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Limited value of cerebrospinal fluid for direct detection of *Toxoplasma gondii* in toxoplasmic encephalitis associated with AIDS

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Abstract The diagnosis of acquired immunodeficiency syndrome-associated toxoplasmic encephalitis (TE), a typically focal disease resulting from reactivation of tissue cysts, relies mainly on indirect diagnostic methods. In a prospective study, we investigated the value of detection of *Toxoplasma gondii* in cerebrospinal fluid (CSF) by using the polymerase chain reaction and the mouse inoculation test. Twenty-four patients with 26 episodes of TE, 2 HIV-infected patients with primary acute *Toxoplasma* infection, and 38 HIV-infected control patients with latent *Toxoplasma* infection were investigated. Detection of *T. gondii* in CSF by both methods was possible in only 3 of the TE patients (11.5%), the remaining patients being negative with either of the methods. In contrast, *T. gondii* DNA was detected in both of the acutely infected patients, indicating that in primary acute toxoplasmosis parasites may easily be found in the CSF, whereas in the majority of TE cases in immunocompromised patients, *T. gondii* parasites do not gain access to the CSF drawn by lumbar puncture.

Key words Acquired immunodeficiency syndrome · Toxoplasmosis · Polmerase chain reaction · Cerebrospinal fluid · Central nervous system diseases

Introduction

Toxoplasmic encephalitis (TE) is the most common opportunistic infection of the central nervous system in patients suffering from the acquired immunodeficiency syndrome (AIDS); it has been demonstrated in 22–46% of autopsied patients in Europe [17]. TE may mimic other focal brain lesions such as lymphoma, tuberculoma, fungal abscess and even progressive multifocal leukoencephalopathy (PML), as well as ischaemic stroke [12, 18]. Brain biopsy seems to be the only way to obtain a definite diagnosis but is potentially hazardous and not always conclusive [18]. The polymerase chain reaction (PCR) has been successfully applied to detect *Toxoplasma* DNA or RNA in the cerebrospinal fluid (CSF) of small groups of AIDS patients with TE [7, 9, 11, 13, 14]. Following the establishment of a more sensitive PCR method for the demonstration of *Toxoplasma* DNA in clinical specimens [7], we wanted to determine in this study the value of this method for the diagnosis of TE from CSF in a larger series of AIDS patients.

Patients and methods

PCR for detection of *Toxoplasma* DNA was prospectively performed with the CSF samples of HIV patients who underwent lumbar puncture for evaluation of possible TE. Patients were diagnosed as having definite TE if histopathology of autopsy or biopsy material demonstrated parasites with a surrounding inflammatory reaction. A circumstantial diagnosis of TE was made when all of the following five criteria were fulfilled: (1) clinical evidence of CNS disease (encephalopathic or focal findings), (2) computed tomography (CT) or magnetic resonance imaging (MRI) revealing multiple or solitary focal brain lesions, (3) demonstration of spe-

cific antibodies to *Toxoplasma*, and (4) *Toxoplasma*-specific therapy resulting in clinical and radiological improvement. To rule out the possibility of a cerebral lymphoma being mistaken for toxoplasmosis, we additionally postulated that, if steroids were also being administered, the patient had to be alive for at least 4 months after the diagnosis had been made (see Discussion).

One CSF sample was drawn from each TE case. In 2 patients (cases 11 and 22) the CSF was of ventricular origin. The CSF of 38 AIDS patients with *Toxoplasma*-specific antibodies who had final diagnoses other than TE served as controls. Diagnoses included lymphoma (5), HIV-encephalitis (5), cryptococcosis (4), PML (2), *Cytomegalovirus*-encephalitis (2), previous TE (2). In addition, 2 patients with acutely acquired toxoplasmosis were tested. The individuals investigated were patients of the university hospitals of Hamburg or Würzburg.

Microbiological examinations of specimens were performed in Würzburg. Serum and CSF of all patients were tested for the presence of *Toxoplasma*-specific antibodies essentially as previously described by means of direct agglutination, dye test, complement fixation test and immunoblot [3, 8, 15, 19]. CSF for microbiological analyses was divided into three aliquots of at least 500 µl/aliquot. The first aliquot was injected intraperitoneally into *Toxoplasma*-antibody-negative mice to confirm the PCR result. Sera from these mice were drawn 3-4 weeks later to test them for seroconversion [20]. To exclude the presence of PCR-inhibitory substances within the CSF, approximately 10 *Toxoplasma gondii* parasites of strain RH were added to the second CSF aliquot (control aliquot). The third aliquot was centrifuged and the supernatant was used for antibody determination. The pellet of this aliquot as well as the pellet of the control aliquot were both resuspended in a buffer consisting of 10 mmol TRIS-Cl (pH 8.3), 1.5 mmol $MgCl_2$, 50 mmol KCl, 0.1 mg/ml gelatine, 0.5% Tween 20 and 20 mg proteinase K. Following incubation for 1 h at 55°C, proteinase K was heat-inactivated, and the suspension was centrifuged at $10,000 \times g$ for 10 min. Subsequently, 2×10 µl of the supernatant was used for two separate PCR reactions performed in parallel essentially as described [7]. For this purpose, two sets of primer pairs derived from the *B1* gene of *T. gondii* [2] were used. The first PCR reaction consisted of the primer pair 1 and 2 (1: 5′-TCT-TTA-AAG-CGT-TCG-TGG-TC-3′; 2: 5′-GGA-ACT-GCA-TCC-GTT-CAT-GA-3′), and the second PCR reaction was performed using the primer pair 3 and 4 (3: 5′-CAG-CGT-ATT-GTC-GAG-TAG-AT-3′; 4: 5′-AAC-GGA-ACT-GTA-ATG-TGA-TA-3′). The PCR was judged as being positive only when both sets of primer pairs amplified the expected gene fragment of either 192bp (primer pair 1/2) or 517 bp (primer pair 3/4). All PCR reactions were repeated at least once.

Results

TE group

A synopsis of the investigated patients with proven TE is given in Table 1. Twenty-six episodes of TE could be demonstrated in 24 patients. Seven cases were proven by autopsy and one by biopsy. In 18 cases, the diagnosis was based on the criteria outlined in Patients and methods. In all but one (no contrast given) of these patients CT or MRI revealed contrast enhancement. TE was the cause of death in 4 patients. All patients had *Toxoplasma*-specific antibodies indicative of a latent infection. None of these patients had serological evidence for an acutely acquired toxoplasmosis.

Toxoplasma DNA could be detected in the CSF of only 3 of the TE patients (11.5%). Mouse inoculation yielded viable *Toxoplasma* organisms in these 3 patients but was negative in the patients with negative PCR. No PCR-inhibiting factors were present in any of the 26 cases.

Case 11 had a necrotizing ventriculoencephalitis with obstructive hydrocephalus [5]. *Toxoplasma* organisms were detected by routine cytology of his ventricular fluid but not in the CSF of the other 23 cases. Case 21 did not markedly differ from the other TE cases: the time lapse between initiation of anti-toxoplasmic therapy and lumbar puncture was comparable with the other cases. CT scans of her and of case 22 did not suggest cerebritis or ventriculitis. High cell count or protein content of CSF did not correlate with positive PCR or culture results. CSF of case 22 was taken by ventricular puncture at autopsy.

Control group

The 38 control patients were all HIV-infected and serologically positive for *Toxoplasma* antibodies. Of these patients 15 had focal brain lesions in CT and 13 had a pathological cell count and/or protein content in the CSF. One patient in this group had a questionable PCR result. He had two contrast-enhancing lesions on CT that proved to be a lymphoma on biopsy. His first CSF sample was positive with only one of the two primer pairs used in PCR and mouse inoculation gave a negative result. Repeat CSF and brain biopsy tissues taken from two different locations were negative by PCR and mouse test.

In addition, the CSF of 2 HIV-positive patients with serological data indicating primary acute toxoplamosis were investigated (Table 2): case 27 underwent routine lumbar puncture although he had no suspicious symptoms. His CD4 count was 520 cells/µl and antibody testing revealed evidence for acute or recently acquired infection with *Toxoplasma* including the demonstration of IgM antibodies. His CSF was PCR positive with both primer pairs but was negative in the mouse test. In the other patient (case 28), investigation of serial blood samples demonstrated *Toxoplasma*-specific seroconversion. He had previously been healthy with no current signs or symptoms of disease and was admitted for serological evidence of syphilis. His CD4 count was 190 cells/µl. Ten days after lumbar puncture and in close temporal relationship with *Toxoplasma*-specific seroconversion, he developed a 2-day episode of organic psychosis with an inflammatory CSF (61 cells/µl, protein 1.3g/l). PCR of his CSF demonstrated *Toxoplasma* DNA while mouse inoculation was negative. MRI of the brain was normal. A CSF sample drawn 4 weeks later showed less inflammatory alterations. PCR was now negative. No *Toxoplasma*-specific therapy was initiated, whereas penicillin was given for *Treponema pallidum*.

Table 1 Patients with toxoplasmic encephalities *(TE)* (*Atov* atovaquone, *Clin* clindamycin, *Cot* cotrimoxazole, *OH* obstructive hydrocephalus, *Pyr* pyrimethamine, *Ster* corticosteroids, *Sulf* sulphadiazine)

Pat/case no.	Age (years) and sex	Clinical presentation [focal (f) and/or encephalopathic (e)]	CDC stage prior to TE	CD4 cell count n/mm^3	CSF findings			Neuroradiology (CT or MRI)		Therapy	Outcome	Neuropathology	Time between first dose of therapy and lumbar puncture	PCR	Mouse inoculation
					Leukocytes n/mm^3	Cytology predominately mononucleic (m) or granulocytic (g)	Protein g/l	Lesions single or multiple	Contrast enhancement						
1	41 m	f, e	IV C 1	10	3	ND	0.33	Multiple	Little	Pyr, Sulf, Clin, Ster	Improved	ND	4 weeks	neg	neg
2	32 m	f	IV C 1	20	52	m	0.92	Single	Ring	Pyr, Sulf→Clin, Ster	Improved	TE	1 day	neg	neg
3	24 m	f	IV C 1	40	20	78% ly, 21% g	0.45	Multiple	Yes	Pyr, Sulf, Ster	Improved	TE	1 day	neg	neg
4	33 m	f	IV C 1	< 100	116	m	1.75	Single	Yes	Pyr, Sulf, Ster	Improved	ND	5 days	neg	neg
5	61 m	f, e	IV C 1	10	1	ND	5.3	Single	Ring	Cot, Ster	Died	TE	2 days	neg	neg
6	64 f	f	III	90	4	ND	0.56	Single	Ring	Pyr, Sulf, Ster	Improved	ND	10 days	neg	neg
7	55 m	f, e	IV C	10	5	g	0.33	Multiple	No	No specific treatment	Died	TE	–	neg	neg
8	44 m	f	IV C 1	86	1	ND	0.55	Single	Ring	Pyr, Clin, Ster	Improved	ND	–5 days	neg	neg
9	41 m	e	III	50	35	65% m, 35% g	2.4	Multiple	Little	Pyr, Sulf	Improved	ND	–3 days	neg	neg
10	41 m	e	IV C 1	40	9	m	0.84	Single	Little	Pyr, Clin, Ster	Improved	TE	–1 day	neg	neg
11	32 m	OH	IV C 1	0	80	g[c]	3.0	Multiple	No	No specific treatment	Died	TE	–	pos	pos
12	28 m	e	IV C 1	ND	13	m	0.95	Multiple	Ring	Pyr, Clin, Ster	Improved	ND	2 days	neg	neg
13	55 m	f, e	IV C 1	120	66	m	ND	Multiple	Ring	Pyr, Sulf	Improved	ND	0.5 days	neg	neg
14	44 m	f, e	III	30	5	m	0.92	Multiple	Yes	Pyr, Sulf	Improved	ND	0 days	neg	neg
15	34 m	f, e	IV C 1	10	2	ND	1.4	Multiple	Yes	Pyr, Clin	Improved	ND	0 days	neg	neg
16	34 m	e	IV C 1	0	4	g	2.45	Single	Little	Pyr, Clin, Ster	Improved	ND	–2 days	neg	neg
17	35 m	f	III	20	9	m	0.88	Multiple	Ring	Pyr, Sulf	Improved	ND	1 day	neg	neg
18	66 f	e	III	130	12	m	1.24	Multiple	ND	Pyr, Sulf→Clin	Improved	ND	0.5 days	neg	neg
19	31 m	f	III	60	25	m	0.55	Multiple	Ring	Pyr, Sulf→Clin→Atov	Improved	ND	–2 days	neg	neg
20	36 m	f	III	20	1	ND	0.58	Single	Ring	Pyr, Clin	Improved	ND	4 days	neg	neg
21	40 f	f, e	IV C 1	260	28	m	1.26	Multiple	Yes	Pyr, Sulf, Ster	Improved	ND	1 day	pos	pos
22	45 m	e	IV C 1	10	ND[b]	ND	ND	Multiple	Yes	No specific treatment	Died	TE	–	pos	pos
23	34 f	f	IV C 1	13	2	52% m, 48% g	1.2	Single	Yes	Pyr, Sulf, Ster	Improved	ND	1 day	neg	neg
24	27 m	f, e	IV C 1	87	1	ND	0.44	Single	Yes	Pyr, Sulf, Ster	Improved	ND	0 days	neg	neg
25	32 m	e	IV C 1	28	1	ND	1.05	Multiple	Yes	Pyr, Sulf, Ster	Improved	ND	6 days	neg	neg
26	25 m	f, e	IV C 1	20	ND	ND	0.59	Single	Yes	Pyr, Sulf, Ster	Improved	TE	14 days	neg[a]	neg[a]

[a] PCR and mouse inoculation from biopsy material positive
[b] CSF taken at autopsy by ventricular puncture
[c] Toxoplasma parasites detected by routine microscopy

Table 2 Cases with primary acute toxoplasmosis

Case no.	Age (years) and sex	Clinical presentation	CDc stage	CD4 cell count n/mm^3	CSF-findings			Neuroradiology (CT or MRI)	Therapy	Out-come	PCR	Mouse inocu-lation
					Leuko-cytes n/mm^3	Cytology pre-dominantly mononuclear or granulocytic	Protein g/l					
27	28 m	No signs or symptoms, routine lumbar puncture	II	520	27	Mononuclear	0.77	MRI normal	None		Positive	Negative
28	57 m	Lumbar puncture for suspected neurosyphylis. No complaints at this time. Ten days later 2-day episode of organic psychosis	II	190	61	Mononuclear	1.30	MRI normal	Penicillin, no specific *Toxoplasma* therapy	Self-limiting course	Positive	Negative

Discussion

The diagnosis of TE in patients suffering from AIDS generally requires clinical and radiological evidence, unless a biopsy is performed. Owing to immunosuppression, serology is usually not conclusive. Since the diagnosis of TE therefore relies on indirect evidence, there is a need for a practicable method to directly demonstrate the *Toxoplasma* parasite. We and others have successfully applied the PCR to detect *Toxoplasma* DNA in the CSF of a proportion of TE patients [7, 11, 13, 14]. However, case numbers have been low and most authors have not attempted to cultivate the organism for verification of the PCR result. By testing CSF samples of 26 TE episodes in 24 HIV patients both by PCR and by cultivation using the mouse inoculation test, we obtained a positive result with both methods in only 3 TE cases (11.5%). The remaining 23 cases were negative with either of the methods.

In studies aiming to establish a new method for diagnosing a disease there is the need for a reference group in which the diagnosis can be regarded as unequivocal. The "gold standard" for the diagnosis of TE is the histopathological demonstration of typical lesions. Owing to the lack of biopsy material in most clinical series, however, the diagnosis of TE is commonly based on indirect evidence such as the presence of *Toxoplasma*-specific antibodies, indicative radiological imaging and improvement on therapy specific for toxoplasmosis. Lymphoma may mimic TE and may regress when steroids are administred. In order to confirm the clinical diagnosis of TE, we therefore required that lymphoma be reasonably ruled out by follow-up for at least 4 months, according to published survival times of non-radiated but steroid-treated lymphoma [1]. We therefore considered the diagnosis of TE to be sufficiently certain in those 18 patients in whom no neuropathology was performed. Furthermore, we identified 6 pathologically proven TE cases with negative PCR results obtained from CSF. Notably, a brain biopsy specimen of one of these patients (case 26) was tested by PCR and mouse inoculation and both tests were positive.

The PCR procedure we used relies on the amplification of the *B1* gene of *T. gondii* that exists in at least 35 copies within the genomic DNA [2]. The sensitivity of this method as performed by us was proven to be less than 10 parasites in specimens rich in contaminating cells [7]. In addition, we excluded the possibility of the presence of PCR-inhibiting substances by the use of positive controls derived from the patient's CSF. By conducting a one-step PCR using two sets of primer pairs simultaneously rather than nested PCR, we reduced the risk of contamination by sample handling. In conjunction with the use of proteinase K digestion, which has been shown to increase the sensitivity, we designed the PCR procedure to be, at the same time, sensitive and relatively safe in terms of avoiding false-positive results. Lebech et al. [11] and Oester-

gaard et al. [13] obtained positive PCR results in all of their seven TE patients, whereas Parmley et al. [14] found a positive result in only four of their nine cases.

While most authors did not perform a parallel mouse inoculation test on their CSF samples, the correctness of our three PCR-positive results was confirmed by demonstration of viable *Toxoplasma* organisms by cultivation in the mouse test. Evidence from animal and extracerebral human toxoplasmosis using blood suggests that the culturing methods have a higher [10] or comparable sensitivity than PCR [4]. We therefore consider our PCR- and culture-negative results to be true negatives in the sense that the CSF samples did not contain detectable amounts of *Toxoplasma* DNA or viable organisms.

Some of our TE patients were treated before the CSF was drawn, thus raising the question whether treatment might lead to clearance of the spinal CSF from *Toxoplasma* parasites. However, this hypothesis is in contrast to the report of Dupouy-Camet at al. [4], who found that PCR might become positive only after initiation of treatment.

Among the control group of 38 patients, one patient with biopsy-proven lymphoma had a questionable PCR result. Since the mouse test in this patient was negative, and since the PCR from CSF was positive with only one of the two primer pairs, this patient was designated as *Toxoplasma*-negative, as could be proven by negative PCR results with subsequently drawn CSF and brain biopsy. Therefore, although no autopsy was performed and the coexistence of lymphoma and TE cannot be ruled out [1], there was no indication of TE.

In addition, 2 AIDS patients with primary acute toxoplasmosis were investigated. Case 27 presented a positive PCR result: serology demonstrated the presence of IgM and IgA antibodies being compatible with a recently acquired primary toxoplasmosis. The lack of clinical overt disease and the negative mouse inoculation test might be explained by the fact that his immune system was well preserved and capable of eliminating the parasites. In addition, as was shown previously in a case of congenital toxoplasmosis, discordant results between PCR and mouse test might be explained by the presence of either non-viable or IgG-opsonized parasites within the CSF that facilitate phagocytosis of *T. gondii* by murine peritoneal macrophages, thus leading to the elimination of parasites in mice [6]. In case 28, who showed *Toxoplasma*-specific seroconversion and the presence of IgA antibodies, a primary toxoplasmosis can be assumed. Although there was serological evidence of neurosyphilis, the inflammatory signs of CSF and the psychotic episode might as well be attributed to cerebral invasion of *Toxoplasma* parasites leading to an acute diffuse encephalitis. Since he was not treated for toxoplasmosis, the course of this infection seemed to be self-limited, possibly owing to the preserved immunological function. This might also explain why the mouse inoculation test was negative, probably because of the presence of non-viable or antibody-opsonized parasites within the CSF sample. Judging from the serological data, this patient and case 27 were still at the stage of generalized toxoplasmosis, while the TE of immunocompromised patients manifests as abscess-like foci probably due to focal reactivation of *T. gondii* cysts.

The CSF of 2 of the 3 PCR positive TE patients was of ventricular rather than lumbar origin. This observation is in accordance with that of dos Santos Neto [16] who, in congenital toxoplasmosis, detected the parasite only in ventricular CSF. We therefore suggest that in primary acute toxoplasmosis, parasites may be easily detected in lumbar CSF, whereas in the majority of TE cases in immunocompromised patients, *T.gondii* parasites do not gain access to the CSF drawn by lumbar puncture. Because of the potential risk of lumbar puncture with space-occupying lesions and in view of the disappointing results of the present study we suggest that lumbar puncture is performed only in those cases where cultures or cytology are needed for differential diagnosis.

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References

1. Baumgartner JE, Rachlin JR, Beckstead JH, Meeker TC, Levy RM, Wara WM, Rosenblum ML (1990) Primary central nervous system lymphomas: natural history and response to radiation therapy in 55 patients with acquired immunodeficiency syndrome. J Neurosurg 73: 206–211
2. Burg JL, Grover CM, Pouletty P, Boothroyd JC (1989) Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. J Clin Microbiol 27: 1787–1792
3. Desmonts G, Remington JS (1980) Direct agglutination test for diagnosis of *Toxoplasma* infection: method for increasing sensitivity and specificity. J Clin Microbiol 11: 562–568
4. Dupouy-Camet J, Lavareda de Souza S, Maslo C, Paugam A, Saimot AG, Benarous R, Tourte-Schaefer C, Derouin F (1993) Detection of *Toxoplasma gondii* in venous blood from AIDS patients by polymerase chain reaction. J Clin Microbiol 31: 1866–1869
5. Eggers C, Vortmeyer A, Emskötter T (1995) Cerebral toxoplasmosis in a patient with the acquired immunodeficiency syndrome presenting as obstructive hydrocephalus. Clin Neuropathol 14: 51–54
6. Gross U, Müller J, Roos T, Schrod L, Heesemann J (1992) Possible reasons for failure of conventional tests for diagnosis of fatal congenital toxoplasmosis: report of a case diagnosed by PCR and immunoblot. Infection 20: 149–152

7. Gross U, Roggenkamp A, Janitschke K, Heesemann J (1992) Improved sensitivity of the polymerase chain reaction for detection of *Toxoplasma gondii* in biological and human clinical specimens. Eur J Clin Microbiol Infect Dis 11: 33–39
8. Gross U, Roos T, Appoldt D, Heesemann J (1992) P30 specific IgM- and IgA-immunoblot for routine diagnosis of *Toxoplasma gondii* infection. J Clin Microbiol 30: 1436–1441
9. Guay JM, Dubois D, Morency MJ, Gagnon S, Mercier J, Levesque RC (1993) Detection of the pathogenic parasite *Toxoplasma gondii* by specific amplification of ribosomal sequences using comultiplex polymerase chain reaction. J Clin Microbiol 31: 203–207
10. Hitt JA, Filice GA (1992) Detection of *Toxoplasma gondii* parasitemia by gene amplification, cell culture and mouse inoculation. J Clin Microbiol 30: 3181–3184
11. Lebech M, Lebech AM, Nelsing S, Vuust J, Mathiesen L, Petersen E (1992) Detection of *Toxoplasma gondii* DNA by polymerase chain reaction in cerebrospinal fluid from AIDS patients with cerebral toxoplasmosis. J Infect Dis 165: 982–983
12. Navia BA, Petito CK, Gold JWM, Cho ES, Jordan BD, Price RW (1986) Cerebral toxoplasmosis complicating the acquired immune deficiency syndrome: clinical and neuropathological findings in 27 patients. Ann Neurol 19: 224–238
13. Oestergaard L, Nielsen AK, Black FT (1993) DNA amplification on cerebrospinal fluid for diagnosis of cerebral toxoplasmosis among HIV-positive patients with signs or symptoms of neurological disease. Scand J Infect Dis 25: 227–237
14. Parmley SF, Goebel FD, Remington JS (1992) Detection of *Toxoplasma gondii* in cerebrospinal fluid from AIDS patients by polymerase chain reaction. J Clin Microbiol 30: 3000–3002
15. Sabin AB, Feldman HA (1949) Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (Toxoplasma). Science 108: 660–663
16. Santos Neto JG dos (1975) Toxoplasmosis: a historical review, direct diagnostic microscopy and report of a case. Am J Clin Pathol 63: 909–915
17. Strittmatter C, Lang W, Wiestler OD, Kleihues P (1992) The changing pattern of human immunodeficiency virus-associated cerebral toxoplasmosis: a study of 46 postmortem cases. Acta Neuropathol 83: 475–481
18. Wanke C, Tuazon CU, Kovacs A, Dina T, Davis DO, Barton N, Katz D, Lunde M, Levy C, Conley FK, Lane HC, Fauci AS, Masur H (1987) *Toxoplasma* encephalitis in patients with acquired immune deficiency syndrome: diagnosis and response to therapy. Am J Trop Med Hyg 36: 509–516
19. Warren J, Sabin AB (1942) The complement fixation reaction in toxoplasmic infection. Proc Soc Exp Biol Med 51: 11–14
20. Werner H (1966) Der Nachweis von *Toxoplasma gondii* durch den Tierversuch. Z Tropenmed Parasitol 17: 328–336