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Clinicopathological indicators of survival among patients with pulmonary carcinoid tumor

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Background: Pulmonary carcinoids (PC) are rare malignant neoplasms that cover approximately 1% of all lung cancers. PCs are classified by histological criteria as either typical (TC) or atypical (AC). Histological subtype is the most studied prognostic factor. The aim of this study was to evaluate if other tissue or clinical features are associated with patient outcomes.

Material and methods: We retrospectively reviewed clinical records of 133 PC patients who underwent operation in the Helsinki University Hospital between 1990 and 2013. Tissue specimens were re-evaluated, processed into tissue microarray format and stained immunohistochemically with serotonin, calcitonin, adrenocorticotropic hormone (ACTH), thyroid transcription factor-1 (TTF-1) and Ki-67. Survival and risk analyses were performed.

Results: Based on histology, 75% (n = 100) of the tumors were TCs and 25% (n = 33) ACs. TCs had higher 10-year disease-specific survival (DSS) rate than ACs (99% (95% CI, 93–100%) for TCs vs. 82% (95% CI, 61–92%) for ACs). Hormonally active tumors expressing serotonin, calcitonin or ACTH were noted in 53% of the specimens but hormonal expression was not associated with DSS. TTF-1 was positive in 78% of the specimens but was not associated with DSS. Ki-67 index varied between <1% and 15%. Ki-67 ≥ 2.5% was associated with shorter DSS (p = .004). The presence of metastatic disease (p = .001), tumor size ≥ 30 mm (p = .021) and atypical histology (p = .011) were also associated with disease-specific mortality.

Conclusions: We conclude that PCs are uncommon tumors. When resected, the long-term survival is in general favorable. In this consecutive, single-institution cohort of patients, presence of metastatic disease, tumor size, histological subtype and Ki-67 index were associated with shorter disease-specific survival. As TC and AC have different clinical behaviors, the correct tumor classification at the time of diagnosis is a necessity.

Introduction

Pulmonary carcinoid (PC) tumors are rare malignant neoplasms that cover approximately 1% of all lung cancers [1,2]. PCs belong to neuroendocrine tumors that fall into four subtypes based on their increasing biological aggressiveness. Typical carcinoid (TC) and atypical carcinoid (AC) are low- and intermediate-grade malignancies, respectively, while large-cell neuroendocrine carcinoma and small-cell lung cancer are high-grade tumors [3].

The incidence of pulmonary carcinoids has risen significantly in recent decades [4,5]. This rise results primarily from improved detection methods and diagnostic protocols as well as increased awareness among clinicians. Contrast enhanced CT scan serves as the diagnostic gold standard, but histopathological examination remains necessary for obtaining a correct classification [6,7]. Surgery represents the primary therapy and only curative option, although metastatic disease is also treated with cytotoxic agents [6,8]. Typical carcinoids rarely metastasize, and the five-year survival rate ranges from 87% to 94% [2,9]. In contrast, atypical carcinoids have a greater tendency for lymph node involvement and metastatic disease, with a lower five-year survival rate (44–71%) [2,10].

Neuroendocrine tumors often express at least one metabolically active peptide or amine [11]. Pulmonary carcinoid tumor cells secrete adrenocorticotropic hormone (ACTH), serotonin and calcitonin [11–14]. However, clinical syndromes
such as Cushing’s syndrome and carcinoid syndrome are rare and affect only 1–6% of PC patients [6]. Some reports [15] indicate that hormone secreting PCs might be more aggressive than the ones without this feature but to our knowledge, comprehensive reports on the issue are lacking.

Thyroid transcription factor-1 (TTF-1) belongs to the NKX2 gene family. It plays a role in normal lung development but also contributes to the pathogenesis of lung cancer [16]. Studies show TTF-1 expression in a significant subset of PCs, but to our knowledge, reports on its correlation to survival are limited [17–19].

In this study, we report the clinicopathological features of pulmonary carcinoid patients treated surgically at our institution over a 20-year period. We also explored whether ACTH, serotonin, calcitonin, TTF-1 or Ki-67 expression associates with PC patient survival. The aim was to increase the understanding of the prognostic factors related to these rare tumors.

Material and methods

Patients and tumor specimens

This study was based on a series of 133 consecutive pulmonary carcinoid patients who were surgically treated at the Department of Surgery, Helsinki University Hospital, between January 1990 and August 2013. The patients were identified from the registry of the Department of Pathology, Helsinki University Hospital. The inclusion criteria were histologically confirmed diagnosis of pulmonary carcinoid, availability of clinical data and a whole tumor specimen obtained at surgery. Altogether, we found 146 patients who fulfilled the abovementioned criteria. However, 12 tissues samples were so scarce that we could not use them without compromising possible future diagnostics.

All histological specimens were re-evaluated by a single pathologist with a special expertise in pulmonary pathology (KS). Tumors were classified according to the World Health Organization (WHO) 2015 classification criteria, where TC is defined as a tumor with a carcinoid morphology, with <2 mitosis per 2 mm², and a lack of necrosis [3]. AC is a tumor with carcinoid morphology, showing 2–10 mitosis per 2 mm² or necrosis. Both tumors had to be more than 0.5 cm in size. Immunohistochemical staining against chromogranin A, synaptophysin and pan keratin was used to confirm neuroendocrine differentiation.

The patient characteristics, treatment details, histopathological information and survival data were obtained from the patient records, the Population Register Center, and Statistics Finland. Epidemiological data were obtained from the Finnish Cancer Registry. The study was approved by the Surgical Ethics Committee of the Helsinki University Hospital.

Tissue microarrays (TMA)

The tissue specimens were retrieved through Helsinki Biobank. After identifying the most suitable formalin-fixed paraffin-embedded tissue blocks per case, fresh slides were sectioned, stained with H&E, and digitized with a Pannoramic scanner (3DHISTECH, Budapest, Hungary). Annotations for the TMAs were marked on the digitized slides in the CaseViewer software (3DHISTECH) in accordance with the following principles: two cores from the middle of the tumor, two cores from the tumor border, two cores from the non-tumor area and one core from the bronchus, if applicable.

The TMAs were constructed with TMA Grand Master (3DHISTECH) using 1 mm punches. As a result, five TMA blocks representing altogether 100 typical carcinoids and 33 atypical carcinoids were created.

Immunohistochemistry

Immunohistochemical stainings against chromogranin A, synaptophysin, pan keratin, Ki-67, TTF-1, ACTH, calcitonin and serotonin were performed in the clinical pathology laboratory (Department of Pathology, Helsinki University Hospital). Briefly, multiple four-micrometer sections were cut with a microtome from each TMA block on TOMO adhesion slides (Matsunami Glass Ind., Ltd., Osaka, Japan). Stainings of slides were performed in BenchMark XT (Ventana Medical Systems, Inc., Tucson, AZ) or AutoStainer 480 instrument (Lab Vision Corp., Fremont, CA). The sections were deparaffinized and antigen retrieval was performed using CC1 reagent (Ventana Medical Systems) and Protease 3 (Ventana Medical Systems). The primary antibodies were incubated, and the immunoreactions were detected using ultraView or OptiView Universal DAB Detection Kit (Ventana Medical Systems) or EnVision Detection Systems (Dako, Agilent Pathology Solutions, Santa Clara, CA). All slides were counterstained with Mayer’s hematoxylin (Dako). Detailed information on primary antibodies and staining protocols are presented in Table 1.

Table 1. Features of the antibodies and staining protocols used for immunohistochemistry.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Supplier</th>
<th>Clone</th>
<th>Dilution</th>
<th>Incubation (min)</th>
<th>Pre-treatment</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromogranin A</td>
<td>Dako</td>
<td>DAK-A3</td>
<td>1:800</td>
<td>32</td>
<td>CC1 std</td>
<td>UltraView</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>Ventana¹</td>
<td>SP11</td>
<td>RTU</td>
<td>32</td>
<td>CC1 mild + Protease 3 12 min</td>
<td>OptiView</td>
</tr>
<tr>
<td>Pan Keratin</td>
<td>Ventana¹</td>
<td>AE1/AE3 &amp; PCK26</td>
<td>RTU</td>
<td>12</td>
<td>CC1 std</td>
<td>UltraView</td>
</tr>
<tr>
<td>Ki-67</td>
<td>Dako</td>
<td>MIB-1</td>
<td>1:100</td>
<td>32</td>
<td>CC1 std</td>
<td>UltraView</td>
</tr>
<tr>
<td>TTF-1</td>
<td>Novocastro¹²</td>
<td>SPT24</td>
<td>1:100</td>
<td>30</td>
<td>None</td>
<td>EnVision</td>
</tr>
<tr>
<td>ACTH</td>
<td>NeoMarkers¹³</td>
<td>Polyclonal</td>
<td>1:200</td>
<td>30</td>
<td>None</td>
<td>EnVision</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Dako</td>
<td>Polyclonal</td>
<td>1:2500</td>
<td>30</td>
<td>None</td>
<td>EnVision</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Dako</td>
<td>SHT-H209</td>
<td>1:10</td>
<td>30</td>
<td>None</td>
<td>EnVision</td>
</tr>
</tbody>
</table>

RTU, ready-to-use; TTF-1, thyroid transcription factor-1; ACTH, adenocorticotropic hormone.

¹Ventana Medical System.
²Novocasta, Leica Biosystems, Nussloch GmbH, Germany.
³Lab Vision.

\[\text{\textsuperscript{3DHISTECH}}\]
Stained slides were digitized with a Pannoramic scanner (3DHISTECH). The core drop-out rate was less than 5%.

**Evaluation of stainings**

The immunostaining results were evaluated from the digitized slides. Chromogranin A, synaptophysin and pan keratin were considered positive if >90% of the neoplastic cells showed at least moderate intensity (Figure 1). TTF-1 was scored positive if >10% of the nuclei of neoplastic cells were positive and the staining intensity was moderate or strong. ACTH, calcitonin and serotonin stainings were considered positive if there was at least one neoplastic cell with a strong cytoplasmic staining (Figure 2). The proliferation index was evaluated according to the Ki-67 immunoreactivity in the nuclei of the highest labeling region of at least 2000 cells both manually and with QuPath software [20].

The scoring was performed by two independent investigators (JA and TV) who did not have knowledge of the clinical data and patient outcome. In the case of different scores, a consensus score was discussed and determined.

**Statistical analyses**

Differences in the dichotomous or nominal variables between the groups were calculated with the Fisher’s Exact Test.
The cumulative survival probabilities were estimated with the Kaplan–Meier method, which was also used to graphically display the disease-specific survival (DSS) curves. The exact 95% confidence intervals (CI) were calculated for the survival rates. Differences in hazard rates (HR) by age, gender, histologic subtype, tumor size, presence of metastatic disease and immunohistochemical findings were tested with univariable Cox regression. If the regression did not converge, the Firth penalized Cox regression was calculated. Receiver operating characteristics (ROC) curves were used to estimate a cutoff value for Ki-67 as a prognostic indicator, accomplished by penalized Cox regression. If the regression did not converge, the Firth penalized Cox regression was calculated. Receiver operating characteristics (ROC) curves were used to estimate a cutoff value for Ki-67 as a prognostic indicator, accomplished by maximizing the Youden’s index (sensitivity + specificity – 1). Survival was calculated from the date of the surgery to the last date of follow-up or death, and non-disease-specific deaths were censored to obtain DSS. A significant difference was predetermimed to be a p value less than .05. Two-tailed tests were used. Analyses were performed using IBM SPSS Statistics 24 (IBM, Armonk, NY) and SAS for Windows 9.4 (SAS Institute Inc., Cary, NC).

Results

Epidemiology and histological subtypes

According to the Finnish Cancer Registry, 13,140 patients were diagnosed with lung cancer between January 1990 and December 2012 in the Hospital District of Helsinki and Uusimaa. Of these, approximately 20% underwent surgical resection. In the same time period, PC diagnosis was given to 146 patients, which was 1.1% of all lung cancers. All but five PC patients were treated with surgery.

Pulmonary carcinoid tumor resections increased during the years. Between 1990 and 1994 there was on average 3.8 resections per year. Between 1995 and 1999, as well as between 2000 and 2004, the average number of resections was 4.4 per year. Between 2005 and 2009 the number increased to seven resections per year. The highest number was found between 2010 and 2013: the average number of resections was 9.7 per year.

Our cohort consisted of 47 male and 86 female patients, with a median age of 54 years (range 19–84). Based on histological re-evaluation, 30% of the primary diagnoses changed. Twenty-seven TCs were re-classified as ACs, and 13 ACs were re-classified as TCs. One AC was re-evaluated as small-cell lung cancer and was excluded from this study. Altogether, after re-evaluation, typical carcinoid was observed in 100 patients (75%, 30 male, 70 female) and atypical carcinoid in 33 patients (25%, 17 male, 16 female). The patients’ characteristics are summarized in Table 2.

<table>
<thead>
<tr>
<th>Location</th>
<th>Left</th>
<th>Upper lobe</th>
<th>Lower lobe</th>
<th>Main bronchus</th>
<th>Whole lung</th>
<th>Right</th>
<th>Upper lobe</th>
<th>Lower lobe</th>
<th>Main bronchus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean</td>
<td>52</td>
<td>23</td>
<td>17</td>
<td>0</td>
<td>60</td>
<td>17</td>
<td>23</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>53</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>21</td>
<td>8</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Mean diameter</td>
<td>17 mm (range 5–50 mm)</td>
<td>10 mm (range 5–40 mm)</td>
<td>15 mm (range 5–50 mm)</td>
<td>8 mm (range 5–20 mm)</td>
<td>21 mm (range 5–74 mm)</td>
<td>8 mm (range 5–50 mm)</td>
<td>15 mm (range 5–50 mm)</td>
<td>5 mm (range 5–24 mm)</td>
</tr>
<tr>
<td></td>
<td>AC percentage</td>
<td>36%</td>
<td>27%</td>
<td>22%</td>
<td>2%</td>
<td>64%</td>
<td>22%</td>
<td>31%</td>
<td>24%</td>
</tr>
</tbody>
</table>

Location and tumor size

The carcinoid tumors were more often located in the right lung (61%), and the most frequent site was the right middle lobe. The tumor diameter, as determined by the pathologist, varied between 5 and 50 mm. The mean diameter of the typical carcinoids was 17 mm (range 5–40 mm), and 19 mm in atypical carcinoids (range 5–50 mm; p = .640). In nodal spread or distant metastatic disease, primary tumor was more often ≥30 mm in diameter (six out of 15, 40%) than in local disease (12 out of 118, 10%, p = .006).

Treatment and tumor spread

All 133 patients were treated with surgery, most with a lobectomy with or without a bronchoplastic procedure (Table 2). The lymph nodes were examined in 104 patients (78%), and mediastinal lymph node involvement was noted in 10 patients (10%, four with TC and six with AC). No operative or perioperative 90-day mortality was observed.

At the time of diagnosis, two AC patients had metastases either in the liver or pleura, and five other AC patients developed metastases in the bones, liver, brain or mediastinum during follow-up. In addition, three TC patients developed later a recurrent disease in mediastinal nodes.

Of the 133 patients reviewed, one AC patient received radiation, and another received radiation and chemotherapy as a neoadjuvant treatment. Three AC patients received post-operative adjuvant chemotherapy. None of the TC patients received neoadjuvant or adjuvant therapy.
Table 3. Immunohistochemical profiling of pulmonary carcinoid tumors.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>TC pos</th>
<th>AC pos</th>
<th>All pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>21 (21%)</td>
<td>6 (18%)</td>
<td>27 (21%)</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>25 (26%)</td>
<td>6 (18%)</td>
<td>31 (24%)</td>
</tr>
<tr>
<td>Serotonin</td>
<td>39 (40%)</td>
<td>14 (42%)</td>
<td>53 (41%)</td>
</tr>
<tr>
<td>TTF-1</td>
<td>81 (82%)</td>
<td>22 (67%)</td>
<td>103 (78%)</td>
</tr>
<tr>
<td>Ki-67 &lt;1%</td>
<td>44 (46%)</td>
<td>10 (30%)</td>
<td>54 (42%)</td>
</tr>
<tr>
<td>Ki-67 1–2%</td>
<td>45 (47%)</td>
<td>18 (55%)</td>
<td>63 (49%)</td>
</tr>
<tr>
<td>Ki-67 &gt;2%</td>
<td>7 (7%)</td>
<td>5 (15%)</td>
<td>12 (9%)</td>
</tr>
</tbody>
</table>

ACTH: adrenocorticotropin hormone; TTF-1: thyroid transcription factor-1.

Immunohistochemical profiling

Immunohistochemical staining against either ACTH, calcitonin or serotonin was positive in 69 specimens (53%). Serotonin expression was the most common and was seen in 53 specimens (41%), whereas calcitonin and ACTH were detected in 27 (21%) and 31 (24%) specimens, respectively (Table 3). There was no significant difference in the peptide expression between the TC and AC tumors. All three peptides were expressed in 10 tumors of which nine were TCs (90%) and one was AC (10%). This difference was not significant (p = .450).

Of the 133 specimens, 103 (78%) stained positively for TTF-1 (Table 3). The TCs showed positivity in 82% of cases (n = 81) while 67% of the ACs were positive (n = 22; p = .089). The Ki-67 proliferation index varied between <1% and 15%, with a median value of 1% (Table 3). There was no difference in the Ki-67 proliferation index between typical and atypical carcinoids (p = .183). All tumors were positive for chromogranin A, synaptophysin and pan keratin.

Follow-up and survival

The patient follow-up ended on 15 March 2017, with a median time of observation of 9.6 years (mean 11.6, range 1.1–26.7). Of the 133 patients, 23 died. Six of them showed evidence of the disease (five AC patients, one TC patient) and 17 died from unrelated causes. The average survival time for the patients who died from the disease was 3.6 years (range 1.1–8.7). Three TC patients, who developed recurrent disease in mediastinal nodes, as well as two AC patients with distant metastases, were still alive in the last follow-up. Their survival times were as follows: AC 8.4 years, TC 9.6 years, TC 15.2 years, TC 19.2 years and AC 19.5 years. No patients were lost to follow-up.

The disease-specific 5- and 10-year survival rates for all pulmonary carcinoid patients were 96% (95% CI, 91–98%) and 95% (95% CI, 88–98%), respectively. The 5-year DSS rate for the TC patients was 99% (95% CI, 93–100%) and 87% (95% CI, 69–95%) for the AC patients. The 10-year DSS survival rate was 99% (95% CI, 93–100%) for the TCs and 82% (95% CI, 61–92%) for the ACs.

According to Cox regression, four factors were associated with shorter DSS (Figure 3, Table 4): atypical histology, the presence of metastatic disease, tumor size ≥30 mm, and Ki-67 proliferation index ≥2.5%. Gender, age at operation, ACTH, serotonin, calcitonin or TTF-1 expression were not associated with DSS.

Cox regression analyses were also performed on a subset of patients (n = 77) who had been operated at least 10 years before ending the follow-up (Table 4). Within this subset of patients the same features associated with shorter DSS.

Discussion

We retrospectively reviewed a consecutive cohort of 133 PC patients who had been treated surgically at our institute between January 1990 and August 2013. This series represents one of the largest studies on pulmonary carcinoid tumors in recent times. The aim was to evaluate if specific histological or clinical features associated with patient outcomes.

Between 1990 and 2012, pulmonary carcinoid tumors accounted for 1.1% of all lung cancers in our hospital district, which is in accordance with literature [1,2]. Incidence of PCs has increased during the recent years [4,5], which was shown also in our study as an increased number of resected patients per year. In our patient series, typical carcinoids were more frequent than atypical carcinoids; 75% of the patients had TC. This finding corresponds to previous studies performed on similar-sized patient cohorts [21–23]. In addition, we found that pulmonary carcinoids were more common in females than in males (65% vs. 35%), which is comparable to previous studies on more than 1000 PC patients (Filoso et al. [9] 64% vs. 36% and Hobbins et al. [24] 62% vs. 38%). However, when focusing on AC patients, the difference in the gender distribution almost disappeared, and AC was slightly more common in males than in females (52% vs. 48%). Others have reported the same phenomenon [2,10,25].

Since TC and AC show different clinical behaviors and long-term prognosis, the correct tumor classification is important, and a re-evaluation of archived tumors thus highly recommended. Most pulmonary carcinoid series published in the twenty-first century are re-evaluated based on the prevailing WHO classification. However, register-based studies, specifically, lack re-evaluation [2,26].

In our series, 42 different general pathologists had provided the primary diagnoses recorded in the patient files. In the re-evaluation process, performed by a single pathologist with a special expertise in pulmonary pathology, 30% of the primary diagnoses changed. However, atypical carcinoid diagnosis was not introduced in our clinical pathology laboratory until 2001. If calculating only diagnoses recorded after this time point (n = 90), 33% of them changed. To our knowledge, only a few studies have reported re-evaluation effects on the histological subtype in pulmonary carcinoids. Daddi et al. described that in their series, 2–4% of the primary diagnoses changed [25].

As shown by others, histological subtype of pulmonary carcinoid tumor has a significant impact on patient outcome [23,26–27]. TC patients present less lymph node involvement as well as distant metastasis. Thus they tend to have a better 10-year survival rate compared to ACs. Our study strengthened this finding.
It is also known from previous studies that the presence of metastatic disease has a negative impact on survival \([21,24–25]\). In addition to this, we noticed that tumor size associates with patient outcome: patients with a tumor more than 30 mm in diameter had worse prognosis. Beasley et al. \([10]\) published similar results with a 35 mm cutoff, while Okereke et al. found no correlation \([22]\). Interestingly, in our cohort, the tumor size was not significantly different between

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**Table 4.** Analysis of potential risk factors for disease-specific mortality using univariable Cox regression.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>All patients</th>
<th></th>
<th>Patients with &gt;10 years follow-up</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( p )</td>
<td>HR 95% CI</td>
<td>( p )</td>
<td>HR 95% CI</td>
</tr>
<tr>
<td>Histological type (AC vs. TC)</td>
<td>( .011 )</td>
<td>16.18 1.89–138.53</td>
<td>( .041^* )</td>
<td>27.46 3.11–3606.93</td>
</tr>
<tr>
<td>Presence of metastatic disease (yes vs. no)</td>
<td>( .001 )</td>
<td>40.93 4.78–350.73</td>
<td>( .009^* )</td>
<td>69.50 7.86–9130.09</td>
</tr>
<tr>
<td>Tumor size (mm) (( \geq 30 ) vs. &lt;30)</td>
<td>( .021 )</td>
<td>6.58 1.33–32.64</td>
<td>( .010 )</td>
<td>10.58 1.77–63.37</td>
</tr>
<tr>
<td>Ki-67 index (( \geq 2.5 ) vs. &lt;2.5)</td>
<td>( .004 )</td>
<td>10.51 2.12–52.13</td>
<td>( .004 )</td>
<td>13.61 2.27–81.57</td>
</tr>
<tr>
<td>Gender (female vs. male)</td>
<td>( .450 )</td>
<td>0.54 0.11–2.67</td>
<td>( .821 )</td>
<td>0.81 0.14–4.87</td>
</tr>
<tr>
<td>Age at operation (years) (( \geq 54 ) vs. &lt;54)</td>
<td>( .344 )</td>
<td>2.27 0.41–12.49</td>
<td>( .369 )</td>
<td>2.27 0.38–13.61</td>
</tr>
<tr>
<td>ACTH expression (pos vs. neg)</td>
<td>( .820 )</td>
<td>0.78 0.09–6.67</td>
<td>( .519 )</td>
<td>0.04 0.00–820.18</td>
</tr>
<tr>
<td>Serotonin expression (pos vs. neg)</td>
<td>( .189 )</td>
<td>3.12 0.57–17.03</td>
<td>( .349 )</td>
<td>2.35 0.39–14.08</td>
</tr>
<tr>
<td>Calcitonin expression (pos vs. neg)</td>
<td>( .564 )</td>
<td>1.65 0.30–8.99</td>
<td>( .972 )</td>
<td>1.04 0.12–9.31</td>
</tr>
<tr>
<td>TTF-1 expression (pos vs. neg)</td>
<td>( .741 )</td>
<td>1.44 0.17–12.30</td>
<td>( .818 )</td>
<td>1.29 0.14–11.58</td>
</tr>
</tbody>
</table>

AC: atypical carcinoid; TC: typical carcinoid; ACTH: adrenocorticotropic hormone; TTF-1: thyroid transcription factor-1.

*The Firth’s penalized regression.
have observed similar results [28–31]. The usefulness of this finding is, however, limited because the histological subtype seems to have even a stronger association with survival. Nonetheless, the Ki-67 index might be an important prognostic factor in cases that are difficult to classify as TC or AC.

Ki-67 values were in general lower than in previous studies [28,30–31] which might be partly due to the use automated image analysis software. They are known to yield lower Ki-67 values than manual methods [30,32]. It is also possible that the TMA technique in some cases misses the areas of the strongest nuclear labeling, even if our punching method considers tissue heterogeneity. To address this, we reviewed the primary histology reports (n = 83, data not shown) to find original Ki-67 values. Based on them, 84% of the samples showed a Ki-67 value of 0–5%. In correspondent TMA samples, the percentage was 96%. However, original Ki-67 values were based on the arbitrary evaluation of 42 general pathologists. As studies have shown, inter-observer and even inter-laboratory variation in Ki-67 scoring is substantial [33–34]. Immunohistochemical staining methods have also evolved over the years.

According to a recent meta-analysis of 17 studies, including 2235 patients, TTF-1 overexpression indicates a favorable prognosis for non-small cell lung cancers and for atypical carcinoids [35]. Another study showed in a small patient series (n = 34) that TTF-1 might be a prognostic factor in PCs [18]. We, however, were unable to find any associations between TTF-1 and patient outcome.

Pulmonary carcinoids are known to secrete ACTH, calcitonin and serotonin. Cushing’s syndrome, caused by overproduction of ATCH, occurs in 1–6% of PC patients [6]. Some investigators report that PCs associated with Cushing’s syndrome are more aggressive [15]. However, the opposite reports also exist [36,37]. Serotonin is the key component in carcinoid syndrome, affecting 2–5% of PC patients, while calcitonin causes diverse symptoms [6,11,13]. We found four patients with Cushing’s syndrome due to a PC tumor. These tumors also immunohistochemically expressed ACTH. No carcinoid syndrome was observed.

To the best of our knowledge, this is the first report to study the expression of ACTH, calcitonin and serotonin as prognostic markers in pulmonary carcinoids. However, we did not find any association with patient outcome. Similarly, neither gender nor age did affect disease-specific survival in our study, although some investigators have observed these to be prognostic factors [9,25–27].

The strength of this study is the number of well-characterized patients according to the histological and immunohistochemical findings as well as reliable clinical follow-up and survival data. With our TMA method, adequate amount of tumor tissue was stained homogenously. However, our study presented only a limited number of disease-specific deaths, despite a relatively long follow-up. Based on this low number (six) of disease-specific deaths, a reliable multi-variate analysis could not been performed, and a larger study is required to obtain more detailed results.

In conclusion, we found that histological subtype, the presence of metastatic disease, tumor size and Ki-67 proliferation index were associated with disease-specific survival in patients with resected pulmonary carcinoid. Resected PCs were, however, generally characterized by a favorable long-term survival. We confirmed that TC and AC have different clinical behavior and long-term prognoses, warranting correct tumor classification at the time of diagnosis.

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