Association of Primary *Pneumocystis carinii* Infection and Sudden Infant Death Syndrome

Sergio L. Vargas,[1] Carolina A. Ponce,[1]
Walter T. Hughes,[6] Ann E. Wakefield,[7] Juan C. Weitz,[1]
Sergio Donoso,[1] Ana V. Ulloa,[1] Patricio Madrid,[1]
Stephen Gould,[8] Juan J. Latorre,[2] Ricardo Avila,[3]
Samuel Benveniste,[4] Miriam Gallo,[5] José Belletti,[5]
and René Lopez[5]

From the [1]Program in Microbiology, Instituto de Ciencias Biomedicas, Universidad de Chile, [2]Department of Pathology, Luis Calvo Mackenna Children's Hospital, [3]Department of Pathology, Roberto del Rio Children's Hospital, [4]Department of Pathology, Exequiel Gonzalez Cortés Children's Hospital, and [5]Medico Legal Institute, Santiago, Chile; [6]Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee, USA; and Departments of [7]Pediatrics and [8]Cellular Pathology, John Radcliffe Hospital, University of Oxford, Oxford, United Kingdom

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.

There is well-documented serological evidence that up to 94% of normal immunocompetent children have detectable antibody to *Pneumocystis carinii* by 30 months to 4 years of age [1, 2], which indicates that primary *P. carinii* infection is one of the most common infections in humans and that exposure occurs early in life. Experimental evidence shows that the host response pattern associated with primary *P. carinii* infection in otherwise healthy animals is milder [3–5] than that associated with the usually massive infection seen in an immunocompromised host. Data for humans are scant: *P. carinii* in low numbers or mild focal pneumonitis has rarely been reported as an autopsy finding for presumably immunocompetent children, and then the infection has been judged to be latent, incidental organisms [6]. In contrast, there are abundant autopsy reports of interstitial plasma cell pneumonia in certain groups of debilitated, under-nourished, or premature infants, and reports of *P. carinii* pneumonia in infants and children with primary and secondary immunodeficiency syndromes [7, 8].

Cross-sectional studies of children's lungs at autopsy might provide histological support for the assumption, based on serology, that primary *P. carinii* infection is a common occurrence in small children. Furthermore, a correlate with diagnosis before death might provide insight to the clinical presentation of primary infection in immunocompetent infants and children.

Because specific stains are needed to identify *P. carinii* in tissue samples, we undertook a prospective search for *P. carinii* by studying autopsy lung specimens from 534 consecutive children (those with AIDS and malignancies were excluded) that were obtained over a 6-year period at 2 major pediatric hospitals in Santiago, Chile. *P. carinii* was detected more frequently in infants who were dead on arrival at the emergency department and had an autopsy diagnosis compatible with sudden infant death syndrome (SIDS) than in children who died of multiple conditions at the hospital. These findings prompted us to expand our study to include an additional 134 infants in Chile who died at home and had a postautopsy diagnosis of SIDS, and 27 infants in Oxford, United Kingdom, who died of SIDS.

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Methods

Lung specimens. A total of 695 autopsy lung samples were studied from infants and children in 4 different series. Series 1

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 methenamine–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 lipidosis, 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
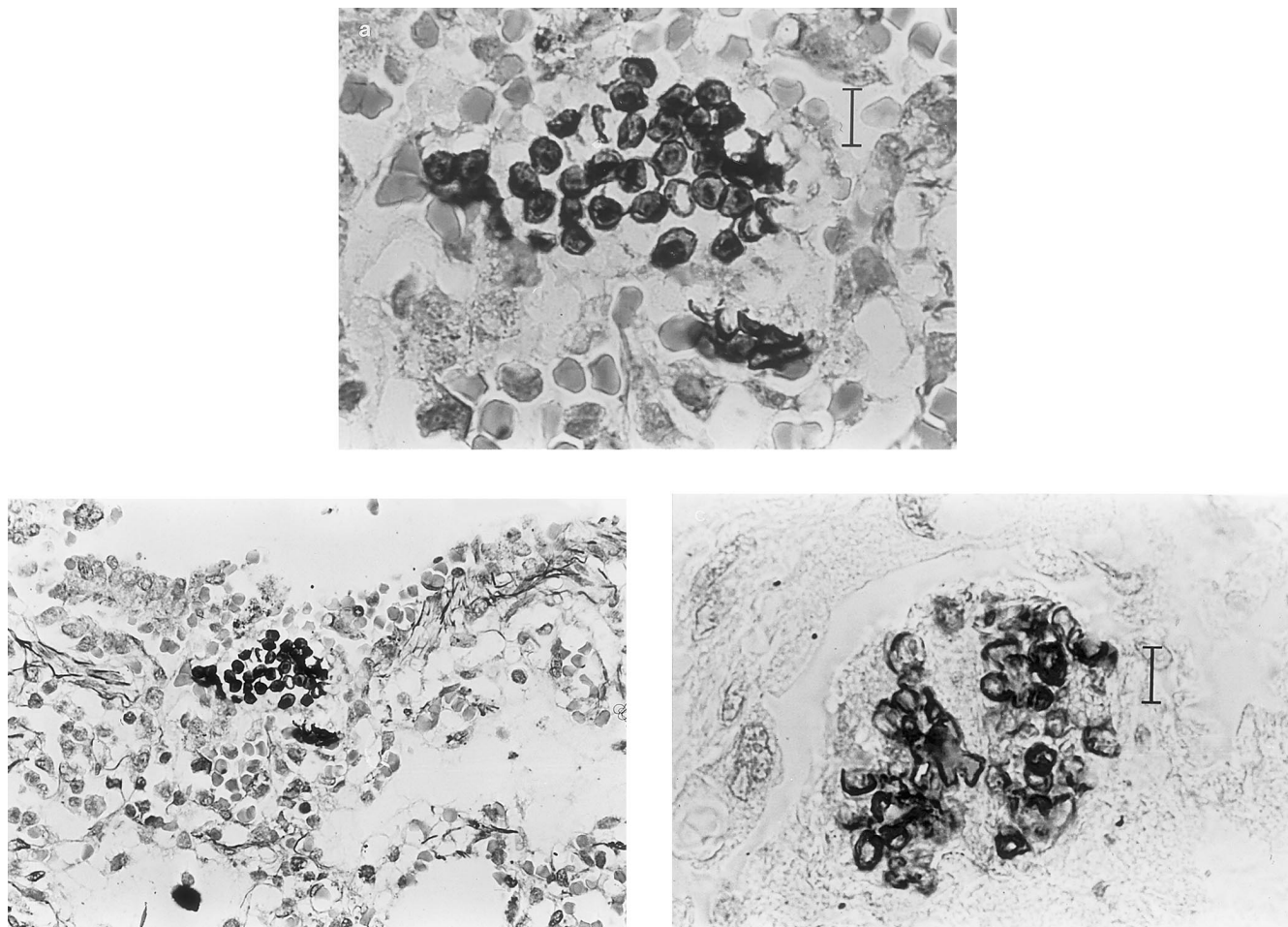
Figure 1. *A, Pneumocystis carinii* clusters in lung tissue specimen from a 2-month-old infant diagnosed with sudden infant death syndrome (SIDS) (Grocott-Gomori methenamine–silver nitrate stain; original mag, ×330; bar, 10 μm). *B,* Smaller magnification (×60) of *A,* illustrating that number of *P. 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, ×330; bar, 10 μm). 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.

cases were categorized as stages 1 and 2, respectively (together, 88.1%), and 5 (11.9%) were categorized as stage 3, which suggests previous symptomatic disease.

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.

Primary autopsy diagnosis	No. of patients per age at time of death (no. positive for *P. carinii*)					
	<5 d	5 d to 1 y	1–2 y	>2 y	NA	Total
Pulmonary (bronchopneumonia and others)	8	68 (5)	10	8	2	96 (5)
Heart (congenital and others)	8	111	5	12	4	140
CNS	3	9	2	2	1	17
Gastrointestinal	2	14	1	2	2	21
Various immunodeficiencies	0	4 (3)	0	4 (1)	0	8 (4)
Prematurity	32	39	0	0	2	73
SIDS	0	16 (4)	0	0	0	16 (4)
Others	6	47 (1)	3	7	4 (1)	67 (2)
NA	13	50 (1)	4	5	24	96 (1)
Total	72	358 (14)	25	40 (1)	39 (1)	534 (16)

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.

cases were not matched with respect to underlying health status, prior drug therapies, and awareness of the diagnosis by investigators examining slides. The samples from Oxford (series 4) demonstrated that these findings were not a local phenomenon restricted to Chile; and so have much wider implications.

Small numbers of *P. carinii* organisms found in infants with SIDS do not necessarily represent the onset of primary *P. carinii* infection; these organisms could correspond to a phase of *P. carinii* clearance that may take up to 1 year [13]. Therefore, a well-defined control group is needed in further studies to document the relevance of a potential pathogenic role of *P. carinii* in a proportion of SIDS cases.

A possible explanation for the pathogenic role of *P. carinii* in some infants with SIDS is that it reduces the level of pulmonary surfactant. This hypothesis is supported by evidence in both experimental models and studies of humans that showed that *P. carinii* infection leads to a decrease in the level of pulmonary surfactant [14–16]. Furthermore, a decreased surfactant level has been recently shown in an animal model to be directly related to *P. carinii* growth and occurs at early stages

of *P. carinii* development [14]. Relevant to this study, infants with a postautopsy diagnosis of SIDS also have been consistently found to have decreased levels of surfactant [17–19]. In 1985, Talbert and Southhall [20] first hypothesized that a decrease in surfactant level may be a mechanism that triggers sudden death in infants when it occurs at a critical stage of their lung development. Our documentation of *P. carinii* in a relatively high proportion of SIDS cases suggests that *P. carinii* might play a role, alone or as an accompanying pathogen, in the decreased level of pulmonary surfactant that is documented in SIDS cases. Whether other pathogens that possess activity against phospholipase A_2 or other mechanisms could be implicated needs to be determined [18].

Most (37 [88.1%] of 42) infants with SIDS who were positive for *P. carinii* and were evaluated by the clinicopathologic correlate developed by Price and Hughes [9] were categorized in stage 1 or 2, thus providing postmortem evidence that primary infection was asymptomatic in most SIDS cases. In agreement with this evaluation, the load of *P. carinii* organisms in SIDS cases was mild, and clusters were difficult to find histologically.

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

Autopsy series, patient group	No. of *P. carinii*–positive patients/ total no. of hospital deaths (%)	No. of *P. carinii*–positive patients/ total no. of patients with SIDS	P^a
1, cases[b]	12/518 (2.3)	4/16 (25.0)	
Controls[c] vs. SIDS cases[c]	10/342 (2.9)	4/16 (25.0)	.002
2, SIDS cases[d]		35/94 (37.2)	
3, SIDS cases[d]		12/40 (30.0)	
Controls[c] vs. 2 and 3 SIDS cases[d]	10/342 (2.9)	47/134 (35.1)	.0000001
4, cases		4/27 (14.8)	

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 aged from 5 days to 1 year in series 1 had bronchopneumonia as a primary autopsy diagnosis. This proportion, which agrees with findings in other studies [26, 27, 29, 30], suggests that *P. carinii* infection should be included in the differential diagnosis of bronchopneumonia in presumably immunocompetent infants. Whether a clinically identifiable pattern is present in mild forms of primary infection occurring in infants who spontaneously recover is not known.

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.

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