

## *Pneumocystis carinii* Antigenemia in Acquired Immunodeficiency Syndrome

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The present study was conducted to determine the prevalence and significance of *Pneumocystis carinii* antigenemia in patients with acquired immunodeficiency syndrome (AIDS) and clinically or invasively diagnosed *P. carinii* pneumonitis. Single serum specimens from 20 AIDS patients invasively examined for *P. carinii* organisms and 106 AIDS patients with a clinical diagnosis only of *P. carinii* pneumonitis were blindly tested for *P. carinii* antigenemia by a counterimmunoelectrophoresis assay. In the 20 specimen-documented cases, the antigen test demonstrated a sensitivity of 75% and a specificity of 90%. The positive predictive value of the test was 90%, while the negative predictive value was 70%. In AIDS patients with specimen-documented *P. carinii* pneumonitis, the prevalence of *P. carinii* antigenemia coincided almost exactly with the prevalence of positive invasively obtained specimens (60 and 59%, respectively). In patients with a clinical diagnosis only of *P. carinii* pneumonitis, half as many (30%) were found to exhibit antigenemia. Sequential *P. carinii* antigen titers determined by a new latex agglutination technique on three AIDS patients with specimen-documented *P. carinii* pneumonitis demonstrated the influence of specific therapy upon *P. carinii* antigenemia and its potential prognostic application.

*Pneumocystis carinii* pneumonitis is the most common serious opportunistic infectious disease observed to occur in conjunction with the acquired immunodeficiency syndrome (AIDS) and has contributed significantly to the high mortality rate in AIDS patients. To date, approximately 59% of all reported AIDS patients initially sought medical attention as a result of clinical symptoms associated with *P. carinii* infection (1, 2). Forty percent of these individuals did not respond to anti-*Pneumocystis* drug therapy (8), and many of those who responded and survived suffered an increased incidence of trimethoprim-sulfamethoxazole (TMP-SMX)-related allergy and other adverse reactions (7). In addition, AIDS patients with *P. carinii* pneumonitis required lengthier therapy and exhibited a higher relapse rate than other subjects with *P. carinii* pneumonitis and cancer, for example, or other underlying conditions (10, 14, 15).

Since very little information is available concerning *P. carinii* antigenemia in AIDS, the objectives of the present study were to attempt to provide a data base that would reveal (i) the prevalence of this phenomenon in AIDS, (ii) how it correlates with clinical observations, and (iii) how antigenemia correlates with the presence of *P. carinii* organisms in specimens obtained via invasive procedures.

### MATERIALS AND METHODS

Sera from 126 AIDS patients with clinically diagnosed or biopsy-documented *P. carinii* pneumonitis from 50 institutions were referred to our laboratory for the *P. carinii* antigen test. Criteria on which clinical diagnoses of *P. carinii* pneumonitis in AIDS patients were made included pulmonary symptoms, chest radiograph, and arterial blood gas determinations consistent with those observed in specimen-documented *P. carinii* pneumonia.

Freshly collected, coded sera (0.2 to 5.0 ml) were sent by overnight air express on dry ice to the *P. carinii* serologic

reference laboratory, and clinical profiles were correlated with antigen test results after the latter had been reported to the referring physician. All sera were obtained prior to the initiation of treatment, since TMP-SMX can prevent *P. carinii* antigenemia when administered prophylactically. Therapeutic doses have often resulted in antigen clearance as early as 24 h after treatment was begun (19).

Twenty patients with AIDS and *P. carinii* pneumonitis were ultimately biopsied by a variety of methods on the basis of a clinical diagnosis of *P. carinii* pneumonitis. A clinical diagnosis of *P. carinii* pneumonitis alone was available for 106 of the AIDS patients tested. With the exception of two patients, all specimens were tested blindly in order to avoid inadvertent bias.

All specimens were concentrated by removal of water from the serum with dehydrated polyacrylamide gel (16) (Lyphogel; Gelman Instrument Co., Ann Arbor, Mich.) and were tested in duplicate with antigen-positive control sera from specimen-documented cases of *P. carinii* pneumonitis and with normal, negative control serum samples.

The technique employed for antigen detection was that of counterimmunoelectrophoresis (CIE), which involves electrophoresis of the patient's serum specimen against antibody prepared against cell culture-grown *P. carinii* organisms (18, 19), derived originally from cortisone acetate-treated Sprague-Dawley rats (5). Specificity of the antiserum has been demonstrated via absorption, growth inhibition of *P. carinii* in cell culture (18), and more recently by experiments employing Ouchterlony technique, enzyme-linked immunosorbent assay and by latex particle agglutination (9, 21, 23).

Sequential serum specimens from three male homosexual patients (A, B, and C) with AIDS and specimen-documented *P. carinii* pneumonitis were titrated for *P. carinii* antigen by a latex particle agglutination technique that uses latex beads (0.81  $\mu\text{m}$  in diameter) coated with rabbit antisera raised against cell culture-grown *P. carinii* organisms (9, 18). This technique has been described in detail in a study of pediatric

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TABLE 1. Prevalence of *P. carinii* antigenemia in AIDS patients with clinical or pulmonary specimen-documented *P. carinii* pneumonitis

Patients with AIDS and clinical <i>P. carinii</i> pneumonitis	n (%)	No. (%) of patients with <i>P. carinii</i> organisms		No. (%) of patients with <i>P. carinii</i> antigenemia	
		Present	Absent	Present	Absent
Not invasively tested for <i>P. carinii</i>	106 (84)	ND <sup>a</sup>	ND	32 (30)	74 (70)
Invasively tested for <i>P. carinii</i>	20 (16)	12 <sup>b</sup> (60)	8 <sup>c</sup> (40)	10 (50)	10 (50)

<sup>a</sup> ND, Not determined.

<sup>b</sup> Of these 12 patients, 9 had *P. carinii* antigen and 3 did not. *P. carinii* organisms were found in all 12.

<sup>c</sup> Of these eight patients, one had *P. carinii* antigen and seven did not. *P. carinii* organisms were not found in any of the eight.

AIDS patients with documented or clinically suspected *P. carinii* pneumonitis (21).

*P. carinii* immunoglobulin G (IgG) antibodies were titrated in the same three AIDS patients by a recently described enzyme-linked immunosorbent assay that involves the use of Microtiter plates coated with cell culture-derived *P. carinii* antigen (26). *P. carinii* pneumonia was confirmed in patients A and C by bronchoalveolar lavage and was confirmed in patient B by open-lung biopsy. In all three cases, *P. carinii* cysts and trophozoites were identified by toluidine blue O and Giemsa stains, respectively. All three patients were initially placed on intravenous TMP-SMX therapy and all were changed to pentamidine because of failure to respond clinically or because of severe allergic reactions, apparently provoked by TMP-SMX.

Interpretations of test results were made according to the formulae and methods described by Dierksheide (4) as follows: sensitivity = true positives/(true positives + false negatives), specificity = true negatives/(true negatives + false positives), positive predictive value = true positives/(true positives + false positives), negative predictive value = true negatives/(true negatives + false negatives), and prevalence = (true positives + false negatives)/(true positives + false negatives + true negatives + false positives).

## RESULTS

A single, freshly collected specimen from each of 126 AIDS patients with specimen-documented or clinically diagnosed *P. carinii* pneumonitis was tested by CIE for the presence of *P. carinii* antigen (Table 1). A total of 106 of the specimens tested were from AIDS patients with clinically diagnosed *P. carinii* pneumonitis, while 20 patients had clinically diagnosed *P. carinii* pneumonitis and were ultimately invasively examined for *P. carinii* organisms. Of those individuals without invasive diagnosis but in whom *P. carinii* pneumonitis was clinically diagnosed, 32 (30%) were antigen positive, while 74 (70%) did not exhibit antigenemia. Of those who were invasively examined for *P. carinii* organisms, 12 (60%) were positive, and 8 (40%) were negative (Table 1).

In those individuals on whom invasive procedures were carried out, antigen and specimen results were in agreement, i.e., both were positive or both were negative, in 80% (16 of 20) of cases and were in disagreement in 20% (4 of 20). In three individuals, antigen data were negative, while the pulmonary specimens were positive. In only one patient, antigen data were positive, while the invasively obtained

specimen was negative for *P. carinii* organisms. With the formulae given in Materials and Methods, the sensitivity of the CIE test for *P. carinii* antigenemia was 75%, while the specificity was 90%. These values were calculated on the basis of antigen tests performed on single serum specimens from invasively documented cases of *P. carinii* pneumonitis in AIDS patients. In all calculations, the standard for comparison ("gold standard") consisted of the biopsy or other invasively obtained specimen results. The positive predictive value of the test was 90%, and the negative predictive value was 70% (4). Prevalence was determined to be 60%.

Patient A (who had both AIDS and *P. carinii* pneumonitis), for whom *P. carinii* antigen-antibody titers were determined on 14 serially collected serum specimens, remained consistently antigenemic by latex particle agglutination assay over a period of 23 days (Fig. 1). His antigen titer peaked on day 12 at 1:32 and remained at 1:16 for approximately 1 week before his death. His antibody titer peaked at 1:2,048 on days 11 and 17 and was measured at 1:1,024 1 day before he expired. The highest antigen titer of patient A corresponded with his lowest antibody titer on days 12 through 14. Numerous *P. carinii* organisms were observed in his postmortem lung imprint slides stained with Gomori methenamine-silver nitrate technique.

Patient B was shown initially, by open-lung biopsy, to have *P. carinii* and was immediately placed on intravenous TMP-SMX, which was discontinued after he failed to improve by day 5 (Fig. 1). As his clinical condition worsened, his antigen titer, as determined by latex particle agglutination, rose from 1:4 to 1:8 and then became negative after 5 days of therapy with pentamidine. When pentamidine therapy was discontinued after 14 days, the antigen titer of patient B reverted to positive and continued to rise to a peak of 1:16 when he expired on hospital day 38. At no time did patient B ever exhibit measurable antibodies to *P. carinii*. CIE and latex particle agglutination *P. carinii* antigen results on serum from patient B coincided exactly, although this was not the case in a recent study of renal transplant recipients (9).

The serum of patient C converted from *P. carinii* negative to positive at 1:2 8 days after TMP-SMX therapy was terminated because of allergic reactions, and pentamidine was begun. Pentamidine therapy was continued for a total of 11 days, during which the antigenemia of the patient completely resolved. He was discharged on hospital day 18 with a *P. carinii* antibody titer of 1:128.

## DISCUSSION

The results of this study support those of previous investigations that provided evidence validating the concept that *P. carinii* is probably a saprophyte or a commensal organism of human lungs. Seroepidemiologic studies have revealed that 80 to 90% of children have specific IgG antibodies to *P. carinii* by age 2 to 4 (12, 19), and antigenemia appears to be a common phenomenon observed in a variety of immunocompromised individuals (11), including those with cancer (20, 25) and organ (9, 10) or bone marrow (13) transplants. In contrast, only 2 to 3% of normal adults, even those in regions with high risk for AIDS, i.e., San Francisco, demonstrated a positive test for *P. carinii* antigen (3). Whether or not these represent false or legitimate positive results is unknown, since neither pulmonary specimens nor medical histories were available for these individuals. Another recent study has shown, however, that although most AIDS patients and male homosexuals have IgG antibodies to *P. carinii*, mem-

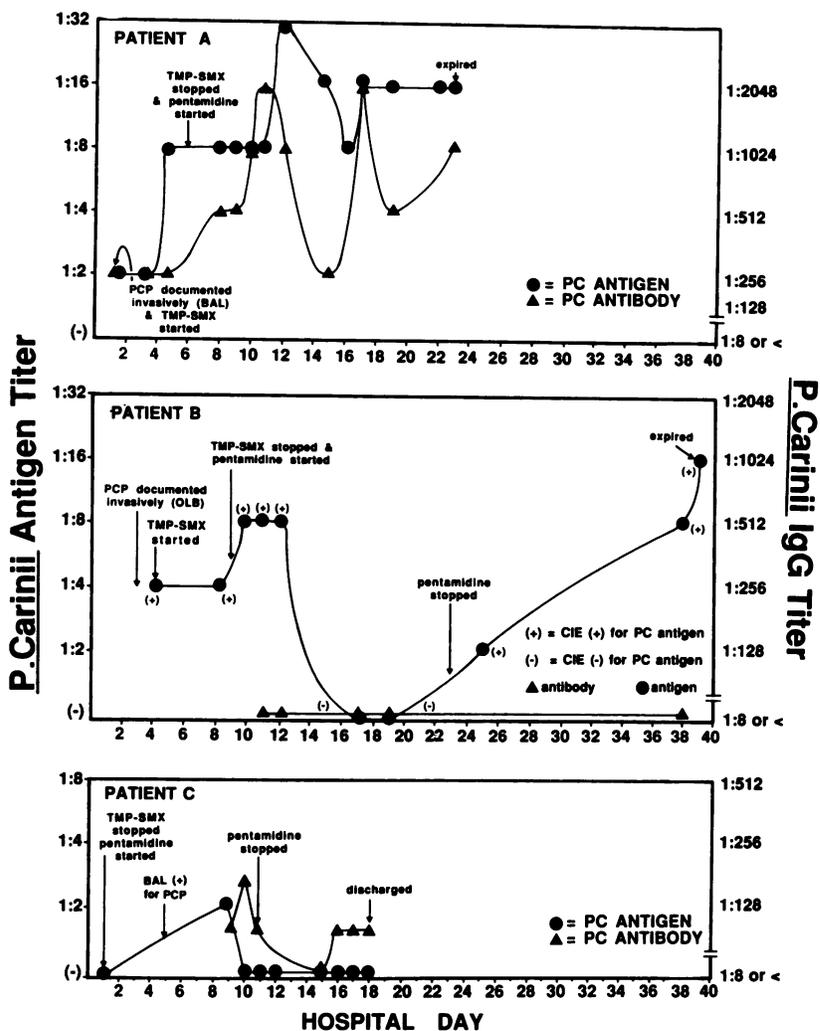


FIG. 1. Sequential *P. carinii* antigen and antibody titers in AIDS patients with fatal and nonfatal *P. carinii* pneumonitis. Abbreviations: PCP, *P. carinii* pneumonitis; PC, *P. carinii*; BAL, bronchoalveolar lavage; OLB, open-lung biopsy.

bers of these groups have substantially lower titers than controls (17, 22, 24; L. L. Pifer, H. B. Niell, B. J. Morrison, J. M. Freeman, and J. D. Counce, Jr., Program Abstr. 22nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 21, 1982).

Although antigenemia correlates fairly well with the documented presence of *P. carinii* organisms in lungs and with clinical symptoms of *P. carinii* pneumonitis, it is important to emphasize that antigenemia alone does not necessarily imply the presence of acute clinical *P. carinii* pneumonitis. That antigenemia provides presumptive evidence, in perspective with all available clinical and laboratory data, of *P. carinii* pneumonitis is strongly suggested by the finding that 95% (19 of 20) of pediatric cancer patients with specimen-documented *P. carinii* pneumonitis yielded positive tests for *P. carinii* antigens (19). In that same study, supporting evidence for the relationship between *P. carinii* colonization and *P. carinii* antigenemia was provided by the observation that none of the subjects receiving prophylactic TMP-SMX were antigenemic, while 27% of the same high-risk group receiving placebos yielded tests positive for *P. carinii* antigens.

Although the test reflected a sensitivity of 75%, its high predictive value is most likely due to the high prevalence of

*P. carinii* infection in AIDS patients. As stated by Dierksheide (4), simple answers are not easily available concerning how high sensitivity or specificity must be before a test may be considered clinically useful. At least three elements complicate potential clinical application of antigen test results in *P. carinii* pneumonitis patients. (i) *P. carinii* is a ubiquitous opportunist to which Koch's postulates do not strictly apply. (ii) *P. carinii* pneumonitis is almost universally fatal if untreated. (iii) At least 40% of patients treated with therapeutic doses of TMP-SMX fail to improve or treatment must be discontinued due to severe drug reactions. Thus, more intensive data must be gathered to assess the practical value of *P. carinii* antigen assays.

The results of this study suggest some revealing epidemiologic findings concerning the status of AIDS patients with pneumonia and with regard to *P. carinii*. That nearly one-third of these patients exhibited *P. carinii* antigenemia is not surprising, since the overall *P. carinii* pneumonitis attack rate in AIDS patients is 70 to 80%. Since the CIE test does not provide a means for differentiating subclinical infection from acute *P. carinii* pneumonitis, it will be necessary to expand these investigations to include the newly developed quantitative latex particle agglutination test for *P. carinii* antigenemia (9, 23). Initial studies in organ transplant recip-

ients (9) and in pediatric AIDS patients (21) suggested that the latex test may prove considerably more useful in the presumptive diagnosis of *P. carinii* pneumonitis, as well as in formulating an accurate prognosis in the patient with *P. carinii* pneumonitis. In the study of pediatric patients with AIDS and *P. carinii* pneumonitis, the latex test exhibited high sensitivity and specificity and an overall accuracy of 94% (21).

Finally, it is of interest to note that of the 20 patients invasively tested for *P. carinii*, only 60% were actually documented as having the organism in their lungs or pulmonary secretions. It is not possible to discern whether this was primarily due to the fact that *P. carinii* exhibits no pathognomonic features, and is thus more readily confused with pneumonias of other etiologies, or if the invasive procedures used resulted in a low yield of organisms. That clinical and laboratory data do not always correlate, especially with regard to *P. carinii* pneumonitis in AIDS patients, was emphasized by the report of Goodman and Tashkin (6), who described *P. carinii* in an AIDS patient with a normal roentgenogram and blood gas profile.

Although patient A (who had AIDS and *P. carinii* pneumonitis), on whom serial serologic studies were performed, had a substantial IgG antibody titer to *P. carinii* when his acute *P. carinii* pneumonitis was diagnosed, it was still below the geometric mean titer of 402 previously determined for healthy heterosexual controls (22; Pifer et al., 22nd ICAAC). An eightfold rise in IgG titer, which was otherwise erratic, measured on days 11 and 17 in conjunction with a steadily rising *P. carinii* antigen titer accompanied the failure of the patient to respond sufficiently to either TMP-SMX or pentamidine. Similar data in renal allograft recipients were recently shown to signal a poor prognosis that was usually followed by death from *P. carinii* pneumonitis (9). Conversely, clearance of circulating antigen by patient C coincided with his clinical improvement and ultimate discharge. The implications of these data must, however, be viewed conservatively until more extensive long-term data become available on patients with AIDS and *P. carinii* pneumonitis.

Although antibody titers did not appear to influence recovery, decisive data on the presence and role of specific immune complexes are in the process of being gathered (22). Until these studies are complete, it is not possible to determine the relative importance of antibody in the ultimate outcome of *P. carinii* pneumonitis in AIDS.

The present report represents an initial attempt to gain insight into the relationship between the epidemiology and prevalence of subclinical *P. carinii* infection in AIDS patients. More extensive, sequential studies will be necessary to obtain a clear, overall concept of the natural history of *P. carinii* in the AIDS patient and the clinical significance of relative antigen and antibody and *P. carinii* immune complex titers during subacute, acute, and convalescent stages of infection.

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