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Authors: La Rosa S, Volante M, Uccella S, Maragliano R, Rapa I, Rotolo N, Inzani F, Siciliani A, Granone P, Rindi G, Dominioni L, Capella C, Papotti M, Sessa F, Imperatori A

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ACTH-producing tumorlets and carcinoids of the lung. Clinico-pathologic study of 63 cases and review of the literature

Stefano La Rosa¹ (ORCID: 0000-0003-1941-2403), Marco Volante², Silvia Uccella³, Roberta Maragliano³, Ida Rapa², Nicola Rotolo⁴, Frediano Inzani⁵, Alessandra Siciliani^{6,7}, Pierluigi Granone⁶, Guido Rindi⁸, Lorenzo Dominioni⁴, Carlo Capella³, Mauro Papotti⁹, Fausto Sessa³, Andrea Imperatori⁴

¹Institute of Pathology, University Hospital and University of Lausanne, Lausanne, Switzerland

²Department of Oncology, University of Turin, San Luigi Hospital, Orbassano, Italy

³Pathology Unit, Department of Medicine and Surgery, University of Insubria, Varese, Italy

⁴Center for Thoracic Surgery, Department of Medicine and Surgery, University of Insubria, Varese, Italy

⁵ UOC Ginecopatologia e Patologia Mammaria, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy

⁶ UOC Chirurgia Toracica, Fondazione Policlinico Universitario A. Gemelli IRCCS-Università Cattolica del Sacro Cuore, Roma, Italy

⁷Division of Thoracic Surgery, Sant'Andrea Hospital, Faculty of Medicine and Psychology, University of Rome 'Sapienza', Roma, Italy

⁸UOC Anatomia Patologica, Fondazione Policlinico Universitario A. Gemelli IRCCS- Università Cattolica del Sacro Cuore, Roma, Italy

⁹Department of Oncology, University of Turin, City of Health and Science, Torino, Italy

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Corresponding author:

Stefano La Rosa
Institut Universitaire de Pathologie, CHUV
25 rue du Bugnon
CH-1011 Lausanne
Switzerland
Tel: +41 (0)21 3147162
Fax: +41 (0)21 3147205
e-mail: stefano.larosa@chuv.ch

Abstract

ACTH-secreting lung carcinoids represent the principal cause of ectopic Cushing syndrome, but the prevalence of ACTH expression and the association between ACTH production and Cushing syndrome in lung carcinoids have scarcely been investigated. In addition, available information on the prognostic meaning of ACTH production is controversial. The aims of this multicentric retrospective study, also including a review of the literature, were to describe the clinico-pathologic features of ACTH-producing lung carcinoids, to assess recurrence and specific survival rates, and to evaluate potential prognostic factors. To identify ACTH production in 254 unselected and radically resected lung carcinoids, we used a double approach including RT-PCR (mRNA encoding for pro-opiomelanocortin) and immunohistochemistry (antibodies against ACTH and β -endorphin). Sixty-three (24.8%) tumors produced ACTH and 11 of them (17.4%), representing 4.3% of the whole series, were associated with Cushing syndrome. The median follow-up time was 71 months. The 10-year overall and specific survival rates were 88.5% and 98.2%, respectively with no difference between functioning and nonfunctioning tumors, nor between ACTH-positive and ACTH-negative carcinoids. At univariate analysis, histological type (typical or atypical) and Ki67 index significantly correlated with tumor recurrence. The literature review identified 175 previously reported patients with functioning ACTH-secreting lung carcinoids and the meta-analysis of survival showed that 92% of them were alive after a mean follow-up time of 50 months. Our results demonstrate that ACTH-producing lung carcinoids are not rare, are not always associated with Cushing syndrome, and do not represent an aggressive variant of lung carcinoid.

Keywords: ACTH; adrenocorticotrophic hormone-producing lung carcinoid; Cushing syndrome; prognostic factors; Ki67 index

INTRODUCTION

The spectrum of lung neuroendocrine neoplasms includes neoplastic proliferations ranging from indolent tumorlet (TL) to low-grade typical carcinoid (TC), intermediate grade atypical carcinoid (AC), and high-grade small- and large-cell neuroendocrine carcinomas [1]. Pulmonary carcinoids have been considered for a long time as "benign" tumors due to their slow growth and excellent prognosis, with a 5-year survival of 85-100%. However, nowadays they are considered malignant since it is recognized that all carcinoids are potentially able to metastasize. Atypical histology (2-10 mitotic figures per 2mm² and/or presence of necrosis) and lymph node metastases have been confirmed to be negative prognostic factors [2-5]. More recently, the Ki67 proliferative index has emerged as a new promising prognostic marker [6, 7]. A recent consensus conference suggested a uniform terminology to classify neuroendocrine neoplasms at any anatomical location: well differentiated neoplasms, which include lung TCs and ACs, are defined neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine neoplasms are defined neuroendocrine carcinomas (NECs) [8]. TC corresponds to NET G1 and AC to NET G2. However, this approach is not currently officially integrated in the WHO classification of lung neuroendocrine neoplasms [1].

Some authors have considered that adrenocorticotrophic hormone (ACTH)-secreting carcinoid of the lung, associated with ectopic Cushing syndrome (CS), is an aggressive subtype of bronchopulmonary carcinoid [9-11], although this has not been confirmed by others [12-14]. Since ACTH-secreting carcinoids have mainly been described as single case reports or small series, the morphological, immunohistochemical, and clinical features of ACTH-expressing lung carcinoids are still not well established and several issues remain unresolved. In particular, the prevalence of ACTH-producing carcinoids among lung carcinoids is not known, as well as it is not clear whether the ACTH production is always associated with CS or not. Furthermore, the relationship between the presence of CS, or the simple expression of ACTH, and prognosis has still to be defined. In this context, we

have planned a multicentric retrospective study with the aim to describe the clinical, pathological, and immunohistochemical features of ACTH-producing lung carcinoids, to assess recurrence and specific survival rates of these patients and, eventually, to identify useful prognostic markers.

MATERIALS AND METHODS

Cases

The surgical pathology and clinical databases were retrospectively analyzed to identify lung TLs and carcinoids (TCs and ACs) resected between 1995 and 2011 in the following Italian institutions: ASST dei Sette Laghi/University of Insubria in Varese (68 cases), San Luigi Hospital/University of Turin in Orbassano (101 cases), and Policlinico Gemelli/Catholic University in Rome (85 Cases). Cases not resected with curative intent were excluded. In line with other studies [9-11], we decided to exclude patients who did not undergo radical resection for the following reasons: (i) to reduce confounding factors in the survival analysis, (ii) to standardize treatment (surgery) and stage, and (iii) to have optimal diagnostic material on which morphological, immunohistochemical and molecular evaluations could be performed without artifactual biases. We are aware that this choice may represent a limitation of the study in terms of the epidemiological description of the disease, as it may result in a significant loss of nonfunctioning ACTH-producing tumors. In addition, as unresectable cases belong to patients with the worst prognosis, the results of the survival analysis should be considered keeping in mind this methodological approach.

The following clinico-pathological data were collected: age, gender, symptoms at diagnosis, presence or absence of CS, type of lung surgery and of lymphadenectomy, tumor site and size, histologic type (TL, TC, AC), stage according to the 8th Edition of UICC TNM classification [15] and postoperative mortality. Follow-up data including tumor recurrence and patients' survival were

obtained from hospital medical files, from general physician or referring specialist, and from local Tumor Registry (when available). Patients were followed-up for at least 36 months after surgery.

Morphology

All tumor tissues were fixed in buffered formalin (formaldehyde 4% wt/vol and acetate buffer 0.05 M) and routinely processed to paraffin. All cases were stained with hematoxylin-eosin for the morphologic evaluations and were revised according to the diagnostic criteria proposed in the WHO classification of lung neoplasms published in 2015 [1]. Representative sections were selected for immunohistochemical and molecular studies.

Immunohistochemistry

For immunohistochemistry, 3- μ m-thick sections of all cases were mounted on poly-L-lysine-coated slides, deparaffinized, and hydrated through graded alcohols to water. After endogenous peroxidase activity inhibition, performed by dipping sections in 3% hydrogen peroxide for 10 minutes, incubation with primary antibodies (Table 1) was done at 4°C for 18 to 20 hours, followed by the avidin-biotin complex procedure. Immunoreactions were developed using 0.03% 3,3'-diaminobenzidine tetrahydrochloride, and then sections were counterstained with Harris hematoxylin.

RNA isolation and quantitative real-time PCR (RT-PCR)

Fifty-six cases positive for ACTH at immunohistochemistry with leftover tissue material for RNA extraction, as well as 88 unselected ACTH-negative cases were analyzed for ACTH precursor pro-opiomelanocortin (POMC) transcript expression by means of quantitative real time PCR. One sample of normal pituitary as well as a pool of four non-tumoral lung parenchyma samples were used as

controls. Ten μm -thick sections were cut in RNase-free conditions from paraffin embedded tissue blocks and representative tumor areas were identified and isolated from hematoxylin-eosin stained slides by means of stereomicroscopy-assisted microdissection using a scalpel. RNA isolation was performed by commercially available RNA extraction kits designed for paraffin material, according to the manufacturer's instructions (High Pure RNA paraffin kit; Roche Applied Science, Germany). Complementary DNA was transcribed using 500 mg/ml oligodT (Roche Applied Science) and M-MLV RT (200 U/ml; Invitrogen, Carlsbad; CA, USA) according to standard protocols. Primers for β -actin were previously reported [16]. For POMC, a commercially available TaqMan gene expression assay (HS01026050_m1; Applied Biosystems) was used according to the manufacturer's instructions. Expression levels for POMC and the internal reference gene (β -actin) were examined using a fluorescence-based real-time detection method (ABI PRISM 7900 Sequence Detection System—Taqman; Applied Biosystems, Foster City, CA, USA). To define POMC expression in individual tumors, the target gene expression levels were normalized to the internal reference gene as ratios (differences between the Ct values), and then compared to a calibrator (commercial total RNA; Stratagene, La Jolla, CA) using the $2^{-\Delta\Delta\text{Ct}}$ formula.

Statistical analysis

Descriptive statistics were performed to investigate the sample characteristics; mean (\pm standard deviation, SD) or median (with interquartile range, IQR) were chosen to summarize continuous variables, while absolute and relative frequencies (no., %) were used for categorical variables. These variables were compared by χ^2 test or Fischer's exact test. Comparison between groups was made using Student's t test or the Mann–Whitney U test for continuous variables. Overall, specific and disease-free survival (DFS) were investigated by Kaplan–Meier method and log-rank test was applied to compare survival curves according to clinico-pathological factors. Univariate Cox proportional-

hazards regression was performed to estimate the hazard ratios of selected potentially prognostic factors. We included all factors into the multivariable model with a p-value <0.20 from the univariate model. Differences were considered statistically significant with a p-value <0.05. Statistical analysis was performed with MedCalc Statistical Software version 18.5 (MedCalc Software bvba, Ostend, Belgium). Correlation between ACTH mRNA and protein expression was determined using the Spearman's correlation test.

Review of the Literature

The PubMed database of the National Center for Biotechnology Information (NCBI) of the US National Library of Medicine was searched using the following string: ACTH-secreting [AND/OR] ACTH-producing lung carcinoid [AND/OR] lung neuroendocrine neoplasm. All articles written in English were included. In addition, since a heterogeneous terminology has been used during the last years to define neuroendocrine neoplasms of the body, we revised the references lists of each paper selected in the PubMed database, with the aim to reduce this risk of missing cases not appropriately defined. For each identified article, the reported cases were singularly identified and the following parameters were considered: sex, age, uni- or multifocality, location within the lungs (laterality, lobe, central or peripheral), presence and type of hormone hypersecretion syndrome, site, size, presence of metastases, type (TC or AC), presence/absence of adrenal hypertrophy, serum ACTH level, Ki67-related index, and follow-up data.

RESULTS

Clinico-pathological and surgical features

A total of 254 well-differentiated neuroendocrine neoplasms of the lung were retrieved from institutional archives. According to the WHO classification they were subdivided as follows [1]: 12

TLs, 200 TCs, and 42 ACs. Among the 254 selected cases, 63 (24.8%) were ACTH positive (Table 2). Eleven out of these 63 cases (17.5%) were functioning neoplasms associated with CS (8 TCs, 3 ACs). These patients were significantly younger (40 ± 14 vs 63 ± 11 years, $p < 0.001$) and slightly more frequently of male sex (55% vs 27%, $p: 0.088$) than the 52 patients with nonfunctioning ACTH-expressing tumorlets/carcinoids. The vast majority of nonfunctioning ACTH-producing tumorlets/carcinoids (89%) was incidentally found whilst all the 11 patients with functioning ACTH-producing carcinoids presented with symptoms related to ectopic ACTH-dependent CS, which was confirmed by the clinico-biological workup. In all 11 CS-associated carcinoids, the presence of a corticotroph pituitary adenoma or other ectopic ACTH-secreting tumors was excluded by imaging analyses.

The surgical approach was substantially homogeneous among the three centers; forty-eight patients had lobectomy, 2 pneumonectomy, and 2 segmentectomy; eleven patients underwent a non-anatomic lung wedge resection. Ipsilateral radical hilar and mediastinal lymph node dissection was performed in 86% of cases, namely in all CS patients and in 83% of nonfunctioning ACTH-producing tumorlets/carcinoids. There were no postoperative deaths.

ACTH-producing carcinoids were relatively small (mean diameter + SD: 2.1 ± 1.2 cm) with no differences between functioning and nonfunctioning cases (Table 2). The majority of patients (82.5%) presented with stage I disease. However, stage distribution was significantly different ($p: 0.003$) between functioning and nonfunctioning cases with stage I in 45.5% and 90% of cases, respectively. Indeed, among CS cases, five patients had lymph node metastases and one was staged pT3, due to the presence of two tumors in the same lobe. Three out of eleven (27.3%) functioning carcinoids were multifocal, as compared to 7/52 (13.5%) nonfunctioning tumors ($p: 0.49$).

The histopathological review confirmed the diagnosis of well-differentiated neuroendocrine tumors in all cases. Mean mitotic count was < 1 mitosis $\times 2\text{mm}^2$ without any statistical difference

between functioning and nonfunctioning carcinoids. Vascular invasion was detected in 17 cases: 3 functioning (1 TC and 2 ACs) and 14 nonfunctioning (9TCs and 5 ACs).

Immunohistochemical results

Immunohistochemical results are summarized in Table 3 and showed in Figure 1. Functioning ACTH carcinoids had a significantly higher median percentage of ACTH-immunoreactive cells than nonfunctioning cases (60%, IQR 14-99% vs 4%, IQR 2-11% respectively; $p < 0.001$). However, among CS-associated ACTH-producing carcinoids three cases showed very low percentage of ACTH-positive cells ranging from 2% to 5% of cells. In 56 cases with available tumor tissue, we also investigated the expression of β -endorphin, which is one of the protein products deriving from the POMC cleavage and is supposed to be co-expressed with ACTH. Immunoreactivity for β -endorphin was observed in 51/56 (91%) cases, including 9/10 (90%) functioning and 42/46 (91%) nonfunctioning tumors. The mean percentage of β -endorphin immunoreactive cells was 14.9% (48.5% in functioning and 7.6% in nonfunctioning tumors; $p: 0.005$). TTF1 was expressed in 15/52 (28%) ACTH-producing tumorlets/carcinoids, namely 6/10 (60%) functioning and 9/42 (21.4%) nonfunctioning cases ($p: 0.04$).

Since galectin 3, BCL10, and CD117 have been identified in pituitary corticotroph adenomas [17-19] we investigated their expression in our series of ACTH-producing tumorlets/carcinoids. Galectin 3 immunoreactivity was found in 4/10 (40%) functioning and 7/47 (15%) nonfunctioning cases ($p: 0.17$). CD117 expression was substantially negative in all the 57 cases investigated (10 functioning and 47 nonfunctioning) except in two nonfunctioning typical carcinoids showing less than 10% of positive cells. All tumorlets and carcinoids investigated were BCL10 negative.

The median Ki67% proliferative index in all series was 2.7% and it was statistically higher in CS-associated carcinoids than in nonfunctioning ones (4.9%, IQR 1-5% vs 2.1%, IQR 1-2% respectively; p: 0.038).

Quantitative RT-PCR

Of the 56 tested cases, three were excluded due to a poor RNA quality. Of the remaining 53 cases, POMC mRNA was heterogeneously expressed in a range from 0 to 55.800-fold changes as compared to calibrator (control values of fold changes of control tissues vs calibrator: pooled non-tumoral lung parenchyma 27.3, normal pituitary 39.367). Indeed, POMC mRNA and ACTH protein expression were significantly and positively correlated each other (Spearman r value 0.65, $p < 0.0001$). All ACTH-negative cases at immunohistochemistry showed no significant POMC mRNA expression, with a single case with detectable POMC transcript expression significantly below the levels of pooled non-tumoral lung parenchyma (Figure 2).

Follow-up and prognostic factors

Follow-up information was available for 60 out 63 (95.2%) cases and the median follow-up time was 71 months (95% confidence interval, 55-101). Five patients (2 with functioning and 3 with nonfunctioning ACTH-producing neoplasms) had tumor recurrence. Two patients died of disease: one patient with a nonfunctioning ACTH-producing atypical carcinoid with Ki67 index of 15% presented early recurrence and died at 12 months, whereas the other patient, who had a typical carcinoid associated with CS with a Ki67 index of 2%, showed liver and bone metastases at 264 months from surgery and died after 272 months. The 10-year overall survival for the whole cohort was 88.5%. The specific survival rate for carcinoids was 98.2% without statistically significant difference between functioning and nonfunctioning tumors. The 10-year disease free survival was

94.6%: 90% in CS patients and 95.7% in nonfunctioning tumors (p: 0.234) (Figure 3). In addition, no statistically significant different overall and disease-free survival was observed between ACTH-positive and ACTH-negative carcinoids (Figure 4).

At univariate Cox proportional-hazards regression analysis, recurrence was significantly correlated with histologic type (p: 0.029) and with increasing Ki67 proliferative index (p: 0.002) (Table 4). At multivariable analysis, only Ki67 index expression slightly parallels with tumor recurrence (p: 0.072) (Table 5). Interestingly, the presence of CS did not correlate with worse prognosis, neither with tumor recurrence nor with patient outcome.

Review of the Literature

Eighty-three articles, published between 1961 and 2018, were retrieved from the PubMed database. Fifty-eight were single case reports and the remaining 25 were small series. A total of 183 CS-associated carcinoids, tumorlets and diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (including our 11 cases) have been reported and the relative details are listed in Supplemental Table 1, whereas Table 6 summarizes their clinico-pathological features. All cases were diagnosed in the setting of a CS. The mean age at diagnosis was 42 years with a slight prevalence in females. Most cases were single tumors with a mean diameter of 1.5 cm, more frequently peripheral, right located, and not associated with metastases. The average time between the clinical onset of CS-related symptoms and tumor diagnosis was 19 months. Follow-up information was available for 125 patients and 115 of them (92%) were alive after a mean follow-up time of 50 months.

DISCUSSION

ACTH is a 39-amino-acid peptide hormone, which stimulates adrenal cortex with consequent secretion of glucocorticoid. Functionally active ACTH peptide derives from the cleavage of a larger precursor protein called pro-opiomelanocortin (POMC). The tissue-specific post-translational processing of POMC protein at different cleavage sites results in a number of biologically active hormone peptides, including ACTH, β -LPH, and the amino terminal fragment of POMC. In animals with a well-developed intermediate lobe of the pituitary gland (e.g. rats) further cleavage of ACTH gives rise to α -MSH and CLIP (corticotrophin-like intermediate lobe peptide), a process not observed in humans where these two peptides are not secreted as separate hormones. Within the sequence of β -LPH, the amino acid sequence γ -LPH and β -endorphin are found, which contain the sequences of β -MSH and met-enkephalin, respectively (Figure 5).

To demonstrate the production of ACTH in our series of lung TLs and carcinoids we used a double approach, including RT-PCR to detect the mRNA encoding for POMC and immunohistochemistry to identify the expression of its derivate proteins ACTH and β -endorphin (Figure 5). Although immunohistochemical expression of these two proteins is not *per se* indicative of POMC cleavage into biologically active peptides, it demonstrates their production by tumor cells. Using this double approach, we were able to identify 63 ACTH-producing TLs/carcinoids in a series of 254 cases, with a prevalence of 24.8%. Interestingly, we observed that the majority of ACTH-expressing TL/carcinoids was not associated with CS. Based on this finding, we can state that ACTH-expressing lung neuroendocrine tumors can be either functioning - as it has been well known for more than 50 years [20] - or nonfunctioning. This feature parallels the clinical behavior of pituitary corticotroph adenomas, which, in turn, can be either functioning or nonfunctioning (silent) [21], whereas this phenomenon is very rare in pancreatic ACTH-producing neuroendocrine tumors, which are frequently associated with CS and aggressive behavior [22]. Intriguingly, although CS-associated carcinoids showed, as a group, a higher percentage of ACTH-expressing neoplastic cells than

nonfunctioning tumors, the functional status of some carcinoids was unrelated to the number of ACTH-immunoreactive cells. Indeed, we found some functioning carcinoids with a very low percentage of ACTH-positive cells and some clinically silent tumors with a number of immunoreactive cells comparable to the ones with CS. The lack of correspondence between the immunohistochemical expression of ACTH and the biological activity observed in these tumors can be explained by four hypotheses: i) the hormonal product secreted by neoplastic cells of nonfunctioning tumors may be biologically inactive, despite being immunohistochemically detectable; ii) the anti-ACTH antibody may identify the non-cleaved and non-biologically active ACTH sequence present into the longer POMC precursor; iii) in functioning tumors the apparently low ACTH expression in neoplastic cells may be due either to rapid hormone release or (iv) to the low sensitivity of the antibodies employed, as recently demonstrated in functioning ACTH-secreting pancreatic neoplasms [22]. The last hypothesis regards the clinical presentation of CS; indeed, depending on the amounts of glucocorticoids secreted, the clinical spectrum of CS ranges from slightly attenuated diurnal cortisol rhythm to complete full-blown syndrome. Patients with subclinical CS lack the classical stigmata of hypercortisolism and can be difficult to identify, especially when ACTH is ectopically secreted [23]. This clinical situation may represent a limit of our study because we cannot totally exclude the possibility that in our series of nonfunctioning ACTH-producing carcinoids there were some cases of subclinical CS.

In line with previously reported data [10], we observed that patients with CS-related carcinoids had a younger age at diagnosis, in contrast to patients with nonfunctioning tumors. This could be related to an anticipation of the diagnosis, due to the presence of clinical signs and symptoms of hypercortisolism. Lymph node metastases were significantly more frequent in CS-related carcinoids than in nonfunctioning ones, suggesting a higher metastatic potential of functioning tumors. By contrast, tumor location (peripheral or central), tumor size, and histological

subtyping (typical and atypical carcinoid) were not statistically different between functioning and nonfunctioning ACTH-producing carcinoids. Even though functioning tumors showed a higher rate of lymph node metastases, a lower percentage of stage I tumors, and a higher Ki67 proliferative index than nonfunctioning ACTH-producing carcinoids, they still showed a high cure rate. Moreover, both disease-specific and disease-free survival rates were not significantly different between the two groups. Indeed, we did not observe statistically significant different survival between functioning and nonfunctioning ACTH-producing cases as well as between ACTH-positive and ACTH-negative carcinoids, is in line with previously reported findings [12-14] although this observation was not confirmed by others [9-11].

Among the various clinico-pathological parameters analyzed, only the histological subtype and the Ki67 proliferative index were prognosticators for tumor recurrence in ACTH-producing carcinoids. This result further increases the burden of evidence supporting the prognostic role of Ki67-related proliferation index in bronchopulmonary NENs [24].

The literature review allowed us to identify 172 ACTH-producing lung carcinoids, two tumorlets and one diffuse idiopathic pulmonary neuroendocrine cell hyperplasia associated with CS. In all these cases other sources of ACTH hypersecretion (e.g. pituitary adenomas) have been excluded. Thus, when we add our 11 CS-associated ACTH-producing carcinoids, a total of 183 carcinoids have been reported until now. The clinico-pathological features of the cases reported in literature depict the physiognomy of an indolent entity, showing no significant difference, in terms of patients' outcome, when compared with silent ACTH-producing carcinoids and with ACTH-negative carcinoids, despite the presence of aggressive clinico-pathological features is somewhat more frequent in CS-associated carcinoids.

In conclusion, this study demonstrates that ACTH expression is not a rare event in lung carcinoids and it is not necessarily associated with signs and symptoms of hypercortisolism. Although

CS-associated carcinoids present higher tumor stage and higher Ki67 proliferative index than nonfunctioning ACTH-producing carcinoids, they do not represent an aggressive variant of well-differentiated neuroendocrine lung tumor.

Author Contribution: All authors have made a substantial contribution to conception and execution of the study including acquisition of data, processing material, analysis and interpretation of data, as well as writing and reviewing the manuscript. All authors have approved the manuscript.

Compliance with ethical standard: This study was performed according to the clinical standards of the 1975 and 1983 Declaration of Helsinki and was approved by the Ethical Committee of the ASST dei Sette Laghi.

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Conflict of Interest: The authors declare no conflicts of interest

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FIGURE LEGENDS

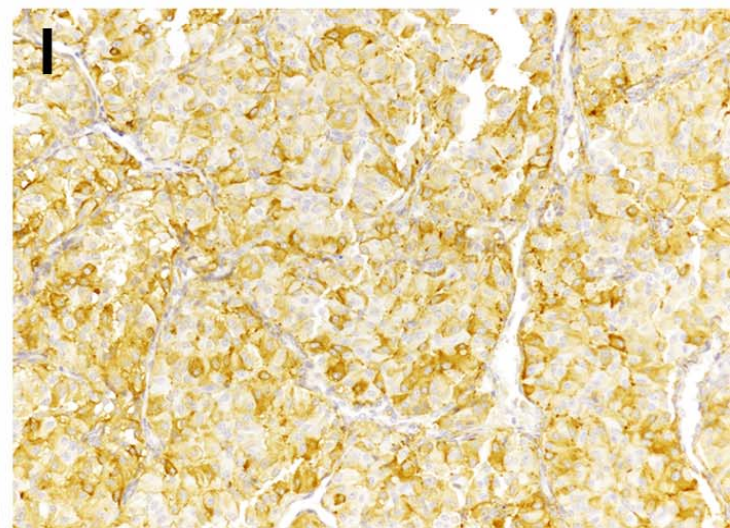
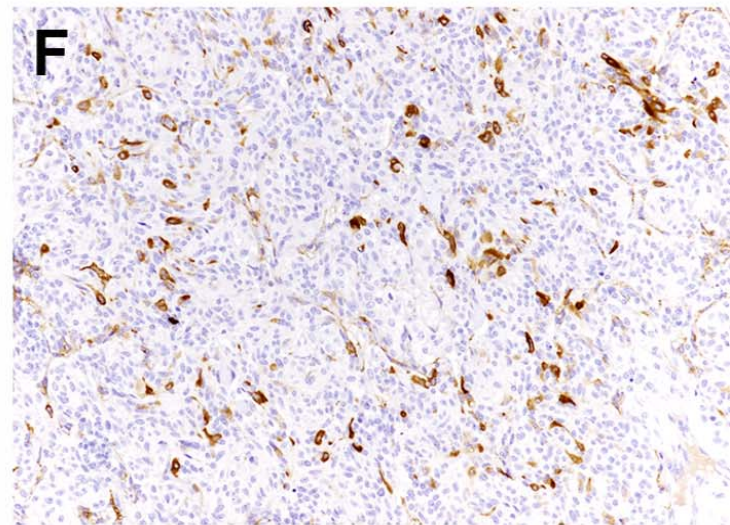
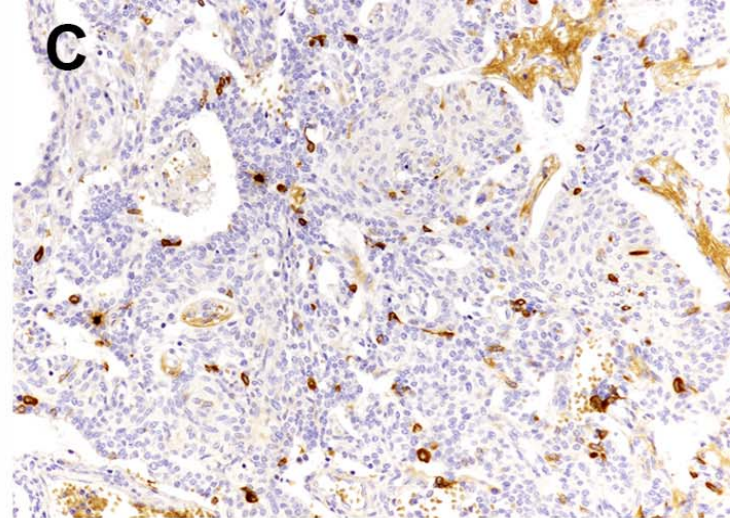
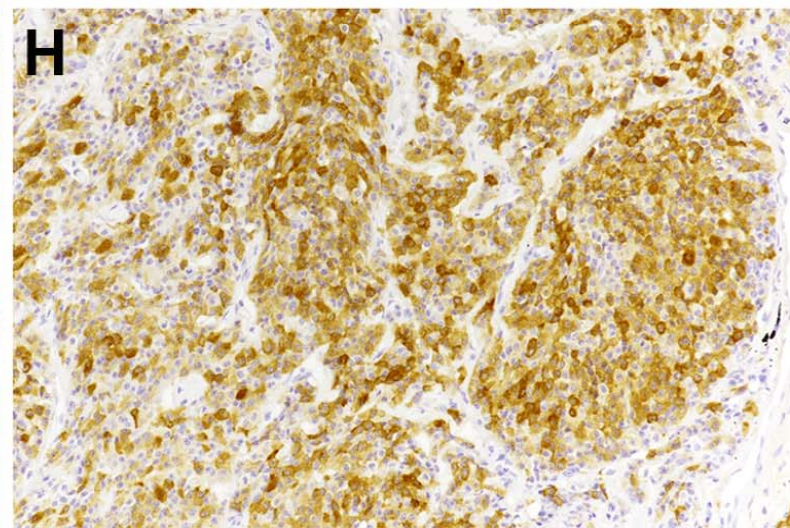
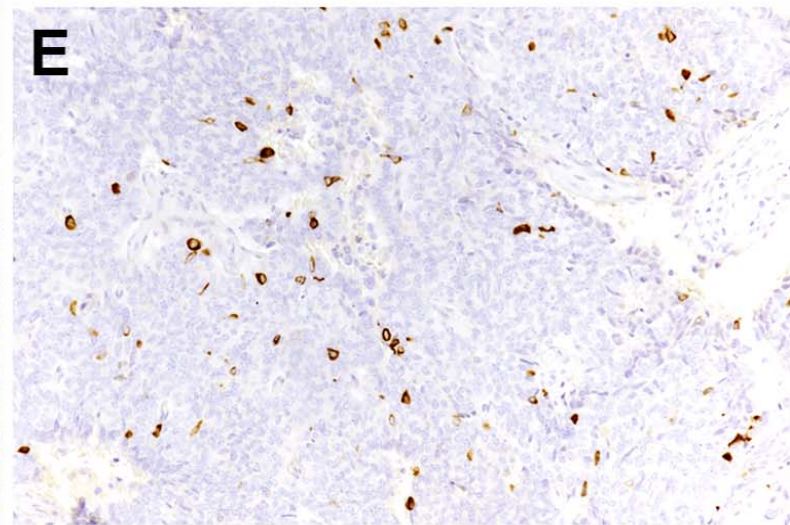
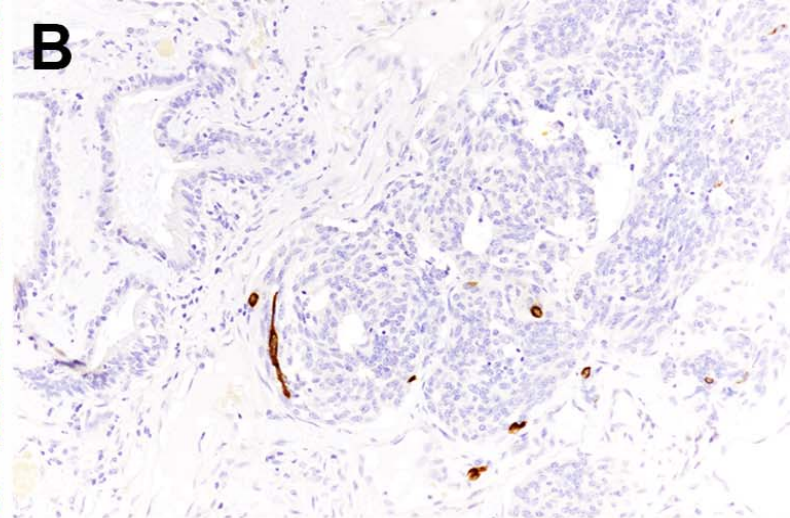
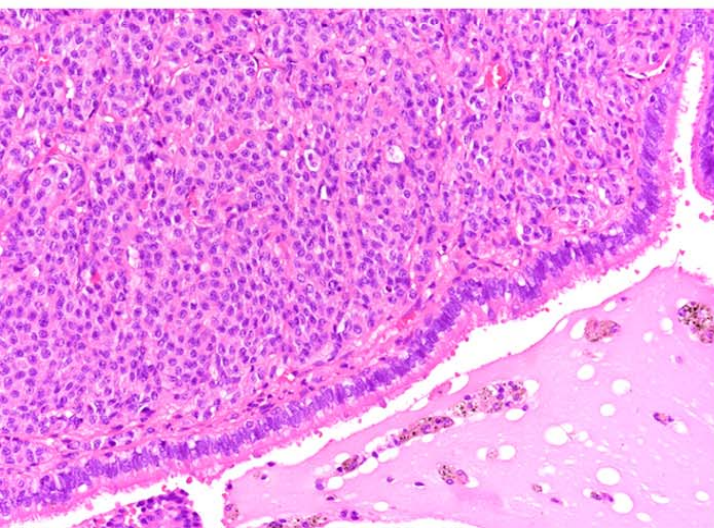
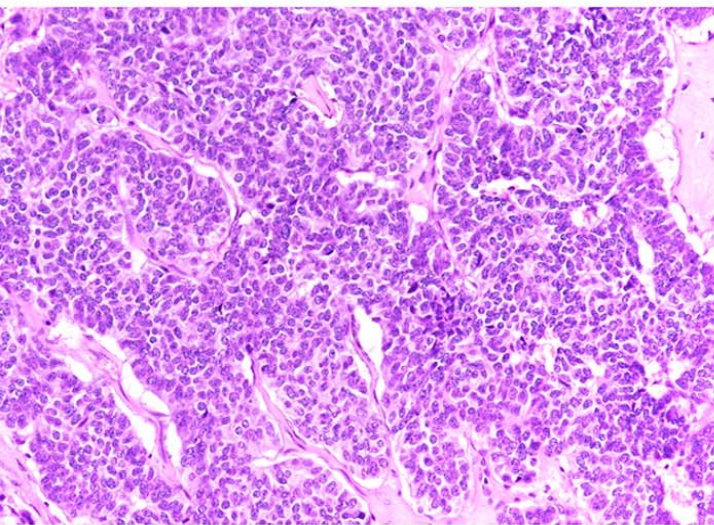
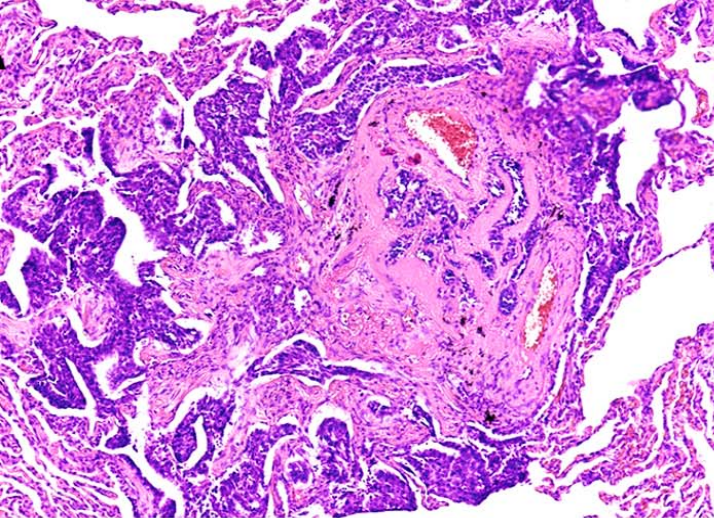
Figure 1. Nonfunctioning tumorlets (A-C) and nonfunctioning carcinoids (D-F) generally show a lower percentage of ACTH positive (B and E) and β -endorphin positive (C and F) cells than functioning carcinoids (G-I), in which ACTH (H) and β -endorphin (I) are positive in the majority of cells.

Figure 2. POMC mRNA expression in ACTH-protein positive and negative cases. *: fold changes are represented in a linear scale; in cases marked with * the bars are not representative of the fraction exceeding 100-fold change

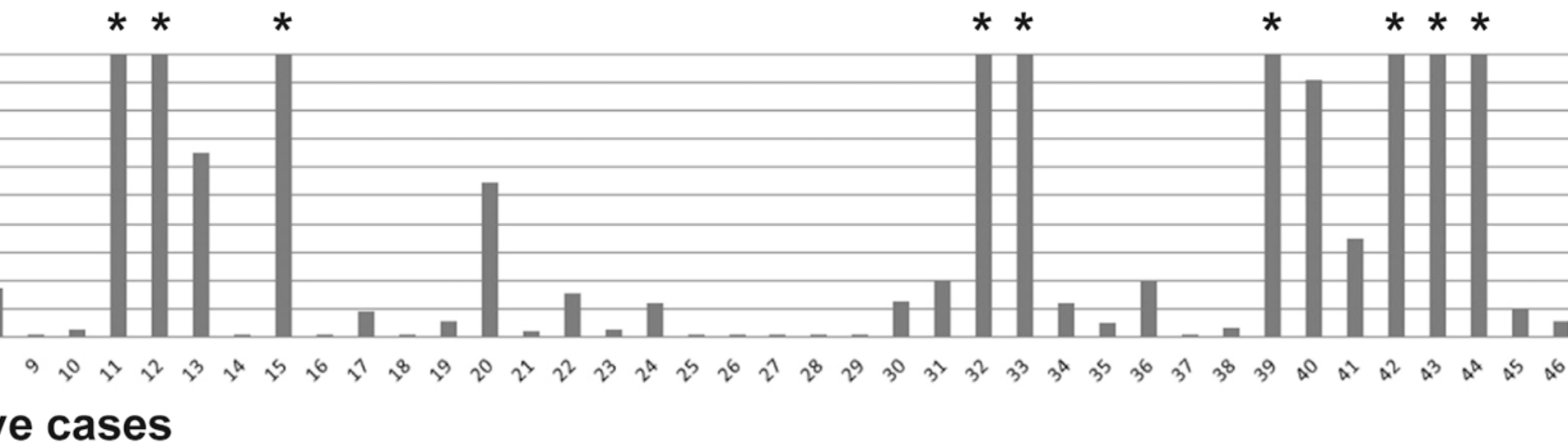
Figure 3. Kaplan-Meier disease free survival curves of functioning and nonfunctioning ACTH-producing carcinoids. No statistical different survival ($p:0.234$) was found.

Figure 4. Overall (A) and specific-disease (B) survivals of patients with ACTH-positive and ACTH-negative lung carcinoids. No statistically significant differences were observed.

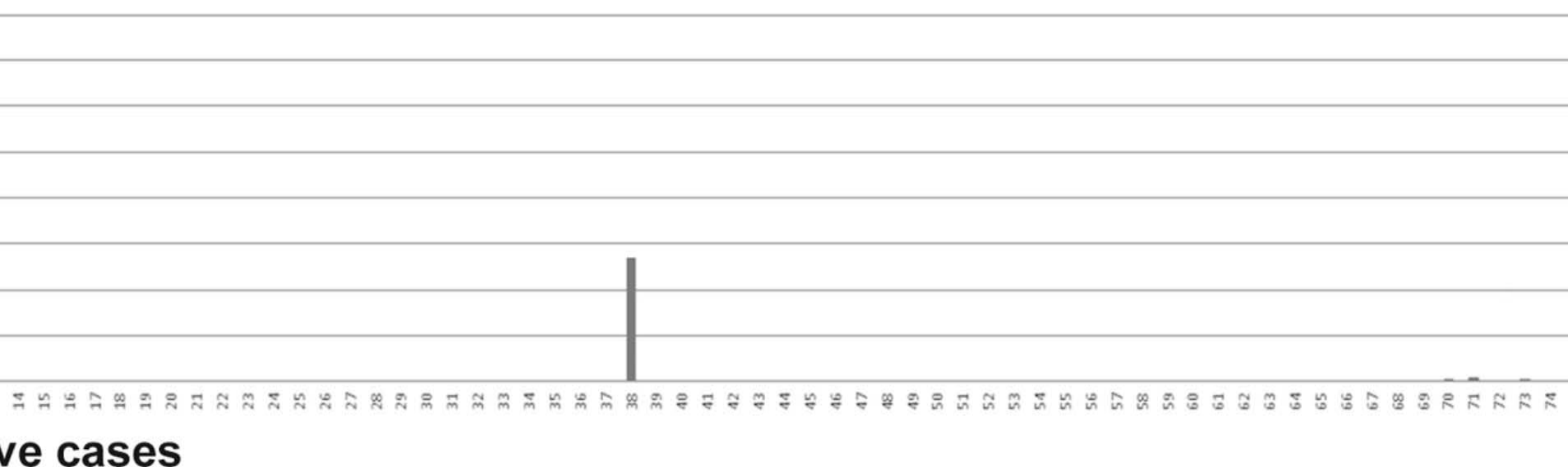
Figure 5. The processing of pro-opiomelanocortin (POMC) into its derivate peptides. To demonstrate ACTH production in our series of lung carcinoids we performed RT-PCR to detect POMC mRNA and immunohistochemistry to detect both ACTH and β -endorphin.



POMC mRNA expression levels

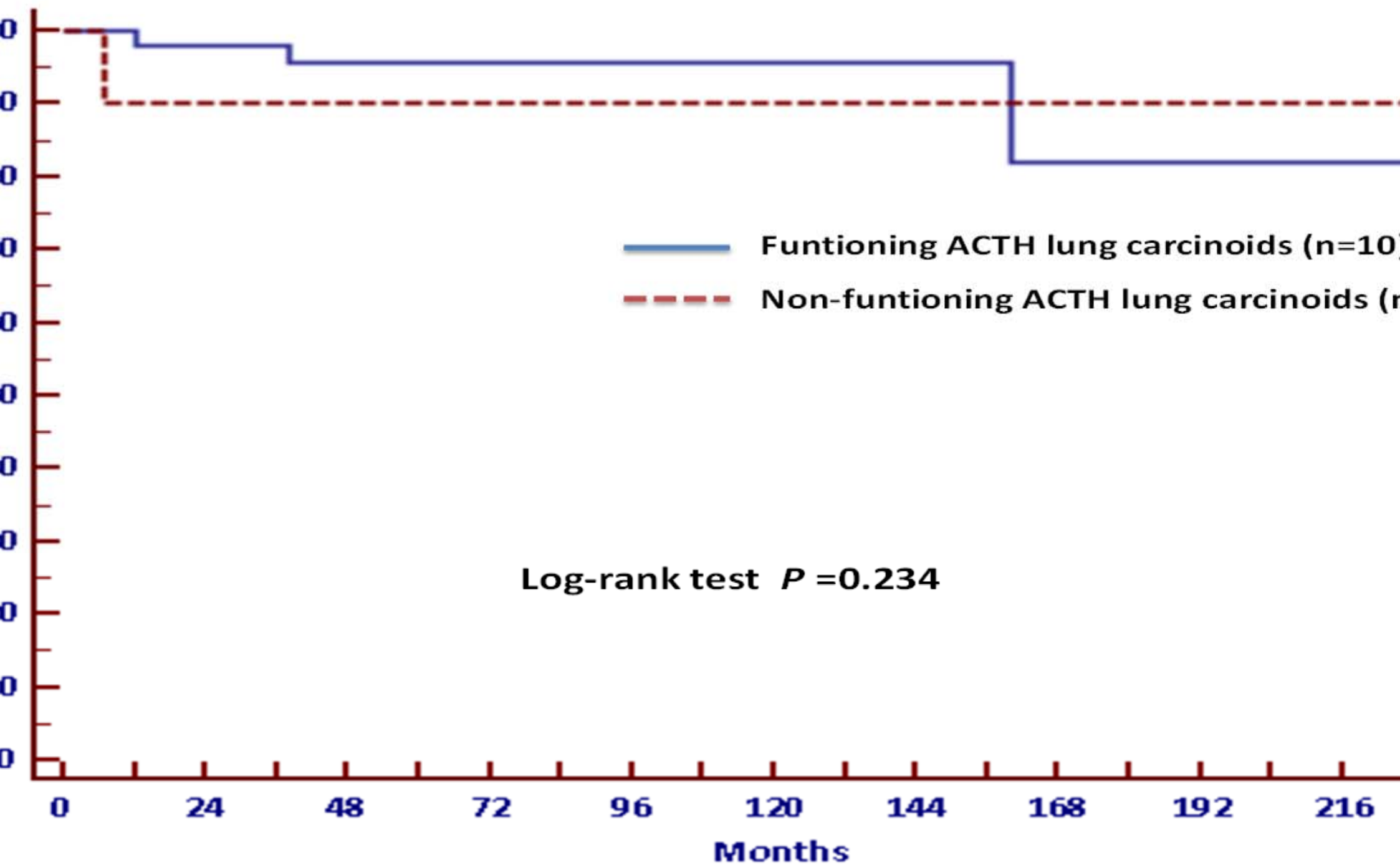


ve cases

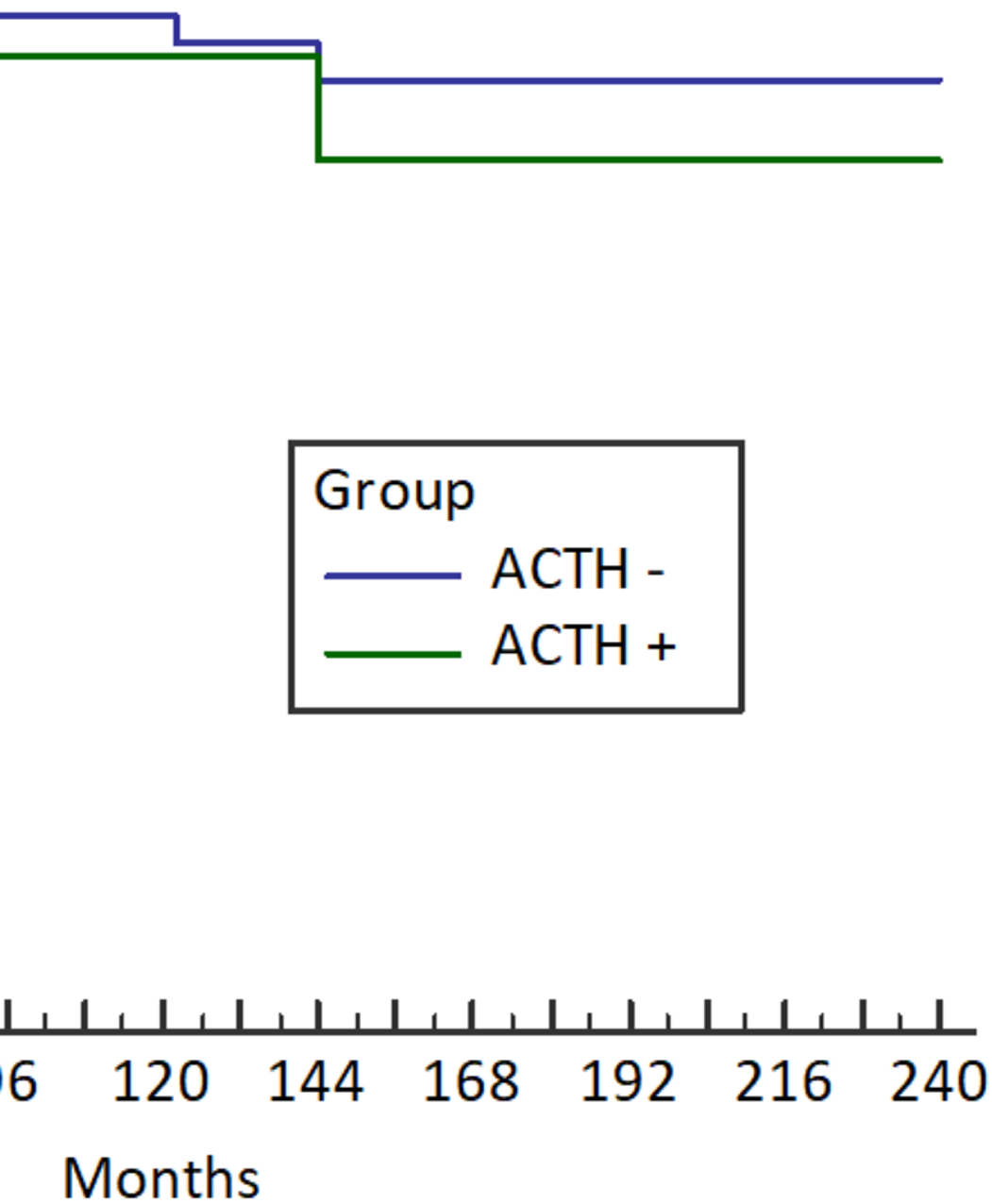


ve cases

Disease free survival



Survival ACTH+ vs ACTH-



Specific disease s

