

## EVALUATION OF THE CRYPTOCOCCAL ANTIGEN TEST AS A DIAGNOSTIC TOOL OF AIDS-ASSOCIATED CRYPTOCOCCOSIS IN RWANDA

par

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**Summary.** — To evaluate the latex test, two different retrospective studies were undertaken. A positive culture for *Cr. neoformans* was used as the golden standard of active cryptococcal infection.

- 439 sera selected at random sent to the NSP laboratory for screening of HIV antibody were tested as well as
- 71 CSF from patients with meningeal symptoms sent to the laboratory of the Centre Hospitalier de Kigali

In total, two discrepancies were found: two CSF samples from ancient cases of cryptococcosis under treatment were positive with the latex test and negative by culture.

If it stands to reason that the antigen test cannot differentiate between active and inactive cryptococcal diseases, the persistence of small amounts of soluble antigens in a CSF implies that the patient must remain under surveillance, a relapse being very frequent in AIDS patients.

As a conclusion, the latex test is a fast, easy to perform and quite fiable test for the diagnosis of cryptococcosis.

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**KEYWORDS:** Cryptococcosis; AIDS; Laboratory diagnosis; Latex test; Rwanda.

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### 1. Introduction

Disseminated including meningeal cryptococcosis is the fourth most commonly recognized cause of life-threatening infections in AIDS patients in Europe and the USA (11). Its prevalence in those patients living in or recently emigrated from Central Africa can be as high as 13 to 35% (9, 15). These figures are much higher than those reported from the USA where apparently only 6% of AIDS patients acquire cryptococcosis (14).

Mycological diagnosis will rest on direct microscopy, isolation from biological specimens, occasionally on histology or on the latex cryptococcal antigen test, an agglutination test that allows the detection of soluble antigens in the serum or in the cerebrospinal fluid (CSF) of the patients.

In Rwanda, at the Centre Hospitalier de Kigali (CHK), two new cases of AIDS-associated cryptococcal meningoencephalitis are diagnosed weekly (19). Direct examination and culture of the CSF are performed in each patient with unexplained headache or altered consciousness, meningeal signs and/or fever.

In order to assess the diagnostic yield of the latex cryptococcal antigen test (LCAT), a study was initiated on serums and CSF samples collected at the CHK.

A positive culture for *Cr. neoformans* from CSF for meningeal cryptococcosis, from sputum or bronchoalveolar lavage fluid for pulmonary cryptococcosis was used as the golden standard of active cryptococcal infection.

## 2. Material and methods

The screening of soluble cryptococcal antigen was carried out with a commercial kit (Latex-Crypto-Antigen-Detection-System/Immuno-Mycologics Inc.). This is a slide agglutination test containing latex particles sensitized with anticryptococcal rabbit polyclonal globulin and pronase to eliminate interference factors due to the presence of the rheumatoid factor (1, 4, 8, 17). 300  $\mu$ l of sérum or CSF are dispensed into a test tube containing 50  $\mu$ l of pronase. The tubes are placed in a waterbath at 56 °C for 30 min. A screening test is performed on each sample by placing 25  $\mu$ l of latex suspension on a slide to which is added 25  $\mu$ l of the treated serum or CSF, allowing it to mix on a rotator for 5 min.

Tests for positive and negative controls are included on the slide. A quantitative test is performed on those samples which are positive with the screening test. Doubling dilution (two-fold) will be performed on these samples to determine the antigen titer.

Two different retrospective studies were undertaken during a 6 weeks period in July and August 1990: 439 sera, selected at random and sent to the National AIDS Surveillance Program (NSP) laboratory for screening of HIV antibody (ELISA and Western Blot confirmation) were tested and all the CSF from patients with meningeal symptoms i.e. 71 sent to the Department of Microbiology of the CHK for cryptococcal diagnosis, were controlled for soluble cryptococcal antigen. Interpretation of the results was blinded.

## 3. Results

### a) Antigen detection in 439 sera

The results are summarized in table 1.

Out of 439 sera sent for HIV serological testing, 226 were negative and 213 positive. No cryptococcal antigen was found in any of the 226 sera from HIV negative patients. Nine out of 213 HIV positive patients (4.2%) were positive for cryptococcal antigen.

All of them were known cases already diagnosed by culture. The titers were: 16 (1 $\times$ ); 32 (2 $\times$ ); 2,048 (2 $\times$ ); 8,192 (2 $\times$ ); 16,384 (2 $\times$ ).

The patient with a titer of 16 had only pulmonary cryptococcosis, didn't show any sign or symptom suggestive for meningitis, his CSF was negative. *Cr. neoformans* was only isolated from bronchoalveolar lavage fluid. The eight others showed signs and symptoms suggestive for meningitis, their CSF had been positive by culture.

TABLE 1  
Antigen detection in 439 sera  
from HIV positive or negative patients

HIV	+	-	
Ag. crypto			
+	9	0	9
-	204	226	430
	213	226	439

b) *Antigen detection in 71 CSF*

Cryptococcal antigen was found in 21 CSF from patients with meningeal symptoms (30%). Direct examination and culture had been performed on the 71 CSF. The results are summarized in tables 2 a and b.

TABLE 2  
Antigen detection in 71 CSF from patients  
with symptoms of cryptococcal infection

a) *Compared with the results of the direct examination*

Dir. Ex.	+	-	
Ag. crypto			
+	17	4	21
-	1	49	50
	18	53	71

b) *Compared with the results of the culture i.e. golden standard*

culture	+	-	
Ag. crypto			
+	19	2	21
-	0	50	50
	19	52	71

Yeasts had been seen by direct examination in 18 of the 71 CSF. In 17 of the 18 cases, the morphology of the yeast was compatible with *Cr. neoformans*, showing the presence of a capsule and the culture was positive. Antigen was detected in those 17 CSF. The last CSF sample yielded non-encapsulated yeasts which were proved to be a *Candida guilliermondii*. The latex cryptococcal antigen test was negative.

Antigen was detected in four of the 53 (7.5%) samples of which direct examination was negative.

Two of them were new cases, the titers were 256 and 512 and the cultures in process were later positive. The other two were ancient cases under

treatment. The titers were respectively 8 and 32 and the cultures remained negative. Compared with the golden standard, the sensitivity was 100% (19/19) and the specificity was 96% (50/52).

#### 4. Discussion

The results obtained on serum confirm that the HIV infection remains the most important predisposing factor for cryptococcosis in Rwanda (19), as 100% of the patients with a positive LCAT were also HIV positive patients against 47% (204/430) of patients with a negative LCAT. This difference was, however, not statistically significant ( $\chi^2 = 2.53$ ).

As the study was retrospective, we could immediately establish that there was no discrepancy between the results of the latex test and cultures and that the 9 patients with soluble antigen in the serum were known cases of cryptococcosis already diagnosed by culture.

From the results obtained on CSF, we can say that the specificity of the latex test was corroborated by its negativity for a CSF yielding *Candida guilliermondii* and not *Cryptococcus neoformans*. Four of the 53 CSF samples negative by direct examination were positive for the latex test, two of the 4, being positive by culture, the other two, negative by culture, being ancient cases under treatment. This confirms a well known observation that even if the direct examination remains a useful test, its sensitivity is slightly lower than that of the LCAT (2, 7). From this point of view, culture is better but takes two days. However, two samples from ancient cases were positive with the LCAT and negative by culture. If it stands to reason that the antigen test cannot differentiate between active and inactive cryptococcal diseases, the persistence of small amounts of soluble antigens in a CSF rings the alarm showing that the patient must remain under surveillance (3). Relapses are indeed very frequent after treatment, especially if there is no maintenance therapy (2). Staib and Seibold (16) have drawn attention to the mycological diagnostic follow-up examinations in a case successfully treated so that the yeast was no longer viable in the CSF and lung.

At the autopsy, the fungus could nevertheless be isolated from the prostate which was found to have been completely colonized by *Cr. neoformans* but there was no growth in material from the CSF or lung.

The Latex-Crypto-Antigen-Detection System/Immuno-Mycologics kit is a very sensitive polyclonal latex kit. It detects the capsular polysaccharide in concentration of approximately 5  $\mu\text{g/ml}$  or greater following the form joined to the kit.

And indeed in the laboratory of mycology of the Institute of Tropical Medicine, this kit has been used for years on several hundreds of sera and CSF, compared with other kits (Calas and Slidex Crypto-Kit, unpublished results) and we are convinced that it remains one of the best polyclonal latex tests on the market (15). Nevertheless, as well as for all the other latex kits, cross-reactions are noted in sera of patients with trichosporon infections (12, 13). Let us mention that trichosporosis in comparison with cryptococcosis especially in AIDS patients, occurs very rarely so that the existence of cross-reactions doesn't justify a delay in treating if a latex test turns positive in a severely ill patient. Nevertheless, therefore, a positive serology must always be followed by a culture for confirmation.

A monoclonal latex test (5) and an ELISA test (6, 10, 18) have recently been proposed. But even if the first assays were promising, more evaluation still remains to be done.

In conclusion, how useful is the LCAT? It is a fast, easy to perform and quite fiable test. Providing costs are no problem (around 100 BF), the test can be included in the series of controls performed on the serum of each HIV suspected patient. In other socially less favorable environments where the test is less easily available, the LCAT should be included among the investigations performed on the serum of each HIV seropositive patient with unexplained headache, altered consciousness, fever, pulmonary infiltrates.

It should also be performed on the CSF of all patients with suspected cryptococcal meningitis and a negative direct examination. The LCAT is also useful to monitor the progress of patients under treatment even if their CSF cultures become negative.

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#### **Evaluation du Test de Recherches d'Antigènes Cryptococciques dans le diagnostic de la cryptococcose associée au SIDA au Rwanda.**

*Résumé.* — Afin d'évaluer le test de recherches d'antigènes cryptococciques, deux études rétrospectives ont été entreprises. L'obtention d'une culture positive pour *Cr. neoformans* a été choisie comme témoin de l'existence d'une cryptococcose active.

- 439 sérums sélectionnés au hasard, envoyés au laboratoire NSP pour le dépistage des antigènes HIV ont été testés, ainsi que
- 71 liquides céphalo-rachidiens envoyés au laboratoire du Centre Hospitalier de Kigali provenant de patients présentant des signes méningés.

Au total, on remarque 2 résultats discordants. Il s'agit de deux échantillons de liquides céphalo-rachidiens provenant d'anciens cas de cryptococcose encore en traitement, positifs avec le test au latex et négatifs en culture.

S'il est évident que le test de recherche des antigènes cryptococciques ne permet pas de faire la distinction entre une cryptococcose active et une inactive, la présence de faibles quantités d'antigènes dans le liquide céphalo-rachidien implique que le patient doit rester sous surveillance, étant donné les nombreuses rechutes observées chez les patients SIDA.

In conclusion, le test au latex pour le dépistage de la cryptococcose est rapide, facile à réaliser et fiable.

#### **Evaluatie van de Cryptococcus Antigeen Test voor de diagnose van met AIDS geassocieerde cryptococcosis in Rwanda.**

*Samenvatting.* — Om de Cryptococcus antigeen test te evalueren, werden er twee verschillende retrospectieve studies ondernomen.

Een positieve cultuur voor *Cryptococcus neoformans* werd als gouden standaard voor actieve cryptococcose beschouwd.

- 439 sera, willekeurig geselecteerd en opgestuurd naar het NSP laboratorium voor HIV antigeen opsporing, werden getest
- 71 stalen van lumbaal vocht afkomstig van patiënten met meningeale verschijnselen, opgestuurd naar het laboratorium van de Centre Hospitalier de Kigali werden eveneens getest.

In totaal werden er twee discrepanties gevonden. Twee stalen van lumbaal vocht van vroegere cryptococcose gevallen, die nog steeds onder behandeling waren, werden positief met de latex test en negatief in cultuur.

Alhoewel het vanzelfsprekend is dat de antigeen test het verschil niet kan aantonen tussen actieve en inactieve cryptococcose, impliceert de aanwezigheid van kleine hoeveelheden oplosbaar antigeen in een lumbaal vocht dat de patiënt onder toezicht moet blijven, aangezien de hoge frequentie hervallen bij AIDS-patiënten.

In conclusie is de latex test een snelle, gemakkelijke en betrouwbare test voor de diagnose van cryptococcose.

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