PATHOGENESIS, DIAGNOSTIC AND MANAGEMENT OF TOXOPLASMOSIS

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ABSTRACT
Toxoplasma gondii is an obligate intracellular parasite of protozoa groups, can infect humans and all warm-blooded animals, are found in almost all locations around the world. Infection generally occurs orally through the consumption of animal products that are not perfectly cooked infected oocyst, parasite containing foods in the form of bradyzoite, contact with cat’s feces containing oocysts or vertical transmission occurring through hematogenous placenta. Toxoplamosis can occur in acute or chronic. It divided into five categories, namely, toxoplasmosis in patients immunocompetent, toxoplasmosis in pregnancy, congenital toxoplasmosis, toxoplasmosis in immunocompromised patients and ocular toxoplasmosis. In each category of clinical manifestations of toxoplasmosis are often non-specific. Methods of diagnosis and interpretation are often different for each category. Toxoplasmosis can be diagnosed through a series of tests such as serology, PCR, histology parasites and parasite isolation. Treatment management of this disease requires a long time. Therapy depends on the category of infections as well as individual therapeutic response. The combination of pyrimethamine with sulfadiazine is the drug choice for toxoplasmosis.

Key words: Toxoplasma gondii, toxoplasmosis diagnostic, toxoplasmosis management, PCR, parasite

INTRODUCTION
Toxoplasmosis is a zoonosis disease causing by Toxoplasma gondii.1,2 Toxoplasma gondii was founded by Nicola and Manceaux in 1908 on lymphatic and liver of Ctenodactylus gondii in Tunisia Africa and in a rabbit in Brazil.3 Toxoplasmosis spread around the worldwide and mostly without symptoms. Generally, infection happen orally from consume animal product that infected oocyst and not cook properly, food that contain parasite like bradyzoite, contact with cat’s feces that contain oocyst or vertical spreading in a hematogen from placenta.4,5,6
Immunocompromised condition such as AIDS object, fertility and tissue transplanted resipien have high risk of Toxoplasma infection. Build this disease diagnostic in clinic and laboratory is very important to determine therapy and prognosis plan. It is depend on the knowledge about epidemiology, pathogenesis and clinical manifestation.

EPIDEMIOLOGY

Toxoplasma gondii almost can found in worldwide and has been infected more than 50% human population in the world.\(^2\)\(^4\) About 10–15% inhabitant in United States shown the positive result in serology check up.\(^7\) Seropositif in HIV-Aids patients estimate about 10–45%.,\(^1\)\(^2\) Checkup result of IgM and IgG anti Toxoplasma in Indonesia, human about 2–63%, cat 35–73%, pig 11–36%, goat 11–61%, dog 75% and the other livestock under 10%.

ETHIOLOGY

Toxoplasma gondii is a parasite obligate intracellular, there are three type, tachyzoite (proliferative form), cyst (contain bradyzoite) and oocyst (contain sporezoite).\(^4\)\(^6\) Tachyzoite form look like sickle moon with pointed point, and the other point about rounded. Length 4–8 micron, width 2–4 micron, has membrane cell and one nucleus in center.

Cyst formed in host cell if tachyzoite who splits have formed a wall. A cyst has varying size, there is a small that only contain some bradyzoite and there is a 200 micron contain about 3000 bradyzoite. Cyst in host body can found in lifetime especially in brain, heart muscle and striated muscle. Constitute rested stage from T. gondii.\(^1\)

Oocyst has the shape ovale, 11–14 9–11 micron. Oocyst has a wall, contain one sporoblast that split into two sporoblast. In the next development, both sporoblast forming wall and being sporocyst. Every sporocyst contain four sporezoite that having size about 82 micron.\(^1\)\(^4\)

LIFE CYCLE AND THE TRANSMISSION WAY

Toxoplasma gondii has two life cycles. Sexual cycle happen on cat as definitive host, while asexual cycle happen in other mamalia (include in human) and various bird strain.\(^1\)\(^2\) This life cycle consist of three forms, tachyzoite and bradyzoite that forming in host mediator and oocyst stage that forming in definitive host epithelial gut cell. Parazite invades erythrocytes then forming microgamete and macrogamete. Zygot or oocyst that produced then come out with feces. Oocyst undergo meiosis outside cat’s body. Oocyst endure for many years in moist condition.\(^7\) Then oocyst consumed by host mediator and forming tachyzoite inside digestion track that causing acute infection.\(^8\)

Acute infection can be cronic if tachyzoite change into bradyzoite. Bradyzoite go into host tissue (brain, heart, muscle and retina) and stay in there for host lifetime in dorman condition.\(^2\)\(^4\) The changes of tachyzoite stage into bradyzoite depend on multiplication speed, pH, area temperature and the existence of anti mitochondria Nitric Oxide (NO) in host body. If human consume meat or drinking water that contaminate with oocyst so bradiizoit or spozoite that resistance with acid pH and enzyme digestive will reach gut, invaded epithelial cell and after several hours change into tachyzoite.\(^4\)

PATHOGENESIS AND IMMUNE RESPONSE

Toxoplasmosis can take an acute or chronic. Acute infection is associated with proliferative forms (tachyzoite), whereas chronic infections associated with tissue cyst forms. During the acute process, tachyzoite invades all cells in the body except host nucleated cells such as red blood cells.\(^4\) Tachyzoite enters the host cell via active penetration into the host plasmalemma or by phagocytosis. Parazites adhere to micronema are able to recognize and target cells, produce enzymes to mature rhoptries parasitophorous vacuoles.\(^5\) In vitro replication of intracellular tachyzoite occur every 6-9 hours. Having collected 64–128 parasites in each cell the parasite will be out to infect neighboring cells. With the host immune system, can turn into a subpopulation tachyzoite bradyzoite.\(^4\)

Macrophages, NK cells, fibroblasts, epithelial cells and endothelial cells become activated by T.gondii infection in the host body, so it can be inhibited parasite proliferation. Non-specific immune response depends on the ability of IL - 12 produced by macrophages and dendritic cells to stimulate NK cells produce IFN - γ. TNF - α also increases the ability of IL - 12 to induce NK cells to produce IFN - γ. IFN - γ inhibit the replication of the parasite because it induces macrophages to release nitric oxide (NO), which kills the parasite. IFN - γ also increases the activity of indoleamine 2,3 dioxygenase that destroys tryptophan which is a substance necessary for the growth of the parasite.\(^9\)

These parasites will induce immunity 4 types of T cells, namely cell-mediated immune response as T.gondii are intracellular parasites.\(^6\) IL - 12 produced by macrophages also strengthen the work of CD4 + cells producing IFN - γ in. CD8 + cells also induces the release of IFN - γ, interferon γ (IFN - γ) plays a role in cyst formation by inhibiting replication in macrophages tachyzoite mice and induce antigen specific for bradyzoite. The humoral immune system has a small role in the fight against toxoplasmosis but is of significant importance in the diagnosis of toxoplasmosis in humans. Antibodies produced by the humoral immune system is able to kill extracellular T.gondii in and through the activities of its complement can inhibit parasite multiplication.\(^6\)
Pathogenesis of toxoplasmosis in the immunocompromised host such as HIV - AIDS patients is influenced by many things, among others, a decrease in CD4 + cell count, the failure of production of IL - 12, IL - 2 and IFN - γ and cytotoxic activity of T - Lymphocyte is declining. Cells infected with the HIV virus to inhibit the formation of IL - 12 and IFN - γ, leaving them vulnerable to infection toxoplasmosis. Levels of IFN - γ usually decrease in patients with AIDS and it could lead to reactivation of chronic toxoplasmosis.4

THE DIAGNOSIS OF TOXOPLASMOSIS

The diagnosis of toxoplasmosis can be established through a series of tests such as serology, polymerase chain reaction (PCR), histological examination of the parasite (imunoperoksidase) and the isolation of the parasite.9

Serology Test

The combination of serology is often necessary to determine whether the patient is really infected or not and to determine the acute or chronic infection lasts. The panel of serological tests or toxoplasma serological profile (TSP) includes sabin - Fieldman dye test (DT), Double sandwich IgM enzyme linked immunosorbsent assay (ELISA), ELISA IgA, IgE ELISA and aglutination test (AC/HS test).9

IgG can be checked by engineering sabin Fieldman DT (Gold standard), indirect fluorescent antibody (IFA) or ELISA. IgG appeared in the first 1–2 weeks of infection and usually can last for years or a lifetime.9 However, in immunocompromised patients IgG levels can not be detected.10 IgG positive indicates that the patient has been exposed by T. gondii but can not indicate whether the newly infected patients or long-term infection.11 IgG avidity has been widely used as additional tests to determine if ongoing infection is acute or chronic. High avidity IgG titer indicates that the infection lasts approximately 4 months earlier while a low titer indicates acute infection.11 12

IgM can be examined by the technique of double sandwich ELISA, IFA and immunosorbsent agglutitation assay (ISAGA). IgM appeared soon after infection and disappears within a few months.11 In some cases IgM can be detected for > 12 years, therefore the serum IgM positive results still need other tests to determine whether the infection is acute or chronic lasted. The sensitivity and specificity of serology varies greatly depending on the lab and the techniques used. A study comparing 6 of tests IgM ELISA found that sensitivity ranged from 93–100 %, and a specificity of 99.1 % 77,5,14

IgA was detected in acute infection in adults and congenital infection. IgA can exist for approximately 1 year. In the examination of congenital toxoplasmosis infection is more sensitive IgA. IgE was detected by ELISA in acute infections in adults and congenital infection and serve as additional tests to identify acute infection.3,13

Tests AC / HS uses two antigen preparations, namely methanol -fixed tachyzoites (AC antigen) indicating acute infection and formalin -fixed tachyzoites (HS antigen) that indicates chronic infection. The ratio of the AC and HS ratio may indicate acute results, equivalence or non-reactive.9

Serologic tests for toxoplasmosis in immunocompromised patients often do not provide a diagnosis for IgG levels in these patients is often low or even undetectable, whereas for the IgM test is often negative. Examination of antigen in the circulation of patients with AIDS have been investigated but have low sensitivity.10,14 A definitive diagnosis can be established if the formation tachyzoite obtained on biopsy results.15

PCR

PCR could detect DNA T. gondii in brain tissue, cerebrospinal fluid, amniotic fluid, aqueous humor and vitreous fluid and Bronchoalveolar Lavage (BAL). In patients with toxoplasmic encephalitis sensitivity of PCR in the CSF of approximately 50-60 %, a specificity of approximately 100 %. PCR on blood samples had a low sensitivity.8

Histology Examination

Immunoperoxidase staining technique can show tachyzoite formation in tissue sections or infected body fluids. Multiple tissue cysts with necrotic inflammation surrounding areas can indicate the presence of an acute infection or reactivation of latent infection. This examination is not routinely performed.2,9

Isolation of T. gondii

A definitive diagnosis of toxoplasmosis can be established by isolation of the parasite from the body fluids (blood, CSF, BAL) or tissue biopsy. This examination is not practical because of the culture of the sample takes approximately 6 months.8

CATEGORIES OF TOXOPLASMOSIS

For clinical purposes, toxoplasmosis is divided into five categories, namely (1) toxoplasmosis in patients immunocompetent, (2) toxoplasmosis in pregnancy, (3) congenital toxoplasmosis, (4) toxoplasmosis in immunocompromised patients, (5) ocular toxoplasmosis.9

1) Toxoplasmosis in Immunocompetent Patients

1. Clinical Manifestation

Only 10–20% of toxoplasmosis in children and adults who have symptoms.2 In immunocompetent patients with toxoplasmosis often without symptoms or only mild symptoms and provide non-specific as fever, enlarged lymph nodes, myalgia, stiff neck, painful swallowing or abdominal pain.6,9

2. Examination Supporting

Examination of IgM and IgG performed for initial evaluation on suspicion of toxoplasmosis. Parallel examination performed 3–4 weeks after the first examination. Results of IgM and IgG were negative excluding the diagnosis of toxoplasmosis. Acute
infection occurs when there is an increase in titer of more than 4-fold compared to titers at baseline examination. Examination of the panel such as Toxoplasma Serological Profile (TSP) or IgG avidity to distinguish whether the infection to occur acute or chronic.9

3. Management

Treatment is not necessary in cases of asymptomatic except in children < 5 years.2 Only immunocompetent patients who have symptoms are treated. Pyrimethamine were given 100 mg loading dose, then 25–50 mg / day in combination with sulfadiazine 2–4 g / day in divided doses 4 times / day for 2–3 weeks or can also be combined with clindamycin 300 mg 4 times / day for 6 weeks. Sulfadiazine and clindamycin can be replaced with azithromycin 500 mg / day or 750 mg atovaquone 2 times / day. Another alternative that can be given is Trimethoprim (TMP) of 10 mg / kg / day, sulfamethoxazole (SMX) 50 mg / kg / day for 4 weeks.7

2) Toxoplasmosis in Immunocompromised Patient

1. Clinical Manifestation

In the immunocompromised host such as patients with AIDS, hematologic malignancies, bone marrow transplant recipients, solid organ transplant (including the heart, liver, liver, kidney), toxoplasmosis can cause encephalitis, meningoencephalitis, myocardiitis, and pneumonitis.6,9,17 The incidence of toxoplasmosis in allogenic transplant recipients was 40%, the mortality rate reaches 60–90%. CNS infections occur in 5–10% of transplant recipients.15,17 Toxoplasmic encephalitis (TE) is the most frequent manifestations in immunocompromised patients.9 In 58–89% of cases occur in sub-acute clinical manifestations in the form of focal neurologic abnormalities, in 15–25% of cases with more severe clinical manifestations of seizures and cerebral hemorrhage. Other clinical manifestations such as loss of consciousness, meningismus, cerebellar signs, neuropsychiatric disorders, dementia, agitation.2 In HIV patients the risk of CNS infection associated with CD4+ < 200 cells / mm3, the number of CD4+ < 200 / mm3 were accompanied by opportunistic infections and malignancies. Regimens used can be given TMP - SMX (trimethoprim - sulfamethoxazole).8 The dose of TMP - SMX is one double strength tablet (DS) (160 mg trimethoprim, 800 mg sulfamethoxazole) 2 times / day (14 DS tablets / week).20

In acute infections may be given a combination of pyrimethamine and sulfadiazine. This regimen is the standard regimen for the treatment of TE. Pyrimethamine initial dose of 200 mg / day next 50-75 mg / day plus sulfadiazine 4–8 g / day for 6 weeks then referred to a lifelong suppressive therapy or to improve the immune system.18 In some of the studies mentioned combination of pyrimethamine - clindamycin and trimethoprim - sulfamethoxazole as effective as the use of a combination of pyrimethamine – sulfadiazine.7 Clindamycin can be given at a dose of 600 mg PO / IV, 4 times / day for 3–6 weeks. The dosage for suppressive therapy 300–450 mg PO every 6–8 hours.2,21 The combination of atovaquone with pyrimethamine or sulfadiazine also provide high effectiveness. These drugs are able to eliminate bradyzoite in experimental animals. Can be administered at a dose of 750 mg (5 mL) PO when eating for 21 days.2,21 In some studies this regimen gives good results on the clinical and radiological picture of 77% within 6 weeks of treatment and recurrence rate of 5% in the maintenance period.8 Maintenance therapy (secondary prophylaxis) can be started after completion of therapy in the acute phase is given, which used the same regimen as in the acute phase but with a half dose.8 Primary prophylaxis can be stopped if the CD4 count after the use of antiretroviral (ARV) increased > 200 / mm3 were settled for approximately 3 months, with an examination of the amount of virus negative.8,22 Secondary prophylaxis was stopped if the patient had
undergone treatment of acute and showed clinical improvement is characterized by loss of the signs and symptoms of toxoplasmosis and improvement of the immune system after treatment with HAART are characterized by increased CD4 + > 200 / mm³ were settled for for about 6 months.8,22

### 3) Congenital Toxoplasmosis

#### 1. Clinical Manifestation

Cases of congenital toxoplasmosis have been reported in Indonesia. Lazuardi et al (1989) reported *T.gondii* antibodies in 44.6% of children with mental retardation, 44.6 % in children with ocular lesions and 9.5% in children with common symptoms.1 The risk and severity of congenital toxoplasmosis symptoms more severe if infection occurs early in pregnancy.23 Classic triad of congenital toxoplasmosis is chorioretinitis, hydrocephalus, and intracranial calcification. The involvement of neurological and ocular systems often arise later if not found at the time of birth. Seizures, mental retardation, and rigidity is the common sequelae.2

#### 2. Examination Improving

IgM positive is strong evidence of congenital infection, but a negative IgM does not exclude the diagnosis. Serum IgA is more sensitive for detecting congenital toxoplasmosis than IgM.9 When symptoms and serological evidence of toxoplasmosis is detected during pregnancy, infection of the fetus can already be enforced by IgM detection and isolation of parasites from fetal blood or amniotic fluid at 18 weeks of gestation. Examination before 20 weeks gestation is difficult to enforce because of the immunological response of the fetus is still low. PCR on amniotic fluid can more accurately diagnose infection in the fetus before 20 weeks gestation.9 The sensitivity of this test is 64% with a negative predictive value of 87.8%, specificity and positive predictive value of 100%.9 Antenatal Ultrasound can identify abnormalities in the fetus is infected. Approximately 36% of fetuses with abnormalities can be identified. Abnormalities that can be found are bilaterally symmetrical ventricular dilatation, intracranial calcification, increased placental thickness, hepatomegaly and ascites.9

#### 3. Management

In newborns with toxoplasmosis, can be given a combination of pyrimethamine 1 mg / kg per day for 2 months followed by 1 mg / kg every 2 days for 10 months, sulfadiazine 50 mg / kg body weight per day, as well as folic acid 5–10 mg 3 times week to prevent the side effects of pyrimethamine2. In addition to the provision of drugs are also required regular follow-up. A complete blood count 1–2 times per week to daily dosing of pyriemetamine and 1–2 times per month for the dosing of pyrimethamine performed every 2 days to monitor the toxic effects of the drug. Also required a complete pediatric examination, including ophthalmologic examination every 3 months until the age of 18 months and then once a year, as well as neurological examination every 3–6 months to 1 year of age.2

### 4) Ocular Toxoplasmosis

#### 1. Clinical Manifestation

Toxoplasmic chorioretinitis can occur because of congenital or postnatally acquired infection. Infection occurs in 2/1000 pregnancies America, with an average of transplacental infection 150%.25 Seventy percent of infants with congenital infection showed a scar on korioretina.24 Symptoms include blurred vision, scotoma, fotofobi and pain. Of ophthalmology examination obtained focal necrotizing retinitis formation that resembles a yellowish white cotton, with unclear boundaries. In congenital infection are often bilateral lesions in infections acquired while generally unilateral.2

#### 2. Examination Improving

Serologic tests are often unhelpful because the diagnosis is often obtained with the IgG titers were low, often undetectable IgM. Increased levels of IgG 4 times the initial levels within 4 weeks showed primary infection. Other tests that can be done is the amplification of parasite DNA from the aqueous or vitreous humor.9

#### 3. Management

Treatment depends on several factors such as the location of lesions, degree of inflammation, the threat of blindness and immune status of the patient. If the infection is not on the optic disc and macula is only accompanied by mild inflammation, treatment is not required.10 Pyrimethamine most effective for this infection, given the loading dose of 25 mg 3 times / day followed by 25 mg / day. This drug should be combined with sulfadiazine with further loading dose of 2 g 1 g 4 times / day. Therapy is done for 6–12 weeks. Treatment response was indicated by the disappearance of a yellowish white cotton, with clear and atrophic scars korioretina being demarcated. Another drug option is clindamycin 300 mg 3–4 times / day for 3–4 weeks, then 150 mg four times / day for the next 3–4 weeks. Spiramycin is the drug most commonly used and has the least amount of side effects among other drug options, can be administered in a dose of 1 g 2 times / day.11

### 5) Toxoplasmosis in Pregnancy

#### 1. Clinical Manifestation

Most pregnant women with acute acquired infection do not experience specific symptoms. Some have symptoms of malaise, subfebris, lymphadenopathy. The frequency of vertical transmission to the fetus increased with increasing gestational age.25

#### 2. Examination Improving

Examination of IgG and IgM should ideally be done in the first trimester of pregnancy. Serum IgG and IgM
negative by showing that pregnant women not infected, face further investigation performed during pregnancy to anticipate the occurrence of seroconversion. On the positive results of IgG but negative IgM in pregnancy < 18 weeks showed an infection occurred in the past, while in gestation > 18 weeks of this result is difficult to interpret whether the infection is acute or chronic lasted so avidity required examination. In the results were negative but IgG positive IgM examination should be repeated in 1–3 weeks later, if the result remains the same mean positive IgG has no clinical significance, whereas in case of seroconversion of IgG becomes positive which indicates that the infection occurs during pregnancy so that the fetus is at high risk affected by congenital toxoplasmosis. On examination of the IgG and IgM positive follow-up examination to confirm acute or chronic infections such indispensable avidity test. High avidity IgG indicates that infection occurred > 16 weeks in advance, so that the examination in the first trimester of pregnancy showed an infection occurs before conception reduces the risk of transmission and the risk of fetal defects is low.

3. Management

Spiramycin is Drug Of Choice for maternal toxoplasmosis. Dose of 3 g / day PO in divided doses 24 times / day for 3 weeks, stopped for 2 weeks and then repeated the cycle of 5 weekly during pregnancy. If PCR positive amniotic fluid regimen should be replaced with pyrimethamine 50 mg / day and sulfadiazine 3 g / day in 2–3 divided doses for 3 weeks interspersed with the provision of spiramycin 1 g 3 times / day for 3 weeks or can be given pyrimethamine 25 mg / day and sulfadiazine 4 g / day in divided doses 2-4 times / day was given until delivery.

PREVENTION

Prevention of toxoplasmosis can be made by cooking the meat until done, wash your hands thoroughly after handling raw meat, wash vegetables and fruits before eating, wash clean kitchen equipment after use, pregnant women should wear gloves when gardening and wash hands afterwards, avoid contact with cat feaces, the primary and secondary prophylaxis should be administered to patients with AIDS.

PROGNOSIS

In immunocompromised patients reactivation of chronic toxoplasmosis are common. Suppressive therapy and improving the immune system may reduce the risk of recurrent infection. Infants with ocular toxoplasmosis acquired have a good prognosis and in the next four years have the same development as uninfected infants. Immunocompetent patients have a good prognosis, lymphadenopathy and other symptoms disappear within a few weeks after infection.

SUMMARY

Methods of diagnosis and interpretation are often different for each category. The diagnosis of toxoplasmosis can be established through a series of tests such as serology, PCR, histology parasites and parasite isolation. Management the treatment of this disease requires a long time. Therapy depends on the category of infections as well as individual therapeutic response. The combination of pyrimethamine with sulfadiazine is the drug of choice for toxoplasmosis.

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