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SEROLOGICAL FOLLOW-UP OF PATIENTS WITH PARACOCCIDIOIDOMYCOSIS TREATED WITH ITRACONAZOLE USING DOT-BLOT, ELISA AND WESTERN-BLOT.

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SUMMARY

Twenty-seven mycologically proven cases of paracoccidioidomycosis (PCM) were treated with itraconazole (100-200 mg/day in month 1 and 100 mg/day until month 6-8) and evaluated clinically and serologically, up to 3.5 years post-therapy, using Dot-blot and ELISA for measuring the titers of IgG, IgA and IgM anti-*P. brasiliensis* antibodies and Western-blot for determining IgG, IgA and IgM antibodies against the antigen components of the fungus. Before treatment, 81.5% (Dot-blot) and 84% (ELISA) of the patients presented elevated IgG anti-*P. brasiliensis* antibody titers which dropped slightly with treatment. On the other hand, the percentages of pre-treatment high-titered sera for IgA and IgM anti-*P.brasiliensis* were lower (51.9% and 51.8%: Dot-blot; 16.5 and 36%: ELISA, respectively) but the titers tended to become negative more frequently with treatment. Prior to treatment, the percentages of positivity for IgG, IgA and IgM anti-*P.brasiliensis* antibodies in Western-blot were 96%, 20.8% and 41.6%, respectively. Antigens with molecular weights varying from 16-78 kDa, from 21-76 kDa and from 27-78 kDa were reactive for IgG, IgA and IgM antibodies, respectively. The most frequently reactive antigenic components had molecular weights of 27, 33 and 43 kDa for IgG, and 70 for IgA and IgM antibodies. During the period of study, the patients responded well to treatment. The present data confirm the diversity and complexity of the humoral response in PCM, and the importance of utilizing different serological tests to detect IgG, IgA and IgM anti-*P. brasiliensis* antibodies.

KEYWORDS: Paracoccidioidomycosis; *Paracoccidioides brasiliensis*; Serology; Itraconazole.

INTRODUCTION

Paracoccidioidomycosis (PCM) is a deep mycosis caused by *Paracoccidioides brasiliensis*, endemic to the central region of São Paulo State, where the town of Botucatu is located. The clinical manifestations of the mycosis are polymorphic with the frequent involvement of the lungs, mucocutaneous areas and the mononuclear phagocytic system[14].

The nature, intensity, extent and dissemination of the lesions vary considerably, which is related to the characteristics of the fungus as well as to some inherent factors of the host[7,14].

The therapeutic arsenal has increased in recent years, particularly with the introduction of azolic derivatives such as ketoconazole, itraconazole and fluconazole[9]. The recent standardization and enhanced sensitivity of serological techniques, such as Dot-blot, Western-blot and ELISA, have contributed to the therapeutic monitorization and follow-up of patients[2,3,10,11,17].

The initial objective of this work was the standardization of Dot-blot for serological diagnosis and monitoring of patients with PCM before, during and after treatment with itraconazole. In addition we compared the immune humoral response (IgG, IgA and IgM antibodies) using Dot-blot and ELISA, and determined by Western-blot the components of *P. brasiliensis* relevant to the humoral response during the anti-fungal therapy.

MATERIAL AND METHODS

Patients - A total of 27 patients, 2 females and 25 males, were followed since 1989. The age of patients ranged between 11 and 60, with the majority being between 30 and 40 years of age.

From a clinical standpoint, the patients presented either the chronic mixed progressive pulmonary form (77.8%), the isolated progressive pulmonary form (14.8%) or the progressive acute-subacute form (7.4%)[14].

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Therapy - Patients were treated with itraconazole 100 (26 patients)- 200 (1 patient) mg/day during the first month and 100 mg/day until the 6th - 8th month. A dose of 100 or 200 mg/day in the first month depended on the initial severity of the clinical features. Itraconazole was not used in severely ill patients whose life were at risk.

Diagnosis and Clinical-Serological Follow-up - Dot-blot, ELISA and Western-blot techniques were used in accordance with protocols previously described[1-3,6,10,15,17,18].

Antigen - A complex antigen was used which was a mixture of culture filtrate and somatic antigen of yeast cells of the fungus. The isolates Bt_1 and Bt_2 (Culture Collection of the Department of Pathology, Faculty of Medicine of Botucatu, UNESP), 18, 113, 192 and 265 (Culture Collection, Laboratory of Medical Microbiology, Faculty of Medicine, USP) were cultured in a partially synthetic liquid at 37° C, for 7 days, with constant agitation[16]. The cells were killed with 0.2 g/l merthiolate and filtered through Whatman filter paper no. 3. They were resuspended vol/vol in 0.85% saline solution (SS), sonicated 20 times with 100 watts and centrifuged. The supernatant was dialyzed for 48 h at 4°C against distilled water. The supernatant of the culture (exoantigen) was concentrated 20 times and dialyzed as described above. After dialysis, the antigens were mixed in equal parts (culture filtrate + somatic antigen), separated into 2 ml aliquots, lyophilized and stored at -20°C.

Dot-blot - A 0.45 µm Millipore nitrocellulose membrane was used as a support for the antigen in a protein concentration of 5 µg/ml[6,8]. The membranes were sensitized with antigen for 1 h at room temperature. Subsequently they were immersed in 5% skim milk in phosphate buffered saline (PBS-M) for 2 h with constant agitation to block the unbound sites of the membrane. The sensitized membranes were processed immediately or stored at -20° C.

At the moment of use, they were set up in the Bio-Rad Dot-blot device and incubated with patients sera diluted 1:100 or 1:16 for the detection of IgG or IgA and IgM antibodies, respectively, for 30 - 60 min. The cut-off dilutions were determined in a block titration of the control sera. After slow vacuum aspiration, they underwent three 30 min washes in PBS-M with constant agitation. Samples were incubated with goat anti-human IgG, IgA or IgM peroxidase labelled conjugates (Sigma), diluted 1:4000, 1:1000 and 1:1000 respectively, for 2 h at room temperature with constant agitation.

After a new series of washes, the membranes were immersed in a substrate comprised of 50 mM Tris-HCl buffer (pH 7.4)- 35 ml, diaminobenzidine- 11 mg and H_2O_2 - 35 µl. The reaction was stopped by distilled water and recorded as negative or positive.

Enzyme-linked immunosorbent assay-ELISA - The procedure was previously described by COLE et al.[4]. The antigen was fixed (solid phase) overnight on 96 well microtitre plates using a protein concentration of 10 µg/ml (100 µl/well) at room temperature. Afterwards, PBS-M was added for 2 h at room temperature to block the unbound sites. The test sera were added in twofold dilutions beginning at 1:100 and incubated for 2 h, at 37°C. The cut-off dilution was determined in a block titration of the control sera. The plates were then washed with 200 µl of PBS-M, 3 washes of 3 min each. They were incubated with the peroxidase labeled conjugates (IgG 1:4000, IgA 1:1000 or IgM 1:1000; 100 µl/well), for 2 h at 37°C. After washing, 100 µl/well of substrate, comprised of citrate-phosphate buffer pH 5.0- 25ul, H_2O_2 - 10 µl and orthophenylenediamine- 10 mg were added. The reaction was stopped after 8 min by adding 50 µl/well of 4N H_2SO_4. Samples were evaluated at a 492 nm wavelength in a Multiscan Titertek ECM-340 ELISA microreader.

For both serological tests, the percentage of sera with decreasing titers (decrease of equal or more than 2 twofold dilutions) were determined for each period of observation up to 24 months.

Western-blot - The antigen was run in SDS-PAGE, for 4 h, at 125 V [12]. The antigen bands were then transferred to Millipore nitrocellulose membrane which was stained with Ponceau S and cut into strips. Strips were incubated individually with sera diluted 1:50 in PBS-M for 2 h at room temperature with constant agitation. Afterwards samples were washed 5 times for 5 min each with PBS-M and incubated with the peroxidase labeled anti-human IgG, IgA or IgM conjugates, diluted 1:10000, for 2 h at room temperature. After washing with PBS-M and twice with 0.05% SS-Tween 20, the strips were developed with a substrate comprised of PBS- 60 ml, diaminobenzidine- 10 mg and of 30% H_2O_2-300 µl. The reaction was stopped with distilled water. Visual readings of bands were recorded and photographed.

Clinical follow-up of the patients- All patients presented clinical improvement during treatment and even remission of the specific symptomatology. Later two patients died of unrelated diseases.

RESULTS

Dot-blot - The distribution of sera according to the reactivity and IgG, IgA and IgM antibody titers in Dot-blot are shown in Tables 1 and 2. Before treatment, 81.5% of patients presented with elevated IgG antibody titers, equal to or greater than 1:800. After 1 year this percentage dropped to 61% and after 3 years to 50%.

The majority of patients presented IgA antibodies before treatment, with 51.9% having titers equal to or greater than 1:512. After 1 year, this percentage fell to 22.2% and to 16.6% after 3 years.

Before treatment, 51.8% of IgM antibody titers were equal to or greater than 1:256. The percent dropped to 38.9% after 1 year and to 33.3% after 3 years.

TABLE 1

Distribution of sera of paracoccidioidomycosis patients during treatment with itraconazole according to anti-*P. brasiliensis* IgG antibody titers and geometric means measured by Dot-blot. Titers are expressed as the reciprocal of the maximum dilution with reactivity.

Period of treatment (months) (n=no. of sera)	Number of sera (Percentage) TITERS								
	Negative	100	200	400	800	1600	3200	6400	Means
0	1	1	1	2	13	6	3	-	820.8
(n=27)	(3.7)	(3.7)	(3.7)	(7.4)	(48.2)	(22.2)	(11.1)	-	
6	1	4	1	3	7	5	3	1	658.9
(n=25)	(4)	(16)	(4)	(12)	(28)	(20)	(12)	(4)	
12	4	1	2	-	3	3	5	-	544.3
(n=18)	(2.22)	(5.5)	(11.1)	-	(16.6)	(16.6)	(27.7)	-	
18	-	-	3	3	3	1	4	-	678.7
(n=14)	-	-	(21.4)	(21.4)	(21.4)	(7.2)	(28.6)	-	
24	3	-	-	1	5	2	2	-	550.8
(n=13)	(23)	-	-	(7.7)	(38.5)	(15.4)	(15.4)	-	
30	1	-	1	2	3	1	-	-	436.2
(n=8)	(12.5)	-	(12.5)	(25)	(37.5)	(12.5)	-	-	
36	1	2	-	-	1	1	1	-	356.4
(n=6)	(16.7)	(33.3)	-	-	(16.7)	(16.7)	(16.6)	-	
42	-	-	3	1	2	-	-	-	356.4
(n=6)	-	-	(50)	(16.7)	(33.3)	-	-	-	

TABLE 2

Distribution of sera of paracoccidioidomycosis patients during treatment with itraconazole according to IgA and IgM anti- *P. brasiliensis* antibody titers and geometric means measured by Dot-blot. Titers are expressed as the reciprocal of the maximum dilution with reactivity.

Period of treatment (months) (n=no. of sera)	Number of sera (Percentage) TITERS											
	Negative		16		128		512		2048		Means	
	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM
0	2	2	7	2	4	9	11	12	3	2	145.5	203.5
(n=27)	(7.3)	(7.3)	(26.0)	(7.4)	(14.8)	(33.4)	(40.8)	(44.4)	(11.1)	(7.4)		
6	4	2	2	4	16	8	1	9	2	2	91.8	151.2
(n=25)	(16.0)	(8.0)	(8.0)	(16.0)	(64.0)	(32.0)	(4.0)	(36.0)	(8.0)	(8.0)		
12	3	1	-	4	11	6	3	5	1	2	118.5	138.2
(n=18)	(16.7)	(5.6)	-	(22.2)	(61.1)	(33.3)	(16.7)	(27.8)	(5.5)	(1.1)		
18	2	-	5	5	4	5	3	3	-	1	55.2	99.9
(n=14)	(14.3)	-	(35.7)	(35.7)	(28.6)	(35.7)	(21.4)	(21.4)	-	(7.2)		
24	-	-	6	3	4	5	3	3	-	2	67.5	167.1
(n=08)	-	-	(12.5)	(12.5)	(50.0)	(37.5)	(37.5)	(37.5)	-	(12.5)		
36	1	-	1	2	3	2	-	1	1	1	90.5	128.0
(n=06)	(16.7)	-	(16.7)	(33.3)	(50.0)	(33.3)	-	(16.7)	(16.7)	(16.7)		
42	3	-	1	-	2	2	-	4	-	-	22.6	322.5
(n=06)	(50.0)	-	(16.7)	-	(33.3)	(33.3)	-	(66.7)	-	-		

The IgA and IgM antibody titers tended to become negative more frequently than the IgG antibody titers.

ELISA - The distribution of sera according to the reactivity and IgG, IgA and IgM antibody titers are expressed in Tables 3 and 4. Prior to treatment, 84% of the patients presented with titers equal to or greater than 1:800 for IgG antibodies. The percentage diminished to 70.6% after 1 year, to 53.9% after 2 years and maintained at 66.8% after 3 years of treatment.

Prior to treatment, 16% and 36% of patients presented with titers equal to or greater than 1:800 for IgA and IgM antibodies, respectively. After 1 year of treatment, the great majority of patients became serologically negative or presented with a significant decrease in antibody titers.

The positivity indices for IgG, IgA and IgM anti-*P.brasiliensis* antibodies prior to treatment in Dot-blot (96.3%, 92.6%, 92.6%) were higher than those in ELISA (84%, 44%, 48%).

During treatment, the decreasing behavior of IgM antibody titers was similar to that of IgA antibodies in both serological tests (Table 5).

Examples of patients who presented decreasing levels of anti-*P. brasiliensis* antibodies during the serological follow-up with both Dot-blot and ELISA are shown in Figure 1.

Western-blot - The distribution of sera according to IgG, IgA and IgM antibody reactivity against antigenic components of *P. brasiliensis*, evaluated by Western-blot, are shown in Table 6.

Prior to treatment, the percentages of positivity for IgG, IgA and IgM antibodies were 96.0%, 20.8% and 41.6%, respectively. Antigens with molecular weights varying from 16 - 78 kDa, from 21 -76 kDa and from 27 - 78 kDa were reactive for IgG, IgA and IgM antibodies, respectively. In the global analysis of 25 patients' sera prior to treatment, the percentages of positivity for IgG, IgM and IgA antibodies against gp43 kDa were 80%, 12.5% and 4.2%, respectively. The molecular weights of the most frequently reactive antigenic components were 27, 33 and 43 kDa for IgG antibodies, and 70 kDa for IgA and IgM antibodies (Fig. 2).

Table 7 depicts the evolutive prolife of three patients for IgG anti-*P.brasiliensis* antibodies detected by Western-blot. After 12-24 months of treatment, the number of labeled bands decreased, with tendency of persistence of reactivity to gp43.

TABLE 3

Distribution of sera of paracoccidioidomycosis patients during treatment with itraconazole according to IgG anti - *P. brasiliensis* antibody titers and geometric means measured by ELISA. Titers are expressed as the reciprocal of the maximum dilution with reactivity.

Period of treatment (month) (n=no. of sera)	Number of sera (Percentage) TITERS									
	Negative	100	400	800	1600	3200	6400	12800	25600	Means
0	4	-	-	2	1	2	-	8	9	4149.9
(n=25)	(16)	-	-	(8)	(4)	(8)	-	(28)	(36)	
6	5	1	1	1	1	-	3	4	7	2439.8
(n=23)	(21.7)	(4.3)	(4.3)	(4.3)	(4.3)	-	(13)	(17.3)	(30.4)	
12	3	2	-	1	1	1	3	1	5	2043.5
(n=17)	(17.7)	(11.2)	-	(5.9)	(5.9)	(5.9)	(17.7)	(5.9)	(29.4)	
18	6	1	-	1	-	3	-	2	1	538.4
(n=14)	(42.8)	(7.2)	-	(7.2)	-	(21.4)	-	(14.2)	(7.2)	
24	4	2	-	1	2	-	2	-	2	646.3
(n=13)	(30.7)	(15.4)	-	(7.7)	(15.4)	-	(15.4)	-	(15.4)	-
30	2	-	-	2	1	1	-	-	2	800.0
(n=08)	(25)	-	-	(25)	(12.5)	(12.5)	-	-	(25)	
36	2	-	-	1	1	1	-	-	1	800.0
(n=06)	(33.2)	-	-	(16.7)	(16.7)	(16.7)	-	-	(16.7)	
42	2	1	-	-	1	1	-	1	-	504.0
(n=06)	(33.2)	(16.7)	-	-	(16.7)	(16.7)	-	(16.7)	-	

TABLE 4

Distribution of sera of paracoccidioidomycosis patients during treatment with itraconazole according to IgA and IgM anti - *P. brasiliensis* antibody titers and geometric means measured by ELISA. Titers are expressed as the reciprocal of the maximum dilution with reactivity.

Period of treatment (month) (n=no. of sera)	Number of sera (Percentage) TITERS																			
	Negative		100		400		800		1600		3200		6400		12800		25600		Means	
	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM
0	14	13	7	3	-	-	1	-	1	3	1	3	-	2	1	-	-	1	114.9	256.7
(n=25)	(56)	(52)	(28)	(12)	-	-	(4)	-	(4)	(12)	(4)	(12)	-	(8)	(4)	-	-	(4)		
6	13	18	4	1	2	-	1	-	3	2	-	2	-	-	-	-	-	-	119.8	100.0
(n=23)	(56.5)	(78.3)	(17.4)	(4.3)	(8.7)	-	(4.3)	-	(13.1)	(8.7)	-	(8.7)	-	-	-	-	-	-		
12	11	11	5	-	-	-	-	-	-	2	-	4	-	-	1	-	-	-	85.0	200.0
(n=17)	(64.7)	(64.7)	(29.4)	-	-	-	-	-	-	(11.7)	-	(23.5)	-	-	(5.9)	-	-	-		
18	13	13	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	61.0	70.7
(n=14)	(92.8)	(92.8)	-	-	-	-	(7.2)	-	-	-	-	-	-	(7.1)	-	-	-	-		
24	9	9	4	1	-	-	-	-	-	3	-	-	-	-	-	-	-	-	61.9	61.9
(n=13)	(69.2)	(69.2)	(30.8)	(7.7)	-	-	-	-	-	(23.1)	-	-	-	-	-	-	-	-		
30	4	4	2	-	1	-	-	1	1	2	-	1	-	-	-	-	-	-	118.9	282.8
(n=08)	(50)	(50)	(25)	-	(12.5)	-	-	(12.5)	(12.5)	(25)	-	(12.5)	-	-	-	-	-	-		
36	4	5	1	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	89.1	89.1
(n=06)	(66.6)	(83.3)	(16.7)	-	-	-	(16.7)	-	-	(16.7)	-	-	-	-	-	-	-	-		
42	3	2	3	-	-	1	-	-	-	3	-	-	-	-	-	-	-	-	70.7	400.0
(n=06)	(50.0)	(33.3)	(50.0)	-	-	(16.7)	-	-	-	(50.0)	-	-	-	-	-	-	-	-		

TABLE 5

Percentages of sera with decreasing anti-*P.brasiliensis* antibody titers (decrease of equal or more than 2 twofold dilutions) at month 6, 12, 18 and 24 post-therapy measured by Dot-blot and ELISA

Essay		Percentages of sera Month			
		6	12	18	24
Dot-blot	IgG	25%	25%	22%	11%
	IgA	56.5%	27%	50%	33%
	IgM	35%	27%	62.5%	33.5%
ELISA	IgG	28%	31%	44%	0%
	IgA	43%	20%	25%	0%
	IgM	62.5%	0%	0%	0%

DISCUSSION

The anti-*P. brasiliensis* serological tests represent an excellent laboratory tool for diagnosis and follow-up of patients with PCM. The high sensitivity of the immunoenzymatic assay makes them appropriate for elaboration of serological curves which guarantee a good prognostic evaluation for the patients.

The Dot-blot assay, used in screening hybridomas[12], has also been tested in the serological diagnosis of infectious and parasitic conditions such as schistosomiasis and leishmaniasis[1,15]. The pioneering work of TABORDA & CAMARGO[18] standardized the technique for the detection of IgG anti-gp43 antibodies, with and without treatment with methaperiodate, in the diagnosis and follow-up of patients with

TABLE 6

Western-blot: IgG, IgA and IgM antibodies against antigenic components of *P. brasiliensis* in paracoccidioidomycosis patients during treatment with itraconazole. Results are expressed as numbers (n) of reactive sera with antigenic bands of different molecular masses in each period of study.

Molecular Mass	Period of study (month) 0	6	12	18	24	30	36	42	48
IgG									
Kda	(n=25)	(n=22)	(n=16)	(n=14)	(n=13)	(n=07)	(n=06)	(n=06)	(n=4)
16 - 20	4	1	2	0	1	1	2	1	0
21 - 29	19	12	5	4	3	3	6	1	1
30 - 39	24	1	8	2	2	2	4	1	0
40 - 49	24	19	12	13	12	6	6	5	3
50 - 59	13	7	11	6	4	1	2	1	1
60 - 69	23	11	10	6	10	7	9	2	2
70 - 79	15	7	11	5	7	4	0	3	2
negative	1	5	4	4	5	2	1	4	1
IgA									
Kda	(n=24)	(n=18)	(n=15)	(n=12)	(n=13)	(n=07)	(n=06)	(n=06)	(n=04)
20 - 29	2	0	0	0	1	1	1	1	0
30 - 39	1	0	0	0	0	0	0	0	0
40 - 49	1	1	0	0	1	1	1	1	0
50 - 59	2	0	0	1	1	0	1	0	0
60 - 69	6	6	3	1	5	5	1	1	0
70 - 79	8	7	2	0	6	6	2	2	0
negative	19	14	10	10	9	4	4	5	4
IgM									
Kda	(n=24)	(n=20)	(n=16)	(n=13)	(n=13)	(n=07)	(n=06)	(n=06)	(n=03)
20 - 29	2	0	0	0	0	0	0	0	0
40 - 49	3	0	1	0	1	0	0	0	0
50 - 59	9	4	4	1	8	4	2	4	0
60 - 69	17	11	8	3	6	4	2	5	1
70 - 79	19	9	6	4	5	5	2	4	1
negative	14	14	12	9	10	5	5	3	2

TABLE 7

Evolutive profile of IgG anti-*P.brasiliensis* antibodies detected by Western-blot in patients with paracoccidioidomycosis, during therapy with itraconazole. Distribution of numbers of reactive bands according to the molecular weights.

Patient #	Duration of treatment (month)	Reactive bands 10-29 kDa	30-49 kDa	50-79 kDa
1	0	1	4	3
	12-24	0	1	2
2	0	2	2	4
	12-24	1	2	1
3	0	1	2	3
	12-24	1	1	0

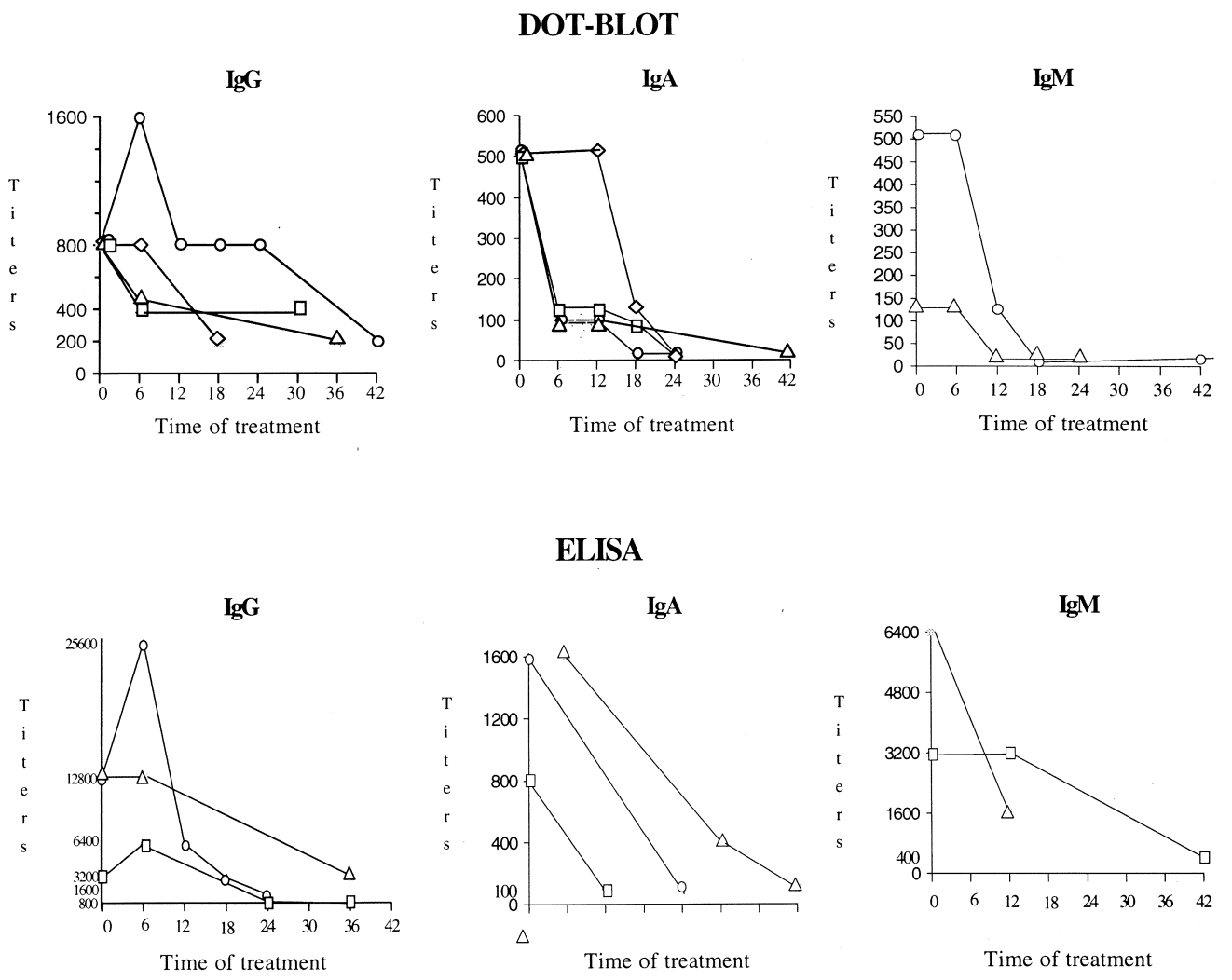

Fig. 1 - Serological curves of patients with PCM treated with itraconazole for anti-*P. brasiliensis* IgG, IgM and IgA antibodies as detected by Dot-blot and ELISA.

PCM. The sensitivity of the test was 100% with pre-treatment sera. Cross reactions with other mycoses were observed only with sera of patients with Jorge Lobo mycosis (31.3%). However, when gp43 was used after partial deglycolization with methaperiodate, the cross reactivity was eliminated.

In the present study, a complex antigen was prepared from various isolates of the fungus. The slightly lower sensitivity of our assay for IgG antibodies, before treatment, compared to the results of TABORDA & CAMARGO[18] (percent positivity 96.2% vs. 100%), might be related to differences in the methodology or to the complex nature of the antigen used, which possibly contained lower concentration of gp43. After treatment, the persistence of a serological scar, as described in other serodiagnostic methods, is likely a consequence of quiescent infectious foci which persist in the host. Anti- *P. brasiliensis* IgA and IgM antibodies were more sensitive for detection of descending serological curves with a tendency to become negative in patients under treatment.

ELISA has been utilized in PCM serodiagnosis since the pioneering work of MENDES-GIANNINI & CAMARGO[10]. The sensitivity and specificity reported with the use of complex antigens are high; sensitivity decreases when the dilution *cut-off* is elevated and specificity increases when the sera are absorbed with yeast-like cells of *Histoplasma capsulatum* and *Candida albicans*. When gp43 was

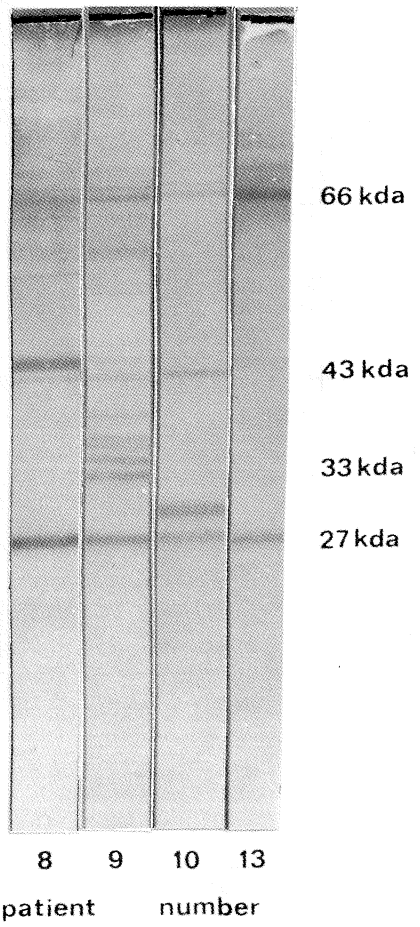

Fig. 2 - Western-blot: IgG reactivity against antigenic components of *P. brasiliensis*. Patients sera prior to treatment.

employed, the test sensitivity was 100%, especially among the patients with the acute or subacute form of the disease.

In the present work, even using a high *cut-off* titer, the sensitivity of the ELISA-IgG test was elevated. The percentages of positivity of the ELISA- IgA and ELISA- IgM were not very much increased in the initial phase of the disease, but nevertheless the titers diminished with treatment.

Western-blot applied to serodiagnosis of PCM and the correlation of results with the clinical features and response to therapy were initially described by MENDES-GIANNINI et al.[17] They also used a complex antigen and observed reactivity of IgG anti-gp43 antibodies, in the majority of sera. These pioneering findings were later confirmed by CAMARGO et al.[2] who detected IgG anti-gp43 and anti-gp70 antibodies in 100% and in 86% of patients, respectively. The reactivity diminished significantly with treatment suggesting that antibodies against these antigenic fractions could be utilized as disease related markers. Accordingly, later on, FERREIRA-DA-CRUZ et al.[5] described IgG anti-gp45 antibodies, antigen which may possibly correspond to gp43, in 90.6% of patients with active disease. In our study, the sensitivity of the test was slightly lower, probably due to methodological peculiarities or because all study samples were processed at once using previously manipulated stored sera.

During the period of study, the patients responded well to treatment and presented no relapses, which did not allow a correlation between the serological data and the recurrence of signs and symptoms.

RESUMO

Seguimento sorológico de pacientes com paracoccidioidomicose tratados com itraconazole, utilizando Dot-blot, Elisa e Western-blot

Vinte e sete pacientes portadores de paracoccidioidomicose (PCM) foram tratados com itraconazole (100-200 mg/dia no primeiro mês e 100 mg/dia até 6-8 meses) e avaliados sob o ponto de vista clínico e sorológico, até 3 e meio anos após o início do tratamento, utilizando-se os testes de Dot-blot e ELISA para medir os títulos de anticorpos IgG, IgA e IgM anti-*P. brasiliensis,* e Western-blot para determinar os anticorpos IgG, IgA e IgM contra os componentes antigênicos do fungo. Antes do tratamento, 81,5% (Dot-blot) e 84% (ELISA) dos pacientes apresentaram títulos elevados de anticorpos IgG anti-*P. brasiliensis*, que decresceram levemente com o tratamento. Por outro lado, as porcentagens de soros pré-tratamento com títulos elevados para anticorpos IgA e IgM foram menores (51,9% e 51,8%: Dot-blot; 16% e 36 %: ELISA, respectivamente); com o tratamento, entretanto, estes títulos tenderam mais frequentemente a se negativar.

Antes do tratamento, as porcentagens de positividade para anticorpos IgG, IgA e IgM, avaliados por Western-blot, foram 96%, 20,8% e 41,6%, respectivamente. Componentes antigênicos de massas moleculares variando entre 16-78 kDa, 21-76 kDa e 27-78 kDa reagiram com anticorpos das classes IgG, IgA e IgM, respectivamente, As frações antigênicas com massas moleculares de 27, 33 e 43 kDa foram as mais frequentemente reativas com anticorpos da classe IgG, e a de 70 kDa para anticorpos IgA e IgM. Todos os pacientes apresentaram remissão da sintomatologia com o tratamento, durante o período de estudo. Os dados do presente trabalho confirmam a diversidade e a complexidade da resposta humoral dos pacientes com PCM e reforçam a importância de se utilizar diferentes testes sorológicos para se detectar anticorpos IgG, IgA e IgM anti- *P. brasiliensis.*

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