VOLUME - XVI
ISSUE - XCIV
JUL/AUG 2019

The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

CONTENTS

1 Editorial
2 Disease Diagnosis
13 Interpretation
15 Troubleshooting
16 Bouquet
17 Tulip News

Editorial

Seeing is believing! Does that hold true for HIV as well? Perhaps yes, as most diagnostic methodologies available do have certain limitations. False positive as well as false negative results are known to occur. Seeing HIV virions require electron microscope - not possible to have at every diagnostic setup.

Besides being expensive, the process is cumbersome and time consuming. What then? Well, we have to know and understand the limitations of existing methodologies. Malignancies, pregnancy, tuberculosis, other retroviral infections AND MANY MORE do and can give false positive results with the in-use HIV diagnostic systems. The answer is to test multiple samples with multiple methodologies for the same patient. The stigma and the seriousness and certainties associated with HIV should make us think twice before signing out an HIV positive report. False positive HIV reports though are rare.

INTERPRETATION section in this issue talks about all existing methods available to diagnose HIV, it very clearly elucidates their limitations or negative aspects. The “GOLD STANDARD” will always be “SEEING IS BELIEVING” i.e., growing the HIV virion and seeing it under the electron microscope. The indirect evidences like existence of antibodies etc will take care of most but the rare cases where interfering antibodies are present.

The **TROUBLE SHOOTING** segment is also devoted to HIV here. The article delves deep into the issues relating to the Rapid HIV Diagnostic formats (RDTs). Where and under what circumstances should one employ them and what are their capabilities is adequately covered under the heading. Until we identify a treatment or a workable vaccine, HIV shall always stay on the 'front burner'. team understands the problems faced by laboratarians and constantly strives to inform you about the remedies. The patient must be informed about the test and also about its limitations. This alone can guard us all because false positives just can not be wished away, this because interfering antibodies exist and shall remain so in future too.

DISEASE DIAGNOSIS is again caught up with fever and infection one that usually spares the immunocompetent ones but creates numerous complications for the immunocompromised ones. You have guessed it right! It is toxoplasmosis. Complete clinico-diagnostic details are provided. Serology, PCR and histopathology as related to toxoplasmosis is cited in detail.

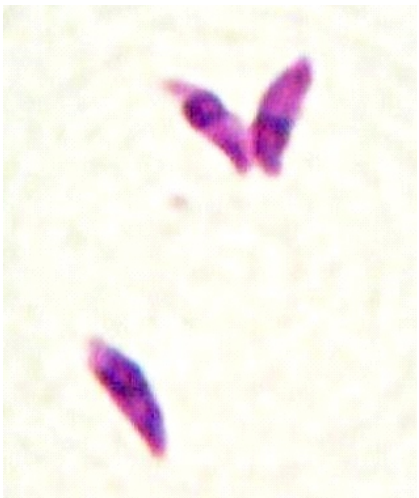
Let's see if **BOUQUET** can motivate you.! At the end of it all, just laugh it off. Forget your worries and straighten the creases on your forehead. After all, life goes on, doesn't it.

DISEASE DIAGNOSIS

TOXOPLASMOSIS

Background

Toxoplasmosis is caused by infection with the protozoan *Toxoplasma gondii,*an obligate intracellular parasite. The infection produces a wide range of clinical syndromes in humans, land and sea mammals, and various bird species. *T gondii* has been recovered from locations throughout the world, except Antarctica (see the image below).



Nicolle and Manceaux first described the organism in 1908, after they observed the parasites in the blood, spleen, and liver of a North African rodent, *Ctenodactylus gondii*. The parasite was named *Toxoplasma* (arclike form) *gondii* (after the rodent) in 1909. In 1923, Janku reported parasitic cysts in the retina of an infant who had hydrocephalus, seizures, and unilateral microphthalmia. Wolf, Cowan, and Paige (1937-1939) determined that these findings represented the syndrome of severe congenital *T gondii* infection. There are 3 major genotypes (type I, type II, and type III) of *T gondii*. These genotypes differ in their pathogenicity and prevalence in people. In Europe and the United States, type II genotype is responsible for most cases of congenital toxoplasmosis. *T gondii* infects a large proportion of the world's population (perhaps one third) but uncommonly causes clinically significant disease. However, certain individuals are at high risk for severe or life-threatening toxoplasmosis. Individuals at risk for toxoplasmosis include fetuses, newborns, and immunologically impaired patients. (See Etiology and Pathophysiology and Epidemiology.) Congenital toxoplasmosis is usually a subclinical infection. Among immunodeficient individuals, toxoplasmosis most often occurs in those with defects of T-cell–mediated immunity, such as those with hematologic malignancies, bone marrow and solid organ transplants, or acquired immunodeficiency syndrome (AIDS).In most immunocompetent individuals, primary or chronic (latent) *T gondii* infection is asymptomatic. A small percentage of these patients eventually develop retinochoroiditis, lymphadenitis, or, rarely, myocarditis and polymyositis.

PATIENT EDUCATION

Primary prevention based on prenatal education could be an effective strategy to reduce congenital toxoplasmosis. Educate the public in toxoplasmosis-prevention methods, such as protecting children's play areas from cat litter. Mothers with toxoplasmosis must be completely informed of the disease's potential consequences to the fetus.

Etiology and Pathophysiology

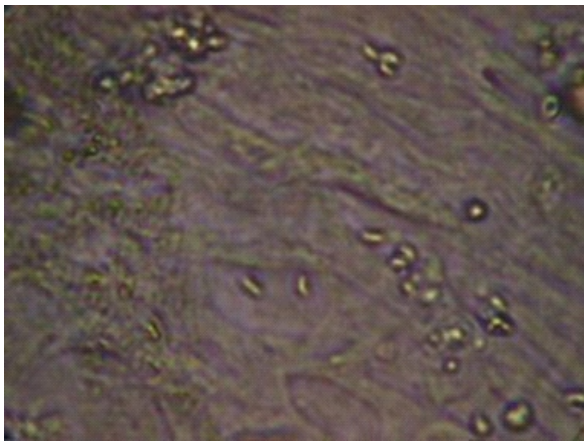
Life cycle of Toxoplasma gondii

T gondii has 2 distinct life cycles. The sexual cycle occurs only in cats, the definitive host. The asexual cycle occurs in other mammals (including humans) and various strains of birds. It consists of 2 forms: tachyzoites (the rapidly dividing form observed in the acute phase of infection) and bradyzoites (the slowly growing form observed in tissue cysts). A cat becomes infected with *T gondii* by eating contaminated raw meat, wild birds, or mice. The organism's sexual cycle then begins in the cat's gastrointestinal (GI) tract. Macrogametocytes and microgametocytes develop from ingested bradyzoites and fuse to form zygotes. The zygotes then become encapsulated within a rigid wall and are shed as oocysts. The zygote sporulates and divides to form sporozoites within the oocyst. Sporozoites become infectious 24 hours or more after the cat sheds the oocyst via feces. During a primary infection, the cat can excrete millions of oocysts daily for 1-3 weeks. The oocysts are very strong and may remain infectious for more than one year in warm humid environments. *T gondii* oocysts, tachyzoites, and bradyzoites can cause infection in humans. Infection can occur by ingestion of oocysts following the handling of contaminated soil or cat litter or through the consumption of contaminated water or food sources (eg, unwashed garden vegetables). Transmission of tachyzoites to the fetus can occur via the placenta following primary maternal infection. Rarely, infection by tachyzoites occurs from ingestion of unpasteurized milk or by direct entry into the bloodstream through a blood transfusion or laboratory accident. Transmission can also occur via ingestion of tissue cysts (bradyzoites) in undercooked or uncooked meat or through transplantation of an organ that contains tissue cysts. (Slaughterhouse workers and butchers may be at increased risk of infection.) In Europe and the United States, pork is the major source of *T gondii* infection in humans. The seroprevalence of *T gondii* antibodies in the human population varies geographically, with prevalence rates approaching 90% in some European countries, while seropositivity rates in the United States have been estimated to fall between 10% and 15%. Infection with the human immunodeficiency virus (HIV) does not seem to effect *T gondii* seropositivity, and there does not appear to be any difference in the rate of toxoplasmosis infection among patients with AIDS with and without cats.

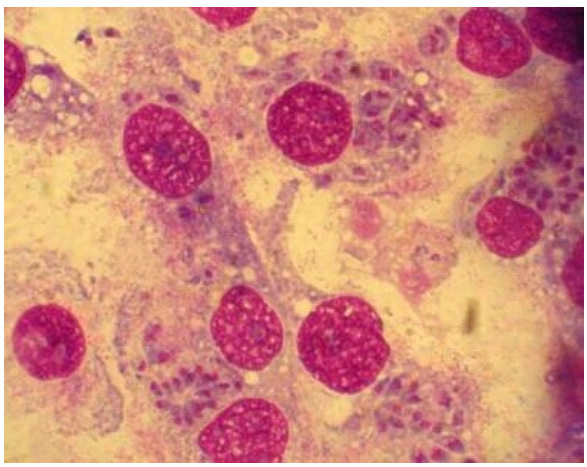
Cellular invasion

As previously stated, *T gondii* oocysts are ingested in material contaminated by feces from infected cats. Oocysts may also be transported to food by flies and cockroaches. When *T gondii* is ingested, bradyzoites are released from cysts or sporozoites are released from oocysts, and the organisms enter gastrointestinal cells. Host cell receptors consisting of laminin, lectin, and SAG1 are involved in *T gondii* tachyzoite attachment and penetration. Tachyzoites multiply, rupture cells, and infect contiguous cells. They are transported via the lymphatics and are disseminated hematogenously throughout the tissues. The ability of *T gondii* to actively penetrate host cells results in formation of a parasitophorous vacuole that is derived from the plasma membrane, which is entirely distinct from a normal phagocytic or endocytic compartment. Following apical attachment, the parasite rapidly enters the host cell in a process that is significantly faster than phagocytosis. The vacuole is formed primarily by invagination of the host cell plasma membrane, which is pulled over the parasite through the concerted action of the actin-myosin cytoskeleton of the parasite. During invasion, the host cell is essentially passive and no change is detected in

membrane ruffling, the actin cytoskeleton, or phosphorylation of host cell proteins. (See the images below.)



Toxoplasma gondii tachyzoites in cell line.



Toxoplasma gondii in infected monolayers of HeLa cells (Giemsa stain).

Tachyzoites proliferate, producing necrotic foci surrounded by a cellular reaction. Upon the development of a normal immune response, tachyzoites disappear from tissues. In immunodeficient individuals and in some apparently immunologically healthy patients, the acute infection progresses, resulting in potentially lethal consequences such as pneumonitis, myocarditis, and necrotizing encephalitis. Tissue cysts form as early as 7 days after infection and remain for the lifespan of the host. The tissue cysts are up to 60µm in diameter, each containing up to 60,000 organisms. They produce little or no inflammatory response but cause recrudescent disease in immunocompromised patients or retinochoroiditis in congenitally infected older children.

Changes in T-lymphocyte levels

Alterations in subpopulations of T lymphocytes are profound and prolonged during acute acquired *T gondii* infection. These have been correlated with disease syndromes but not with disease outcome. Some patients with prolonged fever and malaise have lymphocytosis, increased suppressor T-cell counts, and a decreased helper-to-suppressor T-cell ratio. These patients may have fewer helper cells even when they are asymptomatic.

In some patients with lymphadenopathy, helper-cell counts are diminished for more than 6 months after infection onset. Ratios of T-cell subpopulations may also be abnormal in asymptomatic patients. Some patients with disseminated toxoplasmosis have a very marked reduction in T cells and a marked depression in the ratio of helper to suppressor T lymphocytes. Depletion of inducer T lymphocytes in patients with AIDS may contribute to the severe manifestations of toxoplasmosis observed in these patients.

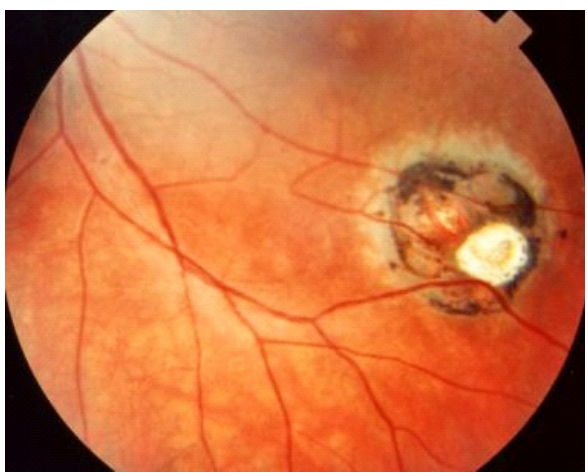
Retinochoroiditis

Retinochoroiditis usually results from reactivation of congenital infection, although cases have been recorded that were part of acute infection.

There are 5 hypotheses related to the inflammatory process of ocular toxoplasmosis, as follows :

- Infection and inflammatory response after spontaneous cyst rupture
- Parasitic toxic mediators released from *T gondii*
- Lytic effect of inflammatory mediators
- Delayed-type hypersensitivity reaction to antigens of *T gondii*
- Cell-mediated immunity against retinal antigens

When the organism reaches the eye through the bloodstream, depending on the host's immune status, a clinical or subclinical focus of infection begins in the retina. As the host's immune system responds and the tachyzoites convert themselves into bradyzoites, the cyst forms. The cyst is extremely resistant to the host's defenses, and a chronic, latent infection ensues. If a subclinical infection is present, no funduscopic changes are observed. The cyst remains in the normal-appearing retina. Whenever the host's immune function declines for any reason, the cyst wall may rupture, releasing organisms into the retina, and the inflammatory process restarts. If an active clinical lesion is present, healing occurs as a retinochoroidal scar. The cyst often remains inactive within or adjacent to the scar. (See the image below.)



Inactive retinochoroidal scar secondary to toxoplasmosis.

Toxoplasma parasites are rarely identified in aqueous humor samples from patients with active ocular toxoplasmosis. This suggests that parasite proliferation occurs only during the early phase of infection and that the retinal damages are probably caused by subsequent inflammatory responses. When human retinal pigment epithelium (RPE) cells are infected with *Toxoplasma gondii*, there is an increased production of several cytokines, including interleukin 1beta (IL-1ß), interleukin 6 (IL-6), granulocyte-macrophage colony-stimulating factor, and intercellular adhesion molecule (ICAM). Patients with acquired toxoplasmic retinochoroiditis exhibit higher levels of IL-1 than asymptomatic patients. It appears that IL-1 gene polymorphisms, in particular genotypes that are related with a high production of IL-1a, may be associated with recurrence of toxoplasmic retinochoroiditis. IL-10 polymorphisms associated with a low production of IL-10 also appear to be associated with the occurrence of toxoplasmic retinochoroiditis. In

contrast, tumor necrosis factor (TNF)–alpha gene polymorphism has not been found to be associated with the occurrence or recurrence of toxoplasmic retinochoroiditis.

Congenital toxoplasmosis

Approximately 10-20% of pregnant women infected with *T gondii* become symptomatic. The most common signs of infection are lymphadenopathy and fever. If the mother was infected prior to pregnancy, there is virtually no risk of fetal infection, as long as she remains immunocompetent. When a mother is infected with *T gondii* during gestation, the parasite may be disseminated hematogenously to the placenta. When this occurs, infection may be transmitted to the fetus transplacentally or during vaginal delivery. If the mother acquires the infection in the first trimester and it goes untreated, the risk of infection to the fetus is approximately 14-17%, and toxoplasmosis in the infant is usually severe. If the mother is infected in the third trimester and it goes untreated, the risk of fetal infection is approximately 59-65%, and involvement is mild or not apparent at birth. These different rates of transmission are most likely related to placental blood flow, the virulence and amount of *T gondii* acquired, and the immunologic ability of the mother to restrict parasitemia. The most significant manifestation of toxoplasmosis in the fetus is encephalomyelitis, which may have severe results. Approximately 10% of prenatal *T gondii* infections result in abortion or neonatal death. In approximately 67-80% of prenatally infected infants, the infection is subclinical and can be diagnosed using only serological and other laboratory methods. Although these infants appear healthy at birth, they may develop clinical symptoms and deficiencies later in life. Congenital toxoplasmosis caused by atypical genotypes is more severe than that caused by typical genotypes. Some infants with more severe congenital infection appear to have *Toxoplasma* antigen–specific lymphocytic anergy, which may be important in the pathogenesis of their disease. Monoclonal gammopathy of the immunoglobulin G (IgG) class has been described in congenitally infected infants, and IgM levels may be elevated in newborns with congenital toxoplasmosis. Glomerulonephritis with deposits of IgM, fibrinogen, and *Toxoplasma* antigen has been reported in congenitally infected individuals. Circulating immune complexes have been detected in sera from an infant with congenital toxoplasmosis and in older individuals with systemic, febrile, and lymphadenopathic forms of toxoplasmosis. However, these complexes did not persist after signs and symptoms resolved. Total serum levels of IgA may be diminished in congenitally infected babies, but no predilection toward associated infections has been noted. The predilection toward predominant involvement of the central nervous system (CNS) and retina in this congenital infection has not been fully explained.

Infection in immunocompromised patients

Most cases of toxoplasmosis in immunocompromised patients are a consequence of latent infection and reactivation. In patients with AIDS, *T gondii* tissue cysts can reactivate with CD4 counts of less than 200 cells/μL; with counts of less than 100 cells/μL, clinical disease becomes more likely. Without adequate prophylaxis or restoration of immune function, patients with CD4 counts of less than 100 cells/μL who are *T gondii* IgG-antibody positive have a 30% risk of eventually developing reactivation disease. Although toxoplasmosis in immunocompromised patients may manifest as retinochoroiditis, reactivation disease in these individuals is typically in the CNS, with brain involvement being common. Toxoplasmic encephalitis and brain abscess present most commonly as headache, but focal neurologic deficits and seizures are as common. With significant disease, patients may also demonstrate the signs and symptoms of elevated intracranial pressure. Cerebral toxoplasmosis is generally identified on computed tomography (CT) scan as multiple ring-enhancing lesions; however, solitary lesions may be seen, and negative CT or magnetic resonance imaging (MRI) scans should not rule out the diagnosis of CNS toxoplasmosis. Aside from CNS toxoplasmosis, other conditions commonly identified in immunocompromised patients include toxoplasmic pneumonitis, myocarditis, and disseminated toxoplasmosis. Toxoplasmic pneumonitis typically presents with symptoms typical for an infectious pulmonary process, including fever, dyspnea, and cough. Chest radiography is often nonspecific, but findings may have an appearance similar to that of *Pneumocystis* (*carinii*) *jirovec*ipneumonia. Diagnosis is established via bronchoalveolar lavage. Most patients with extra-CNS manifestations of toxoplasmosis will also be noted to have CNS lesions when appropriate radiographic studies have been performed.

Effects of toxoplasmosis on mental disorders

Recent investigations have suggested that chronic toxoplasmosis may play several roles in the etiology of different mental disorders. Numerous clinical studies have evaluated the prevalence of anti-*Toxoplasma* antibodies in patients with schizophrenia and other forms of severe psychiatric disorders. The most probable mechanism by which *T gondii* could cause schizophrenia is by affecting neurotransmitters in brain areas known to be involved in schizophrenia. According to these studies, bradyzoites of *T gondii* affect dopamine and other neurotransmitters in rodents and humans. A few studies have also investigated the association between *T gondii* infection and Parkinson and Alzheimer diseases.

Epidemiology

International occurrence

In many populations, such as those in El Salvador and France, the seropositivity rate to *T gondii* is as high as 75% by the fourth decade of life. As many as 90% of adults in Paris are seropositive. Approximately 50% of the adult population in Germany is infected. Women of childbearing age in much of Western Europe, Africa, and South and Central America have seroprevalence rates of greater than 50%. Based on serologic studies, estimates suggest the incidence of primary maternal *T gondii* infection during pregnancy ranges from about 1-310 cases per 10,000 pregnancies in different populations in Europe, Asia, Australia, and the Americas. The incidence of prenatal *T gondii* infection within the same or similar populations has been estimated to range from about 1-120 cases per 10,000 births. The prevalence of immunocompromised patients is higher in some nations as a function of HIV/AIDS infection and also organ transplantation and immunomodulatory medication prescribing. In individuals with HIV infection, the seropositivity rate to *T gondii* is approximately 50-78% in certain areas of Western Europe and Africa. Toxoplasmic encephalitis is the AIDS-defining diagnosis in 16% of patients with AIDS. In France, 37% of patients with AIDS have evidence of toxoplasmic encephalitis at autopsy.

Age-related demographics

With the exception of *T gondii* retinochoroiditis, older individuals are more likely to manifest clinically evident reactivation of *Tgondii* infection. Congenitally acquired *Tgondii* retinochoroiditis is more likely to recur in persons older than 40 years.

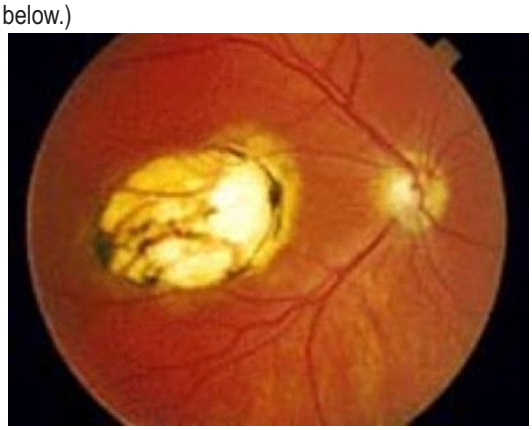
Prognosis

Immunocompetent patients have an excellent prognosis, and lymphadenopathy and other symptoms generally resolve within weeks of infection. Toxoplasmosis in immunodeficient patients often relapses if

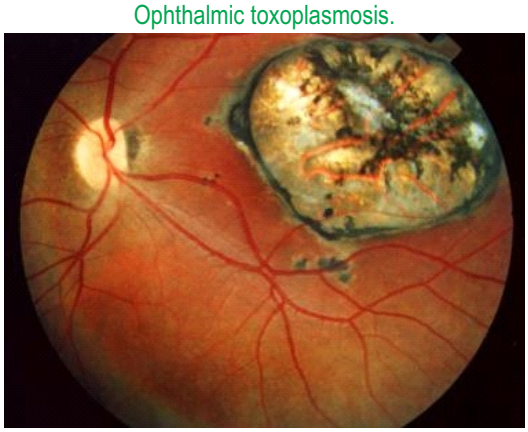
treatment is stopped. Suppressive therapy and immune reconstitution significantly reduce the risk of recurrent infection. Multiple complications may occur in persons with congenital toxoplasmosis, including mental retardation, seizures, deafness, and blindness. Treatment may prevent the development of untoward sequelae in symptomatic and asymptomatic infants with congenital toxoplasmosis. Infants with congenitally acquired toxoplasmosis generally have a good prognosis and are on average developmentally identical to noninfected infants by the fourth year of life. Toxoplasmic encephalitis and brain abscess can result in permanent neurologic sequelae, depending on the location of the lesion and the extent of local damage and inflammation. Basal ganglia seem to be preferentially involved. Seizure disorder or focal neurologic deficits may occur in persons with CNS toxoplasmosis.

Ophthalmic complications

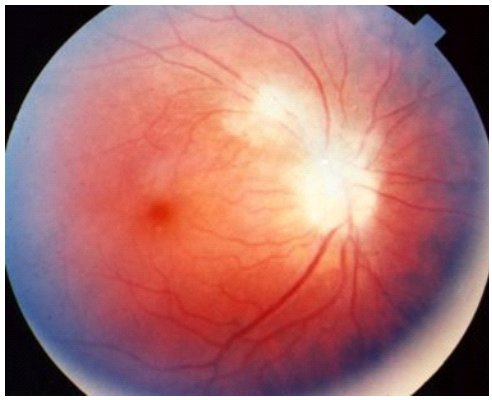
Toxoplasmosis is the most common cause of intraocular inflammation and posterior uveitis in immunocompetent patients throughout the world. Toxoplasmosis is responsible for approximately 30-50% of all posterior uveitis cases in the United States. Retinochoroiditis is a relatively common manifestation of *T gondii* infection. Ocular toxoplasmosis occurs when cysts deposited in or near the retina become active, producing tachyzoites. Focal necrotizing retinitis is the characteristic lesion, but retinal scars from prior reactivation are typically present. Presentation usually involves eye pain and decreased visual acuity. Adults who acquired disease in infancy usually present with bilateral eye involvement. Adults with acute infection generally present with unilateral ocular involvement. Depending on the location and severity of toxoplasmic retinochoroiditis, infection can result in permanent retinal scarring and loss of visual acuity. Recurrent episodes are common, resulting in multiple areas of retinal scarring and functional loss. (See the images below.)



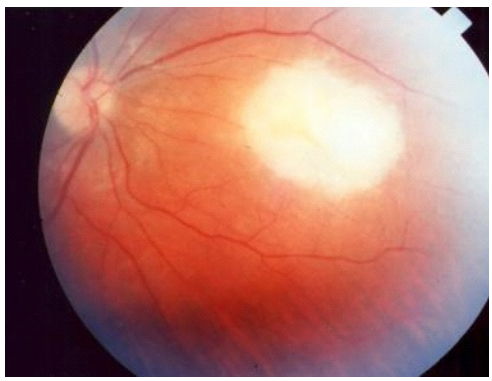
Ophthalmic toxoplasmosis.



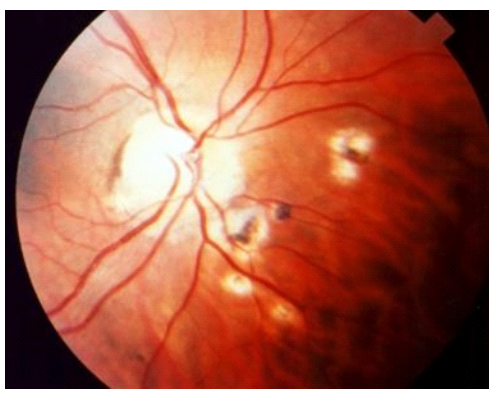
Macular scar secondary to congenital toxoplasmosis. Visual acuity of the patient is 20/400.



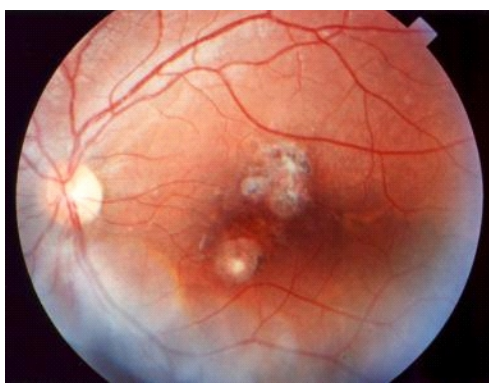
Papillitis secondary to toxoplasmosis, necessitating immediate systemic therapy.



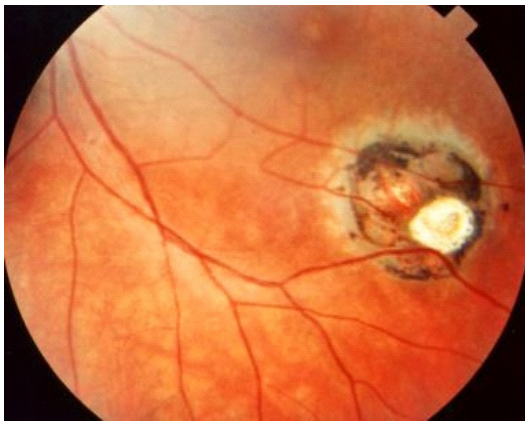
Acute macular retinitis associated with primary acquired toxoplasmosis, requiring immediate systemic therapy.



Peripapillary scars secondary to toxoplasmosis.



Perimacular scars secondary to toxoplasmosis.



Inactive retinochoroidal scar secondary to toxoplasmosis.

Vascular endothelial growth factor (VEGF) has been shown to be a key molecular player in the pathogenesis of choroidal neovascular membrane (CNV). In the current era of anti-VEGF therapy, the extraordinary results obtained in CNV secondary to age-related macular degeneration have been extrapolated to other causes of CNV with apparent good results. Currently available anti-VEGF agents include bevacizumab, ranibizumab, and pegaptanib sodium. Secondary glaucoma may occur with anterior uveitis that is secondary to the obstruction of the outflow channels by the inflammatory cells. This condition may or may not be reversible.

Destruction of the trabecula by chronic inflammation and anterior synechiae may also create a chronic pharmacologically nonresponsive glaucoma.

Other ocular complications include:

- Branch retinal vein occlusion
- Branch retinal artery occlusion
- Tractional retinal detachment
- Cataract
- Posterior synechiae
- Cystoid macular edema
- Retinal perivasculitis
- Optic atrophy
- Epiretinal membrane
- Persistent vitreous opacities

Morbidity and mortality

Acute toxoplasmosis is asymptomatic in 80-90% of healthy hosts. In some apparently immunologically healthy patients, however, the acute infection progresses and may have lethal consequences. Although a relatively small percentage of toxoplasmosis cases are congenital, they tend to account for most acute and fatal infections. In immunosuppressed patients, *T gondii* infection, like other opportunistic infections, can lead to rapidly progressive, fatal disease. Indeed, toxoplasmosis is recognized as a major cause of neurologic morbidity and mortality among patients with advanced HIV disease. However, the incidence of toxoplasmosis (including CNS disease) in patients with AIDS has declined dramatically, likely due to the evolution of highly active antiretroviral therapy (HAART) and the routine use of prophylaxis against *P* (*carinii*) *jiroveci* and *T gondii*. The incidence of CNS toxoplasmosis decreased from 5.4 cases per 1000 person-years between 1990 and 1992 to 2.2 cases per 1000 persons-years between 1996 and 1998. The routine use of cotrimoxazole prophylaxis in the United States and internationally has also likely significantly decreased the incidence of CNS toxoplasmosis.

Toxoplasmosis Clinical Presentation

History

Only 10-20% of toxoplasmosis cases in adults and children are symptomatic. Toxoplasmosis is a serious and often life-threatening disease in immunodeficient patients. Congenital toxoplasmosis may manifest as a mild or severe neonatal disease, with onset during the first month of life or with sequelae or relapse of a previously undiagnosed infection at any time during infancy or later in life. Congenital toxoplasmosis has a wide variety of manifestations during the perinatal period.

Acute toxoplasmosis in immunocompetent persons

See the list below:

- Approximately 80-90% of patients are asymptomatic. Symptomatic disease may be characterized as follows:
- Patients may have cervical lymphadenopathy with discrete, usually nontender, nodes smaller than 3cm in diameter
- Fever, malaise, night sweats, and myalgias have been reported
- Patients may have a sore throat
- Retroperitoneal and mesenteric lymphadenopathy with abdominal pain may occur
- Retinochoroiditis is reported

Acute toxoplasmosis in hosts who do not have AIDS but are immunodeficient

The disease in these patients may be newly acquired or a reactivation. It may be characterized as follows:

- CNS toxoplasmosis occurs in 50% of patients - Seizure, dysequilibrium, cranial nerve deficits, altered mental status, focal neurologic deficits, headache
- Patients may have encephalitis, meningoencephalitis, or mass lesions
- Hemiparesis and seizures have been reported
- Patients may report visual changes
- They may have signs and symptoms similar to those observed in immunocompetent hosts.
- Patients may have flulike symptoms and lymphadenopathy
- Myocarditis and pneumonitis are reported.
- Toxoplasmic pneumonitis can occur - Typical symptoms of a pulmonary infection, mirroring in particular *P* (*carinii*) *jiroveci*, including nonproductive cough, dyspnea, chest discomfort, & fever

Symptoms associated with reactivation toxoplasmosis are dependent on the tissue or organ affected.

Clinical manifestations of toxoplasmosis in patients with AIDS

Brain involvement (ie, toxoplasmic encephalitis), with or without focal CNS lesions, is the most common manifestation of toxoplasmosis in individuals with AIDS.

Clinical findings include the following:

- Altered mental state
- Seizures
- Weakness
- Cranial nerve disturbances
- Sensory abnormalities
- Cerebellar signs
- Meningismus
- Movement disorders
- Neuropsychiatric manifestations

The characteristic presentation is usually a subacute onset, with focal neurologic abnormalities in 58-89% of cases. However, in 15-25% of cases, the clinical presentation is more abrupt, with seizures or cerebral hemorrhage. Most commonly, hemiparesis and/or speech abnormality is

the major initial manifestation. Brainstem involvement often produces cranial nerve lesions, and many patients exhibit cerebral dysfunction with disorientation, altered mental state, lethargy, and coma. Less commonly, parkinsonism, focal dystonia, rubral tremor, hemichorea-hemiballismus, panhypopituitarism, diabetes insipidus, or syndrome of inappropriate antidiuretic hormone secretion may dominate the clinical picture. In some patients, neuropsychiatric symptoms such as paranoid psychosis, dementia, anxiety, and agitation may be the major manifestations. Diffuse toxoplasmic encephalitis may develop acutely and can be rapidly fatal; generalized cerebral dysfunction without focal signs is the most common manifestation, and CT scan findings are normal or reveal cerebral atrophy. Spinal cord involvement manifests as motor or sensory disturbances of single or multiple limbs, bladder or bowel dysfunctions, or both and local pain. Patients may present with clinical findings similar to those of a spinal cord tumor. Cervical myelopathy, thoracic myelopathy, and conus medullaris syndrome have been reported. Pulmonary toxoplasmosis (pneumonitis) due to toxoplasmosis is increasingly recognized in patients with AIDS who are not receiving appropriate anti-HIV drugs or primary prophylaxis for toxoplasmosis. The diagnosis may be confirmed by demonstrating *T gondii* in bronchoalveolar lavage fluid. Pulmonary toxoplasmosis occurs mainly in patients with advanced AIDS (mean CD4 count of 40 cells/µL ±75 standard deviation) and primarily manifests as a prolonged febrile illness with cough and dyspnea. Pulmonary toxoplasmosis may be clinically indistinguishable from *P (carinii) jirovecipneumonia*, and the mortality rate, even when treated appropriately, may be as high as 35%.

Extrapulmonary toxoplasmosis develops in approximately 54% of persons with toxoplasmic pneumonitis. Ocular toxoplasmosis, ie, toxoplasmic retinochoroiditis, is relatively uncommon in patients with AIDS; it commonly manifests as ocular pain and loss of visual acuity. Funduscopic examination usually demonstrates necrotizing lesions, which may be multifocal or bilateral. Overlying vitreal inflammation is often present and may be extensive. The optic nerve is involved in as many as 10% of cases.

Other, uncommon manifestations of toxoplasmosis in patients with AIDS include the following:

- Panhypopituitarism and diabetes insipidus
- Multiple organ involvement, with the disease manifesting as acute respiratory failure and hemodynamic abnormalities similar to septic shock
- Syndrome of inappropriate antidiuretic hormone secretion and possibly orchitis
- Gastrointestinal system invasion of *T gondii* may result in abdominal pain, diarrhea, and/or ascites (due to involvement of the stomach, peritoneum, or pancreas)
- Acute hepatic failure
- Musculoskeletal involvement
- Parkinsonism
- Focal dystonia
- Rubral tremor
- Hemichorea-hemiballismus

Congenital toxoplasmosis

This is most severe when maternal infection occurs early in pregnancy. Approximately 15-55% of congenitally infected children do not have detectable *T gondii* –specific IgM antibodies at birth or early infancy. Approximately 67% of patients have no signs or symptoms of infection. Retinochoroiditis occurs in about 15% of patients, and intracranial calcifications develop in about 10%. Cerebrospinal fluid (CSF) pleocytosis and elevated protein values are present in 20% of patients. Infected newborns have anemia, thrombocytopenia, and jaundice at birth. Microcephaly has been reported. Affected survivors may have mental retardation, seizures, visual defects, spasticity, hearing loss or other severe neurologic sequelae. The prevalence of sensorineural hearing loss is as high as 28% in children who do not receive treatment.

Ocular toxoplasmosis

Patients develop retinochoroiditis (focal necrotizing retinitis). They have a yellowish white, elevated cotton patch with indistinct margins. The lesions may occur in small clusters. Congenital disease is usually bilateral and acquired disease is usually unilateral.

Symptoms include the following:

- Impaired vision - Either sudden or gradual, depending on the site of infection
- Blurred vision
- Scotoma
- Pain
- Photophobia
- Floaters
- Red eye
- Metamorphopsia

Physical Examination

The acquired infection is usually subclinical and asymptomatic. In 10-20% of cases that become symptomatic, the patient develops a flulike illness characterized by fever, lymphadenopathy, malaise, myalgias, and a maculopapular skin rash that spares the palms and the soles. In individuals who are immunocompetent, the disease is benign and self-limited. Hepatosplenomegaly can also occur. Infrequently, patients develop myocarditis, polymyositis, pneumonitis, hepatitis, or encephalitis. The most common form of symptomatic acute toxoplasmosis in immunocompetent individuals is lymphadenopathy. The typical presentation is painless, firm lymphadenopathy that is confined to 1 chain of nodes, most commonly cervical. Ophthalmologic examination reveals multiple yellow-white cottonlike patches with indistinct margins located in small clusters in the posterior pole. A flare-up of congenitally acquired retinochoroiditis is often associated with scarred lesions juxtaposed to the fresh lesion.

Ocular toxoplasmosis (retinochoroiditis)

Symptoms of retinochoroiditis include the following :

- Decreased visual acuity - Other deficits depend on the location of the lesion
- White focal lesions with inflammation of the vitreous humor (the classic "headlight in the fog" appearance) seen on ophthalmoscopic examination
- Recurrent lesions at the border of the retinochoroidal scars

Immunocompromised individuals (AIDS CD4 count < 100 cells/microL)

Host immune function plays an important role in the pathogenicity of toxoplasmosis. Symptoms depend largely on the organ system and tissue involved and may be gradual in onset over a few weeks. They include the following:

- CNS toxoplasmosis - Seizure, mental status change, focal motor deficits, cranial nerve disturbances, sensory disturbances, cerebellar abnormalities, movement disorders, neuropsychiatric findings
- Retinochoroiditis (similar to that seen in immunocompetent individuals)
- Pneumonitis - More common in patients who have undergone bone marrow transplantation and in patients with AIDS; nonproductive

cough, blood-tinged sputum, hypoxia (symptoms indistinguishable from *P [carinii]jiroveci*)
- Septic shock–like presentation

Multifocal, bilateral, and relentlessly progressive lesions characterize the ocular involvement. Because of their immunosuppression, these patients often have problems mounting an inflammatory reaction, which makes the formation of a retinochoroidal scar difficult. Often, the serologic diagnosis is also difficult.

Congenital toxoplasmosis

The classic clinical triad of retinochoroiditis, cerebral calcifications, and convulsions defines congenital toxoplasmosis. Other findings include the following:
- Hydrocephalus
- Microcephaly
- Organomegaly
- Jaundice
- Rash
- Fever
- Psychomotor retardation

Toxoplasmosis Differential Diagnoses

Diagnostic Considerations

Conditions to consider in the differential diagnosis of congenital toxoplasmosis include rubella, encephalopathies, and erythroblastosis fetalis. In the differential diagnosis of toxoplasmic encephalitis, vasculitis and tumor should be considered. The major differential diagnosis of focal CNS lesions in patients with AIDS is CNS lymphoma, which manifests as multiple enhancing lesions in 40% of cases.

Other differentials in the diagnosis of toxoplasmosis include the following:
- Other lesions caused by *Cryptococcus neoformans, Aspergillus* species , *Mycobacterium tuberculosis, Nocardia* species
- Mycosis fungoides
- *Pneumocystis* (*carinii*) *jiroveci* pneumonia
- Sarcoidosis
- Bacterial sepsis
- Syphilis
- Tuberculosis
- Tularemia
- Acute HIV infection
- Cytomegalovirus retinitis
- Cytomegalovirus encephalitis
- Cytomegalovirus ventriculitis
- CNS tuberculosis
- Disseminated tuberculosis
- Leukemia
- Lymphoma
- Progressive multifocal leukoencephalopathy
- Acute retinal necrosis
- Fungal endophthalmitis
- Epimacular membrane
- Intraocular foreign body
- Uveitic glaucoma
- Ocular manifestations of HIV
- Ocular manifestations of syphilis
- Anterior granulomatous uveitis
- Anterior nongranulomatous uveitis
- Fuchs heterochromic uveitis
- Toxocariasis
- Primary intraocular lymphoma
- Pars planitis

Differential Diagnoses

- Brain Abscess
- Cat Scratch Disease (Cat Scratch Fever)
- Cytomegalovirus (CMV)
- Herpes Simplex
- Histoplasmosis
- Epstein-Barr Virus (EBV) Infectious Mononucleosis (Mono)
- Leprosy
- Listeria Monocytogenes Infection (Listeriosis)
- Lymphoblastic Lymphoma
- Metastatic Cancer With Unknown Primary Site

Toxoplasmosis Workup

Approach Considerations

Results from basic laboratory studies such as complete blood cell count (CBC), chemistries, and liver function tests (LFTs) are typically normal, although lymphocytosis may be present.

Direct detection

The diagnosis of toxoplasmosis is confirmed with the demonstration of *T gondii* organisms in blood, body fluids, or tissue. *T gondii* may be isolated from the blood via either inoculation of human cell lines or mouse inoculation. Mouse inoculation may require a longer time to yield results and also is likely to be more expensive. Isolation of *T gondii* from amniotic fluid is diagnostic of congenital infection by mouse inoculation.

Molecular diagnosis and polymerase chain reaction. Molecular diagnostic methods of diagnosing toxoplasmosis include techniques such as conventional polymerase chain reaction (PCR), nested PCR, and real-time PCR for detection of *T gondii* DNA in clinical samples. The original protocol for molecular detection of *T gondii* using conventional PCR targeted the *B1* gene. Studies have also described detection of *T gondii* based on amplification of ITS-1 and 18S rDNA fragments, a method whose sensitivity was similar to the *B1* gene. According to recent studies, the repetitive element of 529 bp in length has shown a sensitivity that is 10-times that of the sensitivity using the *B1* gene. Real-time PCR detection of *T gondii* DNA based on the 529 bp repetitive element is the most frequently used molecular diagnostic approach for toxoplasmosis. Polymerase chain reaction (PCR) assay testing on body fluids, including CSF, amniotic fluid, bronchoalveolar lavage fluid, and blood, may be useful in the diagnosis. However, PCR assay is capable of detecting *T gondii*deoxyribonucleic acid (DNA) in either an aqueous sample or a vitreous sample in only one third of patients with ocular toxoplasmosis.

Indirect detection

Indirect detection is performed in pregnant women and in immunocompromised patients. Detection of immunoglobulin G (IgG) is possible within 2 weeks of infection using the enzyme-linked immunosorbent assay (ELISA) test, the IgG avidity test, and the agglutination and differential agglutination tests. (Acute and convalescent sera have no role in the indirect detection of toxoplasmosis.)

Procedures

The following diagnostic procedures may be performed for toxoplasmosis:
- Lumbar puncture - After imaging to identify evidence of increased intracranial pressure
- Brain biopsy
- Lymph node biopsy

- Amniocentesis - Perform amniocentesis at 20-24 weeks' gestation if congenital disease is suggested
- Bronchoalveolar lavage

Tachyzoites may be demonstrated in tissues or smears obtained from biopsy. They also can be seen in CSF. CSF also shows mononuclear pleocytosis and elevated protein level. Tachyzoites demonstrate acute infection, while tissue cysts and bradyzoites are seen in chronic/latent infection (although they may be present in acute infection/reactivation).

Testing in pregnancy

Although testing in pregnancy may not be indicated and treatment may not have established literature support, a low index of suspicion is needed to identify acute infection in pregnant patients. Suspected congenital infection in a pregnant patient should be confirmed before administering treatment by having samples tested at a toxoplasmosis reference laboratory using tests that are as accurate as possible and correctly interpreted.

Ophthalmic disease

Antibody titers do not correlate with ophthalmic disease. Antitoxoplasmic antibodies may be very low and should be tested in undiluted (1:1) samples if possible. The absence of antibodies rules out the disease; nevertheless, false-negative results do occur. Invasive techniques are usually reserved for difficult cases, such as patients who are immunocompromised. Ocular fluids can demonstrate the presence of intraocular antibody production. Polymerase chain reaction assay can detect the causative organism.

Immunoglobulin Testing

Acute systemic toxoplasmosis has traditionally been diagnosed by seroconversion. Anti-*Toxoplasma* immunoglobulin G (IgG) titers present a 4-fold increase that peak 6-8 weeks following infection and then decline over the next 2 years, although they remain detectable for life. Anti-*Toxoplasma*IgM appears in the first week of the infection and then declines in the next few months. The presence of anti-*Toxoplasma* IgA has also been shown to be detectable in acute infection; however, since the titers can last for more than 1 year, its value in helping to diagnose an acute phase is limited. Detection of IgG is possible within 2 weeks of infection using the ELISA test, the IgG avidity test, and the agglutination and differential agglutination tests. The presence of IgG indicates a likely past infection, while the presence of IgM usually indicates acute infection (particularly in the absence of IgG). However, IgM has, in some cases, been documented to persist for months or years. Lack of IgG and IgM may exclude infection. IgM alone that then transitions to IgG without IgM or both IgG and IgM indicates likely acute infection. There is a significant rate of false IgM positivity. The sensitivities and specificities of the commercially available IgM and IgG tests vary substantially.

Sabin-Feldman dye test

The Sabin-Feldman dye test is a sensitive and specific neutralization test for toxoplasmosis. It is used to measure primarily IgG antibody and is the standard reference test for toxoplasmosis. However, it requires live *T gondii* organisms; therefore, it is not available in most laboratories. (It is used primarily as a confirmatory test in reference laboratories.) High titers suggest acute toxoplasmosis.

Fluorescent antibody test

The indirect fluorescent antibody test is used to measure the same antibodies as the dye test. Titers parallel dye test titers. The IgM fluorescent antibody test is used to detect IgM antibodies within the first week of infection, but titers fall within a few months.

Hemagglutination test

The indirect hemagglutination test is easy to perform. However, it usually does not detect antibodies during the acute phase of toxoplasmosis. Titers tend to be higher and remain elevated longer.

ELISA test

The results from a double-sandwich IgM ELISA are more sensitive and specific than the results from other IgM tests. Enzyme-linked immunofiltration assay (ELIFA) is based on the use of a microporous cellulose acetate membrane in a co-immunoelectrodiffusion procedure. The ELIFA method has a better diagnostic yield than specific IgM and/or IgA detection by immunocapture assay.

IgG avidity test

The results of the IgG avidity test may help to differentiate patients with acute infection from those with chronic infection better than do alternative assays, such as assays that measure IgM antibodies. As is true for IgM antibody tests, the avidity test is most useful when performed early in gestation. IgG produced early in infection is less avid and binds to *T gondii* antigens more weakly than do antibodies produced later in the course of infection. High antibody avidity indicates an older, earlier infection. This test may be helpful in the setting of pregnancy, as the timing of infection has prognostic value. A long-term pattern occurring late in pregnancy does not exclude the possibility that the acute infection may have occurred during the first months of gestation.

Rapid ICT

Recently rapid immunochromatographic tests (ICT) for detecting antibodies to toxoplasma have been developed. The ICT is a qualitative test that simultaneously detects specific *Toxoplasma* IgG and IgM antibodies. The performance of ICT's has found to be comparable to the toxoplasma IgG an IgM estimation on architect in a study by Mahinc et al., where in the sensitivity for toxoplasma IgG was found to be 100% and specificity 98.7%. The sensitivity and specificity for toxoplasma IgM was 100%. The ICT for Toxoplasma IgG-IgM could be promoted as a first-line technique in developing countries and could be particularly interesting for the early follow-up of pregnant women (Begeman et al.). Testing with Toxoplasma ICT IgG-IgM test could bring with it, especially in low- and middle-income settings, the incentive for ideal, more frequent monthly obstetrical care for pregnant women, improving maternal and child health.

Imaging Studies

Head CT scanning in cerebral toxoplasmosis (general)

In most immunodeficient patients with toxoplasmic encephalitis, CT scans show multiple bilateral cerebral lesions. However, although multiple lesions are more common in persons with toxoplasmosis, they may be solitary. Therefore, a single lesion should not exclude toxoplasmic encephalitis as a diagnostic possibility.

Head CT scanning in cerebral toxoplasmosis (in patients with AIDS)

CT scans in patients with AIDS who have toxoplasmic encephalitis reveal multiple ring-enhancing lesions in 70-80% of cases. In patients with AIDS who have detectable *Toxoplasma* IgG and multiple ring-enhancing lesions on CT scans or MRIs, the predictive value for toxoplasmic encephalitis is approximately 80%. Lesions tend to occur at the corticomedullary junction (frequently involving the basal ganglia) and are characteristically hypodense. The number of lesions is frequently underestimated when assessed using CT scan images, although delayed imaging after a double dose of intravenous (IV) contrast material may improve the sensitivity of this modality. An enlarging, hypodense lesion that does not enhance is a poor prognostic sign.

Single-photon computed tomography

Single-photon computed tomography (SPECT) scanning is useful in distinguishing between CNS lymphoma and infection (ie, toxoplasmosis or any other infection).

PET scanning, radionuclide scanning, and MR techniques

Various positron emission tomography (PET) scanning, radionuclide scanning, and magnetic resonance techniques have been used to evaluate patients with AIDS who have focal CNS lesions and to specifically differentiate between toxoplasmosis and primary CNS lymphoma.

MRI

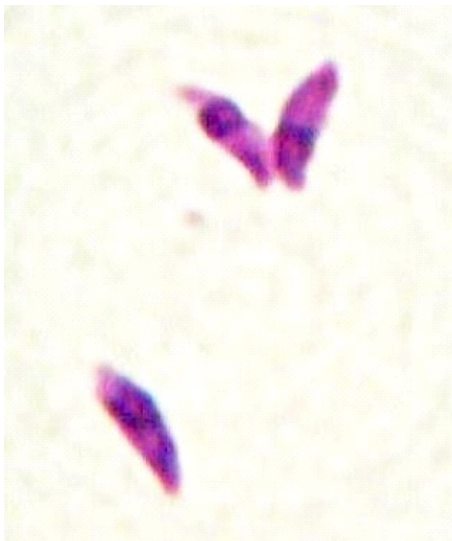
MRI has superior sensitivity (particularly if gadolinium is used for contrast) to CT scanning, and MRI scans often demonstrate a single or multiple lesion(s) or more extensive disease not apparent on CT scans. One study showed that MRI detected abnormalities in 40% of patients whose abnormalities were not detected on CT. Toxoplasmic encephalitis lesions on MRIs appear as high-signal abnormalities on T2-weighted studies and have a rim of enhancement surrounding the edema on T1-weighted, contrast-enhanced images. Hence, MRI should be used as the initial procedure when feasible (and especially if a single lesion is demonstrated on CT scan images). Nevertheless, even characteristic lesions on CT scans or MRIs are not pathognomonic of toxoplasmic encephalitis. The major differential diagnosis of focal CNS lesions in patients with AIDS is CNS lymphoma, which manifests as multiple enhancing lesions in 40% of cases. The probability of toxoplasmic encephalitis falls and the probability of lymphoma rises in the presence of single lesions on MRI scans. Therefore, a brain biopsy may be required to obtain a definitive diagnosis in patients with a solitary lesion (especially if confirmed with MRI). CT scanning abnormalities improve after 2-3 weeks of treatment in approximately 90% of patients with AIDS who have toxoplasmic encephalitis. Complete resolution takes 6 weeks to 6 months; peripheral lesions resolve more rapidly than do deeper ones. Smaller lesions usually resolve completely within 3-5 weeks as shown on MRI, but lesions with a mass effect tend to resolve more slowly and leave a small residual lesion. A radiologic response to therapy lags behind the clinical response, with better correlation by the end of acute therapy.

Ultrasonography

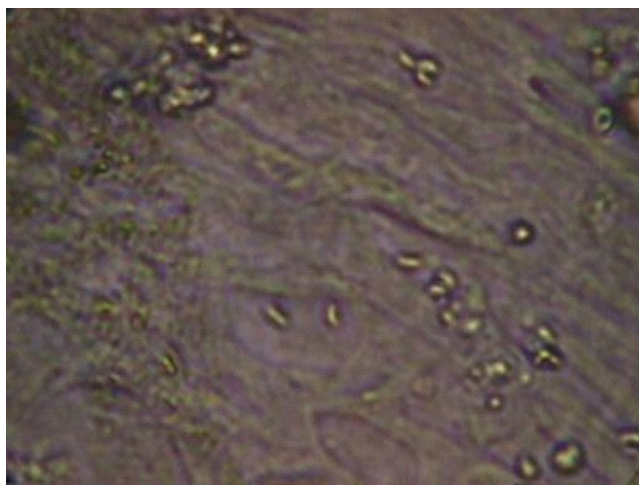
Ultrasonographic diagnosis of congenital toxoplasmosis in a fetus is available at 20-24 weeks' gestation. Fetal or neonatal ultrasonography can be useful in cases of known or suspected maternal acute infection and transplacental infection. Findings are generally nonspecific but include ventriculomegaly and CNS calcifications, particularly in the basal ganglia. Moreover, clinicians should consider a diagnosis of toxoplasmosis in pregnant women with clinical symptoms and signs such as cervical lymphadenopathy and consider CT scanning in fetuses with ultrasonographic abnormalities compatible with TORCH infections (an acronym for toxoplasmosis, other [syphilis, varicella-zoster, parvovirus B19], rubella, cytomegalovirus, herpes infections).

Histologic Findings

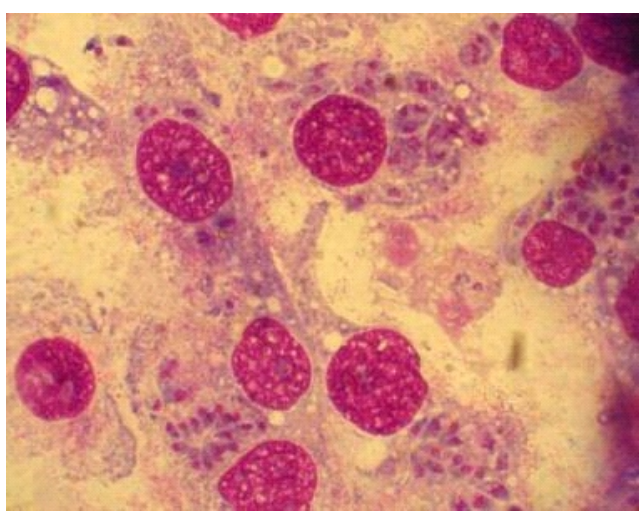
Histopathologic data for human toxoplasmosis has been obtained mostly from autopsy studies in infants and immunodeficient patients with serious infections. Such knowledge in immunocompetent patients is limited. Pathologic findings are usually obtained from lymph node biopsy specimens in these patients. Multiple brain abscesses are commonly found, often involving the cerebral cortex and deep gray nuclei, less often the brainstem and cerebellum, and rarely the spinal cord. (See the following images.)



Toxoplasma gondii tachyzoites (Giemsa stain).



Toxoplasma gondii tachyzoites in cell line.



Toxoplasma gondii in infected monolayers of HeLa cells (Giemsa stain).

View Media Gallery

In acute toxoplasmosis, lesions are composed of central necrotic foci with varying petechiae rounded by acute and chronic inflammation, vascular proliferation, and macrophage infiltration. Tachyzoites and bradyzoites in tissue cysts may be detected at the periphery of the necrotic foci. *T gondii* are commonly found on hematoxylin and eosin or Giemsa stains. However, parasites can be more easily described via immunohistochemical staining. The blood vessels in the area of necrotic lesions may demonstrate distinguished intimal proliferation or frank vasculitis with thrombosis and fibrinoid necrosis.

Chronic lesions are composed of small cystic fields containing a number of lipid- and hemosiderin-laden macrophages with surrounding gliosis. Parasites are difficult to find in older lesions.

Toxoplasmosis Treatment & Management

Approach Considerations

Treatment is usually unnecessary in asymptomatic hosts, except in children younger than 5 years. Symptomatic patients should be treated until immunity is ensured. Outpatient care is sufficient for acquired toxoplasmosis in immunocompetent hosts and for persons with ocular toxoplasmosis. Inpatient care is appropriate initially for persons with CNS toxoplasmosis and for acute toxoplasmosis in immunocompromised hosts. Patients with AIDS who have a CD4 count of less than 100 cells/µL should be commenced on suppressive therapy for *T gondii* until they undergo immune reconstitution.

Consultations

Subspecialty consultation is required for the seriously ill patient, according to organ-specific involvement. Moreover, in the setting of immunocompromise, involvement of one organ system (ie, retina) mandates analysis of further organ system involvement (ie, CNS). In addition to an infectious diseases specialist, the following are recommended consultations:

- Parasitologist
- Ophthalmologist
- Neurologist
- Radiologist
- Gynecologist
- Pediatrician

Follow-up

Follow-up visits should be scheduled every 2 weeks until the patient is stable, and then monthly during therapy. A CBC should be performed weekly for the first month, and then every 2 weeks. Renal and liver function tests should be performed monthly. Infants with confirmed congenital toxoplasmosis should be followed for evidence of developmental delay and should receive ophthalmologic consultation and follow-up.

Activity

The level of activity in patients with toxoplasmosis depends on the severity of disease and the organ systems involved.

Emergency Department Care

Care of the patient in the emergency department should be specific to the presenting manifestations of the disease. Adequate airway, breathing, and circulation must be assessed and treated accordingly. Adequate fluid resuscitation, pain control, and fever control must be ensured. Neuroimaging should be considered for any immunocompromised patient with a new neurologic deficit, cranial nerve abnormality, severe headache, or altered mental status. Because the symptoms associated with acute toxoplasmosis are nonspecific and dependent on the tissues involved, emergency providers must be vigilant and include other infectious and noninfectious etiologies in their differential diagnoses. As such, broad-spectrum antimicrobial therapy is often necessary early in the course of illness, prior to the performance of definitive testing and while the diagnosis may still be uncertain. Emergency consultation with relevant subspecialties may be required for assistance in empiric treatment and the diagnostic workup.

Deterrence and Prevention

Everyone, including immunocompetent patients, should be educated about toxoplasmosis risk factors and ways to minimize the risks. Preventing toxoplasmosis is particularly important in seronegative immunocompromised patients and in pregnant women. Precautions against the disease include the following:

- Avoid eating raw meat, unpasteurized milk, and uncooked eggs, oysters, clams, and mussels.
- Wash hands after touching raw meat.
- Wear gloves when gardening or handling soil and wash hands afterwards.
- Wash fruits and vegetables.
- Avoid contact with cat feces; however, pregnant women and persons with HIV infection who have cats are at no increased risk for toxoplasmosis compared with persons who do not have cats.

Moreover, travel to areas of high endemicity (Western Europe, South America) may increase the risk of exposure. Avoiding transfusions of blood products from a donor who is seropositive to a patient who is seronegative and immunocompromised is prudent, when feasible. If possible, organ recipients who are seronegative should receive transplanted organs from donors who are seronegative. Laboratory workers can become infected via ingestion of sporulated *T gondii* oocysts from feline fecal specimens or via skin or mucosal contact with either tachyzoites or bradyzoites in human or animal tissue or culture. Laboratories should have established protocols for handling specimens that contain viable *T gondii* and for responding to laboratory accidents. Currently, the only effective vaccine against toxoplasmosis is Toxovax, which contains a live attenuated S48 strain and controls congenital infection in sheep. Toxovax decreases the abortion rate but does not eradicate *T gondii* completely. Nevertheless, it is expensive and may be changed into a pathogenic form; for this reason, it is not appropriate for human use. Unfortunately, no licensed vaccine is yet available for humans.

Toxoplasmosis Medication

Medication Summary

Currently recommended drugs in the treatment of toxoplasmosis act primarily against the tachyzoite form of *T gondii*; thus, they do not eradicate the encysted form (bradyzoite). Pyrimethamine is the most effective agent and is included in most drug regimens. Leucovorin (ie, folinic acid) should be administered concomitantly to prevent bone marrow suppression. Unless circumstances preclude using more than 1 drug, a second drug (eg, sulfadiazine, clindamycin) should be added. The efficacy of azithromycin, clarithromycin, atovaquone, dapsone, and cotrimoxazole is unclear; therefore, they should be used only as alternatives in combination with pyrimethamine. The most effective available therapeutic combination is pyrimethamine plus sulfadiazine or trisulfapyrimidines (eg, a combination of sulfamerazine, sulfamethazine, and sulfapyrazine). These agents are active against tachyzoites and are synergistic when used in combination. Careful attention to dosing regimen is necessary because it differs depending on patient variables (eg, immune status, pregnancy). Pyrimethamine may be used with sulfonamides, quinine, and other antimalarials and with other antibiotics.

Nonpregnant patients: Immunocompetent, nonpregnant patients typically do not require treatment.

Treatment of nonpregnant patients is described below.

The 6-week regimen is as follows:

- Pyrimethamine (100mg loading dose orally followed by 25-50 mg/day) plus sulfadiazine (2-4 g/day divided 4 times daily) OR
- Pyrimethamine (100-mg loading dose orally followed by 25-50 mg/day) plus clindamycin (300 mg orally 4 times daily)
- Folinic acid (leucovorin) (10-25 mg/day) should be given to all patients to prevent hematologic toxicity of pyrimethamine
- Trimethoprim (10 mg/kg/day) sulfamethoxazole (50 mg/kg/day) for 4 weeks

Sulfadiazine or clindamycin can be substituted for azithromycin 500 mg daily or atovaquone 750 mg twice daily in immunocompetent patients or in patients with a history of allergy to the former drugs. Consider steroids in patients with radiologic midline shift, clinical deterioration after 48 hours, or elevated intracranial pressure.

Pregnant patients

The diagnosis of acute infection is often difficult to make during pregnancy, and the administration of empiric antimicrobial therapy is discouraged. Substantial controversy exists regarding the efficacy of treatment during pregnancy in terms of reducing the risk of fetal exposure and the subsequent development of clinical disease such as retinochoroiditis or CNS abnormalities. Controversy also exists regarding the optimal regimen for treating maternally acquired infection. Spiramycin and pyrimethamine-sulfonamide are used, but given the infrequency of fetal infection and the asymptomatic nature of most fetal infections, treatment effects are difficult to measure. Spiramycin appears to be somewhat more easily tolerated than pyrimethamine-sulfonamide.

A dosing regimen for pregnant patients is as follows:

- Spiramycin 1 g orally every 8 hours
- If the amniotic fluid test result for *T gondii* is positive: 3 weeks of pyrimethamine (50 mg/day orally) and sulfadiazine (3 g/day orally in 2-3 divided doses) alternating with a 3-week course of spiramycin 1 g 3 times daily for maternal treatment OR
- Pyrimethamine (25 mg/day orally) and sulfadiazine (4 g/day orally) divided 2 or 4 times daily until delivery (this agent may be associated with marrow suppression and pancytopenia) AND
- Leucovorin 10-25 mg/day orally to prevent bone marrow suppression

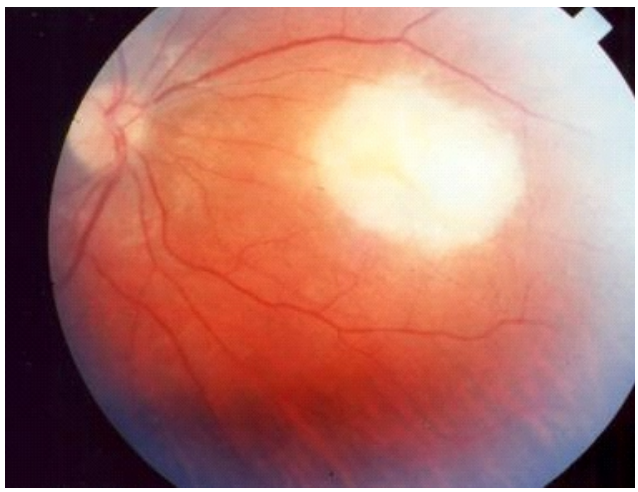
Patients with AIDS

Patients with AIDS are treated with pyrimethamine 200 mg orally initially, followed by 50-75 mg/day orally plus folinic acid 10 mg/day orally plus sulfadiazine 4-8 g/day orally for as long as 6 weeks, followed by lifelong suppressive therapy or until immune reconstitution. Suppressive therapy for patients with AIDS (CD4 count < 100 cells/µL) is pyrimethamine 50mg/day orally plus sulfadiazine 1-1.5 g/day orally plus folinic acid 10 mg/day orally for life or until immune reconstitution. Patients with AIDS, CNS toxoplasmosis, and evidence of midline shift or increased intracranial pressure may also benefit from steroid therapy.

Diagnosing toxoplasmosis in the absence of definitive tissue or culture evidence may be perilous because serology may be misleading and a false-positive IgM result is somewhat common. Consequently, empiric therapy should be avoided.

Retinitis

The mere presence of a focus of retinitis is not always an indication for treatment. Small, peripheral lesions generally heal spontaneously and may be followed conservatively. On the other hand, lesions in the vascular arcade, lesions near the optic disc (Jensen papillitis), lesions in the papillomacular bundle, or large lesions (irrespective of location) are treated. Patients with severe, debilitating vitreitis are also treated aggressively. (See the following image.)



Acute macular retinitis associated with primary acquired toxoplasmosis, requiring immediate systemic therapy.

In a prospective trial, treatment with several regimens failed to shorten the duration of inflammatory activity or to prevent recurrences. However, treatment did reduce the size of the ultimate retinochoroidal scar. In addition, experts differ on their preferred initial treatment. In a report, one third of respondents preferred triple therapy (ie, pyrimethamine, sulfadiazine, prednisone), and a little more than one quarter of respondents preferred quadruple therapy (ie, pyrimethamine, sulfadiazine, clindamycin, prednisone).

Other medicines being used are

- Sulfonamides
- Antibiotics and others (Dapsone)
- Licosamide antibacterials
- Antiprotozoal agents
- Macrolide antibiotics
- Corticosteroids
- Antidotes
- Cycloplegics and Mydriatics

INTERPRETATION

HIV TESTS AND ISSUES RELATED TO THEIR ACCURACY

HIV TESTING

The ELISA, Western Blot and PCR viral load are the most frequently used tests to confirm HIV infections. The ELISA and Western Blot tests detect HIV antibodies in the serum of patients, whereas the PCR Viral Load test is a genetic test that detects small HIV nucleic acid fragments in whole blood. The veracity and reliability of these tests are key to the validity, reliability, quality and accuracy of epidemiological data used by any country. The ELISA test is mainly used to screen for HIV infection in blood donors and for general surveillance, whereas the Western Blot and PCR are generally used as confirmatory tests and in the context of research. All these tests, individually or in combination, are considered by the proponents of the HIV/AIDS theory as important indicators of infection by HIV. The CD4 count is an additional laboratory test used in combination with ELISA to make a diagnosis of AIDS; and with the Viral Load Test to determine the clinical progression of the AIDS disease and the monitoring of the effectiveness of anti-retroviral treatment. The Western Blot test is more expensive and requires a well-developed laboratory infrastructure; it is therefore not affordable for many developing countries. The Western Blot test is not accepted in the United Kingdom as a confirmatory test for HIV infection due to its unreliability.

Co-culturing of virus is used to isolate the virus from the blood of infected AIDS patients. This method is generally used as a research tool as it is too expensive and time-consuming to conduct as a routine surveillance and screening method. It also requires highly specialised staff and infrastructure.

The value of HIV testing and making a diagnosis of HIV infection on the basis of the antibody tests can not be denied. AIDS surveillance was first based on the clinical case definition, and AIDS was initially regarded as just a cluster of diseases. The subsequent discovery of HIV led to the incorporation in the definition of the disease of various clinical and immunological patterns, and, as recently as 1993, the CDC AIDS definition was widened to include a wider spectrum of clinical disease also utilising CD4 counts. European countries do not include the CD4 count in the definition of AIDS.

There is a general lack of standardisation of the definition of AIDS throughout the world. This is because it is possible to diagnose HIV infection by means of non-standardised laboratory tests, as well as by the verification of the presence of clinical symptoms. Since data are compared across countries, there is a need to standardise the definition of AIDS.

After 15 years of research, there is the lack of a 'gold standard' against which to measure the accuracy and reliability of the data generated from the commonly used methods to diagnose HIV infection.

ELISA test

The ELISA test is the most commonly used test for screening blood from donors. Its specificity and high sensitivity make it widely acceptable since it is able to detect all the possible HIV infections. It has also been found to be useful in surveillance. It is generally accepted that a single test cannot be regarded as proof of HIV infection. However, in order to improve the reliability and validity of ELISA, the CDC testing guidelines state that “a test for HIV antibody is considered positive when a sequence of tests, starting with a repeatedly reactive enzyme immunoassay (EIA) and including an additional, more specific assay, such as a Western Blot, are consistently reactive”. Similarly, the WHO testing guidelines require confirmation of samples that are repeatedly reactive by ELISA using the same blood sample but a different ELISA kit. Both testing regimes call for repeated ELISA testing of a single blood sample rather than ELISA testing of more than one blood sample. However, the UNAIDSWHO recommendations state: “An additional blood sample should be obtained and tested from all persons newly diagnosed as seropositive on the basis of their first sample. This will help eliminate any possible technical or clerical error”. Major concerns surrounding the ELISA test, however, include its specificity, reliability and reproducibility, as well as the lack of a comparative 'gold standard'.

Some researchers claim that the HIV ELISA tests are not specific for HIV. They cite the fact that four repeat ELISA tests plus a Western Blot are required for a diagnosis of HIV disease in the USA. Furthermore, be elucidated that there is no standard by which to establish the specificity and sensitivity of the ELISA test. It has also been pointed out that the some PCR kits specifically states that it must not be used as a screening test for HIV nor to diagnose HIV infection .

It has also been pointed out that WHO, in its description of the 34 HIV antibody ELISA tests on the market, uses one antibody test as a gold standard for another It is also opined that mycobacterial and fungal antigens can cross react with HIV ELISAs causing false positives.

It has been argued that no test is perfect and, moreover, that the current generation of ELISA tests is much more sensitive and more specific than in 1984. The current ELISA test uses recombinant proteins made from clones and consequently minimises cross-reactivity by other proteins from the plasma. Immunoassays based on recombinant viral proteins are more specific than tests used previously. All HIV diagnosis should be supported by laboratory tests "and HIV screening should ideally be always followed by two confirmatory tests. All immunoassays should be designed, calibrated, optimised and standardised to the level of their discriminating power which is the power to discriminate between negative and positive cases (ideal being 100%, but high confidence results of 99.9% should be acceptable).

The specificity, reliability and validity of ELISA. based platforms has been questioned from time to time.

The antibody ELISA test is based on the reaction between the unique viral protein (the p24 proteins 'supposedly' from HIV) and serum antibodies from a blood sample. Independent data show that p24 proteins, the basis for the ELISA antibody test, have been found to cross react with a wide variety of uninfected human tissue and blood samples from other disease states. For example, antibodies to candidiasis and mycobacterium infections cross react with p24. Furthermore a warning in the manufacturer's inserts suggests that the ELISA should not be used on its own for HIV diagnosis. It has also been found that many other disease conditions such as leprosy, malaria, leishmaniasis and other viral infections give rise to false positive results in the ELISA test without the concomitant HIV infection. Furthermore, many of the conditions that cause a false positive result in the ELISA test are conditions that are also prevalent in many of the recognised AIDS risk groups. A great deal of scientific data indicates widespread non-specific interactions between what are considered retroviral antigens and unrelated antibodies. A positive HIV ELISA test may also indicate previous antigenic stimulation by other retroviral infection. Another concern is that there is no precedent for the diagnostic utilisation of the ELISA test for other viral diseases. In general, the presence of antibodies specific to a particular disease is a major indicator of potential immune protection by the body, which is not the case with HIV infection, since antibodies to HIV fail to confer any immunoprotection against HIV. The ELISA test may therefore not be a true indicator of infection but an artefact arising from cross reactivity of other naturally occurring viral proteins.

The lack of standardisation of ELISA results, occurs across countries is a source of major concern to some diagnosticians. Results of ELISA tests may be interpreted differently within a single laboratory, between laboratories within one country, and between countries. This may mean that a person that tests positive at one laboratory in say country “A” may test negative at a different laboratory in the same country. Moreover, the lack of standardisation across countries could result in an individual's testing positive in one country and negative in another.

Western Blot

The Western Blot is an antibody test, which, is one of the tests used to confirm the diagnosis of HIV infection in most countries. A positive Western Blot result is synonymous with HIV infection and the attendant risk of developing AIDS. Most clinicians and diagnosticians agree that there was general agreement on the correlation between Western Blot and AIDS and patients that were suffering from AIDS always reacted positively to the Western Blot test.

However, a number of concerns were raised around the specificity, reliability and reproducibility of the Western Blot test.

Some specialists believe that the Western Blot should not be used to confirm and validate the results of the ELISA test since the Western Blot and ELISA tests are based on the same antibody reaction mechanism. As with the ELISA test, another concern over the use of the Western Blot test is its non-specific positive reaction to a number of diseases (including tuberculosis, a variety of parasitic infections and other viral infections) in the absence of HIV infection. The antigens used in the Western Blot test may be similar or identical to other

human proteins, and hence the results of the Western Blot may thus not provide an indication of HIV infection.

Some researchers have reported cross reactivity of a Western Blot test with a number of samples from leprosy, TB and AIDS patients. It appears that the Western Blot results from the different samples are indistinguishable from one another, showing the Western Blot test to be non-specific and unreliable. Many samples test positive, even those from leprosy and TB patients. Furthermore indeterminate results from Western Blot are a definite possibility. The above underlines the fact that the Western Blot test cannot be used as a determinate diagnostic tool.

PCR test for viral load

The PCR viral load test is also used as a confirmatory test. It is based on the amplification of tiny HIV viral particles that are supposed to originate from HIV in the blood. This test is virus specific and specifically detects HIV RNA. It is used to determine the level of viral load in the blood. It is mainly used in the tracking of the clinical progression of advanced HIV infection to AIDS disease, the monitoring of the effect of anti-retroviral treatment and the monitoring of mother-to-child transmission. There is a high correlation between clinical disease progression and the viral load. A high viral load is associated with an increased risk of transmission and the clinical progression to AIDS. Also the level of virus in the blood is directly related to the degree of risk of transmission to uninfected individuals. People with undetectable levels of virus in their blood do not transmit to uninfected partners. Mothers with high viral loads have the highest chance of transmitting the virus to their infants.

Arguments against the use of PCR are that this test is characterised by high variability and lack of reproducibility. In addition, the very wide variability may lead to the erroneous interpretation of results, thus compromising the accuracy and validity of the PCR results. It has been pointed out that the PCR viral load test might not be a legitimate measure of infectious virus. It demonstrates a high level of fluctuation, and the viral load can be increased non-specifically by other viral and bacterial infections (opportunistic infections may also increase viral load). Research results indicate that the viral load test may not always be an indicator for the clinical progression of HIV to AIDS.

Another point of concern that has been raised is the fact that the PCR test was developed for the non-C-clade virus, whereas many countries may have the prevalence of the clade-C virus.

CD4 count

The CD4 count is a determination of the concentration of CD4 T-lymphocytes in the blood. The associated immune deficiency leading to infection by opportunistic infections is ascribed mainly to the depletion of CD4 T-cells. The CD4 count can therefore be regarded as an accurate determination of the robustness and functionality of the immune capability and status to effectively protect the body against general infections. HIV infects and destroys CD4 cells (though some dispute this), rendering the immune system incapable of protecting the body against general infections, hence the resultant immunodeficiency in HIV infection and AIDS. This immunological test is used to monitor the progression of HIV infection to clinical AIDS disease and to monitor the effectiveness of anti-retroviral therapy. The CD4 count can be inversely correlated with the viral load. The higher the viral load, the lower the CD4 count will be. Intermediate progressors (patients who take longer than 10 years to progress from HIV infection to AIDS) consistently maintain the concentration of CD4 within normal range. When the CD4 count drops, it predicts the onset of opportunistic infections. In rapid progressors (those who developed AIDS within 2-4 years after infection), the CD4 drops precipitously, coinciding with the onset of infections and clinical progression to AIDS.

The improvement of the concentration of CD4 during anti-retroviral therapy is used as a surrogate marker for the effectiveness of the treatment.

General recommendations on testing

1. The case definition of AIDS should be standardised for clinical practice.
2. Any positive HIV ELISA result to be repeated with at least two additional blood samples before an HIV diagnosis is confirmed in order to improve the reliability and validity of ELISA.
3. Apply a series of HIV tests of increasing stringency in order to establish the validity, veracity, rigour, reliability and concordance of ELISA, PCR and viral isolation.

BOUQUET

Brain Teasers

1. **What is the normal value of CRP in human blood?**
   A. 0.6 mg/dL
   B. 1.2 mg/dL
   C. 1.8 mg/dL
   D. 2.4 mg/dL.

2. **What is the normal value of ASO in human blood?**
   A. Less than 100 IU/mL
   B. Less than 200 IU/mL
   C. Less than 300 IU/mL

3. **Highest titers of antideoxyribonucleoprotein are found in which of the following disease states?**
   A. Chronic hepatitis
   B. Dermatomyositis
   C. SLE
   D. Scleroderma.

4. **With ANA immunofluorescence what kind of ANA pattern can be observed?**
   A. Peripheral (rim or shaggy)
   B. Speckled
   C. Homogeneous (smooth)
   D. Any of the above

ANSWER: 1. A, 2. B, 3. C, 4. D

TROUBLESHOOTING

RAPID HIV TESTING ISSUES AND USAGE

Wait time reduced from days to minutes

Objectives of this article

- To know the capabilities of rapid HIV testing
- To understand the significance of reactive and nonreactive rapid HIV test results
- To recognize the clinical indications for rapid HIV testing

Introduction

Recent breakthroughs in technology have produced tests for HIV antibody that are highly accurate and easy to use and can give a preliminary result in 20 minutes or less. These rapid HIV tests will be used increasingly in labor and delivery wards, emergency departments, urgent care centers, and the primary care office. They have unique applications for healthcare worker exposures, military operations, public health venues, and developing countries. In this article, the advantages and limitations of rapid HIV testing in various settings are presented. Though based on US (CDC) guidelines, the presentation that ensues may not be acceptable as it is in many different nations, as each nation has set its own diagnostic protocols. By and large, the matter presented can not be refuted by most learned authorities. The conventional HIV testing algorithm starts with a sensitive enzyme immunoassay (EIA). The EIA can be performed with serum, plasma, urine, or oral fluid, and the result is typically available after 3 to 4 days. If the EIA is negative, the result is considered definitive, and no further testing is indicated. A limitation is that HIV antibodies can take up to 3 months to develop after infection occurs. During this window period, antibody tests may remain negative. If the EIA is repeatedly positive, more specific testing, using the Western blot technique, is done for confirmation. The testing process--from the time a specimen is submitted until a final result is available--can take a week or longer.

With rapid HIV antibody testing, a preliminary result is available in 10 to 20 minutes, depending on the brand of test used. The result is comparable in clinical significance to a sensitive EIA result. Patients who have nonreactive rapid tests can be counseled that they are negative for HIV, just as with a negative EIA (including the caveat about the window period). Those who have a reactive rapid HIV test must be advised that this is a preliminary result: it could indicate HIV infection or could be falsely reactive. Confirmation with a Western blot test must be performed before a diagnosis of HIV can be made. This confirmation can take 5 to 7 days. Importantly, the EIA lacks the specificity to be a confirmatory test. Good pretest counseling is critical with rapid testing, and the patient needs to understand that a false-positive test is a possibility.

Conventional testing has two advantages over rapid testing. First, by the time a patient learns the HIV test result, it is definitely positive or negative (except for the occasional indeterminate result). Second, in the case of a positive test, the physician usually has the result a day or two in advance and can be better prepared to discuss it with the patient. A disadvantage of conventional testing is that many patients, especially those in transient care and public health settings, fail to return for test results.

Rapid testing has the obvious advantage of a short turnaround time for obtaining negative or preliminary positive results. This time savings can be critical in clinical situations that require prompt initiation of antiretroviral therapy.

When is a rapid test indicated?

Rapid HIV tests may be indicated and used most often in obstetric wards, healthcare worker occupational exposures, urgent care clinics and emergency departments, military medicine, public health settings, developing countries, and the primary care office.

Obstetric wards: In women with untreated HIV infection, mother-to-child transmission occurs in about 25% of pregnancies. Much of this transmission (60% to 70%) is thought to occur during the birthing process. Knowledge of maternal serostatus is the first step in all measures to decrease mother-to-child transmission.

In urban hospitals many women who present to the labor and delivery ward may have unknown HIV status. The patient has either received no prenatal care or received prenatal care but had no record of an HIV test. An added concern is that patients without prenatal care often have risk factors, such as illicit drug use or history of unsafe sex, that puts them at higher risk for HIV infection. These women can now be offered rapid HIV testing. In the event of a reactive test, the woman can be advised of the possibility of HIV infection and started on prophylaxis to prevent mother-to-child transmission. Fortunately, serious short- or medium-term adverse effects of antiretroviral therapy for the mother or neonate have not been detected. Studies of long-term adverse effects are pending. Pretest counseling of women having a rapid HIV test in labor must be thorough. The patient needs to understand that a false-positive test is a possibility and that HIV therapy will likely be initiated on the basis of a preliminary positive test. Post-test counseling for a reactive test is stressful for both patient and physician. The physician should be able to explain, again, the possibility of a falsely reactive test, the need for confirmatory testing, and the rationale for urgent antiretroviral therapy. In the obstetric setting, the physician should use the most specific test possible to minimize false positives.

Healthcare worker occupational exposure: The number of healthcare workers having a documented HIV seroconversion from occupational exposure is rising steadily. Healthcare exposures can be stressful for everybody involved, including the treating physician. Often, an emergency department physician is called on in the middle of the night to make the decision to treat or not to treat. Confirmation of the source patient's HIV status can be crucial. With a rapid HIV test, the source patient's HIV status can be determined within 20 minutes. If the source patient does not have high-risk behavior and the rapid test is negative, the physician, along with the healthcare worker, might elect not to begin prophylactic therapy. With non-rapid EIA, results might not be available for 3 to 4 anxiety-filled days. An emergency department study has showed that rapid HIV testing is cost-effective in treatment of healthcare worker exposures.

Urgent care clinics and emergency departments: Chart reviews have shown that most patients with newly diagnosed HIV or AIDS have had missed opportunities for earlier diagnosis. Often, these missed opportunities occurred in visits to an urgent care clinic or emergency department. The CDC has long recommended routine HIV testing in urgent care clinics and in emergency departments in areas where HIV prevalence is greater than 1%. Persons who access these episodic care settings are at increased risk for HIV infection because of underinsurance, lack of primary care, and acute medical concerns.

Military medicine: In battle, occupational exposures can be extensive and unavoidable for soldiers and medics. The technical simplicity and portability of rapid HIV testing make it convenient for frontline use. A negative rapid test in the source patient could resolve anxiety and prevent unnecessary post-exposure prophylaxis; a reactive test would be an indication for prophylaxis in the exposed person and for confirmatory testing in the source patient. Rapid testing is not approved by the FDA for screening donated blood, but it could be used for screening emergency blood donations in combat.

Public health settings: A huge problem with publicly funded HIV testing is that 30% to 40% of patients do not return for their test result. Groups least likely to return include adolescents and persons tested at a clinic for sexually transmitted disease. As a consequence, valuable HIV testing resources are squandered and opportunities for timely treatment and prevention counseling are lost.

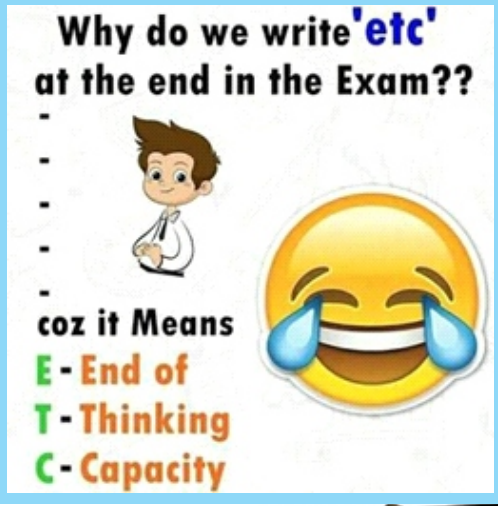
Rapid HIV testing greatly improves the percentage of patients who learn their test result. The average interval between doing the testing and learning the test result is usually about 30 minutes. With this short turnaround time, 99.7% of patients can learn their test result on the spot.

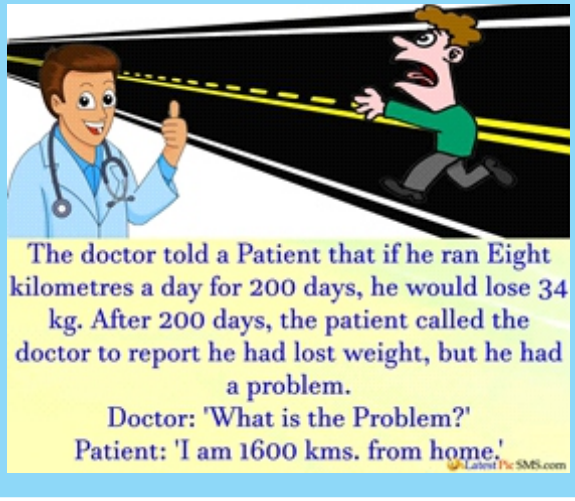
Developing countries: Rapid HIV tests are already used widely in resource-poor countries because the tests are technically simple, accurate, and cost-effective. Also, they can accurately be done by non laboratarians and do not require refrigeration or expensive laboratory equipment. A definitive diagnosis of HIV infection can be achieved by using two or three different rapid HIV tests in combination. These protocols yield sensitivity and specificity equal to those of standard EIA and Western blot methodologies and are recommended by the World Health Organization.

Primary health centers: There are clinical situations where rapid HIV testing makes sense in the primary care setting. A rapid HIV test is recommended for patients with a high probability of not returning (eg, sex workers).

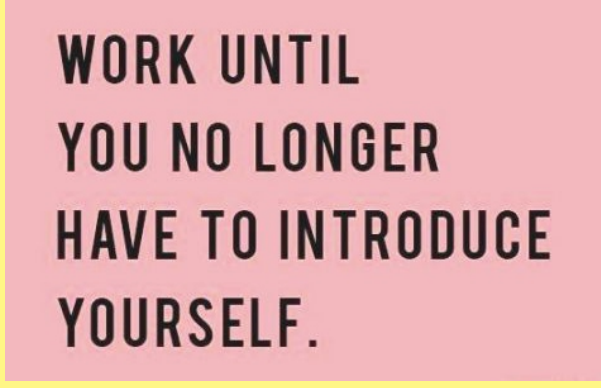
BOUQUET

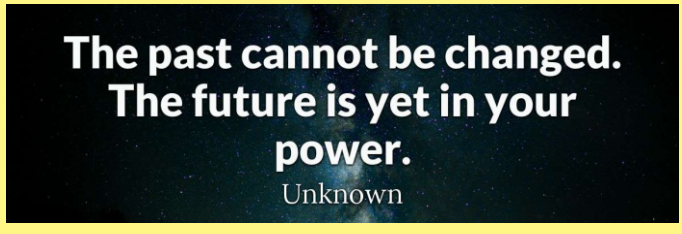
In Lighter Vein



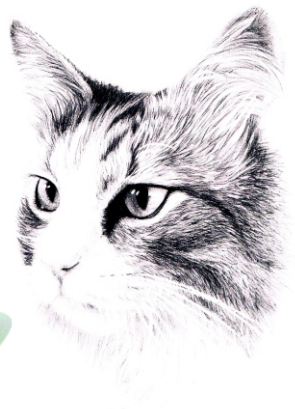


Wisdom Whispers





Expose Toxoplasmosis To PREVENT Congenital Toxoplasmosis



Toxocheck™

- **Employs recombinant surface antigen**
  Immunodominant protein ensures specific detection of antibodies to *T. gondii*
- **Detects IgM & IgG antibodies**
  Helps to differentiate acute and chronic phase of infection
- **Analytical IgG sensitivity : 3 IU/ml**
  Sensitivity equivalent to EIA[4]
- **Excellent correlation with EIA**
  Sensitivity 100 % & Specificity 98.58 %
- **Storage at 4°C - 30°C**
  Suitable for use in most climate conditions
- **Available in 10 Test pack**
  Suitable to use in all laboratories

Toxocheck™ *helps to reduce vertical transmission of toxoplasmosis!*

Printed and published by D.G. Tripathi, Edited by Dr. Ramnik Sood, M.D. (Path.) for and on behalf of Tulip Diagnostics Private Ltd., Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex Post Office, Goa - 403 202, INDIA.
Fax: (0832) 2458544. E-mail: sales@tulipgroup.com Website: www.tulipgroup.com