www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i7.114

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Spectrum of Primary Pulmonary Neuroendocrine Tumors from A Regional Cancer Center in South India

Authors
Dr Usha Amirtham[1], Dr Nikita J.Mulchandani[2], Dr Premalata C.S.[3]
Dr Shankar Anand Bharatnur[4], Dr Rekha V.Kumar[3]
[1]Associate Professor, [2]Postgraduate Student, [3]Professor, [4]Assistant Professor
Department of Pathology, Kidwai Cancer Institute, Bengaluru

ABSTRACT

Objectives: *To determine the clinico-pathologic features of pulmonary neuroendocrine tumors (NET) in a regional cancer center in South India.*

Methods: *89 cases of NETs were identified over a 5-year period from May 2011 to May 2016. The clinico-pathologic and immunophenotypic features of these tumors were analyzed on needle core biopsies and they were classified according to the 2015 World Health Organization Classification of tumors of lung. Cases on which the diagnosis was made on cell blocks of aspirated material were excluded.*

Results: *There were 89 cases of NETs, of which 72 (81%) had lung biopsies and 17 (19%) had biopsies from metastatic sites. Among the NETs, 10 (11.2%) were carcinoid tumors and 79 were neuroendocrine carcinomas (88.8%). Of the 79 cases of neuroendocrine carcinomas, 65 (82%) were small cell lung cancers and 14 (17%) were possible large cell neuroendocrine carcinomas. The mean ages for pulmonary carcinoid and NEC were 44.5 years and 55.5 years respectively. The male to female ratio was 1.5:1 in case of pulmonary carcinoids and 7.9:1 in case of NECs. While 3 (30%) pulmonary carcinoids were central in location, 7 (70%) were peripheral and whereas 16 (20.3%) NECs were central in location, 46 (58.2%) were peripheral. Also, 17 (21.5%) cases presented with metastases in various sites which included cervical and supraclavicular lymph nodes, liver, brain, abdominal wall and bone.*

Conclusion: *Our study was an attempt to analyze the histopathological spectrum of pulmonary NETs, the first of its kind in the Indian literature. We noted marked preponderance of small cell neuroendocrine carcinomas amongst all NETs.*

INTRODUCTION

Pulmonary neuroendocrine tumors are an uncommon group of neoplasms and these tumors are thought to arise from stem cells of the bronchial epithelium which are also known as Kulchitzky cells. These cells are characterized by secretory activity and their ability to take up and decarboxylate the amine precursors (APUD system cells).[1] These neuroendocrine phenotypical and morphological characteristics are present within a broad spectrum of histologies of lung neuroendocrine tumors (NETs), from relatively indolent and low grade typical carcinoids (TCs), to intermediate grade atypical carcinoids (AC) and histologically high-grade biologically aggressive tumors – large cell neuroendocrine carcinomas (LCNEC) and small cell lung cancers (SCLC).[2]

While neuroendocrine tumors (NETs) constitute 20-25% of all invasive lung malignancies, non-small cell lung carcinomas (NSCLC) constitute 75%.[1] In the previous World Health Organization (WHO) classifications of Tumors of the Lung, the carcinoid tumors, SCLC and LCNEC were grouped separately.[3] However, in the current 2015 WHO classification, they are grouped together as neuroendocrine tumors.[2] The tumors are listed in the order of their frequency with SCLC first as it is the most common, followed by LCNEC, carcinoid tumors and diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) which is a preinvasive lesion.[4] In this study, we determine the clinico-pathologic features of pulmonary NETs.

MATERIALS AND METHODS

We analysed 89 cases of pulmonary NETs retrospectively which were identified on small biopsies (core needle) over a 5- year period from May 2011 to May 2016.

Clinical characteristics such as age, gender, smoking history, location, imaging findings, characteristics of lesion and biochemical parameters were obtained from the hospital records. The histological (H&E stained) and immunohistochemical findings of all the cases were analysed. Immunohistochemistry (IHC) was performed by immunoperoxidase staining on formalin fixed paraffin embedded (FFPE) tissue sections of needle core biopsies using Super Sensitive^{TM} polymer-HRP Detection System. The list of antibodies used is summarized in Table 1.

Table 1: Source and other details of antibodies used in IHC

Antigen/Protein	Clone	Antibody	Source	Dilution
Ki67	Mib1	Mouse monoclonal	BioGenex	1:100
CK7	OVTL	Rabbit monoclonal	BioGenex	1:100
CK20	CK20	Mouse monoclonal	Biocare	1:200
p40	ZR8	Rabbit monoclonal	BioGenex	1:200
TTF1	BGX3-97A	Mouse monoclonal	BioGenex	Ready to use
NapsinA	TMU-Ad 02	Mouse monoclonal	Biocare	1:125
CDX2	EP25	Mouse monoclonal	BioSB	1:200
Synaptophysin	SNP88	Mouse monoclonal	BioGenex	1:80
Chromogranin	LK2H10	Mouse monoclonal	BioGenex	1:200
LCA	LCA88	Mouse monoclonal	BioGenex	1:400
PLAP	PL8-F6	Mouse monoclonal	BioGenex	1:100
CD56	123C3.D5	Mouse monoclonal	BioSB	Ready to use

The cases which we studied were diagnosed and classified in accordance with the 2015 WHO classification of tumors of lung.

RESULTS

There were 89 cases of pulmonary NETs among which 10 (11.2%) were carcinoid tumors and 79 were neuroendocrine carcinomas (88.8%). The mean ages for pulmonary carcinoid and NEC were 44.5 years and 55.5 years respectively. The ratio of male to female was 1.5:1 in case of pulmonary carcinoids and 12:1 in case of NECs.

The diagnosis was made using CT-guided, bronchoscopic or thoracoscopic biopsies in 72 (81%) cases and biopsies from metastatic sites which included cervical and supraclavicular lymph nodes, liver, brain, abdominal wall and bone in 17 (19%) cases.

The clinical and radiological features of the NETs in our study are discussed in Table 2.

Table 2: Clinical & radiological features

	TYPICAL CARCINOID	ATYPICAL CARCINOID	SMALL CELL CARCINOMA	LARGE CELL NEUROENDOCRINE CARCINOMA
Mean Age	42 years	47 years	68 years	64 years
Gender predilection	Female	Male	Male	Male
Clinical presentation • Cough • Dyspnea • Hemoptysis	 80% 60% 10%	 100% 80% 40%	 72% 63% 52%	 71% 71% 43%
Smoking association	No	No	Yes (93%)	Yes (100%)
Location: • Central • Peripheral • Metastasis	 60% 40% -	 - 100% -	 21% 58% 21%	 14% 64% 22%

We had 5 (5.6%) cases each of typical and atypical carcinoid tumors. Of the remaining 79 neuroendocrine carcinomas (NEC), 65 (73%) were small cell carcinomas and 14 (16.4%) were possible LCNEC. All 89 patients were subtyped after IHC evaluation (Figure 1).

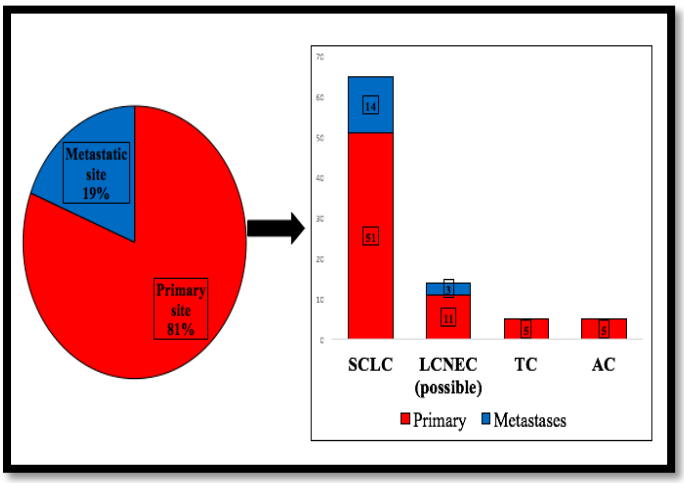

Fig 1: Relative frequency of pulmonary neuroendocrine tumors according to subtype. (SCLC, Small cell lung carcinoma; LCNEC, Large cell neuroendocrine carcinoma; TC, Typical carcinoid; AC, Atypical carcinoid)

All 10 cases of TC and AC showed uniform appearing cells arranged in organoid and trabecular patterns with coarse nuclear chromatin, inconspicuous nucleoli and scant to moderate amount of cytoplasm. While the mitoses in all cases of TC was <2/10 high power fields (hpf), the mitotic index of the AC cases varied from 2-9/10 hpf. Areas of punctate necrosis were noted in 3 cases of AC and none of the TC cases. IHC was performed on all 10 cases and all cases showed expression of both synaptophysin and chromogranin. TTF1 was performed on all cases of pulmonary carcinoids and while none of the TC cases expressed TTF1, 2 out of 5 cases of AC were positive for TTF1. Ki67 labelling index was <5% in all cases of TC and varied from 9-20% in all AC cases. (Figures 2 and 3)

The 65 cases of SCLC in our study showed small cells with neuroendocrine morphology arranged in sheets with a mitotic index of >10/10 hpf in all cases. Areas of necrosis were noted. All cases were confirmed on IHC and were positive for synaptophysin, chromogranin and TTF1. The Ki67 index was >90% in all cases. (Figure 4)

All 14 cases of possible LCNEC were diagnosed on small biopsies and showed large cells with neuroendocrine morphology, coarse chromatin and abundant cytoplasm. Areas of necrosis were present and the mitotic index was >10/10 hpf in all cases. Confirmation by IHC was done and all cases showed positivity for synaptophysin, chromogranin and TTF1. The Ki67 index varied from 50-70%. (Figure 5)

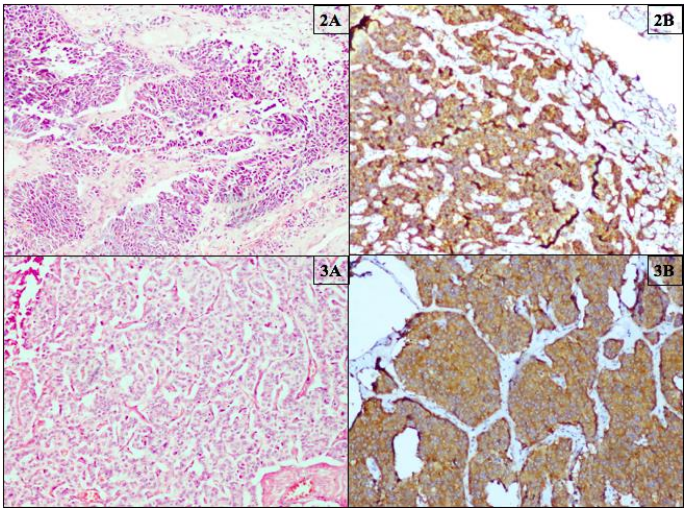

Fig. 2 Typical carcinoid. **A** -Tumor cells in organoid nesting pattern. **B**– Strong cytoplasmic synaptophysin staining.

Fig. 3 Atypical carcinoid. **A**– Three mitoses in the same low power field. **B** - Strong cytoplasmic chromogranin staining.

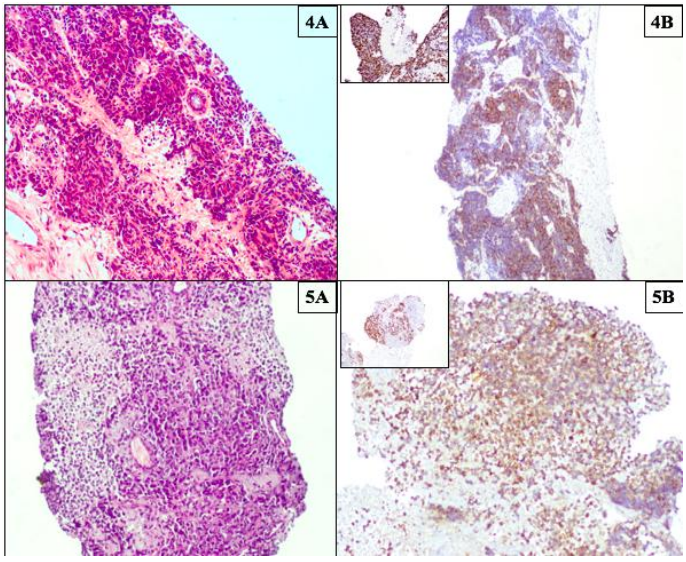

Fig. 4 Small cell carcinoma. A– Densely packed tumor cells. **B**– Cytoplasmic synaptophsyin staining. (Inset) Ki-67 index >90% in tumor cells.

Fig. 5 Large cell neuroendocrine carcinoma. A - Large tumor cells with areas of necrosis. **B**– Cytoplasmic synaptophysin staining. (Inset) Ki-67 index >50% in tumor cells

DISCUSSION

In this study we identified 89 cases of neuroendocrine lung tumors, of which 10 were carcinoid tumors and 79 were neuroendocrine carcinomas. These 89 cases of NET were confirmed on IHC by using a panel of immunomarkers which included synaptophysin, chromogranin in all cases.

Carcinoid Tumors

The respiratory tract is the second most common location for carcinoid tumors after the gastrointestinal tract.[5] While the majority of TCs and ACs arise de novo, a few are also postulated to arise and develop in the setting of proliferating pulmonary neuroendocrine (NE) cells via diffuse idiopathic pulmonary NE cell hyperplasia (DIPNECH).[2,6]

Carcinoid tumors are not early progenitors of high grade NETs.[2] *TP53* and *RB1* mutations are rare in typical carcinoids (TC <5%) and more frequent in atypical carcinoids (AC ~ 20%).[2] Even though they exhibit indolent behavior, these are malignant neoplasms with the potential to metastasize.[2] None of our pulmonary carcinoid cases had either regional or distant metastases or association with paraneoplastic syndromes.

Carcinoid tumors comprised 1.5% of all the lung cancers diagnosed in our institute whereas WHO reports <1% of all primary lung cancers as carcinoids.[2] We report a female to male ratio of 0.7:1 for carcinoid tumors which is in concordance with the study conducted in India by Thomas et al.[7] but is in contrast with other studies in the literature which report a slight female predominance.[8,9] Pulmonary carcinoids often occur in non-smokers[2] and similarly, none of our cases were associated with smoking. Our study showed a higher incidence of peripheral tumors (70%) with all cases of AC being peripherally located which is similar to some of the studies in the literature.[9,10] Similar to our study, Harpole et al. and Fink et al. reported a higher incidence of carcinoid tumors in peripheral locations whereas Thomas et al. identified more cases of centrally located carcinoids.[7,9,10]

On H&E, carcinoids are composed of uniform cells with finely granular nuclear chromatin and inconspicuous nucleoli arranged in organoid and trabecular patterns. AC is differentiated from TC by a higher mitotic activity (<2/10 hpf for TC and

2-10 mitoses/10 hpf for AC) and/or the presence of necrosis. Distinction based on mitotic counts is important as AC has a more aggressive course than TC. All the cases are usually positive for synaptophysin and chromogranin. The Ki-67 proliferative index does not find a place in the criteria given by Travis et al. for the diagnosis of carcinoids nor in distinguishing TC from AC.[2,11]

Small cell carcinomas (SCLC)

SCLC constituted 13% of all lung cancers in our institute which is in concordance with the study by Devessa et al.[12] SCLC, one of the most common pulmonary NETs, is also one of the most distinctive malignancies in the field of oncology with characteristic clinical properties, responsiveness to specific chemotherapy, genetic features and a highly reliable pathological diagnosis.[11]

SCLC occurs almost exclusively in smokers and the affected patients are typically older men.[2] 93% of our cases were smokers with a male to female ratio of 12:1 and the mean age at presentation was 68 years. While SCLC most commonly presents as a mass in the central airways[3], 57% of our cases presented as peripheral lung tumors and 26% presented as large central masses invading or compressing the mediastinum. 17% cases primarily presented with distant metastases. Presenting symptoms can be constitutional, pulmonary, related to extra-thoracic spread or due to paraneoplastic disorders.[11] While cough, dyspnea and hemoptysis were present in the majority of our cases (Table 1), one case showed extra-thoracic extension into the mediastinum.

On H&E, the cells are small in size with scant cytoplasm, finely granular nuclear chromatin with >10 mitoses/2mm^2 and frequent areas of necrosis. The Ki67 index in all our cases was >90%. Diagnostic difficulties can occur due to crush artifact, necrosis or paucity of material.[2] Lesions that can cause confusion in diagnosis include NSCLC, lymphoma, carcinoid or chronic inflammation.[2] IHC can be very helpful in this setting. In our study, IHC was performed in all cases to clinch the diagnosis. The neoplastic cells were also positive for CK and TTF1.

Large cell neuroendocrine carcinomas (LCNEC):

LCNEC was classified as a variant of large cell carcinoma in the previous WHO classification.[3] LCNEC is difficult to diagnose on small biopsies and this is the first WHO classification to provide standardized criteria and terminology for lung cancer diagnosis on small biopsies.[2] Non small-cell carcinomas (NSCC) with NE morphology and positive NE markers, are now called “possible” LCNEC on small biopsies according to the guidelines of the current classification.[2]

LCNECs are rare tumors of the lung; accounting for 3% of cases in surgically resected specimens.[13] In our institute, “possible” LCNECs accounted for 2% of all lung malignancies diagnosed on needle core biopsies during this study period.

LCNECs have more aggressive clinical behavior, are almost always associated with smoking and are rarely associated with paraneoplastic syndrome.[2] All our cases were associated with smoking (100%) and none had associated paraneoplastic syndrome. 64% of the LCNECs we encountered were peripheral in location, whereas WHO mentions a frequency of 84%.[2]

LCNECs are diagnosed according to the following criteria:[2] (i) A tumor with NE morphology, (ii) high mitotic rate: >10 mitoses per 2 mm^2 (median of 70 mitoses per 2 mm^2), (iii) necrosis (often in large zones), (iv) cytologic features of NSCC: large cell size (more than the size of 3 resting small lymphocytes), low nuclear-to-cytoplasmic ratio, vesicular nuclei, coarse or fine chromatin, and/or frequent nucleoli,abundant cytoplasm (v) positive immunohistochemical staining for one or more NE markers.

The Ki67 index varied from 50-70% in all our cases of LCNEC which is similar to the diagnostic criteria suggested by the 2015 WHO.[2] CK and TTF1 were also positive in all our cases of LCNEC.

CONCLUSION

We report the first series of the spectrum of pulmonary NETs from India based on the 2015 WHO classification of lung tumors on needle core biopsies. Small cell carcinoma was the most common pulmonary NET in our study.

IHC is recommended for all cases with suspected NE morphology as accurate histopathological diagnosis is imperative for appropriate therapy and optimal management of patients.

The role of IHC in differentiating NECs form NSCCs cannot be overemphasized when there are no clear adenocarcinoma or squamous features on morphology. The role of Ki-67 is mainly to differentiate high-grade SCLC and LCNEC from carcinoid tumors, especially in small biopsies with crushed tumor cells. The Ki67 labelling index is also an important prognostic marker for the oncologist to individualize patient care.

REFERENCES

1. Fisseler-Eckhoff A, Demes M. Neuroendocrine Tumors of the Lung. Cancers. 2012;4(3):777-798.
2. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. Lyon: International Agency for Research on Cancer, 2015.
3. Travis WD, Brambilla E, Muller-Hermelink HK et al. Pathology and Genetics: Tumours of the Lung, Pleura, Thymus and Heart. Lyon: IARC 2004.
4. Travis W, Brambilla E, Nicholson A, Yatabe Y, Austin J, Beasley M et al. The 2015 World Health Organization Classification of Lung Tumors. Journal of Thoracic Oncology 2015;10(9):1243-1260.
5. Maggard MA, O'Connell JB, Ko CY. Updated Population-Based Review of Carcinoid Tumors. Annals of Surgery. 2004;240(1):117-122.
6. Hendifar A, Marchevsky A, Tuli R. Neuroendocrine Tumors of the Lung: Current Challenges and Advances in the Diagnosis and Management of Well-Differentiated Disease. Journal of Thoracic Oncology. 2017;12(3):425-436.
7. Thomas R, Christopher DJ, Balamugesh T, Shah A. Clinico-pathologic study of pulmonary carcinoid tumours–A retrospective analysis and review of literature. Respiratory medicine. 2008;102(11):1611-4.
8. Hurt R, Bates M. Carcinoid tumors of the bronchus: a 33-year experience. Thorax. 1984; 39:617e23.
9. Harpole DH, Feldman JM, Buchanan S, Young WG, Wolfe WG. Bronchial carcinoid tumors: a retrospective analysis of 126 patients. Ann Thorac Surg. 1992;54:50e5.
10. Fink G, Krelbaum T, Yellin A, Bendayan D, Saute M, Glazer M, et al. Pulmonary carcinoid presentation, diagnosis, and outcome in 142 cases in Israel and review of 640 cases from the literature. Chest. 2001;119:1647e51.
11. W. D. Travis. Advances in neuroendocrine lung tumors. Ann Oncol. 2010;21(7):65-71.
12. Devessa SS, Bray F, Vizcaino AP, et al. International lung cancer trends by histologic type: male:female differences diminishing and adenocarcinoma rates rising. Int J Cancer. 2005; 117:294-9
13. Fasano M, Della Corte CM, Papaccio F, Ciardiello F, Morgillo F. Pulmonary large-cell neuroendocrine carcinoma: from epidemiology to therapy. Journal of Thoracic Oncology. 2015 Aug 31;10(8):1133-41.