ANATOMICAL PATHOLOGY

Neuroendocrine cell proliferations in lungs explanted for fibrotic interstitial lung disease and emphysema

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Summary

Using recently proposed pathological criteria, we determined the incidence of neuroendocrine cell proliferation in a series of explants with lung disease. Cases were defined as NECH (≥ 3 bronchioles with ≥ 5 endocrine cells), borderline diffuse neuroendocrine cell hyperplasia (DPNECH) (1–3 tumourlets with or without NECH), and DPNECH (≥ 3 tumourlets with NECH). Endocrine cells were identified by immunohistochemical staining for synaptophysin. There were 65 explants with interstitial lung disease (57 with non-sarcoid fibrotic lung disease, 8 with sarcoidosis), and 21 with centrilobular emphysema. Over one-third of all explant cases demonstrated histological criteria for NECH. There were three cases of DPNECH in the non-sarcoid fibrotic lung disease group (5%) and 20 cases of NECH (35%). The emphysema group had one case of DPNECH (5%), two cases of borderline DPNECH (10%), and seven cases with NECH (33%). The sarcoidosis group had two cases of DPNECH (25%) and three cases of NECH (38%). NECH is common in interstitial lung disease and emphysema. These results suggest that fibrotic lung disease is a predisposing factor for neuroendocrine cell proliferation, in addition to the known risk of epithelial neoplasms.

Key words: Neuroendocrine cell hyperplasia; NECH; fibrotic lung disease; sarcoidosis; lung transplantation.

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INTRODUCTION

Neuroendocrine cell proliferations of the lung range from neuroendocrine cell hyperplasia (NECH) to malignant neoplasms, namely carcinoids, small cell carcinoma, and large cell carcinoma. The mechanism for the development of well-differentiated lung neuroendocrine tumours—low-grade (typical carcinoid) and intermediate-grade (atypical) carcinoids—is still not well understood. While the majority of cases arise *de novo*, a percentage develop in patients with diffuse idiopathic neuroendocrine cell hyperplasia (DIPNECH), a recently classified preneoplastic lesion in the 2015 World Health Organization (WHO) classification of lung tumours.^{1,2}

Standardised clinical or pathological criteria for the diagnosis of DIPNECH and non-idiopathic neuroendocrine cell

hyperplasia (NECH) have yet to be established. WHO defines DIPNECH as ‘a generalized proliferation of scattered single cells, small nodules (neuroendocrine bodies) or linear proliferations of pulmonary neuroendocrine cells (PNCs) that may be confined to the bronchial and bronchiolar epithelium, include local extraluminal proliferation in the form of tumourlets, or extend to the development of carcinoid tumors’.² However, no quantitative criteria, such as the minimum number of PNCs to indicate a ‘proliferation’ or the number of involved airways for a ‘generalised’ process, are provided. It is also unclear under what circumstances the presence of NECH should be classified as idiopathic versus secondary.

This can pose a diagnostic problem for pathologists as NECH and carcinoid tumourlets are not uncommon in lung surgical specimens.^{3,4} NECH has primarily been described as case reports in association with emphysema and idiopathic pulmonary fibrosis.^{5,6} Most studies have emphasised the presence of NECH in patients with carcinoid tumours and have found symptoms of DIPNECH to be rare, although 10% have multiple tumourlets.⁷ The incidence of NECH in emphysema or idiopathic pulmonary fibrosis is unknown.

Definitions for NECH and DIPNECH have recently been proposed by Marchevsky *et al.*⁸ Using the proposed definitions, we sought to determine the incidence of neuroendocrine cell proliferations in chronic lung disease requiring transplantation.

MATERIALS AND METHODS

We performed a retrospective review on consecutive lung specimens explanted for fibrotic lung disease and emphysema between December 2013 and June 2016. Haematoxylin and eosin slides of each case were reviewed by a pathologist, and two representative blocks with the greatest potential for pulmonary neuroendocrine cell proliferations were selected for synaptophysin staining. Immunohistochemical detection of synaptophysin (27G12) was performed on 4 μ m sections of formalin fixed, paraffin embedded (FFPE) tissue mouse monoclonal predilute antibody from Leica (Germany). Staining was done on the Leica Bond III automated immunohistochemistry slide stainer using online heat induced epitope retrieval method in ER2 buffer (Leica) for 20 min at pH 9.0. The staining was visualised using the Bond Polymer Refine Detection kit (Leica). The slides were subsequently counterstained with haematoxylin. Appropriate positive and negative controls were included in each staining run.

Cases were defined as proposed by Marchevsky *et al.* as NECH (≥ 3 bronchioles with ≥ 5 endocrine cells), borderline diffuse neuroendocrine cell hyperplasia (DPNECH) (1–3 tumourlets without NECH or 1–2 tumourlets with NECH), and DPNECH (≥ 3 tumourlets with NECH). The synaptophysin stained slides were reviewed by two pathologists, and consensus was

achieved in all cases. We also looked for obliterative bronchiolitis, described by Aguayo as neuroendocrine cell clusters obliterating the walls of terminal bronchioles and protruding into the lumen of associated air spaces.⁹

RESULTS

There was a total of 86 explants, 57 with non-sarcoid fibrotic lung disease, eight with sarcoidosis, and 21 with centrilobular emphysema. The majority (54/57) of non-sarcoid fibrotic lung disease specimens were explants for usual interstitial pneumonia (UIP), with two non-specific interstitial pneumonia cases, and one with end-stage diffuse alveolar damage.

In the non-sarcoid fibrotic lung disease cohort, there were 40 men and 17 women, with a mean age at transplant of 60 years. The sarcoidosis cohort had three men and five women, and a mean age at transplant of 57 years. The emphysema cohort had nine men and 12 women and a mean age at transplant of 62 years (Table 1).

Over one-third (35%) of all explant cases had NECH. In the non-sarcoid fibrotic lung disease group, there were three cases of DPNECH (5%), as well as 20 cases of NECH (35%), and no cases of borderline DPNECH. The emphysema group had one case of DPNECH (5%), two cases of borderline DPNECH (10%), and seven cases with NECH (33%). The sarcoidosis group had two cases of DPNECH (25%) and three cases with NECH (38%), and no cases of borderline DPNECH (Table 2).

One UIP case with DPNECH consisted of a total of 26 tumourlets and 50 bronchioles with NECH (Fig. 1). No significant differences were present among the disease groups, although DPNECH was seen only in the UIP cases of the non-sarcoid fibrotic lung disease cohort. There were no cases of DPNECH in the non-UIP, non-sarcoid fibrotic lung disease cases. No cases had any evidence of obliterative bronchiolitis.

DISCUSSION

Since being described in the first case series by Aguayo *et al.* in 1992,⁹ interest and awareness of DIPNECH and the spectrum of neuroendocrine cell disease has increased. There is evidence to suggest that DIPNECH may be under recognised, with more cases possibly coming to light in present day due to increased use of high resolution imaging and biopsy.¹⁰

Marchevsky *et al.* recently proposed using at least five pulmonary neuroendocrine cells in a minimum of three separate bronchioles associated with three or more carcinoid tumourlets as the minimal criteria for a histological diagnosis of DIPNECH. In their study, they evaluated consecutive lung biopsy and resection specimens diagnosed with multifocal neuroendocrine cell proliferations rather than consecutive lung specimens resected for any reason. They reported 70 (9.7%) cases were diagnosed with multifocal neuroendocrine cell proliferation out of 720 lung specimens with carcinoid

tumours or tumourlets. Of those 70 cases, 30 (43%) demonstrated DIPNECH by their criteria.⁸

The results of our study would suggest that NECH is common in fibrotic lung disease and emphysema. The incidence of NECH has been reported to be as high as 76% in patients undergoing resection for carcinoid tumours, though the results may have been biased due to the association between NECH and neuroendocrine tumours.⁴ It is possible that the relatively high percentage of cases classified as NECH in our study is due to the proposed criteria being too inclusive, and further studies of lung specimens with interstitial lung disease and emphysema are needed to verify the frequency of neuroendocrine cell proliferations.

Of note, we did not identify the histological presence of obliterative bronchiolitis, which also was not included as part of the diagnostic criteria proposed by Marchevsky. Obliterative bronchiolitis has been described as a characteristic finding of DIPNECH in several reports, including the first case series by Aguayo *et al.*⁹ However, only approximately 30% of patients with pulmonary function test data have shown obstructive changes, and none of the cases in the study by Marchevsky *et al.* exhibited obliterative bronchiolitis.^{8,11} Furthermore, in our study of lung cases with known interstitial disease, the finding of obliterative bronchiolitis would not be unexpected.

There have been limited studies examining the frequency of normal pulmonary neuroendocrine cells and pulmonary neuroendocrine cell proliferations in normal lung. Weichselbaum *et al.* demonstrated densities of 65–250 cells per mm² on individual whole mounts of bronchial epithelium.¹² A study by Gosney *et al.* reported approximately six neuroendocrine cells per 10 cm of airway epithelial length by neurone specific enolase immunoreactivity in autopsy lung specimens.¹³ Mean density of pulmonary neuroendocrine cells for individual bronchi have remained difficult to characterise due to the heterogeneous distribution of the solitary cells. Difficulty in obtaining biopsy or resection tissue from healthy, adult, non-smoker lungs poses a problem as well.

To our knowledge, this is the first series utilising neuroendocrine immunostains to determine the frequency of pulmonary neuroendocrine cell proliferations in lung explants as a primary or secondary finding. Descriptions of DIPNECH in the context of lung transplantation have been limited to a few case reports as a potential treatment in patients with deteriorating pulmonary function.^{14,15}

NECH arising in interstitial lung disease has primarily been reported in the paediatric population, and is part of the diagnostic criteria for neuroendocrine cell hyperplasia of infancy (NECHI).^{16–21} In adults, a study by Wilson *et al.* compared lungs with interstitial pneumonitis at necropsy, approximately half of which demonstrated frank honeycombing, to controls without pulmonary disease. Pulmonary neuroendocrine cells were found to be decreased in early fibrosis, and absent in areas of honeycombing, despite the fact that all cases contained numerous strips of well preserved epithelium.²² A study by Gubbelmans *et al.* found no difference in the number of neuroendocrine cells per 10 cm of epithelium between control and anthrasicotic lungs by chromogranin A immunoreactivity.²³ A similar study by Gosney *et al.* also found no significant difference by both protein gene produce 9.5 and chromogranin A, though 10% less cells were identified by chromogranin A staining. The

Table 1 Sex distribution and mean age for patients by underlying disease

Disease process	Male:Female	Mean age $\pm$ SD
Fibrotic lung disease (excluding sarcoid)	40:17	60 $\pm$ 12
Emphysema	9:12	62 $\pm$ 6
Sarcoidosis	3:5	57 $\pm$ 7

Table 2 Neuroendocrine proliferation in lung transplant recipients based on underlying disease

Disease process	<i>n</i>	<i>n</i> with NECH	<i>n</i> with borderline DPNECH	<i>n</i> with DPNECH
Fibrotic lung disease (excluding sarcoid)	57	20 (35%)	0 (0%)	3 (5%)
Emphysema	21	7 (33%)	2 (10%)	1 (5%)
Sarcoidosis	8	3 (38%)	0 (0%)	2 (25%)
Totals	86	30 (35%)	2 (2%)	6 (7%)

Fig. 1 Index case of DPNECH in a patient with idiopathic pulmonary fibrosis. (A) A carcinoid tumour was identified on H&E and (B) found to have NECH in an adjacent bronchiole highlighted by synaptophysin staining. (C) Additional tumourlets and (D) bronchioles with NECH were also seen demonstrating synaptophysin positivity.

increase in neuroendocrine cells was also confined to areas of lung affected by acute bronchitis and bronchopneumonia, leading the authors to speculate that inflammation rather than fibrosis is a causative factor of NECH.²⁴

Aguayo *et al.* reported a ten-fold increase in neuroendocrine cells in eosinophilic granuloma by bombesin-like peptide immunoreactivity compared to smokers and patients with idiopathic pulmonary fibrosis. Normal lung tissue was not examined in the study, however, the frequency of approximately 6–8 cells per 10 cm of airway epithelial length is comparable to the frequency of neuroendocrine cells in normal autopsy lungs as reported by Gosney *et al.*^{13,25} A single case report has described NECH as a rare cause of interstitial lung disease in two patients, both of whom were female non-smokers living above sea level. Radiological features found on imaging, specifically diffuse ground glass infiltrates in a mosaic pattern, were consistent with NSIP or early hypersensitivity pneumonitis. Of note, these were the only cases diagnosed with NECH out of 500 consecutive diffuse interstitial lung disease cases studied.²⁶ Another case report described DIPNECH diagnosed in a patient with UIP and obliterative bronchiolitis, and other literature have reported UIP-like findings on imaging in association with DIPNECH.^{6,27,28}

Literature on NECH as a primary finding in the setting of emphysema is also limited. An early study by Gosney *et al.* reported increased populations of pulmonary neuroendocrine cells in the lungs of patients with chronic bronchitis or emphysema compared to controls.²⁹ A case of DIPNECH arising in a patient with known chronic obstructive disease has also been reported.⁵

A series of case reports identified three patients with bronchial carcinoids and sarcoidosis.³⁰ NECH as a primary finding in association with sarcoidosis otherwise has not been studied.

Overall, NECH is generally a well-accepted process in chronic lung disease, however the literature has been limited mostly to case reports, and there are few studies regarding its incidence and quantification. By utilising the proposed criteria for DIPNECH, this is the first study to quantify NECH by the density of neuroendocrine cells within individual bronchi. The association between NECH and chronic lung disease raises the possibility that carcinoid tumours and neuroendocrine carcinomas would have an increased incidence in patients with fibrotic lung disease and emphysema. A series of case reports identified three patients with bronchial carcinoids and sarcoidosis.³⁰ The series of carcinomas reported by Aubry *et al.* in patients with idiopathic pulmonary fibrosis included

squamous cell carcinomas, adenocarcinomas, and a single spindle cell carcinoma, but no case of neuroendocrine tumour.³¹ We know of no association between idiopathic pulmonary fibrosis and carcinoid tumours or neuroendocrine carcinomas, suggesting that the presence of NECH in and of itself is not a high risk for the development of invasive tumours. Similarly, we know of no association between emphysema and neuroendocrine tumours, independent of smoking.

Our results suggest that fibrotic lung disease is a predisposing factor for neuroendocrine cell proliferation, in addition to the known risk of carcinomas. This is consistent with NECH being recognised as a reactive response to chronic hypoxia, such as in patients living at high altitudes or with a history of cigarette smoking.³² Although not addressed in the current study, it has also been identified in patients with a variety of lung diseases, including bronchopulmonary dysplasia, cystic fibrosis, chronic obstructive pulmonary disease, and eosinophilic granuloma.¹⁰ This is speculated to be due to the fact that PNCs secrete a variety of cytokines and growth factors which can stimulate fibroblasts and airway cell chemotaxis, resulting in bronchiolar fibrosis.³³

Currently, the distinction between DIPNECH and a reactive neuroendocrine cell proliferation is based on histopathological criteria as well as clinical context, and it is unclear whether there is an actual difference in the pulmonary neuroendocrine cells proliferating in DIPNECH versus those reacting to lung injury. More recent studies have identified a different immunohistochemical profile in DIPNECH compared to reactive NECH, specifically a more consistent and earlier expression of cell-cycle proteins p53, Ki-67, and p16 in DIPNECH. Neuroendocrine cell proliferations in DIPNECH are also believed to be more florid than in reactive NECH, so applying the diagnostic criteria for DIPNECH may actually be too restrictive in identifying neuroendocrine cell proliferations in response to lung injury.^{34,35}

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