MAJOR ARTICLE

Residual Pulmonary Abnormalities in Adult Patients with Chronic Paracoccidioidomycosis: Prolonged Follow-Up after Itraconazole Therapy

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Itraconazole effectively controls active paracoccidioidomycosis but appears not to hinder lung fibrosis. Clinical records and chest radiographs from 47 itraconazole-treated patients with prolonged posttherapy follow-up (mean follow-up period, 5.6 years) were analyzed; the radiographs were interpreted following pneumoconiosis standards that consider the lungs as 6 fields and grade damage according to the number of fields involved. Infiltrative lesions were observed at diagnosis in 93.6% of the patients. Fibrosis was observed in 31.8% of the patients at diagnosis and had not cleared at the end of the observation period in any of these patients. Fibrosis also developed de novo in 11 patients (25%), so that by the end of the follow-up period it was seen in 53.2% of patients overall. Fibrosis correlated with severity of infiltrates at diagnosis: fibrosis was present in 83% of patients with very severe infiltration and in 12.5% of patients with minor infiltration. Among patients with severe infiltration, fibrosis was present in 30%; this increased (to 75%) when bullae were concomitantly present at diagnosis. Prompt initiation of treatment is necessary to avoid the development of fibrosis.

Paracoccidioidomycosis (PCM) is an important systemic mycosis in Latin America, especially in Brazil; in Colombia, the disease is also regularly diagnosed. The disorder is caused by the thermally dimorphic fungus *Paracoccidioides brasiliensis,* for which the habitat is as yet undiscovered. PCM is probably acquired by inhalation of infectious particles (conidia and mycelial fragments) produced by the mycelial form that reach the lungs, convert into the yeast form, and initiate primary infection. This process is usually asymptomatic but may result in overt disease over the course of years. When clinical manifestations become apparent, the disease may appear in an acute-subacute form (also called the "juvenile form") or a chronic, adult form. In both of these forms, the progression of the disease results in the involvement of other organs (e.g., lymph nodes, spleen, liver, adrenal glands, mucosae, and skin). The lungs are the main target and the site at which both active and residual lesions appear regularly [1, 2].

Active pulmonary involvement and residual fibrotic lesions are observed in ~80% and ~60%, respectively, of patients with PCM. The fibrotic sequelae alter respiratory function and incapacitate the patient so that normal activities become a burden [3–5]. Thus, the lungs are a significant site of morbidity and mortality in patients with PCM [6].

Despite the frequency with which pulmonary repercussions of this mycosis occur, clinical manifestations and auscultatory findings associated with the disease are minor. However, radiologic abnormalities are often extensive, which results in a dissociation between clinical and radiologic findings [3–5, 7]. The silent course of the disease results not only in late consultation but

Received 7 February 2003; accepted 21 May 2003; electronically published 8 September 2003.

Financial support: Corporación para Investigaciones Biológicas, Medellin, Colombia.

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Clinical Infectious Diseases 2003; 37:898–904

1058-4838/2003/3707-0004$15.00

also in steady progression of lung damage, which affects the patient's health and hinders the determination of disease onset [1, 3, 7]. Physicians must be aware that the lungs are the target organs for *P. brasiliensis* and that, in areas where this organism is endemic, the lungs of patients with chronic, nonspecific constitutional symptoms should be examined radiologically. This inexpensive measure may reveal alterations that are suggestive of the mycosis, hopefully while the disease is in an early stage.

Treatment has profoundly changed the outcome for patients with this mycosis. The first therapeutic agents discovered to successfully treat the disease, sulfonamides, brought improvement in >60% of the patients when prolonged (3–5-year) courses of therapy were administered [8]. Amphotericin B also had a significant impact on more-severe cases [9]. Nonetheless, the most important advance was represented by azole derivatives; both ketoconazole and itraconazole facilitated ambulatory therapy, required shorter courses of treatment, and were associated with fewer secondary effects [9–11]. Itraconazole is a potent antimycotic against *P. brasiliensis,* both in vitro and in vivo. The administration of daily doses of 100–200 mg for 6 months results in clinical and mycological response in 95% of patients, and relapses occur in <5% [9, 11, 12].

Despite the effectiveness of itraconazole therapy for treatment of active disease, the development of fibrotic sequelae does not appear to be modified by such treatment. In the present study, we examined the course of PCM in patients who received this triazole and determined the outcome of pulmonary abnormalities after an extended follow-up period.

PATIENTS AND METHODS

The records for 60 patients in whom PCM was diagnosed and who were treated with itraconazole at the Corporación para Investigaciones Biológicas (Medellin, Colombia) during the period 1982–1990 were analyzed. Follow-up observations had been made for a period of >1 year after the end of treatment for all of these patients; however, 8 patients had to be excluded because of the presence of concomitant pulmonary tuberculosis or irregular posttherapy visits. In addition, 5 patients with the juvenile form of the mycosis were excluded, because the course of their disease (e.g., minor or no apparent lung involvement and the presence of symptoms that were not related to pulmonary disease) differed from that of the remaining patients. Consequently, the study group consisted of 47 patients who underwent radiologic examination of their lungs at diagnosis and through the end of the posttherapy observation period.

All patients were adult men (mean age, 49 years; range, 21–75 years). Thirty-nine patients (83.0%) had the chronic, multifocal, adult form of the mycosis, and the remaining 8 (17.0%) had the chronic, unifocal form. The principle associated factor was a history of smoking (present for 44 [93.6%] of the 47 patients).

The patients were treated with itraconazole, 100–200 mg/day; the duration of treatment was determined by clinical presentation and response to treatment. Twenty-seven (69.2%) of the 39 patients with chronic, multifocal disease received treatment for a mean of 5.9 months (range, 3–6 months); the remaining 12 patients (30.8%) received a more prolonged course (mean, 10.4 months; range, 7–12 months). The 8 patients with the chronic, unifocal form received therapy for a mean of 5.5 months (range, 3–6 months). Two (4.3%) of the 47 patients had relapses during the observation period (<2 months after therapy cessation); they were treated again with itraconazole, and prompt control of the mycosis was achieved. Relapses were not considered to be additional (new) cases.

After itraconazole therapy was stopped, regular follow-up observations were made for a mean period of 5.6 years (range, 1–17 years). The distribution is as follows: for 25 patients, the duration of follow-up was 1–5 years; for 16 patients, 6–10 years; and for 6 patients, 11–17 years. All signs and symptoms (pulmonary or extrapulmonary) exhibited by the patients at diagnosis were recorded and compared with those at the end of the observation period.

Chest radiographs were initially read blindly (with no knowledge of the patient's name or the time at which the film was taken) by 2 members of our team (A.M.T. and M.L.O.), who then analyzed the results and classified the findings according to the extension of both infiltrative and fibrotic lesions with the lungs. The lung area was divided into 6 zones, according to the guidelines of the pneumoconiosis international classification of radiographs [13]. These guidelines trace horizontal lines along the superior, central, and inferior fields and vertical lines between the right and left fields; thus, fields comprise one-third and two-thirds of the vertical distance from the pulmonary apex to the dome of the diaphragm. Lung involvement was minor if only 1–2 fields were affected, severe if 3–4 fields were affected, and very severe if 5–6 fields were affected.

The classification and regression tree system proposed by Breiman et al. [14] was used to define factors predictive of the development of fibrosis in the patients studied. Two-way analysis of variance was used to determine differences among the groups.

RESULTS

The various signs and symptoms exhibited by the patients at the time of diagnosis and at the end of the posttherapy evaluation period are shown in table 1. Pulmonary alterations (cough, expectoration, and dyspnea) persisted for years in a substantial number of patients (>38.3%). On the other hand,

Table 1. Signs and symptoms in 47 patients with chronic paracoccidioidomycosis.

Sign or symptom	No. (%) of patients with sign or symptom	
	At diagnosis	At the end of the posttherapy period
Cough	37 (78.7)	22 (46.8)
Expectoration	32 (68.1)	18 (38.3)
Dyspnea	29 (61.7)	21 (44.6)
Dysphonia	14 (29.8)	3 (6.4)
Mucosal lesions	29 (61.7)	0
Skin lesions	10 (21.3)	0
Hypertrophied lymph nodes	20 (42.6)	0
Fever	22 (46.8)	0
Dysphagia	14 (29.8)	1 (2.1)
Weight loss	31 (66.0)	0

extrapulmonary complaints (mucosal or skin lesions and hypertrophied lymph nodes) disappeared in almost all patients.

The initial lung radiographs revealed infiltrative lesions in 44 patients (93.6%); no lung abnormalities were observed in 3 patients (6.4%). Infiltrates were most commonly interstitial (in 28 [63.6%] of the 44 patients with such lesions); mixed (alveolar and interstitial) lesions were seen in 16 patients (36.3%). At the end of the posttherapy observation period, a total of 25 patients (56.8%) had become free of infiltrative lesions (14 of those with interstitial lesions [31.8%] and 11 of those with mixed alveolar-interstitial components [25.0%]). The alveolar component cleared after therapy in the 16 patients with mixed infiltrates; however, both components did not completely clear in all patients: in 5, the interstitial lesions remained. In the group of 29 patients with interstitial pathology, 14 had the same lesions at the end of follow-up as at the beginning. Thus, a total of 19 patients (43.2%) with infiltrative lesions had this abnormality at the end of the observation period (table 2). No significant differences were found among the groups in the type of infiltrates or whether the lesions improved; however, a significant value ($P = .001$) was found when an intragroup comparison was made for those patients in whom infiltrates had not resolved by the end of the study. Figures 1 and 2 illustrate the changes that occurred over time.

At diagnosis, the intensity of the infiltrates was classified as minor in 8 (18.1%) of the 44 patients with such lesions, severe in 18 patients (40.9%), and very severe in 18 patients (40.9%). At the end of treatment, 7 patients (15.9%) had no infiltrative lesions visible on radiographs; 12 (27.3%) had persistent infiltrates only, and 7 (15.9%) had both infiltrates and fibrosis (table 3). In all, 19 patients (43.2%) had persistent infiltrative lesions. Resolution of the infiltrative lesions was also minor in patients with severe and patients with very severe infiltrates (11.1% for both groups). More than 33.4% of these patients had persistent infiltrates at the end of the observation period. The frequency of fibrosis increased with the severity of lung infiltration at diagnosis (table 3); thus, among the 18 patients who had shown infiltrative lesions in 5–6 pulmonary fields, 5 (27.8%) had fibrosis in addition to infiltrates and 10 (55.6%) had fibrosis at the end of the observation period ($P = .0001$).

Fibrotic lesions were found at diagnosis in 14 (31.8%) of the 44 patients with infiltrative lesions. The intensity of the fibrotic lesions was minor in 10 patients (22.7%), severe in 3 (6.8%), and very severe in 1 (2.3%). At the end of the study, fibrosis persisted in these 14 patients, and, furthermore, it appeared de novo in 11 patients, for a total of 25 patients (53.2%) with this residual sequela. The simultaneous presence of other residual lesions, such as bullae, was also noted, in 12 patients (27.3%). In addition, indirect signs of pulmonary hypertension, mainly abnormal thickening of the pulmonary hilar arteries, were recorded in 6 patients (13.6%). Twenty-six patients (59.1%) had bullae at the end of the observation period: 12 who had bullae at diagnosis, and 14 who developed it de novo. Signs of pulmonary hypertension persisted in all 6 patients, and 6 more patients developed pulmonary hypertension; in total, 12 patients (27.3%) had this finding at the end of the observation period.

Table 2. Types of infiltrative lesions present at diagnosis in 44 patients with chronic paracoccidioidomycosis and infiltrative lesions who were treated with itraconazole.

Type of lesion	No. (%) of patients		
	All[a]	With lung involvement improved at end of study	With lung involvement unresolved at end of study
Interstitial infiltrates	28 (63.6)	14 (31.8)	14 (31.8)
Mixed infiltrates	16 (36.4)	11 (25.0)	5 (11.4)
Total	44 (100)	25 (56.8)[b]	19 (43.2)[c]

[a] No lesions were seen on the radiographs for 3 patients.
[b] Free of infiltrative lesions only
[c] $P = .0001$, for patients with interstitial infiltrates vs. patients with mixed infiltrates.

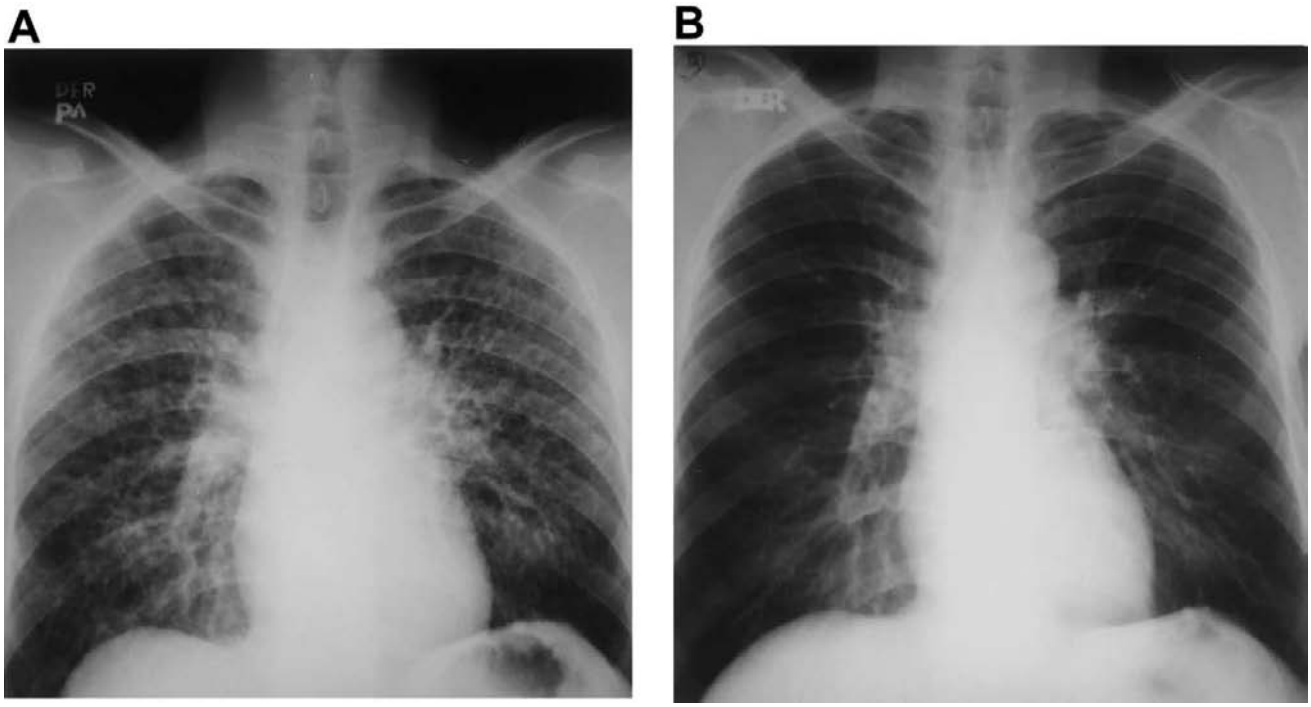

Figure 1. *A,* Chest radiograph at diagnosis showing severe interstitial infiltrates in a 45-year-old patient with paracoccidioidomycosis. *B,* Radiograph for the same patient at the end of the posttherapy follow-up period (12 months) showing clearing of interstitial lesions with no fibrosis in previously involved fields.

Fourteen (66.7%) of the 21 patients who had clinically manifested pulmonary signs (dyspnea and cor pulmonale) at the end of the observation period (table 1) had fibrosis visible on the final radiograph. However, of the 23 remaining patients with no clinically manifested pulmonary signs, fibrosis was present in 11 (47.8%); there was no statistically significant difference among the groups. Attempts were also made to determine the effect of smoking on the radiologic abnormalities detected in these 23 patients, but the attempts failed, because almost all (93.6%) of the patients smoked. In addition, no radiologic differences were found when the group was subdivided into those who had stopped smoking and those who had continued to do so.

In our patients, the intensity of the infiltrative component at time of diagnosis influenced the development of fibrosis, as shown by the classification and regression tree system [14]. Among patients in whom the initial infiltration was very severe (>5 lung fields involved), the possibility of developing fibrosis over time was 83%. By contrast, this possibility was smaller (12.5%) for patients with minor infiltrative involvement (1–2 fields). In the group with a severe infiltrative component (>3 lung areas), the possibility that fibrotic sequelae would be present at the end of the observation period was 30%; however, this figure increased to 75% when bullae were concomitantly present (table 4).

DISCUSSION

Once *P. brasiliensis* infectious propagules reach the lungs and convert into the yeast form in a susceptible host, dissemination throughout the pulmonary structures occurs, frequently producing extensive local damage that, later on, results in respiratory incapacity [3–5, 7, 15, 16]. Residual pulmonary involvement in patients with PCM is characterized by a variety of radiologic abnormalities distributed bilaterally, among which the following are important: infiltrative lesions of both the alveolar and the interstitial types, hypertrophied and calcified hilar and mediastinal lymph nodes, fibrosis, septum thickening, and pseudotumoral and fibronodular masses [3–5, 15]. The first 3 abnormalities were observed in our patients. High-resolution CT findings have emphasized that interstitial abnormalities are the predominant sequela in patients with PCM [16].

It is known that, in an important number of patients, diagnosis of PCM is achieved late during the course of the infection, as a result, in part, of the silent nature of the primary pulmonary symptoms [7]. This circumstance explains why radiologic abnormalities that are consistent with old, established processes, such as fibrosis, are commonly found at diagnosis [4, 5, 15, 16]. This was seen in the present study, in which such alterations were observed in 31.8% of the patients at diagnosis,

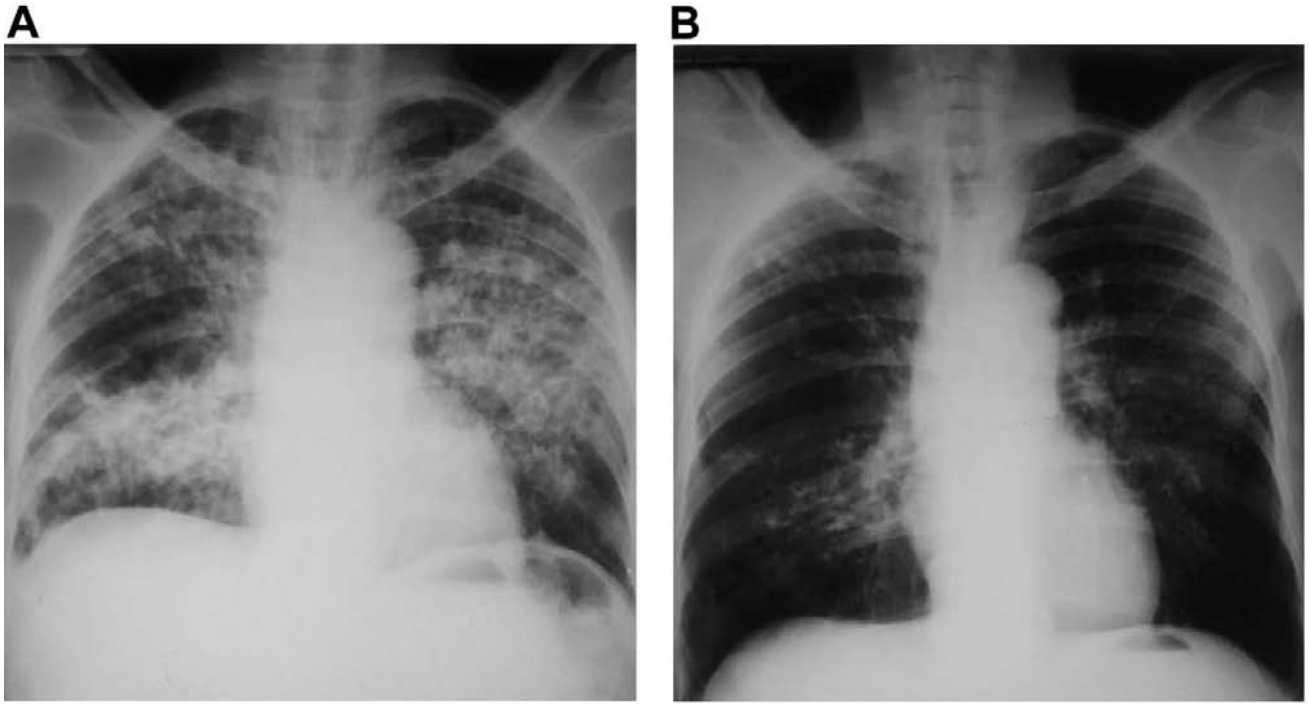

Figure 2. *A,* Chest radiograph at diagnosis showing severe mixed-type infiltrative lesions in a 65-year-old patient with paracoccidioidomycosis. *B,* Radiograph for the same patient at the end of the posttherapy follow-up period (12 months) showing extensive fibrosis and basal bullae.

which is an alarming rate of residual lung damage. Steps should be taken to make physicians aware that, in the population considered at major risk (i.e., men who are employed in areas where PCM is endemic), obtaining radiologic images is mandatory, even in the absence of respiratory symptoms. It would also be desirable to work in cooperation with official campaigns established for the control of tuberculosis, so that pulmonary pathology compatible with this mycotic disorder can be detected.

It has been shown that antifungal treatment effectively controls multiplication of *P. brasiliensis,* thus diminishing the infectious load [9] and allowing the expression of the patient's immune response [17, 18]. In the present study, which emphasized the pulmonary component of PCM, it was observed that, in the 16 patients (36.4%) who had mixed infiltrates, the alveolar component, probably the active lung lesion, disappeared completely. Of the 44 patients with infiltrative lesions, 25 (56.8%) had resolution of the infiltrations during the posttherapy observation period. Nonetheless, at the end of the observation period, only 7 (15.9%) were free of all lesions, and 18 (40.9%) had fibrotic scars, as described below.

At the end of the prolonged posttherapy observation of our patients, fibrosis persisted in those in whom it had been recorded at diagnosis and appeared de novo in some patients, so that 25 (53.2%) of the 47 patients had such sequelae at the end of the observation period. Bullae also persisted, and the frequency increased during the posttherapy follow-up period, until 59% of the patients had this sequela. Signs of pulmonary

Table 3. Findings of chest radiographic examination at follow-up in 44 patients with chronic paracoccidioidomycosis and infiltrative lesions who were treated with itraconazole.

	No. (%) of patients with infiltrative lesions at diagnosis				
No. of pulmonary fields involved at diagnosis	All	With no lesions or fibrosis at end of follow-up	With lesions only at end of follow-up	With lesions and fibrosis at end of follow-up	With fibrosis only at end of follow-up
1–2	8	3 (37.5)	4 (50.0)	0	1 (12.5)
3–4	18	2 (11.1)	7 (38.9)	2 (11.1)	7 (38.9)
5–6	18	2 (11.1)	1 (5.6)	5 (27.8)	10 (55.6)
Total	44	7 (15.9)	12 (27.3)	7 (15.9)	18 (40.9)

NOTE. Three patients had no lung infiltrates.

Table 4. Factors predictive of the development of pulmonary fibrosis in 47 patients with paracoccidioidomycosis.

No. of pulmonary fields with infiltrates at diagnosis	No. of patients			Patients with fibrosis at any time, *n/N* (%)
	Without fibrosis at end of follow-up	Who developed fibrosis during study	With fibrosis at diagnosis	
None	3	0	0	0/3 (0)
1–2	7	1	0	1/8 (12.5)
3–4				
With bullae	2	0	6	6/8 (75)
Without bullae	7	2	1	3/10 (30)
5–6	3	8	7	15/18 (83.3)
Total	22	11	14	25/47 (53.2)

NOTE. Classification and regression trees are described in Breiman et al. [14]. *n/N,* no. of patients with fibrosis at any time/total no. of patients.

hypertension followed the same persistence–de novo course; 27.2% of patients had this sign at the end of the study.

The retrospective nature of the present study, the time (1982) at which the available records began to be analyzed, and the budgetary difficulties encountered when more-precise image studies, such as CT, were requested hindered further examination. Nonetheless, the data obtained indicate that, despite successful results of the use of itraconazole to treat active disease, residual lesions not only persist but increase over time, so that a sizeable proportion of patients have permanent impairment of life quality as a result of dyspnea and/or cor pulmonale.

The present study presented valuable information, demonstrating that the intensity of the infiltrative lesions present at the moment of diagnosis predicts the final outcome of residual lesions. The possibility that a patient will develop pulmonary fibrosis in spite of otherwise successful therapy was much greater when infiltration had been categorized as very severe at diagnosis (83%) than when infiltration was categorized as minor (12.5%). In addition, the simultaneous presence of severe infiltrative changes and of bullae during the first consultation contributed to the marked increase (to 75% of patients) in the frequency of pulmonary sequelae at the end of the observation period. Proper interpretation of lung images at diagnosis and emphasis on the significance of interstitial lesions that are already present may contribute to better patient surveillance. It would also seem desirable to have accessory therapies available that would help avoid or control fibrosis; unluckily, however, earlier experiences with corticosteroids and progesterone reported by clinicians in Argentina and Brazil (R. Negroni, personal communication) have proved unrewarding. Nonetheless, advances in our knowledge of the immune mechanisms involved in the genesis of fibrosis may allow immunomodulation of the patient's tissue response so that the development of sequelae may be prevented [19].

The findings reported above emphasize that, even though treatment is essential to avoid progression of fungal disease and death in patients with PCM, efforts should also be made toward prompter diagnosis, so that undue lung damage attributable to the silent progress of the mycosis can be avoided. Despite the fact that such a course does not allow determination of the role of prompt treatment in avoiding residual lesions, studies in an experimental mouse model of pulmonary PCM have demonstrated that, if animals are treated with itraconazole shortly after challenge with the fungus, disease progression is halted and no fibrosis develops, in contrast with challenged but untreated animals [20]. Thus, if the mycosis were considered earlier in a physician's differential diagnosis, and if the available mycologic and image sources were used appropriately [1, 16], an important decrease in the number of patients with incapacitating fibrosis might be obtained. The cost associated with advancement of molecular biology–based methods of diagnosis may be offset by the time saved in achieving diagnoses [21, 22]. Prospective studies that include CT image analysis are need to define the precise role of previous lung damage in the outcome of residual lesions in patients with PCM.

Acknowledgments

We express sincere appreciation of the patients who participated in this study, for their kindness in keeping up with regular appointments, and to the personnel of the Medical Mycology Diagnostic team of the Corporación para Investigaciones Biológicas, for their cooperation during the study.

References

1. Lacaz CS, Porto E, Martins JEC, Heinz-Vaccari EM, de Melo NK. Paracoccidioidomicose. In: Lacaz CS, Porto E, Martins JEC, et al., eds.

Tratado de Micologia Médica Lacaz. 9th ed. Sao Paulo, Brazil: Sarvier Editores, **2002**:639–729.
2. Bethlem EP, Capone D, Maranhao B, Carvalho CR, Wanke B. Paracoccidioidomycosis. Curr Opin Pulm Med **1999**; 5:319–25.
3. Montenegro M, Franco M. Pathology. In: Franco M, Lacaz CS, Restrepo-Moreno A, Del Negro G, eds. Paracoccidioidomycosis. Boca Raton, FL: CRC Press, **1994**:131–50.
4. Tuder RM, El Ibrahim R, Godoy CE, De Brito T. Pathology of the human pulmonary paracoccidioidomycosis. Mycopathologia **1985**; 92: 179–88.
5. Do Valle ACF, Guimaraes RR, Lopes DJ, Capone D. Thoracic radiologic aspects in paracoccidioidomycosis [in Portuguese]. Rev Inst Med Trop Sao Paulo **1992**; 34:107–15.
6. Coutinho ZF, Silva D, Lazera M, et al. Paracoccidioidomycosis mortality in Brazil (1980–1995). Cad Saude Publica **2002**; 18:1441–54.
7. Correa AL, Franco L, Restrepo A. Paracoccidioidomicosis: coexistencia de lesiones pulmonares y patología pulmonar silente. Descripción de 64 pacientes. Acta Med Colomb **1991**; 16:304–8.
8. Ribeiro DO. Nova terapêutica para a blastomicose. Publ Med **1940**; 12:36–40.
9. Mendes RP, Negroni R, Arechavala A. Treatment and control of cure. In: Franco M, Lacaz CS, Restrepo-Moreno A, Del Negro G, eds. Paracoccidioidomycosis. Boca Raton, FL: CRC Press, **1994**:373–87.
10. Restrepo A, Gomez I, Cano LE, et al. Treatment of paracoccidioidomycosis with ketoconazole: a three-year experience. Am J Med **1983**; 74(Suppl B):48–52.
11. Naranjo MS, Trujillo M, Múnera MI, Restrepo P, Gomez I, Restrepo A. Treatment of paracoccidioidomycosis with itraconazole. J Med Vet Mycol **1990**; 28:67–76.
12. Tobón AM, Gómez I, Franco L, et al. Seguimiento post-terapia en pacientes con paracoccidioidomicosis tratados con itraconazol. Rev Colomb Neumol **1995**; 7:74–8.
13. Guidelines for the use of ILO international classification of radiographs of pneumoconioses. Occupational Safety and Health Series, no. 22. Geneva: International Labour Office, **1980**.
14. Breiman L, Friedman JH, Olshen RA, Stone CJ. Classification and regression trees. Monterey, CA: Wadsworth and Brooks/Cole Advance Books and Software, **1984**.
15. Machado-Filho J, Lisboa RM, Mattos AD, Januzzi A, Miranda JL. Consideraçoes relatives á blastomicose sul-americana. As repercussôes cárdio-vasculares das lesôes pulmonares: dados hemodinâmicos, oximétricos e angiopneumo-gráficos. Hospital (Rio de Janeiro) **1961**; 60:241–7.
16. Funari M, Kavakama J, Shikanai-Yasuda MA, et al. Chronic pulmonary paracoccidioidomycosis (South American blastomycosis): high-resolution CT findings in 41 patients. AJR Am J Roentgenol **1999**; 173:59–64.
17. Calich VLG, Vaz CAC, Burger E. Immunity to *Paracoccidioides brasiliensis* infection. Res Immunol **1998**; 149:407–16.
18. Musatti CC, Peracoli MT, Soares AMVC, Rezkallah-Iwasso MT. Cell-mediated immunity in patients with paracoccidioidomycosis. In: Franco M, Lacaz CS, Restrepo-Moreno A, Del Negro G, eds. Paracoccidioidomycosis. Boca Raton, FL: CRC Press, **1994**:175–86.
19. Antoniou KM, Ferdoutsis E, Bouros D. Interferons and their application in the diseases of the lung. Chest **2003**; 123:209–16.
20. Sahaza J, Cock AM, Urán ME, et al. Modelo murino de paracoccidioidomycosis (PCM): efecto del tratamiento con itraconazol en la respuesta inflamatoria pulmonary. Resúmes V Congreso Colombiano de Enfermedades Infecciosas. Infectio **2001**; 5:118.
21. Gomes GM, Cisalpino PS, Taborda C, Camargo ZP. PCR for diagnosis of paracoccidioidomycosis. J Clin Microbiol **2000**; 38:3478–80.
22. Diez S, Gómez BL, Restrepo A, Hay RJ, Hamilton AJ. *Paracoccidioides brasiliensis* 87-kilodalton antigen, a heat shock protein useful in diagnosis: characterization, purification, and detection in biopsy material via immunohistochemistry. J Clin Microbiol **2002**; 40:359–65.