

Evaluation of polymerase chain reaction for the detection of *Paracoccidioides brasiliensis* DNA on serum samples from patients with paracoccidioidomycosis

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The aim of this study was to demonstrate the DNA of Paracoccidioides brasiliensis in human serum samples of patients with paracoccidioidomycosis using the polymerase chain reaction (PCR). The diagnosis of paracoccidioidomycosis (PCM) was defined by microscopic observation of the fungus on direct exam or histopathology, culture, and serological positivity. DNA from serum of 33 patients with PCM was extracted and submitted to nested-PCR using primers from the gp 43 gene. Only one sample was positive on nested-PCR. We conclude that the prevalence of fungemia in patients with different clinical forms of PCM is low, limiting the use of serum DNA detection as an alternative diagnostic tool.

Key words: paracoccidioidomycosis - *Paracoccidioides brasiliensis* - molecular diagnosis - serum - polymerase chain reaction

Paracoccidioidomycosis (PCM) is a systemic mycosis with occurrence restricted to South and Central America, being rural residents the largest affected group. The diagnosis of PCM has traditionally relied on the finding of characteristic yeast forms on direct examination, culture, or histopathology (Lacaz 1994). Serological tests such as double immunodiffusion and counter-immunoelectrophoresis are also employed for the diagnosis and follow-up of this mycosis (Mendes-Giannini et al. 1994), as well as the detection of a 43-kDa glycoprotein exoantigen (gp43) (Silva et al. 2004).

Studies with blood, cerebrospinal fluid, and sputum specimens from patients with different fungal infections indicated that the polymerase chain reaction (PCR) assay is more sensitive in diagnosing invasive fungal infections than culture methods (Reiss et al. 1998). While several PCR systems have been described, reports on clinical samples from PCM patients are rare. In a study employing sputa specimens, *Paracoccidioides brasiliensis* DNA was detected by gp43-PCR in 100% (11/11) of patients with X-ray proven, pulmonary infection (Gomes et al. 2000). Goldani and Sugar (1998) used primers from a *P. brasiliensis* specific sequence spanning a 62 bp fragment, and were able to show its presence in sera of 5/5 experimentally infected mice. Bialek et al. (2000) developed a highly sensitive and specific nested-PCR detecting a fragment from the gp43 gene on lung homogenates from infected animals and in human tissue samples.

Our objective was to evaluate the use of PCR for detection of *P. brasiliensis* DNA on sera samples from untreated patients with direct evidence of PCM and differ-

ent clinical forms. This study was approved by the Ethics Committee of the Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo.

Thirty-three serum samples from patients with PCM (Table) were submitted to nested-PCR with primers spanning a 196 bp fragment from the gp43 gene as described by Bialek et al. (2000). *P. brasiliensis* genome contains two copies of the gp43 gene (Cano et al. 1998). Amplification of a fragment of 432 bp from the human growth hormone gene was used as a positive control for the whole process according to Meyer et al. (1999). Among the 33 serum samples analyzed, 25 had the HGH fragment amplified, demonstrated DNA adequacy. When DNAs were submitted to the gp43 nested-PCR assay, only one presented the expected amplified fragment. Moreover, all 33 serum samples, when spiked with *P. brasiliensis* cells, presented the corresponding gp43 amplicon, discarding major PCR inhibition. Our results suggest that PCR with DNA extracted from serum, is not useful for the diagnosis of PCM, due to the low prevalence of fungemia in patients with different clinical forms of untreated paracoccidioidomycosis. Itano et al. (2002) were able to show PCR detection of *P. brasiliensis* DNA on 5/20 infected patients with DNA isolated from serum. However these results were obtained only after a third-round of PCR and they obtained no amplification on plasma samples from 78 infected mice after two-rounds of amplification (nested-PCR). An interesting observation of this work was a much higher frequency of positive PCR amplification with DNA extracted from clots in comparison to plasma.

In conclusion, our results show a limited value of nested PCR from serum samples, at least on its present form, for the identification of *P. brasiliensis* DNA. Though disappointing, the low detection rate here reported is corroborated by similar results from heavily infected experimental animals and also from the observation of the rarity of *P. brasiliensis* fungemia and isolation on blood culture from patients and infected mice (Singer-Vermees et al. 1993). Absence of *P. brasiliensis* DNA in sera of PCM patients

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TABLE
Patient backgrounds and the results of Nested-polymerase chain reaction (N-PCR)

Patients	Sex/age	Mycology		Feature(s)	No. of positive samples		
		Site(s)	Result by DME	Clinical	DI	CIE	N-PCR
01	M/54	Oral mucosa	(+)	Multifocal chronic	(+)	(1:16)	(-)
02	M/47	Oral mucosa/laryngea	(+)	Multifocal chronic	(+)	(1:128)	(-)
03	M/57	Mouth	(+)	Multifocal chronic	(+)	(1:8)	(-)
04	M/63	Labial ulcer	(+)	Multifocal chronic	(+)	(1:32)	(-)
05	M/63	Sputum	(+)	Multifocal chronic	(+)	(1:256)	(-)
06	M/49	Ganglia biopsy	(+)	Multifocal chronic	(+)	(1:64)	(-)
07	M/42	Soft palate biopsy	(+)	Multifocal chronic	(+)	(1:8)	(-)
08	M/35	Sputum	(+)	Multifocal chronic	(+)	(1:64)	(-)
09	M/35	Tongue biopsy	(+)	Multifocal chronic	(+)	(1:32)	(-)
10	M/30	Ganglia cervical region	(+)	Juvenile	(+)	(1:1024)	(-)
11	M/47	Ganglia cervical region	(+)	Multifocal chronic	(+)	(1:32)	(-)
12	M/42	Skin	(+)	Multifocal chronic	(+)	(1:256)	(-)
13	F/10	Mouth lesion	(+)	Multifocal chronic	(+)	(1:256)	(-)
14	F/40	Knee biopsy	(+)	Multifocal chronic	(+)	(1:128)	(-)
15	M/43	Tongue biopsy	(+)	Multifocal chronic	(+)	(1:8)	(-)
16	M/66	Mouth biopsy	(+)	Multifocal chronic	(+)	(1:16)	(-)
17	M/28	Skin biopsy	(+)	Juvenile	(+)	(1:2)	(-)
18	F/20	Skin biopsy	(+)	Juvenile	(+)	(1:1024)	(-)
19	M/55	Lip lesion	(+)	Multifocal chronic	(+)	(1:128)	(-)
20	M/52	Alveolar ridge mucosa	(+)	Multifocal chronic	(+)	(1:64)	(-)
21	M/41	Unknown	(+)	Multifocal chronic	(+)	(1:16)	(-)
22	M/58	Lymph node	(+)	Multifocal chronic	(+)	(1:64)	(-)
23	M/49	Skin nose biopsy	(+)	Juvenile	(+)	(1:64)	(-)
24	M/40	Skin biopsy	(+)	Multifocal chronic	(+)	(1:4)	(-)
25	M/64	Orofarinx biopsy	(+)	Unifocal chronic	(+)	(1:128)	(-)
26	M/47	Trachea biopsy	(+)	Multifocal chronic	(+)	(1:32)	(-)
27	M/55	Sputum	(+)	Unifocal chronic	(+)	(1:64)	(-)
28	M/53	Sputum	(+)	Multifocal chronic	(+)	(1:256)	(-)
29	M/20	Orofarinx lesion	(+)	Juvenile	(+)	(1:64)	(-)
30	M/44	Larynx biopsy	(+)	Multifocal chronic	(+)	(1:128)	(-)
31	M/51	Mouth biopsy	(+)	Multifocal chronic	(+)	(1:64)	(+)
32	M/12	Lymph node biopsy	(+)	Juvenile	(+)	(1:256)	(-)
33	M/49	Skin biopsy (nose)	(+)	Multifocal chronic	(+)	(1:32)	(-)

DME: direct microscopic examination; DI: immunodiffusion; CIE: contraimmunoelctrophoresis

deserves further investigation, as it contrasts to other deep mycosis. One possibility is that fungal DNA and/or cells are rapidly fagocytosed by leukocytes, reinforced by a recent observation showing that *P. brasiliensis* cells are readily detected and quantified in the bloodstream of experimental animals by staining with a fluorescence dye (Nishikaku & Burger 2003). We plan to carry a parallel comparison between whole blood and plasma/serum in order to clarify this issue.

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