Received: 16 March 2020 | Accepted: 16 June 2020

DOI: 10.1002/ppul.24910

PEDIATRIC PULMONOLOGY WILEY

Variation in the bombesin staining of pulmonary neuroendocrine cells in pediatric pulmonary disorders—A useful marker for airway maturity

Nagehan Emiralioğlu MD[1] | Diclehan Orhan MD[2] | Güzin Cinel MD[3] | Gökçen Dilşa Tuğcu MD[3] | Ebru Yalçın MD[1] | Deniz Doğru MD[1] | Uğur Özçelik MD[1] | Matthias Griese MD[4] | Nural Kiper MD[1]

[1]Department of Pediatric Pulmonology, Hacettepe University Faculty of Medicine, Ankara, Turkey

[2]Department of Pediatric Pathology, Hacettepe University Faculty of Medicine, Ankara, Turkey

[3]Department of Pediatric Pulmonology, Yıldırım Beyazıt University, Ankara City Hospital, Ankara, Turkey

[4]Division of Pediatric Pneumology, University Hospital Munich & German Center for Lung Research (DZL), Dr. von Hauner Children's Hospital, Munich, Germany

Correspondence
Nagehan Emiralioğlu, MD, Department of Pediatric Pulmonology, Hacettepe University Faculty of Medicine, Ihsan Dogramaci Children's Hospital, Sıhhiye 06230, Ankara, Turkey.
Email: drnagehan@yahoo.com

Abstract

Objectives: Pulmonary neuroendocrine cells (NEC) increase with age due to pulmonary maturity. The aim of this study was to determine whether open lung biopsies from patients with interstitial lung diseases have increased pulmonary NEC compared with neuroendocrine cell hyperplasia of infancy (NEHI). Our second aim was to assess pulmonary NECs in the lung autopsy of children without lung disease who died from different causes.

Methods: Lung tissue of 5 infants with NEHI; 21 patients with pediatric interstitial lung disease (chILD); 17 lung autopsies of infants at varying age without lung disease were included. The percentage of the airways containing neuroendocrine cells, the average percentage of neuroendocrine cells (NECs) per airway, and the number of neuroendocrine bodies (NEBs) in each case were analyzed.

Results: The mean percentage of the airways containing neuroendocrine cells were 95% in the NEHI group, 30% in the chILD group, 89% under Intrauterine 37 weeks, 70% between intrauterine 37 to 40 weeks, 52% at postnatal 4 days to 6 months of autopsy ages. In the *NEHI group*, diffuse NE cell distribution and large NEBs were noticed in the lung biopsy. In the *chILD group*, neuroendocrine cells were dispersed, did not form clusters and NE cells showed solitary distribution. In the *lung autopsy group*, linear NE cells were detected at younger aged fetuses and solitary distribution of NE cells was detected with the older increasing age.

Conclusions: Our findings confirm that NECs are seen in many other childhood interstitial lung diseases; NE cell hyperplasia may be a marker of decreased pulmonary development and NE cells decrease with the increasing age of the fetus during Intrauterine life.

KEYWORDS

bombesin staining, interstitial lung disease, lung biopsy, neuroendocrine cell

1 | INTRODUCTION

Pulmonary neuroendocrine cells (PNECs) are epithelial cells and they are involved in the differentiation and maturation of the fetal lung and lung regeneration. Single PNECs first appear at about 8 to 9 weeks of gestation, with peak numbers including neuroepithelial bodies (NEBs) at about midgestation (19 weeks to about 23 weeks). After mid-gestation, PNECs normally decline relative to other cells as they are diluted by the more rapid expansion of other epithelial cells.[1-3] They produce vasoactive substances (bombesin-like peptide, calcitonin, and serotonin) that can cause bronchoconstriction, epithelial differentiation, and proliferation of mesenchymal cells.[4,5] Pulmonary neuroendocrine cells produce gastrin-releasing peptide (GRP), a mammalian homolog of amphibian bombesin. Gastrin releasing peptide and bombesin is referred to as bombesin like peptides; because of the same physiologic effects at mammalian bombesin/GRP-receptors. Bombesin like peptides appear to play a role in oxygen sensing and they promote growth and maturation of developing fetal lung in humans. Furthermore, PNEC degranulation and bombesin like peptide secretion are known to be induced by hypoxia, which is also associated with increased reactive oxygen species in postnatal lung diseases.[2,3] Number of PNECs continues to decline in the first year of life.[6-8]

Children´s interstitial lung diseases (ILD) comprise a large group of different entities. Several histopattern of interstitial pneumonitis can be differentiated when lung biopsies are assessed.[9,10] In contrast, neuroendocrine cell hyperplasia of infancy (NEHI) is an entity of unknown etiology, clinically presenting with persistent tachypnea, crackles, and sometimes failure to thrive, which is part of the spectrum of ILD in children.[11,12] The diagnosis is made histologically with hyperplasia of neuroendocrine cells of the distal airway epithelium in otherwise normal lung tissue and this finding is confirmed with bombesin antibody staining.[4,13] The presence of PNECs is not specific for NEHI diagnosis. Hyperplasia of pulmonary NEC has also been reported in some pediatric pulmonary diseases with parenchymal involvement including bronchopulmonary dysplasia (BPD), airway injury, pulmonary hypertension.[8] Morphometric quantitation of the increased number of pulmonary neuroendocrine cells and neuroendocrine bodies can be used to distinguish NEHI from other diseases when lung biopsy was performed. Interstitial pneumonia other than NEHI have also associated with hyperplasia of pulmonary NECs and variably increased levels of bombesin like peptides and bombesin staining in the lower respiratory tract.[12,14,15]

In this study, we had two hypotheses; the first one was the confirmation that pulmonary NEC hyperplasia is not only related to NEHI, increased pulmonary NECs may also be obtained in the ILD subgroups. The second hypothesis was an association of more immature lung with more pulmonary NEC hyperplasia. Thus we determined whether open lung biopsies from patients with interstitial lung diseases have increased pulmonary NEC, in comparison to children with NEHI and secondly we assessed pulmonary NECs in the lung autopsy of children without lung disease who died from different causes.

2 | METHODS

This study was conducted as a retrospective case-control study within the lung biopsy of patients aged 0 to 18 years with ILD and NEHI. From our pathology database, all cases between September 1993 and November 2015 were retrieved and reviewed again if they were diagnosed with any of the following histopathologic entities: Nonspecific interstitial pneumonia (NSIP), lymphoid interstitial pneumonia (LIP), bronchiolitis obliterans organizing pneumonia (BOOP), usual interstitial pneumonia (UIP), chronic pneumonitis of infancy (CPI). We obtained lung biopsy before starting of any drug treatment. For comparison, lung tissues from children with autopsies who died without lung disease and had no history of mechanical ventilation were also assessed. The cause of death in the autopsy group and clinical characteristics are given in the supplementary table.

Histopathology of all lung tissue samples was assessed on haematoxylin and eosin (H&E) stained slides and for the presence of neuroendocrine cells (NE) cells by immunohistochemical staining for bombesin.

We made the histologic diagnosis of NEHI when all of the following criteria were fulfilled: (a) neuroendocrine cells in at least 75% of total airway profiles, (b) neuroendocrine cells representing at least 10% of epithelial cells in individual airway profiles, (c) large and/or numerous neuroepithelial bodies, and (d) absence of other significant airway or interstitial disease.[4,16]

Immunohistochemistry for bombesin was performed on formalin-fixed paraffin sections to identify NE cells. Polyclonal rabbit anti-bombesin antibody (ABA) (gastrin-releasing peptide antibody; Dako, Glostrup, Denmark), was undertaken using a PT Link Dako Autostainer Plus (envision FLEX system). Before immunohistochemistry staining, paraffin-embedded tissue sections were not subjected to enzymes or heat-mediated antigen retrieval (HMAR). Two sections were taken for immunohistochemistry staining—one was stained with the ABA, and the other was stained with diluent only and was used as a negative control.

In each case all bronchioles were counted on the available sections of the lung, using microscopy of sections stained with H&E and bombesin immunostaining. An experienced pediatric pathologist, blinded to cases and controls, counted the percentage of the airways containing neuroendocrine cells, the number of all respiratory epithelial cells staining with bombesin in the distal airways, expressed as a percentage of all respiratory epithelial cells in each distal airway. Then we calculated the average percentage of NECs per airway and the number of neuroendocrine bodies (NEBs) in each case.

The present study was approved by the local institutional review board and supported in part by the FP7-project 305653-chILD-EU.

2.1 | Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 23.0 (SPSS Inc., Chicago, IL).

Continuous variables were presented as a mean ± standard deviation (SD) for normal distribution or median (min-max) for nonnormal distribution.

3 | RESULTS

There were five infants diagnosed with NEHI, 21 patients diagnosed with different histopathological subgroups of pediatric ILD (LIP, BOOP, NSIP, CPI, UIP) other than NEHI and 17 lung autopsies of infants at varying gestational age without any lung disease in this cohort. Table 1 shows the subgroups of patients in interstitial pneumonia, NEHI, and lung autopsy group.

In all cases increased NE cells were demonstrated within airways, however with different numbers and distribution in the tissue.

3.1 | Neuroendocrine cell hyperplasia of infancy—NEHI group

The mean percentage of the airways containing neuroendocrine cells was 95%. Diffuse NE cell distribution and large NEBs were noticed in the lung biopsy of patients with NEHI (Figure 1A,B). Neuroendocrine bodies per airway were detected with 5.8%; percentage higher than in the other two groups.

3.2 | Interstitial pneumonia group

The median age of the patients at diagnosis of ILD was 7.8 years (6 months-14.5 years). We found that the mean percentage of the airways containing neuroendocrine cells was 30% in the patients with ILD. Neuroendocrine cells were dispersed and did not form clusters in patients with interstitial pneumonia. This group also showed solitary NE cell figuration. Neuroendocrine body per airway was detected with 2.9% in the patient group with interstitial pneumonia.

3.3 | Autopsy group

The median death age of the patients with lung biopsies was Intrauterine (IU) 37 weeks (range IU 20 weeks-6 months). Autopsy lung tissues had significantly higher percentages of NE cells than interstitial pneumonia. Lung autopsies were evaluated according to different developmental lung ages. The mean percentage of the airways with NECs was 89% under 37 weeks, 70% between 37 to 40 weeks, and 52% postnatal 4 days-6 months of autopsy age. The neuroendocrine body was mostly seen at a younger age of patients. The solitary distribution of NE cells was detected with increasing age in lung autopsies. Linear NE cell staining was detected at a younger age. Neuroendocrine body per airway was detected with 2.9% under 37 weeks, 1.6% between 37 to 40 weeks, and 0.05% postnatal 4 days-6 months of autopsy age (Figure 1C-F). The mean percentage of the airways with NECs and neuroendocrine bodies per airway increased with the younger age of autopsies (Table 2).

Figure 2 summarizes the comparison of the percentage of airways with pulmonary neuroendocrine cells and the percentage of neuroendocrine bodies per airway in each group.

4 | DISCUSSION

Neuroendocrine cells are characteristically found during normal human lung development and it was suggested that they induce proliferation of airway epithelial and mesenchymal cells with the differentiation of alveolar type II cells.[17] The percentage of NEC declines with age increasing pulmonary maturity. Immunohistochemistry for bombesin is a good marker of NECs in the airways and it is suggested to be a key

TABLE 1 Demographic characteristics of patients in the interstitial pneumonia group, neuroendocrine cell hyperplasia group, and lung autopsy group

	n	%	Median age, y	Min-max, y
Interstitial pneumonia group (Histopathology of lung biopsy subgroups)				
Lymphoid interstitial pneumonia (LIP)	2	9.5	11.5	9-14
Bronchiolitis obliterans organizing pneumonia (BOOP)	3	14.2	12	8-15
Nonspecific interstitial pneumonia (NSIP)	10	47.5	10.5	1-13
Usual interstitial pneumonia (UIP)	4	19	13.5	12-14
Chronic pneumonitis of infancy (CPI)	2	9.5	2	1-3
Neuroendocrine cell hyperplasia group	5	100	3	1-4
Lung autopsy group				
Intrauterine 20-36 wk	8	47	35 wk	20-36 wk
Intrauterine 37-40 wk	6	35.3	38 wk	37-40 wk
Postnatal 4 d-6 mo	3	17.7	3.5 mo	4 d-6 mo

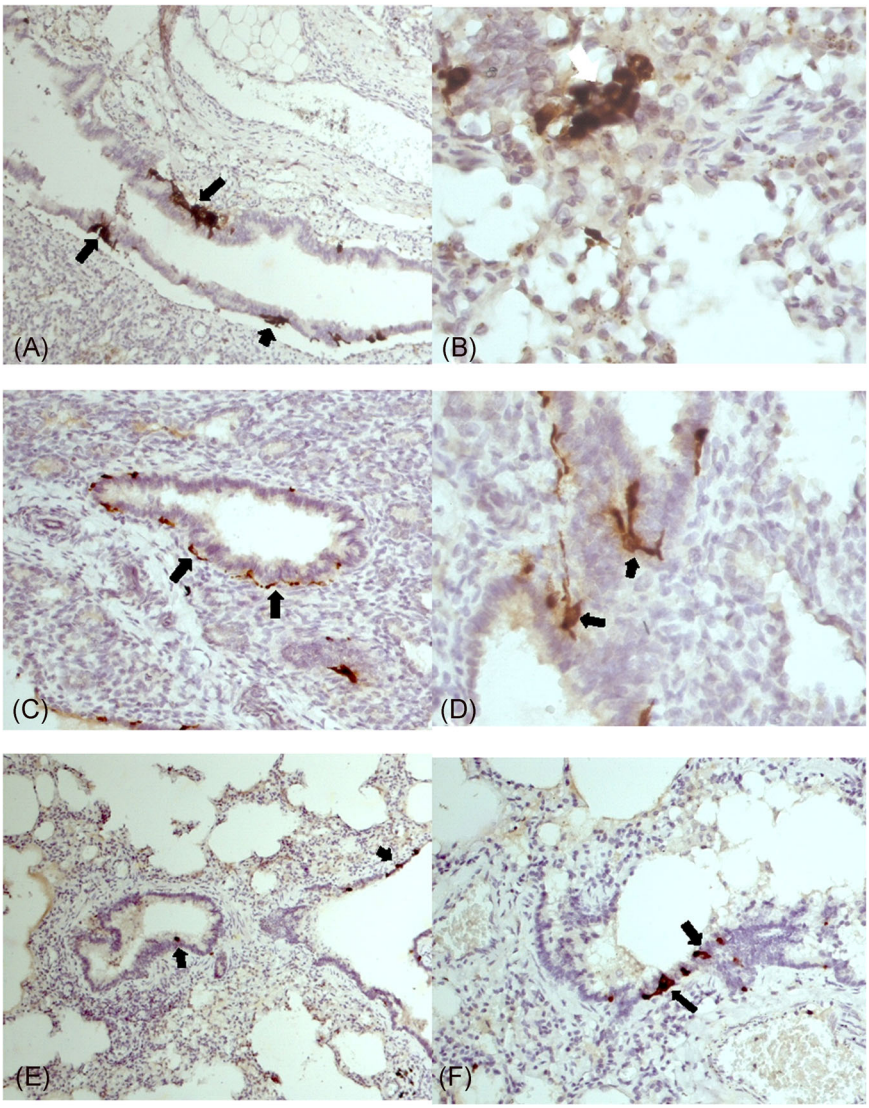

FIGURE 1 A-B, Lung biopsy findings of patients with NEHI. Diffuse neuroendocrine cell distribution (black arrow) (bombesin, ×25) with large neuroepithelial bodies (white arrow) (bombesin, ×50). C-D, Lung autopsy findings of the patient at the age of 20 weeks. Linear neuroendocrine cell distribution (black arrow) (bombesin, ×50). E-F, Lung autopsy findings of the patient at age of 3.5 months. Decreased dispersed neuroendocrine cell distribution with solitary scattered cells (black arrow). (bombesin, ×25, 50). NEHI, neuroendocrine cell hyperplasia of infancy [Color figure can be viewed at wileyonlinelibrary.com]

diagnostic factor for pulmonary NE cell hyperplasia.[5] Here, we report that NECs are also seen in many other childhood ILDs and their marked increased numbers in autopsy samples especially at a younger age during intrauterine period. These findings showed us the relationship between NECs and pulmonary development.

Pulmonary NECs are granulated epithelial cells in the conducting airways and sometimes forming small clusters in the lobular parenchyma, i.e. the neuroepithelial bodies (NEB).[8,17] In our cohort we also evaluated the distribution of NECs and NEB. NEHI had characteristic features with a diffuse neuroendocrine cell distribution and large neuroepithelial bodies. Of interest, in the other ILD subgroups, NECs were dispersed and did not form clusters.[18,19] In autopsies, samples from younger aged fetuses more NEB and a linear neuroendocrine cell distribution demonstrated less mature airways. The solitary distribution of NECs was detected with the older age at lung autopsy. Pulmonary NECs were widely present during fetal life, a time when NECs are thought to play a role in regulating of lung development.

Yancheva et al reported the percentage of NE cells in 73 biopsies from children with a wide range of chILD. There were seven cases of NEHI, with expected increased NE cells and percentages of airways with NEHI cells. Bombesin positive cells were also seen in follicular bronchiolitis and inherited surfactant dysfunction disorders to a similar extent, whereas they are less common in nonspecific interstitial pneumonia (NSIP), children with pulmonary infections and vascular disease presenting as chILD.[12,20] The average percentage of NEC levels in NEHI reported were 6.5% by Deterding et al[4] 5.4% by Young et al[13] and 6.6% by Yancheva et al[12] The average percentage of NEC levels in our NEHI cohort was similar to that in recent reports (5.8%).

Our study differs from others as we evaluated pulmonary NECS in lung autopsies at different ages. In our cohort; lung autopsy of the fetus during the intrauterine period showed that the number of NECs decrease with the age, independent of the underlying nonpulmonary disease. We identified a two to threefold difference in the average percentage of NECs per airway at

TABLE 2 Presence of neuroendocrine cells (NECs) in the lung autopsy of patients

Age	Percentage of airways with NECs (%)	Average percentage of NECs/ airway (%)	Neuroendocrine body/airway (%)
Age at autopsy (age of death)			
IU 20 wk	98	5.3	5.0
IU 33 wk	95	4.35	4.0
IU 34 wk	90	3.2	2.1
IU 35 wk	90	3.9	3.7
IU 35 wk	85	3.2	3.4
IU 36 wk	89	2.8	2.1
IU 36 wk	83	2.9	1.8
IU 36 wk	82	2.3	1.7
IU 37 wk	80	2.3	1.2
IU 38 wk	85	2.4	2.6
IU 38 wk	94.8	3.08	3.0
IU 38 wk	91	2.7	1.6
IU 39 wk	40	1.1	1.3
IU 40 wk	32	1.0	0.03
Postnatal 4 d	60	1.8	0.04
Postnatal 3.5 mo	52	1.4	0.07
Postnatal 6 mo	45	1.4	0.06

Abbreviation: IU, intrauterine.

IU 20 weeks compared to postnatal 3.5 months. Mean percentage of airways with NECs was 89% under 37 weeks, 70% between 37-40 weeks and 52% over 40 weeks; these findings were similar to those reported by Yancheva et al[12] and Young et al[13] Together, we suggest that NEC hyperplasia may represent an adaptive response to hypoxia or lung injury.[21] Recent data suggest that NEBs function as airway-oxygen sensing cells and respond to airway hypoxia.[21,22] The difference of neuroendocrine cell distribution, percentage of NECs and NEBs within the intrauterine lung autopsies in our cohort may also be related to the relationship of immature lung development. Intrauterine vascular adaptation which develops with the maturity of the lung may be the reason for decreased pulmonary NECs in the older autopsy age groups.

We hypothesize that in patients with persistent tachypnea of infancy the percentage of neuroendocrine cells, their distribution, and NEBs may vary depending on their stage of the clinical disease. Due to the continuous improvement of signs and symptoms in the subjects, it is not justified to perform repetitive lung biopsies and thus we cannot easily test this hypothesis.

Previous reports showed increased NECs in BPD.[2,23] Infants who died of acute respiratory distress syndrome (1-7 days of life) had markedly decreased numbers of positive bombesin immunoreactive neuroendocrine cells compared to controls, whereas infants labeled as BPD (2 weeks-6 months of life) had marked increase in the number of bombesin immunoreactive cells.[23] We suggest also considering the natural and gradual decline of PNECs with age throughout the first month. In another report, Ashour et al[2] observed increased numbers of NECs in infants dying with BPD. The authors suggested increased reactive oxygen species, lung injury, and overproduction of bombesin like peptides by PNECs, to trigger the cascade leading to arrested alveolarization and induce interstitial fibrosis in BPD. The main limitations of our study are its retrospective nature and the small number of cases diagnosed with NEHI and ILD.

In conclusion, our findings support the notion that NE cell hyperplasia may be a marker of immature lung development, consistent with decreased NE cells with increasing fetal age during the intrauterine period.

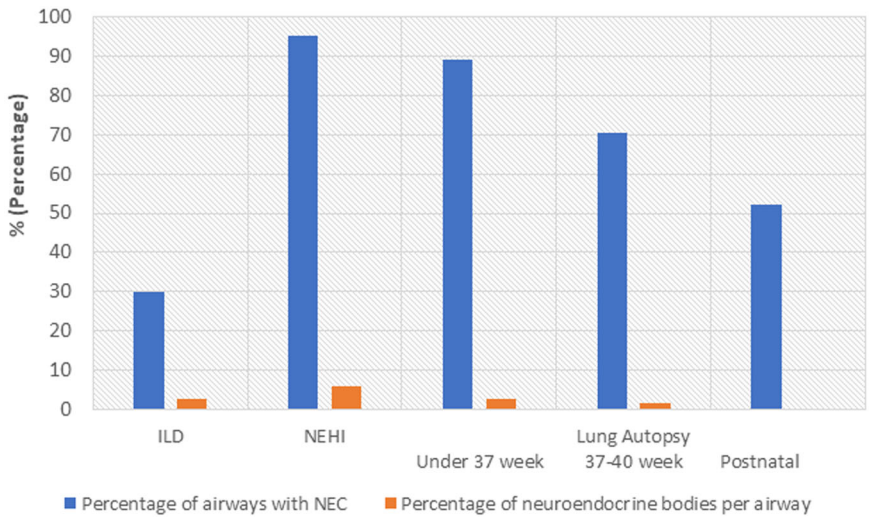

FIGURE 2 Percentage of airways with pulmonary neuroendocrine cells and neuroendocrine bodies per airway in NEHI, ILD and lung autopsies of infants at varying gestational age. ILD, interstitial lung diseases; NEHI, neuroendocrine cell hyperplasia of infancy [Color figure can be viewed at wileyonlinelibrary.com]

ACKNOWLEDGMENT

This project was funded by FP7-HEALTH-2012-INNOVATION-1, chILD-EU-Orphans Unite: chILD better together-European Management Platform for Childhood Interstitial Lung Diseases (Project number: 305653).

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ORCID

Nagehan Emralioğlu http://orcid.org/0000-0002-1405-8401
Diclehan Orhan http://orcid.org/0000-0003-3637-5392
Güzin Cinel http://orcid.org/0000-0002-6209-196X
Gökçen Dilşa Tuğcu http://orcid.org/0000-0002-9804-1200
Ebru Yalçın http://orcid.org/0000-0002-7325-1981
Deniz Doğru http://orcid.org/0000-0001-9931-9473
Uğur Özçelik http://orcid.org/0000-0003-1587-5216
Matthias Griese http://orcid.org/0000-0003-0113-912X
Nural Kiper http://orcid.org/0000-0003-1261-7393

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Emralioğlu N, Orhan D, Cinel G, et al. Variation in the bombesin staining of pulmonary neuroendocrine cells in pediatric pulmonary disorders—A useful marker for airway maturity. *Pediatric Pulmonology*. 2020;55:2383–2388. https://doi.org/10.1002/ppul.24910