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Detection of *Toxoplasma gondii* by PCR and Tissue Culture in Cerebrospinal Fluid and Blood of Human Immunodeficiency Virus-Seropositive Patients

MICHEL DUPON,[1]* JEAN CAZENAVE,[2] JEAN-LUC PELLEGRIN,[3] JEAN-MARIE RAGNAUD,[1] ALAIN CHEYROU,[2] ISABELLE FISCHER,[2] BERNARD LENG,[3] AND JEAN-YVES LACUT[1]

Services de Médecine Interne et Maladies Infectieuses, Hôpital Pellegrin,[1] Hôpital du Haut-Lévêque,[3] and Centre Hospitalier Universitaire de Bordeaux, and Laboratoire d'Analyses Biologiques Médicales Ruffié et Associés,[2] Bordeaux, France

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To investigate whether both tissue culture and PCR on a sequence from the repetitive rDNA could contribute to the diagnosis of toxoplasmosis, blood samples and, if they were available, cerebrospinal fluid (CSF) and aqueous humor samples from 72 human immunodeficiency virus-seropositive patients with suspected toxoplasmosis were prospectively tested. For 10 patients with fever of unknown origin but without confirmed toxoplasmosis, no *Toxoplasma gondii* was detected. For two patients with confirmed toxoplasmic uveitis, only PCR of aqueous humor samples was positive. Of 60 patients (48 with CSF samples) with neurological signs, 25 (from 13 of whom CSF samples were available) had confirmed cerebral toxoplasmosis and 10 had a positive PCR of CSF and/or blood samples, while for 1 patient culture of the CSF sample was also positive. Unlike tissue culture, PCR of rDNA is of value for the detection of cerebral toxoplasmosis in human immunodeficiency virus-seropositive patients, provided that both CSF and blood samples are available (sensitivity, 76.9%; specificity, 100%).

Among the opportunistic infections of human immunodeficiency virus (HIV)-infected individuals, toxoplasmosis encephalitis (TE) is the leading cause of focal brain disease, despite chemoprophylaxis. The diagnosis of TE is difficult since no sensitive and specific noninvasive diagnostic test is available (41). The practice of presumptive therapy for HIV-infected patients with clinical signs (referable to central nervous system [CNS] and/or fever) with characteristic findings on computerized axial tomography (CAT) scan or magnetic resonance imaging (MRI) and a positive serology for *Toxoplasma gondii* is widely accepted because of a high predictive value (except in intravenous drug users, in whom other processes are prevalent in the CNS) (43). Clinical improvement should be clearly evident within 14 days after the initiation of a specific therapy (with first-line agents such as sulfadiazine or clindamycin and pyrimethamine), and a neuroradiographic study performed within 3 weeks after the initiation of therapy should reveal improvement (with cautious interpretation for patients who have received prolonged corticotherapy) (21, 42). Nevertheless, abnormalities may be completely absent in patients with diffuse TE at an early stage, although MRI has a higher sensitivity than CAT scan in detecting lesions (10). TE can be definitely diagnosed by histology (visualization of tachyzoites) or by isolation of *T. gondii* organisms from brain biopsy material. Brain biopsy requires a surgical intervention which may be difficult to achieve because of the location of the lesion and the large size of the brain specimen needed. It is an aggressive technique associated with morbidity and is reserved for use in those with mass lesions occurring during the early stages of AIDS when there is no response after 2 weeks of specific antitoxoplasmosis therapy and because of possible associated brain disease (5). Isolation of the pathogen in cerebrospinal fluid (CSF) and fluid from bronchoalveolar lavage (BAL) has been found to be useful for the diagnosis of TE and toxoplasmic pneumonitis, respectively (11, 23, 24). Isolation of *T. gondii* from patient blood has also been reported, although this method is limited by previous specific therapy. It reflects active infection and thus strongly suggests that even when it is confined, such as in patients with encephalitis or pneumonitis, toxoplasmosis may be a systemic disease (21, 31). Mouse inoculation is the "gold standard" for the isolation of *T. gondii*, but it is cumbersome and time-consuming and requires the use of animals. Recently, tissue culture systems on MRC5 fibroblasts (3, 22, 23) or THP1 myeloid cells (10, 57) have been useful for readily identifying by indirect immunofluorescence assay *T. gondii* organisms from clinical specimens within a few days; moreover, it was found to be as sensitive as mouse inoculation when purified tachyzoites were added directly to cell culture or were inoculated into mice (23). More recently, other possible diagnostic methods have used amplification of nucleic acid sequences specific to *T. gondii*, such as B1 (9), p30 (54), TGR1 genes (18), or the 18S rDNA (12), as targets. The specificity of the ribosomal target was verified by the absence of cross-amplification with either human or murine DNA and with samples containing *Plasmodium*, *Cryptosporidium*, *Pneumoscystis*, or *Cryptococcus* spp. Conversely, amplification of 47 different strains of *T. gondii* was obtained (12). Only very closely related organisms such as *Eimeria* or *Hammondia* spp., which are other coccidian genera, gave positive amplifications, but they are not involved in human pathology (16). The direct sensitivity of the reaction was assessed to be 10 fg (i.e., 0.1 parasite), according to the natural amplification of the target gene; revelation by Southern blot hybridization increases the direct sensitivity 100-fold. In another study with different primers chosen from rDNA sequences, the level of sensitivity obtained from a crude cell lysate allowed the detection of as few

* Corresponding author. Mailing address: Service de Maladies Infectieuses et Médecine Interne, Hôpital Pellegrin, Tripode, Place Amélie Raba-Léon, 33076 Bordeaux Cedex, France. Phone: (56) 79-55-36. Fax: (56) 79-60-36.

as one parasite (29). In biological samples overloaded with quantified *T. gondii*, the sensitivity was assessed to be less than five parasites (12, 14). Comparison with amplification of p30 or B1 genes showed that the sensitivity was at least equivalent or better (15). Detection of *T. gondii* rDNA in 400 amniotic fluid samples by this technique was useful in the diagnosis of congenital toxoplasmosis, with a positive predictive value of 90% and a negative predictive value of 99% (13). However, partly because of the limited volume (0.1 to 0.2 ml), the sensitivity of this technique was limited in the diagnosis of ocular toxoplasmosis when it was used to assay more than 300 aqueous humor samples. A combination with the Witmer-Desmonts coefficient, which assesses the intraocular production of antitoxoplasma antibodies, led to a sensitivity of 73% compared with a sensitivity of 50% by use of the Witmer-Desmonts coefficient alone (1, 13). PCR amplification has been used with success in blood (25, 26), brain biopsy (33, 34), CSF (19, 39, 45, 46, 55), liver biopsy (19), and BAL (7, 19) samples obtained from AIDS patients with TE. It has also successfully detected *T. gondii* DNA in amniotic fluid samples from congenitally infected fetuses (16, 28) and in the aqueous humors of patients with ocular toxoplasmosis (1, 2, 8). Toxoplasma gene amplification has been found to be more sensitive than cell culture and to have a high degree of specificity (30).

The aim of the study described here was to evaluate concurrently the respective contribution of PCR and tissue culture for the detection of *T. gondii* from blood and CSF samples from HIV-infected patients with suspected toxoplasmosis.

TABLE 1. Baseline characteristics of 72 HIV-infected patients at time of inclusion in the study

Characteristic	Value
Mean age (yr [range])	39 (14–72)
Sex (no.)	
Male	56
Female	16
Risk factor for HIV infection (no. [%] of patients)	
Homosexuality	26 (36.2)
Intravenous drug abuse	22 (30.5)
Blood product	14 (19.4)
Heterosexual contact	7 (9.7)
Not definite	3 (4.2)
Stage of HIV infection (no. [%] of patients)[a]	
A3	6 (8.3)
B3	19 (26.4)
C3	47 (65.3)
Mean ± SD CD4 count (cells/μl)	54 ± 71
Toxoplasma serology (no. [%] of patients)[b]	
IgG positive (all levels)	72 (100.0)
IgG positive (level, ≥800 UI/ml)	7 (9.7)
IgM positive	3 (4.2)

[a] According to the 1993 classification system (17).
[b] Indirect immunofluorescence assay.

MATERIALS AND METHODS

Subjects (recruitment, monitoring, evaluation). Seventy-two HIV-infected patients were recruited into the present prospective study from January to December 1993. All patients were hospitalized in three infectious disease departments of two Bordeaux University hospitals, Bordeaux, France (Pellegrin Hospital and Haut-Lévêque Hospital).

All had antibodies to HIV type 1, as confirmed by Western blotting (immunoblotting), and were classified according to the 1993 AIDS case definition (17). The absolute lymphocyte count in whole blood was obtained by using an automated counter (Bayer Diagnostics). The percentage of $CD4^+$ cells was determined by using fluorescein-labelled monoclonal antibodies on whole blood and flow cytometric analysis (Fac-Scan; Becton-Dickinson). The baseline characteristics of the 72 HIV-infected patients are described in Table 1.

All patients had baseline and periodic clinical and laboratory evaluations with a detailed neurological examination in order to detect signs of TE as described previously (21, 40, 43, 47, 50, 56). CAT or MRI scans were done as soon as possible (within 48 h of admission into the hospital) and were repeated if the findings were abnormal. All patients had a fundus eye examination and a retinal angiography if an abnormality was detected. Clinical and radiological evaluations of efficacy were assessed according to the semiquantitative rating scale of the California Collaborative Treatment Group (21). The patients were followed up for 6 months unless death occurred.

Diagnostic criteria. According to previous studies (21, 35, 43, 56), a presumptive diagnosis of TE was made in patients with signs and symptoms of CNS dysfunction and typical lesions on CAT or MRI scans of the brain. A probable diagnosis of TE was made in patients with presumptive TE who showed neurological signs and improvement on CAT or MRI scan after 2 weeks of treatment. A definite diagnosis required either that *T. gondii* be isolated from brain tissue, blood, or CSF or that tachyzoites be seen on histologic evaluation of a brain biopsy specimen or at autopsy. A probable diagnosis of ocular toxoplasmosis was made in patients who complained of decreased visual acuity (i) by observing the characteristic fundus lesion (i.e., massive coagulation necrosis of the retina with a granulomatous inflammation of the underlying choroid) with an ophthalmologic examination and angiography, (ii) by excluding other infectious diseases that might cause necrotizing lesions of the fundus (principally, syphilis, cytomegalovirus infection, herpetic infection, tuberculosis, cryptoccocis, toxocarosis by thorough history, physical examination, blood count and chemistries, and, if needed *Herpes viridae* DNA PCR of a aqueous humor sample), and (iii) after clinical improvement assessed by ophthalmoscopic examination after appropriate therapy. A definite diagnosis was made by detecting an elevated level of antibodies against *T. gondii* in the aqueous humor or isolating *T. gondii* from blood or an aqueous humor sample (2, 35). A definite diagnosis of disseminated toxoplasmosis was established if *T. gondii* was isolated from blood.

Toxoplasmic serological investigation. Specific anti-*T. gondii* immunoglobulin G (IgG) or IgM antibodies were detected by indirect immunofluorescence assays starting at a dilution of 10 IU/ml. For all patients, IgG and IgM levels in serum were determined, and for some of them, IgG and IgM levels in CSF samples were determined if CSF samples were available.

All patients had serum IgG antibodies against *T. gondii*, with a high level (≥800 IU/ml) found in seven patients, six of whom had a probable diagnosis of TE. Three of the six patients also had detectable IgM antibodies (Table 1).

Samples. All HIV-infected patients with neurological signs, fever of unknown origin, or uveitis underwent blood sampling. CSF sampling was done if there was no contraindication. If a patient with neurological signs was at high risk (mass lesion syndrome), lumbar puncture was performed with the utmost care to avoid herniation. Causes of infection other than *T. gondii* were screened by cytologic, microbiologic (bacterial, fungal, viral), and serologic laboratory tests. Samples were taken before specific anti-*T. gondii* therapy if possible. Informed consent for blood and CSF sampling was obtained from the patients or their parents.

Two 5-ml blood samples were drawn from each patient; one was collected in a sterilized tube with lithium heparinate and was used for tissue culture, and the other was collected in a sterilized tube with EDTA for amplification by PCR. When lumbar puncture could be performed, one 5-ml CSF sample was collected in a sterilized tube without additive for tissue culture and PCR amplification. For patients with uveitis, an anterior chamber puncture was performed, and about 0.1 ml of aqueous humor was taken after a limbic paracentesis. All samples were immediately stored at 4°C for rapid processing. The buffy coat (the erythrocyte-free supernatant containing leukocytes and platelets) was collected from blood samples after centrifugation; one sample was immediately used for tissue culture, and the other was suspended in 100 μl of conservation Tris-EDTA buffer (pH 7.5; 10 mM Tris-HCl, 1 mM EDTA) and stored at 4°C for later PCR within 6 days. CSF samples were separated into two aliquots; one was used for immediate tissue culture after centrifugation, and the other was centrifuged, after which the pellets were suspended in 100 μl of Tris-EDTA buffer for subsequent PCR. Aqueous humor samples were processed as described above for the CSF aliquots, but aqueous humor samples were used only for subsequent PCR.

Preparation of DNA from patient samples. For CSF and aqueous humor samples, DNA was purified by a previously described procedure (36). Briefly, after centrifugation the pellets were digested for 1 h at 37°C in 1.5 μl of a buffer containing 5 mM Tris-HCl (pH 7.5), 3.4 mM sodium dodecyl sulfate (0.01%), 40 mM dithiothreitol, and 50 μg of proteinase K per ml. They were then heated in boiling water for 10 min.

For blood samples, DNA was obtained by a different purification method. After centrifugation at 8,000 × *g* for 5 min, the pellets were resuspended in 900 μl of guanidine isothiocyanate and vortexed. Then, 40 μl of silica marbles (silicon dioxide) was added, and the specimens were vortexed and incubated for 10 min. The silica-bound DNA was washed with guanidine isothiocyanate, then twice with 70% ethanol, and next with acetone before drying under vacuum. Finally, the DNA was eluted by resuspending the silica-DNA mixture in 60 μl of distilled

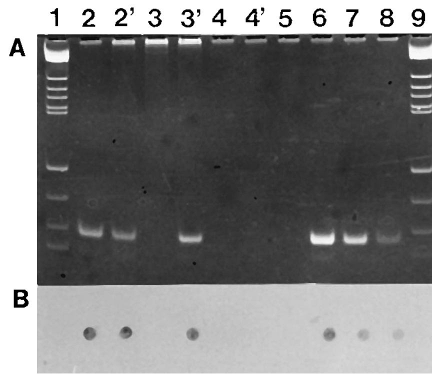

FIG. 1. Detection of *T. gondii* by rDNA amplification and hybridization. The ethidium bromide-stained gel (A) and the autoradiograph of the amplicons after dot blotting (B) are shown. Lanes 1 and 9, PBR322-*Hae*III molecular weight standards; lane 2, positive assay; lanes 3 and 4, negative assays; lanes 2′, 3′ and 4′, inhibition controls (showing an inhibitor in the assay in lane 4′); lane 5, negative control; lanes 6 to 8, positive controls (using purified *T. gondii* DNA concentrations corresponding to 100, 10, and 1 *T. gondii* isolate, respectively).

water and centrifuging it. The supernatant was used for PCR: 30 μl was used for the direct amplification test and 100 fg of *T. gondii* DNA was added to 30 μl for inhibitory control of amplification.

Target and primers for PCR. DNA in CSF or blood was detected by a PCR amplification in which the target was part of an rDNA repetitive gene, initially described by RNA sequencing (37) and exactly defined by DNA sequencing, as described previously (14). An 88-bp segment was amplified with the synthetic oligonucleotide primers 5′-GGCATTCCTCGTTGAAGATT-3′ and 5′-CCTTG GCCGATAGGTCTAGG-3′ (12). The identity of this amplified product was verified previously by restriction analysis with *Nsi*I and by internal probe hybridization (20-bp oligonucleotide, 5′-^{32}P labelled with T4 polynucleotide kinase; 5′-GATAGATTATTGCAATTATT-3′) (12).

PCR amplification and revelation. DNA was amplified in a 50-μl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 16 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 1 mg of bovine serum albumin per ml, 200 μl of each of the four desoxyribonucleoside triphosphates, 1 μM (each) the two oligonucleotide primers, and 1 U of *Taq* polymerase (Cetus). Amplification in an automated thermal cycler (Perkin-Elmer Cetus 9600) consisted of 35 cycles (denaturation step, 95°C for 30 s; annealing step, 55°C for 10 s; synthesis step, 70°C for 100 s). Ten microliters of each amplification reaction mixture was analyzed on a 10% polyacrylamide gel, and the DNA fragments were visualized by staining with ethidium bromide under UV illumination. Dot blot hybridization was used only to determine the specificity of the PCR product. Each amplification run contained several controls (inhibition control with 100 fg of *T. gondii* DNA sample; negative controls with extraction mixture and with the amplification reaction mixture). The sensitivity of the PCR was examined by using three semiquantitative categories and three purified *T. gondii* DNA concentrations corresponding to 100, 10, and 1 parasites (Fig. 1). Strict methods to avoid contamination were used, such as strict spatial partitioning of the different steps in three separate rooms, the use of pipettes with disposable tips and shielded pistons, and frequent changes of gloves. All specimens were analyzed in duplicate.

Tissue culture. The human embryonic fibroblast cell line MRC5 (Bio-Merieux, Marcy l'Etoile, France) was used for tissue culture by a procedure reported by others (23, 53) and previously used for the prenatal diagnosis of congenital toxoplasmosis with amniotic fluid samples (4, 22). Leukocyte pellets from blood samples and cell pellets from CSF or aqueous humor samples were added on coverslips in shell vials with confluent MRC5 cell lines, and the vials were incubated at 37°C in 5% CO_2. After 4 to 7 days, the coverslips were examined microscopically to search for tachyzoites after May-Grünwald-Giemsa staining and by indirect immunofluorescence with polyclonal rabbit antiserum to *T. gondii* and then to fluorescein-labelled anti-rabbit IgG (Diagnostics Pasteur, Marnes La Coquette, France).

Statistical methods. The sensitivity and specificity of the PCR were derived from the use of binomial distribution confidence intervals.

RESULTS

Patient samples. Of the 72 patients, 60 had neurological signs, 10 had fever of unknown origin, and 2 had uveitis (Fig. 2). Blood and CSF samples were available from all except 12 of the 60 patients with neurological signs because of lumbar punc-

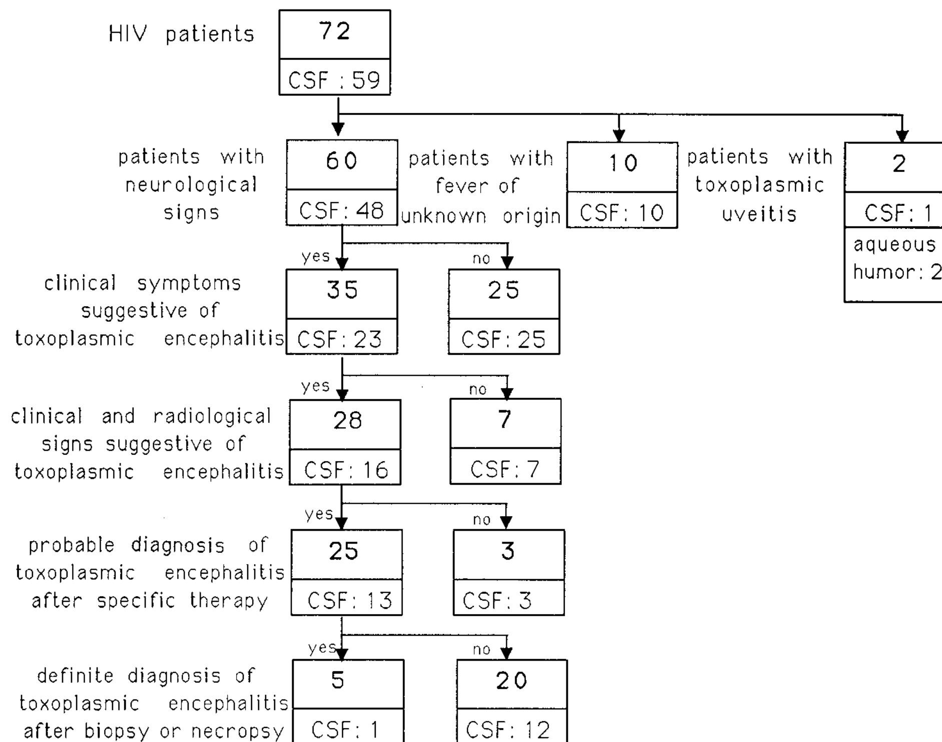

FIG. 2. Classification of the 72 HIV-infected patients according to presenting symptoms, physical findings, brain imaging, outcome with specific antitoxoplasmosis therapy, and CSF sampling.

TABLE 2. Correlation between positive results of *T. gondii* PCR amplification and tissue culture

Category of patients	No. of patients	PCR			Tissue culture		
		Blood	CSF	Ocular	Blood	CSF	Ocular
Patients with accepted TE (n = 10)	3	+	−	ND[a]	−	−	ND
	4	−	+	ND	−	−	ND
	1	−	+	ND	−		ND
	2	+	+	ND	−	−	ND
Patients with accepted TU[b] (n = 2)	1	−	ND	+	−	ND	ND
	1	−	−	+	−	−	ND

[a] ND, not determined.
[b] TU, toxoplasmic uveitis.

ture contraindication and except 1 of the 2 patients with uveitis. Moreover, three patients with neurological signs received antitoxoplasma maintenance therapy treatment, and five other subjects received presumptive treatment (for 48 h in four patients and 12 days in one patient) with a sulfadiazine-pyrimethamine combination at the sample time. Of the 10 patients with fever, a diagnosis of toxoplasmosis was not established in any of them after evaluation and follow-up.

The two patients with uveitis had a probable diagnosis of ocular toxoplasmosis and underwent anterior chamber puncture.

Results of PCR and tissue cultures. Among the patients with neurological signs, 5 of 60 blood samples and 7 of 60 CSF samples tested by PCR were positive, and an 88-bp amplification product was found. All blood cultures were negative and only one CSF culture was positive. All of these positive results were for 10 patients with probable TE; correlations are given in Table 2. It is noteworthy that for three patients only PCR of blood was positive, for four patients only PCR of CSF was positive, and for two patients PCRs of both blood and CSF were positive. For two patients without highly suggestive radiologic signs of TE, positive PCR led to the prompt initiation of specific antitoxoplasma therapy, with dramatic improvement (one patient with cerebellar syndrome and fever; one patient with headache, fever, and pulmonary interstitial syndrome without abnormal findings on BAL examination).

Fourteen patients underwent cerebral biopsy or necropsy, and five had a definite diagnosis of TE. Blood and CSF samples were available for 10 of these 14 patients; there was no false-positive result, but there was one false-negative result in a female with definite TE who had been under presumptive specific therapy for 12 days.

For the two patients with toxoplasma uveitis, only PCR of the aqueous humor was positive.

Among patients with fever of unknown origin, none had positive results for blood and CSF samples.

Diagnostic value of PCR. The sensitivity and specificity of the PCR were calculated (Table 3). The positive and negative predictive values for an HIV-infected patient to have or not to have one or more positive PCR results when both CSF and blood samples were available are also given in Table 3.

DISCUSSION

During HIV infection it is estimated that 25 to 37% of patients ultimately develop TE (40, 43). In most instances TE develops when the $CD4^+$ count falls below 200 cells per μl, with a major risk of TE developing when the $CD4^+$ count falls below 100 cells per μl (40, 47, 50). The mean $CD4^+$ level of 47 ± 65 cells per mm^3 in our survey is in accordance with such data. In France CNS toxoplasmosis was present in 13.8% of AIDS patients at the time of the official declaration of AIDS (cumulative data by 30 June 1994) (52). In the Cohorte Aquitaine, a regional cohort followed in the main hospitals, we found that 272 (8.7%) of 3,119 HIV-infected subjects (22% of AIDS patients) had had a presumptive diagnosis of TE by 11 July 1993 (50, 51). In contrast to patients who are immunocompromised because of other diseases, involvement of extra-CNS tissues has not frequently be reported in HIV-infected individuals. The risk of extra-CNS toxoplasmosis ranged between 1.5 and 2% in a French national survey from January 1990 to September 1992 (49).

Presumptive therapy poses some risks. One should consider that the use of such a response is inherently nonspecific because other organisms might respond to antitoxoplasma therapy (*Babesia* spp., for example). Adverse reactions to first-line drugs in patients with AIDS leads to discontinuation and hampers therapeutic evaluation. An undue delay in pursuing an alternative diagnosis (43) accessible to specific therapy (tuberculosis, cryptococcosis) is also to be feared.

In our study IgG antibodies against *T. gondii* were present in the blood of all patients, as detected by immunofluorescence. This finding is consistent with those of other studies in which all patients with TE had a positive serology, indicating that toxoplasmosic cysts were already present in the tissue (45). In another study, 22% of the patients had undetectable antitoxoplasma IgG antibodies by indirect immunofluorescence, while enzyme-linked immunosorbent assay appeared to be more sensitive (47). The IgG level was increased in only a few people (6 of 27 [22.3%] patients with probable TE or toxoplasmic uveitis), and IgM determination did not provide a sufficient contribution (positive in only three patients), as described previously (60). For diagnosis, compared with blood serologic testing, measurements of the intrathecal production of antibodies against *T. gondii* gave better, although low, sensitivity (63%) and specificity (68%) in earlier surveys (6, 48). When assessed in our patients, CSF was positive for antibodies, but they were present at low levels, and we did not evaluate the ratio of the level of albumin in CSF to that in serum or the total IgG level to confirm local antibody synthesis. As shown in a recent study, the in vitro production of toxoplasma-specific antibodies could improve the diagnosis of TE in HIV-infected patients (59).

Until now, studies concerning the detection of *T. gondii* by PCR and tissue culture have been limited, have often been retrospective, or have been applied to a few samples, and they have never been conducted with the pragmatic assessment of sensitivity and specificity and the usefulness of the two techniques.

Tissue culture is technically easier than PCR but requires

TABLE 3. Diagnostic value of *T. gondii* PCR amplification in 48 HIV-infected patients with neurological signs who underwent blood and CSF sampling (contingency table)[a]

PCR result	Probable TE (no. of patients)		
	Yes	No	Total
Positive	10	0	10
Negative	3	35	38
Total	13	35	48

[a] Sensitivity, 10 of 13 patients = 76.9% (95% confidence interval, 46.2 to 95.0); specificity, 35 of 35 patients = 100% (95% confidence interval, 90.0 to 100.0); positive predictive value, 10 of 10 patients = 100%; negative predictive value, 35 of 38 patients = 92.1%.

viable cells, and samples must be rapidly processed for inoculation. It allows detection of active infection, while PCR detects DNA from cysts or tachyzoites. However, parasitemia may be transient, and the specific prophylaxis decreases the parasite burden. Moreover, the number of parasites used to inoculate cultures together with inhibitory factors (especially in the buffy coats of leukocytes and platelets) may interfere with the infection of the monolayer (25). The latter considerations explain the low level of sensitivity of blood culture: 14 to 38.7% for the diagnosis of TE (3, 21, 57) and 10% for patients with fever and unproven organic infection but improvement after specific therapy (57). Whereas tissue culture was promising in a retrospective study (22), it was not contributor to the diagnosis in our series and for the surveillance of HIV-infected patients at risk of TE (despite the use of serial samples, which should theoretically be more effective for detecting intermittent shedding of *T. gondii* in blood) (20). The only positive result that we found was for a CSF sample with a positive PCR result, while blood culture and PCR were negative. Such a discrepancy has already been reported (10) and underlines the usefulness of local fluid samples (CSF for TE, BAL for *Toxoplasma* pneumonia) (19).

Compared with tissue culture, PCR amplification can be done on frozen erythrocyte-free pellets. With strict measures taken to avoid contamination, it is interesting that, like others (32, 44), we found no false-positive results in our series. Although superior to tissue culture, the reported sensitivity of PCR of CSF is less impressive and has ranged from 35.3 to 100% for HIV-infected patients (27, 44, 45, 46, 55). In a survey of 29 patients (55), the 95% confidence intervals for specificity (71 to 100%) and sensitivity (41 to 86%) resulted in positive predictive value ranges of from 0.38 to 1.00 and negative predictive value ranges of from 0.74 to 0.94. This was based on an estimated 30% prevalence of TE among *T. gondii*-infected patients with AIDS and on a negative control group of nine patients. Our values for specificity (90 to 100%) are higher because of a high number of negative controls: 35 patients without false-positive results. The low level of sensitivity of PCR might be due to inhibitory factors such as porphyrin and *Toxoplasma* DNA destruction before amplification, which can be avoided with adapted measures as described previously (27, 58). PCR amplification with a gene more repetitive than the B1 gene such as rDNA and testing of several sequential samples could improve the sensitivity, which resulted in a 95% confidence interval of 46.2 to 95.0% in our study with a single sample (18, 32, 55). In a recent study with B1 gene amplification, a higher diagnostic accuracy was achieved when lumbar puncture was performed in the first week of anti-*Toxoplasma* therapy (44). Moreover, CSF is unlikely to be positive for deep brain lesions. By combining the use of CSF and blood samples, our study demonstrates a better sensitivity of PCR, especially in patients with few signs of presumptive TE (3 of 10 patients had only a positive PCR of blood, compared with 2 of 15 patients with a positive PCR of blood in a recent study with B1 gene amplification [38]).

Of note is the positivity of PCR amplification in two patients with toxoplasmic uveitis. In former studies, DNA amplification could be positive while the Witmer-Desmonts coefficient was negative (1).

The absence of positive results in the group of patients with unexplained fever may be due to their low numbers.

We conclude that PCR amplification of rDNA is more sensitive than tissue culture on MRC5 cells for the diagnosis of TE in HIV-infected patients. Combined use of CSF and blood sampling is required for better sensitivity. Serial sampling warrants further examination.

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