ORIGINAL ARTICLE

Persistent Tachypnea of Infancy
Usual and Aberrant

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Abstract

Rationale: Persistent tachypnea of infancy (PTI) is a specific clinical entity of undefined etiology comprising the two diseases neuroendocrine cell hyperplasia of infancy (NEHI) and pulmonary interstitial glycogenosis. The outcome of typical NEHI is favorable. The outcome may be different for patients without a typical NEHI presentation, and thus a lung biopsy to differentiate the diseases is indicated.

Objectives: To determine whether infants with the characteristic clinical presentation and computed tomographic (CT) imaging of NEHI (referred to as "usual PTI") have long-term outcome and biopsy findings similar to those of infants with an aberrant presentation and/or with additional localized minor CT findings (referred to as "aberrant PTI").

Methods: In a retrospective cohort study, 89 infants with PTI were diagnosed on the basis of clinical symptoms and, if available, CT scans and lung biopsies. Long-term outcome in childhood was measured on the basis of current status.

Measurements and Main Results: Infants with usual PTI had the same respiratory and overall outcomes during follow-up of up to 12 years (mean, 3.8 yr) as infants who had some additional localized minor findings (aberrant PTI) visualized on CT images. Both usual and aberrant PTI had a relatively favorable prognosis, with 50% of the subjects fully recovered by age 2.6 years. None of the infants died during the study period. This was independent of the presence or absence of histological examination.

Conclusions: PTI can be diagnosed on the basis of typical history taking, clinical findings, and a high-quality CT scan. Further diagnostic measures, including lung biopsies, may be limited to rare, complicated cases, reducing the need for an invasive and potentially harmful procedure.

Keywords: children's interstitial lung disease; diffuse parenchymal lung disease; infants; neuroendocrine cell hyperplasia of infancy; pulmonary interstitial glycogenosis

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*A complete list of members may be found before the beginning of the References.

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At a Glance Commentary

Scientific Knowledge on the Subject: Persistent tachypnea of infancy is a characteristic but frequently undiagnosed chronic lung disorder. A definitive diagnosis and precise categorization as either neuroendocrine cell hyperplasia of infancy or pulmonary interstitial glycogenosis can be achieved only by lung biopsy. There is uncertainty regarding the long-term outcome of a larger cohort of these conditions and about the necessity and value of a lung biopsy for their diagnosis.

What This Study Adds to the Field: Infants with persistent tachypnea and characteristic ground-glass opacities (usual) and with some additional local and minor findings (aberrant) visualized by computed tomography had the same favorable respiratory and overall outcomes. Lung biopsies may be useful only in rare, complicated cases.

Diffuse lung diseases in children comprise a heterogeneous group of rare pediatric disorders (1). The current categorization system lists four groups as more prevalent among infants than in other life stages: diffuse developmental disorders of the lungs, abnormalities of alveolar growth, disorders related to surfactant dysfunction, and a group called "specific conditions of undefined etiology" (2). The last group comprises two diseases: neuroendocrine cell hyperplasia of infancy (NEHI) (3) and pulmonary interstitial glycogenosis (PIG) (2).

NEHI presents in infants similarly to an interstitial lung disease, with symptoms of tachypnea, hypoxemia, crackles, retractions, and failure to thrive (3–9). Initially, the condition was labeled "persistent tachypnea of infancy" (PTI) (5) or "chronic idiopathic bronchiolitis of infancy" (9). The histology of NEHI is mostly normal or has minor abnormalities (8, 9). Because this condition is associated with increased numbers of neuroendocrine cells (NECs) (3), lung biopsies became an important diagnostic procedure. However, it soon became clear that hyperplasia of those cells was not a specific feature of NEHI (i.e., PTI), as this presentation has been described in a variety of other rare pediatric lung diseases (3, 10).

Within the first months of life, PIG presents with rapid and difficult breathing and hypoxemia (11). Histological examinations show oval, glycogen-rich mesenchymal cells expanding the interstitium. No fibrosis or inflammatory reaction is seen (11–20). Of interest, NECs are also hyperplastic (10).

The prognosis of infants categorized as having specific conditions of undefined etiology is commonly considered favorable, making it difficult to argue for open lung biopsy to confirm the diagnosis. Therefore, chest imaging has been of major interest for diagnosis because most patients with NEHI have typical computed tomographic (CT) findings with ground-glass opacities confined to the middle lobe, lingula, and paramediastinal lung areas. Some patients, however, have additional CT abnormalities, such as consolidation, bronchial wall thickening, and interlobular septal thickening. Sometimes ground-glass opacities are absent or have a different distribution (3, 6, 7, 21). With regard to biopsy-proven PIG, the few published CT studies have shown mainly ground-glass opacities in a subsegmental, segmental, or diffuse distribution (11, 15), very similar to what was found in NEHI (11): interlobar septal thickening or scarring, hyperinflation, air trapping, multifocal atelectasis (7), and multiple air-filled cystic changes of variable size (12).

Thus, relevant questions arise regarding whether infants with the characteristic presentation of PTI and atypical CT imaging have the same biopsy findings and, more important, have the same long-term outcomes as infants with PTI with typical CT imaging findings. If that is the case, lung biopsies will be needed only for severe cases with uncertain diagnosis, reducing the need for an invasive and potentially harmful procedure.

Methods

Study Cohort

The Kids Lung Register collects cases of rare pediatric lung diseases, with a focus on diffuse lung diseases (www.kids-lung-register.eu) (22). For all children included in the present study, underlying immune deficiency, cystic fibrosis, primary ciliary dyskinesia, infections, and congenital heart disease were ruled out. The diagnosis of diffuse parenchymal lung disease (DPLD) was made in accordance with the clinical guidelines of the American Thoracic Society (1). All patients included had clinical DPLD defined by (*1*) typical symptoms or signs such as tachypnea and/or dyspnea, crackles, retractions, digital clubbing, failure to thrive, or respiratory failure; (*2*) hypoxemia; and (*3*) diffuse radiological abnormalities present at diagnosis with a minimum duration of 4 weeks. The children were categorized into the distinct DPLD categories and subcategories (22). Between 2001 and 2015, 89 children were allocated to a group referred to as "infant persistent tachypnea of unknown etiology," which is the same as specific conditions of undefined etiology. Allocation was based on the presence of persistent tachypnea; CT changes with mainly ground-glass opacities and only minor other abnormalities; and exclusion of diffuse developmental disorders, deficient alveolarization, surfactant dysfunction disorders, or other conditions that occur in all age groups (see Methods section in the online supplement).

Retrospective information from patient files was collected on breathing frequency, crackles, retractions and/or dyspnea, failure to thrive, chest wall abnormalities such as pectus excavatum noted on CT scans, desaturation or respiratory insufficiency, and age at onset of symptoms. PTI was diagnosed if there was an increased respiratory rate at the onset of symptoms or at the time of diagnosis and documented in medical files during infancy on at least two additional occasions more than 3 weeks apart and persisting within that period based on the judgment of subjects' caregivers. Age-matched normal respiratory rates were taken from the percentile curves reported by Fleming and coworkers (23). The age at the end of tachypnea and the last follow-up status were obtained cross-sectionally in 2015 by structured telephone contact with caregivers of the children or with the children's treating physicians.

Radiological Characterization

Chest CT scans were systematically reevaluated using a scoring system based on the results of a previous CT study to allow comparisons with other studies using the same instruments (7). Ground-glass opacities were regarded as typical when

they were located in the middle lobe, the lingula, and the parahilar and paramediastinal distributions (i.e., centrally) (6). Ground-glass opacities in other distributions were considered atypical. Similarly, ground-glass opacities were judged as atypical when they were demonstrated on expiratory images only, irrespective of location. All additional findings on CT images, including bronchial wall thickening, architectural distortion, pleural effusion, interlobular thickening, focal consolidation (excluding dependent atelectasis), parenchymal cysts, bronchiectasis, and honeycombing, were considered atypical. Because of an insufficient number of expiratory scans (27 of 77 CT scans), air trapping could not be investigated systemically. All infants with PTI and typical CT findings were categorized as having usual PTI, and those with atypical CT findings were classified as having aberrant PTI.

Lung Biopsies and Immunohistochemistry

The presence of NECs was determined by immunohistochemistry for bombesin, chromogranin A, and synaptophysin in consecutive formalin-fixed, paraffin-embedded sections.

All tissue sections were counted, and quantification was performed with the observer blinded to case or control status and reviewed independently by another board-certified pathologist (S.R.). For each tissue section, the number of bronchioles, the number of immunopositive NECs, and the number of bronchiolar epithelial cells were counted manually. The number of immunostained NECs was expressed as a percentage of the total epithelial cells of the bronchiole. The number of bronchioles with immunostained NECs was expressed as a percentage and total number. All bronchioles with at least 10% NECs were counted and expressed as a total number.

Statistical Analysis

Patient and control groups for histology were compared using two-way analysis of variance with the Bonferroni *post hoc* test. Clinical parameters and staining results were compared using Kruskal–Wallis analysis of variance with Dunn's *post hoc* test or Fisher's exact test. Survival analyses were performed using a log-rank (Mantel–Cox) test. For all analyses, $P \leqslant 0.05$ was considered statistically significant.

Ethics Statement

All participants or their parents provided written informed consent to participate in the Kids Lung Register consultation and diagnosis program. The Ethics Commission at the Ludwig Maximilians University of Munich approved the study (EK 026-06, 257-10, 111-13).

Results

Study Population

Between 2001 and 2015, 1,105 children with suspected DPLD were included in the Kids Lung Register. Among them, 89 were categorized as having specific conditions of undefined etiology or infant persistent tachypnea of unknown etiology (Figure 1). Chest CT scans obtained at diagnosis were retrieved for 80 of these infants with chronic tachypnea at the onset of their pulmonary symptoms. The infants were grouped as having usual PTI based on the presence of ground-glass opacities in typical locations (middle lobe, lingula, paramediastinal, and/or parahilar) or as having no other abnormalities (usual PTI) or aberrant PTI if ground-glass opacities were present in atypical locations and/or if additional abnormalities were observed (aberrant PTI) (Figure 1). Lung biopsies with immunohistochemical staining were performed in 20 of the subjects with usual PTI and 13 of the subjects with aberrant PTI (Figure 1).

Clinical Characteristics

Infants with usual PTI were older at the onset of any symptoms than infants with aberrant PTI (3.5 mo vs. 1.3 mo) ($P < 0.001$) (Table 1, Figure 2A). Most of the subjects in both groups presented at symptom onset with retractions, hypoxemia, and a need for oxygen (Table 1; *see also* Figure E1 in the online supplement). A few subjects presented with chest wall abnormalities such as pectus excavatum. Crackles occurred more often in infants in the usual PTI group than in infants in the aberrant PTI group ($P < 0.01$). Also, more subjects with usual PTI had failure to thrive ($P < 0.05$).

During the disease course, about 10% of infants in both groups were exposed to passive smoking. Asthma was diagnosed in 6% of the usual PTI group and 20% of the aberrant PTI group, but this association was not significant. About one-third of the subjects presented with other nonrespiratory diseases (Table 1). Additionally, half of the subjects in both groups had a history of upper and lower respiratory tract infections and pneumonia.

The mean ages of the subjects at last follow-up were 4.2 years in the usual PTI group and 3.2 years in the aberrant PTI category. The oldest age at last follow-up was 10.5 years in the usual PTI category and 12.0 years in the aberrant PTI group. More than 80% of the children in both groups were healthy or had symptoms that had weakened in intensity and/or number ("sick—better") at last follow-up, and only two patients with usual PTI and six patients with aberrant PTI still had the same symptoms with the same intensity and number ("sick—same") at their last follow-up. None of the children had a higher number of symptoms and/or more intense symptoms ("sick—worse"), and none had died (Table 1). Most of the children had improved or become asymptomatic ("healthy"); in both of these groups, 50% had become healthy by age 2.6 years (Figures 2B and 2C). There were no differences in the duration of oxygen therapy or the flow of oxygen delivered (Table 1, Figure E1).

Considering the tachypnea symptom alone, 34.8% of the patients with usual PTI and 53.6% of the patients with aberrant PTI were still tachypneic at last follow-up (Table 1). The time course of normalization of respiratory rate was initially the same, as 50% of all subjects had overcome their tachypnea by age 2.6 years (Figure 2D). However, tachypnea tended to persist longer in subjects with aberrant PTI ($P = 0.179$). The mean ages at the end of tachypnea were 2.4 years in the usual PTI group and 3.7 years in the aberrant PTI group (not significant) (Table 1).

Many but not all of the respiratory rates measured and documented by physicians at the onset of disease were above the 99th percentile. During observation, respiratory rates decreased but in many cases were above the 50th percentile (*see* Figure E2).

Radiological Characteristics

Among 80 subjects, according to their CT scans, 50 had usual PTI and the other 30 were classified as having aberrant PTI. Of the 30 subjects with aberrant PTI, 14 had ground-glass opacities in other locations and/or other significant abnormalities, such as focal consolidation, parenchymal cysts,

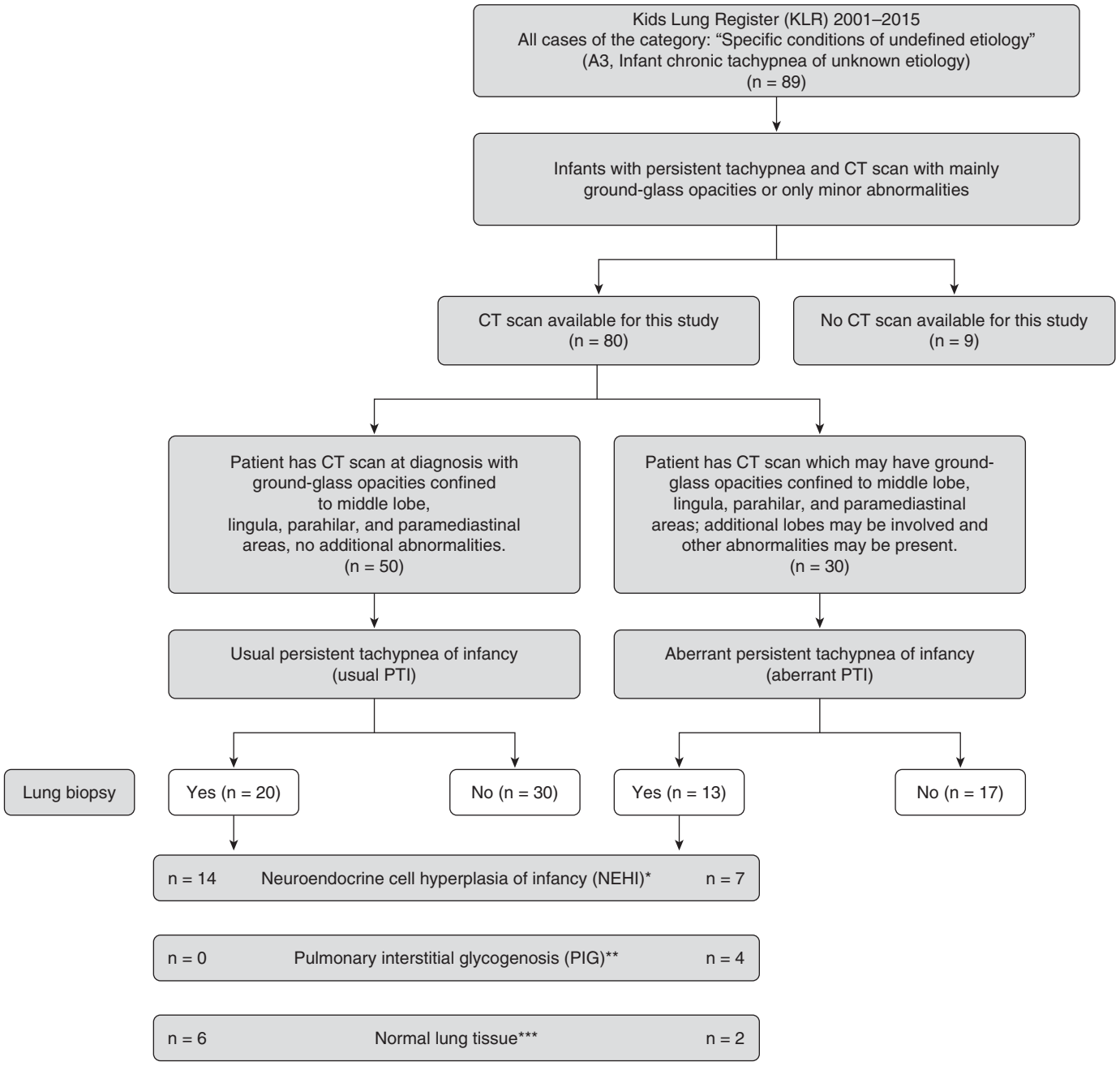

Figure 1. Subject allocation flow. *Neuroendocrine cell hyperplasia of infancy is hyperplasia of neuroendocrine cells (NECs) in lung tissue, defined as immunopositive NECs in more than 70% of bronchioles and at least 10% NECs in at least one bronchiole. **Pulmonary interstitial glycogenosis is periodic acid–Schiff stain–positive glycogen with thickening of alveolar septa. ***Normal lung tissue is no hyperplasia of NECs in lung tissue. CT = computed tomography; PTI = persistent tachypnea of infancy.

pleural effusion, architectural distortion, interlobular septal thickening, and bronchial wall thickening (Table 2). Ground-glass opacities were seen on expiratory images only, in two subjects. After separating the two PTI groups into those with and those without biopsies, no differences in CT findings were identified.

Histopathology

Not considering special stains for NECs, we observed that lung biopsy samples were normal or had only minor abnormalities (*see* Table E1). We found that 4 of 20 samples from subjects with usual PTI and 1 of 13 samples from those with aberrant PTI looked completely normal. There were no differences between groups. In a few samples, discrete chronic bronchiolitis or lymphocytic inflammation of airways was noted. Some subjects had mild to moderate alveolar septal thickening or intraalveolar macrophage accumulation. In four subjects, the most prominent findings were round, glycogen-rich mesenchymal cells that

Table 1. Clinical Characterization and Outcomes of Children with Persistent Tachypnea of Infancy

	Usual PTI			Aberrant PTI		
	All (*n* = 50)	With Biopsy (*n* = 20)	Without Biopsy (*n* = 30)	All (*n* = 30)	With Biopsy (*n* = 13)	Without Biopsy (*n* = 17)
At onset of any symptoms						
Age, mo	3.5 (0–20)*	3.9 (0–20)	3.3 (0–11)	1.3 (0–10)*	0.4 (0–3)	1.9 (0–10)
Tachypnea	50 (100)	20 (100)	30 (100)	30 (100)	13 (100)	17 (100)
Crackles	43 (86.0)†	18 (90.0)	25 (83.3)	16 (53.3)†	7 (53.8)	9 (52.9)
Retractions	41 (82.0)	14 (70.0)	27 (90.0)	26 (86.7)	11 (84.6)	15 (88.2)
Failure to thrive	31 (65.9)‡	14 (73.7)	17 (58.6)	10 (41.7)‡	6 (75.0)	4 (25.0)
Chest wall abnormalities	11 (22.0)	6 (30.0)	5 (16.7)	3 (10.0)	2 (15.4)	1 (5.9)
Hypoxemia, desaturation (respiratory insufficiency)	44 (88.0)	16 (80.0)	28 (93.3)	22 (73.3)	11 (84.6)	11 (64.7)
Oxygen supplementation	39 (78.0)	14 (70.0)	25 (83.3)	21 (70.0)	11 (84.6)	10 (58.8)
Fever	7 (14.0)	3 (15.0)	4 (13.3)	1 (3.3)	0 (0)	1 (5.8)
Preterm birth	1 (2.8)	0 (0)	1 (4.0)	4 (15.4)	2 (22.2)	2 (11.8)
At biopsy						
Age, yr	1.2 (0.3–4.4)	1.2 (0.3–4.4)	—	1.1 (0.2–4.3)	1.1 (0.2–4.3)	—
At end of tachypnea						
Age, yr	2.4 (0.7–4.6)	2.7 (0.7–4.6)	2.2 (0.8–3.2)	3.7 (0.3–10.6)	4.5 (2.0–8.2)	3.0 (0.3–10.6)
Until last follow-up						
Passive smoking	2 (10.5)	0 (0)	2 (20.0)	1 (10.0)	0 (0)	1 (14.3)
Lowest oxygen saturation, %	87.4 (74.0–100.0)	87.8 (80.0–100.0)	87.2 (74.0–97.0)	87.5 (69.0–100.0)	83.9 (70.0–93.0)	90.1 (69.0–100.0)
Duration of oxygen therapy, yr	1.5 (0–5.3)	1.8 (0–5.3)	1.3 (0–4.8)	1.0 (0–3.7)	1.4 (0–3.7)	0.7 (0–2.6)
Nasal oxygen flow, L/min	0.8 (0.3–2.0)	0.7 (0.3–1.1)	0.8 (0.4–2.0)	1.4 (0.1–2.3)	1.4 (0.7–2.3)	1.5 (0.1–2.3)
Physician-diagnosed asthma	3 (6.0)	0 (0)	3 (10.0)	6 (20.0)	2 (15.4)	4 (23.5)
Other nonrespiratory diseases	15 (30.0)	8 (40.0)§	7 (23.3)§	16 (43.3)	8 (61.5)§	5 (29.4)§
Upper respiratory tract infections	24 (48.0)	7 (35.0)	17 (56.7)	12 (42.9)	4 (36.4)	8 (47.1)
Lower respiratory tract infections	34 (68.0)	14 (70.0)	20 (66.7)	15 (53.6)	7 (63.6)	8 (47.1)
History of pneumonia	23 (46.0)	10 (50.0)	13 (43.3)	12 (42.9)	5 (45.5)	7 (41.2)
At last follow-up						
Age, yr	4.2 (0.7–10.5)	4.5 (0.8–10.5)	4.0 (0.7–8.3)	3.2 (0.3–12.0)	3.6 (0.3–12.0)	2.9 (0.3–11.3)
Health status						
Healthy	25 (50.0)	11 (55.0)	14 (46.7)	11 (36.7)	2 (15.4)	8 (47.1)
Sick—better‖	23 (46.0)	8 (40.0)	15 (50.0)	13 (43.3)	8 (61.5)	6 (35.3)
Sick—same¶	2 (4.0)	1 (5.0)	1 (3.3)	6 (20.0)	3 (23.1)	3 (17.6)
Physician-reported tachypnea	16 (34.8)	5 (29.4)	11 (37.9)	15 (53.6)	7 (63.6)	8 (47.1)
Failure to thrive	3 (6.1)	0 (0)	3 (10.3)	3 (10.7)	2 (18.1)	1 (5.9)

Definition of abbreviation: PTI = persistent tachypnea of infancy.

Data are presented as mean (range) or number of cases (%). Statistical comparisons were made between usual PTI, all, and aberrant PTI, all. Other parameters were not significantly different between usual PTI, all, and aberrant PTI, all. No differences with or without biopsy within a PTI group were found (Fisher's exact test, Mann–Whitney *U* test).

*$P < 0.001$ (Mann–Whitney *U* test).

†$P < 0.01$ (Fisher's exact test).

‡$P < 0.05$ (Fisher's exact test).

§Gastroesophageal reflux disease, Beckwith-Wiedemann syndrome, muscular hypotonia, developmental disorder, myocardiopathy, iron deficiency anemia, strabismus, Langerhans cell histiocytosis, hearing impairment, hypermobility syndrome, atopic dermatitis, tubular proteinuria, inguinal hernia, Hunter syndrome, ventricular septal defect, hypoxic ischemic encephalopathy, autism, Gilbert–Meulengracht syndrome, hydronephrosis, pharyngomalacia, multicystic dysplastic kidney.

‖"Sick—better" is defined as signs and symptoms of the disease having weakened in intensity and/or number.

¶"Sick—same" is defined as same signs and symptoms with same intensity and number.

widened the interstitial walls, which was confirmed by periodic acid–Schiff stain and provided the basis for the diagnosis of PIG.

Immunohistochemistry

In the control group with healthy tissue, the maximum frequency distribution of NECs and/or bronchiolar cells (expressed as a percentage) was in the range of 1.00–2.99% based on bombesin staining, whereas the maximum for chromogranin and synaptophysin staining was in the range of 0.00–0.99% (Figures 3A–3C; *see also* Table E2). NEHI was previously defined by immunopositive NECs in more than 70% of all bronchioles and at least 10% NECs in at least one bronchiole (8). The first part of this definition was not very specific in our hands, as in the healthy control group bombesin and chromogranin stains showed more than 70% immunopositive NECs (*see* Figure E3D), demonstrating significant overlap. The second part was suitable in our cohort, as none of the control subjects had bronchioles with at least 10% NECs of total bronchiolar cells (*see* Figure E3C).

Among the 20 subjects with usual PTI, 14 had bronchioles with at least 10% NECs among total bronchiolar cells and were confirmed as having a histological NEHI,

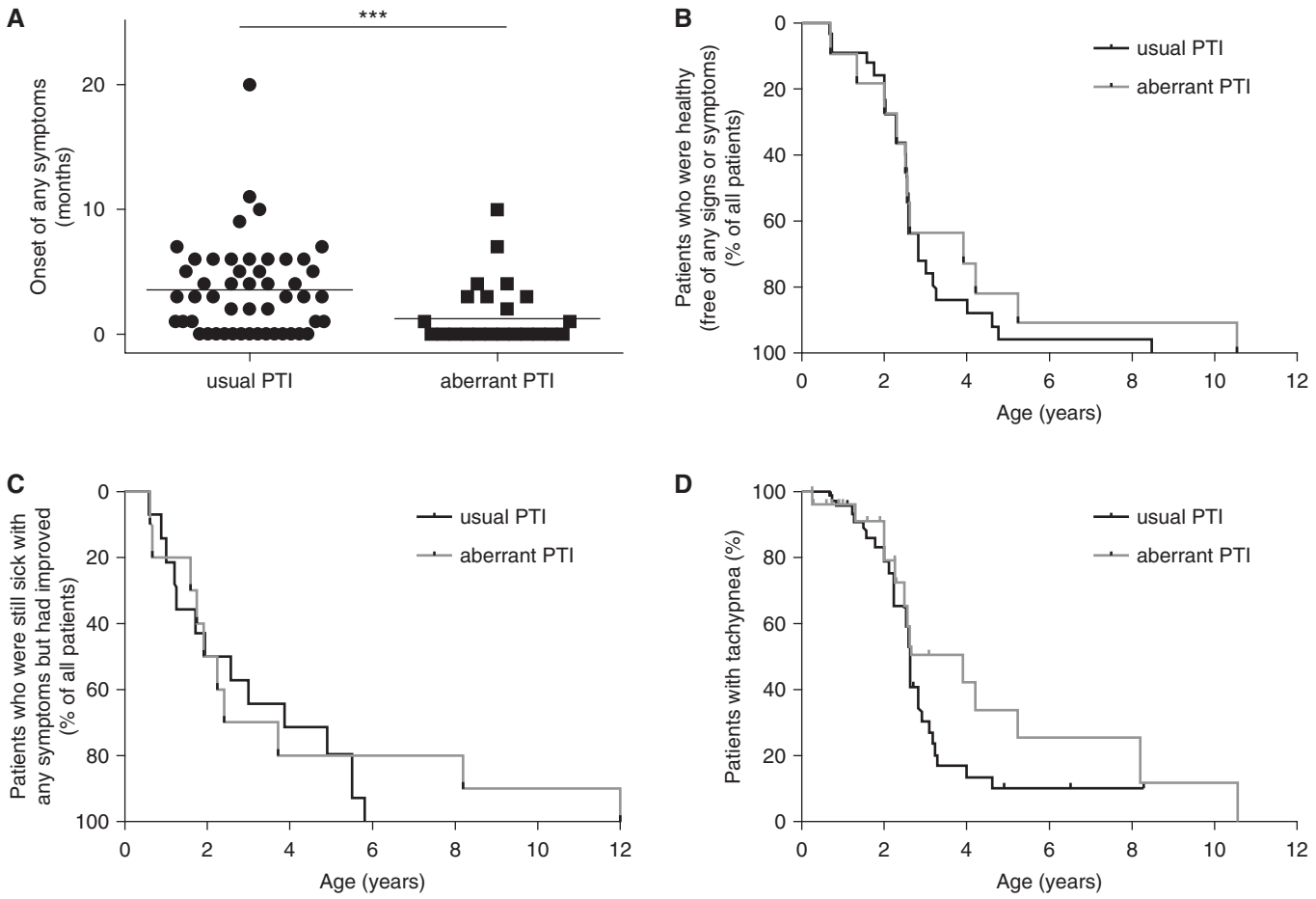

Figure 2. Onset of symptoms and tachypnea and disease status over time. (*A*) Differences between the groups were calculated by Mann–Whitney *U* test. Significant differences are indicated by *horizontal bars* (***$P < 0.001$). (*B*) Differences between the groups were calculated by log-rank (Mantel–Cox) test. No significant differences were observed between usual persistent tachypnea of infancy (PTI) and aberrant PTI. (*C*) Differences between the groups were calculated by log-rank (Mantel–Cox) test. No significant differences were observed between usual PTI and aberrant PTI. (*D*) Differences between the groups were calculated by log-rank (Mantel–Cox) test. No significant differences were observed between usual PTI and aberrant PTI.

whereas the other 6 subjects were not (Figure 1).

Among the 13 subjects with aberrant PTI, 11 had NEC hyperplasia and 2 did not. Four of the subjects with NEC hyperplasia also had thickening of alveolar septa and increased periodic acid–Schiff–positive mesenchymal cells and were consequently characterized as having PIG (Figure 1).

Only in bombesin staining was the number of bronchioles with more than 10% NECs among total bronchiolar cells significantly higher in the usual PTI group ($P < 0.01$) and the aberrant PTI group ($P < 0.001$) (*see* Figure E3B and Table E2). There was no significant difference between the control group and the patient groups in chromogranin or synaptophysin staining.

The mean number of NECs among bronchiolar cells (as a percentage) and the mean percentage of bronchioles with at least 10% NECs among bronchiolar cells were highest on bombesin stains, and bombesin was superior to chromogranin and synaptophysin staining. No significant differences were observed between the usual PTI and aberrant PTI groups in any staining (*see* Figure E3 and Table E2).

Bombesin in Urine

The urine concentration of bombesin and the ratio of bombesin to creatinine did not differ between control subjects, subjects with usual PTI, and subjects with aberrant PTI (*see* Figure E4 and Table E3).

Discussion

Our longitudinal study shows that respiratory and overall outcomes of more than 80 infants with PTI were not dependent on CT imaging or lung biopsy findings. Importantly, outcomes were rather favorable, improving over time, and no deaths were observed, confirming a previous small set of long-term data (4).

Two strengths of the study were the detailed clinical characterization of the infants and the correlation to the other variables. Comprehensive analysis of the CT scans clearly showed two types of findings. On the one hand, CT scans with ground-glass opacities were located only in the middle lobe, the lingula, and the parahilar and paramediastinal distributions. This pattern was described earlier as being typical of NEHI (7) and in our study was defined as infants with usual PTI. On the other hand, infants were classified as having aberrant PTI when the ground-glass opacities were

Table 2. Chest CT Findings in Study Groups

Group	Number of Subjects	Age at CT (*mo*)	Normal Chest CT	Ggo in ML, Lingula, Parahilar, and Paramediastinal Distribution	Ggo in Other Distribution	Ggo in NEHI Typical Location Only on Expiratory Images*	Focal Consolidation	Parenchymal Cysts	Pleural Effusion	Architectural Distortion	Interlobular Septal Thickening	Bronchial Wall Thickening†
Usual PTI												
All	50	10.2 ± 0.8‡	0 (0)	50 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
With biopsy	20	8.3 ± 1.1	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Without biopsy	30	11.5 ± 1.1	0 (0)	30 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Aberrant PTI												
All	30	7.0 ± 1.4‡	0 (0)	9 (30.0)	14 (46.7)	2 (22.2)	17 (56.7)	5 (16.7)	1 (3.3)	2 (6.7)	7 (23.3)	3 (10.0)
With biopsy	13	6.7 ± 7.8	0 (0)	5 (38.5)	6 (46.2)	1 (16.7)	10 (76.9)	1 (7.7)	1 (7.7)	0 (0)	2 (15.4)	0 (0)
Without biopsy	17	7.3 ± 1.8	0 (0)	4 (23.5)	8 (47.1)	1 (33.3)	7 (41.2)	4 (23.5)	0 (0)	2 (11.8)	5 (29.4)	3 (17.6)

Definition of abbreviations: CT = computed tomography; Ggo = ground-glass opacities; ML = middle lobe; NEHI = neuroendocrine cell hyperplasia of infancy; PTI = persistent tachypnea of infancy.

Data are means ± SEM or number of cases (%). Statistical comparisons were between usual PTI (all) and aberrant PTI (all).

*Expiratory images were available for only 27 subjects.

†Other findings not typically associated with NEHI and not present in our cohort were bronchiectasis and honeycombing.

‡Significant difference ($P < 0.01$) (Mann–Whitney *U* test). No differences between corresponding variables in the subgroups with and without biopsy were found (Mann–Whitney *U* test).

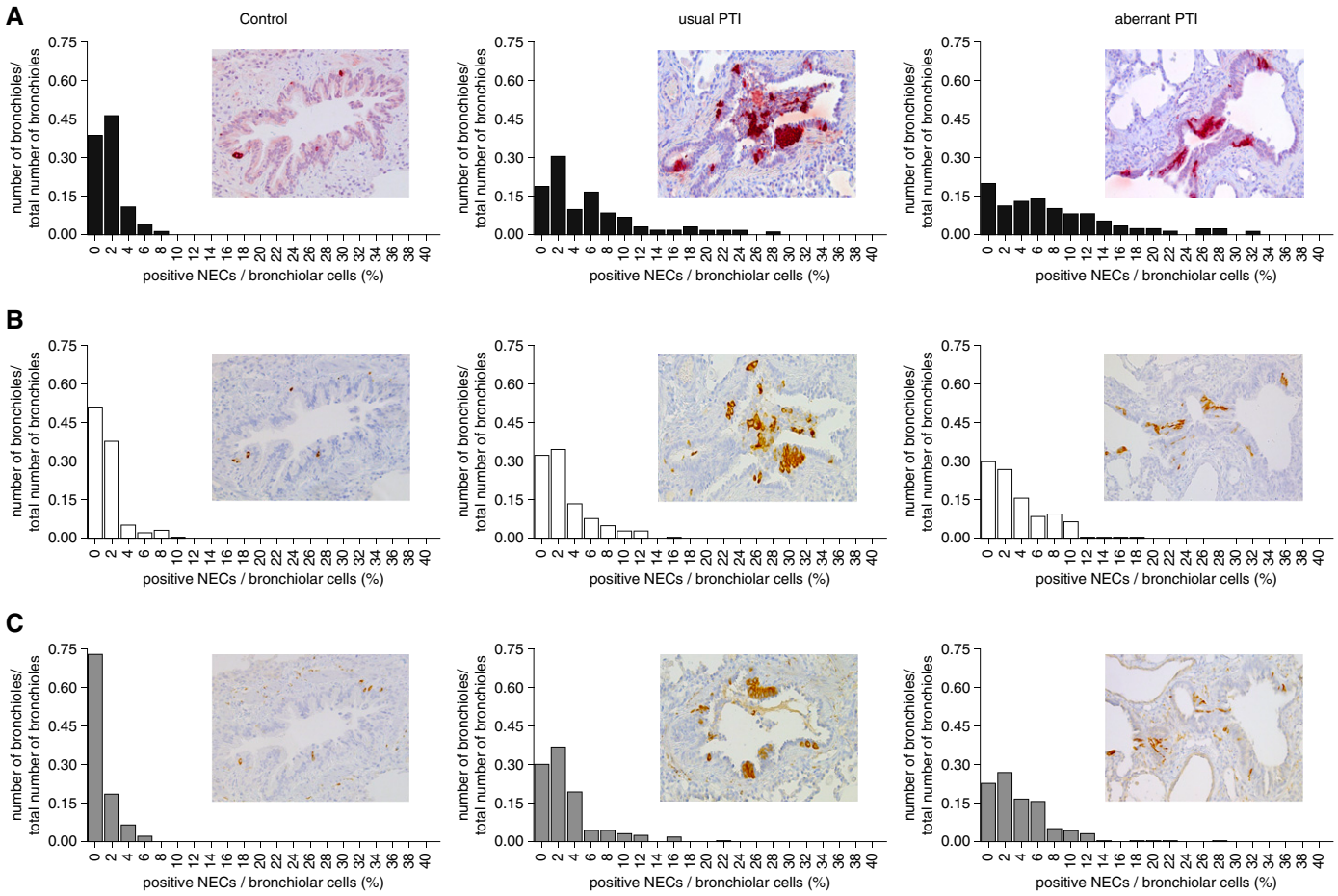

Figure 3. Frequency distribution of neuroendocrine cells (NECs) as a percentage of total bronchiolar cells in the three study groups (columns) for the three different stains (rows) applied to consecutive slides. Each bin represents the number of values within a certain range, whereby each bin is labeled with the midpoint of the bin (e.g., bin 2 = 1.0–2.99). (*A*) Bombesin stain. (*B*) Chromogranin stain. (*C*) Synaptophysin stain. PTI = persistent tachypnea of infancy.

in nontypical locations and/or when additional abnormalities or, rarely, no abnormalities were detected.

Whereas the usual and aberrant groups were similar in most clinical signs and symptoms, and in particular their resolution over time, three important differences were noted. First, infants with aberrant PTI had an earlier onset of symptoms. This was supported but not explained by the infants with PIG, all of whom were allocated to the aberrant PTI group on the basis of both CT findings and histology. All four patients with PIG were symptomatic in the first few weeks of life and underwent lung biopsies at 2 or 3 months of age. After omitting the PIG cases from the calculations, the onset of symptoms was still earlier in the aberrant PTI group ($P < 0.0025$). Second, infants with aberrant PTI less frequently had crackles, a characteristic sign commonly associated with pneumonia and interstitial lung disease. Again, in all four subjects with PIG, no crackles were ever auscultated. Of interest, more than 86% of the infants with usual PTI had crackles at diagnosis in addition to tachypnea, and the triad of hypoxemia, crackles, and tachypnea was most characteristic of usual PTI. Third, failure to thrive at diagnosis was less common in subjects with aberrant PTI. This may be explained in part by a significantly shorter period available for deviation from the growth chart until symptoms were noted.

Clearly, CT imaging of high quality is an important tool in the diagnostic workup of PTI. It is of interest to note that the CT findings leading to the aberrant PTI classification were not diffuse or small in extent and may be secondary to previous infections or sometimes atelectasis due to the anesthesiology or sedative procedure. Thus, CT scans with patterns deviating from those reported here should point to a need for further investigations.

Another strength of this study is the systematic analysis of the lung biopsies. In comparing the results of three different immunostaining methods for NECs, we found that bombesin was the most informative (Table 3). It showed the greatest number of immunopositive cells and was superior to chromogranin and synaptophysin staining. Only bombesin staining was significantly different between the control group and the patient groups in regard to the mean percentage of bronchioles with at least 10% NECs among bronchiolar cells. Consistent with the publication by Young and coworkers, the control and patient groups showed immunopositive NECs in more than 70% of all bronchioles, suggesting that this criterion may not be specific for the diagnosis of NEHI (8).

Our data showed no clinical differences between two groups of children, one with and one without biopsies. This

Table 3. Comparison of Positive Staining for NEC Hyperplasia

	NEC Hyperplasia	No NEC Hyperplasia
Bombesin, n	18	3
Usual	11	2
Aberrant	7 (3 with PIG)	1
Chromogranin, n	8	11*
Usual	5	6
Aberrant	3	5 (3 with PIG)
Synaptophysin, n	7	14*
Usual	3	10
Aberrant	4 (1 with PIG)	4 (2 with PIG)

Definition of abbreviations: NEC = neuroendocrine cell; PIG = pulmonary interstitial glycogenosis. "NEC hyperplasia" was defined as present when at least 70% of the bronchioles were positive with NECs and when at least one bronchiole was present with at least 10% NECs.
*$P = 0.0014$ by Fisher's exact test. In two subjects, chromogranin stains were missing.

suggests random sampling of biopsied cases and a homogeneous underlying pathophysiology in sampled and nonsampled cases. For example, biopsies were done not just in the more severely ill patients but also depended on the diagnostic approach of the treating center. This may compensate for a weakness of our study (i.e., that we did not have biopsies for all infants investigated). Of importance, both groups of infants presented with the same histopathological pattern, and we did not find an association between the frequencies of CT abnormalities and abnormal histopathological findings (Fisher's exact test; data not shown). This result was expected based on the scattered distribution of the additional minor CT findings, and it was consistent with the study by Young and colleagues, who demonstrated no relationship between the number of NECs and the radiographic appearance of the region from which the biopsy specimen was taken (8).

NEC hyperplasia was found in patients with usual PTI, aberrant PTI, and PIG. Such NEC hyperplasia is well known in many other disorders among children with interstitial lung disease (10). Thus, we can conclude that lung biopsy does not appear to be helpful in the diagnosis of PTI when there is no clinical need to differentiate the condition further into NEHI or PIG. On average, the clinical courses were indistinguishable. However, rarely, PIG can present as very severe respiratory failure with pulmonary hypertension and can be lethal in individual cases (16, 24). Thus, the defining diagnosis for our series may encompass less severe PIG cases clinically indistinguishable from PTI, a potential selection bias that should be considered. Symptoms since birth, very severe course, or CT findings not fitting usual PTI are red flags and indicate a need for closer analysis, including biopsy in individual cases.

Bombesin level in urine did not differ between control subjects and patients and was not helpful for diagnosis. This also suggests that NECs are unlikely to play a pathogenic role; however, additional studies are required to analyze more markers.

Although our study was not focused on treatment, we made some important observations in this context. At presentation, up to 90% of the included infants had partial respiratory failure with many desaturations during a day and transcutaneous oxygen saturation values as low as 69%. Therefore, many infants received oxygen supplementation. Age at onset of oxygen supplementation was somewhat earlier in aberrant PTI ($P = 0.054$) (Figure E1A), which is in agreement with an earlier start of lung symptoms in this group. However, flow of oxygen needed and duration of oxygen therapy were not different. Oxygen therapy corrected hypoxemia and, more important, decreased rates of failure to thrive. Clinical data showed that both groups of children had a rather favorable prognosis. Half of the subjects had fully recovered by age 2.6 years, and no deaths have been reported so far.

On the basis of the data presented, we suggest the following diagnostic algorithm. The diagnosis of PTI can be made by typical history taking, clinical findings, and a high-quality CT scan. Red flags that trigger further diagnostic measures include a very early onset (age younger than 4 wk), CT pattern deviating from the findings reported here for usual or aberrant PTI, failure to thrive despite oxygen therapy, additional signs or symptoms in other organ systems, a family history of interstitial lung disease, and a deteriorating clinical course other than respiratory tract infections during long-term follow-up. In the absence of these red flags, biopsy, lavage, genetic testing, or other specialized tests are not likely to be helpful. ■

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