Original Article

Pulmonary neuroendocrine carcinoid tumors: Is there a predictive role to the Ki 67 index?

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Abstract:

INTRODUCTION: There are several factors predicting evolution in carcinoid tumors (CT) to date including the Ki67 role.

AIMS: The aim of this study is to identify a KI67 cut-off point for a population of CT and determine its prognostic implication in global and disease-free survival.

METHODS: Hematoxylin-eosin slides of 102 CT were revised. The percentage of cells expressing Ki 67 was determined manually.

STATISTICAL ANALYSIS: The variables were compared with the *t*-test or the Wilcoxon test according to their distribution, the categorical ones with Chi-square or Fisher's test. The best cut-off point was established by constructing receiver operating characteristic curves, then using that value as a dichotomous variable.

RESULTS: 72 typical carcinoids (TC) and 30 atypical carcinoids (AC) were analyzed; 66% were female. Median age (TC 38 vs. AC 51, $P = 0.001$), Ki67 expression (TC 0.63 vs. AC 2, $P = 0.003$), tumor size (TC 2.5 vs. AC 2.6, $P = 0.001$), the percentage relapse (TC 3.4% vs. AC 23%, $P = 0.006$), and the number of deaths (TC 1 vs. AC 4, $P = 0.042$) were significantly higher in the AC subgroup. The best cut-off point for Ki 67 was 0.755 (area under the curve AUC 0.564, 95% confidence interval 0.270–0.857), with no significant differences found in the disease-free and overall survival curves when considering values < or ≥ at the established cut-off point. The best cut-off point of the Ki-67 when exclusively analyzing AC was 1.18. When using this value as a predictive variable, a marginal statistical association was observed between Ki-67 expression, mortality ($P = 0.077$), and the frequency of relapses ($P = 0.054$).

CONCLUSIONS: Histological type is the best predictor of prognosis in the carcinoid tumor group. In the AC subgroup, the marginal association between mortality, frequency of relapses and Ki values 67 ≥ 1.18 has clinical relevance future analyses are required to determine the real predictive value of this variable.

Keywords:

Ki 67 index, lung carcinoids, predictive value

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Neuroendocrine tumors of the lung (NET) are extremely rare and represent 1%–2% of all bronchopulmonary neoplasms. However, carcinoid tumors (CTs) (typical and atypical) represent 25% of the so-called well-differentiated NET.[1] Although they share morphological, ultrastructural, immunological, and molecular characteristics, their epidemiology, biological behavior, and evolution are markedly different.[1-5] The presence of necrosis and the mitotic count has been the fundamental references for the histological diagnosis between TC and atypical carcinoids (AC).[2] The Ki 67 expression index has been extremely useful

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in differentiating CTs) from small-cell lung cáncer (SCLC), however, the power of discrimination of this determination for the diagnostic among the subtypes of TCs has been underestimated.[3] MKI 67 is a nonhistone nuclear protein, which is present during the G1, S, and G2 phases of the cell cycle, never in G0.[4] Its identification with immunohistochemical techniques with the MIB1 (IgG1) antibody correlates with the degree of cell proliferation.

Given that, the biological behavior of CT is not homogeneous and assuming that the Ki 67 expression index is a surrogate for cell replication, it is possible that there is some cut-off point that can predict the clinical evolution of patients, especially in the AC group that have higher mortality and local relapse rate.

Aims

Identify a KI67 cut-off point for a cohort of surgically treated carcinoids tumors and determine their prognostic implication in overall and disease-free survival (DFS) in both histologic subgroups.

Methods

Retrospective cohort on 102 patients with a diagnosis of lung carcinoid tumor according to the WHO histological classification (2015) treated surgically from January 1981 to December 2018 at Hospital de Rehabilitación Respiratoria María Ferrer and Instituto Médico Especializado Alexander Fleming.

Pathological determination

Immunohistochemistry staining was performed on paraffin block sections of surgical specimens using previously diluted rabbit anti-Ki67 monoclonal antibody (Clone MIB-1) with positive and negative controls according to standard laboratory protocol. Positive nuclei were quantified in 2000 consecutive tumor cells in the areas of greatest activity (HOT SPOT) with a ×40. The percentage of cells that expressed Ki 67 was determined manually by two specialized pathologists (Clone MIB-1) with positive and negative controls according to the standard laboratory protocol.

Statistical analysis

Continuous variables were compared with the *t*-test or the Wilcoxon test according to their distribution, and the categorical variables with the Chi-square test or Fisher's exact test. For the analysis, the time to relapse or death related to the disease, not derived from other pathologies, was estimated. Logistic regression analysis was performed and receiver operating characteristic (ROC) curves were constructed to assess the general predictive capacity according to the histological subgroup, identifying the best cut-off point using the Youden index. Once this point was identified, the regression analyzes were repeated using the Ki-67 as a dichotomous variable (equal to or greater than the cut-off point versus less). All tests are two-tailed and a value of $P < 0.05$ was considered statistically significant. The analysis was performed with the R: A Language and Environment for Statistical Computing program version 3.5.1 (Vienna, Austria).

Results

General characteristics of the population

Seventy-two TC and 30 AC were analyzed; 66% of the cases were women and the median age was 43 years (range 30–58) [Table 1]. The median follow-up time reached 12 months with an interquartile range of 3.8–24 months. Subgroup analysis showed that patients with TC were younger (CT 38 vs. CA 51, $P = 0.001$), had smaller tumors (CT 2.5 vs. CA 2.6, $P = 0.001$), and had a percentage of relapses (CT 3.4% vs. CA 23%, $P = 0.006$) and disease-related deaths (CT 1 vs. CA 4, $P = 0.042$) significantly lower than AC. The DFS (CT 94.8% CA 18.9%, $P = 0.001$) and the 5-year survival (CT 97.1% vs. CA 53.8%, $P = 0.002$) were lower in the subgroup of AC and that difference was statistically significant [Table 1 and Figures 1, 2].

Type of surgery

We performed: 45 lobectomies, 30 lobectomies plus bronchoplastic procedures, 5 pneumonectomies, 1 extended pneumonectomy, 3 sleeve resections, 1 wedge resection, 1 segmentectomy, 1 exploratory thoracotomy and it was not possible to collect data in five patients. Due to the long time analyzed, there were significant modifications in the surgical technique: the traditional approach was thoracotomy from 1981 to January 2018, and the bronchoplasty, if necessary, were performed with separate points of monofilament absorbable material. From January 2018, the approach in all cases was video-assisted thoracoscopy (by one or two ports) in a systematic way, and the bronchoplastic procedure, if required, was performed with a continuous suture. The anatomical surgical extension was different between both subgroups, with a greater number of pneumonectomies being performed among the ACs ($P = 0.001$).

With the exception of one patient with several lung nodules whose surgical biopsy revealed the diagnosis of a carcinoid tumor and tumorlets, the rest of the tumors did not present with associated carcinoid syndrome. In this single case, who presented with severe postoperative bronchospasm, refractory to treatment with bronchodilators and who required mechanical ventilation, the treatment with somatostatin analogs controlled the symptoms and inhibited the radiological progression of the lesions.

Table 1: Patients features

Variables	Total	TC	AC	*P*
n	102	72	30	
Sex, female, *n* (%)	66 (64.7)	45 (62.5)	21 (70.0)	0.621
Age, median (IQR)*	43.5 (30.0-57.8)	38.0 (29.0-55.0)	51.0 (37.0-63.0)	<0.001
Smoke history, *n* (%)	25 (28.1)	15 (24.6)	10 (35.7)	0.406
Incidental finding, *n* (%)				
No	72 (72.7)	59 (81.9)	13 (48.1)	0.002
Yes	27 (27.3)	13 (18.1)	14 (51.9)	
Diagnostic procedures, *n* (%)**				
FBC alone	23 (26.4)	15 (23.8)	8 (33.3)	0.722
FBC with biopsy	54 (62.1)	40 (63.5)	14 (58.3)	
Others	10 (11.5)	8 (12.7)	2 (8.3)	
Follow up (median months, IQR)	12.0 (3.8-24.0)	12.0 (7.0-24.0)	12.0 (3.0-12.0)	<0.001
Tumoral size cm, median (IQR)	2.5 (1.8-3.5)	2.5 (1.8-3.5)	2.6 (1.7-3.5)	<0.001
Nodes metastases, *n* (%)	17 (17.5)	9 (13.0)	8 (28.6)	0.379
Perviascular invasion, *n* (%)	16 (19.0)	13 (20.3)	3 (15.0)	0.751
Ki-67, median (IQR)	0.86 (0.5-3.3)	0.63 (0.44-1.9)	2.0 (0.7-4.0)	0.003
Relapses, *n* (%)**	9 (10.1)	2 (3.4)	7 (23.3)	0.006
5 years disease free survival, % (CI 95%)	81.7 (70.5-94.7)	94.8 (88.0-0.00)	18.9 (3.6-100)	<0.001
Deaths, *n* (%)**	5 (5.6)	1 (1.7)	4 (13.3)	0.042
5 years survival, % (CI 95%)	90.2 (81.2-100)	97.1 (91.5-100)	53.8 (22.9-100)	0.002

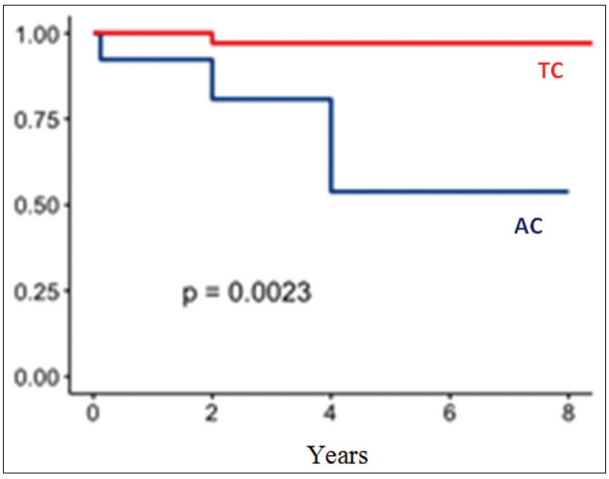

Figure 1: Overall survival according histologic type

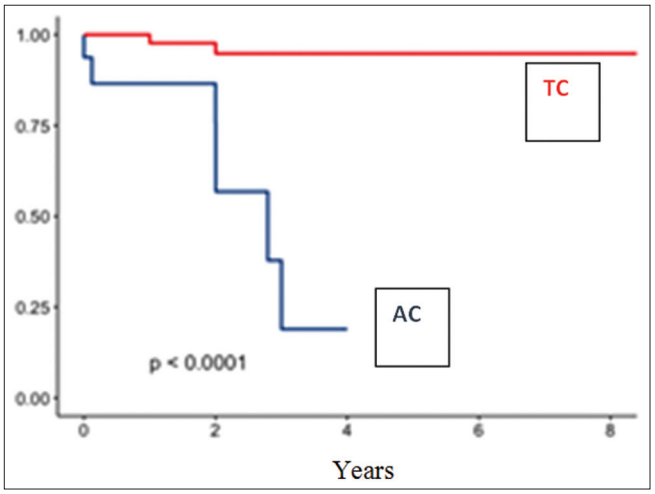

Figure 2: Disease-free survival according histologic type

Ki 67 index

The median percentage of Ki67 expression was statistically higher among AC (TC 0.63 vs. AC 2, $P = 0.003$). The best cut-off point to assess the prognostic value of Ki 67 expression was determined through the construction of a ROC curve and was 0.755 (area under curve 0.564, 95% confidence interval 0.270–0.857) [Figure 3]. However, the distribution of Ki 67 frequencies by histological subtype presented several points of overlap, which reflects the little discriminatory power of this determination in our population of CTs [Figure 4].

No statistically significant differences were found in the DFS and overall survival curves when considering values < or ≥ at the established cut-off point [Figures 5 and 6]. Because the number of events (both deaths and relapses) was higher in the AC subgroup, the comparative analysis of mortality and relapses was repeated for values ≤ or >0.755 in this histological subtype, finding no significant differences (deaths 6.3% vs. 33%, $P = 0.184$; relapses: 13.3% vs. 50%, $P = 0.115$) (data are not shown).

Given the adverse evolution of the AC group, we construct a new ROC curve only for this histological group. The best cut-off point of the Ki-67 when exclusively analyzing patients with AC was 1.18. Using this value as a predictive variable, a marginal statistical association was observed between Ki-67 expression and mortality ($P = 0.077$) as well as the frequency of relapses ($P = 0.054$) (data are not shown).

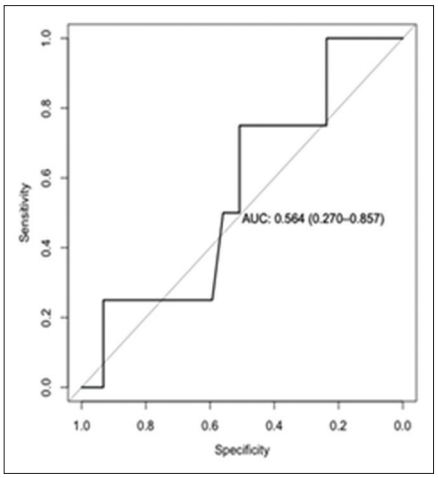

Figure 3: Receiver operating characteristic curve

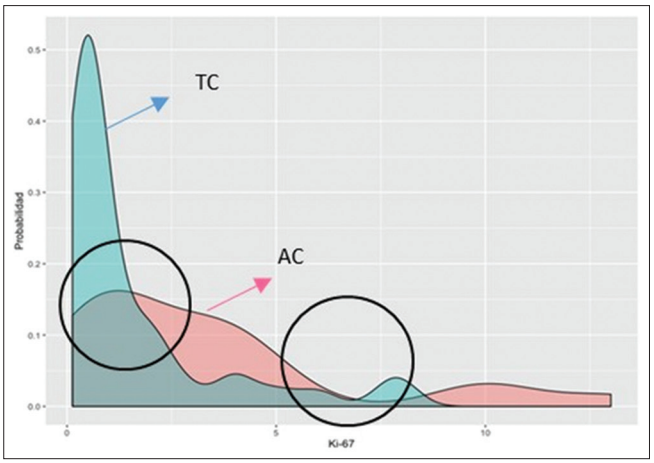

Figure 4: Ki 67 frequency chart by histology

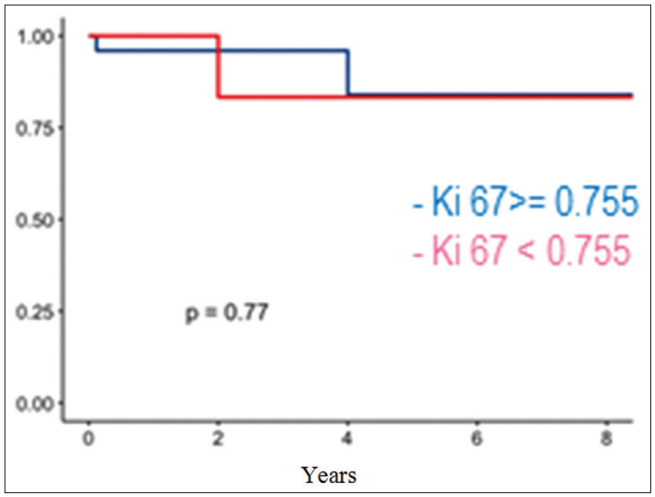

Figure 5: Overall survival according cut-off Ki 67 index

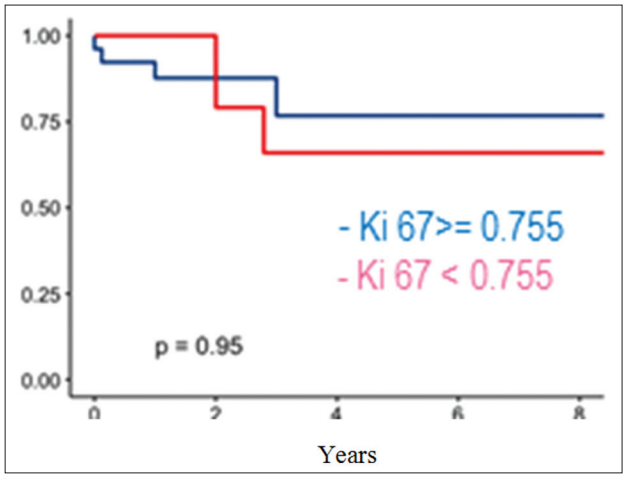

Figure 6: Disease-free survival according Ki 67 index

Discussion

According to the WHO (2015) classification, bronchopulmonary NET have been divided into two groups that include four histological categories. The *Well-differentiated group* comprises typical carcinoid (low-grade carcinoma) and AC (intermediate grade carcinoma). The high-grade tumors which comprise the large-cell neuroendocrine carcinomas (LCNEC) and SCLC are included in the *poorly differentiated group*.[3] Despite this classification, the Ki 67 expression index has not been useful for the differential diagnosis of lung carcinoids, although it allows discriminating high-grade tumors from those of intermediate grade, even in small biopsy samples.[3]

Traditional staging systems-classifying CT in anatomical stages and the classifications in histological grades have not been useful as predictive tools and present poor reproducibility for pulmonary CT.[4,5] In the 7th ed.ition of the TNM classification Travis *et al*.[6] studying the new categories of descriptors T, N, and M in the neuroendocrine lung tumors. The 1437 cases analyzed provided useful information to evaluate the extent of the disease in CT and differentiate the prognostic pattern of each of the stages, even without the need to discriminate the histological subtype. Although the number of cases analyzed is high, there was no possibility of reviewing the histological preparations with the WHO-2004 classification criteria, and other NET may be included in the analysis group. On the other hand, only 56 patients died from causes attributable to the tumor, and although survival curves fall as the stage increases, the total number of events analyzed is scarce, revealing the less aggressive biological behavior of these tumors. No conclusion could be drawn about the relationship between typical, AC and survival due to insufficient data.[6] To date, there is no evidence that the extent of the disease in patients with lung CT has prognostic value, given that these are slow-growing tumors, which will always be widely favored by surgical treatment.[7,8] In

our experience, the extension of resection, preferably pneumonectomies or extended pneumonectomies, has been performed more frequently in the group of patients with AC. Although by definition, NETs are characterized by having a central anatomical location, it is highlighted from our results the greater frequency with which this form of presentation has affected the AC subgroup and the fact that these tumors presented with hilar lesions making performing bronchoplastic procedures impossible.

Of the 102 patients analyzed, only one presented with carcinoid syndrome. This is generally observed in the presence of diffuse neuroendocrine cell hyperplasia (DIPNECH) and/or tumorlets.[9] DIPNECH is observed in patients without predisposing conditions (hypoxia, bronchiectasis, etc.), and has been associated with tumorlets and carcinoid tumors.[7] Only 5.4% of patients with a diagnosis of a carcinoid lung tumor present DIPNECHT/tumorlets, and they have generally peripheral lesions with a low degree of malignancy (TC).[9] Although our incidence is lower than published data (<1%), in the only patient who presented with symptoms, the diagnosis was made by thoracoscopic biopsy of one of the many peripheral lesions under another presumptive diagnostic.[10]

The role of Ki 67 has been pondered in gastrointestinal (GI) NET; however, there are controversial publications about its diagnostic and prognostic value in neuroendocrine lung tumors in general and among CT in particular. Several authors have highlighted histology, presence of lymph node metastases, and age as some variables of interest to be considered as prognostic factors.[11-15] Walt *et al*. particularly evaluated the role of Ki 67 expression and found that although the average index was significantly different (TC 3.7 vs. AC 18.8, $P < 0.001$), the distribution of frequencies by histological subtype showed overlap, and it could not be concluded that for the 5% cut-off point identified by them the KI 67 index had prognostic implications.[4] At the same time, Pelosi *et al*. concluded that CT should continue to be classified according to histology and that the Ki-67 analysis did not add relevant information due to the overlapping of values in the biologically close categories.[15] Similar findings emerge from our analysis.

Rindi *et al*. have proposed a new classification in three grades of pulmonary NETs, based on the mitotic count, percentage of necrosis, and Ki 67 expression in an attempt to standardize the classification used for GI-NET.[16] Based on a multicenter retrospective cohort of 399 cases, they included all NET of the lung surgically treated in three different centers in Italy and analyzed the expression of various clinicopathological variables, TNM staging (2010), the classification in degrees of the GI-NETs (ENETS/WHO 2010) and the Ki 67 index. All histological specimens were reviewed to assess the consistency of the diagnosis with the WHO 2004 histological classification. Unlike our findings, the distribution of Ki 67 was significantly different between TC versus AC, AC versus LCNEC and between LCNEC versus SCLC ($P < 0.001$). The specific cut-off point to differentiate TC from AC was established by constructing a ROC curve, and its value was > 3%, with an AUC of 0.71. New curves were constructed to establish cut-off points that distinguish AC of LCNEC (>20%) and LCNEC of SCLC (>60%). As the histological spectrum advances, the discriminative power of each cut-off point achieves better sensitivity and specificity values and greater discrimination power, with an excellent correlation between manual or computerized counting of this determination, both being considered the gold standard.[16] Because the variables of interest (mitosis, necrosis, and the Ki 67 index) exhibited high collinearity, the authors constructed five nonnested multivariate models, in which the mitotic count (>2; 2–47 or > 47), the necrosis percentage (0; <10; >10%) and Ki67 (<4%; 4_25%; ≥25%) expressed in tertiles allowed an adequate classification in three grades: G1, G2 or G3, according to which two out of three indicators belong to that level. This allowed all TC to be reclassified as G1, 29 AC as G1 and 45 as G2, 86 LCNEC were G2 and 78 G3 and of the 82 SCLC, 6 were G2 and 76 G3. This promising classification requires external validation, and according to current recommendations, the histological classification prevails, and the percentage of expression of Ki 67 must be reported.[17,18]

Despite the small number of patients in our series, the evolution of the 30 AC analyzed has been distinguished from their counterpart group, the TC, but also, by repeating the construction of a ROC curve for the AC, the cut-off point presented a marginal statistical relationship with the number of relapses and death, a result that may be conditioned by the paucity of the population analyzed, but which is clinically relevant. It is possible that the classification in degrees proposed by Rindi explains the heterogeneity in the behavior of tumors of the same histological type.

Joseph *et al*. evaluated the prognostic utility of Ki 67 in CT as a variable combined with mitotic count and tumor size on 48 patients treated consecutively for 25 years, adding a sub-analysis among those who presented with metastases versus the subgroup of nonmetastatic cases 19. Although Ki 67 expression was higher in those with a diagnosis of AC and in the subgroup of cases that presented with metastases, this difference did not reach statistical significance. In the univariate analysis, only the histology and the number of mitoses (>2/10 high-magnification fields) showed a significant relationship with the presence of metastases. Although

this statistical significance was still observed when combining the analysis with mitotic count or tumor size plus the Ki 67 index it is possible that, in both cases, these variables act as confounders factors of the relationship of Ki 67 index and metastatic disease, and this cannot be controlled by the absence of multivariate analysis.[19]

Our work has several limitations: It is retrospective and does not include all pulmonary NET, but focuses its analysis on the TCS subgroup. It is possible that the low number of cases have influence the results about the prognostic value of KI 67, especially in tumors of intermediate or high grade.

Conclusions

The best Ki 67 cut-off point of 0.755 has not had predictive value in the population analyzed. Histological type is the best predictor of prognosis in the carcinoid tumor group and AC had worse overall and DFS. When modifying the cut-off point of Ki 67 by constructing a new ROC curve it was observed that AC had statistically marginal association between mortality, frequency of relapses, and Ki 67 value ≥ 1.18.

Although these results do not present statistical significance, we consider that they present clinical relevance and future analyses are required to determine the real predictive value of this variable.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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