A HUMAN IMMUNODEFICIENCY VIRUS INFECTED *TOXOPLASMA* SERONEGATIVE PATIENT WITH PRESUMED TOXOPLASMIC ENCEPHALITIS: A CASE REPORT AND LITERATURE REVIEW

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Abstract. Human Immunodeficiency Virus (HIV) infected patient with toxoplasmic encephalitis usually have positive serology for *Toxoplasma*, but some cases with negative serology occur. A 39-year-old man presented to the hospital with a 1-week history of headache, nausea, and vomiting, and a 6-month history of 15 kg weight loss. Magnetic resonance imaging (MRI) of the brain showed multiple ring-enhancing lesions with perifocal edema consistent with the appearance of toxoplasmosis of the brain. His HIV test was positive, but his serology of *Toxoplasma* was negative. He was treated for toxoplasmosis of the brain, and a follow-up MRI showed a marked decrease in the size of the lesions. However, after one month of treatment, the patient developed headache and a repeated MRI of the brain showed enlarging lesion with increased perifocal edema. Repeat testing of *Toxoplasma* serology was again negative. The patient was continued on treatment for toxoplasmosis of the brain, and by 5 months of treatment, a repeat MRI of the brain showed complete resolution of the lesion. The patient's course suggests he had seronegative toxoplasmosis of the brain. Immunosuppressed patients with symptoms and MRI findings consistent with toxoplasmosis of the brain should be treated as such in spite of *Toxoplasma* serology result. Further studies are needed to determine the course of this phenomenon.

Keywords: case report, HIV, toxoplasmic encephalitis, *Toxoplasma gondii*, seronegative

INTRODUCTION

The protozoan parasite *Toxoplasma gondii* is widely distributed and approximately 25-30% of the human population is infected with *T. gondii* (Montoya and Liesenfeld, 2004). The clinical manifestation of *Toxoplasma* infection varies based on the immune status; being an asymptomatic in most of immunocompetent patients to mononucleosis-like syndrome in some immunocompetent patients or

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severe toxoplasmosis in immunodeficient patients (Robert-Gangneux and Dardé, 2012). Although the incidence of toxoplasmic encephalitis (TE) among human immunodeficiency virus (HIV)-infected patients has decreased due to the use of highly active anti-retroviral therapy (HAART) and the broad use of trimethoprim-sulfamethoxazole prophylaxis, TE is still the most important opportunistic disease of the central nervous system of HIV-infected patients (Luft and Remington, 1992; Abgrall *et al*, 2001; Bonnet *et al*, 2005). Among HIV-infected patients who develop toxoplasmosis, 80% have CD4 cell counts <100 cells/μl (median range: 25-50 cells/μl) (Porter and Sande, 1992; Renold *et al*, 1992; Luft *et al*, 1993; Nissapatorn *et al*, 2004). Nearly all HIV-infected patients with TE are seropositive for anti-*Toxoplasma* immunoglobulin G (IgG) antibodies (Raffi *et al*, 1997; Rosenow and Hirschfeld, 2007). *Toxoplasma*-seronegative testing among patients with TE is uncommon, but cases do occur (Raffi *et al*, 1997; Rosenow and Hirschfeld, 2007). However, there are no reports on the management of *Toxoplasma*-seronegative TE patients. We report here a case of *Toxoplasma*-seronegative TE in a patient as his initial presentation with acquired immunodeficiency syndrome (AIDS) and discuss the challenges faced while treating this patient.

CASE REPORT

A 39-year-old man presented to the hospital with a 1-week history of headache, nausea, and vomiting. He also had a 6-month history of 15 kg weight loss. He denied any limb weakness or unstable gait. On history taking, he denied contacting with cats or recent consumption of undercooked or raw meat or seafood. He was initially admitted to the neurosurgery ward with an initial diagnosis of increased intracranial pressure (IICP) due to unknown etiology.

His initial vital signs were as follows: a blood pressure of 156/124 mmHg, a pulse of 71 bpm, a respiratory rate of 18 rpm, and a temperature of 35.6°C. His initial Glasgow Coma Scale score was 15. No other sign of decreased muscle power was noted. On laboratory examination, his white cell count was 5.78×10^9/l with 68% of granulocytes, 17.3% of lymphocytes, and 8% of monocytes. His hemoglobin concentration was 17.8 g/dl, the mean corpuscular volume was 87.2 fl, and the platelet count was 150,000/mm^3. Magnetic resonance imaging (MRI) on the day of admission showed multiple enhancing lesions with marked perifocal edema in the basal ganglia, corticomedullary junction and posterior fossa, resulting in a mass effect (Fig 1A).

A lumbar puncture was not performed because of the space-occupying lesion with mass effect in the brain. An HIV test was performed and it was positive. An HIV viral load test (Cobas Amplicor HIV-1 Monitor Test™ version 1.5, Roche Diagnostics, Indianapolis, IN) was performed and it was 115,000 copies/ml. His CD4 count was also measured (FACSFlow, Becton Dickinson, Immunocytometry Systems, Oxford, United Kingdom), and was found to be only 13/μl. Tests for hepatitis B surface antigen, hepatitis C antibodies, and serum Cryptococcus antigen were all negative. Testing for *Cytomegalovirus* IgG was positive. Testings for anti-*Toxoplasma* IgG and IgM antibodies (Architect Toxo IgG and IgM assays, Abbott, Wiesbaden, Germany) were negative.

Although the patient was *Toxoplasma*-seronegative, he was empirically treated

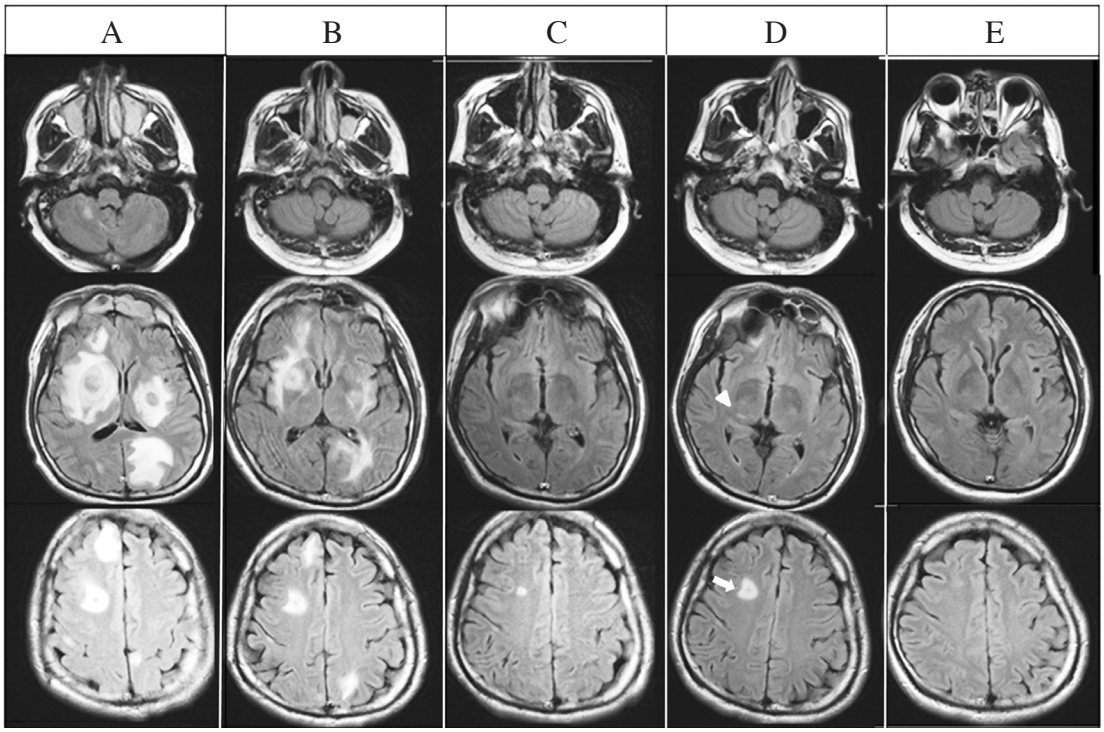

Fig 1–MRI of the brain in the study patient. **A**. MRI on admission revealing multiple enhancing lesions with marked perifocal edema in the right frontal lobe, bilateral parietal lobes, bilateral temporal lobes, bilateral occipital lobes, bilateral basal ganglia, right thalamus, and bilateral cerebellum. **B-C.** MRI showing improvement in mass lesions with empiric anti-*Toxoplasma* treatment. **D.** MRI taken when the patient had a worsening headache during treatment showing mild enlargement of the mass lesions with perifocal edema over the right hemisphere (arrows) and right basal ganglia (arrowhead). **E.** MRI taken after 5 months of anti-*Toxoplasma* therapy showing nearly complete resolution of the lesion and edema.

for TE with anti-*Toxoplasma* agents because the MRI findings were more consistent with TE than with primary central nervous system lymphoma (PCNSL). Since PCNSL could not be completely ruled out based on brain MRI findings alone, we closely monitored the therapeutic response and avoided steroid use so as not to mask the potential diagnosis of PCNSL. Because sulfadiazine is not widely available in Taiwan, we treated the patient with pyrimethamine 200 mg once followed by 50 mg daily, clindamycin 600 mg every 6 hours and leucovorin 30 mg daily (Table 1) starting on the 3rd day of admission. The patient's symptoms of headache, nausea and vomiting greatly improved by day 3 of treatment. A repeat MRI of the brain on the 15th day of admission revealed a marked decrease in the size of the lesions and the perifocal edema (Fig 1B). The patient was discharged home from the hospital three weeks later and was followed up as an outpatient.

Owing to intolerance of adverse reactions (nausea and upset stomach) and the high pill burden of clindamycin, his treatment was changed to pyrimethamine 50

Table 1
Medication treatment regimen in study patient.

	Highly active antiretroviral therapy		
Duration	7 days	To the present	
Regimens	Kivexa 1 tablet daily plus efavirenz 1 tablet daily	Atripla 1 tablet daily	
	Anti-*Toxoplasma* regimens		
Duration (days)	32	116	154
Regimens	Pyrimethamine 25 mg, 2 tablets once daily Leucovorin 15 mg, 2 tablets once daily Clindamycin 150 mg, 4 tablets every 6 hours	Pyrimethamine 25 mg 2 tablets once daily Leucovorin 15 mg, 2 tablets once daily TMP/SMX 80/400 mg 5 tablets every 12 hours	TMP/SMX 80/400 mg 2 tablets every 12 hours

HAART, highly active antiretroviral therapy; TMP-SMX, trimethoprim-sulfamethoxazole.

mg, daily trimethoprim-sulfamethoxazole (TMP-SMX) 80/400 mg, and leucovorin 30 mg daily (Table 1). A repeat MRI of the brain showed further resolution of mass lesions and perifocal edema (Fig 1C). However, the patient developed headache again 2 months later, and a repeat MRI of the brain was performed (Fig 1D) showing enlargement of the mass lesions with increased perifocal edema in the right basal ganglia and right hemisphere. Repeat testing for anti-*Toxoplasma* IgG and IgM antibodies remained negative. We explained the uncertainty of the diagnosis to the patient and discussed performing stereotactic brain biopsy for differentiation from other focal neurological disorders, such as PCNSL or paradoxical immune reconstitution inflammatory syndrome of TE, or treatment failure, but the patient refused. The patient continued the same treatment regimen and the headache gradually resolved. A repeat MRI of the brain performed 6 weeks later (Fig 1E) showed complete resolution of mass lesions. An additional 5-month course of maintenance therapy for TE with TMP/SMX was then prescribed (Table 1). At the time of writing the report, the patient is currently physically and neurologically normal.

The patient was concomitantly treated with HAART for his HIV using Kivexa (abacavir and lamivudine) plus efavirenz beginning on the 17th day of admission. This regimen was changed to Atripla (efavirenz, emtricitabine, and tenofovir) to reduce the pill burden and improve compliance on the 23th day of admission (Table 1). The immunological and virological responses to the HAART treatment are shown in Table 2.

DISCUSSION

Toxoplasma-seronegative TE is unusual, but not rare, in HIV-infected patients (Porter and Sande, 1992). The recommendations for TE management in HIV-infected patients are based on studies among *Toxoplasma*-seropositive patients (Porter and Sande, 1992; Derouin *et al*, 1996). Whether these recommendations can be applied to *Toxoplasma*-seronegative TE patients is unclear.

Table 2
CD4 and HIV viral loads in study patient during toxoplasmosis encephalitis treatment.

Testing series	CD4 count and HIV viral loads	
	CD4 count (/μl)	Viral load (copies/ml)
1st test	13	115,000
2nd test	23	332
3rd test	130	760
4th test	129	<40
5th test	176	<40
6th test	286	<40
7th test	306	<40
8th test	201	<40
9th test	273	<40

HIV, human immunodeficiency virus.

There are several challenges to treat such patients. First is the diagnosis of toxoplasmosis. Definitive diagnosis requires demonstration of tachyzoites in stained tissue sections or cysts containing *T. gondii* surrounded by an inflammatory reaction (Porter and Sande, 1992). However, in clinical practice, most patients with multiple ring-enhancing lesions who are seropositive for *Toxoplasma* species are treated empirically (Mamidi *et al*, 2002). The diagnosis of TE can be supported by a rapid therapeutic response to an anti-*Toxoplasma* regimen (Porter and Sande, 1992). In seronegative cases, the absence of anti-*Toxoplasma* IgG antibodies makes diagnosis of toxoplasmosis difficult (Raffi *et al*, 1997; Rosenow and Hirschfeld, 2007). Other methods are needed to support the presumptive diagnosis of *Toxoplasma*-seronegative TE, especially when treating an HIV-infected patient with a central nervous system mass lesion. Although Cytomegalovirus-related encephalitis and white matter disease, such as AIDS dementia complex and progressive multifocal leukoencephalopathy, can be differentiated from TE and PCNSL on imaging (Skiest, 2002), differentiation of TE from PCNSL based on imaging alone is not always easy. Radiographic findings of TE are characterized by the presence of multiple ring-enhancing lesions with surrounding edema in the basal ganglia and at the corticomedullary junction, but considerable overlap in appearance with PCNSL exists (Ammassari *et al*, 1996; Miller *et al*, 1998). The thallium index of each lesion, measured as the ratio of the mean uptake in the lesion to that of the corresponding contralateral side, may help differentiate between the two diseases (Young *et al*, 2005). However, this technique is not widely available. PCR testing to detect *T. gondii* DNA in body fluids may also be helpful, but the sensitivity is not high (Cinque *et al*, 1997), the test is not widely available, and lumbar puncture is usually contraindicated in patients with a space-occupying lesion with mass effect in the brain. Because of these difficulties, a good response to 2-week empiric anti-*Toxoplasma* treatment is often used to support the diagnosis of

TE (Porter and Sande, 1992). However, it is unclear whether response to treatment differs between *Toxoplasma*-seronegative and seropositive patients. This study patient improved clinically within 3 days and radiologically within 12 days with anti-*Toxoplasma* therapy. A case series of *Toxoplasma*-seronegative patients needs to be conducted to determine if this rapid response is to be expected in similar seronegative patients.

The rates of serum *Toxoplasma* IgG seronegativity among patients with TE ranges from 0% to 16% (Luft *et al*, 1984; Porter and Sande, 1992; Raffi *et al*, 1997; Nissapatorn *et al*, 2004). There are several possible explanations for this seronegativity. First, a recent infection may have occurred. However, most cases of TE in AIDS patients result from reactivation of a latent infection rather than a newly acquired infection (Robert-Gangneux and Dardé, 2012). Additionally, no anti-*Toxoplasma* IgM antibodies were found in this patient, suggesting the infection was probably not acute. Second, the serological results may vary based on the method used. Currently available serological methods to diagnose toxoplasmosis include a modified agglutination test, an enzyme-linked immunosorbent assay, an indirect fluorescent antibody test, a chemiluminescent microparticle immunoassay (CMIA), and a dye test; each having different reported sensitivities and specificities (Shaapan *et al*, 2008; Sickinger *et al*, 2008). In this case, we used a CMIA (ARCHITECT Toxo IgG and IgM, Abbott), which is designed for quantitative determination of *T. gondii*-specific IgG antibodies reactive to *T. gondii* recombinant antigen P30 (SAG1) and P35 (GRA8) coated microparticles (Sickinger *et al*, 2008). The reported specificity and sensitivity for this assay are 99.6% and 99.7%, respectively (Sickinger *et al*, 2008). We tested for the patient's serum anti-*Toxoplasma* IgG at presentation and at recovery of CD4 count and both results (1.1 and 0.6 IU/ml, respectively) were negative. Although we were unable to confirm our results using a different method because of depleted sample, the discordant result should be very low (Gay-Andrieu *et al*, 2009). Third, because of gradual impairment of immune status, antibody production tends to be poor in immunocompromised individuals and *Toxoplasma*-specific antibody titers may fail to rise at the time of diagnosis (Derouin *et al*, 1996; Mechain *et al*, 2000). HIV-infected patients, often have lower anti-*Toxoplasma* IgG titers making disease phase definition and therapeutic decisions difficult (Spausta *et al*, 2003).

The diagnosis of TE in our subject may be questionable because the recovery of CD4 counts under HAART did not result in seroconversion. Although initiation of HAART can restore all CD4 T-cell subsets and most CD8 T-cell subsets, this is not true for all B-cell subsets (Shaapan *et al*, 2008; Gay-Andrieu *et al*, 2009). Only naïve, not memory B-cell subsets increase on HAART (Furco *et al*, 2008; Machala *et al*, 2009), possibly explaining why improvement of the CD4 T-cell counts in our patient from 13/μl to 176/μl did not result in positive serology. Moreover, a high initial anti-*Toxoplasma* IgG titer is associated with a lymphocyte proliferative response to *Toxoplasma* antigen, which is associated with a lower chance of TE relapse after anti-*Toxoplasma* therapy (Fournier *et al*, 2001; Furco *et al*, 2008). Thus, *Toxoplasm*-seronegative patients may require chronic maintenance therapy to prevent TE relapse.

Although the present case is limited by a lack of pathological evidence for a diagnosis of TE, characteristic findings

on brain MRI supplemented with a rapid therapeutic response to anti-*Toxoplasma* therapy support the diagnosis of TE. After a 5-month course of anti-*Toxoplasma* therapy, the MRI of the brain showed nearly complete resolution of mass lesions. The patient remained disease-free for an additional 5-month course of chronic maintenance therapy. However, the speed of response to anti-*Toxoplasma* therapy and the duration of treatment in seronegative TE patients to prevent relapse of TE should be investigated in further studies.

In conclusion, a negative serological result does not exclude the diagnosis of TE and should not be allowed to cause a delay in empiric therapy of TE in AIDS patients with findings consistent with TE. However, the diagnosis and treatment of *Toxoplasma*-seronegative TE is challenging among HIV-infected patients. While the current recommendations for TE management of HIV-infected patients seem appropriate for *Toxoplasma*-seronegative patients, further studies are needed among similar patients to determine if this hold true.

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