Survival and Predictors of Death for Patients with Bronchopulmonary Carcinoid at a Danish Tertiary NET Centre

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Abstract. *Background/Aim: Bronchopulmonary carcinoids comprise of typical carcioids (TC) and atypical carcinoids (AC). We present characteristics and associated mortality in patients with TC and AC followed-up at a Danish NET Centre between 2000-2014. Patients and Methods: Data were obtained from medical records, computed tomographic scans, and pathology reports. Mortality was investigated by Kaplan–Meier survival analysis. Results: We included 68 patients, 55 with TC (81%) and 13 with AC (19%). Median follow-up was 5.6 years (range=1.0-26.3). Fourteen patients died; 13 were smokers, and nine had a Ki67 index >2%. Metastatic disease, smoking and male gender were significantly associated with risk of dying within the first 5 years (log-rank, all p<0.05), while Ki67 index >2% showed a trend for being associated with risk of death (p=0.11). Conclusion: Survival is strongly associated with tumor type and current/previous smoking. Metastatic disease at diagnosis and male gender are predictors of death within 5 years.*

Bronchopulmonary neuroendocrine tumors (BP-NETs) account for 20-25% of all neuroendocrine tumors and 20-25% of all lung cancer. BP-NETs comprise of typical carcinoids (TC), atypical carcinoids (AC), large-cell neuroendocrine carcinomas (LCNEC) and small-cell lung carcinomas (SCLC). Bronchial carcinoids (TC 80%, AC 20%) represent 1-2%, LCNEC <1%, and SCLC 10% of all lung cancer (1-4). TCs are considered low-grade and ACs

intermediate-grade NETs while LCNECs and SCLCs are high-grade. Survival rates for each subtype reflect these differences. Five-year survival in a retrospective study of 12,345 patients diagnosed with BP-NET between 1978-1997 was 87% for TC, 44% for AC, 15% for LCNEC, and 2% in SCLC (5). However, other studies described 5-year survival of 87-100%, 61-88%, 15-57% and <5% for TC, AC, LCNEC and SCLC, respectively (1-3).

Studies on cytogenetics found distinct chromosomal imbalances in BP-NET but to a lesser degree than SCLC. Genetic alterations were less widespread and involved narrower gene regions in BP-NET compared to SCLC, but several alterations were identical for both BP-NET and SCLC (6, 7).

Studies investigating an association between smoking and development of AC and TC are conflicting. Several studies concluded that smoking was not associated with bronchial carcinoid development, whereas two other studies found higher smoker prevalence among AC patients compared to healthy individuals or patients with TC (8-11). An association between smoking status and survival has not been demonstrated (3, 9, 12, 13). Other predictors of mortality are histological type and stage at diagnosis (3, 9), whereas female gender was a negative predictor in one study (3).

The aim of the present study was to describe demographic, immunohistochemical, and biochemical characteristics, survival, and predictors of death for all patients diagnosed with bronchial carcinoid and referred to our ENETS Centre of Excellence at Department of Hepatology and Gastroenterology, Aarhus University Hospital, Denmark in the period 2000-2014.

Patients and Methods

This is a retrospective cohort study. All patients from Western Denmark diagnosed with TC and AC followed at the ENETS Centre of Excellence at Aarhus University Hospital, Denmark, between 2000-2014 were included. According to the Danish "Act on Research Ethics Review of Health Research Projects", act number 593 of 14 July 2011, section 14,1 only health research studies are notified to the Committees. The Committees did not consider this study to be a health research study (section 2,1) (Ethics journal no: 179/2015).

Patients were evaluated in a multidisciplinary setting. All patients were hospitalized for disease staging and had an octreotide scintigraphy or Ga[68]- 1,4,7,10-tetra-azacyclododecane tetra-acetic acid 1-Nal3-octreotide (DOTANOC) positron-emission tomography (PET)–computed tomographic (CT) scan performed. Our multidisciplinary team decided if patients were suitable for surgery, radiofrequency ablation, chemo-embolization, medical treatment, chemotherapy, stereotactic radiotherapy, peptide receptor radionuclide treatment, or other experimental treatments. Patients were followed-up at our Outpatient Clinic after 1 month and subsequently every 3-6 months during follow-up. CT scan and plasma chromogranin A (CgA) determination preceded every visit. Plasma CgA was measured by a specific assay at Rigshospitalet, Copenhagen, Denmark as previously described (14).

Demographic, clinical, biochemical, and immuno-histochemical data were obtained. TNM classification, histopathological (TC and AC) and immunohistochemical [CgA, synaptophysin, CD56, tyrosine transcription factor-1 (TFF-1), and Ki67-index] data were examined based on pathology reports retrieved from a national Danish pathology database. An experienced pathologist with expertise in neuroendocrine tumors evaluated all biopsies.

Demographic and clinical features. We examined the following demographic and clinical features: patient age at diagnosis, gender, smoking status at diagnosis, primary resections, somatostatin receptor imaging (In[111] pentetreotide scintigraphy (SPECT) or Ga[68]-DOTANOC PET) combined with CT scans, and plasma CgA. All data were obtained from patients' medical records. Smoking status was divided into non-smokers, previous smokers (more than 6 months since quitting), and current smokers (ongoing smoking and <6 months since quitting).

Pathological features. Histopathological diagnosis was established by a specialized pathologist employing light microscopy according to The 2004 WHO Classification of Lung Tumors (15). TC was defined as a tumor ≥0.5 cm with carcinoid morphology, <2 mitoses per 2 mm^2 [10 high-power fields (HPF)], and lack of necrosis. AC was defined as a tumor with carcinoid morphology and 2 to <10 mitoses per 2 mm^2 or necrosis. Ki67 index, a marker of proliferating cells, was estimated according to the most agreed-upon procedure (16). TNM classification was determined according to the seventh edition of the TNM Staging for Lung Cancer (17) based on data obtained from pathology reports (from biopsies or resections from primary surgery), medical records, and CT scan reports.

Statistics and survival analysis. The chi-square test and two-sample Wilcoxon test were used to test the distribution of binomial and continuous data as appropriate. Categorical data are shown as absolute numbers (valid cases, percentages). Continuous variables are presented as medians with range unless otherwise noted. To investigate association between specific risk factors, we calculated total mortality and constructed unadjusted Kaplan–Meier curves. Log-rank statistics was used to test associations. Tests were considered statistically significant when *p*-values were less than 0.05 (two-sided test). Statistical analyses were performed using Intercooled STATA version 11 (StataCorp LP, College Station, TX, USA).

Results

Demographic, clinical and biochemical features. Demographic, clinical and biochemical features are summarized in Table I. We included 68 patients, 24 men (35%) and 44 women (65%). Median follow-up was 5.6 years (range=1.0-26.3 years). The median age at diagnosis was 61 (range=6-79 years) with no difference between men and women [56 (range=32-76) *vs.* 62 (range=6-79) years, respectively, (*p*=0.28). The median age was significantly higher for patients with AC compared to patients with TC. Seventy-eight percent (n=53) of the study population underwent primary surgery, while 22% (n=15) had advanced disease or significant comorbidity at diagnosis contradicting surgery. We found no difference in primary surgery rates by tumor type or by gender. Thirty percent (n=19) had a positive somatostatin receptor imaging at the first hospitalization at our department for disease staging. This was not associated with death, tumor type, gender, smoking status, or metastatic disease at diagnosis.

The median CgA level was not significantly different in patients with TC from that in patients with AC (92.5 pmol/l *vs.* 153 pmol/l; *p*=0.44).

Smoking status. The frequency of current or former smokers and gender distribution of smokers was similar in with TC and AC groups (*p*=0.54). Current/former smokers were, however, overall significantly older than non-smokers (log rank, *p*=0.016).

Pathological features. The study population was subdivided according to tumor type. Fifty-five patients (81%) were diagnosed with TC and 13 (19%) with AC. There was a non-significant distribution of women between TC and adenocarcinoma groups; the female to male ratio in those with TC was 1.6:1, whereas that for AC was 3.3:1. The median Ki67 index was significantly lower at 2% in patients with TC than that in patients AC (10%; *p*<0.0001).

In our cohort, 64% (n=35) had T1 disease, 27% (n=15) T2 disease and 9% (n=5) T3 disease at diagnosis. There was no difference in T-class distribution between TC and AC, but we observed a higher T-class in men compared with women (*p*=0.01).

Seventy-one per cent of our study population had non-metastatic disease (N0, M0), 13% N1, 13% N2 and 3% N3 disease at diagnosis. There was a significant difference in N-class distribution between TC and AC (*p*=0.02) with a larger fraction of patients with N>1 in AC than TC. We found no difference in gender distribution between N-classes (*p*=0.84). Twelve percent (n=8) of all patients had distant metastases (M1a/M1b) at diagnosis. There was no significant difference in distribution of M-classification between tumor types (*p*=0.56) or genders (*p*=0.14).

Table I. *Demographic, clinical, and biochemical description and TNM classification of patients with typical carcinoid (TC) and atypical carcinoid (AC).*

Characteristic	TC (N=55)	AC (N=13)	*p*-Value
Gender, n (%)			0.31
Men	21 (38)	3 (23)	
Women	34 (62)	10 (77)	
Female:male ratio	1.6:1	3.3:1	
Median age at diagnosis (range), years	56 (6-79)	65 (54-74)	0.026
Men	55 (32-76)	58 (57-74)	0.13
Women	61 (6-79)	67 (54-73)	0.10
T-classification, n (%)			0.17
T1	28 (67)	7 (54)	
T2	10 (24)	5 (38)	
T3	4 (9)	1 (8)	
N-classification, n (%)			0.020
N0	37 (74)	10 (77)	
N1	8 (16)	0	
N2	5 (10)	1 (8)	
N3	0	2 (15)	
M-classification, n (%)			0.61
M0	48 (87)	12 (92)	
M1	7 (13)	1 (8)	
Ki67 index, n (%)			<0.001
<2	27 (57)	0	
3-20	20 (43)	13 (93)	
Median pCgA (range), pmol/l	92.5 (27-7,600)	153 (44-20,500)	0.44
Receptor scintigraphy/Ga-DOTANOC PET-CT, n (%)			0.56
Positive	16 (31)	3 (23)	
Negative	35 (69)	10 (77)	
Smoking status, n (%)			0.54
Non-smoker	2 (40)	4 (31)	
Current/previous smoker	33 (60)	9 (69)	

Survival analysis. Fourteen patients died during follow-up (21%). Twenty-nine percent (n=7) of men died compared to 16% (n=7) of women. Sixteen percent (n=9) of patients with TC died compared with 38% (n=5) of those with AC.

There was no significant survival difference between patients with TC and those with AC at 5-years follow-up (*p*=0.49), while survival was significantly higher in the TC group compared with the AC group when analyzing the entire follow-up period (*p*=0.02) (Figure 1). Male gender was associated with higher risk of dying within 5 years (*p*=0.04), however, there was no significant survival difference between men and women for the entire follow-up period (*p*=0.20) (Figure 2).

During the entire follow-up period, 13 current/previous smokers died compared with only one non-smoker. Current/previous smoking status was associated with a significantly higher risk of dying both within 5 years and after the full follow-up period (*p*=0.03 and 0.01, respectively) (Figure 3).

The risk of dying for male current/previous smokers over the entire follow-up period was significantly higher compared to the rest of the study population (*p*<0.001).

A Ki67 index of >2% was associated with a trend towards greater risk of dying within 5 years (*p*=0.11). This association was significant for the full follow-up period (*p*=0.04). Male gender combined with current/previous smoking status and Ki67 index >2% was significantly associated with death within 5 years of follow-up compared with the rest of the study population (*p*<0.0001).

Metastatic disease at diagnosis was a negative survival predictor both within 5 years of diagnosis and for the entire follow-up period (both *p*<0.01). No patient with non-metastatic disease at diagnosis died within the first 5 years of follow-up (Figure 4).

The median CgA level was higher in patients who died, but the association was not significant (*p*=0.22).

Discussion

In this retrospective study of patients with bronchial carcinoids from a Danish NET Centre, we observed a strong association between survival and current/previous smoking status. In accordance with previous studies, we found a higher mortality for patients with AC compared to patients
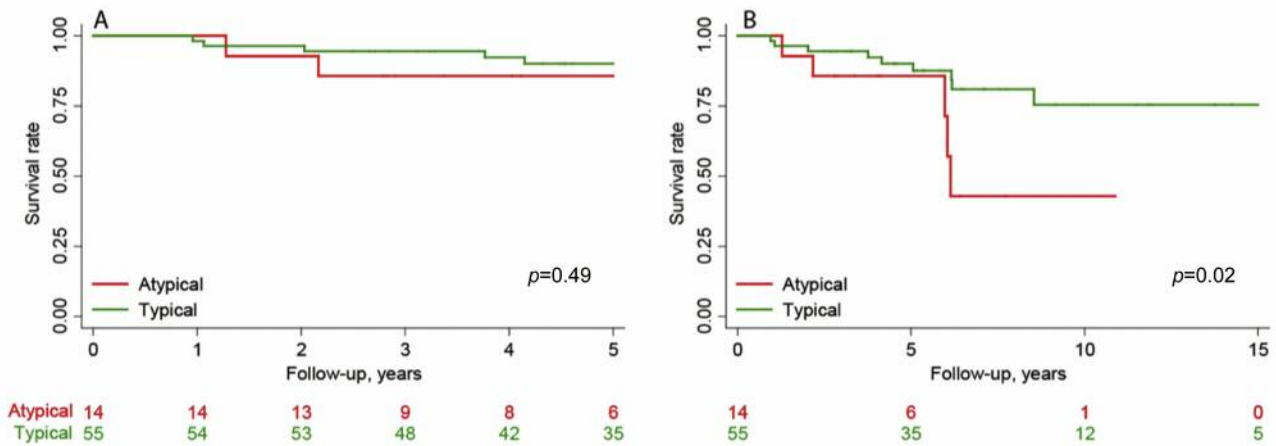
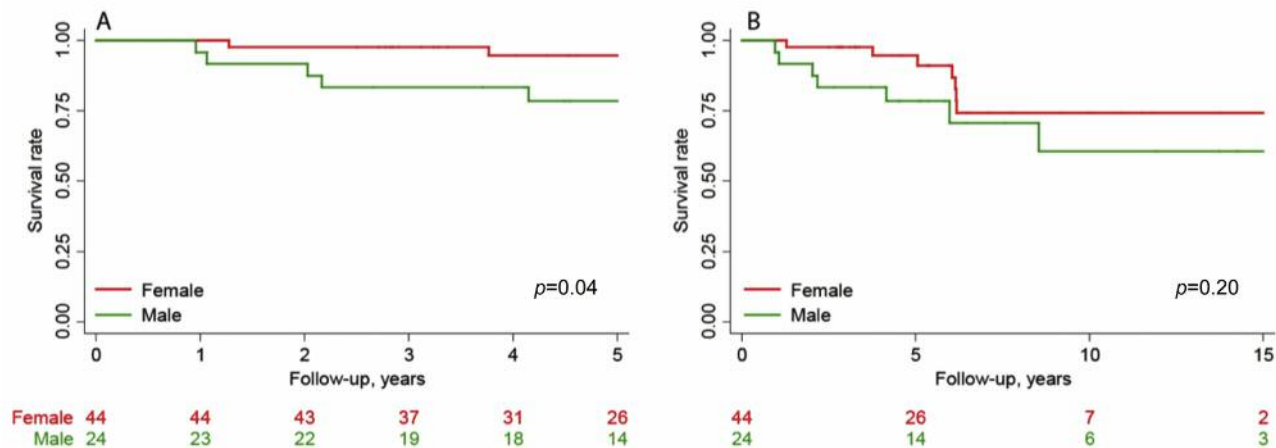Figure 1. *Survival rates for up to 5 years (A) and for full follow-up period (B) based on tumor type.*

Figure 2. *Survival rates for up to 5 years (A) and for full follow-up period (B) based on gender.*

with TC and for patients with Ki67 index >2%. Metastatic disease at diagnosis and male gender were additional mortality predictors.

We did not demonstrate any difference in distribution of non-smokers and previous/current smokers in TC compared to AC. However, prevalence of current smokers in both TC and AC in our study (60% and 69%, *p*=0.54) was considerably higher than prevalence in the general Danish population (approximately 20% in 2013) (18). This may indicate that smoking is a risk factor for developing bronchial carcinoid. Several studies investigated this question, and results are conflicting. Patients with SCLC and LCNEC are frequently heavy-smokers. However, previous studies have been unable to demonstrate any clear association with the development of AC and TC (1, 12, 13).

An Israeli study of 142 patients found the prevalence of smoking in the TC group to be almost that of the general population, whereas the prevalence was twice as high among patients with AC (8). Similarly, Froudarakis *et al.* described a higher prevalence of smoking, especially heavy smoking, in AC compared with TC patients (9). In our retrospective study of patients with bronchial carcinoid, we convincingly demonstrate that current and previous smoking status is associated with a poor survival. As far as we are aware, this has never been described previously. During the entire follow-up period, only one single patient died from the non-smoker group compared with 13 in the current/previous smoker group. Smoking status thereby seems to be the strongest single predictor of mortality for patients with bronchial carcinoids.
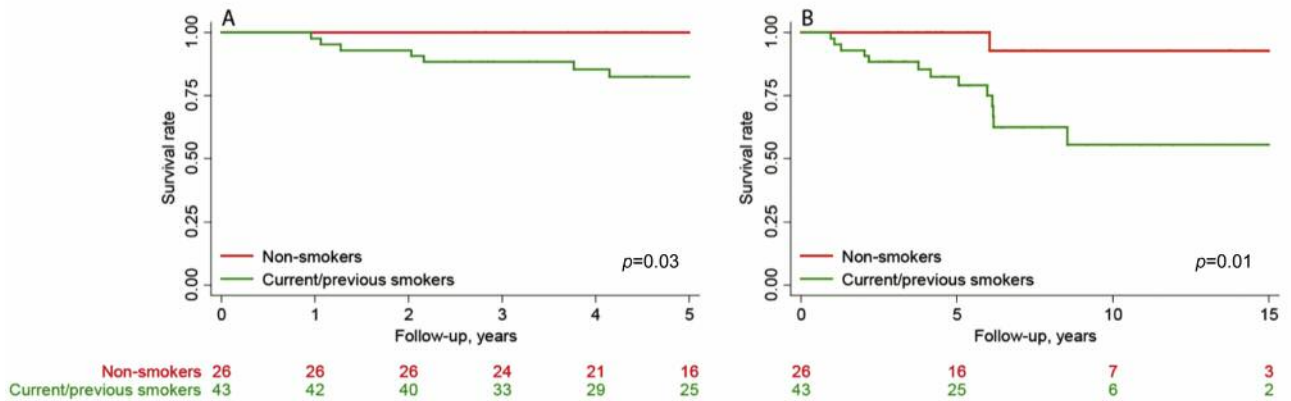
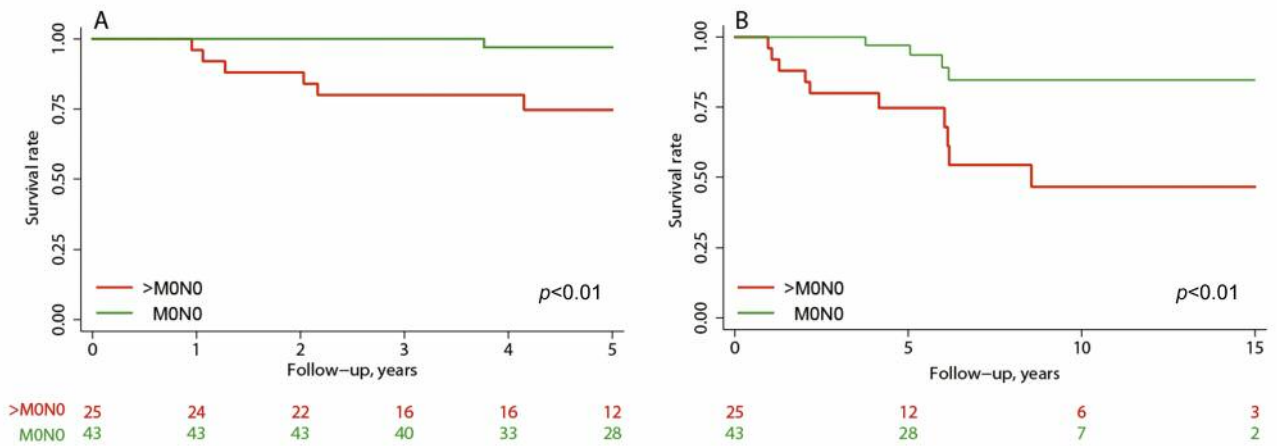Figure 3. *Survival rates for up to 5 years (A) and for full follow-up period (B) based on smoking status.*

Figure 4. *Survival rates for up to 5 years (A) and for full follow-up period (B) for metastatic (>M0N0) versus non-metastatic (M0N0) disease at diagnosis.*

In the study of Froudarakis *et al*., histological type, stage at diagnosis, and the presence of distant metastases were significant predictors of mortality, whereas age, gender, smoking habits, tumor size, and lymph node involvement were not (9). Beasley *et al*. found that increased numbers of mitoses, tumor size >3.5 cm, and female gender were negative predictors of survival, whereas smoking was not (3). The same is found in several other studies regarding smoking (12, 13). In the present study, survival was associated with gender, Ki67-index, and metastatic disease at diagnosis. We found no difference in survival between TC and AC at five years of follow up. As found in previous studies, survival of AC patients was poorer looking at the entire follow-up period ($p<0.05$) (1-3, 5). Zahel *et al*. questioned the reliability of prognosis, when dividing

patients according to diagnosis (TC/AC). They found a stronger association with prognosis when dividing patients according to the grading system for gastrointestinal neuroendocrine tumors (G1: <2 mitoses per 2 mm^2 and/or Ki67-index ≤2; G2: 2-20 mitoses per 2 mm^2 and Ki-67-index between 3-20) (19). This supports our findings of Ki67-index being a stronger prognostic factor of survival than the TC/AC diagnosis.

Strengths of the present study are the number of well-characterized patients according to clinical, biochemical, and pathological features as well as imaging. Patients were followed in a highly specialized NET center. Patients had a similar distribution of age, gender, and tumor type compared with previous studies. (4, 8, 9, 13, 20). Hence, they were representative of the population with bronchial carcinoids.

Since all patients diagnosed with TC and AC from Western Denmark in the period 2000-2014 were referred to our NET Centre, we do not consider selection bias to be a problem.

However, our study has limitations. It is a retrospective study with a limited number of deaths, despite a relatively long follow-up period. This probably explains lack of difference in survival between TC and AC within the first five years. Analyzing the full follow-up period revealed a significantly poorer prognosis of patients with AC. However, numbers at risk later than five years of follow-up were very low, and data for the entire follow-up period should, therefore, be interpreted with caution.

In conclusion, we observed that current or previous smoking, metastatic disease at diagnosis and male gender are strong predictors of mortality in patients with bronchial carcinoids. Prospective studies with larger study populations and longer follow-up periods are required to further investigate these associations.

Conflicts of Interest

The Authors declare no conflicts of interest in regard to this study

Acknowledgements

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