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Flávia A. Chaves-Borges
Universidade Federal de Uberlândia

Maria A. Souza
Universidade Federal de Uberlândia

Deise A.O. Silva
Universidade Federal de Uberlândia

Lloyd H. Kasper
Geisel School of Medicine at Dartmouth

José R. Mineo
Universidade Federal de Uberlândia

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DETECTION OF *Toxoplasma gondii* SOLUBLE ANTIGEN, SAG-1(p30), ANTIBODY AND IMMUNE COMPLEX IN THE CEREBROSPINAL FLUID OF HIV POSITIVE OR NEGATIVE INDIVIDUALS

Flávia A. CHAVES-BORGES(1), Maria A. SOUZA(1), Deise A. O. SILVA(1), Lloyd H. KASPER(2) & José R. MINEO(1)

SUMMARY

Active infection by *T. gondii* was evaluated by immunoassay for soluble SAG-1 (p30), the major surface antigen from *T. gondii*, specific antibodies and immune complexes in human cerebrospinal fluid (CSF) samples. A total of 263 samples of CSF were collected from hospitalized patients presenting neurological disorders and analyzed for antibodies to HIV. Patients were divided into two groups: HIV positive (n = 96) or HIV negative (n = 167). The results of the assays showed that 45% of all samples were positive for soluble SAG-1. *Toxoplasma* Ag/Ab immune complexes were detected in 19% of the CSF samples and 62% were positive for *T. gondii*- specific IgG. A combination of these assays in the presence of clinical findings consistent with active *Toxoplasma* infection may predict the presence of toxoplasmic encephalitis. Moreover, detection of soluble SAG-1 in the CSF of these individuals appears consistent with active infection.

KEYWORDS: *T. gondii*; SAG-1 (p30) antigen; AIDS; Encephalitis; Human cerebrospinal fluid

INTRODUCTION

Toxoplasma gondii, an intracellular coccidian, infects a wide range of eukaryotic cells and is an important opportunistic pathogen in humans and animals. The infection is frequently encountered without any symptom but there are two groups of high-risk individuals, the human fetuses and the immunosuppressed persons, particularly those with acquired immunodeficiency syndrome (AIDS), that frequently develop fatal toxoplasmic encephalitis (TE)²⁹. In these patients, the diagnosis relies on a compatible clinical presentation, serologic evidence of exposure to *T. gondii*, and the presence of cerebral lesions by imaging (either MRI or CT). The diagnosis is confirmed if a response to specific treatment is observed within 20 days, as determined by radioimaging scan. However, there are limiting factors to this treatment strategy. For example, the level of antibody may not be correlated with active infection and furthermore unnecessary therapy can be dangerous because of the potential for bone marrow toxicity with anti-*T. gondii* drugs. Therefore, accurate diagnosis of toxoplasma infection is essential for improved prognosis.

Toxoplasma gondii has several molecular components that have been evaluated as possible diagnostic tools. A group of proteins from 26-38kDa can be detected, by Western blot, in cerebrospinal fluids and serum of patients, during toxoplasmosis⁴¹. The major surface protein of the parasite, SAG-1 (p30) is included among these proteins²³. This protein belongs to a group of excreted-secreted antigens (ESA)^{3,7,35} and is involved in host immunity³⁸. Polyclonal antibodies to p30 have been

identified in the serum of acute and chronically infected patients and in congenitally infected individuals.

P30 antigen is stage-specific in that it is expressed exclusively by tachyzoites that are the fast replicative form of the parasite. This molecule is involved in parasite attachment^{14,31} and could represent an important indicator of active infection since it can be a circulating antigen^{7,37}.

Immunoenzymatic assays, ELISA (Enzyme-linked Immunosorbent Assay), have been used in the diagnostics of toxoplasmosis^{6,12,30}. Quantification of antigenemia has been studied by ELISA and immunoblot method^{1,2,4,16}.

The polymerase chain reaction (PCR) has been successfully applied to detect *Toxoplasma* DNA or RNA in the cerebrospinal fluid of groups of AIDS patients^{15,26}. However, it has been recently demonstrated that PCR, although diagnostics for primary infection, may be of a limited value for diagnosis of toxoplasmic encephalitis in AIDS patients¹⁰. Detection of parasite nucleic acid by PCR may be not correlated with active infection since chronic infection may be associated with intervals of parasitemia during asymptomatic phases of the disease^{9,24}.

In the present study, we describe an ELISA system for simultaneous detection of soluble SAG-1 (p30) antigen, IgG antibodies to *T. gondii* and immune complex in human cerebrospinal fluid (CSF) samples in those who are HIV seropositive.

(1) Laboratory of Immunology, Department of Pathology, Universidade Federal de Uberlândia, 38400-902 Uberlândia, MG, Brazil.

(2) Department of Medicine (Neurology) and Microbiology, Dartmouth Medical School, Lebanon, New Hampshire, USA, 03756.

Correspondence to: Dr. J. R. Mineo, Department of Pathology, Universidade Federal de Uberlândia, 38400-902 Uberlândia, MG, Brazil. E-mail: jrmineo@ufu.br

MATERIALS AND METHODS

Cerebrospinal fluid samples. This work was conducted in accordance with the guidelines of the Ethical Committee at the General Hospital at University of Uberlândia.

A total of 263 cerebrospinal fluid (CSF) samples were randomly obtained at the Clinical Laboratory of the General Hospital of the Federal University of Uberlândia, from July 1994 to April 1996. All samples were kept at -20°C until be tested.

After being tested for simultaneous detection of soluble SAG-1 (p30) antigen, IgG antibodies to *T. gondii* and immune complex, the results of these samples were retrospectively analyzed with the clinical data presented at the files from those patients.

Production of anti-P30 monoclonal antibodies. BALB/c mice were immunized i.p. with 1.5×10^7 tachyzoites of *T. gondii* RH strain previously treated by acetone at 30% for 72 h at 4°C , at days 0, 14, 28 and 42. At day 46, the spleen was removed and the spleen cells were fused with SP₂O/Ag 14¹⁷. The supernatants of hybridomas growing under the HAT selection were screened for anti-*T. gondii* antibodies by an indirect ELISA. The positive hybridomas were expanded and the cells were cloned by limiting dilution, being further assessed by another screening round. The positive clones were characterized by Western blot against SDS *T. gondii* lysates, demonstrating reactivity to a band of 30 kDa (p30). Further characterization was carried out by isotyping of anti-p30 monoclonal antibodies using the Mouse Typer-Bio-Rad Kit (Hercules, USA). 6A8 mAb to p30, which was obtained as described, was used as a positive control³¹.

Excreted-secreted antigens (ESA) *T. gondii* RH strain tachyzoites were harvested from mice peritoneal exsudates 2 days after the inoculation of parasites. The suspension was selected when parasite containing ranged from 10^7 to 10^8 parasites/ml, followed by centrifugation at 1000g for 15 min. The sediment was resuspended in 10 ml of sterile PBS at 4°C and centrifuged again at the same conditions as above described. The sediment was resuspended in 0.5 ml of sterile PBS, and incubated at 37°C for 45 min under eventual stirrings and centrifuged at 1000g for 15 min. The supernatant was filtered in 0.2 μm membrane (Sterile Acrodisc 13, Gelman Sciences, USA) and was stored at -20°C . The protein concentration of the ESA was determined by using bovin serum albumin as standard²⁸. The electrophoretic profile of the ESA proteins was analyzed by SDS-PAGE²⁵ in 15% gel without reducing conditions and stained by silver stain¹³. The p30 relative concentration in the ESA preparations was estimated by densitometry in different samples of the metabolic antigen. The determination was performed in a densitometer at 525 nm (Helena J. France S.A., Saint-Leula, Forêt, France).

P30 antigen detection. Monoclonal anti-p30 antibodies (6A8 and 6E9) were coated onto wells of polyvinylchloride microtiter plates (Falcon, Oxnard, USA) at concentration of 50 $\mu\text{g}/\text{ml}$. The volume added was 50 μl /well and the plates were incubated in a humid chamber, for 18 h at 4°C . After this incubation, the plates were washed three times for 5 minutes with PBS-T 0.05% (Sigma, St. Louis, USA). Following washing, 50 μl /well of each CSF sample was added and incubated for 2 h at 37°C . The negative controls consisted of CSF samples obtained from patients negative for anti-HIV and for p30 antigen, anti-*T. gondii*

antibodies and immune complex. ESA was added to samples with this same profile in order to obtain positive controls. Plates were incubated for 2 h at 37°C , in a humid chamber and were washed again three times. A volume of 50 μl /well of the biotinylated mouse IgG anti-*T. gondii* was added after diluted 1/200 in PBS-T 0.05%. The reaction was incubated for 45 min at 37°C and then washed in the same conditions described above. The avidin-biotin system (ABC-Vectastain Kit, Vector Laboratories, Inc., Burlingame, CA, USA) was used according to manufacturer instructions and, after incubation for 45 min and washes, the substrate solution was added. This consisted of a solution of H₂O₂ and o-phenylenediamine (Merk, Schardt, Germany) in 0.1M citrate-Na₂HPO₄ buffer, pH 5.5. The reaction was stopped after 15 min with 2N H₂SO₄. The reading was carried out at 492 nm in a spectrophotometer microwell reader system (Organon Teknika, Belgium) at 492 nm.

Anti-*T. gondii* IgG detection. Polyvinylchloride microplates were coated with 50 μl of ESA antigen (5 $\mu\text{g}/\text{ml}$) for 18 h at 4°C . The plates were washed three times with phosphate-buffered saline 0.01M pH 7.2 containing 0.1% Tween-20 (PBS-T) and blocked during 30 min at room temperature with 5% goat serum in PBS-T. After a new cycle of washes, 50 μl of undiluted CSF samples were added in duplicate and incubated for 45 min at 37°C . As controls, it was used negative and positive CSF samples for IgG anti-*T. gondii* antibody, and controls for conjugate and substrate, as well. Microplates were washed three times and 50 μl of goat IgG anti-human IgG peroxidase (Sigma, St. Louis, USA), diluted 1/4000 in PBS-T, were added and incubated for 45 min at 37°C . After another series of washes, as described above, the plates were incubated with substrate solution consisting of H₂O₂ and o-phenylenediamine in 0.1M citrate-Na₂HPO₄ buffer (pH 5.5) for 15 min at room temperature. The reaction was stopped with 2N H₂SO₄ and the absorbance was determined at microwell reader system (Organon Teknika, Belgium) at 492 nm.

Immune complex detection. Polyvinylchloride microplates were coated for 18 h at 4°C with 50 μl of IgG obtained from supernatants of 6A8 and 6E9 mAbs (50 $\mu\text{g}/\text{ml}$). After three washes with PBS-Tween 20 (0.05%), 50 μl /well of undiluted CSF samples were added and incubated for 2 h at 37°C . Positive and negative CSF samples to immune complex were used as control. The positive CSF samples consisted of a positive sample for anti-*T. gondii* IgG which was added of ESA and pre-incubated for 1 h at 37°C . After incubation for 45 min at 37°C and a new cycle of washes, it was added the conjugate goat IgG anti-human IgG-peroxidase. After incubation for 45 min at 37°C and another three washes, the substrate solution was added (50 μl /well) and the reaction was stopped as previously described.

ELISA sensitivity and specificity for P30 antigen detection. To determine the sensitivity of the P30 ELISA, the CSF samples were substituted by ESA solutions diluted in PBS-T 0.05%. The end point was obtained with the maximum dilution in which the absorbance values were higher than those obtained with the negative controls, determined as cut off values. The sensitivity was calculated based in P30 concentrations, obtained by densitometry as above described. The specificity of the P30 ELISA was determined by assessing 29 different antigenic extracts of microorganisms widely spread in nature, obtained by preparation of soluble extracts as generally used for intradermic cutaneous tests (Alerbras and Alergofar, Rio de Janeiro, Brasil): Candidina,

Escherichia coli, PPD, Tricofitina, Estreptoquinase/Estreptodornase, *Aspergillus sp.*, *Alternaria sp.*, *Cladosporium sp.*, *Rhizopus sp.*, *Penicillium sp.*, *Rhodotorula sp.*, *Diplococcus pneumoniae*, *Streptococcus viridiam*, *Streptococcus dyogene*, *Streptococcus faecalis*, *Neisseria catarrhalis*, *Staphylococcus sp.*, *Aerobacter aeruginosa*, *Proteus vulgaris*, *Salmonella* group, *Klebsiella pneumoniae*, *Shigella* group, *Staphylococcus toxoids*, *Streptococcus toxoids*, *Haemophilus influenzae*, *T. cruzi*, *Leishmania amazonensis*, cetonic extract of Dengue virus and soluble extract of *Cysticercus cellulosae*. All these preparations had a protein concentration varying from 20 to 25 µg. ESA preparation (19.5 µg) and PBS were used, as positive and negative controls, respectively.

Indirect ELISA for anti-HIV IgG detection. The reactions for the detection of anti-HIV IgG in the CSF samples were carried out using anti-HTLV-III Kit (“Vironostika” Organon Boxtel,) following the instructions of the manufacturer. The CSF samples were assayed undiluted at one volume of 50 µl.

Statistical analysis. The results of the studied tests were analyzed by the “Statistic for Windows- Release 4.5 A” program (Statesoft, Inc. 1993). χ^2 , McNemar and Fisher’s exact tests were performed for evaluation of the differences between two or more proportions. The null hypothesis was rejected when $p < .05$.

RESULTS

Characterization of the assays. Samples of excreted-secreted antigens were obtained (protein concentrations from .38 to 1.7 mg/ml) and analyzed by SDS-PAGE. Following staining and densitometry, the concentration of SAG-1 was determined and the sensitivity of the assay was estimated at a minimum of 5 ng. Specificity was investigated against unrelated antigens, with 29 samples comprising a group of pathogens commonly found in nature. By taking the cut off as the medium value of negative controls plus 3 standard-deviations, all these samples displayed absorbances lower than the cut off, thus evidencing the specificity of the test for detection of SAG-1 (p30).

Detection of soluble SAG-1(p30), antibody and immune complex in CSF samples. Among the 263 CSF samples examined, 96 (37%) were diagnosed as positive to HIV and 167 (63%) samples had negative results. A total of 164 (62%) CSF samples were positive to anti-*T. gondii* IgG and 99 (38%) were negative.

Table 1 shows the results of simultaneous detection of the markers P30 /Ab/IC among the test population. Among the 96 HIV seropositive group, 69 (72%) exhibited anti-*T. gondii* IgG whereas among the 167 anti-HIV negative samples, 95 (57%) were positive. There were no significant differences between the prevalence of anti-*T. gondii* IgG antibodies in the HIV positive samples and HIV negative samples ($p = 0.2349$).

P30 was detected in 119 (45%) of the CSF samples. Among the anti-HIV positive and negative samples, the positivity of p30 protein was 54% and 40%, respectively, showing a statistically significant difference between these two groups ($p < 0.05$) (Table 1).

Immune complex (IC) was detected in 50 samples (19%). Its incidence was higher in samples of the group of the HIV positive patients (29%) than in the samples of the HIV negative group (13%) ($p = 0.0009$) (Table 1).

The analysis of the results showed that positive results for both IgG anti-*T. gondii* and p30 antigen were found in 35% of the total CSF samples. Positive results for anti-*T. gondii* and negative results for p30 antigen were found in 27%, whereas negative results for anti-*T. gondii* and positive results for P30 were observed in 10% of the samples ($p < 0.001$) (Table 1).

The incidence of IC was higher in positive IgG anti-*T. gondii* samples (17%) than in negative samples (2%) ($\chi^2 = 16.98$; $p = 0.0001$) and was higher in p30 positive samples (16%) compared to p30 negative samples (3%) ($\chi^2 = 12.29$; $p < 0.001$).

Positive results for the three markers were found in 37 samples (14%) and results entirely negative were found in 72 (27%) of the samples. Positive samples for detection of p30 antigen or antibody were found in 21 (8%) and 65 (25%) of the studied samples, respectively.

Further comparison of these results was done based on the reactivity for HIV infection. It was observed that the incidence of positive results for all markers was higher in the HIV positive group (20%) than in the HIV negative group (11%) ($p < 0.05$). Considering the detection of at least one marker, represented by the SAG-1 (p30) antigen or immune complex, as indicative of active infection, the HIV positive group also showed higher incidence than the HIV negative group ($p < 0.01$). Figure 1 shows the correlation between the quantitative data for p30 and IC assays, demonstrating a significant correlation index for both HIV

Table 1
Results of the detection of molecular markers P30/Ab/IC (p30 soluble antigen, IgG anti-*T. gondii* and immune complex) obtained by immunoenzymatic assays in 263 cerebrospinal fluid samples that were positive or negative for IgG anti-HIV

Detection of P30 antigen, IgG antibody or Immune complex ^a								
	P30/Ab/IC	P30/Ab	P30/IC	P30	Ab/IC	Ab	No detection	Total
HIV+	19	23	4	6	5	22	17	96
HIV-	18	32	2	15	2	43	55	167
Total	37	55	6	21	7	65	72	263

^a (P30) = p30 antigen, major surface antigen of *T. gondii*; (Ab) = IgG antibody to *T. gondii*; (IC) = immune complex

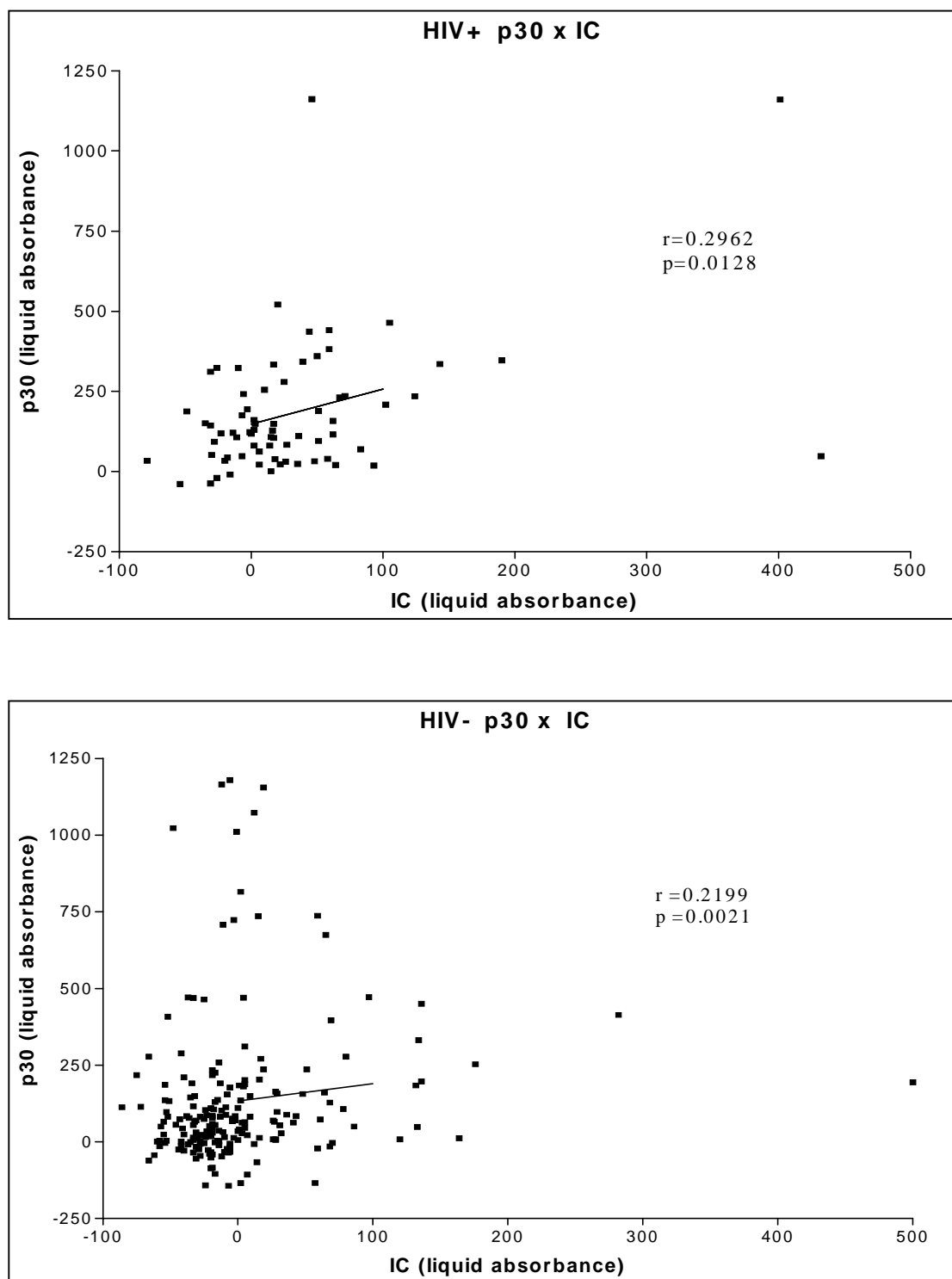


Fig. 1 - Correlation between the detection of P30 soluble antigen and immune complex (IC), obtained by immunoenzymatic assays in 263 cerebrospinal fluid samples from positive or negative IgG anti-HIV patients.

positive and HIV negative samples ($p = 0.0128$ and $p = 0.0021$, respectively).

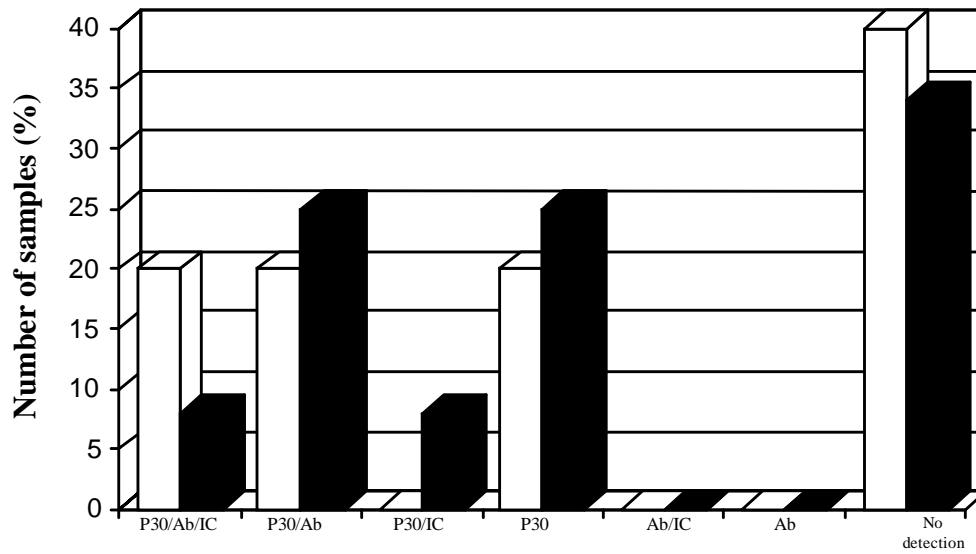
Association of clinical data with the results of the CSF markers. The presence of active infection was defined when the P30 antigen and/or Immune complex assays was/were positive in CSF samples. As demonstrated in Table 2 and according to the clinical data presented in the patient files, it was observed that the active infection was present in all groups, in samples from both HIV positive and negative patients.

Detection of p30 /Ab/IC in CSF samples from children. Figure 2 shows the results of simultaneous detection of the studied markers among 17 CSF samples from children aged from newborn to 12-month old. These samples were also tested for the presence of anti-HIV antibodies. It was found that 65% of these samples were positive for p30 and, among these, 36% were positive exclusively for this marker. The detection of P30/Ab/IC showed no significant difference between the HIV positive and HIV negative groups.

Table 2
Evidence of active *T. gondii* infection among HIV positive and HIV negative CSF samples according to the clinical data presented in the patient files

Clinical Hypothesis	Evidence of active infection ^a	Anti-HIV antibodies	
		Positive	Negative
Cerebral toxoplasmosis (acquired or congenital)	13/26 (50%)	9	4
Other CNS infectious diseases (cysticercosis, syphilis, cryptococcosis, meningococcal or aseptic meningitis)	30/63 (48%)	13	17
Not infectious CNS diseases	44/103 (43%)	15	29
Not available clinical data	39/71 (55%)	20	19
Total	126/263 (48%)	57	69

^aratio between the number of samples presenting active infection and the total number of samples. The presence of active infection was defined when the P30 antigen and/or Immune complex assays was/were positive in CSF samples.



Detection of P30 antigen, IgG antibody or Immune complex

Fig. 2 - Incidence of molecular markers P30/Ab/IC (P30 soluble antigen, IgG anti-*T. gondii* and immune complex) obtained by immunoenzymatic assays among 17 cerebrospinal fluid samples from children under 1 year-old that were positive (white columns) or negative (black columns) for IgG anti-HIV.

Table 3

Association of clinical signs obtained in the files of 17 patients aged from newborn to 12 month-old with the ELISA results to SAG-1 (p30) *T. gondii* antigen, antibody, and immune-complex in cerebrospinal fluid samples

Sample Number	Age	Detection of p30 antigen (P30), Antibody (Ab) and/or Immune-complex (IC)										Clinical signs in the patient files	
		P30/Ab/IC		P30/Ab		P30/IC		P30		No detection			
		HIV +	HIV -	HIV +	HIV -	HIV +	HIV -	HIV +	HIV -	HIV +	HIV -		
1	12m				x								Congenital toxoplasmosis
2	11m		x										Cerebral paralysis
3	12m			x									Hydrocephalus Myelomeningocele
4	3m				x								Hydrocephalus
5	3m				x								Hydrocephalus
6	NB							x					Cerebral malformation, with areas of encephalic absence
7	1m										x		Malnutrition, anemia, dehydration
8	NB	x											Intracranial hemorrhage, hydrocephalus
9	NB									x			NA
10	NB							x					Arm muscle tonus disturbs
11	2m							x					NA
12	12m										x		Treated bacterial meningitis
13	12m										x		Hydrocephalus
14	12m										x		Convulsions
15	12m						x						Cerebral cysts, meningitis
16	1m									x			Marasmus, vomit
17	1m								x				Congenital syphilis

m= month NB= newborn X= association between test results and clinical data NA= not available

Association of clinical data with the results obtained in the CSF samples from children. Table 3 shows that the results expressing active infection accomplished 65% of the samples. Among ten patients presenting at least one symptom related to the Sabin's tetrad, 8 (80%) of them presented CSF samples considered positive for active infection. Five out of nine samples with clinical evidences of other diseases were totally negative for p30, IgG antibody and immune complex. The remaining four samples were positive for only the p30 antigen. In addition, the presence of p30 and IC was occurred in 6/7 children with clinical signs of hydrocephalus and generalized brain affection as compared with 0/10 in other diseases. Even if the two cases without clinical diagnosis will be excluded, it was observed a significant definition of those tests in this group of patients ($p = 0.0014$).

Detection of p30/Ab/IC in multiple CSF samples from AIDS patients. Figure 3 shows the results of simultaneous detection of the markers p30/Ab/IC in multiple samples from 3 patients who were HIV positive and had clinical symptoms of toxoplasma encephalitis. It was observed that the detection of p30 was positive in these individuals. Moreover, there was a significant increase in reactivity of the CSF samples during disease progression. In only one patient the p30 detection was

accomplished by the detection of antibody against *T. gondii* and increasing amounts of immune complex (Figure 3B).

DISCUSSION

The diagnosis of *T. gondii* infection in humans can be determined by a variety of immunological molecular methods. The identification of the parasite in blood and other organic fluids or in tissue can confirm the etiology of the infection. Although presence of tissue cysts and free tachyzoites in brain or in placenta is diagnostic, it is impractical. Current parasitologic methods for the detection of parasites are at insufficient sensitivity for accurate detection of infection in the newborn or compromised host. Therefore, the methods dealing with anti-*T. gondii* antibody or circulating antigen detection are more widely used. The methodology for antibody detection is well established, allowing the identification of serologic profiles, correlated with the stage of the infection^{5,6}. The presence of circulating antigen from the parasite in infected human beings has been intensively investigated and consists in an important indicator of active infection. So far, it has been described that the detection of circulating antigen has limited value in the immunocompromised individuals or newborns²⁷.

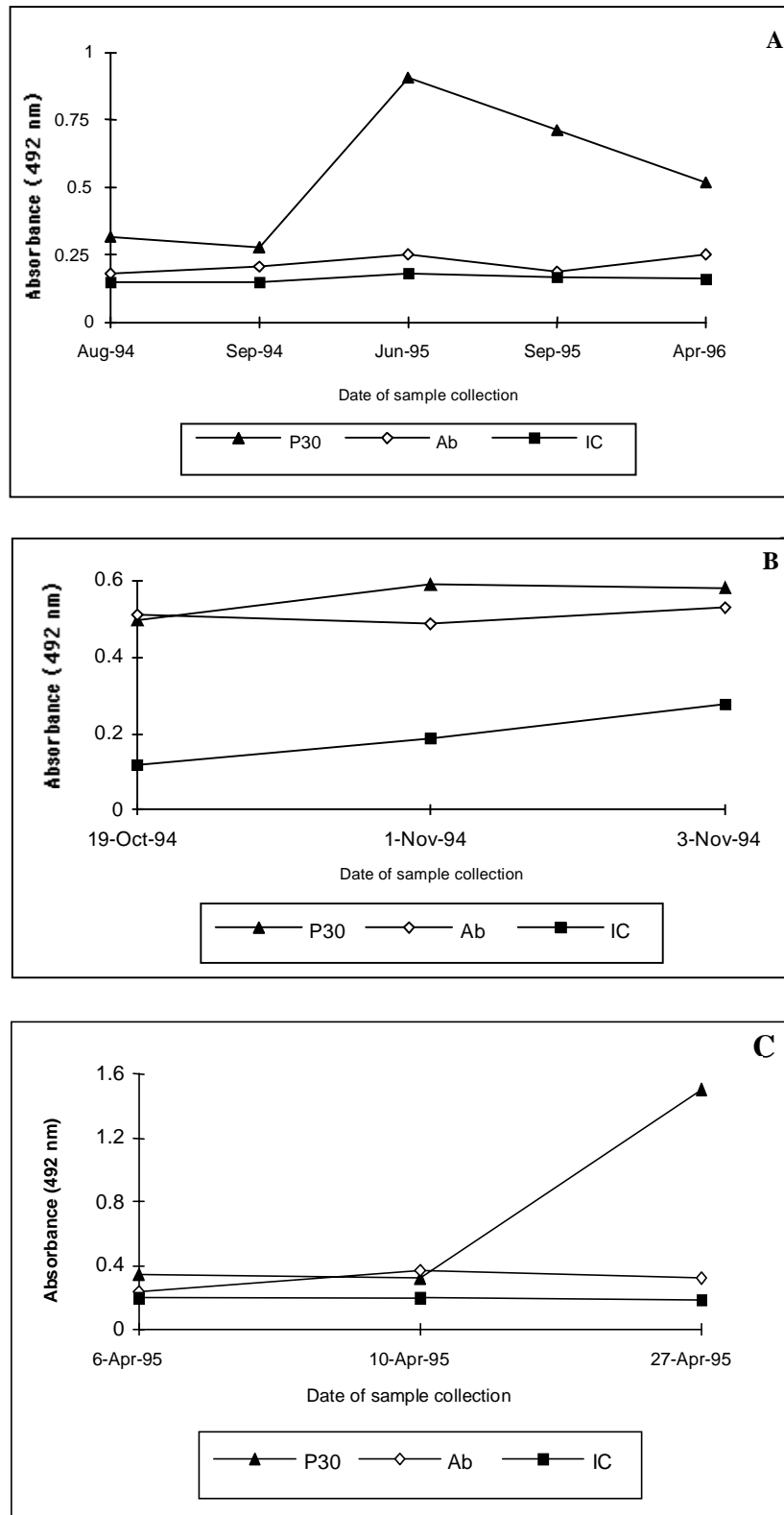


Fig. 3 - Detection of p30 soluble antigen (P30), IgG anti-*T. gondii* (Ab) and immune complex (IC) by immunoenzymatic assays in CSF samples from patients with *Toxoplasma encephalitis*. (A) D.M.F., HIV-seropositive, 35 year-old, female; (B) N.P.S., HIV-seropositive, 48 year-old, male; (C) D.F.S., HIV-seropositive, 18 year-old, male.

The present study characterizes the profile of the simultaneous detection of p30 antigen of *T. gondii*, antibody and immune complex in CSF samples of HIV seropositive and seronegative individuals. Among 263 CSF samples randomly collected, approximately one third came from HIV positive patients. These data demonstrate the high index of HIV patients among those searching for medical assistance, probably because they do have developed neurological disorders, indicated by the fulfillment of lumbar puncture to obtain CSF samples.

The high prevalence of anti-*T. gondii* antibodies (62%) among the samples tested reflects the high degree of the infection in the region. This prevalence did not differ between positive and negative HIV samples. These results are consistent with studies that suggest toxoplasmic encephalitis in AIDS is due to reactivation of latent infection than acquired acute infection²⁹. These results also suggest that the reactivation of the infection may be correlated with p30 levels at CSF. Since p30 is a stage-specific marker for the tachyzoites, it is indicator of active disease. Considering the high prevalence of the *T. gondii* infection among the HIV positive samples, it has to point out the importance of the knowledge of the reactivity profile for toxoplasmosis since that positive results can alert to future complications coming upon from AIDS.

The presence of p30 antigen among HIV positive (54%) and negative (40%) samples was high, indicating that detection of p30 protein has diagnostic value in both groups of patients. Thus, it appears that the rise in p30 levels may be correlated with onset of active infection.

It was shown that p30 antigen was detected in 35% of the CSF samples which also presented IgG anti-*T. gondii* antibodies. From the total p30 positive CSF samples, 10% displayed negative results to anti-*T. gondii* IgG. The absence of anti-*T. gondii* IgG antibodies in serum and/or CSF samples in patients with toxoplasmosis has been reported in the literature²¹. Circulating antigen of this parasite was detected in serological negative samples in patients with toxoplasmosis¹⁶ and antigens of molecular weight of 4 kDa were identified³³. The presence of clinical symptoms suggestive of acquired infection as lymphadenopathy, and negative serology can not exclude the toxoplasmosis diagnosis because the humoral responses in some patients can be delayed creating difficulty in the definitive diagnosis^{8,20}.

Although intrathecal antibody production may establish a specific indicator of toxoplasmic encephalitis, approximately 50% of the patients will not present a detection of antibody in the CSF samples^{34,40}. Important differences related to the avidity of the IgG antibody can be detected when it was compared paired samples of the CSF/serum and constitute in a direct indicator of the IgG intrathecal production³². The presence of anti-*T. gondii* IgG antibodies of low avidity in serum samples of patients presenting recent primary infection was described in the literature¹⁸. Evidence of compartmentalization of anti-*T. gondii* humoral immune response was observed by the synthesis of IgG antibodies of low avidity in aqueous humor samples of patients presenting toxoplasmic retinochoroiditis³⁹.

The blood-brain barrier may represent an obstacle to immunoglobulin and mononuclear cells passage from the blood to the CSF. This data perhaps can justify the non detection of anti-*T. gondii* IgG antibodies in the CSF samples that presented the p30 antigen. However, this antigen

can be transported from the blood or from brain extracellular fluid to the CSF through the cuboid cells of plexus-coroide. Besides molecules that are specifically transported, hydrophobic molecules can exceed the barrier³⁶. Perhaps, this fate explains the high incidence of P30 protein in the CSF samples here examined. It was verified recently, by immunohistochemical studies of the brain, that the incidence of infection of the choroid plexus in AIDS patients with cerebral toxoplasmosis was 53% in a total of 17 cases¹¹.

Presence of immune complex, associated with the absence of detectable levels of antibodies or free P30 can be an indicative of the stage of immune response to the infection. Predominance of immune complex in HIV positive samples may be associated with compromising of the mononuclear phagocytic system responsible for IC clearance from CSF. This could promote a longer persistence of the IC in this and other organic fluids^{19,22}.

Combined positive results for p30/Ab/IC were more frequent in HIV positive samples. Among the 62% of samples with anti-*T. gondii* antibodies, this marker was the only positive reaction in 25% of the total samples, while p30 or IC were also positive in the remaining 37%. These observations support the hypothesis that the search for the three markers (p30/Ab/IC) might add a new piece of information in the pathogenesis of human toxoplasmic encephalitis. Moreover, detection of p30 in 45% of the studied CSF samples indicates a high incidence of active infection in the group here included.

Taken into account the results of these three markers, the presence of active infection was detected in CSF samples from patients presenting various clinical data, but it should be pointed out that some of them the first diagnostic hypothesis was not cerebral toxoplasmosis. This emphasizes the clinical application of these assays, with the objective of helping diagnosis and improving prognosis of those patients infected with *T. gondii*.

RESUMO

Deteção de antígeno solúvel de *Toxoplasma gondii* SAG-1(p30), anticorpo e imuno complexo em amostras de líquido cefalorraqueano de pacientes HIV positivos ou negativos

Foi investigada a presença de infecção ativa pelo *T. gondii* em pacientes hospitalizados apresentando acometimentos neurológicos diversos. Amostras de líquido cefalorraquiano (LCR) foram analisadas em imunoenaios padronizados para a detecção de três marcadores moleculares: antígeno solúvel contendo epítipo imunodominante da superfície parasitária SAG-1 (p30), anticorpos específicos e imunocomplexos. Foram coletadas 263 amostras de LCR que foram inicialmente analisadas quanto a presença de anticorpos anti-HIV. Os pacientes foram divididos em dois grupos: HIV positivos (n = 96) ou HIV negativos (n = 167). Os resultados dos ensaios demonstraram que 45% do total de amostras foram positivas para o antígeno SAG-1 (p30). Imunocomplexos foram detectados em 19% das amostras analisadas e 62% foram positivas para anticorpos IgG anti-*T. gondii*. A associação destes resultados com os achados clínicos consistentes com a presença de infecção ativa pelo *Toxoplasma* pode ser de importância no diagnóstico de encefalite toxoplásmica.

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