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Detection of *Toxoplasma gondii* in AIDS Patients by the Polymerase Chain Reaction

**Summary:** In recent years, toxoplasmosis has become one of the most frequent and life-threatening opportunistic infections in AIDS patients. Despite strict clinical follow-up and repeated biological examinations, its diagnosis remains difficult to establish in the context of immunodeficiency because of the poor predictive value of serology. The aim of the study was to compare standard methods of diagnosis with the polymerase chain reaction (PCR), in an attempt to investigate the potential usefulness of PCR in the diagnosis of toxoplasmosis. Twelve biological samples (cerebrospinal fluid, bronchoalveolar lavage fluid, one brain biopsy and one liver biopsy) from 11 unselected AIDS patients were tested by PCR. The results showed good correlation (for eight out of 11 patients) between classical methods and PCR, and confirm the value of bronchoalveolar lavage for the diagnosis of toxoplasmosis in AIDS patients. The pathophysiological significance of the presence of *Toxoplasma* in samples tested is discussed.


**Introduction**

The coccidian protozoan *Toxoplasma gondii* is the etiological agent of toxoplasmosis, an infection that occurs worldwide and is historically known for the congenital malformations it causes. In AIDS patients, reactivation of latent toxoplasmic cysts can result in fatal encephalitis as well as disseminated forms of toxoplasmosis. Twenty to 30% of AIDS patients with positive toxoplasma serology will develop a cerebral toxoplasmosis [1]. In these cases, the diagnosis is supported mainly by clinical evidence, e.g. central neurological signs, epilepsy, and computed tomography (CT) scan abnormalities. The diagnosis of disseminated forms (such as lung localization) is, however, more difficult, due to problems with interpretation of serological tests and the absence of specific clinical signs. Direct parasitological diagnosis is necessary, and time-consuming techniques such as *in vitro* and *in vivo* culture are appropriate. More recently, PCR has been used to diagnose congenital toxoplasmosis [2,3] and *Toxoplasma* infection in immunocompromized patients [4]. We are currently investigating the respective contributions of the classic techniques and PCR for the diagnosis of toxoplasmosis in AIDS patients.

**Patients and Methods**

**Patients:** The patient population consisted of 11 unselected AIDS patients clinically surveyed in the Department of Infectious Diseases at the Albert Michallon Hospital in Grenoble, France. For some of these patients, clinical and radiological features and the evolution of illness under specific treatment were not sufficient to make a clear diagnosis. Thus samples were taken for biological examination.

**Samples:** Four cerebrospinal fluid (CSF) samples, six bronchoalveolar lavage (BAL) specimens, one liver biopsy and one brain biopsy were submitted to the Department of Parasitology-Mycology for routine examination and *in vitro* culture of *T. gondii*. At the same time, corresponding sera were tested by classical serological techniques (indirect immunofluorescence assay, enzyme linked immunosorbent assay, immunosorbent agglutination assay). PCR assay was then carried out. For safety reasons, all samples from AIDS patients were incubated at 60°C for 2 h in order to inactivate HIV before PCR treatment.

**Preparation of samples for PCR:** For CSF and BAL specimens, 10–20 μl of the previously inactivated sample was added to one volume of a lysis buffer (KCl 50 mM, TRIS-HCl pH 8.3 10 mM,
MgCl₂ 2.5 mM, gelatin 0.1 mg/ml, Nonidet p40 0.45%, Tween 20 0.45%) [5] and proteinase K, to achieve a 100 µg/ml final concentration. Lysis was performed at 50°C for 2 h and proteinase K was inactivated by incubation at 95°C for 10 min. Ten microliters of this crude lysate were used directly for PCR assays. For the two biopsies, tissue fragments were overlaided with the lysis buffer in an Eppendorf tube, and then finely ground under sterile conditions. Proteinase K was added to give the resulting suspension a 200 µg/ml final concentration. This suspension was then incubated for 2 h at 50°C, and centrifuged for 10 min. at 10,000 x g, to eliminate the non-soluble residues. Ten microliters of the clarified supernatant were used directly for amplification.

In vitro cultures: In vitro cultures were performed using MCR5 embryonic fibroblasts (bioMérieux, Marcy l’Etoile, France). Briefly, after centrifugation (10 min. at 3,000 x g) (CSF, BAL samples) or grinding (biopsies), samples were inoculated into the culture in MEM medium (bioMérieux). The presence or absence of Toxoplasma was revealed after six days of culture by immunofluorescence. To date, this is the most sensitive and the least time consuming technique available for detecting the presence of living T. gondii tachyzoites in clinical samples [6,7].

PCR methods: Ten microliters of each sample were mixed in a 50 µl final volume (KCl 50 mM, TRIS-HCl pH 8.3 8.5 mM, MgCl₂ 1.5 mM, gelatin 0.1%) with each dNTP at 800 µM and each primer at 0.5 µM. Samples were overlaided with 50 µl mineral oil, and then incubated for 7 min. at 95°C. The PCR cycles began after addition of 1.25 units of Taq polymerase per tube. After 30 cycles (1 min. at 94°C, 2 min. at 55°C, 3 min. at 72°C), the amplified products were analysed by gel electrophoresis, transferred onto nylon membranes by Southern-blotting, and then hybridized with a specific radio-labelled oligonucleotide. The two PCR primers corresponded to nucleotides 91–112 and 264–286 of the TGR1E sequence, and the oligonucleotide probe corresponded to positions 192–214 [8]. This probe has revealed all the strains previously studied.

Results
The PCR test results for the 11 AIDS patients are presented in Figure 1; a comparison of clinical and biological results is shown in Table 1. Serology for Toxoplasma was considered negative when no specific IgG or IgM was detected by any of the techniques employed.

Discussion

We attempted to compare the PCR technique with the classic biological and clinical examinations usually used to diagnose toxoplasmosis. Concordance between PCR and the classical methods is observed in 8/11 patients, but some cases require comment.

In our patient series neither classical diagnostic methods nor the PCR technique gave positive results when serology was negative. This result is not surprising: toxoplasmosis in N. diagnose toxoplasmosis. Concordance between PCR and classic biological and clinical examinations usually used to the classical methods is observed in 8/11 patients, but nor the PCR technique gave positive results when serology In our patient series neither classical diagnostic methods some cases require comment.

was suspected but not confirmed by the biological tests. The presence of toxoplasmic DNA when parasites are not detected in culture can be explained by the non viability of the tachyzoites. In fact, in addition to being given para...toxoplasmosis in AIDS patients [9]. Toxoplasmosis can occur in patients with negative serology, but only in the very rare cases of primary toxoplasmic infection [10].

Of the seven patients for whom the diagnosis of toxoplasmosis was not confirmed, only in one case (patient no. 2) was the PCR result positive. Unfortunately, in vitro culture was not available to confirm or invalidate this result. Nevertheless, we speculate that tachyzoites were present in this patient’s BAL specimen. Pneumopathy was observed in this patient and Pneumocystis carinii infection was suspected but not confirmed by the biological tests. Despite the lack of a confirmed diagnosis trimethoprim-sulfamethoxazole treatment was administered, which cured the pneumopathy. Since this treatment is known to act not only against P. carinii but also against T. gondii, the pneumopathy could have been caused by a toxoplasmic reactivation with a parasitic dissemination [11,12].

Of the four cases of proven toxoplasmosis, two were confirmed by PCR results. For patient no. 11 concordance was observed between the in vitro culture and PCR; the post-mortem biopsy, performed after acute parasitic lep...is detected in culture can be explained by the non viability of the tachyzoites. In fact, in addition to being given pyr...tachyzoites by PCR (such as in BAL samples or liver biopsy) confirm the parasite as the cause of clinical symptoms [21–23] since the parasite is not present in these samples in healthy individuals positive for Toxoplasma. The presence of tachyzoites in such samples provides evidence that toxoplasmosis in AIDS patients is due to parasitic dissemination with occurrence of parasitemia. Thus, PCR, together with the routine tests, may be a supplemental test in some especially difficult cases. We therefore propose that further investigations be carried out in order to determine the value of BAL and blood analysis for diagnosis of disseminated toxoplasmosis.

References

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