Morphological and Immunohistochemical Features of Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia: Aguayo - Miller Disease

Prof. Dr. Lutfi ALIA
Centre of Oncological Prevention. Siena – Italy
it.alia47@yahoo.it

Abstract

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) is a rare pulmonary disorder that is characterized hyperplasia of bronchiolar and bronchial pulmonary neuroendocrine cells. The hyperplasia of neuroendocrine cells can be primary or reactive. In the WHO - IASLP classification of lung tumours (1999, 2004, 2005, 2015), DIPNECH is considered a preneoplastic lesion in the spectrum of pulmonary tumours. According to the WHO classification, the definition of DIPNECH is purely histological. The histological appearance of DIPNECH within the lungs takes many forms. It may comprise a diffuse hyperplasia of scattered neuroendocrine cells, small nodules (neuroendocrine bodies), or a linear proliferation in alveolar ducts and alveols. These proliferations are superficial to the basement membrane and confined to the bronchial and bronchiolar epithelium. When there is extension beyond the basement membrane, the neuroendocrine cell proliferations in the multiple nodules < 5 mm diameter are termed tumourlets, but where the tumourlets have > 5 mm diameter are classified carcinoid tumours.

The DIPNECH was initially described in 1992 by Aguayo et al., who reported six non-smoking patients with cough, exertional dyspnea, wheezing, less frequently

hemoptysis and a mixed obstructive/restrictive defect on pulmonary function tests. In the Aguayo – Miller disease, the DIPNECH is a primary proliferation and is associated with tumourlets and carcinoid and with obstructive bronchiolitis. This disease has a predilection for nonsmoking middle-aged women (female to male ratio it's approximately 10:1)

In this study we present a 62-year-old, non-smoker woman, presented with respiratory symptoms ascribable to DIPNECH. After surgery, the morphological study of lung specimens, conferme the DIPNECH, multiple tumourlets, one peripheral carcinoid and obliterative bronchiolitis in the right middle pulmonary lobe.

Key words: *Neuroendocrine pulmonary cell hyperplasia, tumourlets, carcinoid, obliterative* bronchiolitis.

Introduction

Neuroendocrine cells are component of the normal bronchiolar and bronchial epithelium, that comprise about 1 % of epithelial cells in an adult lung. These cells expressed neuroendocrine markers (chromogranin A, synaptophysin, CD 56 etc.) DIPNECH is a clinico-pathological syndrome, as well as an incidental finding on histological examination, althought there are obvious differences between these two scenarios. [1 - 5] When no other pathological pulmonary disease is detected, DIPNECH is idiopathic, instead the reactive neuroendocrine hyperplasia is believed to be a response to hypoxia and chronic obstructive pulmonary disease as interstitial fibrosis, asthma, cystic fibrosis, bronchopulmonary dysplasia, bronchiectasis, chronic obstructive pulmonary disease, and also in the neuroendocrine pulmonary tumours, non endocrine pulmonary tumours, chronic exposure to high altitude, exposure to tobacco smoke etc. [2, 3, 4, 5, 6.] In WHO classification of tumours of the lung (1999, 2004, 2005, 2015), the DIPNECH was defined a preneoplastic lesion, because it is commonly found in patients with tumourlets and carcinoid tumours. [2, 3, 4, 5] According to the WHO classification, the definition of DIPNECH is purely histological. However, DIPNECH encompasses asymptomatic patients with airway disease, as well as symptomatic patients with pulmonary neuroedocrine cell hyperplasia associated with multiple tumourlets and carcinoid tumors. [1, 2, 3, 4, 5]

Histologically, the DIPNECH may manifest as: a. generalised proliferation of scattered neuroendocrine cells; b. tinely nodular aggregates (neuroendocrine body); c. linear proliferation. Althought usually confined to the bronchial and bronchiolar epithelium, these proliferation can extend beyond the basement membrane to form tumourlts, or carcinoid tumours, lesions which have continuity and correlation. [1, 6, 7, 8, 9]

The disease Aguayo – Miller is characterized by diffuse idiopathic hyperplasia of scattered neuroendocrine cells in the distal bronchi and bronchioles, a linear hyperplasia in alveolar ducts and alveols, associated with multiple tumourlets and peripheric carcinoid and peribronchiolar fibrosis obliterating small airways, and by mild chronic lymphocytic inflammation. [1, 3, 4, 5, 6, 7]

This disease was initially described in 1992 by Aguayo et al., who reported six non-smoking patients with cough, exertional dyspnea, wheezing, less frequently hemoptysis and a mixed obstructive/restrictive defect on pulmonary function tests. [1, 2, 6, 8, 9]

There have now been more than 200 cases of this disease, published since the initial description by Aguayo in 1992, in the form of case reports or small case series, but no clear consensus has been established in the literature about the radiological or pathological diagnostic criteria, or the management of this disease. [3, 6, 10]

Case report

A 62-year-old woman, non-smoking, presented with a respiratory tract infection, expressed with cough, exertional dyspnea, and wheezing. The radiography and the high-resolution computed tomography (HRCT) of the chest, showed a mosaic attenuation do to interstitial fibrosis, and presence of some little nodules and a nodule measuring 28 mm, in the right middle pulmonary lobe.

The lung function tests show an obstructive ventilation defect. After the lobe resection, the microscopic examination (histopathology and immunohistochemistry), confirme the diagnosis of diffuse idiopathic pulmonary neuroendocrine cells hyperplasia, multiple tumourlets, one carcinoid tumours and obliterative bronchiolitis with peribronchiolar fibrosis obliterating small airways, and intertstitial chronic inflammation. The patient was followed according to standard follow-up for patients after lobe resection. She is alive and well 4 year after surgery.

Pathologic findings

In the histopathologically examination (haematoxyline & eosine staining) of lung surgical specimens, we have revealed diffuse idiopathic hyperplasia of pulmonary neuroendocrine cells involved distal bronchi and bronchioles with numerous neuroepithelial bodies present within the mucosa. (Figure 1). The neurondocrine cells are round, or oval shape, monomorphe size, with less citoplasme, large nuclei deeple stained, or are fine granular-like, nucleoli are unobvious. Neuroendocrine

cells are scattered in individual ones or at line-like, or form small nests in the bronchiolar epithelium, and even completely replace bronchiolar epiethelium, resulting in narrow lumen but not penetring basament membrane. These neuroendocrine cells hyperplasia is complicated with obliterative bronchiolitis. The residual airway lumen is replaced by nodular and circumfe-rential arrangement of neuroendocrine cells, expressed with peribronchiolar fibrosis obliterating small airways, and associated wit a little interstitial chronic inflamation. (Figure 2) In some fields we have seen the lineare neuroendocrine cells hyperplasia in alveolar ducts and alveoli (Figure 1). No mitotic figures, no areas of necrosis, no cell pleomorphism were detected in the proliferative neuroendocrine cells.

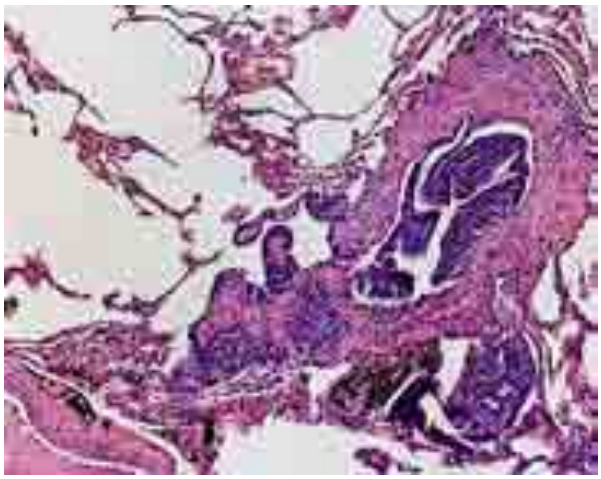

FIGURE 1. Neuroendocrine cells involved bronchioles with numerous neuroepithelial bodies present within the mucosa, and two tumourlets.

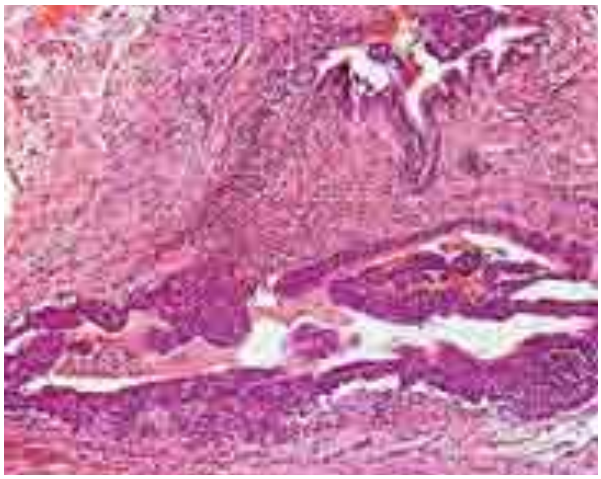

FIGURE 2. The residual airway lumen is replaced by nodular and circumferential arrangement of NEP cells, associated peribronchiolar fibrosis and interstitial chronic inflamation.

FIGURE 3. Immunohistochemical staining. Neuroendocrine pulmonary cells positive for chromogranin A.

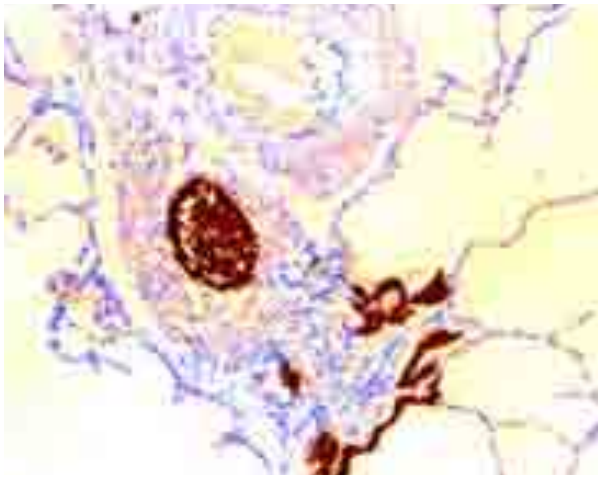

FIGURE 4. Linear proliferation of NEP cells in the alveolar ducts and alveoli, positive for chromogranin A

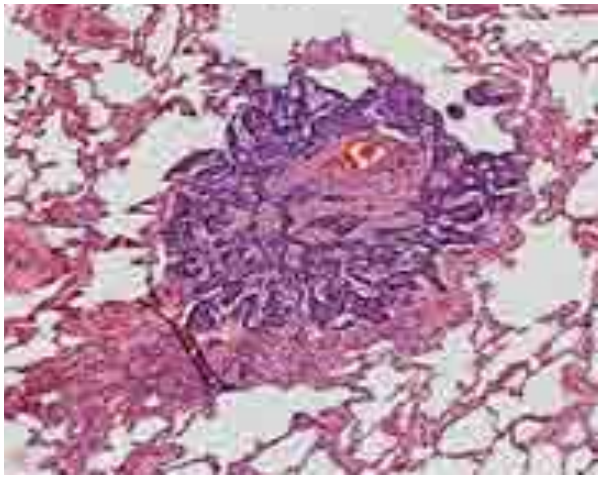

FIGURE 5. Tumourlet: neuroendocrine cells are embedded an dense fibroelastic stroma

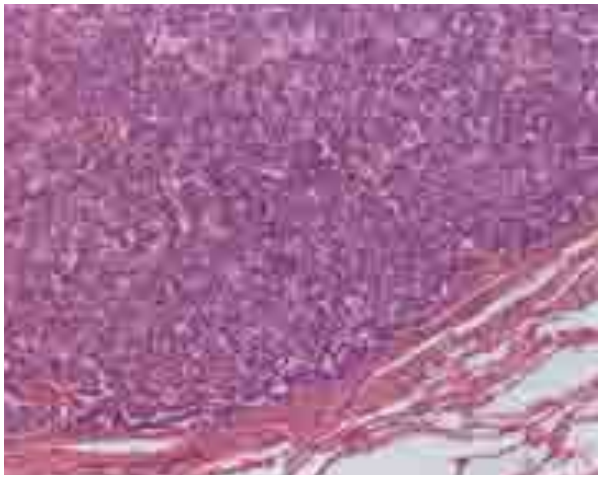

FIGURE 6. Typical carcinoid is circumscribed but not encapsulated.

In the immunohistochemical staining we have revealed the neuroendocrine cells involving distal bronchi and bronchioles (Fig 3) and linear proliferation in the alveolar ducts and alveoli contains neurosecretory granules wich are positive for chromogranin A (Fig. 4), and for synaptophysine, and for CD56, which is a marker of neuroendocrine cell differentiation. At least nine tumorlets, were found in the peribronchial region of the right middle pulmonary lobe, of varying size and morphologic features. The tumourlets are 2 – 4 mm sized nodular hyperplasia of neuroendocrine cells, that are embedded in dense fibroelastic connective tissue. (Figure 5). Movat's pentachrome staining demonstrated a abnormal deposition of disorganized collagen and elastic fibers in both the tumorlets and the submucosa underlying areas of pulmonary neuroendocrine cells hyperplasia in small airways not obliterated by tumorlets.

In the immunohistochemical stains, the neuroendocrine cells of tumourlets presents positivity for chromogranin A (Figure 3 and Figure 4), synaptophysine, Neuron Specific Enolase, CD56, and bombesine. The proliferative activity with Ki67 < 1 % in the tumourlets.

We have detected a typical carcinoid with 28 mm diameter in the parenchima of the right middle pulmonary lobe. The typical carcinoid is circumscribed, but not encapsulated, and have nests of uniform cells, covered in the elegant fibrovascular stroma. (Figure 6). The cytologic apparence of neuroendocrine cells of carcinoid is basically the same: monomorphic cells population with scarce eosinophilic cytoplasm, monomorphic central nucleus with finely granular chromatin as "salt and pepper". The neuroendocrine cells were arranged delimiting nests, without necrosis and with 1 - 2 mitosis in 10 hpf, but without significant atypia.

In the immunohistochemical staining, the neuroendocrine cells of typical carcinoid was positive for chromogranin A, synaptophysine, Neuron Specific Enolase, CD56, also have EGF focal expression, VEGF modest expression, and Bax over expression. Proliferative activity with Ki-67 < 3 %.

Discussion

Pulmonary Neuroendocrine cells are part of normal epithelium of bronchial and bronchiolar anatomy and are thought to play an important role in lung development, as they are frequently found in the airways of fetal and neonatal lungs [1, 3]. Neuroendocrine cells of the lung decrease in number with age and are only present focally in adult airways, where they represent approximately 1% of all epithelial cells in the adult lung [2, 3] Pulmonary neuroendocrine cell hyperplasia can be either primary or reactive. It is important to emphasize, that the DIPNECH is defined as being idiopathic, so existing without any pre-existing chronic lung disease and in the absence of other lung diseases, but reactive pulmonary neuroendocrine cells hyperplasia can occur as a result of a wide spectrum of chronic conditions that are expressed with hypoxia, including pulmonary interstitial fibrosis, asthma, cystic fibrosis, bronchopulmonary dysplasia, bronchiectasis, chronic obstructive pulmonary disease, neuroendocrine lung tumours, non neuroendocrine lung tumours, chronic exposure to high altitude, exposure to tobacco smoke etc. [2, 3, 4, 5, 6, 7.].

The first series of six cases of DIPNECH was published by Aguayo et al., in 1992 [1], describing a clinical condition with respiratory symptoms as well as specific radiological and histological characteristics. The treatments of these patients included the lobe or the lung resection. [1, 2, 3, 5, 8, 9].

The WHO classification 2015 has considered the DIPNECH a precancerous lesion of a lung neuroendocrine tumour, it is defined as within broncchial mucosal epithelium, there is duffuse clustered, linear or nodular neuroencdocrine ccell hyperplasia without basement membrane breaakthrough. [2, 3, 4, 6, 10, 11, 12]. The current definition of DIPNECH by the WHO is exclusively histological. [3, 6, 11, 12, 13, 14]. There have now been more than 200 cases of DIPNECH described since the initial description by Aguayo, in the form of case reports or small case series, but no clear consensus has been established in the literature about the radiological or pathological diagnostic criteria or the management of this condition. [6, 10, 11, 12]. DIPNECH has a predilection for nonsmoking, middle-aged women and is associated with a predominantly obstructive ventilatory pattern on pulmonary function tests seen in obliterative bronchiolar fibrosis. [1, 3, 5, 6, 14]

The diagnosis of DIPNECH is often made several years after the onset of clinical

symptoms, usually following the incidental discovery of a lung nodule on chest radiography or CT scan. The condition progresses slowly, although there have been cases reported of a rapidly progressive and more aggressive clinical course, which has required lobectomy or pulmonectomy, and even lung transplantation. [6, 10, 11, 12, 14].

There seems to be two different modes of clinical presentation of DIPNECH. Onset is occult, there may be no clinical symptoms, or there is long duration of dry cough, and exertional dyspnea, expressed with obstructive – restrictive lung function profile, but these cases clinically are commonly misdiagnosed for bronchial asthma or chronic bronchitis. [5, 6, 9, 12, 13]. DIPNECH is often accompanied with chronic airway inflammation and diseases that can cause severe interstitial pulmonary fibrosis. [4, 6, 10, 11, 12, 15, 16].

Our case have similar clinico-morphological characteristics to symptomatic forme to those published.

Under a light microscope, our case present hyperplasia of pulmonary neuroendocrine cells, confined within bronchial mucosal epithelium and manifest as round, or oval cells, with relatively consistent size and shape showing: hyperplasia, less cytoplasm, large nuclei deeply stained, or are chromatine fine granule-like, nucleoli are unobvious. Neuroendocrine cells are scattered in individual ones, or form small nests at base of bronchiolar epithelium, and even completely replace bronchiolar epithelium, resulting in narrow lumen, but not penetrating basement membrane. These neuroendocrine cells hyperplasia is complicated with obliterative bronchiolitis. In some fields we have seen the lineare neuroendocrine cells hyperplasia in alveolar ducts and alveoli (Figure 1 and Figure 4). The residual airway lumen is replaced by nodular and circumferential arrangement of neuroendocrine cells, expressed with peribronchiolar fibrosis obliterating small airways, and associated with interstitial fibrosis and chronic inflamation. (Figure 2). No atypical mitotic figures, no areas of necrosis, no cell pleomorphism were detected in the proliferative neuroendocrine cells.

In some other fields we have seen the neuroendocrine cells penetrate basement membrane to infiltrate lung interstitium and show nodular growth, which are tumourlets.

In the immunohistochemical staining we have revealed the neuroendocrine cells involving distal bronchi and bronchioles (Figure 3) and linear proliferation in the alveolar ducts and alveoli contains neurosecretory granules wich are positive for chromogranin A (Figure 4), and for synaptophysine, and for CD56, which is a marker of neuroendocrine cell differentiation.

We have identificate nine tumorlets, which were found in the peribronchial region. The tumourlets 2 – 4 mm sized, present nodular hyperplasia of neuroendocrine cells, that are embedded in dense fibroelastic connective tissue.

(Figure 5) In the immunohistochemical stains, the neuroendocrine cells presents positivity for chromogranin A (Figure 3), synaptophysine, Neuron Specific Enolase, CD56, and for bombesine. The nodular proliferation of neuroendocrine cells, formed lesions with a diameter < 5 mm are considered to be tumourlets, but where the nodules are > 5 mm, are classified as typical carcinoid tumour. [3, 5, 6, 7, 8, 9]. Also, we have diagnosed a typical carcinoid tumour (28 mm diameter) in the right middle pulmonary lobe.

In the immunohistochemical study, the neuroendocrine cells of carcinoid are positive for chromogranin A, synaptophysine, Neuron Specific Enolase, CD56, also have EGF focal expression, VEGF modest expression, and Bax over expression. Proliferative activity with Ki-67 < 3 %.

DIPNECH is regarded as a precursor lesion for tumourlets and carcinoid tumors. [3, 6, 10, 14, 15, 16]

The carcinoid is subdivided into: typical carcinoid, which is low grade tumour and atypical carcinoid, which is intermediate grade tumour. [3, 4, 6] In the series by Davies et al.[13], there were three cases associated with atypical carcinoid, one of whom had multiple endocrine neoplasia. This is the first case with a classic clinical picture of DIPNECH with metastatic carcinoid and multiple unrelated tumors. [6, 13]

The morphological diagnosis of our case is: Aguayo – Miller disease: DIPNECCH in the distal bronchial and bronchiolar wall, the linear proliferation confined to the alveolar duct and alveola, presence of multiple tumourlets, one carcinoid tumour and obliterative bronchiolitis, interstitial fibrosis associated with chronic interstitial inflammation.

However, a recent paper by Marchevsky et al. [11] reported on 70 consecutive surgical lung biopsies showing multifocal neuroendocrine proliferations, which had neither histological features of obliterative bronchiolitis nor had they been diagnosed with DIPNECH before histological examination, indicating that the condition can be asymptomatic [13]. This study suggested that the presence of multifocal PNECH combined with more than three tumorlets as the minimum pathological criteria for the diagnosis of DIPNECH, limiting the condition to a pathological entity. [7] A pathology-based approach by Marchevsky aimed at distinguishing DIPNECH from reactive neuroendocrine cell hyperplasia, suggested that the presence of multifocal NECH associated with more three tumorlets could represent a pathological criterion for the diagnosis of DIPNECH.

Wirtschafter and al. [12] evaluated 30 cases of DIPNECH and systematically reviewed 169 cases reported in the English literature and concluded that only 55 (28 %) had obliterative or constrictive bronchiolitis. This same conclusion has been drawn by Davies and al [11, 13], who suggest that even in cases with histological evidence of airway wall thickening, chronic inflammation, and constrictive obliterative bronchiolitis, the majority of patients did not have clinically important

airflow obstruction. [8, 10, 11, 12, 13] With regard to symptomatic cases of DIPNECH, as in the case we have presented, the condition typically presents with a chronic cough, exertional dyspnea, and frequent wheezing, with a clinical presentation predominantly in non-smoking middle-aged women. In the appropriate clinical and radiological setting, a transbronchial biopsy may be sufficient for diagnosis of DIPNECH, although open surgical lung biopsy is considered optimum for diagnosis [3, 9, 10, 12, 13, 14, 16].

The histological features of DIPNECH include constrictive/obliterative bronchiolitis characterized by chronic inflammation, bronchial wall thickening and fibrosis, believed to be the reason for progressive narrowing and/or complete obliteration of the bronchiolar lumen in severe cases. [3, 12, 13, 14, 16, 17, 18, 19 , 20].

The chromogranin A, synaptophysin, and CD56 are the most commonly expressed immunohisto-chemical markers, while p53, Ki-67, and p16 may distinguish DIPNECH from reactive pulmonary NECH. [3].

The patient profile and presentation in this case report fits the typical DIPNECH profile, that of a middle-aged non-smoking female presenting with exertional dyspnea, in association with the discovery of a lung nodule on chest CT [3, 8]. The biopsy findings are those of the histological criteria for DIPNECH diagnosis as defined by the WHO and cited by Marchevsky et al. [3, 7, 9, 11]

The treatment for our case was surgery (lobectomy), but some authors use the somatostatin analogs (SSA) in DIPNECH. [17, 18] Gorshtein et al., in their review of 11 DIPNECH patients, suggested the affirmative role of SSA in the symptom management of DIPNECH. In the American single-center experience, most of their patients responded to treatment with SSA and had significant improvement in their presenting symptoms. [18]

Conclusions

DIPNECH remains a rare pulmonary condition, and considered a preneoplastic lesion in the spectrum of pulmonary tumours. According to the WHO classification, the definition of DIPNECH is purely histological. While most patients experience a relatively uneventful clinical course, this condition may be associated with tumourlets, carcinoid tumour and airway obstruction (Aguayo-Miller disease). Awareness of the condition, imaging, and histopathology are required to make the definitive diagnosis, and close follow-up is important in the more aggressive cases of DIPNECH.

It is possible that DIPNECH is an under-diagnosed pulmonary condition because it is rarely associated with symptoms. This case report has highlighted this rare, but potentially progressive condition and the need for evidence-based management guidelines for DIPNECH.

References

01. Aguayo SM, Miller YE, Waldron JA, Jr, et al. (1992). Brief report: Idiopathic diffuse hyperplasia of pulmonary neuroendocrine cells and airways disease. *New England Journal Medicine*, 327: pp. 1285 – 1288
02. Travis WD, et al. (2004).WHO classification of tumours: Pathology & Genetics: Tumours of the lung, pleura, thymus and heart. Lyon, France: *IARC Press*; pp. 19 - 21
03. Travis WD, Brambilla E, Burke AP, et al. (2015). WHO classification of tumours of the lung, pleura, thymus and heart. Lyon: *IARC Press*; pp. 78–79.
04. Lutfi Alia. (2017). Diffuse idiopathic pulmonary neuroendocrine cell Hyperplasia, multiple tumourlets and Typical carcinoid. 3° International Multidisiplinary Medical Congress. Tirana novembre 2017.
05. Jin, Linfang ; Wang, Zhiqiang ; Qi, Xiaowei. (2018). Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia.Case series and a review of the literature. *Medicine*, Vol. 97, p 13806
06. Giulio Rossi, Alberto Cavazza et. Al. (2016). Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia Syndrome. *European Respiratory Journal*, 47. pp. 1829 - 1841
07. Evangelos Koliakos. (2017). Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia. A Case Report and Review of the Literature. *American Journale Case Report*, 18: pp. 975 – 979
08. Benson RE, Rosado-de-Christenson ML, Martínez-Jiménez S, et al. (2013). Spectrum of pulmo-nary neuroendocrine proliferations and neoplasms. *Radiographics*, 33: 1631 – 1649.
09. Cansız Ersöz C, Cangır AK, Dizbay Sak S. (2016). Diffuse neuroendocrine cell hyperplasia: Report of two cases. *Case Report Pathology*, 34, p. 19725.
10. Nassar AA, Jaroszewski DE, Helmers RA, et al. (2011). Diffuse idiopathic pulmonary neuroendo-crine cell hyperplasia: A systematic overview. *American Journale Respiratory Crit Care Med*, 184: pp. 8 – 12
11. Marchevsky AM, Wirtschafter E, Walts AE. (2015). The spectrum of changes in adults with multifocal pulmonary neuroendocrine proliferations: What is the minimum set of pathologic criteria to diagnose DIPNECH? *Human Pathology*, 46: pp. 176 – 181.
12. Wirtschafter E, Walts AE, Liu ST, et al. (2015). Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia of the lung (DIPNECH): Current best evidence. *Lung,* 193: pp. 659 – 667.
13. Davies SJ, Gosney JR, Hansell DM, et al. (2007) Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia: An under-recognised spectrum of disease. *Thorax,* 62: pp. Pp. 248 – 252.
14. Carr LL, Chung JH, Achcar RD, et al. (2015). The clinical course of diffuse idiopathic pulmonary neuroendocrine cell hyperplasia. *Chest*, 147: pp. 415 – 22.
15. Schnabel PA, Junkel K. (2015). Pulmonary neuroendocrine tumours in the new 2015 WHO classification. Start of beaking new grounds?. *Pathology,* 36: pp. 383 – 392
16. Luis Cabezón - Gutiérrez , Parham Khosravi-Shahi , Magda Palka-Kotlowska, Sara Custodio Cabello, Maria García-Martos. (2019). Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia: Review of the Literature and a Single-center Experience. *Cureus*: pp. 35 - 45
17. Gorshtein A, Gross J, Barak D, Strenov Y, Refaeli Y, Shimon I, Grozinsky-Glasberg S. (2012). Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia and the associated lung neuroendo-crine tumors: clinical experience with a rare entity. *Cancer*, 118: pp. 612 – 619
18. Chauhan A, Ramirez RA: 2015, Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) and the role of somatostatin analogs: a case series. *Lung*, 193: 653 – 657
19. Lutfi Alia. (2019) Hiperplazia difuze e qelizave neuroendokrine pulmonare: "Bazat patologjike të sëmundjeve". Tiranë: Shtepia Botuese "Streha", vol I, ff. 259 – 263
20. Godwin Ofikwu. (2015). A rare Case of Diffuse Idiopathic Pulmonary Neuroendocrine cell Hyperplasia. *Case report in Surgery*. 31, pp. 5 – 8.