test has been used successfully by our group to determine if serologic test results are more likely consistent with infection acquired in the recent or more distant past. The AC/HS test is interpreted as previously described by comparing IgG titers obtained with formalin-fixed tachyzoites (HS antigen) with those obtained with acetone-fixed tachyzoites (AC antigen).

The TSP has been successfully used in the setting of toxoplasmic lymphadenitis, myocarditis, polymyositis, and chorioretinitis and during pregnancy. For sera with positive results in IgG and IgM tests, the discriminatory power of the TSP to differentiate between recently acquired infection and chronic infection is probably superior to any other single serologic test.

Current interpretation of results in the TSP at the Toxoplasma Serology Laboratory at the Palo Alto Medical Foundation Research Institute (TSL-PAMFRI) is as follows: Sera that are positive in the DT, negative in the IgM, IgA, and IgE ELISAs, and reveal a chronic pattern in the AC/HS test are typically found in patients infected in the most distant past. The combination of high titers in the DT, positive IgM, IgA, and IgE ELISAs, and an acute pattern in the AC/HS test is highly suggestive of a recently acquired infection. In contrast, the presence of positive DT and IgM ELISA results but a negative, low-positive, or equivocal result in the IgA and IgE ELISAs and an equivocal pattern in the AC/HS test is more difficult to interpret. In the latter setting, a follow-up sample is usually obtained, the 2 samples are run in parallel, and the serologic test titer results are compared. If the titers obtained in the 2 samples do not change significantly, the infection is most likely to have been acquired in the distant past. In contrast, significant changes (rise or decline) detected in the titers of the 2 samples are considered to be suggestive of a recently acquired infection.

IgG antibodies. The most commonly used tests for the measurement of IgG antibody are the DT, the ELISA, the IFA, and the modified direct agglutination test. In these tests, IgG antibodies usually appear within 1–2 weeks of acquisition of the infection, peak within 1–2 months, decline at various rates, and usually persist for life.

When two different compounds (i.e., acetone and formalin) are used to fix parasites for use in the agglutination test, a “differential” agglutination test (also

known as the “AC/HS test”) results due to the fact that the different antigenic preparations vary in their ability to recognize sera obtained during the acute and chronic stages of the infection. This test has proved useful in helping to differentiate acute from chronic infections [8] but is best used in combination with a panel of other tests (e.g., the TSP).

Recently, a number of tests for avidity of Toxoplasma IgG antibodies have been introduced to help discriminate between recently acquired and distant infection. It has been observed that the functional affinity of specific IgG antibodies is initially low after primary antigenic challenge and that it increases during subsequent weeks and months by antigen-driven B cell selection. Protein-denaturing reagents including urea are used to dissociate the antibody-antigen complex. The avidity result is determined using the ratios of antibody titration curves of urea-treated and untreated samples.

IgM antibodies. IgM antibodies may appear earlier and decline more rapidly than IgG antibodies. The most commonly used tests for the measurement of IgM antibody are double-sandwich or capture IgM-ELISA kits, the IFA test, and the immunosorbent agglutination assay (IgM-ISAGA; available from bioMérieux). False-positive results due to rheumatoid factor and antinuclear antibodies in some IgM-IFA tests are not detected by the most commonly used commercial double-sandwich or capture IgM-ELISAs. Despite the wide distribution of commercial test kits to measure IgM antibodies, these tests often have low specificity, and the reported results are frequently misinterpreted.

An IgM test is still used by most laboratories to determine if a patient has been infected recently or in the distant past, and because of the hurdles posed in interpreting a positive IgM test result, confirmatory testing should always be performed.

In patients with recently acquired primary infection, T. gondii— specific IgM antibodies are detected initially, and in most cases, these titers become negative within a few months. However, in some patients, positive T. gondii—specific IgM titers can still be observed during the chronic phase of infection. Some investigators have reported that IgM antibodies can be detected as long as 12 years after the acute infection. The persistence of these IgM antibodies does not appear to have any clinical relevance, and these patients should be considered chronically infected. Further complicating the interpretation of a positive IgM test result is the fact that several methods for its detection still may result in a relatively high frequency of false-positive results. Thus, a positive IgM test result in a single serum sample can be interpreted as a true-positive result in the setting of a recently acquired infection, a true-positive result in the setting of an infection acquired in the distant past, or a false-positive result.

IgA antibodies. IgA antibodies may be detected in sera of acutely infected adults and congenitally infected infants by use of ELISA or ISAGA. As is true for IgM antibodies to the parasite, IgA antibodies may persist for many months or more than a year. For this reason, they are of little additional assistance for diagnosis of acute infection in the adult. In contrast, the increased sensitivity of IgA assays over IgM assays for diagnosis of congenital toxoplasmosis represents an advance in diagnosis of the infection in the fetus and newborn. In a number of newborns with congenital toxoplasmosis and negative IgM antibodies, the serologic diagnosis has been established by the presence of IgA and IgG antibodies

IgE antibodies. IgE antibodies are detectable by ELISA in sera of acutely infected adults, congenitally infected infants, and children with congenital toxoplasmic chorioretinitis. Their demonstration does not appear to be particularly useful for diagnosis of T. gondii infection in the fetus or newborn when compared with IgA tests. The duration of IgE seropositivity is briefer than that with IgM or IgA antibodies and hence appears useful for identifying recently acquired infections

PCR

PCR amplification for detection of T. gondii DNA in body fluids and tissues has successfully been used to diagnose congenital, ocular, and cerebral and disseminated toxoplasmosis. PCR has revolutionized the diagnosis of intrauterine T. gondii infection by enabling an early diagnosis to be made, thereby avoiding the use of more invasive procedures on the fetus. PCR has enabled detection of T. gondii DNA in brain tissue, cerebrospinal fluid (CSF), vitreous and aqueous fluids, bronchoalveolar lavage (BAL) fluid, and blood in patients with AIDS.

Histologic Diagnosis

Demonstration of tachyzoites in tissue sections or smears of body fluid (e.g., CSF or amniotic or BAL fluids) establishes the diagnosis of the acute infection. It is often difficult to demonstrate tachyzoites in conventionally stained tissue sections. The immunoperoxidase technique, which uses antisera to T. gondii, has proven both sensitive and specific: It has been used successfully to demonstrate the presence of the parasite in the central nervous system (CNS) of AIDS patients. The immunoperoxidase method is applicable to unfixed or formalinfixed paraffin-embedded tissue sections. A rapid, technically simple, and under-used method is the detection of T. gondii in air-dried, Wright-Giemsa—stained slides of centrifuged (e.g., cytocentrifuge) sediment of CSF or of brain aspirate or in impression smears of biopsy tissue. Multiple tissue cysts near an inflammatory necrotic lesion probably establish the diagnosis of acute infection or reactivation of latent infection.

Isolation of T. gondii

Isolation of T. gondii from blood or body fluids establishes that the infection is acute. Attempts at isolation of the parasite can be performed by mouse inoculation or inoculation in tissue cell cultures of virtually any human tissue or body fluid.