Pneumocystis carinii Serologic Study in Pediatric Acquired Immunodeficiency Syndrome

Linda L. Williford Pifer, PhD; Diane R. Woods; Carol C. Edwards; Rebecca E. Joyner, MT; Frank J. Anderson; Kris Arheart

- **Pneumocystis carinii** antigen and IgG antibody profiles were prepared on 17 pediatric patients with acquired immunodeficiency syndrome (AIDS) with pneumonia who were examined by a variety of invasive methods for **P carinii** organisms. Overall, the accuracy of the antigen assay in invasively examined pediatric patients with AIDS with pneumonia was 94% (sensitivity, 100%; specificity, 90%), as antigen and invasive test results agreed in 16 of 17 patients. No statistically significant differences in IgG titer were observed between controls and patients invasively examined for **P carinii**, whether the organism was observed in the specimen or not. Since 38% of all serum samples referred were derived from "blood-borne" cases of AIDS, including patients who contracted AIDS as a result of both transfusion and hemophilia A, this suggests that **P carinii** pneumonia or **P carinii** pneumonia-like pneumonias may be more common in these individuals. (AJDC 1988;142:36-39)

**Pneumocystis carinii** pneumonia (PCP) has played a prominent role in the pediatric acquired immunodeficiency syndrome (AIDS). Fifty-eight percent of all pediatric patients with AIDS whose cases have been reported have experienced PCP. Despite this observation, to our knowledge, no reports have been forthcoming to date describing the serologic response to **P carinii** in the pediatric population with AIDS, although simultaneous sequential **P carinii** antigen and antibody titers have been reported in adult patients with AIDS with PCP.

The present study was undertaken with the goal of providing fundamental information concerning **P carinii** serology in the pediatric population with AIDS. Another objective was that of determining if an enzyme-linked immunosorbent assay (ELISA) for IgG antibody to **P carinii** and a latex particle agglutination (LPA) test for **P carinii** antigenemia might prove useful in the presumptive diagnosis, surveillance, and more knowledgeable management of the pediatric patient with AIDS with, or at risk for, PCP.

**PATIENTS AND METHODS**

Patient Population

Since our group serves as a national serologic reference laboratory for **P carinii** infections in patients with AIDS, cancer, and organ transplants, all specimens, with the exception of controls, were shipped on dry ice by overnight air express with an accompanying clinical profile. To avoid inadvertent bias on our part, it was requested that results of invasive procedures, when available or pending, be withheld from our group until we reported the serologic results to the referring laboratory. The specimens were tested, therefore, in a "blinded" manner.

All patients, whose demographic profiles are described in Table 1, met the Centers for Disease Control (Atlanta) criteria for pediatric AIDS and were positive for antibody to human immunodeficiency virus (HIV) by the Western blot technique. Each patient had specimen-documented and/or clinically diagnosed PCP. Patients who had been treated with sulfamethoxazole and trimethoprim were excluded from the study since treatment for as long as 24 hours, possibly less, may rapidly result in the elimination of **P carinii** antigenemia in patients with a positive prognosis.

Only freshly collected serum samples were accepted for testing, since **P carinii** antigens deteriorate during cold or frozen storage. All specimens had been frozen and thawed only once prior to testing. To ensure valid correlation of antigen data on serologic specimens with invasively obtained material, all serum samples were collected no more than 48 hours before or after invasive sampling.

Control serum samples were derived from healthy, afebrile, age- and sex-matched patients admitted to LeBonheur Children's Medical Center, Memphis, for tests or elective surgery. All specimens were frozen and thawed once prior to testing.

**Methods**

All serum samples were tested in triplicate with positive and negative control serum samples on the day of receipt for **P carinii** antigen, and results were reported by telephone on the same day, resulting in a 24-hour "turnaround time" on antigen test results. Convalescent serum samples were tested at two to three weeks after PCP.

**Use of LPA and ELISA**

Rabbit antibody, raised against in vitro cultured **P carinii** organisms that were originally derived from cortisone acetate-treated Sprague-Dawley rats, was bonded to 0.81-μm latex beads and mixed on ring slides with doubling dilutions of serum (1:2, 1:4, 1:8, etc) as described in previous studies. Positive and negative human serum controls (specimen-documented) were included in each test as well as control latex beads to which normal rabbit serum was bonded. The latter controls were included to test for any nonspecific agglutination. After the slides were rotated for six minutes, the **P carinii** antigen titer was recorded as the highest serum dilution displaying agglutination visible to the unaided eye. Serum samples failing to show agglu-
tion were recorded as negative for the presence of antigen.4

IgG antibody to P carinii was measured by a micro-ELISA technique. This has been described previously in detail.1,9

Statistical Methods

The geometric mean titers (GMTs) of IgG antibody to P carinii were determined by using the log (base 10) of each titer. Log titer variance was tested by one-way analysis of variance and Kruskal-Wallis nonparametric analysis.10 The Student-Newman-Keuls multiple comparison test was used to analyze the significance of any intergroup differences. The normality of the data was analyzed by the Kolmogorov-Smirnov goodness-of-fit test.

RESULTS

Demographic Profiles

A demographic summary of the pediatric patients with AIDS10 whose serum samples were submitted for P carinii serologic study is presented in Table 1. These data are not necessarily representative of pediatric patients with AIDS as a whole because (1) all were clinically suspected of or confirmed as having PCP, and (2) 22 (47%) of 47 patients were from the Southeast, and this reflects proximity to our serology laboratory rather than actual incidence of pediatric AIDS relative to the remainder of the United States.

Nevertheless, these data reflect some interesting findings. For example, the ratio of male to female pediatric patients with AIDS with invasively documented PCP was 6:1. Of all 47 pediatric patients with AIDS with documented or suspected PCP, 32 (68%) were male.

Parents were the source of HIV infection in 57% (24/42) of the patients whose source of infection was known and reported. In most instances, the mother was an intravenous drug abuser and/or a prostitute, or the father was bisexual and/or an intravenous drug abuser. In two cases, a parent was from a high incidence of HIV region outside the United States, and in one case the father had hemophilia.

Therapy for hemophilia A accounted for 23% (10/42) of all pediatric patients with AIDS serologically tested for P carinii in whom the HIV source was identified. This percentage is nearly fivefold greater than the nationwide percentage of pediatric AIDS acquired by therapy for hemophilia A and may represent an important finding regarding the incidence of pneumonia due to or resembling PCP in pediatric patients with hemophilia A and AIDS.

Transfusions were the source in 19% (8/42), and in 11% (5/47), the source of infection was unclear or was not revealed for various reasons. The overall mean age of all pediatric patients with AIDS included in this study was 38 months.

P carinii Antibody Titers

The GMTs of IgG antibody to P carinii as measured by ELISA are expressed in Table 2, along with the P values derived by comparing the various groups for any statistically significant differences.1,11 The results suggest that P carinii antibody titers are noncontributory to the establishment of a diagnosis of PCP. The titers of normal children, pediatric patients with AIDS with pneumonia proved not to be PCP (group 3), and pediatric patients with AIDS who were P carinii antigen negative but not invasively assessed for P carinii organisms all had comparable GMTs that were, respectively, 59, 91, and 82.

Although the patients with specimen-documented PCP had a GMT twice that of controls, this difference was not statistically significant (P = .8). One final observation was that
Table 2.—Comparison of Pneumocystis carinii IgG Antibody Titers* in Pediatric Patients With AIDS and Normal Children†

<table>
<thead>
<tr>
<th>Index Group</th>
<th>N</th>
<th>GMT</th>
<th>Comparison Group</th>
<th>N</th>
<th>GMT</th>
<th>P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal children</td>
<td></td>
<td>25</td>
<td>59</td>
<td>2</td>
<td>7</td>
<td>118 .2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>98</td>
<td>4</td>
<td>11</td>
<td>482 &lt;.001</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>21</td>
<td>83</td>
<td>2 and 4</td>
<td>18</td>
<td>.8</td>
</tr>
<tr>
<td></td>
<td>3 and 5</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>118</td>
<td></td>
<td>3</td>
<td>6</td>
<td>91 .4</td>
</tr>
<tr>
<td>AIDS and specimen-documented PCP§: antigen (+) and specimen (+)</td>
<td>4</td>
<td>11</td>
<td>482 .04</td>
<td>5</td>
<td>21</td>
<td>82 .5</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>91</td>
<td></td>
<td>5</td>
<td>21</td>
<td>82 .001</td>
</tr>
<tr>
<td>AIDS and suspected PCP; antigen (-) and specimen (-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>482</td>
<td></td>
<td>5</td>
<td>21</td>
<td>82 .001</td>
</tr>
<tr>
<td>AIDS and suspected PCP; no invasive specimen obtained; antigen (+) and specimen (?)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td></td>
<td>See data above</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIDS and suspected PCP; no invasive specimen obtained; antigen (-) and specimen (?)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All geometric mean titers (GMTs) are based on acute-phase serum samples.
†AIDS indicates acquired immunodeficiency syndrome; PCP, P. carinii pneumonia.
‡P ≤ .05 (limit of significance).
§Specimen refers to tissue or secretions, usually obtained by a variety of invasive techniques, that may (or may not) contain P. carinii organisms identifiable by light microscopy with appropriate stains.

antigen-positive patients, whether specimen documented or not, had higher anti-P. carinii titers than did antigen-negative individuals. None of these differences, however, was substantially significant or consistent to suggest the utility of P. carinii titers as a sole criterion for diagnosis.

P. carinii Antigenemia

Results of the LPA test for P. carinii antigenemia were as follows. The test was accurate in 94% (16/17) of coded serum samples from invasively tested patients. Seven patients (41%) were biopsy specimen and antigen positive, and nine (53%) were biopsy specimen and antigen negative. There was one false-positive test result (6%) and no false-negatives. None of the 25 controls yielded a positive test for P. carinii antigen.

COMMENT

In summary, 72 children were included in the study. Of these, 25 represented controls and 47 were pediatric patients with AIDS with specimen-documented or clinically suspected PCP. All were tested for P. carinii antigenemia and/or IgG antibody specific for P. carinii.

Seventeen patients were invasively tested for P. carinii organisms, and of these, seven were positive and ten were negative. Assuming that the invasive diagnostic means were usually accurate, the probability of detecting P. carinii organisms in pediatric patients with pneumonia suggestive of PCP is about 41%.

Regarding the demographic data in Table 1, the relatively high proportion of pediatric patients with AIDS with pneumonia and hemophilia A is noteworthy. According to recent statistics, hemophilia accounts for only 5% of all pediatric patients with AIDS, whereas in the present study, 23% (10/42) of pediatric patients with AIDS with confirmed or suspected PCP in whom the source of AIDS was known or disclosed had hemophilia A. This is consistent with reports that PCP is more common in blood-borne cases of AIDS as compared with infection acquired by other means. It may also reflect the possibility that hemophiliacs with AIDS have an increased susceptibility to PCP and/or other types of pneumonia.

Nationwide, 19% of all pediatric patients with AIDS have received blood or blood products. Of these, only 5% were hemophiliacs. In this study, hemophilia A alone accounted for 23% (10/42) of all serum samples from pediatric patients with AIDS referred for P. carinii serologic study. Seventeen percent represented HIV infection acquired from transfusions or blood products used for the treatment of disorders other than hemophilia A. Therefore, a total of 43% (18/42) of pediatric patients with AIDS with pneumonia documented or suspected as PCP had blood-borne HIV infections. The apparently high percentage of pediatric patients with PCP and AIDS in the Southeast reflects the geographic location of the reference laboratory and is not indicative of the actual proportion of documented or suspected PCP in pediatric patients with AIDS in the general southeastern area.

Regarding normal children and P. carinii, it has been known since the late 1970s that 80% to 90% of healthy children develop specific antibody to this agent by age 2 to 4 years.4,14,16 Most histologic14,15,17 as well as serologic evidence points to the probability that the ubiquitous P. carinii organism may be a commensal or symbiont in mammalian lungs.5,8 This would logically explain its emergence as a pathogen in patients with AIDS, cancer, organ or bone marrow transplants, and in congenitally or therapeutically immunocompromised children and adults.

Since pediatric patients with AIDS generally respond poorly to most challenge antigens (pneumococcal and influenza vaccines), the higher P. carinii IgG antibody titers seen in these patients were not anticipated and were not observed in their adult counterparts with AIDS.18,20 Although a full interpretation of this phenomenon will await further investigation, these titers may reflect a response to P. carinii antigen liberated into the circulating blood during episodes of antigenemia. Based on our observations, antigenemia may precede acute clinical PCP...
by as much as two months and perhaps longer. Alternatively, the higher *P. carinii* titers may merely reflect polyclonal B-cell activation often observed in HIV infection.

The higher GMTs in acute PCP in pediatric patients with AIDS appear to suggest that IgG titers might have some diagnostic significance. However, these are composite values and do not reflect the wide range of titers measured in these groups. Interpreted in context with all other available clinical data, antibody titers at best may add support to a diagnosis of PCP but usually cannot be used alone with any degree of confidence.

The LPA assay for *P. carinii* antigenemia was accurate in 94% (16/17) of the cases presented in this study in which biopsy was done. There was one false-positive and no false-negative test results. The sensitivity of the test was 100% (true-positives [7]/true-positives [7] + false-negatives [0]) and the specificity was calculated to be 90% (true-positives [9] + false-positives [1]). These data differ somewhat from data obtained from adult patients with AIDS and PCP, in whom the latex test was 80% accurate, with 5% false-positives and 15% false-negatives. In pediatric AIDS, the sensitivity was 80%, while the specificity was 89%. In general, these data appear to suggest that adult patients with AIDS may have somewhat more difficulty in processing or mobilizing antigen from the lung to the peripheral circulation. This interpretation is supported by the observation that macrophage and monocyte depression are depressed in patients with AIDS.

In conclusion, *P. carinii* antibody titers may be useful from an epidemiologic standpoint and may occasionally prove useful in supporting a diagnosis of PCP. Antigen test results, however, appear to show promise in the noninvasive presumptive diagnosis of PCP in the pediatric patient with AIDS, particularly when invasive procedures are contraindicated. At present, no serologic test, in the absence of other supporting clinical and laboratory data, can be recommended with confidence as providing a definitive diagnosis of PCP. More extensive, sequential antigen and antibody titers will be required to assess the ultimate clinical and epidemiologic value of these potentially useful techniques.

This investigation was supported in part by grant 1K01 CA34897-01 from the National Cancer Institute, National Institutes of Health, Bethesda, Md; by grant PDT-121C from American Cancer Society; by The Thrasher Research Fund; and by Medical Student Research Fellowship grant award T35-AM 07406-55.

Ingrid Heaton provided secretarial assistance.

References