

Proliferation Marker (Ki67) in Sub-Categorization of Neuroendocrine Tumours of the Lung

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ABSTRACT

Objective: The 2015 WHO classification classifies neuroendocrine tumours (NET) of the lung into typical carcinoid, atypical carcinoid, large cell neuroendocrine carcinoma and small cell carcinoma based on morphology alone. Mitosis is the major parameter for this classification, and thus several studies have focused on the role of Ki67 in these tumours but without conclusive results. The aim of the study was to categorize neuroendocrine tumours of the lung based on morphology and to assess the utility of Ki67 in diagnosis.

Material and Method: The study included 42 cases (23 biopsies and 19 lobectomy specimens) of neuroendocrine tumours (excluding small cell carcinoma). Haematoxylin & eosin stained sections, immunohistochemistry for neuroendocrine markers and Ki67 were studied.

Results: Based on WHO criteria, cases were classified as typical carcinoids (83.3%), atypical carcinoids (12%) and large cell neuroendocrine carcinomas (4.7%). The Ki67 index ranged between 1%-10% (mean 2.6%), 10%-30% (mean 19%), 35%-50% (mean 42.5%) in typical carcinoid, atypical carcinoid and large cell neuroendocrine carcinoma respectively. Using the ROC curve, the cut off value of Ki67 for typical and atypical carcinoids was 7.5% (P value<0.001), and for atypical carcinoid/large cell neuroendocrine carcinoma was 32.5% (P value=0.051). On comparing the size and infiltration pattern (both local and lymphovascular invasion) of tumours in resected specimens, there was no association with the proliferation index (P value >0.05).

Conclusion: Morphological features are the gold standard for subtyping of neuroendocrine tumours. Ki-67 is a potentially meaningful marker for sub-categorization of lung NETs, especially in small biopsies. However, the size and infiltrative pattern of the tumours are independent of the proliferation index.

Key Words: Ki67, Lung, Neuroendocrine tumours

INTRODUCTION

The lung is a common site of carcinoid tumours and accounts for 30% of well differentiated neuroendocrine tumours (NETs) in the body (1). The 2015 World Health Organization (WHO) classification recognizes 4 major types of neuroendocrine tumours (NETs) of the lung: typical carcinoid (TC), atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC) and small cell carcinoma (SCC). Excluding SCC, the remaining NETs constitute 5% of lung tumours (2). Currently, WHO classifies these tumours based on the morphologic features of which mitoses and necrosis are of paramount importance. Several studies have tried to evaluate the role of Ki67 similar to other organ sites such as the gastrointestinal tract and pancreas in diagnosing these tumours but without conclusive results. Since these subtypes of lung NETs have markedly different prognostic implications and treatment regimens, the importance of accurate pathologic diagnosis is underscored. The aim of this study was to assess the

utility of proliferation marker Ki67 in sub-categorization of these tumours (excluding SCC).

MATERIAL and METHODS

Forty two cases (23 biopsies and 19 lobectomy specimens) of NETs of the lung excluding SCC of lung were analyzed retrospectively from Jan 1999 to Aug 2014. Sections prepared from paraffin blocks were stained with hematoxylin and eosin and the morphological features were studied.

Morphology

Cases were classified based on morphological criteria used by 2015 WHO classification. Tumours with carcinoid morphology, <2 mitoses per 2mm² and lacking necrosis were classified as TC, those with carcinoid morphology and 2-10 mitoses per 2mm² or necrosis (often punctate) were categorized as AC. The tumours with neuroendocrine morphology, positive immunohistochemical staining for one or more NE markers (other than neuron-specific enolase), high mitotic rate: ≥11 per 2mm², necrosis (often

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Table I: Comparison of Age and M:F ratio in different tumour types.

Tumour type	Age			M:F	
	Range	Mean	Median		
TC	18-72	37.56	38	15.06	
AC	21-65	34.4	31	20.46	
LCNEC	52,60	56		5.66	
					Both males

TC: Typical carcinoid; AC: Atypical carcinoid, LCNEC: Large cell neuroendocrine carcinoma, M:Male, F:Female.

Table II: Values of Ki67 in different tumour types.

Tumour type	Range	Mean
TC	1-10	2.6
AC	10-30	19
LCNEC	35-50	42.5

TC: Typical carcinoid; AC: Atypical carcinoid, LCNEC: Large cell neuroendocrine carcinoma.

large zones) and cytologic features of a non-small-cell lung carcinoma i.e. large-cell size, low nuclear to cytoplasmic ratio, vesicular or fine chromatin, and/or frequent nucleoli were classified as LCNEC.

Immunohistochemistry

Neuroendocrine Markers: Immunohistochemistry for one of the neuroendocrine markers; chromogranin, synaptophysin and CD56 was done in all cases.

Proliferation Index: The Ki-67 proliferative index was determined in all cases on formalin-fixed paraffin-embedded tissue sections using a mouse anti-Ki-67 monoclonal antibody (1:50; clone MIB-1; Dako). Five hot spots i.e. areas of highest proliferation were counted. A total of 1000 cells in each spot were counted and results were expressed in percentage.

Statistical Analysis

Microsoft Excel was used for all the calculations. The Ki67 distribution among WHO 2015 categories (TC, AC, LCNEC) was investigated using non parametric test: the Kruskal-Wallis equality of population rank test and Mann-Whitney tests. Receiver Operating Characteristic (ROC) curve was used to decide the cutoff value of Ki67 in distinguishing these tumours with maximum sensitivities and specificities.

RESULTS

Clinico-Pathological Parameters

The patients were of an age group ranging from 18 to 72 years. Mean age was 37.56 (median 38) for TC, 34.40 (median 31) for AC and 56 years for LCNEC. Patients with LCNEC presented in the 5th and 6th decades whereas patients with AC and TC presented from the 2nd to 7th decades

(Table I). The male-female ratio was 1.15:1 (Table I). All the cases were showing neuroendocrine architecture-organoid nests, trabeculae and rosettes and all were positive for one or more neuroendocrine markers (CD56, synaptophysin and Chromogranin). Based upon morphology, of the 42 cases 35 (83.3%) were TC (Figure 1A-D), 5 (12%) were AC (Figure 2A) and 2 (4.7%) cases were LCNEC (Figure 3A-C).

Proliferation Index

Ki67 index ranged between 1%-10% (mean 2.6%), 10%-30% (mean 19%), and 35%-50% (mean 42.5%) in TC, AC and LCNEC respectively (Figure 1D, 2B, 3D respectively) (Table II). The Kruskal-Wallis equality of population rank test showed a statistically significant difference between the three categories of tumours with a P value <0.001. Using the Mann-Whitney test, a significant difference was found for pairwise comparison between TC and AC with a P value <0.001. However, the results were not found to be significant between AC and LCNEC with a P value 0.051. Using the ROC curve, the cut off value of Ki67 for typical/atypical carcinoids was 7.5% with sensitivity and specificity of 91.4% and 100% (area under curve is 0.9685) (Figure 4 A,B), whereas for atypical carcinoid/large cell neuroendocrine carcinoma, the cut off value was 32.5% with sensitivity and specificity of 100% (Figure 4C,D) (area under curve is 1). Overall the Ki67 cutoffs discriminated with optimal discrimination power between 3 classes.

Resection Specimens

The pneumonectomy/lobectomy specimens were available in 19 cases. Of these, three cases were AC and remaining 16 cases were TC. The size of the tumours ranged from 1 to 9 cm (in the largest dimension). On gross examination, all cases were well circumscribed except four cases (Figure 5). On microscopic examination, 9 cases had infiltrative margins, of which two showed lymphovascular involvement; both were TCs. Of the three ACs, two showed infiltrative margins. Lymph nodes (hilar/peribronchial/subcarinal) were available in 6 cases which were free of tumour. On comparing the size and infiltration pattern (both local and lymphovascular invasion), using the T-test and Pearson Correlation, there was no association with the proliferation index (P value>0.05) as well as tumour type.

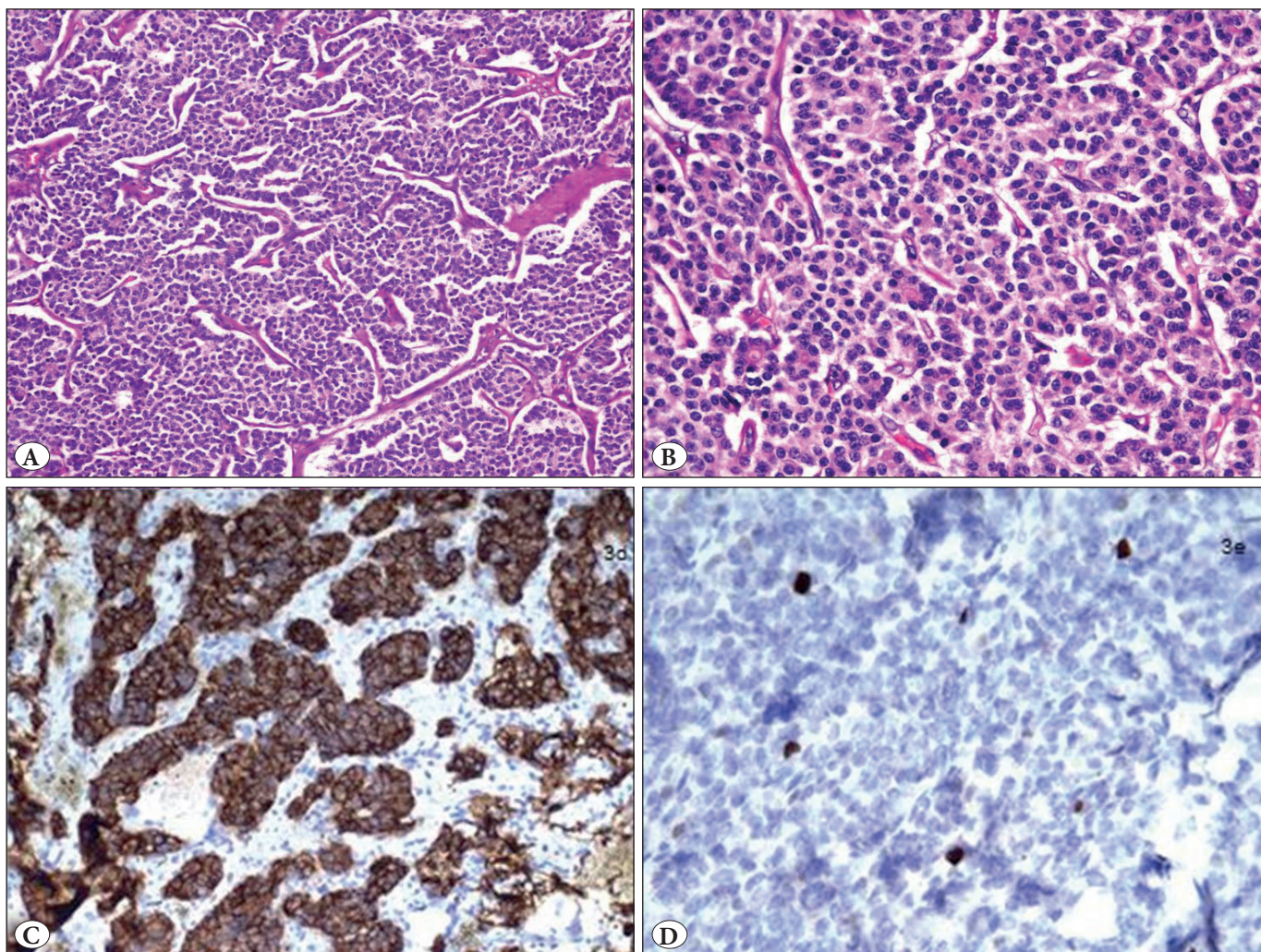


Figure 1: Typical Carcinoid. Photomicrographs showing A-B) Organoid pattern of arrangement (H&E; x100 and x200). C) Cytoplasmic positivity with chromogranin (IHC; x200). D) Ki67 staining nuclei of <1% of cells (IHC; x400).

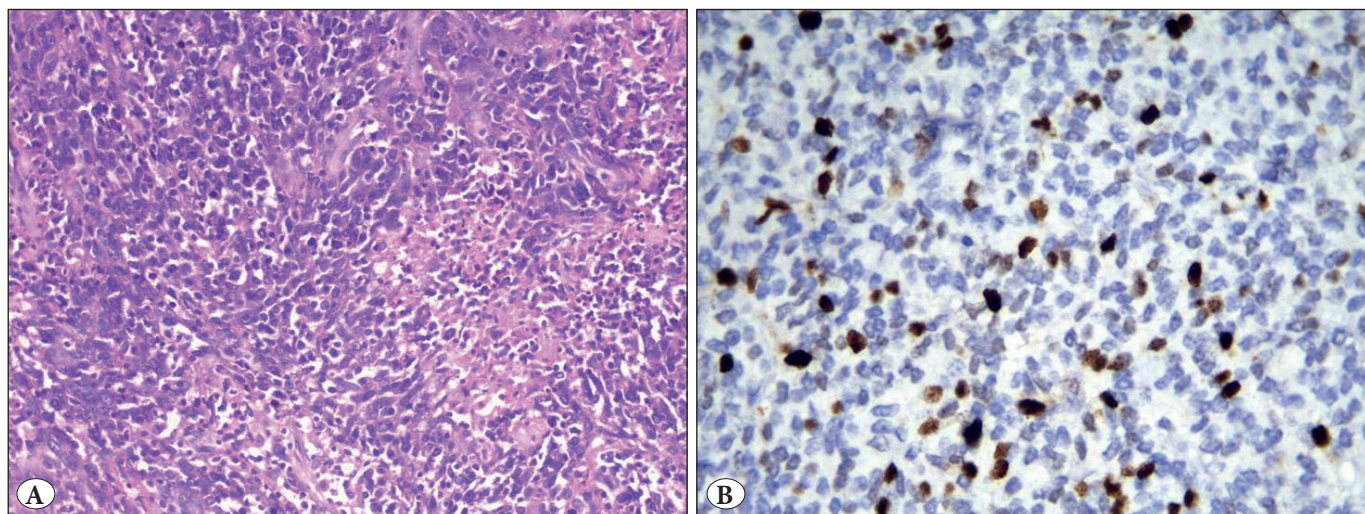


Figure 2: Atypical Carcinoid. A) Photomicrographs showing focal areas of necrosis. (H&E; x10X). B) Ki67 shows nuclear staining in 20% of the tumour cells (IHC; x40).

DISCUSSION

NETs of the lung include four different histologic subtypes as defined by WHO; Typical carcinoid (TC-1-2%), atypical carcinoid (AC-0.1-0.2%), large cell neuroendocrine carcinoma (LCNEC-3%) and small cell carcinoma (SCC-15-20%) (2,3). TCs are associated with a fairly benign behavior and are classified as low grade NET/Carcinoma (Grade 1), ACs are classified as intermediate grade tumour (Grade 2), whereas LCNEC and SCC are grouped together under high grade NET/Carcinoma (Grade 3) (2). Of the high grade tumours, the SCCs have distinct nuclear morphology posing less diagnostic problems, and thus were excluded from the study. The main concern lies in distinguishing low and intermediate grade tumours (TC/AC).

The WHO 2015 classifies these tumours based on morphological features only, which includes carcinoid

morphology, mitosis and/or necrosis. All these tumours have positive immunohistochemical staining for one or more NE markers. The difficulties in classifying these tumours arise in rare tumours that show mitotic activity just slightly or focally exceeding $2/2\text{mm}^2$ or $10/2\text{mm}^2$ which otherwise would qualify as AC or LCNEC respectively (4). Also, in small biopsies with extensive crush artifact it is difficult to count mitotic figures and when the amount of tumour tissue is limited (5,6). To overcome these difficulties, an effective grading system for digestive NETs was recently introduced by the European Neuroendocrine Tumour Society and endorsed by the WHO and the American Joint Cancer Committee (7). This system largely relies on the assessment of the proliferative marker Ki67 which proved accurate and predictive (8). Different studies have assessed the role of proliferation marker Ki67 in lung neuroendocrine tumours but the results are not conclusive (9,10).

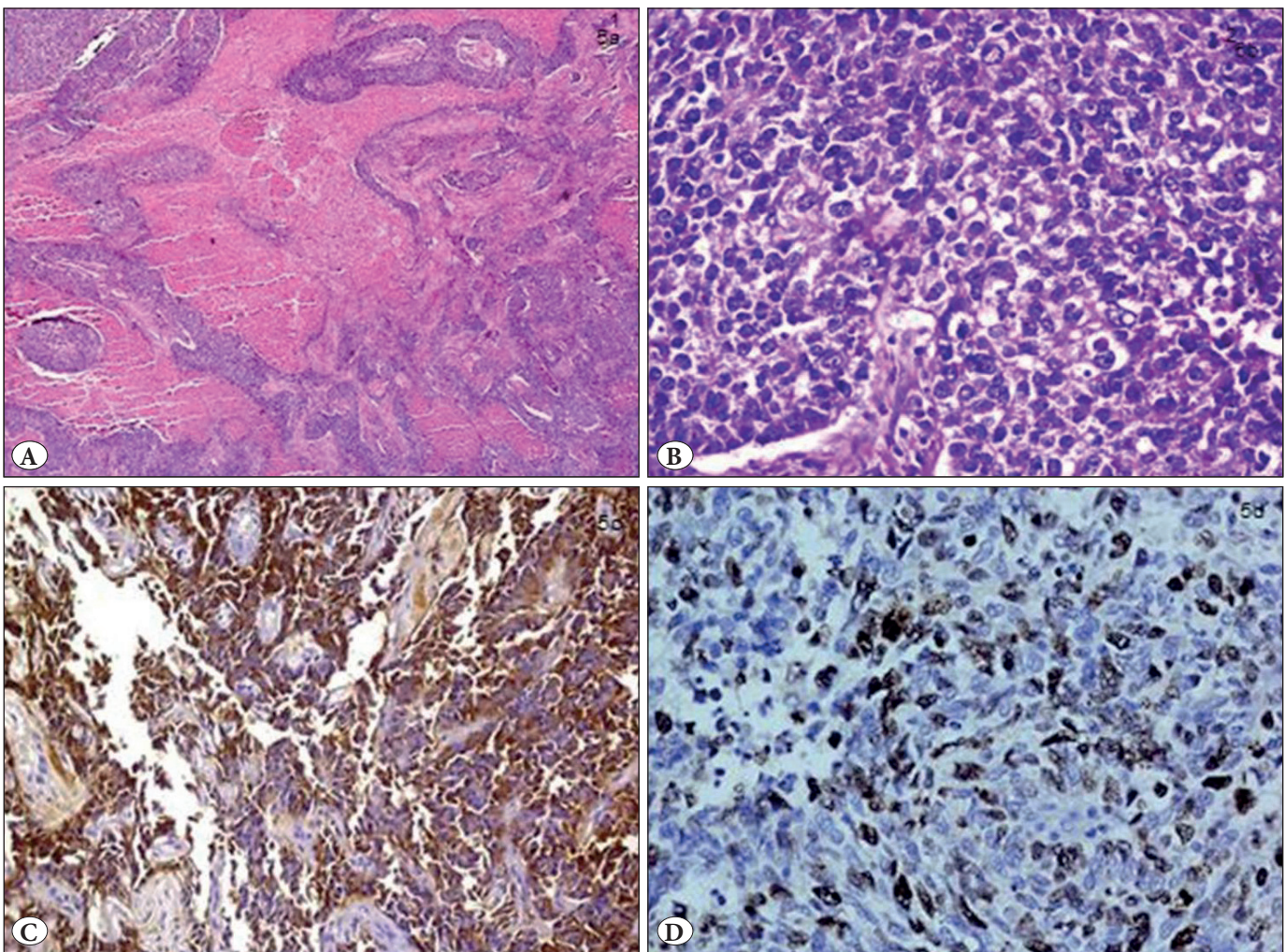


Figure 3: LCNEC. Photomicrographs showing A) extensive areas of necrosis with organoid pattern (H&E; x10). B) Large cell size, vesicular nucleus, prominent nucleolus and frequent mitosis (H&E; x40). C) Cytoplasmic positivity with synaptophysin (IHC; x20). D) Ki67 staining nuclei of 35% of cells (IHC; x40).

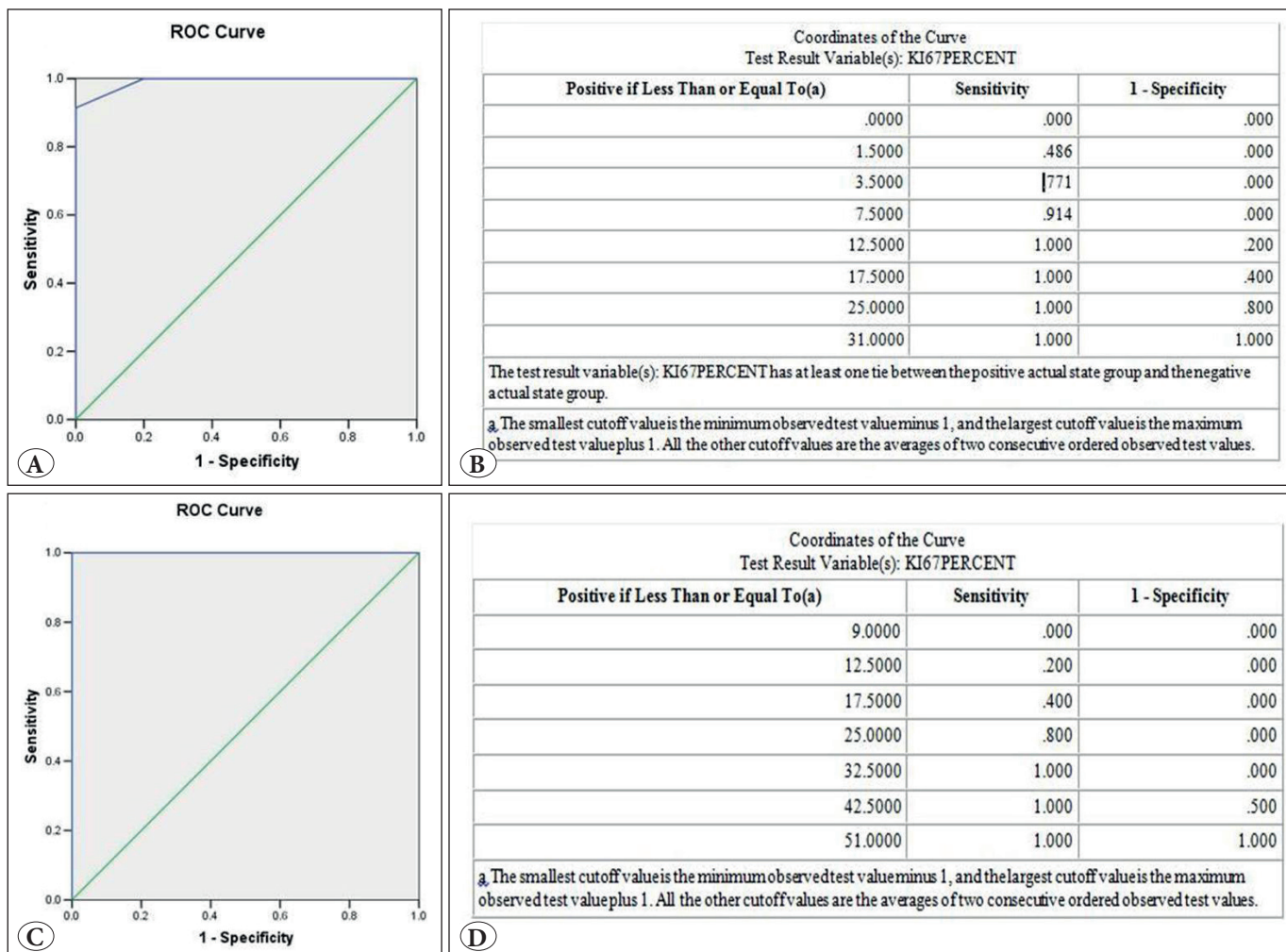


Figure 4: A-B) ROC-Ki67 cutoff values with different sensitivities and specificities for TC/AC. C-D) ROC-Ki67 cutoff values with different sensitivities and specificities for AC/LCNEC.

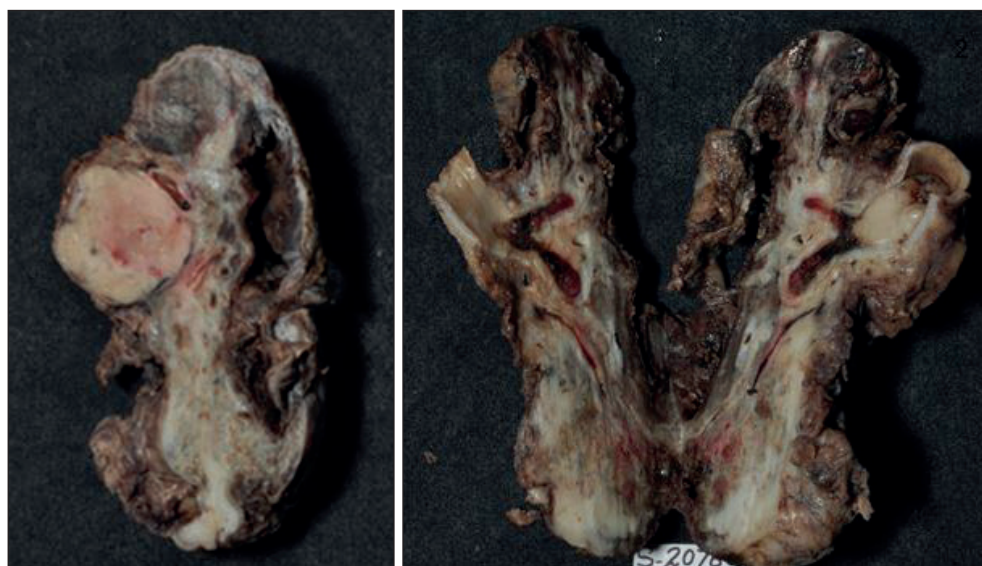


Figure 5: Gross photographs of endobronchial carcinoid tumour; a well circumscribed grayish yellow tumour.

Table III shows average Ki67 values across different studies; however, the results vary (11-14).

The mean values of Ki67 index in the present study are 2.6%, 19%, 42.5% in TC, AC and LCNEC respectively and the cutoff values by ROC curve analysis are 7.5% for TC/AC and 32.5% for AC/LCNEC. Of the 35 cases of TC, 3 cases have Ki67 > 7.5% (10%). The discrepancy in Ki67 index and mitotic count could also arise from the fact that the mitotic phase represents the smallest portion of the cell cycle and Ki-67 which detects cells from mid-G1 through S and G2 phases will detect proliferating cells that do not show mitotic figures. The mean Ki67 proliferative index significantly increases from TC to AC and poorly differentiated NETs (15). Ki67 is thus capable of distinguishing the 3 classes in a significant manner. However, in the present study, pair wise comparison between AC and LCNEC is not significant with a P value of 0.051. This was probably due to very low number of cases of each subtype.

Pelosi et al. (14) reported cases where TC and AC were over diagnosed as SCC on biopsy specimen. In such cases, the Ki67 labeling index is emerging as the most useful ancillary technique. It supports mitotic count to discriminate between low grade versus high grade tumours and also helps to distinguish these cases by immunohistochemical staining of cytological smears (16,17). It may reflect the tumour grade and predicts survival (18).

These tumours have different treatment modalities and survival rates, and a definite and accurate diagnosis is therefore necessary. Though TCs are low grade malignancies, they are capable of regional lymph node metastasis in 10-15% of the cases. ACs have 50% nodal and 20% distant metastases (19). Patients with TC, AC, LCNEC have a survival of 87%, 60% and 15-57% respectively (20-22). Surgery is the mainstay of the treatment of carcinoids. A study by Ducrocq et al. (23) showed that TCs have equivalent survival rate for limited resection versus lobectomy/pneumonectomy. A mediastinal lymph node dissection is recommended in clinical N0 central AC. Patients of AC with a consequent higher rate of nodal involvement and an adequate pulmonary reserve should receive lobectomy (24). Some reports showed that patients with ACs and regional lymph node metastasis have a high likelihood of developing recurrent disease, and if treated with surgical resection alone have a significantly worse outcome. Thus adjuvant treatment, chemotherapy or radiotherapy should be considered in such cases (25). Most LCNEC are poor candidates for surgical resection (14).

Table III: Comparison of average Ki67 values by different authors

Average values of Ki67 (%) by different authors			
	TC	AC	LCNEC
Igarashi et al, 2004 (11)	1.3	8.6	52.2
Zheng et al, 2013 (12)	3	7	60
Skov et al, 2010 (13)	1.8	4	25.5
Pelosi et al, 2005 (14)	2.3	9	47.5
Present study	2.6	19	42.5

To have accurate treatment modalities for these cases, accurate and early diagnosis is very important. Ki67 can act as an important supportive tool along with morphological features in classifying these tumours. However, extensive studies with larger case series to establish the appropriate cutoff values and follow up studies to establish its correlation with nodal and distant metastasis and overall survival are required in order to assess its validity.

In conclusion, morphological features are the gold standard for subtyping of neuroendocrine tumours. Ki-67 is a potentially meaningful marker for sub-categorization of lung NE tumours, especially in small biopsies. The mean values of Ki67 index in the present study were 2.6%, 19%, 42.5% in TC, AC and LCNEC respectively and the cutoff values for differentiating TC/AC and AC/LCNEC were 7.5% and 32.5% respectively. However, the size and infiltrative pattern of these tumours are independent of the proliferation index.

CONFLICT of INTEREST

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