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DETECTION OF *CRYPTOCOCCUS NEOFORMANS* CAPSULAR POLYSACCHARIDE ANTIGEN IN ASYMPTOMATIC HIV-INFECTED PATIENTS

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SUMMARY

Serum samples from 242 HIV-positive persons were studied for the detection of capsular polysaccharide antigen of *Cryptococcus neoformans*; 193 of these patients presented less than 300 CD4+ cells/µl of blood and 49 patients had more than 300 CD4+ cells/µl.
None of them had symptoms or signs characteristic of cryptococcosis.
The capsular antigen of *C. neofarmans* was detected by latex agglutination technique with pronase pretreatment (IMMY, Crypto-Latex Antigen Detection System, Immunomycologics Inc., OK, USA); in 61% of the samples, ELISA technique was also used (Premier, Cryptococcal Antigen, Meridian Diagnostic Inc., Cincinatti, Oh, USA). The comparative study of both methods showed that the results obtained were similar in 96.9% of the cases.
The capsular antigen was detected in 13 out of 193 (6.7%) patients with less than 300 CD4+ cells/µl. Cryptococcosis was confirmed mycologically in 3 of these 13 cases (23%) by the isolation of *C. neoformans* in CSF or blood cultures. Three patients, who had presented negative results of both tests for capsular antigen, suffered disseminated cryptococcosis 4 to 8 months later.
The predictive diagnostic value of capsular antigen detection of *C. neoformans* seems to be low and we believe that it should not be done routinely in asymptomatic HIV-positive persons.

KEYWORDS: Cryptococcosis; Antigenemia; Latex agglutination; ELISA tests.

INTRODUCTION

Cryptococcosis is the potentially fatal systemic mycosis most frequently associated with AIDS. It holds the fourth position among the severe infections affecting this group of patients in Argentina, after pneumocystosis, tuberculosis and infections caused by the *Herpetoviridae* family[1].

In recent experiences carried out at Hospital F.J. Muñiz, we have been able to show that 35% of the patients with AIDS related meningeal cryptococcosis die in the first 4 weeks post diagnosis, despite therapy[8,13]. In 10% of patients, diagnosis was confirmed after their death, since direct microscopic examinations were negative and only CSF cultures were positive[1].

Although there are no official data on the frequency of AIDS related cryptococcosis in our country, we have estimated that approximately 15% of the patients hospitalized at our institution have had this mycosis. These data are consistent with a recent estimation made at Rawson Hospital, in Córdoba City, and they both place Argentina among the countries with high incidence of cryptococcosis, less than in Central Africa but higher than in USA[10].

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The studies carried out for detection of *Cryptococcus neoformans* capsular polysaccharide antigen in blood samples of HIV-infected persons without defined symptoms of cryptococcal infection, have shown different results in Denmark and Zaire[2,4]. The Danish authors examined 530 serum samples and 50 CSF samples from 334 HIV positive patients, most of them with AIDS or AIDS related complex, to determine the presence of *C. neoformans* antigens by latex agglutination. All samples were negative. They also retrospectively studied the serum and CSF of three patients with confirmed cryptococcosis, with positive results. The authors concluded that this reaction should not be done systematically in Denmark, due to the low incidence of cryptococcosis in this country[4].

The investigators from Zaire, using the same technique, examined 450 serum samples from HIV positive or AIDS patients and found 55 positive results (12.2%). They obtained CSF from 44 of the 55 cases and showed the presence of *C. neoformans*, through microscopic evaluation or culture in 29 (66%) of them[2], culture media used in both studies were similar.

A study carried out in an area of intermediate epidemiologic characteristics among both countries, would contribute to some decisions, such as primary prophylaxis for this mycosis.

It seems that the systematic search of antigenemia would be justified in countries with high incidence of cryptococcosis, like Zaire, even in the abscence of the typical signs of cryptococcosis, as well as the indication of primary prophylaxis, conversely, in countries with low incidence of cryptococcosis related to AIDS, the cost-benefit ratio would not justify any of both measures. We do not know which is the case in an area of intermediate epidemiological characteristics.

Despite the seriousness of cryptococcal infection, primary prophylaxis has not been universally accepted, probably due to its high costs and the interaction of the azoles with other medications that many of these patients must take.

This study was aimed at determining the frequency of detection of capsular antigen of *C. neoformans* in blood samples of HIV-infected patients with less than 300 CD4+ lymphocytes/µl, using latex particle agglutination test and ELISA. The predictive value of both techniques in contributing to the final diagnosis of cryptococcosis was also studied.

MATERIALS AND METHODS

Studied subjects

Blood samples of 242 persons with positive HIV serology (presumptive and confirmatory) were analyzed, of them 193 had less than 300 CD4+/µl and 49 had more than 300 CD4+ cells/µl. None of the patients showed symptoms that may lead to suspicion of cryptococcal infection. This study had no exclusion criteria based on sex or age.

The samples were stored at -20° C until processed.

Techniques

All blood samples were studied by latex particles agglutination technique with rabbit immunoglobulin anti-*C. neoformans* capsular polysaccharide, using the IMMY Latex-Crypto antigen detection system, Norman OK, origin USA. This system contains pronase in order to increase the specificity of the test. ELISA technique for detection of *C. neoformans* capsular antigen was also used in 160 samples (66.1%), with Premier^{R} Cryptococcal Antigen system, from Medirian Diagnostic Inc., Cincinnati, Ohio, USA. The equipments used in this study do not specify which antigens were used to produce their respective antibodies and, therefore, their results are not comparable.

Serum samples from 49 HIV positive patients with more than 300 CD4+ cells/µl were considered as controls.

Studies in patients with positive antigenemia

All patients with positive latex particles agglutination or ELISA tests for capsular antigen were submitted to mycologic studies to confirm or discard the diagnosis of cryptococcosis. In total, 11 CSF samples and 12 urine samples were studied, from the 13 cases with positive serum results.

TABLE 1

Results of latex particles agglutination and ELISA techniques, for detection of *C. neoformans* capsular antigen.

CD4 + count	Latex Agglutination		ELISA	
	Negative	Positive	Negative	Positive
> 300/µl	49	0	0	0
< 300/µl	181	12	151	9
TOTAL	230	12	151	9

1. *Blood cultures*. Three samples, 8 ml each, were obtained from the veins of the patients and were placed in sterile tubes containing 1 ml 5% saponin solution with 0.4% sodium sulphonate polyanethol in isotonic saline solution. The tubes were shaken during some minutes for good mixing of the contents and were then kept at 37°C during 15 minutes for lysis completion. They were subsequently centrifugated at 2000 rpm during 10 minutes, the supernatant was discarded and the sediment was seeded in 2 tubes with Sabouraud honey-agar and other 2 tubes with brain heart infusion agar, which were kept at 28° C and 37° C respectively, for 3 weeks.

2. *Mycologic studies of CSF and urine*. Both samples were centrifugated at 2000 rpm during 10 minutes, the sediments were examined as wet smears between the slide and the coverslip, with a drop of diluted India ink. The remaining portions of the sediments were used for culture in Sabouraud honey-agar, brain and heart infusion agar and sun flower seed extract agar with the addition of 100 µg/ml ampicillin. Cultures were incubated at 28° C and 37° C during 3 weeks.

3. *Search of C. neoformans capsular antigen in CSF and urine*. The presence of *C. neoformans* capsular antigen in CSF and urine supernatant was determined by the latex agglutination technique. Only this technique was used in CSF and urine, with the same equipment used for serum samples.We did not use concentrated samples of any material.

4. *Clinical controls*. Patients with positive antigenemia for *C. neoformans* were clinically evaluated and controlled, when possible, in several occasions, every 2 or 3 months during 1 year.

RESULTS

The results of *C. neoformans* capsular antigen determination in blood samples are summarized in Table 1.

The results of latex particles agglutination and ELISA techniques for capsular antigen were similar in 96.9% of the samples examined by both methods. Only one sample which was positive with ELISA was negative with latex agglutination and, conversely, 4 samples which were positive with the latter method, were negative with ELISA.

The presence of *C. neoformans* capsular antigen was detected in 13 cases studied with any of the two techniques used, all corresponding to patients with less than 300 CD4+ cells/µl and only in 3 of them (23%) the diagnosis of cryptococcosis was confirmed by mycologic studies. In nine patients of the remaining group, who could be followed-up frequently, the diagnosis could not be confirmed and 1 patient did not return for new examinations. The studies carried out in this group of patients are summarized in Table 2.

Three patients with negative antigenemia developed systemic cryptococcosis after 4-8 months.

The studies for determination of antigenemia were positive in 6.7% of the HIV-infected patients with less than 300 CD4+ cells/ul; none of the 49 HIV-infected patients with CD4+ counts above this limit had positive antigenemia.

DISCUSSION AND CONCLUSIONS

One study similar to ours, carried out in Zaire, showed 12% of positive antigenemia in HIV-infected patients and in 66% of them, the diagnosis of cryptococcosis was confirmed by culture[2]. On the contrary, a study done in Denmark showed the uselessness of the systematic search of *C. neoformans* antigenemia in HIV-infected patients [4,9]. The results of our study showed 6.7% positive antigenemia in HIV positive persons with less than 300 CD4+ cells/µl; in only 23% of these patients, the diagnosis of cryptococcosis could be confirmed by culture. The results of our study are intermediate in comparison with the other two previous studies, apparently coinciding with an intermediate endemic situation between western developed countries, with low incidence of cryptococcosis, and Central Africa and Southeastern Asia, where incidence of this mycosis is high[12,15].

The studies for antigenemia showed a low predictive value of cryptococcosis associated with AIDS; only 23% of the patients with positive antigenemia had cryptococcosis confirmed by culture and there were 3 cases with negative antigenemia which, after some months, developed this mycosis.

We are not aware of the reason why 10 persons had positive antigenemia for *C. neoformans*; probable causes of false positive results could be *Trichosporon* infection, and *Klebsiella* and DF-2 bacterial infections[5,16]. The system used adding pronase eliminates the possibility of false positive reactions by rheumatoid factor[5,9].

As shown by other authors, the results obtained with latex particles agglutination and ELISA techniques, were similar in over 95% of the samples[3,6,7,8,14]. Based on a cost-benefit relation, we believe that the systematic study of *C. neoformans* antigenemia in HIV positive patients with less than 300 CD4+ cells/µl, is not justified in our country.

This conclusion is based on the low predictive value shown by the search of the capsular antigen of *C. neoformans* in our study. Only 23% of the positive cases effec-

TABLE 2

Results of studies carried out in patients with positive antigenemia.

Study/Cases	1	2	3	4	5	6	7	8	9	10	11	12	13
First study													
a. Antigen													
LA	+	+	+	+	+	+	+	+	+	+	+	+	-
ELISA	+	+	+	+	+	-	-	-	+	-	+	+	+
b. Blood Culture													
1	-	-	-	-	-	-	-	-	-		-	+	
2	-	-	-	-	-	-	-	-	-		-	+	
c. Urine													
Direct ex.	-	-	-	-	-	-	-	-	-		-	-	-
Culture	-	-	-	-	-	-	-	-	-		-	-	-
Antigenuria	-	-	-	-	-	-	-	-	-		-	+	+
d. CFS													
Indian Ink	-	-	-	-	-	-	-	-	-		+		+
Culture	-	-	-	-	-	-	-	-	-		+		+
CSF - Antigen	-	-	-	-	-	-	-	-	-		+		+
1st control													
a. Antigen													
LA	-	+	-	-	-	-	-				+		+
ELISA	-	+	-	-		-	-						
b.Blood Culture						-							
c. Urine Culture						-							
d. CSF													
Indian Ink					-		-						
Culture					-		-						
CSF antigen							-						
2nd control													
a. Antigen													
LA			-			-			-				
ELISA			-										
b. Blood Culture									-				
c. Urine Culture									-				
Antigenuria			-						-				
d. CSF													
Indian Ink			-										
Culture			-										
CSF antigen			-										

References
LA: latex agglutination test
ELISA: enzyme-linked immunoassay
CSF: cerebrospinal fluid
+: positive
-: negative

tively developed cryptococcosis and three patients who had had negative antigenemia, developed cryptococcosis in a period from 4 to 8 months. In a developing country like Argentina, the cost of the equipment needed for this determination is very high and the effectiveness of systematic use was low.

Regarding prophylaxis, which is done empirically in some centers, it is costly, it does not totally avoid the occurrence of disseminated cryptococcosis, and it may cause side effects and drug interactions. For all these reasons, we believe that the treatment of patients based only on the results of the antigenemia is not justified. The presence of the antigen in CSF or the finding of *C. neoformans* are the basis for the indication of therapy.

This study could be interesting in regions of higher endemicity, such as Central Africa or Southeastern Asia.

RESUMEN

Detección del antígeno capsular del *Cryptococcus neoformans* en pacientes asintomáticos infectados por HIV

Fueron examinadas las muestras de suero de 242 personas, HIV positivas, para determinar la presencia de antígeno capsular del *C. neoformans*, 193 de estos pacientes tenían recuentos de células CD4 + inferiores a los 300/µl y 49 pacientes presentaron recuentos superiores a este límite. Ninguno de los enfermos tenía sintomatología que hiciese sospechar criptococosis. El antígeno capsular del *C. neoformans* fue determinado por una técnica de aglutinación de partículas de látex previo tratamiento con pronasa (IMM, latex-Crypto antigen detection system, Immunomycologics, Oh, USA) y 61% de las muestras fueron también examinadas mediante la técnica de ELISA (Premier, Cryptococcal Antigen, Medirian Diagnostic Inc, Cincinatti, OH, USA). Los resultados de ambas técnicas fueron coincidentes en 96.9% de los casos.

Pudo comprobarse la presencia de antígeno capsular del *C. neoformans* en 13 casos entre los 193 pacientes HIV positivos, con recuentos de células CD4+ menores de 300/µl (6.7%), ningún paciente con recuentos superiores a este límite presentó antigenemia positiva. En 3/13 enfermos (23%) pudo confirmarse el diagnóstico de criptococcosis mediante el aislamiento del *C. neoformans* en LCR o hemocultivos. Tres pacientes que habían presentado antigenemias negativas sufrieron 4 a 8 meses después, criptococosis diseminada.

Se considera que el valor diagnóstico predictivo de la detección de antígeno capsular del *C. neoformans* es bajo y que la realización sistemática de esta reacción no se justifica.

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