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THE SENSITIVITY, SPECIFICITY AND EFFICIENCY VALUES OF SOME SEROLOGICAL TESTS USED IN THE DIAGNOSIS OF PARACOCCIDIOIDOMYCOSIS

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SUMMARY

This work reports on the results of double immunodiffusion (ID), counterimmunoelectrophoresis (CIE), complement fixation (CF) and indirect immunofluorescence (IIF) techniques in the serodiagnosis of paracoccidioidomycosis. The study was undertaken on four groups of individuals: 46 patients with untreated paracoccidioidomycosis, 22 patients with other deep mycoses, 30 with other infectious diseases (tuberculosis and cutaneous leishmaniasis) and 47 blood donors as negative controls. Data were obtained using **Paracoccidioides brasiliensis** antigens, i.e.,a yeast culture filtrate for ID, CIE and CF, and a yeast cell suspension for IIF. The sensitivity, specificity and efficiency values were measured according to GALEN & GAMBINO[8].The gel precipitation tests (ID and CIE) showed the greatest sensitivity (91.3 and 95.6%, respectively), maximum specificity (100%) and the highest efficiency values when compared to the CF and IIF tests.

KEY WORDS: Paracoccidioidomycosis; **Paracoccidioides brasiliensis**; Serological tests.

INTRODUCTION

Paracoccidioidomycosis presents multiple clinical manifestations, since the fungal agent **Paracoccidioides brasiliensis**, disseminated through the bloodstream and lymphatic system, may become established in any part of the host organism. Serological procedures applied to paracoccidioidomycosis have allowed its early diagnosis and made it possible to manage the disease appropriately.

The tests employed in the serodiagnosis and cure control of paracoccidioidomycosis have been evaluated according to their efficiency which in turn is, based upon their sensitivity and specificity[8].

The tests most used during the 1950's and 1960's were the classic complement fixation and the precipitin tube, standardized by FAVA NETTO (São Paulo, Brazil)[5, 6]. Later, other reactions in agar and agarose gel and indirect immunofluorescence techniques were added successfully for diagnosis and therapeutic control [7, 9, 11, 12, 13, 14]. A modification of the classic complement fixation test, known as the micro-technique, standardized by C.D.C. Laboratory Branch (Atlanta, USA) was also valuable in the immunological diagnosis of paracoccidioidomycosis[10].

Serological tests are considered to possess high efficiency when both sensitivity and specificity are of the highest degree and when a minimum of false-positive and false-negative results are obtained in the population studied.

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Their predictive value however depends upon the prevalence of the disease in the specific population in question. This report demonstrates the results of certain common serological tests carried out in patients exhibiting active paracoccidioidomycosis and ranks their confiability in the diagnosis of the mycosis infection.

MATERIAL AND METHODS

1. SERA

Sera obtained from four groups of patients were evaluated:

Group I: 46 patients (42 male, 4 female) showing untreated paracoccidioidomycosis, confirmed by direct mycological examination and/or histopathology; 29 patients presented the adult chronic form while 17 exhibited the lymphatic or juvenile type form of the mycosis.

Group II: 22 patients with other proven deep mycoses: 7 cases of histoplasmosis, 6 cases of aspergillosis, 6 of cryptoccocosis, 2 of Jorge Lobo's disease and 1 case of systemic candidiasis.

Group III: sera from 17 patients with tuberculosis and 13 with cutaneous leishmaniasis, kindly supplied by the Parasitology Laboratory, Instituto Adolfo Lutz, São Paulo, Brazil.

Group IV: sera from 47 blood donors, kindly provided by the Fundação Hemocentro, Hospital das Clínicas, São Paulo, Brazil.

2. SEROLOGICAL TESTS

a. Double immunodiffusion (ID) was performed on glass slides (75 x 25 mm) covered with 3 ml of a gel composed of 1% purified agar (Difco Laboratories, USA) in a buffered saline (pH 6.9) containing 0.4% sodium citrate and 7,5% glycine. Antigen(12µl) was placed in the central well and reference serum and patients'sera (12 µl) in the surrounding wells. The slides were incubated in a moist chamber at room temperature for 48 hours. They were then washed in saline, dried and stained in 0.4% Coomassie brilliant blue R (Sigma, USA) in an ethanol-acetic acid-water mixture as the solvent.

b. Counterimmunoelectrophoresis (CIE) was carried out on glass slides (75 x 50 mm) covered with 6 ml of a gel composed of 1% agarose (A-6877, Sigma, USA) in veronal buffered saline pH 8.2 . Electrophoresis was performed for 90 minutes at 120 V. The slides were then washed in saline, dried and stained with the same dye used for the ID test.

Electrophoresis was performed for 90 minutes at 120 V. The volumes of antigen and sera were the same as used for ID (12 µl). The sera were twofold diluted, with buffered saline while the antigen was used undiluted.

Results of both tests were considered positive when one or more bands were detected.

c. Complement fixation (CF) was performed according to the microtechnique recommended by the C.D.C. Laboratory Branch (Atlanta, USA) . Results were considered positive when the titer was 1:8 or greater.

d. Indirect immunofluorescence (IIF) was performed according to the methodology of CANO et al.[3] Results were considered positive when the titer was 1:64 or greater. An anti-IgG-fluorescein isothiocyanate conjugate (Biolab, Brazil) was employed.

3. ANTIGENS

a. The ID, CIE and CF tests were developed with a yeast filtrate antigen obtained from **P. brasiliensis** strain 113, cultured in NGTA medium (neopeptone 1.6%; glucose 1.0%; thiamine 0.01% and asparagine 0.02%)[4]. The cultures were incubated for 20 days at 36° C on a gyratory shaker (50 rpm). After this period, the cultures were filtered through filter paper, concentrated four fold and dialyzed for 48 hours against distilled water. The same batch of antigen was used throghout the study. The antigen was used undiluted for ID and CIE, and diluted 1:256 for the CF test.

b. IIF was performed using a suspension containing three different strains of **Paracoccidioides brasiliensis** (strains 18, SN and 256) prepared as described by CANO et al.[3]

RESULTS

Table I summarizes the results obtained employing the equations defined by GALEN & GAMBINO[8].

The ID test was positive in 42 of the 46 patients with active paracoccidioidomycosis (91.3%). Sera from patients with other mycoses, tuberculosis, leishmaniasis and the healthy controls were non-reactive (100% specificity). The CIE test was positive in 44 of the 46 patients with active paracoccidioidomycosis (95.6%) and it was also 100% specific.

With the CF test, 32 patients from the 46 in group I reacted positively (71.1%) while serum from just one histoplasmosis patient was reactive, providing specificity of 95.4% in relation to group II. Sera from the other groups were non-reactive.

With IIF, positive results were obtained in 30 of the patients with paracoccidioidomycosis (65.2%). A number of sera pertaining to the other groups (II, III and IV) were also reactive, specificity values above 90% always being obtained, however.

The efficiency values of the tests have been expressed separately for each other group (II, III and IV) since such values are dependent on the specificity which has also been obtained separately. It should be noted that, even when the specificity results are equal for the three other groups, slight variation in the efficiency values occurs. This variation results from the fact that the calculation necessary to obtain the efficiency values depends on the size of the group which was variable in our study.

TABLE I -
Sensitivity, specificity and efficiency values of serological tests using antigens from **Paracoccidioides brasiliensis.**

TEST	SENSITIVITY	SPECIFICITY	EFFICIENCY
ID	91.3%	100% (GROUP II)	94.1% (GROUP II)
		100% (GROUP III)	94.7% (GROUP III)
		100% (GROUP IV)	95.6% (GROUP IV)
CIE	95.6%	100% (GROUP II)	97.0% (GROUP II)
		100% (GROUP III)	97.3% (GROUP III)
		100% (GROUP IV)	97.8% (GROUP IV)
CF	71.1%	95.4% (GROUP II)	80.5% (GROUP II)
		100% (GROUP III)	82.6% (GROUP III)
		100% (GROUP IV)	85.8% (GROUP IV)
IIF	65.2%	90.0% (GROUP II)	77.6% (GROUP II)
		96.6% (GROUP III)	80.0% (GROUP III)
		97.8% (GROUP IV)	83.6% (GROUP IV)

(ACCORDING TO GALEN & GAMBINO, 1975)

ID, double immunodiffusion; CIE, counterimmunoelectrophoresis; CF, complement fixation; IIF, indirect immunofluorescence.
Group II, histoplasmosis, aspergillosis, cryptococosis, Jorge Lobo disease, systemic candidiasis.
Group III, tuberculosis, leishmaniasis.
Group IV, control sera.

DISCUSSION

Analysis of the present results suggests the great value of the agar and agarose gel precipitation tests in the serodiagnosis of active paracoccidioidomycosis. Both tests showed high efficiency, CIE being slightly superior to ID. The high specificity values of both tests which were negative in sera of other deep mycoses should be noted.

Regarding the CF test, the results obtained show a lower degree of sensitivity than the precipitation reactions (71.1%); serum from one member of group I gave an anticomplementary reaction (2.1%) and 13 sera showed a negative reaction in this test (26.8%). In this group of 13 sera, two samples were from patients with the chronic form of the disease, one was from a patient infected with HIV and who presented the lymphatic form, and the remaining sera were from patients with the juvenile type of the mycosis.

It is know that during the acute, progressive, juvenile course of paracoccidioidomycosis, the complement fixation test may either yield low antibody titers or even be negative, since such patients are known to be poor producers of CF antibodies[15].

Regarding indirect immunofluorescence, and taking into consideration the titers of 1:64 and above as significant, this test disclosed an efficiency of about 80%, showing higher specificity than sensitivity. The test revealed a low percentage of cross-reactions which occurred mainly with sera of patients exhibiting histoplasmosis and aspergillosis, as already reported in the literature [7,9,14], these cross-reactions were seen only at the cut-off dilution. However, the improvement of specificity did impair slightly the sensitivity of the test. The antigen used in the reaction did not contain strain 113 of **P.brasiliensis** which may have contributed to the difference in sensitivity noted on comparison with other sorological tests.

Based on our data and those from the literature [2, 11, 16], we suggest that medical mycology laboratories give top priority to the agar and agarose gel precipitation tests in the immunological diagnosis of paracoccidioidomycosis.

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RESUMO

Sensibilidade, especificidade e eficiência de alguns testes sorológicos na Paracoccidioidomicose.

O presente trabalho avalia a sensibilidade, especificidade e eficiência da imunodifusão dupla (ID), contraimunoeletroforese (CIE), reação de fixação de complemento (FC) e imunofluorescência indireta (IFI) no diagnóstico da paracoccidioidomicose. Os pacientes portadores da micose, virgens de tratamento, tiveram o diagnóstico confirmado por exame micológico e/ou histopatológico. Utilizou-se como antígenos o filtrado de cultura da fase leveduriforme do **Paracoccidioides brasiliensis** para os testes de ID, CIE, FC e suspensão de células leveduriformes de "pool" de cepas do mesmo fungo para o teste de IFI. O estudo foi realizado em 4 grupos de indivíduos: 46 com paracoccidioidomicose ativa (sem tratamento), 22 com outras micoses profundas, 30 com outras doenças infecciosas (tuberculose e leishmaniose tegumentar) e 47 controles normais. Os valores de sensibilidade, especificidade e eficiência foram obtidos de acordo com a metodologia utilizada por GALEN & GAMBINO (1975). Os resultados revelaram que os testes de precipitação em gel de agar e agarose, representados pela ID e CIE foram os melhores, apresentando maior sensibilidade (91,3% e 95,6%, respectivamente), máxima especificidade (100%) e os maiores valores de eficiência quando comparados à FC e IFI. Tais resultados confirmam dados obtidos por LONDERO et al. (1981), SIQUEIRA (1982), CAÑO & RESTREPO (1987), entre outros.

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