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Evaluation of the hepatobiliary system in patients with paracoccidioidomycosis treated with cotrimoxazole or itraconazole

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Abstract

A prospective study was performed in 200 paracoccidioidomycosis (PCM) patients, 51 presenting the acute/subacute form (AF) and 149 the chronic form (CF), submitted to the evaluation of the hepatobiliary system at admission and during the follow-up treatment with cotrimoxazole (CMX) or itraconazole (ITC). This study aimed to better evaluate the involvement of the hepatobiliary system in PCM and the effect of these antifungal compounds on this system. Serum levels of direct bilirubin (DB), total bilirubin (TB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) were evaluated. At admission, all the variables showed changes with elevated values ranging from 6.2% for TB to 32.6% for GGT. After treatment, the incidence of elevated serum levels ranged from 3.6% for DB to 27.5% for ALT. The course of the alterations during the treatment showed regression to normal values in CMX-treated patients and persistence in ITC-treated patients but without the need to discontinue the therapy. Our findings contribute to the knowledge of the hepatobiliary involvement by *Paracoccidioides* sp. and to a safe follow-up of PCM patients under treatment.

Key words: paracoccidioidomycosis, hepatobiliary, system, cotrimoxazole, itraconazole.

Introduction

Paracoccidioidomycosis (PCM) is a systemic disease caused by dimorphic fungi *Paracoccidioides brasiliensis* and *Paracoccididies lutzii*.[1,2] PCM is the most prevalent systemic mycosis in Latin America. *P. brasiliensis* is heterogeneously distributed; Brazil, Colombia and Venezuela have reported the highest incidence.[3]

A Brazilian study on death certificates from 1980 to 1995 revealed a mortality rate from PCM of 1.45 per million

Table 1. Changes in serum biochemistry of the hepatobiliary system at admission and after treatment with itraconazole or cotrimoxazole.

Disease	Clinical form	*n*	Increased values at admission (%)	Increased values with ITC*	Reference number
PCM	AF+CF	121	AST (16.5), TB (8.5), CB (8.5) and ALP (46.3)	...	14
PCM	AF	24	Bilirubins (25), aminotransferases (25) and GGT (61.9)	...	15
PCM	AF+CF	14	...	ALP	9
PCM	AF+CF	25	...	AST, ALT, and ALP	8
Other mycosis	...	189	...	AST, ALT, ALP, TB, and DB	16

AF, acute/subacute form; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CF, chronic form; CMX, cotrimoxazole; DB, direct bilirubin; GGT, gamma glutamyl transferase; ITC, itraconazole; *n*, number of patients; Other mycosis: coccidioidomycosis, cryptococcosis, aspergillosis, blastomyces, histoplasmosis; PCM, paracoccidioidomycosis; TB, total bilirubin; () prevalence in percentage.
*There is no study with CMX.

inhabitants and identifies this mycosis as the eighth leading cause of death by predominantly chronic or recurrent infectious and parasitic diseases.[3] A study performed in São Paulo State (Brazil) between 1985 and 2005 reported a PCM mortality rate of 2.66 per million inhabitants, 1.58 of which from the disease itself and 1.08 from associated causes, and in Botucatu region (São Paulo State), these numbers reached 8.73, 4.89, and 3.84, respectively, demonstrating the hyperendemic status of this area, which registers,[4] on average, 15 new cases per year in the Infectious Diseases Service of the University Hospital of Botucatu Medical School.

Active PCM presents two main clinical forms—the acute/subacute form (AF) and the chronic form (CF). The AF predominates in children, adolescents, and young adults, presents a shorter duration of symptomatology, affects both genders (male to female ratio of 1.7:1.0), and the clinical manifestation are related to the involvement of the phagocytic-mono-nuclear system (lymph nodes, liver, spleen and bone marrow). The pulmonary involvement is rare in the AF (~5%) and that of the oral mucous membranes is few observed (~17%). The CF predominates in adult patients over 30-years old presents a longer duration of symptomatology, usually higher than 4 months, sometimes higher than 6 months, predominates in males (male to female ratio of 22.2:1.0), and the clinical manifestations show high prevalence of pulmonary (~90%) and mucous membranes (~60%) involvement. The involvement of lymph nodes is usually limited to the cervical chains.[5]

Itraconazole (ITC) and the sulfamethoxazole-trimethoprim (cotrimoxazole, CMX) are the main antifungal compounds used in PCM treatment, which should be maintained for a long time.[6–13]

In order to better evaluate every PCM patient and to monitor the treatment, several laboratory tests are necessary: acute phase reactions, erythrocyte sedimentation rate, blood chemistry evaluating the hepatobiliary and kidney system, and metabolic alterations. However, few studies have evaluated the hepatobiliary system of PCM patients which are limited by few patients and short follow-up periods (Table 1).

Thus, this study aimed to better evaluate the hepatobiliary system of PCM- patients at admission; before introduction of the treatment as well as to evaluate the effects of the antifungal compounds ITC and CMX on these functional parameters.

Methods

This study was performed at the Infectious Diseases Service of the University Hospital of the Faculdade de Medicina de Botucatu, São Paulo State University (UNESP, Botucatu, SP, Brazil).

Study design

A prospective and quasi experimental study was conducted in 200 PCM- patients submitted to the evaluation of hepatobiliary system at admission at our institution and during the treatment follow-up with the CMX or ITC. One hundred forty-nine patients presented with the chronic form (CF), and 51 presented the acute/subacute form (AF). Thirty-one patients were treated with ITC, and 169 were treated with CMX. ITC was orally administered in capsule formulation once a day at a dose of 200 mg, and CMX was administered with 400 mg of sulfamethoxazole and 80 mg of trimethoprim using 3 tablets twice each day throughout the study period.

Clinical and blood chemistry evaluations were performed before starting the treatment, defined as moment 0 (M_0), and periodically until the patients exhibited clinical

cure and regression of the erythrocyte sedimentation rate (ESR) to normal values, called the final moment- M_3 (18–23 weeks). Clinical cure was defined as the disappearance of the symptomatology previously exhibited by the patient. Between these two moments, the patients were evaluated at intervals defined in relationship to the number of weeks of treatment: M_1: 4 to 6; M_2: 7 to 10.

Research Ethics Committee

The study protocol was reviewed and approved by the institutional ethics committee the Faculdade de Medicina de Botucatu – São Paulo State University (UNESP), Faculdade de Medicina de Botucatu, Campus Botucatu, Brazil. Written informed consent for participation was provided by the patient or their parents.

Study population

The criteria for enrollment in the study included an age of 12 years or more and PCM confirmed by both a suggestive clinical picture and the identification of typical *P. brasiliensis* yeast forms in clinical materials (confirmed cases) or a suggestive clinical picture associated with serum antibodies determined by the double agar gel immunodiffusion (DID) test (probable cases). The patients included in the study were admitted between 1988 and 2011 and were treated with CMX or ITC. Patients were excluded if they had other systemic diseases of infectious, inflammatory or neoplastic etiology, if they were pregnant or lactating, if they had a previous history of hypersensitivity or severe side effects to azoles or CMX, or if they were taking medications that affect the pharmacokinetics of these antifungal compounds or that can interact with them.

Classification of clinical forms

The classification of the clinical forms was carried out according to Mendes[5] and was done by the infectious diseases medical doctor responsible for attending the patients.

Outcomes

The prevalence, incidence, intensity, and evolution of the hepatobiliary alterations were evaluated for patients treated with each antifungal compound. Comparisons between the groups were also performed.

Methods

The clinical and laboratory information of each patient was obtained from the attending physician from the patients' clinical records, and the patients were always supervised by the same professor leading the study.

Serum levels of direct bilirubin (DB), total bilirubin (TB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) were evaluated at the Central Laboratory of the University Hospital.

Prevalence and incidence

The prevalence indicates the percentage of patients with altered variables at admission and incidence the percentage of patients who developed alterations during the period of study, that is, variables initially normal but altered after introduction of the antifungal compound or variables initially altered which increased the alterations after treatment.

Criteria of drug-induced liver injury

The criteria of drug-induced disorders used in the present study were proposed in an international consensus with a small modification and has been briefly presented.[17]

All of the liver tests were expressed as a ratio between the value measured and the upper limit of the normal range (N). In the evaluation of its intensity the highest value observed after introduction of the antifungal compound was used. When the increase in the liver test results was between N and 2N, the finding was considered an "abnormality", as it was suggested by the consensus[17] or a mild injury.

Liver injury was classified as hepatocellular, cholestatic, or mixed according to the biochemical profile. Hepatocellular injury was defined as an: increase of ALT alone or $R \geq 5$, where R is the ratio between the serum activity of ALT and the serum activity of ALP, measured at the same time and expressed as multiple of N. Cholestatic injury was defined as the: increase of ALP above 2N alone or $R \leq 2$. Mixed injury was diagnosed: when both ALT (above 2N) and ALP were increased and when R was between 2 and 5 ($2 < R < 5$). Mild mixed injury was diagnosed: when both ALT and FA were between 1.1 and 1.9 and when $R < 2$N. An AST/ALT ratio $\geq$2 was considered suggestive of alcohol-induced liver injury.

De Ritis ratio

The ratio of AST to ALT, also called De Ritis quotient, which normally is 1.0 or slightly more, was also evaluated. De Ritis quotient >1.5 is suggestive of hepatitis caused by alcohol intake.[18]

The erythrocyte sedimentation rate (ESR) was determined by the Westergreen method employing blood anticoagulated with EDTA. The results were measured as

millimeters in the first hour, and the upper limits of the reference values for men and women, respectively, are as follows: (a) below age 50 years: 15 and 20; (b) between ages 50 and 85 years: 20 and 30; and (c) above 85 years: 30 and 42.[19–20]

Statistical analysis

The ordinal measurements were compared by the Kruskal–Wallis test; the analyses of paired samples were performed by the Wilcoxon test, while the unpaired samples were analyzed by the ***t*** test. The categorical variables were analyzed by the χ^2 test or Fisher exact test. Comparison of moments regarding to the clinical forms and the treatment were performed by the Friedman and the Mann–Whitney test. Significance was set at $\boldsymbol{P} \leq .05$. The risk for hepatotoxicity was evaluated by the relative risk and the odds ratio. All the analyses were performed using the program SAS version 9.2.

Results

Homogeneity of the groups. The patients treated with ITC did not differ from those who received CMX as to age (ITC: Md = 42.6; CMX: Md = 43.0; $P = .19$), sex (male: ITC: Md = 84.3%; CMX: Md = 88.7%; $P = .49$), clinical form (ITC: AF = 34.3%, CF = 65.7%; CMX: AF = 24.0%, CF = 76.0%; $P = .10$) and severity (ITC: Mild CF = 6.2%, moderate CF = 53.2%, severe CF = 6.2%, moderate AF = 28.2%, severe AF = 65.7%; CMX: mild CF = 3.6%, moderate CF = 56.9%, severe CF = 15.6%, severe AF = 21.6%; $\boldsymbol{P} = .41$).

The results will be presented using tables with the number of individuals evaluated for every variable since not all tests were carried out on all patients.

The evaluation of the hepatobiliary system upon admission to service (M_0). The prevalence of lab tests with elevated values ranged from 6.2% for TB serum levels to 32.6% for GGT serum levels (Table 2A). In addition, the prevalence of evaluated serum levels of DB, ALP and GGT was higher in AF patients than in CF patients (Table 2A).

A comparison of the intensity of the elevated variables between the two PCM clinical forms revealed that the ALP and GGT values were higher in AF patients than in CF patients (Table 2B).

Furthermore, comparison variables, two by two, showed that ALT elevations were higher than those of AST, GGT than ALP, and finally, ALP than ALT (Table 3).

The evaluation of De Ritis quotient in 181 patients revealed no difference between clinical forms- CF:135 patients, Md = 1.07 [IQR 0.78;1.48]; AF: 46 patients, Md = 1.14 [IQR 0.67; 1.63]; $P = .59$. In addition, the evaluation of 42 patients with increased serum aminotransferases levels, showed no difference between clinical forms- CF: 28 patients, Md = 0.63 [IQR 0.44, 1.39]; AF: 14 patients, Md = 0.83 [IQR 0.57, 1.27]; $P = .61$.

Progress of variables evaluating the hepatobiliary system in patients with previously increased variables. CMX-treated patients exhibited reduction of AST, TB, DB, GGT to normal values, and decreased ALT and ALP serum levels, but they did not reach normal values (Table 4). On the contrary, ITC-treated patients showed a decrease of all the variables, but never reached normal values (Table 4).

Incidence of laboratory side effects. The study of patients that presented increase in the serum levels of variables evaluating the hepatobiliary system showed alterations in all of them, ranging from 3.6% for DB to 27.5% for ALT (Table 5). The incidence of increased variables did not vary as to clinical form. Nevertheless, the incidence of DB and TB was higher in ITC-treated patients than CMX-treated patients (Table 5). The intensity of the elevated serum levels was higher in the AF for DB than those with the CF. Nevertheless, the incidences of elevations did not vary as to antifungal compound (Table 5).

The relative risk and the odds ratio for hepatotoxicity were higher for ITC than for CMX, mainly for DB, TB and ALP (Table 6). In addition, the risk of hepatotoxicity was higher for patients with the AF than those with the CF (Table 6).

The predominant alteration in the pattern of the hepatobiliary system differed depending on the treatment used – hepatocellular for CMX (64.7% of the cases) and mixed/mild for ITC (60.0%).

Discussion

Several autopsy studies have demonstrated the paracoccidioidal involvement of the hepatobiliary system.[21–28] Taken together, 44.6% of the 314 autopsied PCM patients exhibited hepatic and/or bile duct injury.

This study aimed to evaluate the hepatobiliary system of PCM patients before and during treatment with CMX or ITC, routinely performed.

Few studies have addressed the changes in the hepatobiliary system of PCM patients before treatment. Some authors have presented a case report,[27] and others have focused on histopathological examination in biopsied tissue. Therefore, our findings related to the influence of the disease on the hepatobiliary system will be compared with only two previous studies.[14–15]

Our study identified increases in all the parameters evaluating the hepatobiliary system, with prevalences ranging from 6.2% in TB to 32.6% in GGT and intensities from 1.15 for AST to 14.95 for DB.

Table 2A. Analysis of increased serum levels of tests evaluating the hepatobiliary system of patients with active paracoccidioidomycosis before treatment (moment M_0).

Evaluation of the prevalence as to clinical form					
			Patients with increased values altered/total (%)		
Variables	Patients (total)	Global prevalence (%)	Acute/subacute form	Chronic form	*P* value
DB	175	12.0[BC]	10/46 (21.7)	11/129 (8.5)	<.05
TB	178	6.2[C]	4/46 (8.7)	7/132 (5.3)	.20
AST	181	12.7[BC]	10/46 (21.7)	13/135 (9.6)	.05< *P* < .10
ALT	181	18.2[BC]	11/46 (23.9)	22/135 (16.3)	>0.10
ALP	181	26.0[AB]	23/46 (50.0)	34/135 (17.8)	<0.001
GGT	181	32.6[A]	22/46 (47.8)	27/135 (24.7)	<.02

Teste de Cochran ($P = .00001$). Capital letters compare frequencies in the same column; frequencies with the same letter do not differ ($P > .05$); frequencies with different letters indicate statistically significant difference ($P \leq .05$); Comparison between clinical forms was performed by using the χ^2 test. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirrubin; GGT, gamma-glutamyl transferase; *n*, number of patients. (), prevalence in percentage; TB, total bilirrubin.

Table 2B. Median, 1st and 3rd quartiles of the increased serum levels of tests which evaluate the hepatobiliary system of patients with active paracoccidioidomycosis before treatment (moment M_0). Comparison of intensity as to clinical form.

	Clinical Form				
Variable	Acute/ subacute	Patients (*n*)	Chronic	Patients (*n*)	*P* value
DB	4.75[2.00; 14.95]	10	2.00 [1.30; 9.50]	11	.22
TB	6.02 [3.51; 9.27]	4	1.28 [1.13; 1.73]	7	.07
AST	2.40 [1.15; 3.25]	10	1.25 [1.19; 1.77]	13	.13
ALT	2.17 [1.47; 4.58]	11	1.64 [1.17; 1.83]	22	.06
ALP	1.97 [1.35; 3.52]	23	1.31 [1.08; 1.70]	24	.001
GGT	3.02 [1.61; 4.86]	22	1.75 [1.15; 2.45]	37	.02

[], 1st and 3rd quartiles. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; GGT, gamma-glutamyl transferase; *n*, number of patients; TB, total bilirubin. Mann–Whitney *U* test.

Table 3. Comparative study of the intensity of the altered variables of the hepatobiliary system of patients with active paracoccidioidomycosis before treatment (moment M_0).

Comparison 1st versus 2nd	Patients (*n*)	1st variable	2nd variable	*P* value
AST vs ALT	41	1.05 [0.74; 1.56]	1.50 [1.06; 2.08]	.02
ALP vs GGT	81	1.07 [0.66; 1.64]	1.38 [0.96; 3.01]	< .001
ALP vs ALT	67	1.18 [0.80; 1.96]	1.06 [1.42; 1.71]	.05

Comparison of 2 × 2 variables, when both were increased. Data presented as median, 1st and 3rd quartiles. Paired-sample testing of continuous data (Wilcoxon test). ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; *n*, number of patients.

Our findings confirm previous studies as to variables altered and its prevalence. Nevertheless, the intensity of the alterations was evaluated only in our study.

Increased serum levels of aminotransferases, at admission, may be explained by hepatocellular injury caused by the fungus of the *Paracoccidioides* genus. [21–29]

Elevated DB and ALP are the result of intra-hepatic cholestasis caused by paracoccidioidal injuries to the liver parenchyma, including granulomas, or by the extrinsic compression of extra-hepatic bile ducts .[21–25]

The increase of GGT serum levels may be explained by injuries to the canaliculi of liver cells and to the epithelial cells of the bile ducts caused by PCM.[21,23,24,25,26,27,28]

The prevalence of elevated ALP, DB, and GGT serum levels in AF patients was higher than in CF patients, suggesting that the predominance of cholestatic injury as well as damage to the cell membrane could be related to the involvement of organs rich in the mononuclear phagocytic system, such as in the liver of patients with the juvenile form.[5]

Table 4. Influence of the treatment on the courses of hepatic enzymes of paracoccidioidomycosis-patients treated with itraconazole (ITC) or cotrimoxazole (CMX) as to moment and clinical form. Evaluation of patients with increased serum levels at admission to the Service (M_0). Data presented as median, 1st and 3rd quartiles. Friedman test.

		Moments of evaluation				
	Clinical form (*n*)	M_0	M_1	M_2	M_3	*P* value
AST	CMX (19)	1.35 [1.20; 0.75]^{a}	0.60 [0.54; 1.19]^{ab}	0.90 [0.67; 1.62]^{ab}	0.90 [0.46; 1.19]^{b}	.01
	ITC (06)	1.30 [1.15; 2.75]^{a}	1.20 [0.90; 1.40]^{ab}	1.05 [0.61; 2.71]^{ab}	1.05 [0.35; 1.05]^{b}	.04
	AF (11)	1.35 [1.19; 2.65]^{a}	0.80 [0.55; 1.36]^{b}	0.90 [0.67; 1.56]^{ab}	0.97 [0.43; 1.16]^{b}	<.001
	CF (14)	1.25 [1.2; 1.76]^{a}	0.55 [0.50; 0.61]^{b}	0.83 [0.55; 1.20]^{ab}	0.78 [0.36; 1.05]^{b}	<.001
ALT	CMX (28)	1.69 [1.29; 2.25]^{a}	0.78 [0.61; 1.33]^{ab}	0.89 [0.41; 1.39]^{b}	1.06 [0.67; 1.44]^{ab}	.005
	ITC (06)	1.78 [1.56; 2.17]	1.50 [1.11; 2.13]	1,11 [0.92; 3.16]	1.28 [0.61; 1.50]	.32
	AF (12)	2.06 [1.50; 4.28]	2.14 [1.17; 4.04]	1.11 [0.64; 2.39]	1.24 [0.67; 2.68]	.18
	CF (22)	1.64 [1.17; 1.83]^{a}	0.72 [0.61; 0.83]^{b}	0,78 [0.41; 1.33]^{b}	0.97[0.61; 1.28]^{b}	.007
TB	CMX (08)	1.39 [1.05; 3.05]^{a}	0.65 [0.40; 2.80]^{ab}	1.00 [0.90; 1.08]^{ab}	0.55 [0.40; 1.15]^{b}	.04
	ITC (04)	3.51 [1.21; 9.05]	0.82 [0.58; 1.18]	0.50 [0.50; 0.80]	1.19 [0.60; 1.67]	.15
	AF (05)	5.80 [1.16; 7.76]^{a}	1.06 [0.61; 3.00]^{ab}	0.70 [0.50; 1.00]^{ab}	0.50 [0.28; 1.52]^{b}	.019
	CF (07)	1.28 [1.12; 1.73]	0.65 [0.50; 1.30]	1.00 [0.75; 1.93]	1.00 [0.52; 1.23]	.19
DB	CMX (17)	2.33 [1.3; 11.96]^{a}	0.80 [0.50; 7.25]^{b}	0.80 [0.35; 2.45]^{b}	0.60 [0.04; 2.13]^{b}	<.001
	ITC (07)	3.00 [1.48; 9.23]	3,25 [2.00; 4.00]	1.00 [0.65; 2.55]	2.35 [1.48; 4.75]	.51
	AF (11)	3.00 [2.00; 14.04]	1.67 [0.61; 5.81]	1.67 [0.46; 2.46]	2.00 [0.46; 7.45]	.14
	CF (13)	2.00 [1.28;5.50]^{a}	1.50 [0.50; 6.25]^{ab}	0.80 [0.50; 6.67]^{b}	1.00 [0.00; 1.88]^{b}	.004
ALP	CMX (44)	1.53 [1.16;2.26]^{a}	1.25 [0.98; 1.84]^{ab}	1.13 [0.81; 1.37]^{bc}	1.05 [0.71; 1.61]^{c}	<.001
	ITC (08)	1.30 [1.10;2.24]	1.30 [1.10; 2.24]	1.30 [1.10; 2.24]	1.30 [1.10; 2.24]	.65
	AF (29)	2.18 [1.33;3.68]^{a}	1.75 [1.24; 3.13]^{ab}	1.27 [0.81; 1.47]^{b}	1.09 [0.74; 2.05]^{b}	<.001
	CF (26)	1.22 [1.08; 1.68]^{a}	1.09 [0.95; 1.33]^{b}	1.10 [0.91; 1.26]^{b}	0.87 [0.72; 1.21]^{b}	.001
GGT	CMX (55)	1.79 [1.19; 3.44]^{a}	1.20 [0.72; 2.15]^{b}	0.96 [0.58; 1.46]^{b}	0.86 [0.54; 1.37]^{b}	<.001
	ITC (11)	2.21 [1.21; 6.31]	2.04 [0.93; 5.01]	1.57 [0.70; 1.81]	1.14 [0.70; 3.85]	.30
	AF (26)	3.02 [1.56; 4.86]^{ab}	1.88 [0.82; 4.26]^{b}	1.00 [0.46; 2.23]^{bc}	0.80 [0.53; 2.11]^{c}	<.001
	CF (40)	1.57 [1.12; 2.11]^{a}	1.07 [0.72; 1.73]^{b}	0.93 [0.60; 1.47]^{b}	0.99 [0.65; 1.63]^{b}	<.001

[], 1st and 3rd quartiles. Small letters compare frequencies in the same line; frequencies with the same letter do no differ ($P > .05$); frequencies with different letters indicate statistically significant difference ($P \leq .05$). ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CMX, cotrimoxazole; DB, direct bilirubin; GGT, gamma-glutamyl transferase; ITC, itraconazole; Moment (M): $M_{0:}$ before treatment, M_1: 4 to 6, M_2: 7 to 10; and M_3: clinical cure and regression of the erythrocyte sedimentation rate to normal values (18–23) weeks after beginning treatment. *n*, number of patients; TB, total bilirubin.

Few studies are known to have addressed the follow-up of the hepatobiliary system in PCM patients after the introduction of antifungal therapy. Some authors reported that the treatment with CMX or ITC did not increase the serum levels of variables evaluating the hepatobiliary sytem[6,7,10,11,12]. Only three studies reported alterations of variables evaluating the hepatobiliary system, but with no information as to incidence or intensity.[8,9,16]

In the current study, all parameters used to evaluate the hepatobiliary system after the introduction of antifungal treatment showed different degrees of increase. The incidence varied from 8.5% for TB serum levels to 33.0% for ALT serum levels.

The antifungal compound did not influence the incidence of elevated variables. Nevertheless, the incidence of elevated serum levels of DB was higher in AF patients than in CF patients.

The evaluation of ITC in 189 patients suffering from different systemic mycoses revealed an overall incidence of elevated levels of 1.0% for bilirubin, 5.0% for aminotransferases, and 2.0% for ALP after the use of daily doses of 400 mg ITC in 167 patients and 50 to 200 mg ITC for the remaining patients; for 88.4% of such patients, the mean treatment time was 5 months.[16] The incidence of changes in this study was much smaller than with should in the current study.

The changing behavior of the study parameters varied depending on the clinical form of the patients. In CF, all of the parameters that evaluated the hepatobiliary system normalized by the end of initial treatment, except for DB, whereas in AF, the DB, ALT, and ALP levels remained high at the end of treatment. Such findings suggest that hepatic injury is more intense and/or more persistent in AF than in CF and should be taken into account during patient follow-up.

In the current study, the course of the parameters used to evaluate the hepatobiliary system during the initial treatment also varied according to the antifungal agent used.

Table 5. Incidence and intensity of increased blood chemistry tests evaluating the hepatobiliary system of paracoccidioidomycosis patients after introduction of antifungal treatment. Evaluations as to clinical form and antifungal compound. Incidence presented as percentage and intensity as median, 1st and 3rd quartiles. χ^2 test and Mann–Whitney test.

	Clinical form				Treatment			
	Acute subacute/form		Chronic form		CMX		ITC	
Variable	Incidence	Intensity	Incidence	Intensity	Incidence	Intensity	Incidence	Intensity
DB	(12.0)	7.00[2.70;18.50]	(11.1)	1.90[1.45;4.0]	(7.4)	3.00[1.75;12.58]	(32.3)	2.35[1.67;4.00]
TB	(5.9)	1.73[1.35;3.38]	(2.8)	1.37[1.25;1.77]	(1.8)	1.90[1.45;4.0]	(12.9)	1.39[1.29;1.73]
ALP	(9.8)	2.37[1.22;6.36]	(9.5)	1.28[1.12;1.42]	(10.2)	1.36[1.22;1.65]	(6.3)	1.08[1.03;1.12]
AST	(21.6)	1.43[1.15;1.63]	(13.4)	1.42[1.17;1.56]	(17.3)	1.42[1.15;1.58]	(6.3)	4.03[1.25;6.80]
ALT	(29.4)	1.67[1.28;2.42]	(26.8)	2.06[1.29;3.21]	(28.6)	1.67[1.28;2.81]	(21.9)	1.83[1.50;2.83]
GGT	(20.0)	1.71[1.15;2.63]	(12.8)	2.63[1.22;6.62]	(14.3)	1.59[1.14;3.82]	(16.1)	2.21[1.75;8.19]

Variable	AF vs CF		CMX vs ITC		Variable	AF vs CF		CMX vs ITC	
	Incidence	Intensity	Incidence	Intensity		Incidence	Intensity	Incidence	Intensity
DB	0.86	0.03	<0.001	0.64	AST	0.16	1.00	0.11	0.40
TB	0.30	0.40	0.002	0.63	ALT	0.72	0.38	0.44	0.56
ALP	0.48	0.13	0.48	0.06	GGT	0.21	0.42	0.79	0.17

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CMX, cotrimoxazol; DB, direct bilirubin; GGT, gamma- glutamyl transferase; ITC, itraconazole; TB, total bilirubin; (), incidence in percentage; [], 1st and 3rd quartiles.
Statistical analysis: ***P*** values obtained in different comparisons.

Table 6. Evaluation of risk and chance of hepatotoxicity induced by CMX and ITC, in 39 patients with paracoccidioidomycosis and liver biochemistry normal initially.

	Antifungal compounds				Clinical form			
	R_A-ITC	R_A-CMX	RR	OR	R_A-AF	R_A-CF	RR	OR
DB	0.33	0.03	8.4	12.2 [2.30; 64.33]	0,09	0,91	0,1	0,009 [0.001; 0.0086]
TB	0.08	0.01	6.3	6.80 [0.39; 117.06]	0,09	0,01	6,8	7,40 [0.42; 127.85]
ALP	0.16	0.09	1.8	2.00 [0.35; 10.85]	0,18	0,09	2,0	2,22 [0.39; 12.38]
GGT	0.08	0,08	1.0	1.04 [0.11; 9.56]	0,27	0,05	5.4	6,84 [1.30; 36.20]
AST	0.08	0.17	2.1	2.30 [0.26; 19.41]	0,27	0,14	1,9	2,25 [0.51; 9.81]
ALT	0.08	0.20	2.4	2.79 [0.33; 23.39]	0,09	0,19	2,1	2,41 [1.26; 36.2]
Global Evaluation	0.58	0.30	1.9	3,20 [0.94; 10.82]	0,54	0,42	1,3	1,60 [0.44; 5.69]

Comparison of 32 patients treated with CMX and seven with ITC.
AF, acute/subacute form; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CF, chronic form; CMX, cotrimoxazole; DB, direct bilirubin; GGT, gamma-glutamyltransferase; ITC, itraconazole; OR, odds ratio; R_A, absolut rate; RR, risk ratio; TB, total bilirubin.

Patients treated with CMX exhibited regression in the serum levels of AST, TB, DB, and GGT to the normal values; the levels of ALT and ALP regressed from their peak but continued to be higher than normal. In contrast, patients who received ITC did not exhibit changes in the serum levels of ALT, TB, DB, ALP, or GGT but did exhibit a wild decrease in AST. These findings suggest the relatively higher safety of CMX. Additionally, the knowledge of the behavior of each antifungal compound is essential to patient follow-up.

The results of De Ritis ratio showed that the alterations of the aminotransferases were not caused by alcohol intake, when this ratio should be higher than 1.5. In addition, it is important to consider that alcoholism is frequent among our PCM patients, and that they usually withhold this information from the physician.

CMX is widely used to treat bacterial infections,[30] *Pneumocistis jirovecci* pneumonia,[31] and PCM[11–13] and is usually well-tolerated after oral and intravenous administration, although hepatic injury has been reported.[32–34]

Evaluation of the CMX-induced hepatotoxicity in PCM patients is relevant due to the wide use of this compound and the long treatment length.

Hepatocellular injury has been the most frequent type of hepatic lesion caused by CMX,[33,35,36] although the development of a cholestatic pattern has frequently been reported.[34,37,38,39] Our results, evaluated by blood chemistry variables, indicated a strong predominance of the hepatocellular injury, some cases with mixed injury and few cases of cholestatic reaction, which is consistent with the literature. Cholestatic injury could be related to older patients, as a study reported that 9 out of 10 patients were older than 48 years.[34]

The histopathologic findings in patients with hepatocellular lesions are characterized by multiple disseminated focal areas of necrosis or massive necrosis in fatal cases.[40]

The histopathologic findings in patients with cholestatic liver injury include centrilobular cholestasis (perivenular, zone III), mild-to-moderate portal inflammation and feathery degeneration.[34] The ultrastructural demonstration of characteristic lysosomal changes in the liver further suggests drug-related hepatic injury.[34] Hepatic phospholipidosis, defined as the accumulation of characteristic lamellated, membranous deposits in newly formed lysosomes, can also be observed in patients receiving CMX therapy.[34]

The biochemical mechanisms of CMX liver injury are not completely known. Sulfamethoxazole (SMZ) is conjugated by hepatic N-acetyltransferases, forming predominantly the water-soluble N^4-acetylsulfamethoxazole and, to a lesser degree, the highly lipid-soluble N^1-acetylSMZ.[41] Thus, patients with increased N^1-acetylSMZ formation could exhibit cholestasis and phospholipidosis by injury to cell membranes and accumulation in lysosomes.[35] Genetic polymorphism in the acetylation of sulfanilamide drugs is well known and could lead to increased formation of the highly lipid-soluble N^1-acetylSMZ.[35,43,44] Phospholipidosis could also be caused by inhibition of lysosomal enzyme phospholipase A_1, as was demonstrated with other drugs.[44,45] Although sulfamethoxazole has been considered responsible for liver injury induced by CMX, the recurrence of jaundice after challenge with trimethoprim alone has been reported.[46]

Both types of hepatic toxicity, cholestatic and hepatocellular, could be ascribed to acquired hypersensitivity, as is suggested by the past history of previous contact with sulfonamide compounds. A patient with a previous history of long contact with sulfa compounds that he mixed daily into hog feed presented with cholestatic hepatitis after ingestion of CMX for 7 days.[37] Another patient, a 70-year-old man, developed two episodes of skin rash after CMX intake and recurrence of these signs, in addition to jaundice, one day after taking two pills of this medication.[36] Upon hospitalization, the patient was deeply icteric, with very high aminotransferases serum levels, and his condition progressed to hepatic failure and death. The autopsy revealed a cut surface with a diffuse mottled pattern of patchy necrosis. Microscopic examination revealed, in some areas, massive hepatic necrosis with the disappearance of lobules, collapsed reticulum framework, inflammatory cells, and proliferating bile ducts. Lymphocytes, monocytes and a notable number of eosinophils constituted the inflammatory infiltrate.[36] The metabolic pathway through CyP450 leads to the formation of reactive metabolites, which, after covalent binding, is transformed into a substance which functions as a haptene.[42] A latent period between the start of drug intake and the onset of clinical manifestations ranges from 5 to 14 days,[40] but in subsequent administrations it may be less than one day.[36] A poorer prognosis of the acute hepatocellular disease in the elderly could be explained by the sluggish regenerative potential of the liver in these patients.[36]

CMX-induced hepatotoxicity in PCM patients is not rare, frequently is not intense, is predominated by the hepatocellular type and is reversible while this antifungal compound is maintained. However, its mechanism remains unclear and must be investigated.

The mechanism of ITC-induced liver damage is not well known, but ketoconazole-induced hepatotoxicity may be due to metabolic idiosyncrasy.[47]

Considering that phenobarbital, an inducer of cytochrome P450 (CyP450), prevents ITC hepatotoxicity and that SKF525A, an inhibitor of CyP450, increases ITC hepatotoxicity, we can conclude that this enzymatic system plays a role in the detoxification of ITC and/or its reactive metabolites.[48,49] However, it remains unclear which molecule is responsible for the hepatotoxicity observed clinically and laboratorially in patients treated with ITC.

The decrease of ATP was observed with low concentrations of ITC and was shown to be dose-dependent. It is possible that lower doses of ITC could cause inhibition of mitochondrial respiration, whereas the higher ones could be responsible for the disruption of the whole hepatocyte.[49]

The prolonged use of ITC in rats' results in histopathological alterations in the liver, typically necrosis, bile duct hyperplasia, and biliary cirrhosis, accompanied by liver enlargement and increased AST and ALT activities.[50]

Thus, it is possible that ITC can cause an auto-inhibition of its metabolism, thereby inducing hepatotoxicity.[49] Inhibition of CyP450 could lead to the use of an alternative route for metabolism of ITC, such as the flavin-containing monooxygenase (FMO), as has been suggested for ketoconazole.[47] The cytochrome P450-mediated monooxygenases are known to metabolize many compounds and are genetically regulated.[51]

ITC-induced hepatotoxicity in PCM patients is not rare, frequently is not intense, is predominantly the mixed mild type and is not reversible while this antifungal compound is maintained. However, its mechanism remains unclear, although some evidence exists that CyP450 plays a key role. The CyP450 isoenzyme involved in the hepatotoxicity observed in human patients treated with ITC must be investigated.

Our study showed that hepatotoxicity caused by CMX and ITC is not rare but not intense, and present different patterns; however, CMX-induced alterations are reversible during the treatment, while those caused by ITC are not. In addition, in any case the treatment had to be discontinued due to the side effects caused by ITC. These findings should be considered in the choice of the antifungal compound to treat PCM patients.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. Franco M, Peraçoli MT, Soares A, Montenegro R, Mendes RP, Meira DA. Host-parasite relationship in paracoccidioidomycosis. *J Med Vet Mycol*. 1987; **25**: 5–18.
2. Theodoro RC, Teixeira MM, Felipe MSS, Paduan KS. Genus *Paracoccidioides*: species recognition and biographic aspects. *PLoS One*. 2012; **7**: 1–15.
3. Coutinho ZF, Silva DD, Lazera M et al. Paracoccidioidomycosis mortality in Brazil (1980–1995). *Cad Saude Publica*. 2002; **18**:1441–1454.
4. Santo AH. Paracoccidioidomycosis-related mortality trend, state of São Paulo, Brazil: a study using multiple causes of death. *Rev Panam Salud Publica*. 2008; **23**: 313–324.
5. Mendes RP. The gamut of clinical manifestations. In: Franco M, Lacaz CS, Restrepo Moreno A, Del Negro G. *Paracoccidioidomycosis*. *Boca Raton, FL: CRC*, 1994: 233–258.
6. Restrepo A, Gomez I, Robledo J, Patiño MM, Cano LE. Itraconazole in the treatment of paracoccidioidomycosis: a preliminary report. *Rev Infect Dis*. 1987; **9**: 51–56.
7. Negroni R, Palmieri O, Koren F, Tiraboschi N, Galimberti RL. Oral treatment of paracacoccidioidomycosis and histoplasmosis with itraconazole in humans. *Rev Infect Dis*. 1987; **9**: 47–50.
8. Naranjo MS, Trujillo M, Munera MI, Restrepo P, Gomez I, Restrepo A. Treatment of paracoccidioidomycosis with itraconazole. *J Med Vet Mycol*. 1990; **28**: 67–76.
9. Shikanai-Yasuda MA, Bernard G, Higaki Y et al. Randomized trial with itraconazole, ketoconazole and sulfadiazine in paracoccidioidomycosis. *Med Mycol*. 2002; **40**: 411–417.
10. Queiroz-Telles F, Goldani LZ, Schlamm HT, Goodrich JM, Espinel-Ingroff A, Shikanai-Yasuda MA. An open-label comparative pilot study of oral voriconazole and itraconazole for long-term treatment of paracoccidioidomycosis. *Clin Infect Dis*. 2007; **45**: 1462–1469.
11. Barbosa W, Vasconcellos WMP. Action of sulfamethoxazole associated with trimethoprim in the treatment of South American blastomycosis. *Rev Patol Trop*.1973; **3**: 329–339.
12. Furtado T, Marcos AR, Maia FAA. South American blastomycosis therapy by the combination trimethoprim-sulfamethoxazole. *Folha Med*. 1975; **71**: 275–279.
13. Pedrosa PN, Wanke B, Coura JR. Use of the sulfamethoxazole-trimethoprim combination in the treatment of paracoccidioidomycosis (South American blastomycosis). *Rev Soc Bras Med Trop*. 1974; **8**: 160–165.
14. Fiorillo MA, Martinez R, Moraes CR. Injuries to the digestive system. In: Del Negro G, Lacaz CS, Fiorillo AD. *Paracoccidioidomycosis blastomycosis South American. Hoboken, NJ: Sarvier*, 1982; 180–193.
15. Ferreira MS. Paracoccidioidomycosis: clinical and terapeutic study of the juvenil form of the disease. *Rev Pat Trop*. 1993; **22**: 267–406.
16. Tucker RM, Haq Y, Denning DW, Stevens DA. Adverse events associated with itraconazole in 189 patients on chronic therapy. *J Antimicrob Chemother*. 1990; **26**: 561–566.
17. Bénichou C. Criteria of drug induced liver disorders. Report of an International Consensus Meeting. *J Hepatol*. 1990; **11**: 272–276.
18. Botros M, Sikaris K. The De Ritis ratio: the test of time. *Rev Clin Biochem*. 2013; **34**: 117–130.
19. Bottiger LE, Svedberg CA. Normal erythrocyte sedimentation rate and age. *Br Med*. 1967; J.**8**: 85–87.
20. Zauber NP, Zauber AG. Hematologic data for healthy very old people. *American Med Assoc*. 1987; **257**: 2181–2184.
21. Raphael A, Campana AO, Waiman J. Hepatic coma in South American blastomycosis. *Rev Assoc Med Bras*. 1964; **10**: 151–154.
22. Montenegro MR, Franco MF. Pathology. In: Franco M, Lacaz CS, Restrepo-Moreno A, Del Negro G. *Paracoccidioidomycosis*. Boca Raton, FL: **CRC1994**: 131–150.
23. Brass K. Observations on the pathological anatomy, pathogenesis and evolution of paracoccidioidomycosis. *Mycopathol Mycol Applic*.1969: **37**: 119–138.
24. Boccalandro I, Mello e Albuquerque FJ. Jaundice and hepatic impairment in South American blastomycosis: about 10 cases. Literature review. *Rev Paul Med*. 1960; **56**: 350–366.
25. Salfelder K, Doehnert G, Doehnert HR. Paracoccidioidomycosis: anatomic studies with complete autopsies. *Virchows Arch Abt A Path Anat*. 1969; **348**: 51–76.
26. Teixeira R, Gayotto LC, Brito T. Morphological patterns of the liver in South American blastomycosis. *Histopathology*. 1978; **2**: 231–237.
27. Goffi FS, Ferriarini E. Obstructive jaundice due to blastomycotic choledochal granuloma. *Rev Paul Med*.1960; **57**:12–21.
28. Brito T, Castro RM, Shiroma M. Hepatic biopsy in South American blastomycosis. *Rev Inst Med Trop*. 1968; **10**: 188–191.
29. Pinto WP. Contribution to the study of liver involvement in paracoccidioidomycosis. Master Dissertation. Faculdade de Medicina da Universidade de São Paulo, 1980.
30. Brumfitt W, Pursell R. Trimethoprim-sulfamethoxazole in the treatment of bacteriuria in women. *J Infect Dis*. 1973; **28**: 657–663.
31. Winston DJ, Lau WJ, Gale RP, Young LS. Trimethoprim-sulfamethoxazole for the treatment of *Pneumocystis carinii* pneumonia. *Ann Inter Med*. 1980; **92**: 762–769.
32. Dossing M, Andreasen PB. Drug-induced liver disease in Denmark: an analysis of 572 cases of hepatotoxicity reported to the Danish board of adverse reactions to drugs. *Scand. J Gastroenterol*. 1982; **17**: 205–211.
33. Colluci CF, Cicero ML. Hepatic necrosis and trimethoprim-sulfamethoxazole. *J Am Med Assoc*. 1975; **233**: 952–953.
34. Muñoz SJ, Martinez-Hernandez A, Maddrey WC. Intrahepactic cholestasis and phospholipidosis associated with the use of trimethoprim-sulfamethoxazole. *Hepatology*. 1990; **12**: 342–347.
35. Horak J, Mertl L, Hrabal P. Severe liver injury due to sulfamethoxazole-trimethoprim and sulfamethoxydiazine. *Hepatogastroenterology*. 1984; **31**: 199–200.

36. Ronsohoff DF, Jacobs G. Terminal hepatic failure following a small dose of sulfamethoxazole-trimethoprim. *Gastroenterology*. 1981; **80**: 816–819.
37. Thies PW, Dull WL. Trimethoprim-sulfamethoxazole-induced cholestatic hepatitis. *Arch Intern Med*. 1984; **144**: 1691–1692.
38. Nair SS, Kaplan JM, Levine LH, Geraci K. Trimethoprim-sulfamethoxazole-induced intrahepatic cholestasis. *Ann Inter Med*. 1980; **92**: 511–512.
39. Abi-Mansur P, Ardiaca MC, Allam C. Trimethoprim-sulfamethoxazole–induced cholestasis. *Am J Gastroenterol*. 1981; **76**: 356–359.
40. Dujovne CA, Chan CH, Zimmerman HJ. Sulfonamide hepatic injury: review of the literature and report of a case due to sulfamethoxazole. *N England J Med*. 1967; **277**: 785–788.
41. Vree TB, Helster YA, Damama JE, Van Der KE, O´Reilly WJ. Pharmacokinetics of N\'-acetylsulphamethoxazole in man. *Clin Pharmacokinet*. 1979; **4**: 310–319.
42. Shear NH, Spielberg SP, Grant DM, Tank BK. Differences in metabolism of sulfonamides predisposing to idiosyncratic toxicity. *Ann Inter Med*. 1986; **105**: 179–184.
43. Barraviera B, Pereira PC, Mendes RP, Machado JM, Lima CR, Meira DA. Evaluation of the acetylador phenotype, renal function and serum sulfadiazine levels in patients with paracoccidioidomycosis treated with cotrimazine (a combination of sulfadiazine and trimethoprim). *Mycopathologia*. 1989; **108**: 107–112.
44. Hostetler KY, Reasor M, Yasaki PJ. Chloroquine-induced phospholipid fatty liver. *J Biol Chem*. 1985; **260**: 215–219.
45. Hostetler KY, Giordano JR, Jellison EJ. *In vitro* inhibition of lysosomal phospholipase A_1 of rat lung by amiodarone and desthylamiodarone. *Biochim Biophys Acta*. 1988; **959**: 916–921.
46. Tanner AR. Hepatic cholestasis induced by trimethoprim. *Br Med J*. 1986; **293**: 1072–1073.
47. Rodriguez RJ, Alcosta DJ. N-deacetyl ketoconazole-induced hepatotoxicity in a primary culture system of a rat hepatocytes. *Toxicology*. 1997; **117**: 123–131.
48. Somchit N, Wong CW, Zuraini A et al. Involvement of phenobarbital and SKF 525A in the hepatotoxicity of antifungal drugs itraconazole and fluconazole in rats. *Drug Chem Toxicol*. 2006; **29**: 237–253.
49. Somchit N, Ngee CS, Yaakob A, Ahmad Z, Zakaria ZA. Effects of cytochrome P450 inhibitors on itraconazole and fluconazole induced cytotoxicity in hepatocytes. *J Toxicol*. 2009; **20**: 1–7.
50. Somchit N, Norshahida AR, Hasiah AH, Zuraini A, Sulaiman MR, Noordin MM. Hepatotoxicity induced by antifungal drugs itraconazole and fluconazole in rats: a comparative in vitro study. *Hum Exp Toxicol*. 2004; **23**: 519–525.
51. Nebert DW, Jensen NM. The Ah locus: genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochrome *P*-450 mediated monooxigenases. *CRC Crit Rev Biochem*. 1979; **6**: 401–437.