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Persistent Tachypnea of Infancy Is Associated With Neuroendocrine Cell Hyperplasia

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Summary. We sought to determine the clinical course and histologic findings in lung biopsies from a group of children who presented with signs and symptoms of interstitial lung disease (ILD) without identified etiology. Patients were identified from the pathology files at the Texas Children's Hospital who presented below age 2 years with persistent tachypnea, hypoxia, retractions, or respiratory crackles, and with nonspecific and nondiagnostic lung biopsy findings. Age-matched lung biopsy controls were also identified. Their clinical courses were retrospectively reviewed. Biopsies were reviewed, and immunostaining with antibodies to neuroendocrine cells was done. Fifteen pediatric ILD patients and four control patients were identified for inclusion in the study. Clinically, the mean onset of symptoms was 3.8 months (range, 0–11 months). Radiographs demonstrated hyperinflation, interstitial markings, and ground-glass densities. Oxygen was initially required for prolonged periods, and medication trials did not eliminate symptoms. After a mean of 5 years, no deaths had occurred, and patients had improved. On review of the lung biopsies, all had a similar appearance, with few abnormalities noted. Immunostaining with antibodies to neuroendocrine cell products showed consistently increased bombesin staining. Subsequent morphometric analysis showed that immunoreactivity for bombesin and serotonin was significantly increased over age-matched controls. In conclusion, we believe this may represent a distinct group of pediatric patients defined by the absence of known lung diseases, clinical signs and symptoms of ILD, and idiopathic neuroendocrine cell hyperplasia of infancy. These findings may be important for the evaluation of ILD in young children. **Pediatr Pulmonol. 2005; 40:157–165.** © 2005 Wiley-Liss, Inc.

Key words: interstitial lung disease; lung disease; persistent tachypnea; pediatric; neuroendrocrine.

INTRODUCTION

Infants who present with persistent tachypnea, hypoxia, retractions, and respiratory crackles are suspected of having interstitial lung disease (ILD). Unlike adults who generally have well-described forms of ILD,[1] pediatric patients with ILD have a broader array of often poorly defined and more heterogeneous disorders.[2] In some instances, infants and young children with signs and symptoms of ILD have nondiagnostic lung biopsies with only minor but consistent histology abnormalities, a condition that we call persistent tachypnea of infancy (PTI).[3] Because initial histology reviews of lung biopsy material suggested increased airway ''clear cells'' consistent with possible pulmonary neuroendocrine cells (PNEC), we wondered if the clinical course or histologic findings of pulmonary neuroendocrine cell (PNEC) hyperplasia could better define this group of patients.

PNECs are granulated epithelial cells distributed in the conducting airways and occasionally as small clusters in the lobular parenchyma as neuroepithelial bodies (NEB). They produce bioactive products (bombesin-like peptide, serotonin, and calcitonin) capable of bronchoconstriction, vasoactivity, epithelial differentiation, and smooth muscle alteration. PNECs are present most abundantly in fetal life, when they are thought to play a role in regulating lung development.[4] Bombesin-positive cells peak at midgestation, and by birth, numbers decline rapidly to the low levels seen in adults.[5] PNEC hyperplasia has been described after the newborn period in children and in adults with a wide variety of known lung diseases. In fact, an adult form of ILD associated with idiopathic diffuse

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hyperplasia of PNEC and airway disease (IDHPNC) was reported.[6]

The evaluation, treatment, and prognosis of adult patients with ILD has been guided by the identification of characteristic clinical features and the classification of abnormalities in lung histology.[1] Although a specific classification for pediatric ILD (pILD)[2] was suggested, details of clinical features and abnormalities in lung histology are less advanced than are those that characterize adult ILD. Insights gained from lung biopsies in children, such as the recently reported new variant of neonatal interstitial lung disease associated with evidence of pulmonary interstitial glycogenosis (PIG), will be critical to understanding pILD.[7]

In this report, we describe the presentation, clinical course, and more specific lung biopsy findings of a cohort of pediatric patients diagnosed with pILD. The recognition of PNEC hyperplasia in this group of symptomatic infants and young children with ILD and without features of a known pulmonary disease may allow us to better classify and understand these patients.

MATERIALS AND METHODS

Study Population

Sixteen patients were identified from the consultation files of the Pathology Service at Texas Children's Hospital between 1993–1997 who presented below age 2 years with persistent tachypnea, hypoxia, or respiratory crackles, and who had lung biopsies that showed similar but minor nondiagnostic abnormalities. The lung histology for all patients was characterized by mildly increased airway smooth muscle, increased alveolar macrophages, and often an increase in ''clear cells'' in the distal airways on hematoxylin-and-eosin (H&E) stain. No histologic changes characteristic of a known pulmonary process were identified, and no biopsy showed significant inflammatory changes. Biopsies were obtained by video-assisted thoracoscopic (VATS) or open lung biopsy, and biopsy location was determined by respiratory crackles, high-resolution computed tomography (HRCT), and the intraoperative observations of the surgeon. The patients had no evidence of genetic disease, congenital heart disease, immune dysfunction, or an identified cause of persistent respiratory disease. We previously labeled patients described in this population as having persistent tachypnea of infancy (PTI).[3]

Lung samples from four age-matched controls (Table 1) without acute or chronic inflammatory or reactive changes were selected from the pathology files at Texas Children's Hospital as a comparison group for immunostaining and morphometric analysis. Two control patients had a diagnosis of congenital lobar overinflation; however, the sections used as control material were not from the overexpanded left upper lobe, but were from the normal lingula. Lung histology from both the PTI and control groups was reviewed together. Historical controls from a published autopsy series of pediatric patients, with a similar age spectrum as our subjects (3–24 months), were compared to disease and study control subjects to further evaluate the percent of bombesin-immunoreactive airway area.[8]

The clinical histories for patients with PTI were retrospectively reviewed from patient charts and from information provided at the time of lung biopsy to determine the clinical presentation, evaluation, treatment, and outcome. Limited clinical information was available for the comparison group. Age of onset was defined as the earliest report of tachypnea or retractions. This was listed as 0 or 1 month if symptoms were noted from birth or early infancy, respectively. The referring physicians determined the evaluation for each patient. The University of Colorado Institutional Review Board approved this study.

Immunohistochemistry

Immunohistochemistry was performed using antibodies to neuron-specific enolase (NSE) (BBS/NC/V1-H14, Dako), calcitonin (polyclonal, Dako), synaptophysin (Snp88, Biogenex), chromogranin (DAK A3, Dako), bombesin (polyclonal, ImmunoStar), and serotonin (5HT-HT09, Dako). The primary antibodies employed were monoclonal mouse anti-human NSE (dilution 1:250), polyclonal rabbit anti-human calcitonin (dilution 1:5,000), monoclonal mouse anti-synaptophysin (dilution 1:800), monoclonal mouse anti-human chromogranin (dilution 1:500), monoclonal mouse anti-serotonin (dilution 1:4,000), and polyclonal rabbit anti-bombesin (gastrin-releasing peptide antibody) (dilution 1:1,000). Immunostaining was performed using a Ventana ES automatic immunostainer following antigen retrieval,

TABLE 1—Control Patient Characteristics Ordered by Patient Age at Time of Biopsy

Patients/sex	Altitude	Agent biopsy (months)	Condition
1/M	No	4	Congenital lobar overinflation
2/M	No	4	Ventilator and steroids
3/M	No	5	VSD and congenital lobar overinflation
4/F	No	12	VSD and pulmonary hypertension

VSD, ventricular septal defect.

according to a standard protocol employing either Tris EDTA or citrate buffer, depending on the antibody, and a steamer for 30 min.

Ten serial sections were obtained from each block of formalin-fixed, paraffin-embedded tissue for immunostaining from all cases and controls. However, only one section from each series was immunostained with bombesin. Standard methods for immunostaining were used.[9] The 10 serial sections included one each of the five antibodies employed and negative controls incubated with nonimmune buffer and run concurrently.

Morphometric Analysis

Each section was initially evaluated and subjectively rated for intensity (absent, mild, moderate, or strong) and amount/pattern (rare, focal, or prominent) of immunoreactivity by light microscopy. Morphometry was performed on case and control lung sections immunostained for bombesin and serotonin because of the more consistent reactivity initially observed with these antibodies. Computer-assisted morphometry was then performed using a Bioquant System IV package with a Summagraphics digitizing tablet. Using a Nikon Optiphot microscope and an attached Sony CCD/RGB color video camera, areas were traced using the 20× objective, and cell counts were done at whatever magnification was required for clear identification of individual cells. For each tissue section, measurements included a count of the number of bronchioles and NEBs, as well as the area of each section. For each bronchiole, the area of immunoreactive neuroendocrine cells and the area of the bronchiolar epithelium were obtained, as were the number of immunopositive cells and the number of all bronchiolar epithelial cells. For each NEB, the number of cells was counted and the area was measured. This data were summed for each case to provide the total area of the biopsy, total bronchiolar epithelial area, total immunoreactive bronchiolar epithelial area, and total NEB area, number of bronchioles and NEBs, and total number of epithelial cells and immunoreactive cells in bronchioles and NEBs. Calculations provided percentages of immunoreactive bronchioles, immunoreactive bronchiolar epithelium, and immunoreactive area within each biopsy, as well as mean NEB area. Cartilage-containing airways were not included in the morphometric evaluation. Calculations were performed using methods similar to those previously published.[9,10]

Statistics

Patient demographic data were analyzed using descriptive statistics, and morphometric data were analyzed using Levene's test for equality of variances followed by a *t*-test with equal variances not assumed. Analysis of variance and unadjusted and Bonferroni-adjusted *t*-tests for multiple comparisons were performed to determine differences in percent bombesin immunoreactivity between subjects with disease (PTI), study controls, and published historical controls. Analysis of covariance was used to analyze age as a covariate in different groups.

RESULTS

Clinical Characteristics in NEHI

One patient was excluded, as there was insufficient tissue for analysis. The remaining 15 children (Table 2) consisted of 12 males and 3 females. Ten resided in or near Denver, Colorado at an approximate altitude of 1,600 m, and 5 lived at altitudes closer to sea level. Four age-matched comparison samples were taken from 3 males and 1 female (Table 1).

The mean age of onset for symptoms in study patients was 3.8 months (range, 0–11 months). Most lung biopsies, however, were performed much later, at a mean age of 15 months (range, 4–34 months). Control subjects had an approximate mean age of 6.0 months (range, 4–12 months) at time of lung biopsy. Historical autopsy control subjects ranged from 3–24 months in age at time of death.[9]

All study patients had some degree of persistent retractions, tachypnea, and daytime or nighttime hypoxia noted. Table 2 lists other findings of individual patients. Eighty percent of patients were born at term. None of those born prematurely had neonatal respiratory problems associated with chronic lung disease. Only 3 children had needed brief neonatal periods of oxygen, and none received supplemental oxygen for longer than 36 hr. Thirty-three percent of patients ($n = 5$) were exposed to second-hand smoke, and 66% ($n = 10$) had a family history of asthma. The majority had persistent crackles on chest auscultation. In contrast, only a few patients had wheezing; cough, if present, was not prominent.

The majority of study patients had complete evaluations prior to lung biopsy, including EKG, echocardiogram, immunoglobulins, sweat test, upper gastrointestinal (UGI) radiographs, chest radiographs, HRCT scans, bronchoscopy, and bronchoalveolar lavage (BAL) with cultures and evaluation of lipid- and hemosiderin-laden macrophages. Many patients had UGIs or pH probes that suggested gastroesophageal reflux (GER); however, when examined, BAL was negative for significant numbers of lipid-laden macrophages. A few patients had organisms recovered from BAL which were not considered to be persistent pathogens or responsible for persistent clinical symptoms. Minor abnormalities are listed in Table 2.

All study patients had chest radiographs, and many had HRCT to visualize the location and severity of pulmonary parenchymal abnormalities. Chest radiographs consistently showed hyperexpansion. A representative chest X-ray is shown in Figure 1. HRCT findings included

TABLE 2— NEHI Patient Characteristics Ordered by Patient Age at Time of Biopsy

Patient/ sex	Altitude	Age of onset (months)	Age of biopsy (months)	Birth issues	Asthma History	Cough	Crackles	Wheeze	Other issues
1/M	Yes	1	4	Term	No	No	Yes	No	Poor growth
2/M	Yes	0	7	Term	Yes	No	Yes	No	GER; Nissen
3/M	Yes	3	9	Term	No	Yes	Yes	No	Wt gain poor; rhinovirus on BAL
4/M	Yes	6	10	Term	Yes	No	Yes	No	Borderline IGG levels, normal immune response
5/F	Yes	4	11	Term, O_2[1]	No	No	Yes	Occ	GER; Nissen; aberrant right subclavian
6/M	No	6	12	Term	Yes	NK	NK	NK	
7/M	Yes	2	13	Term	No	No	Yes	No	GER; RVH; Nissen
8/M	No	3	13	Term, O_2[1]	Yes	Occ	Yes	Occ	Wt gain poor, *M. catarralis* on BAL
9/M	No	9	14	33 wk; vent[2]	Yes	Yes	Yes	No	PDA ligated
10/M	No	1	15	36 wks	Yes	No	Yes	No	
11/M	Yes	1	17	NK	Yes	No	No	No	*H. influenza* on BAL
12/M	Yes	0	18	Term	Yes	Yes	Yes	No	
13/F	Yes	8	21	37 wk	Yes	Yes	Occ	Occ	GER
14/M	Yes	2	22	Term	No	Yes	Yes	Yes	
15/F	No	11	34	NK	Yes	No	Yes	No	TB skin test +; cultures −

[1]Oxygen for less than 6 hr.
[2]Ventilated for 12 hr and then O_2 for 36 hr.
NK, not Known; Occ, occasional; BAL, bronchoalveolar lavage; PDA, patent ductus arterosus; wt, weight; GER, gastroesophageal reflux; vent, ventilation; wk, weeks; M, male; F, female.

pulmonary hyperexpansion, ground-glass densities in a subsegmental or segmental distribution involving one or two lobes, or atelectasis. A representative HRCT is shown in Figure 1C.

PNEC Hyperplasia in Lung Tissue

Histologic findings on lung biopsy were similar in all study patients. There was no significant inflammation, or other specific diagnostic features. There were no changes of pulmonary hypertension in the vasculature. Specifically, there was no evidence of medial thickening of small pulmonary arteries, or extension of arterial smooth muscle into intralobular vessels. All showed quite mild and nonspecific changes, including increased numbers of alveolar macrophages (Fig. 2A) and mild airway smooth muscle hyperplasia (Fig. 2B), as well as occasional prominent clear cells in the airway epithelium (Fig. 2B). Clear cells were not prominent in the control group (Fig. 2C).

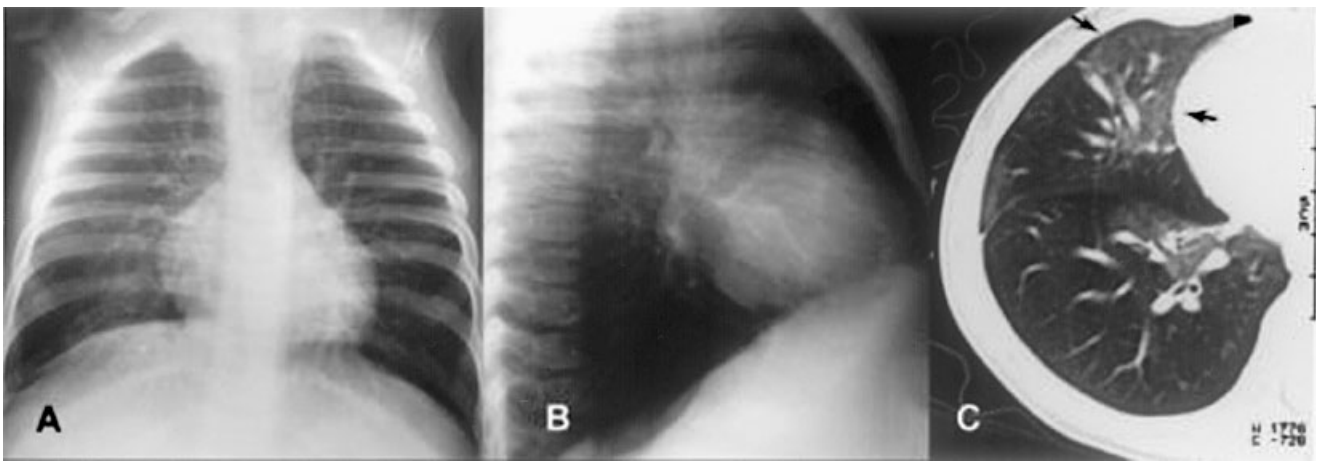

Fig. 1. A, B: Chest radiographs demonstrate hyperinflation and increased interstitial markings. C: HRCT section shows increased segmental ground-glass densities (arrows).

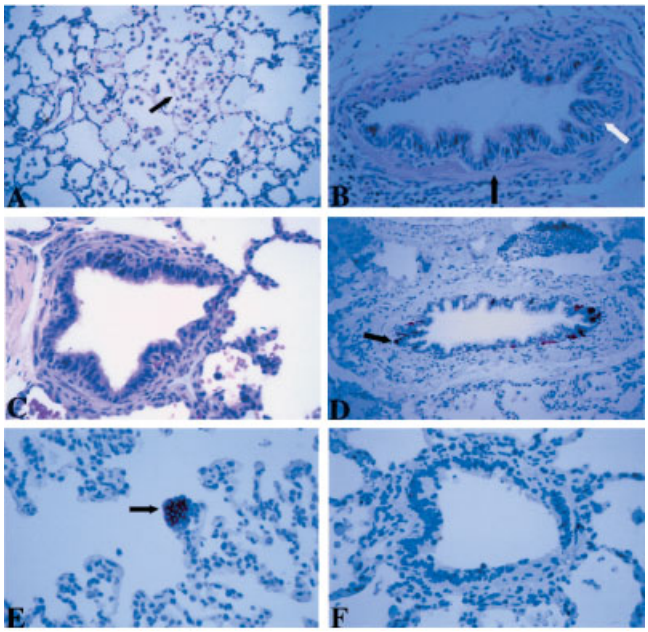

Fig. 2. Representative photomicrographs of lung tissue. A: Study patient biopsy with nonspecific changes, including increased alveolar macrophages (arrow) (original magnification ×25; H&E stain). B: Bronchiole with increased clear cells in epithelium (white arrow) and nonspecific airway smooth muscle (black arrow) in NEHI lung biopsy (original magnification ×50; H&E stain). C: Bronchiole from control lung with normal-appearing bronchiolar epithelium without identifiable clear cells (original magnification ×50; H&E stain). D: Immunopositive cells (arrow) in bronchiole from NEHI patient biopsy (original magnification ×25; immunostain for bombesin). E: Cluster of immunopositive cells and neuroepithelial body (arrow) in lobular parenchyma of lung biopsy from NEHI patient (original magnification ×50; immunostain for bombesin). F: Bronchiole of control patient shows no immunopositivity (original magnification ×50; immunostain for bombesin).

Immunostains for all antibodies were evaluated for intensity and pattern of distribution. Bombesin and serotonin showed the strongest staining intensity and greatest numbers of immunoreactive cells. Staining intensity was comparable in control and study patients, but there were fewer immunoreactive cells in control patients. Study patients had increased numbers of PNECs within the airway epithelium, as well as increased number and size of NEBs in the lobular parenchyma (Fig. 2D,E) compared with controls (Fig. 2F). The NEB changes are somewhat less consistent than those of the airway PNECs.

Morphometric studies were used to determine whether the apparent increase in immunoreactivity was significantly different from controls. Data from the analysis of bombesin and serotonin immunostaining are presented statistically in Table 3. In lung biopsies from study patients, immunoreactivity for bombesin was significantly increased over controls in both the percentage of immunoreactive epithelial area and in the number of NEB immunoreactive cells per unit biopsy area ($P = 0.002$ and $P = 0.018$, respectively). The individual case data for percentage of bombesin immunoreactive epithelial area related to total airway epithelial area are presented graphically in Figure 3. As seen in Figure 3, there was no significant difference between study patients from lower (LP, mean 6.8%) vs. higher (HP, mean 6.4%) altitudes. Immunoreactivity for serotonin (Table 3) was also significantly different. The percentage of immunopositive airway epithelial area, the number of immunopositive cells per airway, the total number of immunopositive cells per unit area of the biopsy, and the number of NEB cells within the lobular parenchyma for the serotonin antibody were significantly increased over controls ($P < 0.005$, $P = 0.025$, $P = 0.011$, and $P = 0.005$, respectively). No significant differences were observed

TABLE 3—Statistical Analysis of Morphometric Data for Bombesin and Serotonin Immunostaining Comparing Study Population With Control Group

Antibody	Measurement	Significance (two-tailed)	Mean difference	Standard Error Difference	95% confidence interval of mean	
					Lower	Upper
Bombesin	% immunoreactive biopsy area	0.164	−0.013	0.009	−0.033	−0.006
	% immunoreactive airway epithelial area	0.002	−3.67	0.907	−5.69	−1.65
	% immunoreactive cells/airways	0.341	−2.481	2.316	−8.773	3.812
	% immunoreactive cells/biopsy	0.071	−1.366	0.646	−2.883	0.151
	NEB cell no./biopsy	0.018	−0.461	0.176	−0.833	−8.90
	Mean NEB area	0.081	−50.855	22.461	−111.242	9.532
Serotonin	% immunoreactive biopsy area	0.166	−0.017	0.0112	−0.041	0.008
	% immunoreactive airway epithelial area	<0.005	−4.75	0.885	−6.65	−2.86
	% immunoreactive cells/airways	0.025	−4.849	1.98	−9.001	−0.698
	NEB cell no./biopsy	0.011	−1.766	0.621	−3.075	−0.457
	Mean NEB area	0.005	−0.411	0.125	−0.676	−0.146

NEB, neuroepithelial body.

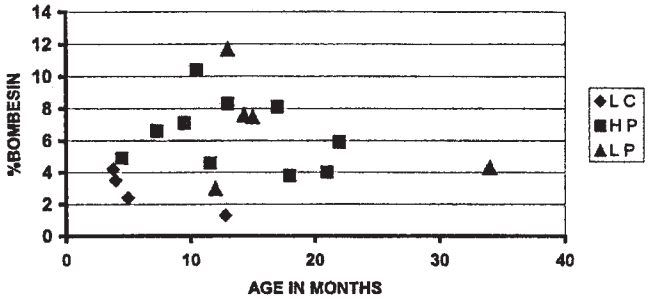

Fig. 3. Graph shows percent of bombesin immunopositive airway epithelial area for each individual patient by age in months at time of biopsy. Control patients from lower altitude (LC) and 15 NEHI patients are represented by different symbols, depending on whether they lived at lower altitude (LP) or higher altitude (HP).

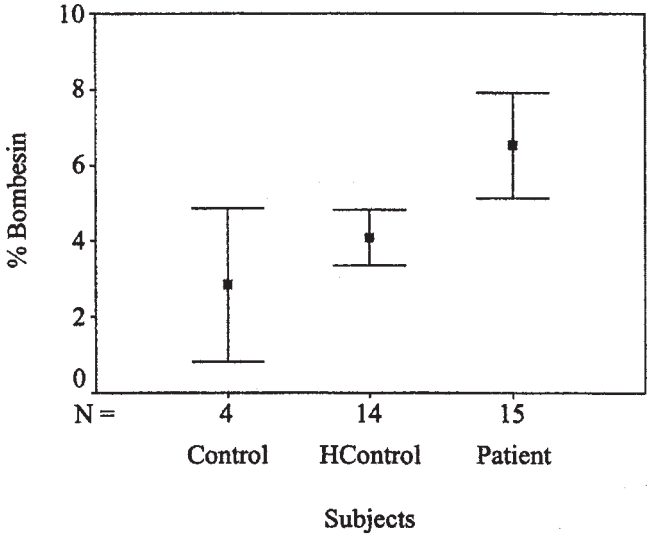

Fig. 4. Graph shows mean and 95% confidence intervals for percent of bombesin immunopositive airway epithelial area by subject group: study controls, historical controls (HControl), and NEHI patients. Study patients had significantly (*$P = 0.004$) higher percent bombesion immunopositive airway epithelial area when compared to Control and HControl groups. Control and HControl were not significantly different.

with the other antibodies studied, all of which showed infrequent staining.

Bombesin immunoreactive epithelial area related to total airway epithelial area (percent bombesin immunoreactivity) for study patients and control subjects was compared to age-matched historical autopsy controls. As age was not a significant predictor of percent bombesin ($P = 0.059$), all ages were pooled for each subject group. There was no significant difference between study biopsy controls (mean, 2.83%) and historical autopsy controls (mean, 3.90%). However, when comparing historical autopsy controls to study patients (disease subjects; mean, 6.52%), statistically significant differences ($P = 0.004$) were noted, as shown graphically in Figure 4.

Clinical Treatment and Outcome

Most study patients received a trial of bronchodilators and systemic glucocorticoids. A few were treated with azathioprine and hydroxychloroquine in an attempt to eliminate symptoms. In those who had evidence of GER, antireflex medication was prescribed. Although it was difficult to determine response to therapy retrospectively, most patients remained partially symptomatic despite the use of medications. In a few cases, physicians commented that systemic glucocorticoids seemed to improve, but not eliminate, hypoxia. All patients received oxygen to maintain adequate oxygen saturation, and most required oxygen for at least 2–3 years.

To date, no study patient is known to have died. Outcome data related to symptoms, medication, and oxygen use were available in 12 of 15 patients. At a mean age of 5 years (range, 3.6–7 years), 11 were off all oxygen. Most were able to discontinue oxygen use between 2–4 years of age, though two continued to require oxygen past age 5 years. Ten patients had occasional evidence of respiratory dysfunction, which included one or more of the following: mild exercise intolerance or intermittent tachypnea, wheezing, and crackles. Eight required intermittent bronchodilators or steroids to treat their symptoms. Lung function evaluated by spirometry after age 5 years was normal in 6 patients and mildly obstructive in one. Two patients with normal spirometry had assessment of lung volumes at 6 and 7 years of age, which showed marked increases in residual volumes.

DISCUSSION

Infants who present with persistent signs and symptoms of pILD pose formidable challenges for physicians and families. Our case series characterizes the clinical presentation, clinical course, and lung histology of a group of infants who presented with signs and symptoms of pILD from an early age. Many patients were treated aggressively with bronchodilators and glucocorticoids without resolution of their symptoms, in contrast to patients who might have asthma. Despite persistent symptoms and prolonged need for oxygen therapy, all patients showed slow improvement and no mortality. This is in contrast to the reported mortality rate of 21% in pILD.[11]

The classic diagnostic spectrum of ILD is vast and provides little information about the incidence and pathogenesis of disease, or the clinical course for most affected infants. To address these issues, reports such as ours have attempted to define distinct clinical disorders in

young children without other known cardiovascular, immunologic, or identified disease.[12] Though descriptive, this work was important for categorizing disease pathology and gaining an understanding of the clinical course. On the severe end, the clinical spectrum of pILD includes surfactant protein B (SPB) deficiencies[13] and some surfactant protein C (SPC) mutations,[14,15] the newly described ATP-binding cassette transporter A3 (ABCA3) mutations,[16] familial desquamative interstitial pneumonitis (DIP), and idiopathic pulmonary fibrosis of infancy,[17] which are associated with very high mortality. Of note, although idiopathic pulmonary fibrosis (IPF) of infancy was used in the literature to describe young children with pILD, the classic pathologic finding of fibroblastic foci in adults with IPF has not been seen in young children, making it difficult to interpret this diagnosis in infants.[18] Although somewhat less severe, chronic pneumonitis of infancy,[19] idiopathic pulmonary hemosiderosis in infancy, bronchiolitis obliterans (BO), and some forms of SPC mutations also have considerable morbidity and mortality. Fortunately, our patients do not fit the classic pathologic findings or common clinical courses described in these severe forms of pILD.

Descriptive reports were also published of infants with milder symptoms and clinical courses. Many of these entities have clinical findings that resemble our patients with NEHI. Most similar to our patient population were 8 infants with ''chronic idiopathic bronchiolitis of infancy.''[20] These infants were strikingly similar in clinical presentation, course, response to therapy, and outcome. Lung biopsies in children with chronic idiopathic bronchiolitis were described as either normal or as showing minimal lymphocytic bronchiolar infiltration, but lung tissue was not evaluated for PNEC hyperplasia. It is possible that NEHI and ''chronic idiopathic bronchiolitis of infancy'' constitute the same entity. Three other described entities, i.e., idiopathic follicular bronchitis (IFB),[21] cellular interstitial pneumonitis in infants,[22] and pulmonary interstitial glycogenosis (PIG),[7] also have clinical similarities to NEHI but different histological features. Although our patients had radiographic evidence of hyperinflation and biopsies with mild airway findings, NEHI is distinctly different from asthma. Unlike asthmatics, NEHI patients do not routinely wheeze or cough, and the process does not clear with bronchodilators or anti-inflammatory therapy. Additionally, biopsy findings are not like those seen in patients with asthma.

We recognize the limitations of the control samples used for morphometric analysis. It is not possible to obtain normal control lung tissue from healthy living infants or children, and infants with sudden infant death syndrome who were shown to have hyperplasia of bombesin immunoreactive pulmonary neuroendocrine cells and neuroepithelial bodies[9] predominate in the autopsy series of previously well infants. Therefore, our control samples were limited, but all were from living children with either pulmonary anomalies or cardiac disease, but without acute or chronic inflammation or reactive changes affecting the airways. Blocks from the most normal-appearing portions of biopsy/lobectomy specimens were used for analysis. Sections from infants with congenital lobar overinflation were not taken from the overinflated areas, but rather from the attached normal lingula. We believed that samples from living children would be the best controls for our patient data.

However, to address the limitation of our small control sample size, age-matched historical autopsy controls from previously published data[9] were compared to our study controls and patient data for percent of bombesin immunoreactive epithelial area. There were significant differences noted between historical controls and our patients, but no differences were noted between historical and study controls. Thus, historical control data further substantiated our morphometric findings.

It is unclear what the cause or effect of PNEC hyperplasia may be in any of the clinical conditions where it has been described. PNECs are present in greatest numbers at birth and then decrease markedly through the first year of life,[23,24] suggesting that they may play a role in lung development, and bombesin content peaks at midgestation and declines to adult levels by birth.[5] There are strong data to suggest that neuroepithelial bodies (NEBs) function as airway-oxygen sensing cells, where they respond to airway hypoxia. However, the function of NEBs in neonatal adaptation and chronic lung disease is unknown.[25,26] Related to lung disease, associations with PNEC hyperplasia were noted in children with Wilson-Mikity syndrome,[10] bronchopulmonary dysplasia (BPD),[27] sudden infant death syndrome (SIDS),[9] cystic fibrosis, mechanical ventilation,[28] and pulmonary emphysema in one child.[29] In adults, PNEC hyperplasia was associated with extreme elevations in altitude (Bolivan natives: 3,500–4,300 m,[30] susceptibility to smoking-related airway disease,[31] and an idiopathic disorder of PNECs.[6] In our patient population, the molecular mechanism for PNEC hyperplasia is unclear. NEHI patients had no definable acute lung injury or known lung diseases associated with PNEC hyperplasia; nor were they consistently exposed to known factors that cause PNEC hyperplasia.

Direct evidence does not exist to suggest that PNEC hyperplasia contributes to the pathogenesis of airway disease in NEHI. However, bombesin and other PNEC secretory products are known to effect epithelial differentiation, fibroblast proliferation, smooth muscle hypertrophy, and cytokine expression by alveolar macrophages.[31] Bombesin is also a potent bronchoconstrictor. Because of these effects, some postulate that PNECs could contribute to airway disease. The most compelling evidence for this was reported by Sunday et al.,

who hypothesized that bombesin-like peptide could mediate BPD.[32] Using a hyperoxic baboon model, these investigators found that elevated levels of urinary bombesin were closely correlated with the severity of BPD, and that the postnatal administration of an anti-bombesin antibody attenuated lung disease.[32] A primary role for PNECs was also proposed in adults with idiopathic diffuse hyperplasia of pulmonary neuroendrocrine cells and airway disease (IDHPNC).[6] Patients with this disorder have diffuse hyperplasia and dysplasia of PNECs, multiple carcinoid tumorlets, and peribronchiolar fibrosis obliterating small airways. As there were no other histopathologic lesions, minimal inflammation, and no obvious secondary process to stimulate PNEC hyperplasia in IDHPNC, these authors suggested that PNECs may contribute to airway fibrosis.

Although our patient population had good clinical outcomes and no deaths, we believe that NEHI may have clinical implications in the future. It is sobering that an adult form of ILD associated with PNEC hyperplasia (IDHPNC) exists. Adults with IDHPNC, who appear to have both a far greater degree of PNEC hyperplasia and airway fibrosis not seen in infants with NEHI, typically present later in life with a long-standing history of cough and exertional dyspnea.[6] We do not know if NEHI is an unrelated process or is an early manifestation of this adult disorder. A 6-year-old child with PNEC hyperplasia and hyperinflation[29] had similarities to NEHI, which suggests that PNEC hyperplasia can also be seen in symptomatic older children. As many of our patients continue to have minor respiratory symptoms, a continuum of clinical disease from infancy to adulthood may exist in some patients with PNEC hyperplasia. Obviously, long-term clinical follow-up is needed to address these issues.

In conclusion, we describe a condition in young children best defined by impressive symptoms of pILD and PNEC hyperplasia, yet relatively minor and non-specific findings on lung biopsy. As it remains difficult to classify pILD in infants solely on clinical presentation, lung biopsy provides a way to identify specific markers of disease and to classify different pILD disorders. We believe that the identification of PNEC hyperplasia in this clinical setting and in the absence of other known diseases may be a useful marker for categorizing this clinical entity that we have termed NEHI. There have been no deaths and mostly only mild, intermittent respiratory symptoms in NEHI patients who were followed for an average of 5 years. Despite this favorable short-term prognosis, long-term follow-up is required to determine if these patients are at risk for future pulmonary morbidity.

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