Rev. Inst. Med. trop. São Paulo
32 (6): 456-460, novembro-dezembro, 1990

COAGGLUTINATION TEST (COA) FOR Cryptococcus neoformans CIRCULATING ANTIGEN DETECTION IN CEREBRAL SPINAL FLUID (CSF)

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SUMMARY

We tested 82 CSF of 24 renal transplanted patients (RT) with cerebral cryptococcosis, 8 CSF of asymptomatic RT patients, 43 CSF of proven cryptococcosis cases (positive control) and 35 CSF of patients with other diseases (histoplasmosis, paracoccidioidomycosis and bacterial infections) as negative control.

The RT CSF were cultured in Sabouraud agar slant added with sunflower seeds and both control and RT CSF were qualitatively examined by cryptococcosis latex test (Crypto-LA test).

The COA test was developed both qualitatively and quantitatively. The highest titre encountered was 1:2048. No false reactions appeared among the controls. The diagnostic value demonstrated by Galen and Gambino's method was: sensitivity - 92.1%; specificity - 92.6% and efficiency - 92.3%. Besides that, the COA proved to be quick, exact and cheap, but it depends on CSF and sera pre-treatment, in order to avoid autoagglutination and increase its sensibility.

KEY WORDS: Coagglutination Test; **Cryptococcus neoformans**; Antigen detection.

INTRODUCTION

C. neoformans is the etiologic agent of cryptococcosis, an opportunistic disease that has been recognized with increase frequency in immunosuppressed people and in AIDS patients in general[8].

The laboratory diagnosis is made in several ways: by direct mycological examination, by means of histopathology and culture or differential culture in Sabouraud agar added with sunflower seeds[16]. When the isolation of the fungus is not possible, serology is the best tool for the correct diagnosis[13]. The LA test is, at the moment, the most specific one[9], but economically it is not cost-effective and the available commercial kits are not profitable. BAVA, in 1986, standardized the Coagglutination technic, used only to demonstrate bacterial antigens, to detect the circulating **C. neoformans** antigen in CSF, sera and urine[1, 5, 7].

The Coagglutination test uses **S. aureus** protein A positive cells (COWAN I), adsorbed with specifically IgG immuneglobulins[5]. This is the support reagent and any kind of antisera can be conjugated to these cells[3].

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The purpose of this study is to standardize the Coagglutination test for the diagnosis and the therapeutic control of immunosuppressed patients cryptococcosis diseased and demonstrate the specificity and sensibility of the test. Otherwise, it shows the qualities of the suitable and available reagents used in the method.

METHODS

CSF samples: 82 CSF obtained from 24 RT patients with cerebral cryptococcosis, 8 CSF from asymptomatic RT patients, 43 CSF from proven cryptococcosis cases (positive control) and 35 CSF from patients with other diseases (histoplasmosis, paracoccidioidomycosis and bacterial infections). The first were cultured in Sabouraud agar (Difco) slant added with sunflower seeds in order to isolate the fungus. Their protein contents were determined by Lowry's method[12].

LA test: Commercial kit — **Crypto-LA test,** available from the International Biological Laboratories, Inc. Dist. Subsidiary of Carter-Wallace, Inc. Cranbury, NJ 08512.

Coagglutination reagent (CR): S. aureus cells protein A positive (COWAN I) were cultured in brain heart infusion broth (BHI broth-Difco) and shaken for 18 hours at 37°C. They were washed in formaldehyde 37% (Merck) diluted at 0.5% in phosphate buffered saline (PBS) pH 7.4 10 mM. The cells pellet was resuspended at a 10% solution in bovine albumin (fraction V-Sigma) 0.1% in PBS pH 7.4 10 mM. In this stock solution we added thimerosal (Sigma) in a final concentration of 1:10.000[1, 11].

C. neoformans antiserum: Prepared according to PALMER et al[15] method. G, A, and M immuneglobulins were demonstrated by Ouchterlony double immunodiffusion.

Rabbit normal serum: It was conjugated with the **CR** and used as a COA control reagent or non-reactive conjugate **(NCR).**

C. neoformans polysaccharide antigen: Prepared by MASIH et al[14] method and used as the main positive control **(MPc).**

Coagglutination conjugate (CC): The specific **C. neoformans** antiserum was adsorbed to protein A containing **Staphilococci,** in this proportion: 1 ml **CR** 10% + 0.15 ml **C. neoformans** antiserum + 9.0 ml PBS pH 7.4 10 mM. The binding time was 1 hour at room temperature being continuously shaken. The solution was centrifugated at 2500 rpm by 10 minutes, and the **CC** was resuspended at 10% in PBS pH 7.4 10 mM[1]. This stock solution was stored in a sterile flask.

Coagglutination reaction: The CSF were inactivated at 80°C during 5 minutes[11]. The test was performed in Boerner slides and 25 microlitres of each CSF and CC were mixed during 1-2 minutes at room temperature in an orbital shaker.

We tested the RT CSF, the negative and positive controls, qualitatively. They were called positive when they agglutinated **CC.** The RT CSF and 10 positive controls were titred (quantitative COA test) and considered positive till the final dilution which agglutinated **CC.** As a reaction control we also tested these alternatives: **NCR + MPc; CC + MPc; CR + MPc; CC + c(+)** and **CC + c(−).** All the CSF were tested by three distinct **CC's** batches to check the reprodutibility of the COA reaction. The **CC** and **NCR** were stained by Evans blue (Difco) at 10 mg% (vol/vol), to assure correct interpretation.

RESULTS

Among the 24 RT patients, 18 were qualitatively positive by Crypto-LA test and 17 by COA. Six of them were negative by LA test and 7 by COA (Table 1).

The qualitative COA test demonstrated that 61 of the RT CFS samples were positive and 21 negative. Four were false-positive (5%) and 8 were false-negative (10%) in relation to the LA test results. This did not occur with the controls. The asymptomatic patients had both COA and LA negative tests: one of them showed yeasts cells by direct examination (Table 1). CSF: L-117137.

Those 61, qualitatively positive RT CSF, were titred. Twenty-three of them agglutinated up to 1:8 and 38 agglutinated up to different dilutions. The highest titre was 1:2048 (Table 1. CSF: 115858). The false-positive and the false-negative were also titred. Among the first only two reac-

TABLE 1

LA test and COA test of the first (A) and last (B) sample's results of each of the 32 RT patients, including the asymptomatic (L) ones.

Patient number	Proteins mg/dl	Qualitative				Quantitative	
		LA test		COAG test		COAG test	
		A	B	A	B	A	B
10 8723	74 - 84	+	+	+	+	1:1	1:16
10 9094 (fn)*	24	+	/	—	/	—	/
11 3538 (fn)*	26 - 43	+	+	—	—	—	—
11 4864	110 - 32	+	+	+	—	1:1	—
11 5003	86	+	/	+	/	1:1	/
11 5073	80 - 32	+	+	+	—	1:1	—
11 5329 (fn)*	70 - 33	+	+	—	—	—	—
11 5515	97	+	/	+	/	1:8	/
11 5858	143 - 57	+	+	+	+	1:2048	1:256
11 5864	131 - 91	+	+	+	+	1:1	1:8
11 5980 (fp)*	80	—	/	+	/	1:1	/
11 6034 (fp)*	45	—	/	+	/	1:6	/
11 6062	105 - 152	+	+	+	+	1:64	1:8
11 6119	39 - 24	—	—	—	—	—	—
11 6294	121 - 72	+	+	+	+	1:8	1:1
11 6355	34	+	/	+	/	1:32	/
11 6364	70 - 56	+	+	+	+	1:16	1:64
11 6374	45 - 26	+	+	+	+	1:32	1:64
11 6461	97 - 66	+	+	+	+	1:64	1:16
11 6465 (fp)*	135	—	/	+	/	1:16	/
11 6627	39	+	/	+	/	1:16	
11 6793	27 - 28	—	—	—	—	—	—
11 6981	31 - 28	+	—	+	—	1:1	—
11 7284	28	—	/	—	/	—	/
L-117135	30	—	/	—	/	—	/
L-117136	18	—	/	—	/	—	/
L-117137	22	—	/	—	/	—	/
L-117238	41	—	/	—	/	—	/
L-117239	25	—	/	—	/	—	/
L-117462	22	—	/	—	/	—	/
L-117548	30	—	/	—	/	—	/
L-117549	23	—	/	—	/	—	/

* (fn) = false negative; * (fp) = false positive.

ted at 1:16 and the further demonstrated agglutination till 1:8 dilution. Two CSF used as positive control showed titre up to 1:1024.

The reaction control did not demonstrate either unspecific agglutination or autoagglutination[11], and both CC, NCR and CR were viable at 4°C during one year.

GALEN & GAMBINO method[6] was employed to show the diagnostic value of the COA test: sensitivity - 92.1%; specificity - 92.6% and efficiency - 92.3%.

In Table 2, there is an example of COA CSF curve from two RT CSF patients. The therapeutic control of all the 24 RT patients with cerebral cryptococcosis was not yet accomplished.

DISCUSSION

In this investigation we had evaluated the usefullness of COA test in the diagnosis and prognosis of cryptococcosis in RT patients. The method appeared to be reliable for the detection of cryptococcal polysaccharide in CSF.As in LA test, it must be controlled because CSF and sera need to be pre-treated (heat incubation or alkali precipitation) to avoid unspecific agglutination[11]. The COA test also provides good results when is used as qualitative or quantitative test.

The low number of false-positive and false-negative among the RT CSF and the non-occurrence among the controls were important evidences for using the COA routinely. Therefore, a combination of two or three tests still results in false-negative rate up to 15%[9]. Table 2, illustrates its sensibility when CSF have been collected from some RT patients in a short time range. Immunosuppressed patients, mainly organ transplant recipients, show a cryptococcal infection rate of 2-3%[8].

TABLE 2
COA test CSF curve example.

Patients	Sample's date	Titer
11 6062	24/08/87	1:64
	07/09/87	1:64
	11/09/87	1:32
	23/09/87	1:8
10 8723	12/06/87	1:1
	09/07/87	1:64
	29/07/87	1:64
	29/08/87	1:16

The results demonstrated the efficiency of COA test and showed it is a cheap, suitable, available and easily feasible method for many laboratories. As the same, the COA demonstrates to be an important tool for a precise and non-time-consuming diagnosis.

RESUMO

Pesquisa do antígeno circulante de Cryptococcus neoformans **em líquido cefalorraqueano pelo teste de coaglutinação.**

Foram utilizadas 82 LCR de transplantados renais (24 pacientes), 43 LCR de pacientes com criptococose comprovada (controles positivos), 35 LCR de pacientes com outras doenças (histoplasmose, paracoccidioidomicose e infecções bacterianas) como controles negativos. Os primeiros foram cultivados em ágar Sabouraud com sementes de girassol e juntamente com os demais examinado pelo teste de látex para pesquisa de antígeno circulante de **C. neoformans,** qualitativamente.

O teste de Coaglutinação foi realizado qualitativamente e quantitativamente, encontrando-se títulos até a diluição 1:2048. Não foram detectadas reações falso-positivas ou falso-negativas entre os controles. Como prova de valor diagnóstico demonstrou: sensibilidade - 92,1%; especificidade - 92,6% e eficiência - 92,3%. Provou também ser um teste rápido, exato e econômico, embora sua escolha dependa do pré-tratamento de LCR (80°C por 3 a 5 minutos) e soros (diluição ou álcali-precipitação) para evitar autoaglutinação e aumentar a sensibilidade da reação.

ACKNOWLEDGEMENTS

We gratefully acknowledge Dr. Gildo Del Negro and Dr. Maria Conceição Rodrigues for correcting the manuscript.

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Recebido para publicação em 21/5/1990.