Medical Mycology September 2005, **43**, 487–493

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Development of an enzyme-linked immunosorbent assay for the serodiagnosis of several clinical forms of sporotrichosis

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We performed a serological study with sera from 92 patients with confirmed sporotrichosis registered between 1999 and 2004 in two hospitals in Rio de Janeiro State, Brazil. The clinical presentation of sporotrichosis was distributed as follows: lymphocutaneous, 67%; fixed cutaneous, 23%; disseminated cutaneous, 8%; and extracutaneous, 2%. Sera were assayed by ELISA against a cell wall antigen of *Sporothrix schenckii*, *Ss*CBF, that we have previously described. The cross-reactivity was determined with 77 heterologous sera. The serological test showed a sensitivity of 90% and a global efficiency of 86%. A group of 55 patients with several clinical presentations of sporotrichosis was clinically and serologically followed-up for at least 6 months. We observed by ELISA data a decrease in the antibody serum titers which correlated with the progress in healing. An HIV-positive patient with meningeal sporotrichosis was serologically followed-up for over 2 years. Serum and cerebrospinal fluid specimens were examined and significant antibodies levels against the antigen *Ss*CBF were detected. Our results strongly suggest that this serological test is valuable for the differential diagnosis and follow-up of all clinical forms of sporotrichosis.

Keywords antigen, cell wall, *S. schenckii*, serodiagnosis, sporotrichosis

Introduction

Sporotrichosis is a subacute or chronic infection caused by the dimorphic fungus *Sporothrix schenckii*. The disease is usually acquired by the traumatic inoculation of fungal elements and has several clinical presentations; the most frequent being the lymphocutaneous form [1–3]. Osteoarticular and visceral involvement occurs mostly in patients who are immunocompromised and who have underlying alcoholism [4–6]. More recently, sporotrichosis has been reported as an emerging mycosis in HIV-positive individuals [6–9]. Although this mycosis has a worldwide distribution, the climatic and geographical conditions such as those existing in Brazil, India and Peru, favour the occurrence of this disease [10].

Sporotrichosis may be misdiagnosed (sporotrichoid lesions) with other diseases [3,11] and some of the currently available methods may not diagnose it immediately, especially the disseminated forms [3,12]. The delay in diagnosis of the extracutaneous forms of this pathology and the variable response to therapy are factors that may lead to fatality [4,6]. Serologic tests are being proposed and applied for the diagnosis of several mycoses, such as cryptococcosis [13], paracoccidioidomycosis (PCM) [14,15] and candidiasis [16],

Received 27 April 2004; Accepted 25 September 2004
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DOI: 10.1080/13693780400019909

and therefore could also be useful for sporotrichosis. Cell-wall antigens and anti-cell-wall antibodies may be the basis for developing specific and sensitive serologic tests [17]. The main challenge to developing a suitable diagnostic test is obtaining a species-specific antigen. This becomes an even more complicated goal because of the high cross-reactivity observed for glycoconjugates present either on the cell wall or in culture filtrate preparations of fungi.

The cell wall of *S. schenckii* is a suitable source of antigens [18]. Several epitopes were described on the cell surface of *S. schenckii* and were related with N- and O-linked oligosaccharides of the cell wall peptido-rhamnomannan [18,19]. The O-linked pentasaccharide was the main epitope identified in the peptido-rhamnomannan fraction [19]. By the presence of 2-O substituted mannose residues in the O-linked oligosaccharides, it was possible to isolate a concanavalin A (ConA)-binding antigen, further denominated *Ss*CBF [20]. This antigen showed a high reactivity when assayed with sera from patients with cutaneous sporotrichosis [21].

In the present work we have performed a serological study of patients with lymphocutaneous, fixed cutaneous, disseminated cutaneous and extracutaneous forms of sporotrichosis. An ELISA test was developed to determine specific antibodies against the cell wall antigen *Ss*CBF on sera and cerebrospinal fluid from these patients.

Materials and methods

Clinical specimens

Ninety-two patients with sporotrichosis confirmed by histopathology, direct KOH examination and/or culture were used in the study. Samples were collected between March 1999 and July 2004 at the Hospital Universitário Pedro Ernesto, of the State University of Rio de Janeiro, and at the Hospital Evandro Chagas, Fiocruz, Rio de Janeiro, Brazil. The patients studied had fixed cutaneous, lymphocutaneous, the cutaneous disseminated and extracutaneous forms of sporotrichosis. Fifty-five of these patients were evaluated at every follow-up appointment and serum samples were still collected after clinical healing. A period of 6–12 months was set for the serological analysis after the treatment was started, depending on the clinical presentation and the therapeutic response. Also, sera from 77 patients with aspergillosis ($n=6$), histoplasmosis ($n=8$), PCM ($n=35$), chromoblastomycosis ($n=10$) and cryptococcosis ($n=18$) were assayed by ELISA. Controls consisted of 30 serum samples from healthy donors from our department. All sera were collected with the authorized consent of the individuals.

An HIV-positive patient diagnosed for cutaneous sporotrichosis that further developed meningitis was followed-up clinically and serologically. Serum and cerebrospinal fluid (CSF) specimens were collected for the ELISA test. In addition, we tested five samples of CSF obtained from patients with other pathologies, who were either HIV positive or negative.

Micro-organism and growth conditions

Sporothrix schenckii, strain 1099-18, was used throughout this study and was originally obtained from the Mycology Section, Department of Dermatology, Columbia University, New York, USA. The micro-organism was routinely maintained on Sabouraud slants and stored at 4°C. The yeast phase of *S. schenckii* was grown in brain–heart infusion broth (BHI; Difco, MD, USA) for 7 days at 37°C in a rotary shaker (150 r.p.m.). Cultures with at least 90% yeast-like cells were used. Cells were killed with 0.01% (W/v) thimerosal (Merck, Darmstadt, Germany) and washed four times with 0.9% NaCl prior to cell wall extraction.

Isolation of cell wall glycopeptides

S. schenckii cell wall peptido-rhamnomannan (CWPR) was isolated from the yeast cell mass as previously described [22]. Briefly, the yeast cell mass was suspended in 0.02 mol/l citrate buffer pH 7.0 and autoclaved for 90 min at 120°C. The crude cell wall extract was further fractionated by Cetavlon (N, N cetyl hexadecyltrimethyl-ammoniun bromide) precipitation in the presence of borate. The CWPR was lyophilized for further purification.

A mannoprotein fraction of *Saccharomyces cerevisiae* was isolated and purified as described above. The MP was used as a negative control antigen for the ELISA assays with the CSF samples.

Isolation of the concanavalin A-binding fraction (SsCBF)

The *Ss*CBF antigen was obtained by affinity chromatography of the CWPR, as previously described [20]. Briefly, the CWPR was applied on a ConA-sepharose 4B column equilibrated with phosphate-buffered saline, pH 7.4 (PBS). The ConA bound material, denominated as *Ss*CBF, was eluted with 0.1 mol/l methyl-α-D-mannopyranoside in PBS and was further fractionated on a Bio-Gel P4 column. The void volume was collected and lyophilized.

ELISA

Human antibodies (IgG class) against the *Ss*CBF antigen were quantified by an indirect solid-phase enzyme-linked immunosorbent assay (ELISA), as previously described, with slight modifications [21]. Wells of polystyrene microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) were coated with *Ss*CBF by passive adsorption overnight at 4°C. After the blocking step, the plates were incubated with each serum sample which was assayed either at a 1:6400 dilution or in serial two-fold dilutions, depending on the assay. After a washing step, the plates were incubated with a goat anti-human IgG-HRP conjugate (Gibco, BRL, St Louis, MO, USA) and revealed with *O*-phenylenediamine 2.5 mg/ml and 0.006% H_2O_2 in 0.01 mol/l sodium citrate buffer, pH 5.6. The reaction was stopped after 20 min with 3 mol/l H_2SO_4 and the optical densities (*OD*) were read at 490 nm in an ELISA reader (ELISA Reader; Bio-Rad). For the CSF analysis, the samples were assayed as above at a 1:200 dilution, with either *Ss*CBF or MP fractions.

Statistical analyses

Values are reported as mean of at least three independent experiments. Comparison between patient and control ELISA data was done by analysis of the variance (ANOVA) with Bonferroni correction for multiple comparisons. All *P*-values of less than 0.05 were considered significant. Sensitivity, specificity and efficiency were determined as described by Crowter [23].

Results

Serological evaluation of patients with cutaneous and disseminated sporotrichosis

The serological reactivity against the antigen *Ss*CBF was determined by ELISA in the sera of patients with different clinical presentations of sporotrichosis. As show in Fig. 1, 67% of the patients examined presented the lymphocutaneous form of sporotrichosis. The other 33% were patients with the fixed cutaneous, the disseminated cutaneous and the extracutaneous forms of sporotrichosis. Individual serum samples of these patients were collected just before treatment was started and were assayed by ELISA at a 1:6400 dilution (Fig. 2). The cut-off point ($A_{490} = 0.23$) was established by the mean plus 2 standard deviations of the ELISA values from sera of healthy donors (control group). The ELISA reactivity was reproducible and determined in duplicates of at least three independent experiments.

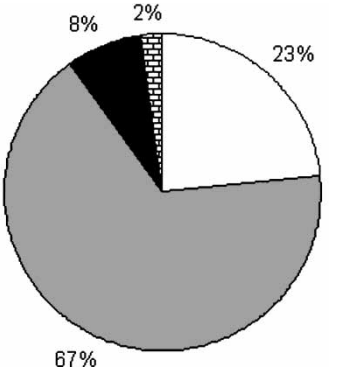

Fig. 1 Distribution of the clinical forms of sporotrichosis as observed for a group of 92 patients serologically evaluated during the period 1999–2004. Lymphocutaneous (■), fixed cutaneous (□), disseminated cutaneous (■) and extracutaneous (▦).

The cross-reactivity was evaluated with sera from 77 patients with other mycosis (Fig. 3). Some degree of cross-reactivity was observed in heterologous serum samples. A low cross-reactivity was observed specifically with sera from patients with chromoblastomycosis. The sensitivity of the test was 90% with a specificity of 80% and a global efficiency of 86%.

Serological follow-up of sporotrichosis patients

The antibody levels against the antigen *Ss*CBF were evaluated during the clinical trial. Serum specimens from 55 patients were analysed before and during treatment and were collected at every follow-up appointment. Fig. 4 shows a representative serological

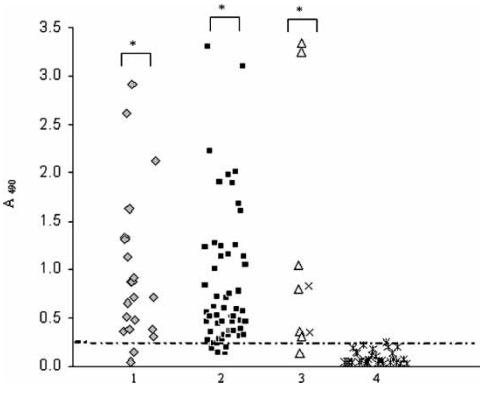

Fig. 2 Reactivity determined by ELISA of serum specimens from 92 patients with confirmed sporotrichosis. The groups studied are indicated by numbers, as follows. (1) Patients with the fixed cutaneous form ($n = 21$); (2) patients with the lymphocutaneous form ($n = 62$); (3) patients with the disseminated cutaneous and the extracutaneous forms ($n = 9$); (4) healthy individuals ($n = 30$). Sera were assayed at 1:6400 dilutions. The horizontal dashed line represents the cut-off point. $^*P < 0.01$ compared with healthy individuals (control group).

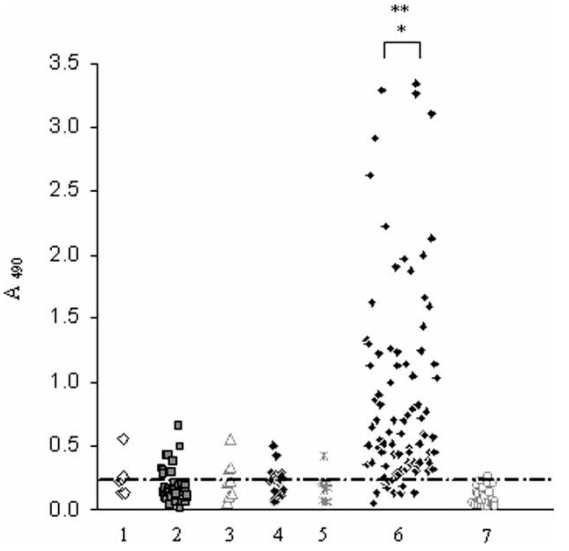

Fig. 3 Indirect ELISA for the evaluation of anti-*Ss*CBF antibodies in sera of patients with several clinical forms of sporotrichosis ($n=92$) and with heterologous fungal infections. Serum specimens from patients with fungal diseases and healthy donors are represented as follows. (1) Aspergillosis ($n=6$); (2) PCM ($n=35$); (3) histoplasmosis ($n=8$); (4) cryptococcosis ($n=18$); (5) chromoblastomycosis ($n=10$); (6) sporotrichosis ($n=92$); (7) healthy volunteers ($n=30$). Sera were assayed at a 1:6400 dilution. The horizontal dashed line represents the cut-off point. $*P<0.05$ compared with groups 2, 3, 4 and 5; $**P<0.01$ compared with the control group.

finding during the follow-up of a patient with lymphocutaneous sporotrichosis. A significant decrease in the serum antibody titer against the antigen *Ss*CBF correlated with the therapeutic response. Similar results were observed for other patients submitted to several therapeutic regimens (data not shown). Independent of the clinical presentation, the ELISA test could detect a decrease in the IgG titers that parallels the therapeutic regimen and clinical healing (Fig. 5). The serological follow-up of an HIV-positive patient diagnosed for cutaneous sporotrichosis who further developed meningeal sporotrichosis showed an increase in the IgG titer which corresponds to the recurrence of episodes (Fig. 5D) described later.

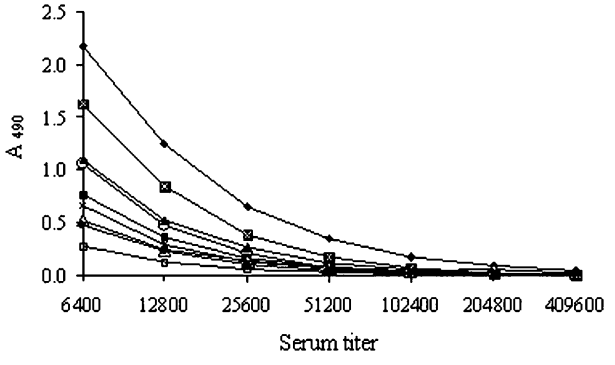

Fig. 4 IgG titers of nine serum samples from a patient with lymphocutaneous sporotrichosis as determined by ELISA with the *Ss*CBF antigen. The patient was monitored over 9 months and serum samples were collected before treatment (◆) and, on the second (⊠), third (▲), fourth (○), fifth (□), sixth (✳), seventh (△), eighth (●), and ninth (■) month after therapy was started.

The mean IgG titers in serum samples for each group of patients with several clinical presentations of sporotrichosis were evaluated before and after treatment (Fig. 6). In the latter cases, sera were collected 6–12 months after the drug regimen was started and/or the patient was considered clinically cured. A significant decrease in the antibody serum titers was observed for the patients with lymphocutaneous and disseminated forms of sporotrichosis. Although not statistically significant, an unambiguous decrease in the antibody serum titer after clinical healing was also observed for the group of patients with the fixed cutaneous form of this disease.

The titers of two patients were analysed individually. One of these patients was HIV-positive and had disseminated cutaneous sporotrichosis. He showed higher antibody titers starting from 819 200 and decreasing to 51 200 during treatment. He abandoned the treatment after 4 months. The other patient was an immunocompetent individual initially diagnosed for the disseminated cutaneous form of sporotrichosis but showing serum titers of 409 600. She was further diagnosed with osteoarticular sporotrichosis and amphotericin B was introduced. The serum titer decreased to 12 800 after 12 months.

Detection of antibodies against SsCBF in CSF specimens of an HIV-positive patient

A 27-year-old man with fever, lymphadenomegaly, hepatoesplenomegaly and cutaneous lesions was diagnosed as HIV-positive (CD4 = 97 cells/mm^3). Skin biopsy revealed presence of *S. schenckii* at histopathology and culture. Treated initially with itraconazole and retroviral therapy, his clinical status complicated with meningitis, which was treated with amphotericin B (3 g total dose) and maintenance therapy at a day-hospital. Two months after initial treatment, meningitis recurred and was associated with fluconazole. Three months after the second meningeal episode, amphotericin B was stopped because of nephrotoxic side-effects. Once again, meningitis recurred. Meningeal episodes stopped only when viral load reached undetectable levels and CD4 counts raised to 200 cells/mm^3. The patient was serologically followed-up for 26 months. Serum and CSF samples were collected and assayed against the

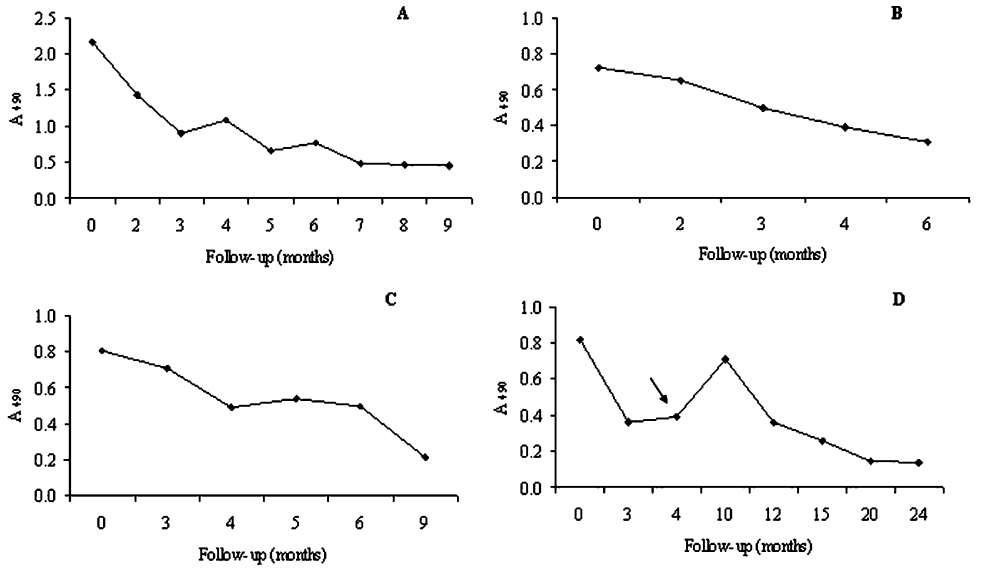

Fig. 5 Findings at follow-up for four patients with different forms of sporotrichosis by indirect ELISA. (A) lymphocutaneous form; (B) fixed cutaneous form; (C) disseminated cutaneous form; (D) extracutaneous form. The black arrow indicates a recurrence of meningeal sporotrichosis.

antigen *Ss*CBF (Fig. 5D, 7). ELISA data of CSF showed an increase in the IgG level with specific peaks at recurrence episodes and amphotericin B therapy discontinuation (Fig. 7). The CSF titer lowered with the improvement of the clinical condition. ELISA assays with an unspecific mannoprotein fraction of *S. cerevisiae* confirmed that the IgG antibodies detected specifically recognized the glycoprotein antigen, *Ss*CBF (data not shown). Cerebrospinal fluid specimens of five patients with other uninformed pathologies were used as controls. No detectable cross-reactivity was observed and the *OD* was lower then 0.07 (data not shown).

Discussion

Currently, a sporotrichosis epidemic is occurring in the State of Rio de Janeiro, involving cats, dogs and humans [24]. The increasing number of infected domestic cats represents an important epidemiological factor as their lesions are rich in yeast-form parasites, which can be directly inoculated in the human host by scratches. This fact could lead to a change in the incidence of more severe forms of sporotrichosis [25], as the yeast phase is highly virulent [26]. Since the 1980s, veterinarians, technicians, handlers and owners of sick cats have been considered as a new risk group for the acquisition of sporotrichosis [1]. Also, sporotrichosis had been reported as an emerging mycosis in HIV-positive individuals [6,27,28]. Because of the absence of specific clinical signs and symptoms, systemic mycoses

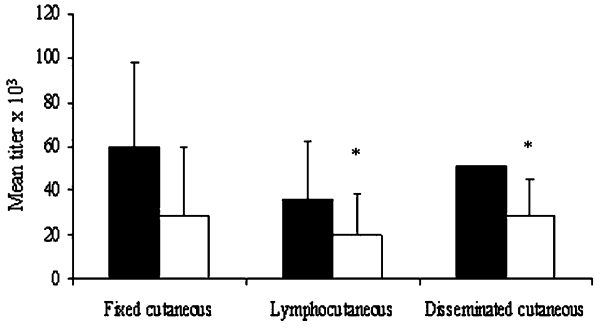

Fig. 6 Mean IgG titer determined by ELISA with the *Ss*CBF antigen in sera of patients with sporotrichosis. Serum samples were collected before (black bars) and after treatment (white bars). $*P < 0.05$ compared with the untreated group.

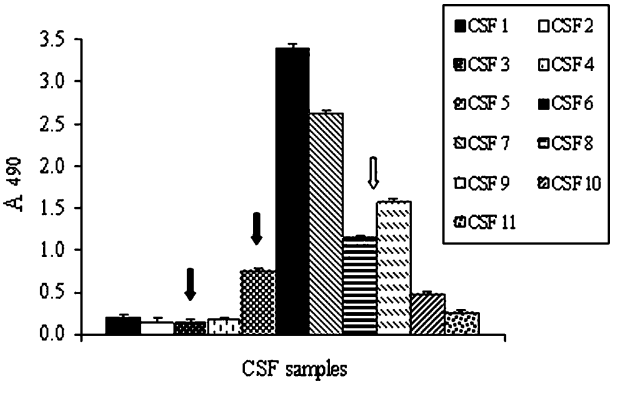

Fig. 7 Determination of IgG titers in the cerebrospinal fluid (CSF) of an HIV-positive patient diagnosed for meningeal sporotrichosis. Eleven CSF samples were collected in 1- to 3-month intervals (CSF 1 to CSF 11) and were assayed by ELISA against *Ss*CBF. The first sample corresponds to the beginning of the therapeutic regimen (CSF 1). The black arrows indicate recurrence episodes of meningeal sporotrichosis and the white arrow indicates the isolation of *S. schenckii* from the CSF.

remain a diagnostic and therapeutic challenge [29]. Serologic tests can rapidly diagnose fungal infections, enabling prompt therapy to be initiated [30]. A great number of serodiagnostic tests currently available are far from satisfactory, lacking both sensitivity and/or specificity [31] and are therefore not helpful in diagnosing relevant invasive mycosis [32].

In a previous work, we described the species-specific *Ss*CBF antigen, which showed a potential application in the serological diagnosis of sporotrichosis [20]. This antigenic fraction gave low cross-reactivity with sera from healthy individuals and from patients with paracoccidioidomycosis, cryptococcosis, candidiasis, aspergillosis or histoplasmosis. Thus, in the present work, we performed a serological study of 92 patients with confirmed sporotrichosis who were clinically followed-up. The validation of the diagnostic test was evaluated with 199 human sera. To evaluate whether or not the sensitivity of the test would vary significantly with the severity and extent of the infection, we appraised antibody titers against the *Ss*CBF antigen of patients' sera presenting different clinical forms of this mycosis. Based on the cut-off value, the serological test was positive for 90% of gold standard patients, illustrating the usefulness of this method for the diagnosis of sporotrichosis. Taking into account the healthy donors and the groups of patients with other mycoses, we could observe a specificity of 80% for the test. The *Ss*CBF antigen has not only the O-linked oligosaccharides we had previously characterized on the cell wall peptido-rhamnomannan but also, the N-linked chains [18,33]. These N-linked epitopes can cross-react not only with other fungi but also with *Streptococcus* species. [18]. Consequently, the low degree of cross-reactivity observed in our test may be due, at least in part, to the presence of N-linked epitopes and the O-linked trisaccharide (α-L-Rha 1→3 α-D-Man 1→2 α-D-Man) in the *Ss*CBF antigen [21]. The cross-reactivity with other pathologies that classically present sporotrichoid lesions, such as cutaneous leishmaniasis and mycobacterium infections, is currently under study. It is interesting to notice that among the control group of sera we had samples from healthy volunteers of our laboratory who had routinely contact with this pathogen. In conclusion, the global efficiency as defined as the percentage of subjects correctly classified as diseased with a given prevalence was 86%.

The clinical evolution face to a given therapeutic regimen can also be potentially appraised by a serological follow-up. The good sensitivity that we obtained in the ELISA test makes us to evaluate its helpfulness as a prognostic tool. During the period of study, patients responded well to treatment, which corresponded with the decrease in their serum IgG titers. Some abandoned the treatment, which did not allow a correlation of 100% between the serological data and the complete healing or the recurrence of signs and symptoms. We observed a clear correlation between clinical improvement and antibody titer lowering. Also, the ELISA test with the cell wall antigen *Ss*CBF was applied to the serological analysis of CSF specimens from a patient with meningeal sporotrichosis. We detected specific antibodies against *Ss*CBF in the CSF of this patient and no reactivity was observed with the cell wall MP fraction of *S. cerevisiae*. Moreover, in blind experiments a clear correlation between the increase of antibody levels in serum and CSF and the recurrence episodes was observed. No cross-reaction with CSF from patients of the control group was observed. A previously report in the literature showed an increase in the antibodies levels in CSF of seven patients with meningeal sporotrichosis in a serological study with a crude antigenic preparation from the culture filtrate of *S. schenckii* [34]. The authors did not show cross-reactivity for the CSF of patients with other heterologous fungal, bacterial and viral infections.

Our findings suggest that the present ELISA assay based on the *Ss*CBF antigen represents a relevant laboratory tool for the differential and/or complementary diagnosis of all clinical forms of sporotrichosis.

Acknowledgements

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (Faperj). A.R.B.E. is supported by Proatec/UERJ. We thank Dr Monica Vidal of Universidade de São Paulo for providing sera from patients with chromoblastomycosis and paracoccidioidomycosis.

References

1 Kauffman CA. Sporotrichosis. *Clin Infect Dis* 1999; **29**: 231–237.
2 De Araujo T, Marques AC, Kerdel F. Sporotrichosis. *Intern J Dermatol* 2001; **40**: 737–742.
3 Morris-Jones R. Sporotrichosis. *Clin Dermatol* 2002; **27**: 427–431.
4 Padhye AA, Kaufman L, Durry E, *et al*. Fatal pulmonary sporotrichosis caused by *Sporothrix schenckii* var. *lurei* in India. *J Clin Microbiol* 1992; **30**: 2492–2494.
5 Howell SJ, Toohey JS. Sporotrichal arthritis in south central Kansas. *Clin Orthop* 1998; **346**: 207–214.
6 al-Tawfiq JA, Wools KK. Disseminated sporotrichosis and *Sporothrix schenckii* fungemia as the initial presentation of human immunodeficiency virus infection. *Clin Infect Dis* 1998; **26**: 1403–1406.

7 Aarestrup FM, Guerra RO, Vieira BJ, Cunha RM. Oral manifestation of sporotrichosis in AIDS patients. *Oral Dis* 2001; **7**: 134–136.
8 Carvalho MT, de Castro AP, Baby C, *et al*. Disseminated cutaneous sporotrichosis in a patient with AIDS: report of a case. *Rev Soc Bras Med Trop* 2002; **35**: 655–659.
9 Donabedian H, O'Donnell E, Olszewski C, *et al*. Disseminated cutaneous and meningeal sporotrichosis in an AIDS patient. *Diagn Microbiol Infect Dis* 1994; **18**: 111–115.
10 Bustamante B, Campos PE. Endemic sporotrichosis. *Curr Opin Infect Dis* 2001; **14**: 145–149.
11 Agudelo SP, Restrepo S, Velez ID. Cutaneous New World leishmaniasis-sporotrichosis coinfection: report of three cases. *J Am Acad Dermatol* 1999; **40**: 1002–1004.
12 Queiroz-Telles F, McGinnis MR, Salkin I, Graybill JR. Subcutaneous mycoses. *Infect Dis Clin North Am* 2003; **17**: 59–85.
13 Stockman L, Roberts GD. Specificity of the latex test for cryptococcal antigen: a rapid, simple method for eliminating interference factors. *J Clin Microbiol* 1992; **16**: 965–967.
14 Camargo ZP, Berzaghi R, Amaral CC, Silva SH. Simplified method for producing *Paracoccidioides brasiliensis* exoantigens for use in immunodiffusion tests. *Med Mycol* 2003; **41**: 539–542.
15 Diez S, Gómez BL, McEwen JG, *et al*. Combined use of *Paracoccidioides brasiliensis* recombinant 27-kilodalton and purified 87-kilodalton antigens in an enzyme-linked immunosorbent assay for serodiagnosis of paracoccidioidomycosis. *J Clin Microbiol* 2003; **41**: 1536–1542.
16 Kondori N, Edebo L, Mattsby-Baltzer I. *Candida albicans* cell wall antigens for serological diagnosis of candidemia. *Med Mycol* 2003; **41**: 21–30.
17 Lopez-Ribot JL, Casanova M, Murgui A, Martinez JP. Antibody response to *Candida albicans* cell wall antigens. *FEMS Immunol Med Microbiol* 2004; **41**: 187–196.
18 Travassos LR. Antigenic structures of *Sporothrix schenckii*. *Immunol Ser* 1989; **47**: 193–221.
19 Lopes-Alves L, Travassos LR, Previato JO, Mendonca-Previato L. Novel antigenic determinants from peptido-rhamnomannans of *Sporothrix schenckii*. *Glycobiology* 1994; **4**: 281–288.
20 Lima OC, Lopes-Bezerra LM. Identification of a concanavalin A-binding antigen of the cell surface of *Sporothrix schenckii*. *J Med Vet Mycol* 1997; **35**: 167–172.
21 Penha CVL, Lopes-Bezerra LM. Concanavalin A-binding cell wall antigens of *Sporothrix schenckii*: a serological study. *Med Mycol* 2000; **38**: 1–7.
22 Lloyd KO, Bitoon MA. Isolation and purification of a peptido-rhamnomannan from the yeast form of *Sporothrix schenckii*. Structural and immunochemical studies. *J Immunol* 1971; **107**: 663–671.
23 Crowter JR. *Methods in Molecular Biology. The ELISA Guidebook*, vol. 149. New Jersey: Humana Press, 2001.
24 de Lima Barros MB, Schubach TM, Gutierrez Galhardo MC, *et al*. Sporotrichosis: an emergent zoonosis in Rio de Janeiro. *Mem Inst Oswaldo Cruz* 2001; **96**: 777–779.
25 de Lima Barros MB, de Oliveira Schubach A, Galhardo MC, *et al*. Sporotrichosis with widespread cutaneous lesions: report of 24 cases related to transmission by domestic cats in Rio de Janeiro, Brazil. *Int J Dermat* 2003; **42**: 677–681.
26 Fernandes KS, Coelho AL, Lopes-Bezerra LM, Barja-Fidalgo C. Virulence of *Sporothrix schenckii* conidia and yeast cells, and their susceptibility to nitric oxide. *Immunology* 2000; **101**: 563–569.
27 Durden FM, Elewski B. Fungal infections in HIV-infected patients. *Semin Cutan Med Surg* 1997; **16**: 200–212.
28 Ware AJ, Cockerell CJ, Skiest DJ, Kussman HM. Disseminated sporotrichosis with extensive cutaneous involvement in a patient with AIDS. *J Am Acad Dermatol* 1999; **40**: 350–355.
29 Richardson MD, Kokki MH. New perspectives in the diagnosis of systemic fungal infections. *Ann Med* 1999; **31**: 327–335.
30 de Repentigny L. Serological techniques for diagnosis of fungal infection. *Eur J Clin Microbiol Infect Dis* 1989; **8**: 362–375.
31 Buckley HR, Richardson MD, Evans EG, Wheat LJ. Immunodiagnosis of invasive fungal infection. *J Med Vet Mycol* 1992; **30**: 249–260.
32 Petri MG, Konig J, Moecke HP, *et al*. Epidemiology of invasive mycosis in ICU patients: a prospective multicenter study in 435 non-neutropenic patients. *Intensive Care Med* 1997; **23**: 317–325.
33 Lopes-Alves LM, Mendonca-Previato L, Fournet B, *et al*. O-glycosidically linked oligosaccharides from peptidorhamnomannans of *Sporothrix schenckii*. *Glycoconj J* 1992; **9**: 75–81.
34 Scott N, Kaufman L, Brown A, Muchmore HG. Serologic studies in the diagnosis and management of meningitis due to *Sporothrix schenckii*. *N Engl J Med* 1987; **317**: 935–940.