Association of Primary Pneumocystis carinii Infection and Sudden Infant Death Syndrome

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To delineate clinical and histological features of the first Pneumocystis carinii infection affecting the immunocompetent host, P. carinii–specific histological stains were performed on autopsy lung specimens from 534 consecutive pediatric patients (those with AIDS and malignancies were excluded) in Santiago, Chile. P. carinii clusters were found in 4 (25%) of 16 infants who died of no apparent cause at arrival to the emergency department, and in 10 (2.9%) of 342 infants who died of multiple conditions at the hospital (P = .002, Fisher’s exact test). This prompted us to analyze additional series of infants with sudden infant death syndrome (SIDS). In 161 additional SIDS cases, 47 (35.1%) of 134 infants from Chile and 4 (14.8%) of 27 infants from Oxford, United Kingdom, were found to have P. carinii clusters in the lungs. The quantity of P. carinii cysts was small compared with the numbers seen in immunocompromised hosts with P. carinii pneumonitis. This study provides histological evidence that primary P. carinii infection is associated with SIDS.

Clinical Infectious Diseases 1999; 29:1489–93
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Received 26 February 1999; revised 6 August 1999.
This work was presented in part at the 36th annual meeting of the Infectious Diseases Society of America held on 12–15 November 1998 in Denver, Colorado.

Financial support: This work was supported in part by Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT research grant 1960940), Santiago, Chile, and by the St. Jude International Outreach Program and American Lebanese Syrian Associated Charities, St. Jude Children’s Research Hospital, Memphis, Tennessee.
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comprised 534 consecutive pediatric patients (those with malignancies and AIDS were excluded) who were autopsied between January 1990 and December 1996 at the Department of Pathology, Luis Calvo Mackenna Children’s Hospital, and the Department of Pathology, Roberto del Rio Children’s Hospital, in Santiago. Series 2 comprised 94 infants with SIDS who were autopsied at the Legal Medicine Institute of Chile during 1996 and 1997; series 3, 40 infants with SIDS who were autopsied at the Department of Pathology, Exequiel Gonzalez Cortés Children’s Hospital in Santiago between 1990 and 1993. Series 4 comprised 27 infants with SIDS who were autopsied at the Department of Pathology, John Radcliffe Hospital, University of Oxford, Oxford, from 1996 to 1998.

Formalin-fixed paraffin-imbedded lung specimens were provided by pathologists from each institution. Age, circumstances of death, and postautopsy diagnosis were recorded when available. SIDS was diagnosed if there was no recognized premortem disease, no significant microscopic or macroscopic pathological findings, and toxicology studies were negative.

Control subjects. All 342 infants who were aged between 5 days and 12 months at the time of death at the hospital were identified from the original 534 infants in series 1. These were selected as control subjects for the purpose of statistical comparison with the age-matched infants who died suddenly at home. Newborns aged <5 days were excluded.

Processing of lung specimens and stains. Lung tissue specimens were sectioned (5 μm) and stained with Grocott-Gomori methanamine–silver nitrate and hematoxylin-eosin stains. Slides from series 1 were examined by investigators blind to the diagnosis of SIDS, and slides from series 2 and 3 were examined by investigators aware of the diagnosis of SIDS. Slides in cases from Oxford were examined by investigators blind to the diagnosis of SIDS who also analyzed slides for possibly immunocompromised or immunocompromised patients with unknown P. carinii status. Specimens were examined by 2 different investigators (S.L.V. and J.C.W., C.P., or P.M.) in all cases. Discordant results were discussed, and cases were labeled as positive only if typical P. carinii cysts in clusters of 3 or more organisms were seen by both investigators. A third investigator reviewed positive cases (W.T.H. for Chilean samples, and S.G. for Oxford samples). Positive slides were subsequently stained with monoclonal antibody 3F6 (Dako Diagnostics, Carpinteria, CA), which recognizes an 82-kDa protein present in the cyst wall that is not altered by formalin or paraffin; all positive cases were confirmed by both methods (figure 1).

Clinicopathologic correlation. To gain insight into the clinical history and to better describe the histopathologic pattern of primary P. carinii infection in these children, the criteria described by Price and Hughes [9] for children with malignancies were retrospectively applied to P. carinii–positive patients with SIDS. Briefly, this scale of lung involvement with clinical correlation considers 2 asymptomatic stages and 1 symptomatic stage of P. carinii infection. Asymptomatic stages were described as isolated cysts with no parenchymal reaction of the lung (stage 1) or desquamation of organisms into the alveolar lumen with an increasing number of P. carinii and minimal or no inflammatory response in alveolar septa (stage 2). The symptomatic stage was defined as a host response consisting of alveolar desquamation and lymphocytic and plasma cell alveolar infiltrates (stage 3). Stage 3 was found by these investigators to correlate with clinical symptoms and radiographic signs of P. carinii pneumonitis in children with different types of cancer.

Statistical analysis. To compare the incidence of P. carinii–positive and –negative lung samples among infants who died at home of SIDS with the incidence among those who died at the hospital of multiple conditions, we used Fisher’s exact test (using Epi-Info version 6; Centers for Disease Control and Prevention, Atlanta, GA). Control subjects were compared with infants with SIDS from series 1 and also with infants from series 2 and 3 combined. P < .05 was considered statistically significant.

Results

Series 1. Of 534 lung tissue specimens from consecutive pediatric patients that were blind to investigators with respect to age and diagnosis, 16 (3%) were found to be positive for P. carinii clusters. Primary autopsy diagnoses for these children were as follows: bronchopneumonia, 5 children; SIDS, 4; bronchitis, 1; generalized lipodisso, 1; and no diagnosis available, 1. The following underlying diseases suggestive of an immune defect were present in 4 patients who also had bronchopneumonia as a secondary diagnosis: severe combined immunodeficiency syndrome, congenital medullary aplasia, mucocutaneous candidiasis, and fulminant hepatitis. Age distribution and primary autopsy diagnoses for these patients are shown in table 1. P. carinii was detected in 4 (25%) of 16 infants who were dead at arrival to the emergency department and had a postautopsy diagnosis of SIDS compared with 10 (2.9%) of 342 infants who were aged between 5 days and 1 year and died of multiple conditions at the hospital (P = .002, Fisher’s exact test). On the basis of this observation, we elected to examine a larger number of infants with a postautopsy diagnosis of SIDS.

Series 2–4 and control subjects. We examined an additional 134 infants with a primary autopsy diagnosis of SIDS who were autopsied at different hospitals in Santiago (series 2 and 3) and 27 infants who died of SIDS and were autopsied at a hospital in Oxford (series 4) (table 2). Ages of these infants ranged from 20 to 575 days (mean, 95 days; median, 60 days). Ages of control subjects ranged from 5 to 365 days (mean, 88 days; median, 60 days); control subjects were matched according to the age criterion for the diagnosis of SIDS.

Ten (2.9%) of 342 controls had P. carinii clusters compared with 47 (35.1%) of 134 Chilean infants with an autopsy diagnosis of SIDS in series 2 and 3 (P = .0000001, Fisher’s exact test). Four (14.8%) of the 27 infants who died of SIDS in Oxford were found to have P. carinii clusters by histological analysis (table 2).

Lung reaction, extent of P. carinii infection, and retrospective correlation with clinical manifestations before death. Of 55 P. carinii–positive patients with SIDS, 13 were not evaluable because the specimens had extensive postmortem autolysis. The clinicopathologic correlation criteria developed by Price and Hughes [9] were applied to 42 evaluable cases. Twelve and 25
Association of Primary *P. carinii* Infection and SIDS

Figure 1.  

A. *Pneumocystis carinii* clusters in lung tissue specimen from a 2-month-old infant diagnosed with sudden infant death syndrome (SIDS) (Grocott-Gomori methenamine–silver nitrate stain; original mag, \( \times 330 \); bar, 10 \( \mu \)m).  

B. Smaller magnification (\( \times 60 \)) of A, illustrating that number of *P. carinii* clusters in patients with SIDS is few.  

C. Immunohistochemical analysis with monoclonal antibody 3F6 (Dako Diagnostics, Carpinteria, CA) of lung tissue specimen from a 4-month-old infant diagnosed with SIDS that reveals *P. carinii* cluster filling an alveolus (original mag, \( \times 330 \); bar, 10 \( \mu \)m). Grocott-Gomori methenamine–silver nitrate stain is generally considered standard for detection of *P. carinii* in tissue specimens. It correlates well with immunohistochemical analysis with monoclonal antibody 3F6. *P. carinii* was detected by both techniques in all cases considered positive.

Discussion

This study provides histological evidence of mild infection by *P. carinii* in presumably normal immunocompetent infants, a finding in agreement with serological evidence that most normal children are exposed to *P. carinii* at an early age [1, 2]. Mild, naturally occurring *P. carinii* infection has previously been observed in other mammals shortly after weaning: rabbits [3, 4] and piglets [5]. The young age of the patients and the characteristically mild histological pattern encountered suggest that our findings correspond to primary infection rather than to reactivated or secondary infection which has been histologically well described for the immunocompromised host [7–12].

This study also suggests an association between primary *P. carinii* infection and SIDS (table 2). A small number of reports of cases of mild, focal *P. carinii* pneumonitis in infants with SIDS in Germany, the United States, and Chile in the 1950s [6, 10–12] provide further support of this association. In this study, *P. carinii* was also found in a relatively high proportion of patients with SIDS in Santiago and Oxford. Some innate flaws in the study must be considered. In series 2 and 3, slides were examined by investigators aware of the diagnosis of SIDS. To further assess the statistical significance found in series 1, the proportion of *P. carinii*–positive cases in series 2 and 3 was compared with that of *P. carinii*–positive control subjects (table 2); however, deaths in control subjects were not sudden, and
Table 1. Age distribution, primary autopsy diagnosis, and positivity for *Pneumocystis carinii* for 534 consecutive pediatric patients (those with AIDS and malignancies were excluded) autopsied from 1990 to 1996 at 2 children’s hospitals in Santiago, Chile.

<table>
<thead>
<tr>
<th>Primary autopsy diagnosis</th>
<th>No. of patients per age at time of death</th>
<th>No. positive for <em>P. carinii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;5 d</td>
<td>5 d to 1 y</td>
</tr>
<tr>
<td>Pulmonary (bronchopneumonia and others)</td>
<td>8</td>
<td>68 (5)</td>
</tr>
<tr>
<td>Heart (congenital and others)</td>
<td>8</td>
<td>111</td>
</tr>
<tr>
<td>CNS</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Various immunodeficiencies</td>
<td>0</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Prematurity</td>
<td>32</td>
<td>39</td>
</tr>
<tr>
<td>SIDS</td>
<td>0</td>
<td>16 (4)</td>
</tr>
<tr>
<td>Others</td>
<td>6</td>
<td>47 (1)</td>
</tr>
<tr>
<td>NA</td>
<td>13</td>
<td>50 (1)</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>358 (14)</td>
</tr>
</tbody>
</table>

NOTE. Lung tissue sections were examined by Grocott-Gomori methenamine-silver nitrate staining, and positive specimens were also analyzed by immunohistochemical technique. NA, not available; SIDS, sudden infant death syndrome.

Table 2. Positivity for *Pneumocystis carinii* in lung tissue specimens from infants and children who died at hospital and from infants with SIDS.

<table>
<thead>
<tr>
<th>Autopsy series, patient group</th>
<th>No. of <em>P. carinii</em>-positive patients/total no. of hospital deaths (%)</th>
<th>No. of <em>P. carinii</em>-positive patients/total no. of patients with SIDS</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, cases</td>
<td>12/518 (2.3)</td>
<td>4/16 (25.0)</td>
<td>.002</td>
</tr>
<tr>
<td>Controls’ vs. SIDS casesa</td>
<td>10/342 (2.9)</td>
<td>4/16 (25.0)</td>
<td></td>
</tr>
<tr>
<td>2, SIDS cases</td>
<td>35/94 (37.2)</td>
<td>12/40 (30.0)</td>
<td>.0000001</td>
</tr>
<tr>
<td>3, SIDS cases</td>
<td>47/134 (35.1)</td>
<td>27/40 (67.5)</td>
<td></td>
</tr>
<tr>
<td>Controls’ vs. 2 and 3 SIDS casesd</td>
<td>10/342 (2.9)</td>
<td>4/27 (14.8)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. For a description of series, see text under Methods. SIDS, sudden infant death syndrome.

a Fisher’s exact test.

b Slides were examined by investigators blind to any diagnosis. All pediatric ages (newborn to 16 years) were included. Patients with malignancies and AIDS were excluded.

c From series 1 (controls were aged 5 days to 1 year).

d Slides in series 2 and 3 were examined by investigators aware of diagnosis of SIDS.
Therefore, the terminal event of SIDS cannot be explained by these findings under the current understanding of *P. carinii* disease.

Alternatively, because *P. carinii* is largely a pathogen of the immunocompromised host, finding *P. carinii* more frequently in infants with SIDS might suggest that it marks the presence of an underlying immune defect in SIDS, just as *P. carinii* has served as a marker for HIV infection [21–25].

Previous reports indicate that *P. carinii* can present as pneumonia in immunocompetent infants aged <3 months [26] and also suggest that *P. carinii* pneumonia might be associated with apnea [27, 28]. In our study, 5 (7.3%) of 68 immunocompetent infants with SIDS warranted further investigation.

The data provide histological evidence of primary infection by *P. carinii* in apparently immunocompetent infants and children. They show that *P. carinii* infection is more common in infants aged <1 year who die in the community than in those who die in the hospital setting and that this infection can be asymptomatic. The high prevalence of *P. carinii* infection in infants with SIDS warrants further investigation.

References