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DIAGNOSTICS AND TREATMENT IN BRONCHIAL CARCINOID TUMORS

Ellen M. B. P. Reuling

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Diagnostics and treatment in bronchial carcinoid tumors

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van de Faculteit der Geneeskunde
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door

Ellen Marie Brigitte Paulien Reuling

geboren te Wehl

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Contents

Chapter 1

General introduction and outline of the thesis

Carcinoid tumors

History of carcinoid tumors

In 1907, a German pathologist named Obendorfer, coined the term "karzinoide", or "carcinoma-like" tumor to describe the unique feature of a tumor which behaved like a benign tumor, despite resembling a carcinoma microscopically. The endocrine-related properties where discovered much later in 1948 by Rapport, who isolated serotonin that was related to the Kulchitsky cells of the tumor [1].

Tumor biology

Carcinoids are rare slow-growing neuroendocrine tumors (NET) that originate primarily from enterochromaffin cells (Kulchitsky cells), predominantly located in the lungs and gastrointestinal tract. The diagnosis is based on histological confirmation and a characteristic immunohistochemical profile with markers such as Chromogranin A, Synaptophysin and CD56. Tumor-generated vasoactive substances can enter the circulation to produce carcinoid syndrome, which is very rare in bronchial carcinoid [1]. The most common biologically active substance secreted from carcinoid tumors is serotonin (5-HT), which is produced by the Kulchitsky cells. This peptide is involved in numerous physiological processes including sleep, thermoregulation, learning and memory, pain, (social) behavior, feeding, motor activity and biological rhythm. Carcinoid syndrome consists of symptoms such as flushing, diarrhea, and less frequently, heart failure, vomiting and bronchoconstriction and is often related to disseminated disease [2]. Rarely, a Cushing's syndrome is seen due to hormonal production of ACTH by the tumor [3].

Tumor classification

Recently, the WHO introduced a new classification for neuroendocrine neoplasms (NENs) which is specific for the primary location of the tumor [4]. Lung NENs are subdivided in well-differentiated neuroendocrine tumors (NETs) and poorly-differentiated neuro-endocrine carcinomas (NECs). NETs are low-grade typical carcinoids (TC/Grade 1), atypical carcinoids (AC/Grade 2) and atypical carcinoid with elevated mitotic counts and/or Ki67 proliferation index (Grade 3). NECs consist of large-cell neuroendocrine carcinoma (LC-NEC) and small cell lung carcinoma (SCLC)[5]. Carcinoid tumors are biologically distinct from high-grade neuroendocrine carcinomas, given the significant differences in clinical behavior. TC and AC are slow growing tumors with a lower tendency to metastasize compared to the aggressive high-grade neuroendocrine tumors (NECs). The differentiation of TC and AC is based on the mitotic count and/or the presence of necrosis (Table 1) [4-6].

Well-differentiated neuroendocrine tumor (NET)	Typical carcinoid/NET, grade 1	<2 mitoses/2 mm2 and no necrosis
	Atypical carcinoid/NET, grade 2	2–10 mitoses/2 mm2 and/or necrosis (usually punctate)
	Carcinoids/NETs with elevated mitotic counts and/or Ki67 proliferation index, grade 3	Atypical carcinoid morphology and a higher (>10 mitoses per 2 mm2) mitotic count and/or a higher (>30%) Ki67
Poorly-differentiated neuroendocrine carcinoma (NEC)	Small cell (lung) carcinoma	>10 mitoses/2 mm2, often necrosis and small cell cytomorphology
	Large cell NEC	>10 mitoses/2 mm2 , virtually always necrosis and large cell cytomorphology

Table 1: The World Health Organization (WHO) 2022 epithelial neuroendocrine neoplasms classification for the lung [4].

Organ specific disease

Carcinoids can arise anywhere in the body. However, the small intestine, rectum, and respiratory tract are the most common sites. Gastrointestinal carcinoids account for approximately 75%, and pulmonary carcinoid for 20%, of all neuroendocrine tumors. Carcinoids are incidentally found in the thymus (5%) [1].

Bronchopulmonary carcinoids

Bronchial carcinoids (BC) are highly vascularized polypoid lesions. Both TC and AC predominantly occur in the central airways. BCs are usually asymptomatic until they become large enough to obstruct the involved bronchus, which can cause recurrent or non-resolving pneumonia, wheezing, persistent cough and dyspnea. Because these symptoms are not specific for BC, diagnostic delay is not uncommon. More peripheral located carcinoids are often asymptomatic [7]. In contrast to non-small cell lung carcinoma (NSCLC), bronchial carcinoid is not associated with smoking and usually present at a younger age (40-60 years) [5,7,8]. Carcinoid syndrome is very rare in BC [1].

Epidemiology of bronchial carcinoid tumors

Bronchial carcinoid tumors comprise approximately 1% of all lung cancers [5]. The incidence of 0.2 per 100.000 persons results in approximately 125 TC and 45 AC annually in the Netherlands [9], which is approximately 1% of all lung cancer in the

Netherlands [5]. However, its incidence and prevalence has increased over the last decades, possibly related to an increased use of chest computed tomography (CT) [10,11]. AC is characterized by a higher metastatic rate than TC (23% in AC and 8% in TC), which also translates into poorer prognosis. After curative intent resection, 5-year survival rate for AC is lower than for TC (76% vs 94%) [1].

Diagnosis and staging

Computed tomography
Bronchial carcinoid is classified according to the 8th Edition TNM classification of lung cancer. Prior to treatment, all patients undergo a computed tomography (CT) scan of the chest and bronchoscopy as part of routine workup. CT scanning provides excellent anatomic detail of the lobulated endobronchial and intra –and extraluminal components of the tumor (Figure 1 and 2 B/C). The centrally located BC's are typically located, at least in part, in the lumen of the airway [12]. Contrast-enhanced CT scans frequently show marked enhancement due to the vascular nature of the tumors.

Bronchoscopy
CT imaging cannot distinguish bronchial carcinoid from a carcinoma and therefore histologic biopsy remains mandatory. For central tumors, bronchoscopic biopsy results in the diagnosis in the majority of patients. The most important complication is bleeding which usually can be stopped using endobronchial interventions such as local instillation of cold saline, adrenaline, xylomethazolin, tranexamic acid or compression [13]. In patients with a more peripheral localization, navigation bronchoscopy or transthoracic needle aspiration (TTNA) may establish a diagnosis.

Endobronchial ultrasound
Endobronchial ultrasound (EBUS) is standard in the work up for centrally located NSCLC, or NSCLC with clinical suspected lymph node involvement. However, the role of EBUS in pulmonary carcinoid is unclear [14-16]. Preoperative EBUS with needle aspiration can find nodal metastases not previously identified by imaging [16]. However, hilar (N1) or mediastinal (N2) lymph node involvement in pulmonary carcinoid will in most patients not change treatment, because neo-adjuvant treatment in pulmonary carcinoid has never been shown to be effective in increasing resectability or survival [17,18].

Nuclear imaging
Baseline imaging using one of the somatostatin receptor-based imaging techniques, such as 68Gallium (68Ga) DOTATATE position emission tomography (PET), is generally recommended in patients with advanced NET's. Both as an adjunct to routine cross-sectional imaging, the location of metastasis and because evidence of somatostatin receptor expression (based on a positive scan) can be predictive for clinical response to therapy with somatostatin analogs (reported diagnostic accuracy of >90%) [19-25]. In our study population, the majority of patients had stage I disease

and therefore nuclear imaging was not indicated. [^{18}F]Fluorodeoxyglucose (FDG)-PET/CT is unreliable for diagnosis of carcinoid, due to low uptake of FDG [26-28].

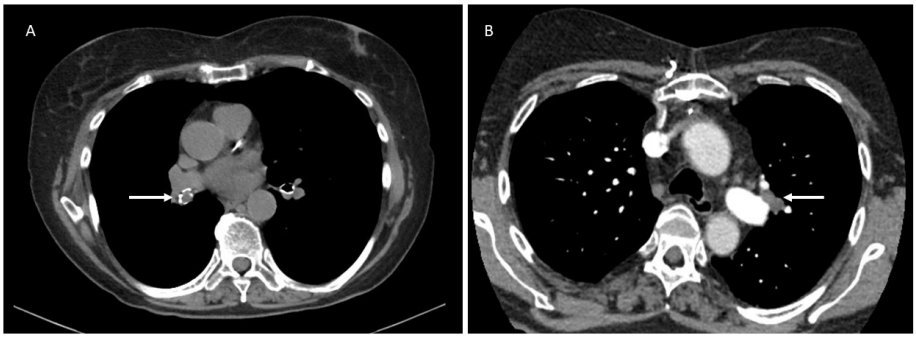

Figure 1: CT images of an intraluminal carcinoid tumor in the right bronchus (A) and an extraluminal carcinoid of the left bronchus (B) (white arrow).

MRI abdomen

Experts debate whether MRI of the liver should be part of the standard pre-operative work-up for BC's. While AC have a greater tendency to metastasize than TC, the diagnosis is almost never made preoperatively. Guidelines recommend liver imaging for all patients with a lung NET [29,30].

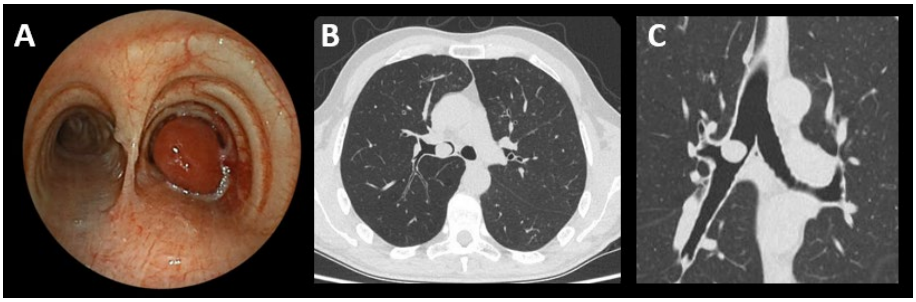

Figure 2: Bronchoscopic (A), axial (B) and coronal (C) CT images of a polypoid typical carcinoid in the right main bronchus.

Biomarkers

Serum levels of chromogranin A (CgA) are lower with lung NETs than they are with NETs at other sites, and they overlap with those seen in patients who have nonmalignant conditions associated with increased CgA level (for example use of proton pomp inhibitors). Thus, this marker is generally of limited use for patients with localized tumors. However, measurement of serum CgA levels can be useful to follow disease activity in the setting of advanced or metastatic disease, including BC's [31].

Treatment

According to current guidelines, surgical resection is the cornerstone of treatment for patients with bronchial carcinoid tumors [30,32]. However, because of the frequently indolent nature of these tumors, especially of typical carcinoid tumors, minimally invasive techniques such as endobronchial treatment (EBT) and parenchyma sparing surgical resection (wedge, segment or (bronchial) sleeve resection) are gaining popularity [13,33-37].

EBT is performed under general anesthesia by an interventional pulmonologist. The removal of the carcinoid tissue is established using laser, cryotherapy, diathermy, mechanical debulking, or a combination of these techniques (Figure 3). To prevent local recurrence, the location where the tumor was attached to the bronchus (base of the tumor) is often treated with cryotherapy (Figure 4) [38]. The excised specimen is sent for pathology for definite histology and classification. To detect residual disease, repeat bronchoscopy and CT scan is typically planned 6 weeks after EBT and biopsies are obtained if residual carcinoid is suspected. Surgery is planned in those patients with residual disease. In patients with minimal residual disease, EBT may be repeated when considered feasible. In the absence of residual disease, patients will be followed with CT scan and bronchoscopy [13].

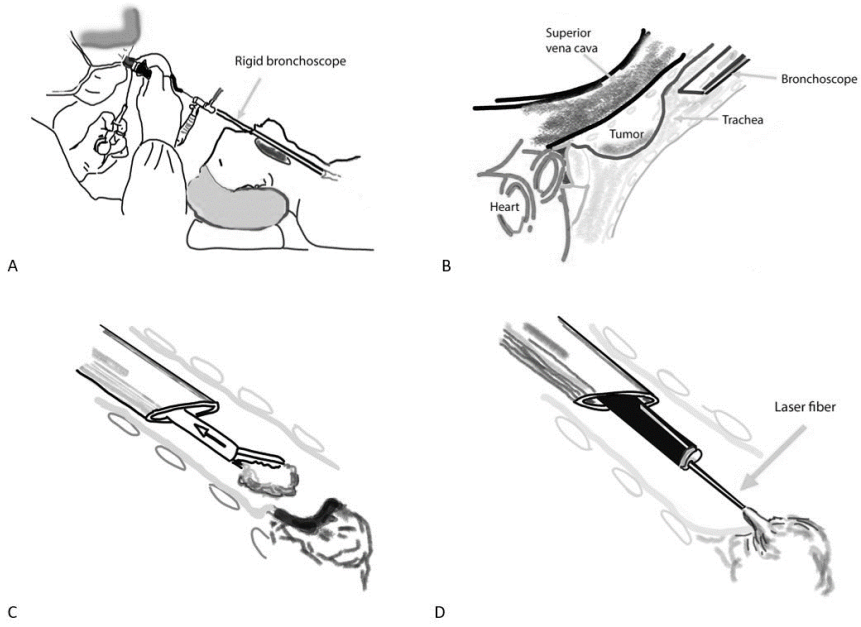

Figure 3: Endobronchial therapy. Rigid bronchoscopy under general anesthesia (A), obstruction of the central airway by a tumor (B), mechanical debulking of a tumor in the central airway (C), removal of central tumor with laser technique (D) [39].

In several cohort studies, EBT and parenchyma sparing surgery have demonstrated comparable oncological outcome with low morbidity when compared to standard surgical procedures [13,33-35,40-43,44-46]. Because no compromise can be made with regard to local control, not all patients qualify for these minimally invasive approaches. A bronchial carcinoid with an extrabronchial component on CT scan or evident during bronchoscopy, is not amenable to EBT and a patient with a relatively aggressive NET may not be a good candidate for limited surgical resection. Accurate patient selection is therefore crucial and depends on patient characteristics (e.g. age, comorbidity) and tumor characteristics such as T stage, N stage and histological classification.

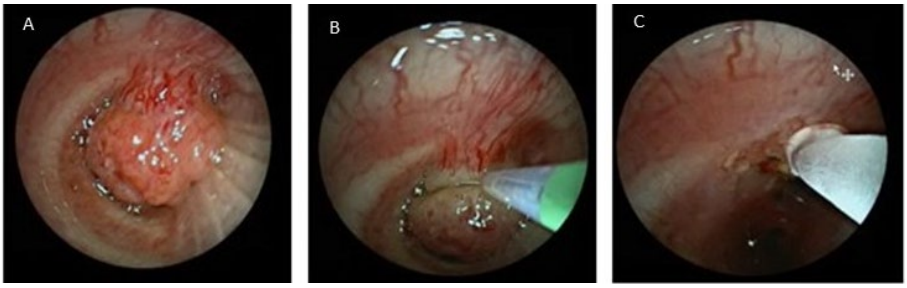

Figure 4. Endobronchial image of a polyp like carcinoid tumor in the left main bronchus (A) treated with endobronchial diathermy (B) and cryotherapy (C).

Outline of this thesis

At the start of this thesis there were several unanswered questions with regard to optimal patient selection for different treatments of bronchial carcinoid. Also, there were unanswered questions with regard to classification of bronchial carcinoid on small histological samples and with regard to prognostic value of several new tissue biomarkers. The aim of this thesis was to further optimize patient selection for parenchyma sparing techniques, and to explore the potential value of biomarkers in the treatment of bronchial carcinoid.

Part one (chapter 2-5) of this thesis describes the classification of bronchial carcinoid on small histological samples and the use of diagnostic and prognostic biomarkers.

In Chapter 2 we analyze patient selection for endobronchial treatment and underscore the importance of radiological assessment of bronchial carcinoids.

Differentiation between typical and atypical carcinoid according to the current WHO classification is accomplished by obtaining the mitotic count and the presence of necrosis. As outlined above, TC is defined as a neuroendocrine tumour with less than 2 mitoses per 2 mm^2 and absence of necrosis, while AC is defined by 2-10 mitoses per 2 mm^2 and/or the presence of (dot-like) necrosis [5,7,8]. However, it is known that small biopsies have limited diagnostic value for the distinction between TC and AC [47]. In Chapter 3 and Chapter 4 we analyzed biopsy-resection paired specimens of patients referred for treatment for bronchial carcinoid to measure accuracy of pretreatment biopsies and analyze the value of tumor biopsy size. Furthermore, we describe the reliability of different immunohistochemical markers on different biopsies and resection specimens, and assessed the impact of specimen size on the accuracy of the diagnosis.

Because the current classification of TC and AC only partially reflects their metastatic potential, there is a need for more accurate prognostic biomarkers for bronchial carcinoids. In Chapter 5 we investigated a combination of morphological and novel immunohistochemical markers for predicting dissemination.

Part two (chapter 6-8) focuses on therapeutic strategies and patient selection for EBT. In Chapter 6 we present the results of a systematic review of the available literature on the feasibility and outcome of endobronchial treatment compared to surgical resection. We studied several endpoints such as overall survival, disease free survival, recurrence rate, complications, quality of life, and healthcare costs.

Where EBT can be curative for patients with small intraluminal carcinoid tumors, tumor debulking prior to surgery may potentially result in less lung parenchyma that

has to be removed during surgery to achieve complete resection of the tumor. In addition, EBT may reduce the need for sleeve resection to achieve radical margins when bulky tumors are removed. It is in Chapter 7 that we investigated whether endobronchial therapy for bronchial carcinoid, if not curative, can reduce the extent of the surgical resection and whether EBT prior to surgery is associated with increased surgical morbidity.

Several letters, in reply to comments from experts in the field of treatment for bronchial carcinoid tumors, regarding published studies by our group, are bundled in Chapter 8.

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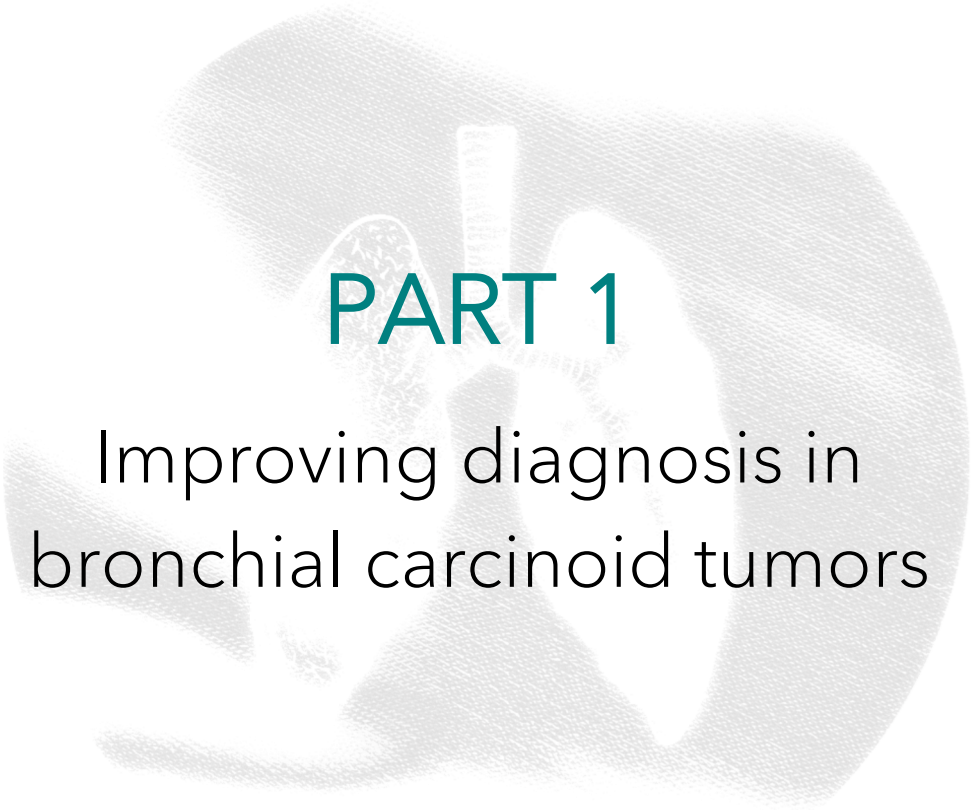

PART 1

Improving diagnosis in bronchial carcinoid tumors

Chapter 2

Endobronchial treatment for bronchial carcinoid: patient selection and predictors of outcome

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Respiration 2018

Abstract

Background

Traditionally, surgical resection is the preferred treatment for typical carcinoids and atypical carcinoids located in the lungs. Recently however, several studies show excellent long-term outcome after endobronchial treatment of carcinoid tumors located in the central airways. This study investigates clinical and radiological features as predictors of successful endobronchial treatment in patients with a bronchial carcinoid tumor.

Objectives

To identify clinical and radiological features predictive of successful endobronchial treatment in patients with bronchial carcinoid.

Methods

This analysis was performed in a cohort of patients with typical and atypical bronchial carcinoid referred for endobronchial treatment. Several patient characteristics, radiological features and histological grade (typical or atypical carcinoid) were tested as predictors of successful endobronchial treatment.

Results

One hundred and twenty-five patients with a diagnosis of bronchial carcinoid underwent endobronchial treatment. On multivariate analysis, a tumor diameter smaller than 15 mm (odds ratio 0.09; 95% confidence interval 0.02-0.5, p=<0.01) and purely intraluminal growth on computer tomography (CT scan) (odds ratio, 9.1; 95% confidence interval 1.8-45.8, p=<0.01) were predictive of radical endobronchial treatment. The success rate for intraluminal tumors with a diameter of <20 mm was 72%.

Conclusions

Purely intraluminal disease and tumor diameter on CT scan seem to be independent predictors for successful endobronchial treatment in patients with bronchial carcinoid. Based on these data, patients with purely intraluminal carcinoid tumors with a diameter <20 mm on CT scan are good candidates for endobronchial treatment, regardless of histological grade. In contrast, all patients with a tumor diameter of ≥20 mm should directly be referred for surgery.

Introduction

Pulmonary carcinoid tumors are included in the group of neuroendocrine tumors (NETs) and derive from pulmonary neuroendocrine cells [1,2]. Pulmonary NETs (pNETs) comprise around 20% of all lung tumors and are divided in 2 groups, based on specific tumor characteristics. The first group consists of high grade small cell carcinoma (SCLC) and large cell neuroendocrine carcinoma (LCNEC), both characterized by a high mitotic rate, aggressive behaviour and a predisposition to metastasize early [2]. The second group consists of atypical carcinoid (AC) and typical carcinoid (TC). These tumors have a more benign morphology, are less aggressive and have a lower tendency to metastasize [3].

Traditionally, surgical resection is the preferred treatment for both TC and AC. Recently however, several studies show excellent long-term outcome after endobronchial treatment (EBT) of carcinoid tumors located in the central airways [4-9]. When compared with surgery, EBT is a minimally invasive and parenchyma-sparing technique. Not all patients with carcinoid located in the central airways are candidates for endobronchial treatment because several part of the bronchial carcinoids have an extraluminal component. In a previous study from our institute, it was reported that EBT is successful in 42% of the patients [5]. If curation cannot be achieved by EBT, other advantages of EBT may include desobstruction of the involved bronchus with ensuing resolution of post-obstructive pneumonia and reduction of the extent of the subsequent surgical resection. However, these unsuccessful beneficial effects have not been proven. Since serious complications can occur during EBT [5] and because EBT attempts can delay curative treatment, it is critical to assess as soon as possible whether or not patients are good candidates for EBT. Identification of those patients with bronchial carcinoid who are good candidates for EBT would facilitate adequate selection of the most appropriate treatment modality. In the current study, we aimed to identify clinical and radiological factors predictive of successful EBT in patients with bronchial carcinoid.

Material and Methods

Study design & methods

With Institutional review board approval (Medical Ethics Review Committee of VU University Medical Center, IRB00002991), a cohort of patients referred to our tertiary referral center for endobronchial treatment of (suspected) bronchial carcinoids was established between June 1991 and December 2015. Details of this patient cohort and the EBT technique have been described before [5]. In short, prior to treatment all patients underwent a Computed Tomography (CT scan) of the chest and bronchoscopy as part of the routine work-up. Radiological images were not stored as part of the prospective cohort study. After informed consent, EBT was performed by experienced interventional pulmonologists and procedural data were registered. The procedure was planned based on information obtained from the chest CT scan and the bronchoscopy. Patients were excluded from EBT in case of evident and significant extraluminal growth, lymph node involvement or evidence of multifocal/disseminated disease on CT scan. EBT was performed under general anesthesia. At the discretion of the interventional pulmonologist, removal of the carcinoid tissue was established using Yttrium Aluminium Garnet (YAG) laser, cryo- or electrosurgery, mechanical debulking, or a combination of these techniques. To prevent recurrences we treated the base with cryotherapy. The excised specimen was sent for pathology for definite histology and classification. To detect residual disease, repeat bronchoscopy and CT-scan were typically planned six weeks after EBT. Biopsies were obtained if residual carcinoid was suspected. Surgery was planned in those patients with extensive residual disease. In patients with minimal residual disease EBT was repeated if deemed feasible. In the absence of residual disease, patients were followed with CT scan and bronchoscopy annually.

For the current analysis, we retrospectively reviewed the patients from the cohort in order to identify clinical and radiological factors predictive of successful EBT. Successful EBT was defined as the absence of residual disease during the first two years of follow-up with CT-scan and bronchoscopy after EBT. Disease seen during follow up after two years was defined as recurrence of disease. The following potentially predictive factors for successful EBT were considered: sex, age, smoking history, ASA (American Society of Anesthesiologists) classification [10], location (central: trachea and main bronchi; peripheral: segmental branches or more distal), diameter of the carcinoid tumor on CT scan, purely intraluminal disease on CT scan, purely intraluminal disease during bronchoscopy and final pathology (typical carcinoid, 0-1 mitosis per mm^2 and atypical carcinoid, ≥2 mitosis per mm^2). Available CT images of sufficient quality were revised by a senior chest radiologist and a resident. Sufficient quality of CT images was defined as the availability of digital images with an axial slice

thickness of ≤5 mm. A structured assessment was made with the following parameters: tumor location, tumor diameter (long axis, axial plane), signs of pneumonia and/or atelectasis, signs of extraluminal disease, enlarged lymph nodes and distant metastases. Patients in whom tumor extension on CT scan could not be clearly assessed, were scored as "indeterminate" by the radiologist and were added to the "possible extraluminal disease" group for the purpose of the statistical analyses.

Analysis

The statistical analyses and calculations were performed with SPSS 22.0 (SPSS Inc., Chicago, Illinois, USA) (ER, VC). Categorical variables were expressed as proportions and continuous variables as means and standard deviations. We tested the univariate associations between the independent prognostic factors (sex, age, smoking, ASA score, location, diameter on CT scan, purely intraluminal disease on CT scan, purely intraluminal disease at bronchoscopy, histology after bronchoscopy (typical/atypical) and the dependent factor "successful EBT", by either the Fisher's Exact Test (dichotomous/nominal variables) or the T-test (continuous variables). Effect sizes for the univariate associations were presented as odds ratios (ORs) or mean differences, both with appropriate 95% confidence intervals. Subsequently, a multivariate logistic regression model with backward selection was fitted. A P value of <0.05 indicated statistical significance.

Results

From June 1991 until august 2015, 154 patients were referred for EBT, of which 125 underwent EBT (Figure 1). Baseline characteristics of patients who underwent EBT are presented in Table 1. Mean age was 48.4 years (SD 14-84) and 56% (n=70) of the patients was female. Patients were classified by the anesthesiologist as ASA I in 48% (n=60), ASA II 34% (n=42) and ASA III in 18% (n=23) of patients. Most patients presented with a (recurrent) pneumonia (n=42, 34%). Other symptoms were cough (n=24, 20%), hemoptysis (n=23, 18%) and dyspnea (n= 23, 18%). Twenty-nine patients (19%) were not eligible for EBT due to significant extraluminal disease reported on CT scan or during bronchoscopy (n=26), or because of the presence of multiple tumor lesions (n=3) (Figure 1). One hundred and twenty-five patients (81%) underwent EBT. The majority of patients were treated in one session (n=77, 62%). A total of two, three, four or even five EBTs were necessary in 26% (n=33), 6% (n=7), 2% (n=3) and 4% (n=5) of patients, respectively. In 61 patients (49%) EBT was successful. EBT was unsuccessful in 64 patients (51%) due to extraluminal disease (n=51, 80%) or histologically confirmed residual disease (n=13, 20%). Patients not eligible for EBT (n=29, 19%) and patients in whom EBT failed and subsequently considered not

eligible for repeat-EBT (n=64, 42%), were candidates for surgical resection (n=93, 60%). Eighty-two of these patients (53%) underwent surgical resection. Operations performed included: 32 lobectomies, 21 bilobectomies, 6 pneumonectomies, 21 sleeve lobectomies, 1 brochus sleeve and 1 segmentectomy. The median time between unsuccessful EBT and surgery was 3 months (interquartile range 9 months). Eleven patients (7%) did not proceed to surgery. Eight patients (5%) were deemed inoperable due to poor physical status/comorbidities and 3 patients (2%) refused surgery. Of all 125 patients treated with EBT, 10 (8%) had residual or recurrence of disease during follow up. Four (3%) patients showed residual disease ≤ 2 years after EBT. One (1%) patient was treated with EBT and three (2%) received a lobectomy. Only 6 (5%) patients showed a recurrence after 2 years after the first EBT. Two (2%) patients refused surgery and are alive with disease. The other 4 (3%) patients received a surgical resection and are still alive without disease (Table 4a and 4b Supplemental data).

Survival

Of all 61 patients in whom EBT was successful, fifty-two (85%) are still alive without signs of disease recurrence. Seven (12%) patients died of another cause, one patient (2%) died of an unknown cause and one patient (2%) was lost to follow-up. Of the 56 patients that underwent surgery after unsuccessful EBT, 47 (84%) are still alive without disease recurrence. Three (5%) patients had recurrence of disease after surgery but are still alive. Two patients (4%) died of another cause, three (5%) patients died related to the bronchial carcinoid (2 metastatic disease and one due to postoperative complications) and one (2%) was lost to follow up (Table 5a and 5b in the Supplemental data). The median follow-up time of all included patients was 82 months (interquartile range 98 months).

Complications

EBT remained uncomplicated in 86% (n=108) of the patients. Complications were bleeding (n=12, 9%), one of which required emergency surgery, bronchospasm (n=1, 1%), a broken tooth (n=1, 1%) due to rigid bronchoscopy, vocal cord paralysis (n=1, 1%), and stricture of the bronchial tree (n=2, 2%).

Factors predicting successful EBT

Table 2 shows the univariate and multivariate analysis of prognostic factors of successful EBT. For the purpose of the multivariate analysis, tumor diameter on CT scan was dichotomized by using the median as cut-off value (15 mm). Smoking (p=0.01), purely intraluminal disease on CT scan (p=<0.01) or during bronchoscopy (p=<0.01) and tumor size on CT scan (p=<0.01) were significantly associated with successful EBT on univariate analysis. Multivariate analysis revealed tumor size (<15 mm) on CT scan and purely intraluminal growth on CT scan as significant independent predictors for successful EBT. No patient with a tumor size of ≥20 mm

was successfully treated by EBT (Figure 2). Agreement between CT scan and bronchoscopy with regard to luminal extension was poor, since 41% of patients with possible extraluminal growth on CT scan were diagnosed as purely intraluminal during bronchoscopy. When combining the significant prognostic factors; tumor diameter and growth pattern on CT, we found that small (<20 mm) purely intraluminal tumors have a success rate of 72% (Table 3).

Discussion

We found that purely intraluminal disease on CT scan and tumor diameter on CT scan are independent predictors of successful EBT, whereas patient characteristics, bronchoscopic findings and histologic grade are not. Tumor diameter is the strongest predictor of successful EBT.

In the current study, we show that patients with a carcinoid tumor of <20 mm as identified on CT scan, can be safely treated with EBT regardless of histological grade. For these patients EBT represents a minimally invasive and parenchyma sparing alternative for surgical resection. Because no patient with a tumor of ≥20 mm was successfully treated with EBT, we suggest that these patients are directly referred for surgery. This would also make sense from a biological standpoint since it has been demonstrated earlier that tumor diameter has prognostic impact. A diameter of ≥30 mm is associated with advanced histological grade and poorer prognosis [11-15].

For patients with carcinoid tumors with possible extraluminal disease on CT scan, EBT is associated with a low success rate. However, it is intriguing that 28% of small tumors (<20 mm) with possible extraluminal growth on CT scan can still be successfully with EBT (Table 3). This means that almost 1 in 3 patients with a small bronchial carcinoid and possible extraluminal disease on CT scan might benefit from EBT. There could be several explanations for this finding. First, tumor growth with obstruction of the involved bronchus can cause inflammation or atelectasis, which makes it difficult for the radiologist to definitively rule out extraluminal growth. Indeterminate tumor growth on CT scan, as scored by our radiologist, was classified as possible extraluminal disease in our analysis. As a consequence, the 'possible extraluminal disease' group might have contained some purely intraluminal tumors. This is the reason that in practice we schedule a follow-up visit six weeks after EBT with bronchoscopy and CT scan. The removal of intraluminal disease and subsequent resolution of atelectasis often makes the second CT scan easier to interpret with regards to extraluminal extension. Radial endobronchial ultrasound can improve the assessment of tumor extent in or beyond the bronchial wall, but this technique was not used in the current study [16,17]. Second, the tumor can invade the bronchial wall

without extending beyond it. With techniques such as cryotherapy it seems feasible to treat bronchial wall involvement or minimal extraluminal disease. Cryotherapy seems a very suitable technique in these patients because it is directed to tissues with high water content, thereby sparing the cartilaginous structures in the bronchial wall. Bertoletti et al. specifically focused on the use of cryotherapy for the treatment of bronchial carcinoid in 18 patients and reported favorable long term outcome [4]. They proposed to treat the base of the carcinoid with cryotherapy to prevent local recurrence. Over the years, this approach has become standard practice at our institution as well.

The need for lymph node resection in TC and AC is an issue of ongoing debate. Previous studies showed that parenchyma sparing therapy (e.g. bronchoscopic resection, bronchial sleeve resections), without lymph-node dissection, did not influence survival in patients with TC and AC [4,5,8,18-20]. In contrast, other studies showed nodal status to be a very important prognostic factor, particularly in AC [11,13-15,21-26]. With this in mind, preoperative staging of TC and AC, with specific attention to lymph node status, is very important, especially when treating patients with minimal invasive techniques like EBT, as lymph nodes are not sampled during this technique. With a specificity of 90-93% for the assessment of nodal involvement in patients with bronchial carcinoids, a preoperative CT scan is a reliable tool to exclude lymph node involvement before EBT, and is therefore mandatory in the work-up for patients with bronchial carcinoid tumors [13,27]. In this study we used 2 years as a cut-off point to define successful EBT and experienced a very low total recurrence rate of 5%. This can help clinicians in informing patients about EBT for bronchial carcinoids. Furthermore, this serve as an practical surrogate endpoint for future prospective studies. Figure 3 shows a suggested flowchart to aid the selection of patients for EBT.

The findings of the present study must be interpreted in the context of several potential limitations. Since the patients were referred to our hospital for treatment with EBT, the population included in this study has been exposed to selection bias. We assume that small tumors located in the central airways were referred to our center while larger tumors were directly referred for thoracic surgery. Furthermore, this is a single center study, with experienced interventional pulmonologists in a tertiary referral center. This makes that results cannot be extrapolated to everyday practice in other hospitals. Because endobronchial treatment of bronchial carcinoids can be technically challenging and complications such as bleeding can occur, we advocate referral to specialized centers with a thoracic surgery department. Another important limitation of the current study is the number of missing CT scans. The cohort was established over a long period of time. We attempted to retrieve CT scans from patients included in the 90s and early 00s from referral hospitals, however most of them were destroyed or of poor quality resulting in a significant amount of CT scans (57%) not available for revision. However, nearly all CT scans of the last ten years were available, which makes our data reflective of the present era where high

quality CT scans are available almost everywhere. In the reports before revision, specific comments about tumor size and intra-or extraluminal growth, were often lacking, which underlines the importance of structured assessment and reporting of CT scans in patients with bronchial carcinoids. This study shows that CT scan has an important role in directing patients towards the most suitable treatment modality. Additional imaging techniques such as FDG positron emission tomography (PET)/ computed tomography (CT) (PET CT) or [68Ga]1,4,7,10-tetraazacyclododecane-N(I-IIII)-tetraacetic acid-(D)-Phe1-Thy3-octreotide (DOTATOC)-PET/CT (68Ga-DOTATATE) are relatively new techniques, and were not evaluated in this study. The clinical role of FDG-PET/CT is related to aerobic glycolysis which is amplified in fast growing tumor cells. Slowly growing tumors, for example bronchial carcinoid, have a lower metabolic rate than most other thoracic malignancies (ie, non-small cell lung carcinoma, small cell lung carcinoma). Multiple studies found that FDG- PET/CT for bronchial carcinoid, and expecially TC, is unrelieable due to low glucose uptake [28-30]. More promising is the recently introduced 68Ga-DOTATATE PET-CT scan. Because carcinoid tumor are rich in somatostatin receptors, this scan is more sensitive and more specific than FDG-PET-CT. Recent studies have shown a sensitivity of 90-97% for a 68Ga-DOTATATE in the detection of bronchial carcinoid [31-35]. 68Ga-DOTATATE PET-CT scan was only recently introduced in our hospital. We are currently investigating the additional value of 68Ga-DOTATATE scan in the diagnostic work-up of patients with bronchial carcinoid.

To minimize the risk of complications and to maximize the potential of EBT, we highly advocate to centralize this treatment to referral centers with experienced radiologists for accurate and systematic CT scan evaluation, interventional pulmonologists and pulmonary surgeons, in order to safely perform endobronchial treatment and manage complications such as airway hemorrhage.

Conclusion

Small tumor size and purely intraluminal disease on CT scan are associated with successful EBT in patients with bronchial carcinoid, independent of the histological grade. Based on these data, CT scan has an important role in directing patients towards the most suitable treatment modality. While patients with a bronchial carcinoid tumor of ≥20 mm in diameter should be referred for surgery, purely intraluminal carcinoids with a diameter of <20 mm can be treated with EBT with a success rate of 72%. Careful analysis of the baseline CT scan, including lymph node status, enables optimal selection of the appropriate treatment modality.

Characteristics	Frequency	Percentage
Number of patients	125	100
Age (SD)	48.4 (14-85)	
Gender		
Female	70	56
Male	55	44
Smoking		
Former/ current smoker	61	49
Never smoker	57	46
Missing	7	5
Comorbidity		
ASA 1	60	48
ASA 2	42	34
ASA 3	23	18
ASA 4	0	0
ASA 5	0	0
Presenting symptoms		
Pneumonia	42	34
Cough	24	20
Dyspnea	23	18
Hemoptysis	23	18
Asthma like disease	3	2
Incidental finding	3	2
Other	5	4
Unknown	2	2

Table 1: baseline data of patients who received bronchoscopic therapy.

Prognostic factors	Successful EBT *n=61* Number of patients (%)	Unsuccessful EBT *n=64* Number of patients (%)	Univariate analyses *(p)*	OR (95%CI)/ mean (diff)*	Multivariate analyses *(p)*	OR (95% CI)
Sex			0.3	0.8 (0.7-2.7)	-	-
• *Male*	29 (48)	26 (41)				
• *Female*	32 (52)	38 (59)				
Smoking			**0.01**	2.7 (1.3-5.6)	NS	-
• *Current or former*	36 (59)	25 (39)				
• *Never smoker*	20 (33)	37 (58)				
• *Unknown*	5 (8)	2 (3)				
Age (mean in years)	49.0	47.9	0.7	1.2 (-4.7 - +7.1)*	-	-
ASA-classification			0.4	no odds†	-	-
• *ASA 1*	28 (46)	32 (50)				
• *ASA 2*	19 (31)	23 (36)				
• *ASA 3*	14 (23)	9 (14)				
Location			0.5	0.9 (0.5-2.5)	-	-
• *Central lesion*	47 (77)	50 (78)				
• *Peripheral lesion*	14 (23)	14 (22)				
Diameter CT scan∞ (mean 15 mm)			**<0.01**	11.3 (6.9-15.7)*	**<0.01**	0.09 (0.02-0.5)
• ≤15 mm	16 (26)	11 (17)				
• >15 mm	2 (3)	25 (39)				
• Indeterminate	18 (30)	22 (34)				
• missing	25 (41)	6 (9)				

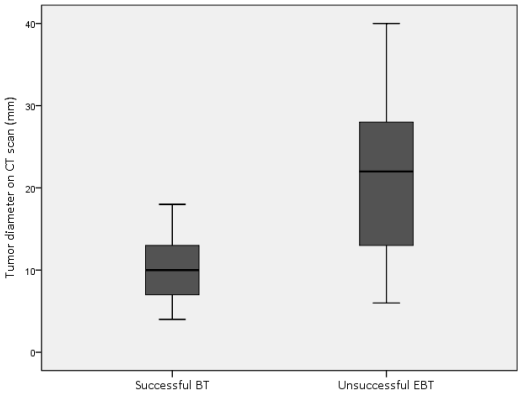
Purely intraluminal disease on CT scan∞			**<0.01**	11.5 (4.3-30.8)	**<0.01**	9.1 (1.8-45.8)
• *Yes*	27 (44)	46 (72)				
• *No*	9 (15)	12 (19)				
• *Missing*	25 (41)	6 (9)				
Purely intraluminal disease at bronchoscopy			**< 0.01**	9.6 (3.6-25.4)	NS	-
• *Yes*	3 (5)	27 (42)				
• *No*	55 (90)	31 (48)				
• *Indeterminate*	3 (5)	6 (10)				
Histology after bronchoscopy			0.3	1.8 (0.7-4.8)	-	-
• *Typical*	54 (89)	51 (80)				
• *Atypical*	7 (11)	12 (18)				
• *No differentiation possible*	0 (0)	1 (2)				

Table 2: Prognostic factors of successful-and unsuccessful EBT. Significant p values are presented in bold. NS=not significant, ∞ n=94, † due to 3 variables.

Tumor diameter/growth pattern tumor	Successful	(%)
<20 mm / purely intraluminal growth	13/18	72
<20 mm / possible extraluminal growth	5/18	28
≥20 mm / purely intraluminal growth	0/20	0
≥20 mm / possible extraluminal growth	0/1	0

Table 3: curation rates of EBT for patients groups based on tumor diameter and growth pattern.

◀ *Figure 1: treatment of patients with bronchial carcinoid.*
** Due to residual or extraluminal disease.*



▶ *Figure 2: tumor diameter as assessed on initial CT scan in patients with successful and unsuccessful endobronchial treatment (EBT).*

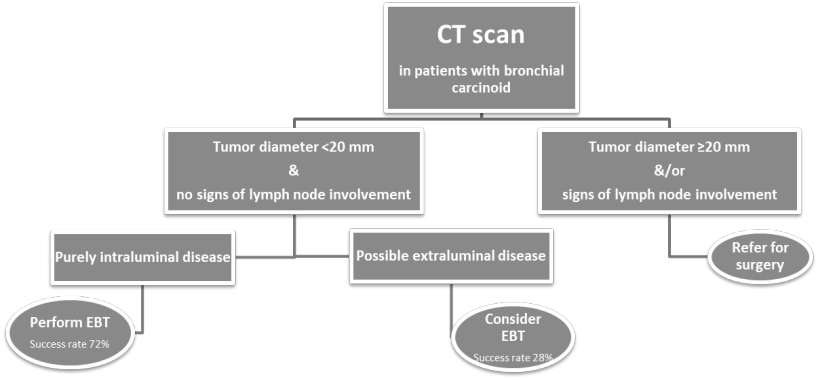


Figure 3: suggested treatment protocol of bronchial carcinoid based on assessment of the baseline CT scan.

Number	Time of residual disease after EBT (yrs)	Treatment of residual disease	Outcome
1	0.3	Lobectomy	Alive without disease
2	1.0	Lobectomy	Alive without disease
3	1.6	Lobectomy	Alive without disease
4	2.0	EBT	Alive without disease

Supplemental Table 4a: residual disease after EBT (≤ 2 years).

Number	Time of recurrence after EBT (yrs)	Treatment of recurrence	Outcome
1	3.7	Lobectomy	Alive without disease
2	5.2	No treatment∞	Alive with disease
3	8.6	Pneumonectomy	Alive without disease
4	9.5	Pneumonectomy	Alive without disease
5	11.9	EBT*	Alive with disease
6	16.5	Bronchus sleeve resection	Alive without disease

*Supplemental Table 4b: recurrences after EBT (>2 years), ∞refused EBT and surgery, *refused surgery.*

Deaths during follow up	Interval death after first EBT (months)	Cause
1	4	Dead other cause
2	26	Dead other cause
3	62	Dead other cause
4	83	Dead other cause
5	120	Dead other cause
6	171	Dead other cause
7	223	Dead other cause
8	unknown	Dead other cause
Mean survival	207 (SD 181-232)	

Supplemental Table 5a: deaths during follow up in patients with successful EBT (n=61).

Deaths during follow up	Interval death after first EBT (months)	Cause
1	12	Postoperative complications
2	24	Dead other cause
3	60	Dead other cause
4	81	Metastatic disease
5	unknown	Metastatic disease
Mean survival	251 (SD 222-281)	

Supplemental Table 5b: deaths during follow up in patients with unsuccessful EBT and surgery (n=64).

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Chapter 3

Diagnosis of atypical carcinoid can be made on biopsies > 4 mm^2 and is accurate

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Abstract

Introduction

In the 2021 WHO Thoracic tumors gradation of lung carcinoids in biopsies is discouraged. We hypothesized that atypical carcinoid (AC) could be reliably diagnosed in larger preoperative biopsies.

Methods

Biopsy-resection paired specimens of carcinoid patients were included, definitive diagnosis was based on the resection specimen according to the WHO 2021 classification. A total of 64 biopsy-resection pairs (26 typical carcinoid (TC) (41%) and 38 AC (59%)) were analyzed.

Results

In 35 patients (55%) tumor classification between the biopsy and resection specimen was concordant (26 TC, 9 AC). The discordance in the remaining 29 biopsies (45%, 29 TC, 0 AC) was caused by misclassification of AC as TC. In biopsies measuring <4 mm^2, 15/15 AC (100%) were misclassified compared to 14/23 AC (61%) of biopsies ≥ 4 mm^2. Categorical concordance of Ki-67 in biopsy-resection pairs at threshold of 5% was 68%. Ki-67 in the biopsy was not of additional value to discriminate between TC and AC, irrespective of the biopsy size. Atypical carcinoid is frequently missed in small bronchial biopsies (<4mm^2).

Conclusion

Our study provides strong arguments to make the diagnosis of AC in case of sufficient mitosis for AC on a biopsy and keep the diagnosis 'carcinoid NOS' for carcinoids with ≤ 1 mitosis per 2 mm^2. If the carcinoid classification is clinically relevant, a cumulative biopsy size of at least 4 mm^2 should be considered. Ki-67 has a good concordance but was not discriminative for definitive diagnosis.

Introduction

Pulmonary carcinoids comprise a subgroup of neuroendocrine tumours and are categorized into low-grade typical carcinoid (TC) and intermediate-grade atypical carcinoid (AC) according to the current WHO classification [1]. Morphologically, TC is defined as a neuroendocrine tumour with 0 or 1 mitoses per 2 mm^2 and absence of necrosis, while AC has 2-10 mitoses per 2 mm^2 and/or dot-like necrosis [2,3]. Ki-67 is a widely accepted marker in the diagnostic pathology of gastrointestinal neuroendocrine tumors [4] and it showed a lower interobserver variability than the mitotic count [5]. However, Ki-67 is currently not used for distinction between TC and AC, but some literature and expert opinion in the current 2021 WHO classification suggest that a Ki-67 ≥5% might be suggestive of AC [1,6]. Accurate identification of AC at time of diagnosis can be clinically relevant as it directs treatment selection. For example, endobronchial treatment is a promising parenchyma sparing procedure for selected patients with centrally growing intraluminal bronchial TC. During this parenchyma-sparing procedure, a rigid bronchoscope is used which allows for larger biopsies and in selected cases even complete resection [7]. Furthermore, diagnostic accuracy might implicate a more aggressive search for potential dissemination as AC tend to metastasize more often than TC [3]. However, in the latest WHO classification of thoracic tumors 2021, classification of carcinoids in the biopsy is discouraged, suggesting a diagnostic term 'carcinoid NOS' in the biopsy [1]. We hypothesized that AC could be reliably diagnosed in larger preoperative biopsies. To test this hypothesis, we investigated the relation of biopsy surface, Ki-67 and accuracy of diagnosing of AC correctly.

Methods

Approval of the institutional review board (Medical Ethics Review Committee of VU University Medical Center, IRB00002991) was retrieved. Patients who underwent surgical resection for centrally located pulmonary carcinoid (stage I-III) between June 1991 and December 2019 at the Amsterdam University Medical Center, were screened for eligibility. Central tumors were defined as tumors situated proximal to the segmental bronchi. Patients who had paired diagnostic biopsies obtained with either flexible (FLB) or rigid (RIB) biopsy, were selected. Samples of central carcinoid tumors were independently evaluated by two pathologists (TR & ET), and scored for mitotic count, presence of necrosis and diagnosis. The Ki-67 scoring was based on an estimated percentage of positive cells in a hotspot region after scanning the whole slide. Ki-67 index was calculated in surgical specimens by counting at least 2000 consecutive tumors cells in hot spot fields at ×40 magnification or 2 mm^2 for consistency with the histological classification [6]. The highest recorded value was taken into account, as described before [8]. Two Ki-67 thresholds of 3 and 5% described in the literature were used [6,9,10]. Tumor classification on the resection specimen was considered as the gold standard, and thus marked as definitive diagnosis. Mitotic figures on biopsies and resection specimen were counted as described previously [11]. In short, the whole slide was first explored for mitotic hotspots and the mitotic count was subsequently performed in the hotspot area. HE stained slides were scanned using Phillips UFS scanner and analyzed with the Philips pathology viewer version 3.2. Histological tumor sample size was digitally measured and defined as tumor surface (mm^2) in the whole histological sample. Areas with cauterization or mechanical artifacts were discarded. The statistical analyses and calculations were performed with IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA) (ER, DN).

Results

Paired biopsy and resection specimens of central pulmonary carcinoids from 64 patients were available. The diagnosis was based on mitotic count, as (dot-like) necrosis was absent. No significant differences were observed between patients with TC (n=26) and AC (n=38) regarding clinic-pathological characteristics, except for a trend of a larger tumor diameter in patients with AC (p=0.05) (Table 1).

The diagnosis was concordant with definitive pathology in 35 out of 64 patients (55%, 26 TC, 9 AC). In the remaining 29 (45%) patients, the biopsy-based diagnosis was TC (n=29), while the diagnosis in the pulmonary resection specimen was AC. If AC was diagnosed in the biopsy, the diagnosis was consistently accurate (9/9, 100%; Table 2). In biopsies measuring <4 mm^2, 15/15 AC (100%) were misclassified as TC and in biopsies ≥4 mm2 14/23 (61%) AC were misclassified as TC (Table 2). Accuracy of correctly identifying AC in the biopsy did not further increase with the biopsy diameter (data not shown).

The Ki-67 proliferation index was assessed with cut off values of 3 and 5% in the biopsy. At a cut off of <3% in the biopsy, 20/37 (54%) AC's where misclassified as TC, compared to 8/17 (47%) ≥3%. In biopsies with a cut off value of <5%, 23/42 (55%) of the AC's where misclassified as AC compared to 5/12 (42%) in biopsies of Ki-67 ≥5% (Table 2B and C). In biopsies >4 mm2, Ki-67 did not increase the diagnostic accuracy for TC or AC (Table 2D).

Figure 1 presents the distribution of diagnoses (TC vs AC), mitotic count and Ki-67 index for flexible biopsy (FLB), rigid biopsy (RIB) and surgical resection specimen respectively. When considering biopsies obtained with FLB and RIB separately, discordance was 59% and 30%, respectively (p=0.021). In total, 38 (59%) cases were identified with definitive AC diagnosis. Nine histological AC diagnoses were made in the biopsies, more often in RIB (7/30, 23%) than in FLB (2/34, 6%) (p=0.07, Fig. 1A). In RIB, a higher number of mitotic figures was demonstrated when compared with FLB (p=0.012). In addition, RIB were significantly larger than FLB (median histological tumor sample size FLB; 3.1 mm^2 (range 0.1-15 mm^2) vs RIB; 29 mm^2, (range 10-145 mm^2), p<0.001).

Concordance in diagnosis between biopsy and resection was associated with increasing biopsy surface area (median sample size biopsy TA (diagnosis in biopsy TC and in resection AC) 3.9 mm^2 (range 0.10 -87 mm^2), TT (biopsy and resection diagnosis TC) 18 mm^2 (range 0.6-145 mm^2), AA (biopsy and resection diagnosis AC) 22 mm^2 (range 5-101 mm^2). Discordant biopsies versus concordant biopsies for typical carcinoid (p=0.009) and atypical carcinoid (p=0.004) where significantly

smaller (Figure 1C). In 68% (43/63) of the cases, the Ki-67 in the biopsy and resection were concordant (concordance category 1: Ki-67 0-5%, concordance category 2: Ki-67 ≥5%), more often in FLB (26/33, 79%) than in RIB (17/30, 57%) (p=0.05, Figure 1D).

Discussion

In the current study we showed that, the diagnosis of AC could be made on the biopsy and if so, the diagnosis was always accurate. Moreover, AC was consistently missed in biopsies <4 mm^2. Biopsies <4mm^2, all taken during flexible bronchoscopy, resulted in 59% of the patients discordantly classified as TC, compared to 30% of biopsies obtained with rigid bronchoscopy. Ki-67 in the biopsy did not show additional value in the discrimination between TC and AC, irrespective of the biopsy size.

Even though the classification of carcinoids in the biopsies is discouraged in the recent WHO 2021 [1], there are clinical situations where identification of AC in the biopsy might be relevant for the treatment choice [12-17]. For example, EBT and parenchyma saving procedures are not preferred in patient with AC. Our data showed that if AC could be diagnosed on a biopsy, the diagnosis was consistently accurate. However, out study also shows AC may be missed, even in larger biopsies. A classification as TC on a biopsy should be interpreted with caution and a diagnosis of 'carcinoid NOS' is more appropriate. The current WHO classification suggests that if the diagnosis AC can be made, this 'may be suggested in a comment'. Our study provides strong arguments to make the diagnosis of AC in case of sufficient mitosis for AC and keep the diagnosis 'carcinoid NOS' for carcinoids with ≤ 1 mitosis per 2 mm^2.

Our data are largely in line with a recent retrospective study analyzing the accuracy of pre-operative biopsies for bronchial carcinoid tumors. The authors reported a 57% discrepancy when diagnosis in the biopsy was compared to postoperative diagnosis with a wider variety of discrepancies [18]. In contrast to our data, only 15/330 (4.5%) AC's were diagnosed in the biopsy of which 6 were reclassified as TC in the resection specimen. However, this real-life study was based on the pathology reports of the national database. In our in depth study of biopsy type and diameter, we provide at least the partial explanation of the discordances in biopsy-resection pairs. Although underdiagnoses of AC was not excluded in larger biopsies, AC was always missed in biopsies <4 mm^2. Conceptually, a preferred cumulative biopsy surface may be estimated that is associated with a higher diagnostic accuracy. We assume that a cumulative surface of 4 mm^2 is equivalent to ± 4 bronchial biopsies of 1 mm^2 tumor

(Figure 2) or 2 biopsies of 2 mm^2 tumor. Therefore, if a preoperative identification of AC patients is of clinical importance, biopsy of ≥ 4 mm^2 should be considered.

This study could not show any additional value of Ki-67 proliferation index in the biopsy for discrimination of TC and AC in the resection, comparable to some previously published studies [10,19]. Published evidence so far has not allowed for definitive cut-off points to be determined for Ki-67 [6,10], although the 5th edition WHO guideline for thoracic tumors currently suggest that a Ki-67 ≥5% is most probably an AC [1]. Therefore, usage of Ki-67 as a diagnostic tool in lung carcinoid might be still debatable. However, Ki-67 might be useful as an independent prognostic marker of dissemination, additional to the mitotic count [6]. Current study did not investigate this aspect of Ki-67. Interestingly, concordance in Ki-67 between the biopsy and resection was higher than for the diagnosis TC or AC (based on the mitotic count), similar as described before [6]. Remarkably, flexible biopsies showed a higher Ki-67 concordance with the resection than rigid biopsies (79% vs 59% respectively). This might be explained by the high rate of tumor debulking during the rigid bronchoscopy (endobronchial treatment) and only a small tumor rest in the resection. Concordance of 79% is therefore probably more reflective of the daily clinical practice worldwide.

Flexible biopsies are easier to obtain and require less sedation compared to rigid biopsies. However, performing 4 biopsies in a relatively high vascularized tumor in non-anesthetized patients is challenging due to a risk of difficult bleeding control. Preferably these biopsies should be performed in a controlled setting under general anesthesia via a rigid bronchoscopy in specialized centers and should be considered whenever clinically relevant; e.g. in patients with central bronchial carcinoid tumors suitable for curative endobronchial therapy, or patients unfit for surgery in whom bronchoscopic debulking could relieve symptoms of dyspnea or post obstructive pneumonia.

Thus, biopsy size does matter, which was previously shown in large cell neuroendocrine carcinoma, where neuroendocrine morphology was more frequently lacking in smaller biopsies (<5 mm) when compared to larger biopsies [20]. In addition, for determination of PD-L1 in lung cancer, a biopsy size of <2 mm is associated with a 14% chance of false negative score [21,22]. These examples, and findings from the current study, underscore the fact that small biopsy samples are associated with 'false negatives' /underdiagnoses.

In our cohort a larger proportion of AC was observed than in the literature [3,15]. A possible explanation may be a selection bias, as our center is a tertiary referral center for endobronchial treatment and more complex surgery. The higher proportion of AC allowed a more precise investigation of diagnostic accuracy for AC in a relatively small patient population, the latter being a potential limitation of this study.

Conclusion

The diagnosis AC is frequently missed in small bronchial biopsies (<4mm^2). If the carcinoid classification is clinically relevant, a cumulative biopsy surface of at least 4 mm^2 should be considered. Our study provides strong arguments to make the diagnosis of AC in case of sufficient mitosis for AC on a biopsy and keep the diagnosis 'carcinoid NOS' for carcinoids with ≤1 mitosis per 2 mm^2. Ki-67 in biopsy-resection pairs had a higher concordance but was of no additional discriminative value for the definitive diagnosis, irrespective of the biopsy size.

Patient characteristics	N=64 (%)	TC (n=26)	AC (n=38)	p-value
Mean age in years (SD)	45 (16.2)	43 (15.8)	46 (16.7)	0.51
Female	40 (62.5)	14 (53.8)	25 (65.8)	0.33
Biopsy				
FLB	34 (53.1%)	12 (46.2%)	22 (57.9%)	0.36
RIB	30 (46.9%)	14 (53.8%)	16 (42.1%)	
Type of surgery	64	26	38	0.10
Pneumonectomy	3 (4.7)	2 (7.7)	1 (2.6)	
Bilobectomy	13 (20.3)	9 (34.6)	5 (13.2)	
Lobectomy	29 (45.2	8 (30.7)	20 (52.7)	
Sleeve lobectomy	17 (26.6)	6 (23.1)	11 (28.9)	
Segmentectomy	1 (1.6)	1 (3.8)	0	
Bronchial sleeve resection	1 (1.6)	0	1 (2.6)	
Tumor diameter				0.05
T1a	38 (59.4)	21 (80.8)	17 (44.7)	
T1b	14 (22)	3 (11.5)	11 (28.9)	
T2a	8 (12.5)	2 (7.7)	6 (15.8)	
T2b	2 (3.1)	0	2 (5.3)	
T3	2 (3.1)	0	2 (5.3)	
T4	0	0	0	
Nodal stage				0.78
N0	59 (92.2)	25 (96.2)	34 (89.5)	
N1	4 (6.3)	1 (3.8)	3 (7.9)	
N2	1 (1.6)	0	1 (2.6)	
Radicality				0.68
R0	58 (90.6)	23 (88.5)	35 (92.1)	
R1	6 (9.4)	3 (11.5)	3 (7.9)	

Table 1: Clinicopathological characteristics of central carcinoid cohort.

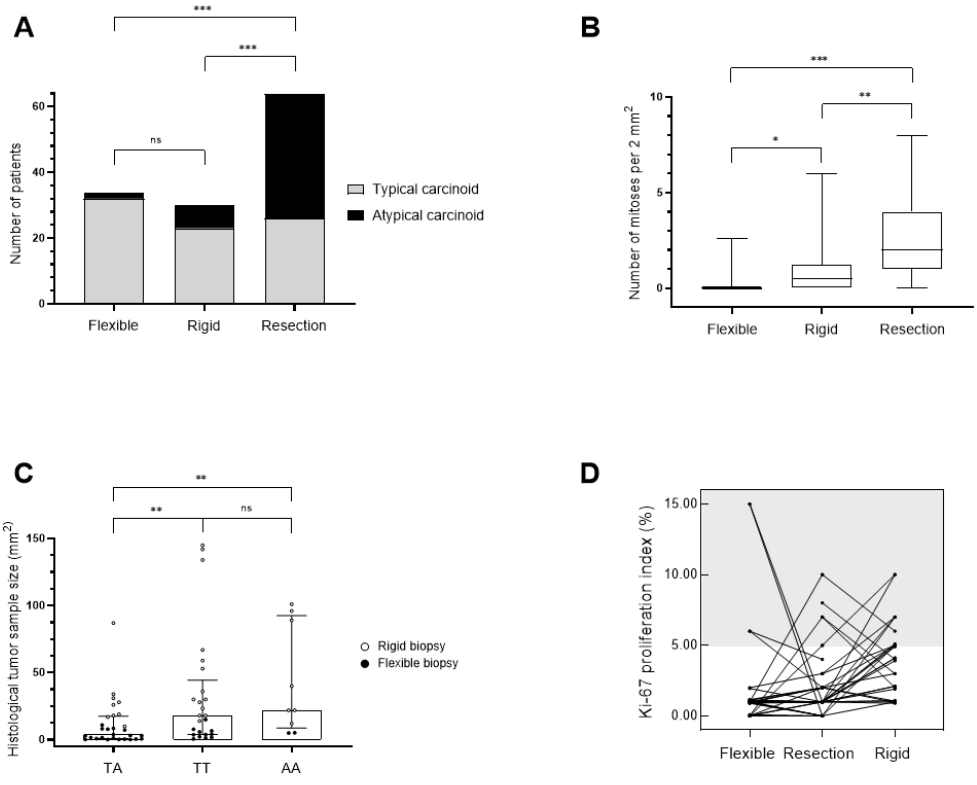

*Figure 1: Outcomes in flexible biopsy, rigid biopsy and resection in relation to typical and atypical carcinoid (A), mitotic count (B). Discordancy and concordancy between biopsy and resection in relation to biopsy sample size; discordant TA: diagnosis in biopsy TC and in resection AC; concordant TT: biopsy and resection diagnosis TC; AA: biopsy and resection diagnosis AC (C) . Concordancy between biopsy (flexible or rigid) and resection in relation to Ki-67 (D). * $p \leq 0.05$; ** $p \leq 0.01$ *** $p \leq 0.001$.*

A

	Diagnosis resection		
	TC	AC	Total
Diagnosis TC biopsy < 4mm²	6	15	21
Diagnosis TC biopsy ≥ 4mm²	20	14	34
Diagnosis AC biopsy < 4mm²	0	0	0
Diagnosis AC biopsy ≥ 4mm²	0	9	9
	26	38	64

B

	Diagnosis resection		
	TC	AC	Total
Diagnosis TC biopsy Ki-67 <3%	17	20	37
Diagnosis TC biopsy Ki-67 ≥3%	9	8	17
Diagnosis AC biopsy Ki-67 <3%	0	4	5
Diagnosis AC biopsy Ki-67 ≥3%	0	5	5
	26	37	63*

C

	Diagnosis resection		
	TC	AC	Total
Diagnosis TC biopsy Ki-67 <5%	18	23	42
Diagnosis TC biopsy Ki-67 ≥5%	8	5	12
Diagnosis AC biopsy Ki-67 <5%	0	5	5
Diagnosis AC biopsy Ki-67 ≥5%	0	4	4
	26	37	63*

D

	Diagnosis resection		
	TC	AC	Total
Biopsy < 4mm² and Ki-67 <5%	5	13	19
Biopsy < 4mm² and Ki-67 ≥5%	1	1	2
Biopsy ≥ 4mm² and Ki-67 <5%	13	15	28
Biopsy ≥ 4mm² and Ki-67 ≥5%	7	8	15
	26	37	63*

*Table 2: Diagnosis in the biopsy versus resection specimen, stratified for the biopsy size < and ≥ 4 mm² and stratified for Ki-67 < and ≥ 3 (B) and 5% (C). Note that none of the AC could be diagnosed in biopsies <4 mm2 and when AC was diagnosed on the biopsy it was always concordant with the section specimen (underscored (A). Ki-67 was not of additional value for discrimination between TC and AC in the biopsy (underscored B and C). Biopsy size combined with a Ki-67 < and ≥ 5% did not increase diagnostic accuracy (D). * Ki-67 missing (n=1).*

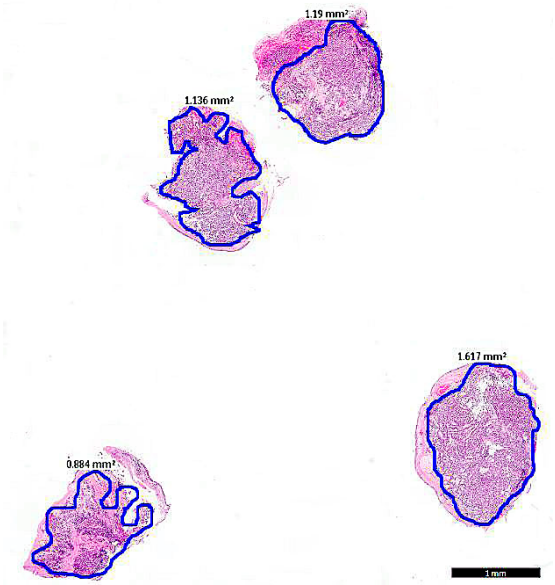

Figure 2: Example of 4 flexible biopsies of 1 mm² with bronchial carcinoid.

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Chapter 4

In-depth analysis of immunohistochemistry concordance in biopsy-resection pairs of bronchial carcinoids

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Abstract

Primary diagnosis of bronchial carcinoids (BC) is always made on biopsies and additional immunohistochemistry (IHC) is often necessary. In the present study we investigated the concordance of common diagnostic (synaptophysin, chromogranin, CD56 and INSM-1) and potential prognostic (OTP, CD44, Rb and p16) IHC markers between the preoperative biopsies and resections of in total 64 BCs, 26 typical (41 %) and 38 atypical (59 %) carcinoid tumors. Synaptophysin and chromogranin had 100 % concordance in all resected carcinoids and paired diagnostic biopsies. Synaptophysin was not affected by variable expression in biopsies compared to chromogranin, CD56 and INSM-1. Notably, INSM-1 IHC was false negative in 8 % of biopsies. Of the novel and potential prognostic markers, only CD44 showed 100 % concordance between biopsies and resections, while OTP showed two (4 %) false negative results in paired biopsies. While Rb IHC was false negative in 8 % of biopsies, no strong and diffuse pattern of p16 expression was observed. In this study, most false negative IHC results (85 %, 22/26) were observed in small flexible biopsies. Taken together, our data suggest excellent concordance of synaptophysin and CD44 on the preoperative biopsy samples, while other neuroendocrine markers, Rb and OTP should be interpreted with caution, especially in small biopsies.

Introduction

Bronchial carcinoids (BCs) are well-differentiated neuroendocrine (NE) neoplasms, representing approximately 1–2 % of all lung malignancies [1,2]. BCs exhibit prototypical NE morphology features, including organoid patterns, palisading arrangements, trabecular formations, and rosettes. BCs are composed of uniform tumor cells with eosinophilic cytoplasm, inconspicuous nucleoli and finely granular chromatin [3]. Besides high levels of immunoreactivity for common NE cell markers in thoracic pathology such as chromogranin A, synaptophysin and CD56, BCs show strong positivity for the novel nuclear INSM-1 marker [3-6].

The current World Health Organization (WHO) classification of thoracic tumors (5th edition; 2021) designates two grades of BCs: low-grade typical (TC) and intermediate-grade atypical (AC) carcinoids [3]. Distinction between TC and AC is based on histologic evaluation of mitotic count (TC 0–1 and AC 2–10 mitoses per 2 mm^2) and presence of focal or punctate necrosis in resection specimens. While proliferative assessment based on Ki-67 immunohistochemistry (IHC) is currently not used as primary diagnostic WHO criterion, it is acknowledged as an adjunctive tool for distinguishing carcinoids from high-grade carcinomas, especially in small or crushed biopsy samples [3].

Grading of BCs based on small biopsies is discouraged as it is considered imprecise for definitive grade, and thus only diagnosis of "carcinoid tumor not otherwise specified" (NOS) is recommended in non-resection material [3,7,8]. While surgical resection is required for the definitive grading of TC or AC, preoperatively obtained biopsies may guide treatment decisions (e.g., endobronchial therapy in TC versus preferred resection in AC) [3,7]. Although lobectomy is the preferred curative treatment for BCs, parenchyma-sparing strategies may be considered for small, centrally located TCs with intraluminal growth [9,10]. These treatment decisions are hampered by the limited concordance between carcinoid grade on paired biopsy-resection specimens [7,8]. In our previous work with biopsy-resection pairs of primary BCs, we showed a more precise differentiation between TC and AC in biopsies >4mm^2 and a higher concordance of Ki-67 compared to mitotic count, both relevant for the classification and differentiation with high-grade neuroendocrine tumors [8].

While there is a large amount of literature on the discordance of the carcinoid grade between the biopsy and resection in BCs, very few studies were devoted to the concordance of commonly used IHC markers between the biopsy-resection pairs. IHC can be used for solving the differential diagnosis with non-small cell lung cancer (NSCLC) and high-grade NE carcinomas. For example, when confirmation of the NE differentiation is necessary, commonly used NE markers can be applied such as

synaptophysin and chromogranin. IHC for retinoblastoma protein (Rb) and p16 might be helpful in differentiating BCs from high-grade carcinomas due to divergent aberrations in the Rb/p16/cyclin D1 pathway in high-grade NE tumors, resulting ultimately in loss of Rb and high p16 expression [11-13]. Novel IHC markers such as orthopedia homeobox (OTP) and CD44 have emerged from gene expression studies as potential predictors of adverse outcomes in BCs [14-16].

The aim of this study was to investigate the IHC concordance in biopsy-resection BC pairs of common diagnostic neuroendocrine markers (synaptophysin, chromogranin, CD56, INSM-1) and potential prognostic markers (CD44, OTP, Rb and p16).

Material and methods

Case selection

This study was approved by the Institutional Review Board (Medical Ethics Review Committee of VU University Medical Center, IRB00002991). As previously described by Reuling et al., a cohort of patients with surgically resected bronchial carcinoids and available paired diagnostic biopsies, acquired during either flexible (FLB) or rigid bronchoscopy (RIB) between June 1991 and December 2020 in the Amsterdam University Medical Center, location VUmc were included [8]. Consent from each patient after full explanation of the purpose of this study was retrieved. Tissue microarrays containing 304 NSCLC resection specimen were used as a control group for NE and potential prognostic (CD44, OTP) markers.

Pathology

Case evaluation and definitive diagnosis

The cases were evaluated and scored by two pathologists (TR & ET) specialized in lung pathology. The diagnosis, presence of necrosis and mitotic count were re-evaluated according to the 5th edition of WHO classification of thoracic tumors. Definitive diagnosis was based on the tumor in the resection specimen. Mitotic count was scored by a specialized laboratory technician (MB) as described previously [17]. In short, the mitotic count in the biopsy was evaluated in an area of 2 mm^2 or whole biopsy if the sample was smaller. In the resection specimen, the whole slide was first explored for mitotic hotspots and the mitotic count was subsequently performed in the hotspot.

Immunohistochemistry

IHC was performed by one laboratory technician (PK). From each block, 4-µm thick sections were cut and mounted on positively charged glass slides. These sections

were stained for chromogranin, synaptophysin, CD56, insulinoma-associated protein 1 (INSM-1), retinoblastoma gene (Rb), p16 protein, orthopedia homebox (OTP) and CD44. The features of antibodies and method for IHC are listed in Supplementary Table 1. H-scoring (range 0–300) was used to describe the IHC staining intensity. In short, the percentage of cells at each staining intensity level (0, 1+, 2+, and 3+) was scored and H-score was calculated using the following formula: 1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+) [18]. IHC in surgical resections was defined as the gold standard and staining intensities were compared in paired biopsy-resection specimens. A difference in H-scoring of >100 in staining intensity in biopsy-resection pairs was considered as potentially clinically meaningful. H-scores < 30 were considered negative. Cases with decreased immunoreactivity of at least two independent IHC markers in the same area of biopsy or resection specimens were considered to be influenced by tissue handling variables: pre-analytical variables e.g. delayed fixation or cauterization.

Statistics

Statistical analyses and calculations were performed with SPSS 26.0 (IBM Corp., Armonk, N.Y., USA). Chi-square and Fisher tests were applied for comparison of clinicopathologic variables. Differences in delta median H-scores between biopsy-resection specimens with and without variation in the pre-analytical phase (i.e. delayed fixation) were tested by the non-parametric Mann-Whitney U test. A p-value < 0.05 was considered statistically significant.

Results

Patient characteristics

We analyzed available paired biopsy-resection specimens of central bronchial carcinoids from 64 patients, of which 40 (62.5 %) were female. Additional clinicopathological characteristics were previously described by Reuling et al. and are summarized in Table 1 [8]. Depending on the mitotic index (no dot-like necrosis was found), final carcinoid classification on surgical resection revealed 26 TC (40.6 %) and 38 AC (59.4 %) diagnoses. Preoperative biopsies were performed using either flexible bronchoscope (n = 34, FLB) or rigid bronchoscope (n = 30, RIB). In our previous work, we described a significantly larger histological tumor sample size of RIB than FLB biopsies in this patient population [8].

IHC for diagnostic neuroendocrine markers in biopsy-resection pairs

IHC results for NE markers in biopsy-resection pairs are summarized in Table 2, Table 3. Overall, only synaptophysin and chromogranin showed positivity in all 64 matched

biopsy and resection specimens, irrespective of material type and carcinoid grade. IHC for CD56 and INSM-1 yielded positive results in 63 out of 64 (98.4 %) resected carcinoids. Of these 63 cases, three corresponding biopsies (2 FLB and 1 RIB), (4.8 %) were false negative for CD56 and five FLB (7.9 %) were false negative for INSM-1. Interestingly, we observed cytoplasmatic staining of INSM-1 in 92.2 % of the resection specimens, including some cases in absence of a nuclear staining (see Supplementary Fig. 1).

Next, we assessed the variability in IHC intensity of NE markers in biopsy-resection pairs. While CD56, chromogranin, and, in particular, INSM-1 showed major variation in IHC intensity within the pairs (35.9 %, 40.6 %, and 53.1 % cases with delta H-score > 100, respectively), synaptophysin was considerably less affected by highly variable expression (7.8 % cases with delta H-score > 100) (see Fig. 1).

Sensitivity and specificity of neuroendocrine markers for bronchial carcinoids

For resected bronchial carcinoids, synaptophysin and chromogranin demonstrated similar sensitivity (100 %) and were the most sensitive NE diagnostic markers in this study cohort. CD56 (95.2 %) and INSM-1 (92.1 %) were slightly less sensitive compared to synaptophysin and chromogranin (see Table 3). In terms of specificity, synaptophysin and chromogranin (99.7 %) were marginally higher than CD56 (98.4 %) and INSM-1 (96.1 %) as only sporadic NSCLC cases stained positive for these NE markers.

IHC for Rb and p16 in biopsy-resection pairs

Results are summarized in Table 2 and Fig. 1. All 64 resected carcinoids showed Rb IHC positivity, mostly in a diffuse and moderate-to-strong manner with an H-score of 100–300 (median: 200). While all paired rigid biopsies yielded positive IHC results, five out of 33 (15.2 %) flexible biopsies were false negative for Rb IHC with intact staining in endothelial cell nuclei (Fig. 2). p16 was found negative in these biopsies. p16 was found weak and partially positive in 11 of 63 (17.5 %) resected carcinoids. Of these, 9 were classified as AC and 2 as TC. No strong and diffuse pattern of p16 expression was observed (maximum H-score of 150, see Fig. 1). All corresponding biopsies, including 8 FLB and 3 RIB, were negative for p16 IHC.

IHC for OTP and CD44 in biopsy-resection pairs

OTP and CD44 IHC was positive in 56 (87.5 %; 30 AC, 26 TC) and 55 (87.3 %; 29 AC, 26 TC, 1 missing) resection specimens, respectively. Of the 56 OTP-positive resections, two corresponding FLB were false negative (3.6 %). Of the 55 CD44 positive resections, no false negative results were observed in corresponding biopsies. In contrast to the consistent expression of CD44 in biopsy-resection pairs (1.6 % cases with a delta H-score > 100), OTP was considerably more variable among

these samples (35.9 % delta H-score > 100) (see Fig. 1). Notably, in NSCLC, positivity for CD44 was more frequently observed (16.0 %) compared to OTP (2.3 %).

Pre-analytical variables

Variation of IHC that was recognized to be associated with pre-analytical handling was observed in 17 FLB (50 %), 15 RIB (50 %) and 27 (42.2 %) resection specimens. The influence of pre-analytical handling on the IHC performance in these biopsy-resection pairs was associated with significant variation in IHC intensities of OTP (median delta H-score 55 versus 0; p = 0.035) and INSM-1 (median delta H-score 100 versus 70, p = 0.039). Other IHC markers were not significantly influenced by pre-analytical handling (data not shown).

Discussion

In this study, we demonstrated that synaptophysin and chromogranin were the most concordant NE IHC markers in biopsy-resection pairs of BC. Of the novel and potential prognostic markers, CD44 showed the highest concordance in biopsy-resection pairs. In contrast to Rb and OTP, CD44 showed no false negative IHC staining results on the preoperative biopsy specimens. Most false negative IHC results were found in small flexible biopsies. In daily clinical practice, diagnostic work-up for BCs is frequently based on preoperatively obtained biopsies, incorporating morphologic features, NE markers and Ki-67. Diagnosis and classification of lung malignancies are, however, hampered by the nature of the biopsy taken (e.g. flexible bronchoscopy) as morphological features are insufficiently recognizable for the pathologist due to either small sample size or crush effects. The addition of IHC into routine diagnostic work, particularly in the context of small biopsies, allows optimization of proper pathologic classification and improvement of diagnostic reproducibility of lung tumors [19]. Reliability of different IHC markers in small bronchial biopsies is therefore an important diagnostic feature in lung pathology. The use of established NE markers in diagnostic work requires awareness of IHC concordance between biopsy-resection pairs.

In line with previous studies, all carcinoids were strongly and diffusely positive for synaptophysin IHC [5,6], irrespective of sample type and size. Moreover, our current study demonstrated that synaptophysin was less affected by variable expression and discordancy between biopsy and resection compared to chromogranin and CD56. Taken together, these results suggests that a single synaptophysin IHC may serve as an unequivocal marker of NE differentiation in biopsies when BC is morphologically suspected and needs to be confirmed. In the context of broader differential diagnosis, multiple NE markers might be a better choice. In terms of novel markers

for NE differentiation, several studies have discussed the potential role of transcription factor INSM-1 as standalone marker for SCLC diagnosis, mainly because of its unique combination of high sensitivity and near-perfect specificity [5,6,20-22]. For carcinoid tumors, the INSM-1 positivity scores in our resected BC cohort were similar to the findings previously reported [5,6]. Despite the high frequency of INSM-1 positive-resection specimens, IHC on paired small biopsies revealed false-negative results in several cases. Therefore, negative INSM-1 IHC results in small diagnostic biopsies should be interpreted with caution. Notably, of all NE markers, INSM-1 showed the highest variability of H-scores in paired biopsy-resection pairs. This discordance in IHC results could be partially attributed to the sensitivity of INSM-1 to (pre)analytical variabilities (i.e. fixation time and epitope degradation), in accordance with other nuclear transcription IHC markers such as TTF1 [23]. Although INSM-1 has emerged as a useful marker of NE differentiation due to the distinct nuclear expression, we observed a more common cytoplasmatic IHC without nuclear accentuation in a few cases. Taken together, synaptophysin might be the most concordant and least variable marker to confirm NE differentiation, even in small biopsies.

Gradation of BC is discouraged in the preoperative biopsies [4,7,8]. In general, small biopsies of pulmonary malignancies challenge accurate assessment of morphologic features, due to sampling error and lack of morphological features in little tissue. Moreover, in pulmonary carcinoids, the grading was proven to be imprecise in the biopsies, mainly due to underdiagnosis [3]. As in some patients the treatment choice can be based on the preoperative biopsy, concordance between biopsy and resection features is essential. The use of IHC might aid more precise grading of BCs in this context. Proliferative index quantified using Ki-67 IHC was shown to have a significant prognostic value for the recurrence free survival in several studies, although the cut-off values for the diagnosis of TC versus AC are not yet established [3]. We and others demonstrated a higher interobserver agreement and concordance of Ki-67 between biopsy-resection pairs compared to mitotic count, also in small biopsies [8,24].

The absence of membranous CD44 and nuclear OTP expression in pulmonary carcinoids were shown to predict metastatic potential, identifying patient subsets at risk of metastasis in whom more aggressive treatment strategies may be applied [14-16,25,26]. Therefore, loss of CD44 and OTP might be incorporated in classification of BCs in the future. IHC scoring may yield less interobserver variability than mitotic count, especially for IHC with binary results, like loss of OTP and/or CD44 in contrast to retained or positive staining. Future studies are necessary to conform these assumptions. Variation in expression of OTP was relatively more pronounced in biopsy-resection pairs compared to CD44, suggesting the widely available CD44 to be a more reliable marker in small thoracic biopsies. While CD44 showed no false negative results in biopsies, two FLB samples stained negative for OTP despite predominant OTP positivity in corresponding resection specimen. This might be the

result of the lower epitope concentration of nuclear transcription factors compared with cytoplasmatic proteins, with increased sensitivity to (pre)analytical variations similar to other nuclear markers such as INSM-1.

Inactivation of the tumor-suppressor gene Rb has been widely reported in large-cell NE carcinoma and small-cell carcinoma as a crucial step in tumor progression, but is rarely observed BCs [11-13,28]. In accordance with previous reports, no resected carcinoids in this study cohort showed loss of Rb. Despite retained Rb protein expression in resection specimens, five corresponding biopsies obtained with the aid of flexible bronchoscope were false-negative for Rb IHC. While Rb function might be helpful in differentiation between BCs and high-grade carcinomas, Rb IHC in small flexible biopsies should be interpreted with caution due to occasional discordant results. A negative p16 IHC could be helpful in this context, as Rb and p16 are involved in a negative feedback mechanism, with strong and diffuse positivity for p16 if Rb is lost. A previous study has indicated that the number of cases with strong and diffuse p16 protein expression progressively increased from lower- to higher-grade neuroendocrine carcinomas, with negative p16 in the majority of TCs and strong p16 positivity in almost all SCLCs [29]. In our data only minority BCs showed low p16 positivity which was not discriminative for the grade.

The findings of the present study must be interpreted in the context of several limitations. First, archival material was included in this study and therefore it cannot be excluded that unexpected negative IHC results were caused by epitope degradation. Second, given the low incidence rate of carcinoids, we could only analyze a limited number of biopsy-resection pairs. It should be noted, however, that this study represents the first comprehensive analysis of IHC concordance of novel and established routine markers in biopsy-resection pairs of BCs. As more markers may be implemented in routine diagnostics and management of BCs, we encourage further research on IHC concordance of these markers in preoperative biopsies and resection specimens.

Conclusion

In summary, of all diagnostic neuroendocrine markers, synaptophysin and chromogranin showed the highest concordance between small biopsies and paired resection specimens of BCs. With respect to prognostic markers, CD44 had the highest concordance in biopsy-resection pairs. Small biopsies with false negative results for Rb IHC were p16-negative, suggesting the added value of both Rb and p16 in the IHC panel for differential diagnosis with SCLC in small or crushed biopsies. Considering the higher frequency of false-negative IHC in significantly smaller flexible biopsies, large biopsies seem to have higher concordance of diagnostic and prognostic markers in biopsy-resection pairs.

Patient characteristics	N=64 (%)	TC (n=26)	AC (n=38)	p-value
Mean age in years (SD)	45 (16.2)	43 (15.8)	46 (16.7)	0.51
Female	40 (62.5)	14 (53.8)	25 (65.8)	0.33
Biopsy				
FLB	34 (53.1%)	12 (46.2%)	22 (57.9%)	0.36
RIB	30 (46.9%)	14 (53.8%)	16 (42.1%)	
Type of surgery	64	26	38	0.10
Pneumonectomy	3 (4.7)	2 (7.7)	1 (2.6)	
Bilobectomy	13 (20.3)	9 (34.6)	5 (13.2)	
Lobectomy	29 (45.2	8 (30.7)	20 (52.7)	
Sleeve lobectomy	17 (26.6)	6 (23.1)	11 (28.9)	
Segmentectomy	1 (1.6)	1 (3.8)	0	
Bronchial sleeve resection	1 (1.6)	0	1 (2.6)	
Tumor diameter				0.05
T1a	38 (59.4)	21 (80.8)	17 (44.7)	
T1b	14 (22)	3 (11.5)	11 (28.9)	
T2a	8 (12.5)	2 (7.7)	6 (15.8)	
T2b	2 (3.1)	0	2 (5.3)	
T3	2 (3.1)	0	2 (5.3)	
T4	0	0	0	
Nodal stage				0.78
N0	59 (92.2)	25 (96.2)	34 (89.5)	
N1	4 (6.3)	1 (3.8)	3 (7.9)	
N2	1 (1.6)	0	1 (2.6)	
Radicality				0.68
R0	58 (90.6)	23 (88.5)	35 (92.1)	
R1	6 (9.4)	3 (11.5)	3 (7.9)	

Table 1: Clinicopathological characteristics of central carcinoid cohort.

	Chromogranin		Synaptophysin		CD56		INSM-1		Rb		p16		OTP		CD44	
NSCLC																
	Resection	*1/304*	*0.33%*	*1/302*	*0.33%*	*5/304*	*1.64%*	*12/304*	*3.95%*	*NA*	*NA*	*NA*	*NA*	*7/304*	*2.30%*	*34/213*	*15.96%*
AC																
	Resection	*38/38*	*100%*	*38/38*	*100%*	*37/38*	*97.4%*	*37/38*	*97.4%*	*38/38*	*100%*	*9/38*	*23.7%*	*30/38*	*78.9%*	*29/37*[∞]	*78.4%*
	Flexible	*22/22*	*100%*	*22/22*	*100%*	*21/22*	*95.5%*	*17/22*	*77.3%*	*18/21*[∞]	*85.7%*	*1/22*	*4.5%*	*15/22*	*68.2%*	*16/21*[∞]	*76.2%*
	Rigid	*16/16*	*100%*	*16/16*	*100%*	*15/16*	*93.8%*	*16/16*	*100%*	*16/16*	*100%*	*0/16*	*0%*	*14/16*	*87.5%*	*13/16*	*81.3%*
TC																
	Resection	*26/26*	*100%*	*26/26*	*100%*	*26/26*	*100%*	*26/26*	*100%*	*26/26*	*100%*	*2/25*[∞]	*8.0%*	*26/26*	*100%*	*26/26*	*100%*
	Flexible	*12/12*	*100%*	*12/12*	*100%*	*11/12*	*91.7%*	*12/12*	*100%*	*10/12*	*83.3%*	*1/12*	*8.3%*	*11/12*	*91.7%*	*12/12*	*100%*
	Rigid	*14/14*	*100%*	*14/14*	*100%*	*14/14*	*100%*	*14/14*	*100%*	*14/14*	*100%*	*0/14*	*0%*	*14/14*	*100%*	*14/14*	*100%*

Table 2: Expression of chromogranin, synaptophysin, CD56, INSM-1, Rb, p16, OTP and CD44 in typical (TC) and atypical carcinoid (AC), and non-small cell lung cancer as control group; NA, not applicable; ∞, one H-score not assessable.

	Sensitivity	Specificity
Chromogranin	100%	99.7%
Synaptophysin	100%	99.7%
CD56	95.2%	98.4%
INSM-1	92.1%	96.1%

Table 3: Sensitivity and specificity of neuroendocrine markers

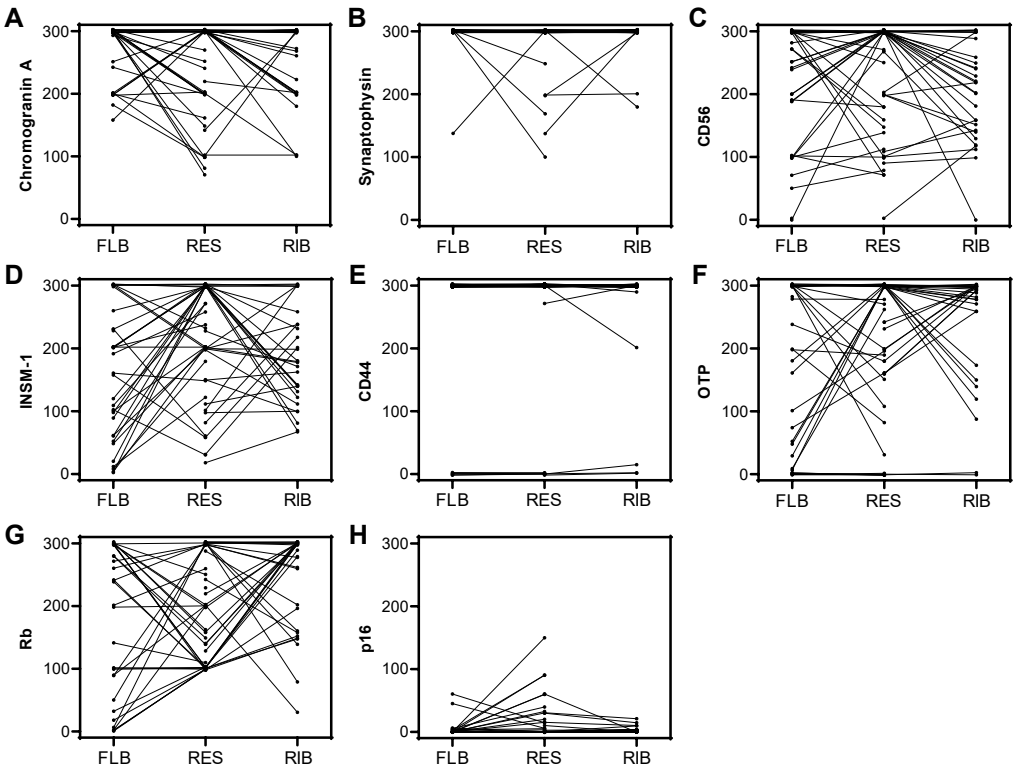

Figure 1: (A-H) Paired dot plots showing H-scores of diagnostic and prognostic IHC markers on flexible biopsies (FLB), resection specimens (RES, gold standard), and rigid biopsies (RIB).

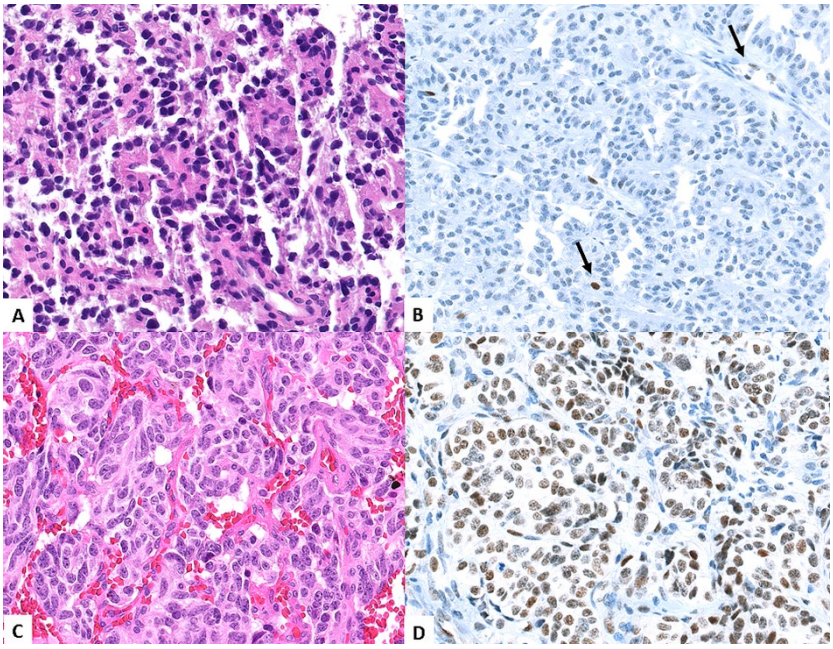

Figure 2: (A and B) HE of a small flexible biopsy with carcinoid (A) and Rb immunohistochemistry without evident nuclear staining of the tumor cells. Note the wild-type staining in endothelial cells and inflammatory cells (arrows); (C and D) HE of the corresponding resection of the same tumor (C) with a clear wild-type nuclear staining of the tumor cells (D).

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Chapter 5

A Multimodal Biomarker Predicts Dissemination of Bronchial Carcinoid

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Abstract

Background

Curatively treated bronchial carcinoid tumors have a relatively low metastatic potential. Gradation into typical (TC) and atypical carcinoid (AC) is limited in terms of prognostic value, resulting in yearly follow-up of all patients. We examined the additional prognostic value of novel immunohistochemical (IHC) markers to current gradation of carcinoids.

Material and methods

A retrospective single-institution cohort study was performed on 171 patients with pathologically diagnosed bronchial carcinoid (median follow-up: 66 months). The risk of developing distant metastases based on histopathological characteristics (Ki-67, p16, Rb, OTP, CD44, and tumor diameter) was evaluated using multivariate regression analysis and Kaplan-Meier method.

Results

Of 171 patients, 7 (4%) had disseminated disease at presentation and 164 (96%) received curative-intent treatment with either endobronchial treatment (EBT) (n=61, 36%) or surgery (n=103, 60%). Among the 164 patients, 13 developed metastases at follow-up of 81 months (IQR 45-162). Univariate analysis showed that Ki-67, mitotic index, OTP, CD44 and tumor diameter were associated with development of distant metastases. Multivariate analysis showed that mitotic count, Ki-67 and OTP were independent risk factors for development of distant metastases. Using a 5% cutoff for Ki-67, Kaplan-Meier analysis showed that the risk of distant metastasis development was significantly associated with the number of risk predictors (AC, Ki-67 ≥5%, and loss of OTP or CD44) (p<0.0001). Six out of seven patients (86%) with all three positive risk factors developed distant metastasis.

Conclusion

Mitotic count, proliferation index and OTP IHC were independent predictors of dissemination at follow up. In addition to the widely used carcinoid classification, a comprehensive analysis of histopathological variables including Ki-67, OTP, and CD44 could assist in the determination of distant metastasis risks of bronchial carcinoids.

Introduction

Pulmonary carcinoids (PCs) are a heterogeneous group of well-differentiated neuroendocrine tumors, representing approximately 1-2% of all lung malignancies [1,2]. As opposed to high-grade large cell neuroendocrine carcinoma (LC-NEC) and small cell lung cancer (SCLC), bronchial carcinoid tumors behave in a relative nonaggressive and benign manner. According to the current World Health Organization (WHO) classification of thoracic tumors (5th edition; 2021), atypical carcinoid (AC) can be distinguished from the low-grade typical carcinoid (TC) based on histologic evaluation of mitotic count (TC 0-1 and AC ≥ mitoses per 2 mm^2) and presence of focal or punctate necrosis in resection specimens [3]. ACs are characterized by a higher metastatic rate and a relatively unfavorable prognosis compared to TCs which is reflected by a lower 5-year survival rate for AC (76% vs 94%) after curative intent resection. Although ACs carry worse prognosis, and present with higher distant disease recurrence rates (range 1-6% vs range 14-29%), the majority of ACs do not disseminate [4]. Although a recent publication of Chiappetta et al. showed a prognostic score for survival in lung carcinoids, scientific evidence in prognostic value of clinical-pathological features such as lymphatic involvement, tumor size, mitotic count and accurate prediction of disease recurrence after curative surgery, is still limited [4-8]. This results in annual follow-up for an extensive period of time. This limitation indicates the need for combined assessment of prognostic markers to further subdivide PCs into relevant clinical categories. While proliferative assessment based on Ki-67 immunohistochemistry (IHC) has emerged as an helpful tool for discriminating PCs from high grade neuroendocrine carcinomas, there is, in contrast to gastroenteropancreatic neuroendocrine tumors, no consensus concerning its prognostic value in low-grade PCs [9-12]. On the basis of recent gene and expression profiling of PCs, novel markers such as orthopedia homeobox (OTP) and CD44 have been identified as potential predictors of adverse outcomes [13]. In addition, IHC of retinoblastoma protein (Rb) and p16 might be helpful in predicting adverse outcomes due to divergent aberrations in the Rb/p16/cyclin D1 pathway among low- and high-grade neuroendocrine tumors [14-16].

The aim of this study was to retrospectively investigate a set of markers as potential predictors of dissemination in our institutional cohort. In particular, we analyzed the value of morphological, morphometric (mitotic count) and immunohistochemical (OTP, CD44, Ki-67, Rb and P16) markers as potential prognostic indicators.

Material and methods

Study design and methods

After approval by the Institutional Review Board (Medical Ethics Review Committee of Amsterdam UMC IRB00002991), patients referred to our tertiary referral center for curative treatment of bronchial carcinoid between 1991 and 2019, with sufficient tissue for additional immunohistochemistry, were included. Patient selection and curative treatment were previously described [17]. After curative treatment, patients were followed up annually with CT scan and, if indicated, with bronchoscopy. For the current analysis, we divided this patient cohort into three groups: 1) curatively treated patients without metastases during follow up, 2) curatively treated patients who developed metastases during follow up, and 3) patients diagnosed with disseminated disease at baseline (stage IV).

Case evaluation and definitive diagnosis

Cases were analyzed and diagnosed (TC/G1 vs AC/G2) by two pathologists specialized in pulmonary pathology, blinded for patient outcome. For stage classification, the 8th edition of the American Joint Commission on Cancer TNM staging system for non–small cell lung cancer was used.

Mitotic count and presence of necrosis

Mitotic count was scored by a specialized pathology laboratory technician (M.A.M. Broeckaert) as previously reported [18]. In short, the whole slide was first explored for mitotic hotspots and the mitotic count was subsequently performed in the hotspot. Presence of necrosis was assessed by one of the pathologists.

Immunohistochemistry

Clones and conditions for IHC staining of Ki-67, Rb, p16, OTP and CD44 are presented in Supplementary Table 1. Immunohistochemical staining intensity was scored using the H-score (range 0–300). In short, the percentage of cells at each staining intensity level (0, 1+, 2+, and 3+) was scored, and H-score was calculated using the following formula: 1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+) [19]. The staining intensity of <30 was considered negative for markers with overall high expression (OTP, CD44, Rb) [20]. For p16, any positive H-score was classified as positive, as it was largely negative in almost all patients. The Ki-67 scoring was based on an estimated percentage of positive cells in the hotspot region after scanning the whole slide. The highest recorded value was taken into account, as described before [21].

Statistical analysis

The statistical analyses and calculations were performed with SPSS 26.0 (IBM Corp., Armonk, N.Y., USA). Chi-square and Fisher tests were applied for comparison of qualitative variables. The area under receiver operating characteristics curve (ROC), abbreviated as AUC, was calculated to identify the discriminatory ability of immunohistochemical markers and mitotic index for development of distant metastases during follow-up. The Spearman's correlation coefficient was used to measure the monotonic association with respect to continuous variables (mitotic and proliferation indices). A binary multiple regression analysis was performed to identify independent risk factors for distant metastases. Survival analysis was performed using the Kaplan–Meier method and log-rank test. Patients with disseminated disease at presentation were excluded from the survival analysis (n=7). A p-value <0.05 denoted statistical significance.

Results

Patient characteristics

A total of 171 patients with bronchial carcinoid were included in the present study (Table 1). The median age at diagnosis was 49 (IQR: 36-61 years) and 97 (56.7%) patients were female. TC was diagnosed in 112 (65%) and AC in 59 (35%) patients. Immunohistochemistry results of both TC and AC are shown in Supplementary Tables 2 and 3. A total of 164 (96%) patients underwent curative-intent treatment with either definitive endobronchial therapy (EBT) (n=61, 36%) or surgery (n=103, 60%). Of 164 curatively treated patients, 13 (8%) developed metastases during follow-up (median FU 81 months, IQR: 45-162). Seven patients (4%) were diagnosed with distant metastases at presentation (liver n=2, pleura n=2, dura mater n=1, skin n=1 and adrenal gland n=1) (Table 1).

Mitotic index and Ki-67

Tumors of patients, who developed distant metastases during follow-up (n=13), showed a higher mitotic rate (median 8; IQR 2-10) compared to patients without metastases at follow-up (median 0; IQR 0-2, p<0.001) (Table 2). Development of metastases was furthermore associated with an increased Ki-67 index (median 6%; IQR 1-11) compared to non-metastatic cases (median 1%; IQR 1-2) (p<0.001). As a result of considerable overlap in frequency distributions of Ki-67, the median Ki-67 indices for TC (1%; IQR 1-3) and AC (2%; 1-4) were not significantly different (p=0.134) (Supplementary Table 2). The ROC analyses demonstrated that mitotic count (AUC 0.87; p<0.0001) and Ki-67 (AUC 0.77; p=0.0012) were discriminatory for development of distant metastases (n=13) (Fig. 1A). The optimal cut-off values from

this analysis were analyzed using the ROC curve. Mitotic count of ≥2 per 2 mm^2 and Ki-67 index of ≥5% were significantly associated with the occurrence of distant metastases (p<0.001). Notably, mitotic count and Ki-67 showed negligible correlation (ρ=0.19, p=0.01) (Supplementary Figure 1).

Immunohistochemistry

Loss of OTP and CD44 expression was observed in 30 cases (18%) (Table 2). In contrast to curatively treated patients without metastases at follow up, loss of OTP (77 vs 12%) and CD44 (69% vs 10%) expression was significantly more common in patients with distant metastases (p=0.004 and p<0.001 respectively). ROC analyses revealed similar area under the curve (AUC) values for OTP (AUC 0.78, p=0.001) and CD44 (AUC 0.82, p<0.001) when development of distant metastases during follow-up was taken as an endpoint (Fig. 1B). Rb was positive in all cases (100%), and 51 cases (30%) cases were positive for p16 staining. Both Rb and p16 were not discriminant for tumor classification (TC vs AC), and no association with metastases at follow up was observed (Supplementary Table 2) (Table 2).

Risk factors distant metastases

Subsequently, multivariate binary logistic regression was employed to identify independent risk factors of distant metastases development during follow up (n=13). Input variables were all first tested in a univariate fashion for association with occurrence of distant metastases. Univariate analysis demonstrated that tumor diameter (p<0.001), CD44 (p<0.001), OTP (p<0.001), mitotic count (p<0.001), and Ki-67 (p<0.001) were significantly associated with distant metastases at follow up (Table 2). Of these significant terms, only mitotic count (p=0.001), OTP (p=0.004), and Ki-67 (p=0.034) were independently associated with a higher risk of distant metastases (Table 2). In comparison to individual markers, this combination of predictors demonstrated a greater performance in terms of discriminatory capacity (AUC 0.90; CI 0.78-1.00; p<0.0001) (Fig. 1C).

Immunohistochemistry profile in patients with disseminated disease at baseline

Univariate analysis revealed that CD44 (p<0.001), Ki-67 (p<0.001) and tumor diameter (p=0.026) were significantly associated with disseminated disease at baseline. These significant variables on univariate analyses became non-significant on multivariate analysis. When discriminatory markers were compared between patients who presented disseminated disease (n=7) and patients who developed metastases during follow-up (n=13), an increased median proliferation index was found for Ki-67 in both groups (10%, IQR 8-15 vs 6%, IQR 1-11; p=0.157) (Fig. 2A). Loss of OTP and CD44 was comparable in both groups, despite a non-significant trend in more frequent loss of CD44 in patients with disseminated disease at diagnosis (Fig. 2B).

Metastasis-free survival

Finally, a survival analysis was performed to assess the influence of independent predictors (Ki-67, mitotic count, OTP) on the development of distant metastases in curatively treated patients. Although not significant in the multivariate analysis, but highly discriminative in the AUC, CD44 was also added to the survival analysis (Fig. 3). The log-rank test showed a significant difference in distant metastasis-free survival in biomarker-based categories of patients ($p<0.0001$). Patients ($n=76$) with carcinoids harboring favorable marker profile (Ki-67 <5%, mitotic count <2 per 2 mm2, and OTP or CD44 positivity) exclusively, did not develop distant metastases during follow-up. Presence of only unfavorable biomarkers (Ki-67 ≥5%, mitotic count ≥2 per 2 mm^2, and OTP or CD44 negativity) was associated with development of distant metastases in 6 out of 7 patients (86%). Patients with at least 1 of these unfavorable prognostic characteristics developed metastases in 7 (6 AC and 1 TC) out of 79 cases (9%). This patient category is further specified in Supplementary Table 3. Figure 4 and 5 shows an example of favourable and unfavourable profile of mitotic count, Ki-67, OTP and CD44.

Discussion

In this study, we demonstrate that combining mitotic count with proliferation index (Ki-67) and novel immunohistochemistry for OTP and CD44 greatly aids the prediction of metastatic dissemination in patients with bronchial carcinoid. None of the TCs with a low Ki-67 expression (<5%) and OTP/CD44 positivity metastasized during follow up. In contrast, patients who presented with disseminated disease at baseline or developed metastases during follow up could be identified by atypical carcinoid morphology in combination with a high proliferation index (Ki-67 ≥5%) and loss of OTP and/or CD44 expression.

In this series of 171 carcinoids, we could show that loss of OTP and CD44 expression was strongly associated with unfavorable disease outcome. Loss of OTP was independently associated with development of distant metastases during follow up. While only OTP was in independent predictor of dissemination at follow up in this cohort, CD44 showed a similar pattern of loss in all but one patient. Therefore, the two biomarkers most probably show comparable prognostic value and future studies should further elucidate this matter. In agreement with our results, gene expression profiling of carcinoids by Swarts et al. revealed strong downregulation of OTP and CD44 in association with poor survival and increased risk of metastases, and subsequent validation using immunohistochemistry confirmed the prognostic value of these promising biomarkers [13,22]. Similarly, Granberg et al. described a decreased progression-free and overall survival in TCs with loss of CD44 [23]. In more recent study by Papaxionis and colleagues, CD44 and OTP were incorporated with carcinoid

gradation in a multivariate model with a similar results [22]. In this study, however, the prognostic value of these markers was enhanced by the addition of proliferative index. The exact mechanisms of the homeobox protein family member contributing to aggressive tumor behavior and the molecular interactions with CD44 remains to be elucidated. Of these novel markers, CD44 is easily applicable in the current clinical practice, because of the wide availability of monoclonal antibodies and the possibilities for standardization of the staining procedure.

Ki-67, together with mitotic count, is already part of a grading system for gastrointestinal neuroendocrine tumors [24]. While this nuclear protein plays an essential role in the control and timing of cell proliferation, Ki-67 was previously not included in the World Health Organization classification scheme of PCs, partly because of inconsistencies in proposed cutoff thresholds and prognostic values [9,11,25]. However the recent 5th edition WHO guideline for thoracic tumors currently suggest that a Ki-67 ≥5% is most probably an AC [3,26]. Here we report an association between Ki-67 ≥5% and development of distant metastases. A similar Ki-67 index cutoff of 5% was described by Dermawan et al. to predict long-term recurrence in PCs [27]. According to several studies, this 5% Ki-67 cut off might enable a more refined 3-tier histologic grading of carcinoids in which PCs are stratified into 3 grades based on histologic grade and Ki-67 immunoreactivity [27,28]. In this carcinoid cohort, Ki-67 did not exceed 20%, in concordance with the suggested diagnostic cut-off value for differentiation between carcinoids and large cell neuroendocrine carcinoma (LCNEC) [29]. Notably, there was no correlation between the mitotic count and proliferation index. A possible explanation for this might be that Ki-67 is expressed in multiple phases of cell division, while mitotic count reflects activity of the mitotic phase only [30]. This is probably the reason for the independent and, in fact, additive prognostic value of Ki-67 and mitotic count.

With respect to the Rb/p16/cyclin D1 pathway, abnormalities in the form of RB and p16 loss were not associated with development of metastases, nor differentiation between TC and AC. A few studies, however, reported loss of tumor suppressor RB in a significant subset of ACs, while RB demonstrated preserved function as low abundant nuclear transcription factor in TCs [15,31]. The p16 protein, which negatively regulates the cyclin D-dependent phosphorylation of Rb, was partially positive in approximately 30% of cases. Interestingly, p16 did not show strong diffuse immunoreactivity, and might therefore function as adjunctive tool in differentiating between PC and LCNEC, in which diffuse positivity of p16 are more frequently observed [16,31].

To place our results in clinical context, we should explore how these outcomes might change the management of patients with bronchial carcinoid. First, biomarkers could be of help in the selection of the most appropriate treatment modality. In the current study, no dissemination of carcinoids harbouring a favourable biomarker profile was detected after EBT or surgery. Therefore, patients with local intraluminal bronchial carcinoid, no signs of lymph node involvement and a favourable biomarkers profile may be good candidates for EBT, while all other patients with local disease should be treated with oncological surgical resection. Of course, prospective trials are required to assess safety and efficacy of such a biomarker-driven treatment strategy. Second, this biomarker set could also change the follow-up after radical treatment. Because local recurrence occurs in a small proportion of patients after EBT, follow-up with bronchoscopy and/or CT is obviously mandatory. After radical surgical resection, however, extensive follow up may be futile in case of a carcinoid with a favourable biomarker profile (low mitotic count, low Ki-67 expression and normal OTP/CD44 expression), which in our cohort was 47%.

The findings of the present study must be interpreted in the context of several potential limitations. First, since the patients were referred to our hospital for treatment with EBT, the population included in this study has been exposed to selection bias. Carcinoids treated with EBT were predominantly small TC tumors compared to the subset of malignancies that were subjected to surgical resection. However, this allowed us to investigate the prognostic value of morphological, morphometric and immunohistochemical characteristics in the context of low T stage and long/intensive follow-up. Second, as previously described, accurate assessment of Ki-67 and mitotic count can be problematic due to interobserver variability [32]. Third, a limited number of distant metastases served as the sample size for the multivariate analyses, reducing the power of the multimodal biomarker and increasing the margin of error. Considering the indolent growth of these tumors and the median follow-up time of 5.5 years in this study, we encourage further analysis of long-term results in an independent study.

Conclusion

Adding OTP, CD44, and Ki-67 to the widely used TC/AC classification, provides a multimodal biomarker strategy that improves prognostic classification. In the future, this strategy may enable risk stratification and hence guide a more tailored approach regarding treatment and follow-up of patients with bronchial carcinoid.

	Curative treatment	Disseminated disease after curative-intent treatment	Distant metastasis at diagnosis	*p-value*§
Number of patients (%)	151 (88)	13 (8)	7 (4)	*NA*
Median age at diagnosis (IQR)	48 (35-60)	57 (44-67)	58 (47-64)	0.088
Gender (M / F)	67 / 84	4 / 9	3 / 4	0.618
Histology				<0.000
TC	107 (71)	1 (8)	4 (57)	-
AC	44 (29)	12 (92)	3 (43)	-
Curative treatment			*NA*	0.002
EBT	61 (40)	0 (0)	-	-
Surgery	90 (60)	13 (100)	-	-
Type of surgery			*NA*	0.190
Pneumonectomy	4 (3)	2 (15)	-	-
Bilobectomy	24 (16)	0 (0)	-	-
Sleeve lobectomy	18 (12)	3 (23)	-	-
Lobectomy	37 (25)	7 (54)	-	-
Bronchial sleeve resection	1 (1)	0 (0)	-	-
Bronchotomy	1 (1)	0 (0)	-	-
Segmentectomy	4 (3)	1 (8)	-	-
Wedge resection	1 (1)	0 (0)	-	-
Tumor stage				<0.000
1a	53 (35)*	0 (0)	0 (0)	-
1b	61 (40)*	6 (46)	0 (0)	-
1c	26 (17)*	4 (31)	0 (0)	-
2a	9 (6)	1 (8)	0 (0)	-
2b	1 (1)	0 (0)	0 (0)	-
3	1 (1)	2 (15)	0 (0)	-
4	0 (0)	0 (0)	7 (100)	-
Median follow up in months (IQR)	66 (35-118)	81 (45-162)	50 (12-80)	0.268

*Table 1: Clinicopathological characteristics of patients treated for bronchial carcinoid (n=171); EBT: endobronchial treatment; IQR: interquartile range; ǂ TNM classification 8th edition; * In EBT cases, T and N status was based on tumor characteristics on CT scan; § calculated using the Kruskal-Wallis test, Chi-Squared or Fisher's Exact Test.*

Variable	Patient categories	IHC positivity (%)	Median (SD)	Range (IQR)	Distant metastases during FU (n=13)	
					Univariate analysis§	Multivariate analysis#
Ki-67						
	Total cohort	*NA*	1 (3.8)	0-20 1-3		
	Curative treatment, no metastases at FU	*NA*	1 (2.5)	0-15 1-2		
	Distant metastases during FU	*NA*	6 (6.9)	1-20 1-11	<0.001	0.034
	Distant metastasis at baseline	*NA*	10 (4.9)	5-20 8-15		
Mitotic index						
	Total cohort	*NA*	1 (2.4)	0-10 0-2		
	Curative treatment, no metastases at FU	*NA*	0 (1.7)	0-8 0-2		
	Distant metastases during FU	*NA*	8 (3.9)	0-10 2-10	<0.001	0.001
	Distant metastasis at baseline	*NA*	1 (2.5)	0-7 0-2		
OTP						
	Total cohort	141/171 (82)	250 (112.9)	0-300 150-300		
	Curative treatment, no metastases at FU	133/151 (88)	260 (102.8)	0-300 160-300		
	Distant metastases during FU	3/13 (23)	0 (131.6)	0-300 0-150	<0.001	0.004
	Distant metastasis at baseline	5/7 (71)	270 (138.3)	0-300 0-300		
CD44						
	Total cohort*	139/169 (82)	300 (115.9)	0-300 300-300		
	Curative treatment, no metastases at FU	134/151 (89)	300 (92.6)	0-300 300-300		
	Distant metastases during FU	4/13 (31)	0 (138.7)	0-300 0-275	<0.001	*ns*
	Distant metastasis at baseline	1/7 (14)	0 (37.8)	0-100 0-0		
Rb						
	Total cohort	171/171 (100)	200 (79.1)	100-300 100-280		
	Curative treatment, no metastases at FU	151/151 (100)	190 (79.5)	100-300 100-280		
	Distant metastases during FU	13/13 (100)	190 (80.5)	100-300 100-265	0.893	*ns*
	Distant metastasis at baseline	7/7 (100)	240 (69.7)	100-300 190-300		
p16						
	Total cohort‡	51/170 (30)	0 (39.3)	0-240 0-5		
	Curative treatment, no metastases at FU	44/150 (29)	0 (34.5)	0-240 0-3		
	Distant metastases during FU	4/13 (31)	0 (74.9)	0-240 0-60	0.558	*ns*
	Distant metastasis at baseline	3/7 (43)	0 (36.5)	0-100 0-20		
Tumor diameter (*mm*)						
	Total cohort	*NA*	15 (11.1)	1-65 10-22		
	Curative treatment, no metastases at FU	*NA*	15 (9.7)	1-65 10-21		
	Distant metastases during FU	*NA*	27 (16.1)	14-65 15-35	<0.001	*ns*
	Distant metastasis at baseline	*NA*	25 (15.2)	11-55 16-36		

*Table 2: Results of morphometric and immunohistochemical markers in different (prognostic) categories of bronchial carcinoid; significant p-values are reported in bold; curatively treated patients were selected as the reference group; IHC: immunohistochemistry; SD: standard deviation; IQR: interquartile range; NA: not applicable; ns: not significant; * two missing CD44 values; ǂ 1 missing p16 value; § calculated using the Mann-Whitney U test; [a] calculated using the binary multiple regression analysis (backward stepwise procedure).*

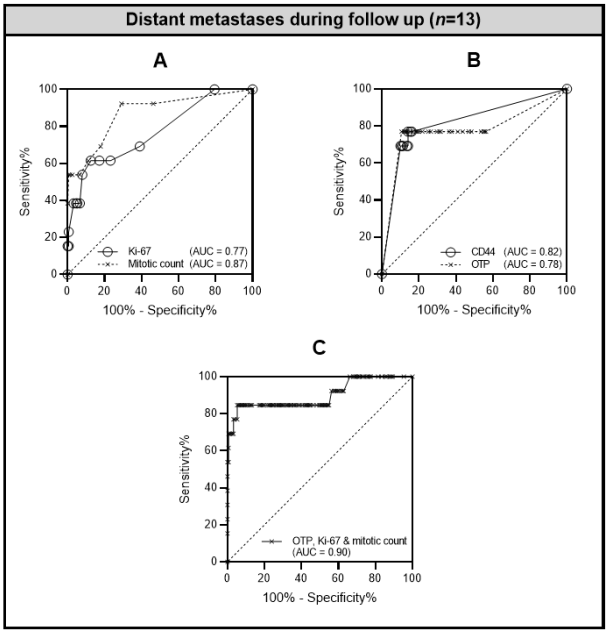

Figure 1: Receiver operating characteristic (ROC) curves for Ki-67, mitotic count, CD44, and OTP; Panels A to C present ROC curves of individual (A: Ki-67, p=0.003, and mitotic count, p<0.000; B: CD44, p<0.000, and OTP, p<0.000) and combined markers (C: OTP, Ki-67 and mitotic count, p<0.000) for distinguishing occurrence of distant metastases (n=13) from no occurrence of distant metastatic disease during follow-up.

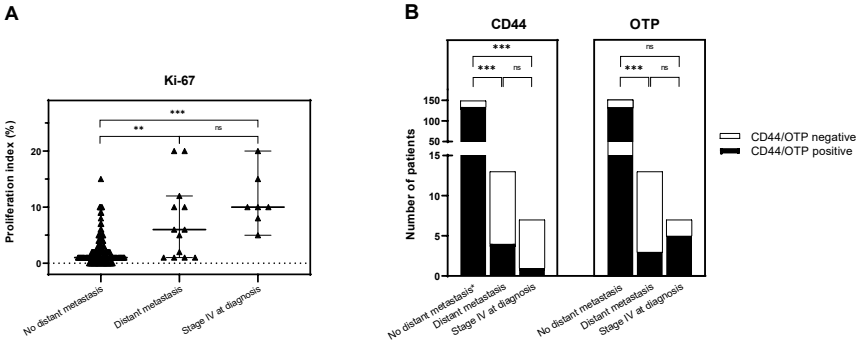

*Figure 2: Immunohistochemical analysis of Ki-67 (A), CD44 and OTP (B) expression in the studied patient categories; significance was determined using the Mann-Whitney U test (A) and Chi-Squared or Fisher's Exact test (B); ns = not significant; **p < 0.01; *** p < 0.001; ns: p > 0.05.*

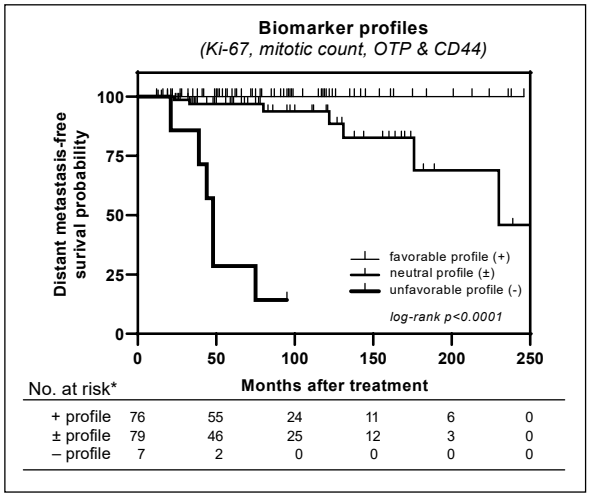

*Figure 3: Distant metastasis-free survival curves estimated by the method of Kaplan-Meier for three biomarker profiles; + (favorable) profile: Ki-67 <5%, mitotic count <2 per 2 mm2, OTP and CD44 positivity; – (unfavorable) profile: Ki-67 ≥5%, mitotic count ≥2 per 2 mm2, and loss of OTP and/or CD44 expression; ± (neutral) profile: 1 or 2 characteristics of (un)favorable profile; * excluding 9 patients: stage IV disease at diagnosis (n=7), missing CD44 values (n=2).*

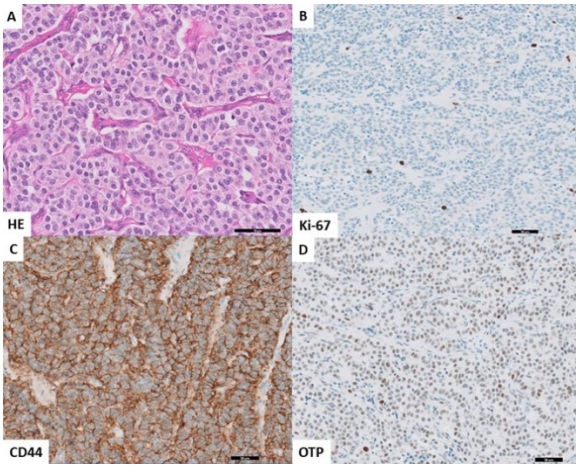

Figure 4: Prognostically favourable profile in histology and immunohistochemistry. A. HE (40x) showing carcinoid with a well differentiated neuroendocrine morphology without mitoses. B. Immunohistochemistry for Ki-67 (20x) showing a proliferation index of <3% in tumorcells. C. Immunohistochemistry for CD44 (20x) with retained strong cytoplasmatic and membranous staining in the tumor cells . D. Immunohistochemistry for OTP (20x) showing a retained nuclear staining in the tumor cells.

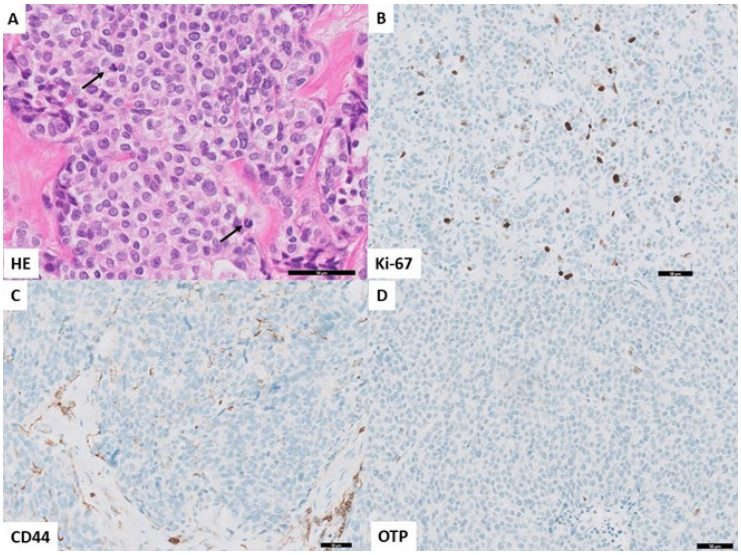

Figure 5: Unfavourable profile in histology and immunohistochemistry. A. HE (40x) showing carcinoid with a mitotic hotspot with readily visible two mitotic figures in one HPF. B. Immunohistochemistry for Ki-67 (20x) showing a proliferation index of >5% in tumorcells. C. Immunohistochemistry for CD44 (20x) showing loss of cytoplasmatic and membranous staining in the tumor cells D. Immunohistochemistry for OTP (20x) showing loss of nuclear staining in the tumor cells.

Antibody	Company	Clone	Species	Reference number	Dilution	IHC incubation time (min)	Detection method
CD044	Ventana	SP37	Rb	06364985001	RTU	32	Optiview
MIB 1	Dako	MIB1	Mo	M7240	1/50	32	Optiview
OTP	Sigma-Aldrich	Polyclonal	Rb	HPA059342	1/50	64	Optiview
RB	BD Biosciences	G3-245	Mo	554136	1/1000*	32	Optiview
p16	Ventana	E6H4	Mo	06695248001	1/2	32	Optiview

*Table S1: Characteristics of antibodies used for immunohistochemistry; IHC: immunohistochemistry; RTU: ready-to-use; * in Dako Antibody Diluent.*

Marker	Number of IHC positive cases (%)∞			Median H-score / Ki-67 score (SD)		
	TC	AC	*p*-value§	TC	AC	*p*-value^a
Rb	112/112 (100)	59/59 (100)	*NA*	180 (77)	200 (82)	0.102
p16	30/111* (27)	21/59 (36)	0.246	0 (32)	0 (49)	0.126
OTP	96/112 (86)	45/59 (76)	0.123	240 (106)	300 (126)	0.608
CD44	96/111* (86)	43/58* (74)	**0.046**	300 (104)	300 (134)	0.055
Ki-67	*NA*	*NA*	*NA*	1 (3)	2 (4)	0.134

*Table S2: Immunohistochemistry results for TC and AC; IHC: immunohistochemistry; TC: typical carcinoid; AC: atypical carcinoid; * one missing p16 or CD44 value; ∞ excluding three cases with missing mitotic figures; § calculated using the Chi-Squared test; ^{a} calculated using the Mann-Whitney U test; significant p-values are reported in bold.*

Variable	Total (%)	TC (%)	AC (%)	*p*-value*
OTP positive	57 (72)	16 (20)	41 (52)	0.001
OTP negative	22 (28)	15 (19)	7 (9)	
CD44 positive	62 (78)	19 (24)	43 (54)	0.003
CD44 negative	17 (22)	12 (15)	5 (6)	
Ki-67 <5%	59 (75)	15 (19)	44 (56)	<0.001
Ki-67 ≥5%	20 (25)	16 (20)	4 (5)	
No distant metastasis	72 (91)	30 (38)	42 (53)	0.236
Distant metastasis†	7 (9)	1 (1)	6 (8)	

*Table S3: Characteristics of patients with neutral biomarker profile (n=79); TC: typical carcinoid; AC: atypical carcinoid; † occurrence of distant metastasis during follow up; * calculated using the Chi-Squared or Fisher's Exact test.*

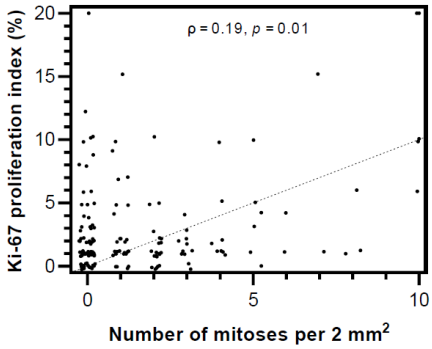

*Figure S1: Spearman's rho correlation between Ki-67 proliferation index and number of mitoses per 2 mm^2; **ρ**: Spearman rank-order correlation coefficient.*

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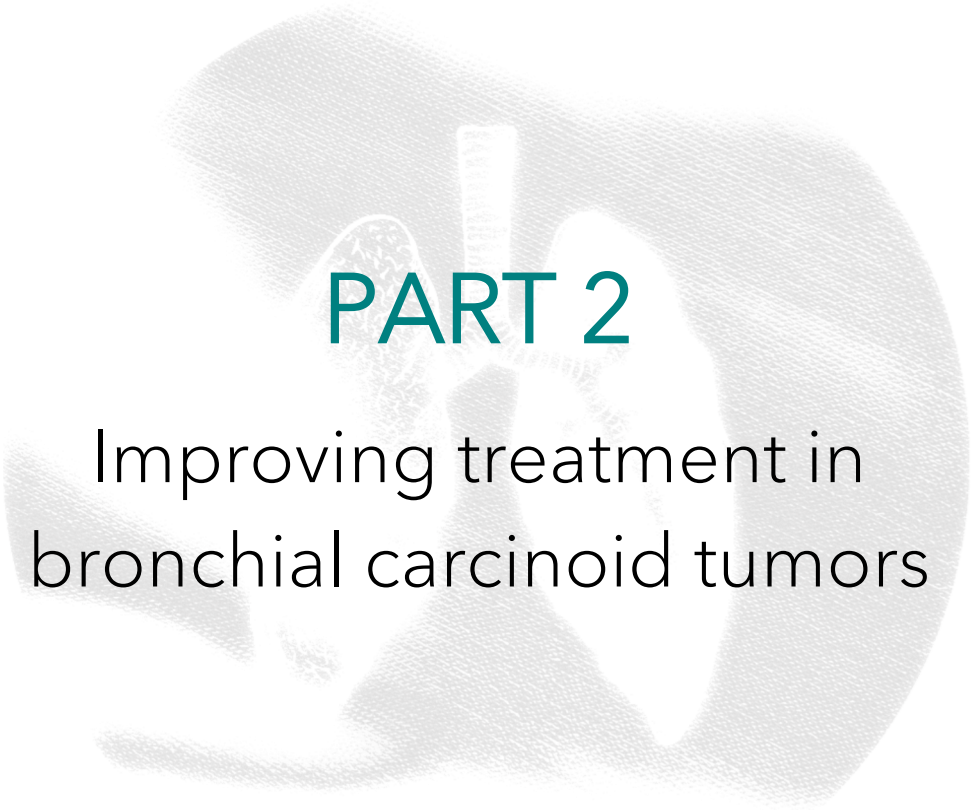

PART 2

Improving treatment in bronchial carcinoid tumors

Chapter 6

Endobronchial and surgical treatment of pulmonary carcinoid tumors: a systematic literature review

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Lung Cancer 2019

Abstract

Introduction

The treatment of pulmonary carcinoid has changed over the last decades. Although surgical resection is still the gold standard, minimally invasive endobronchial procedures have emerged as a parenchyma sparing alternative for tumors located in the central airways. This review was performed to identify the optimal treatment strategy for pulmonary carcinoid, with a particular focus on the feasibility and outcome of parenchyma sparing techniques versus surgical resection.

Material and Methods

A systematic review of the literature was carried out using MEDLINE, Embase and the Cochrane databases, based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement. Two separate searches of publications in endobronchial and surgical treatment in patients with pulmonary carcinoid, were performed. Outcomes were overall survival, disease free survival, recurrence rate, complications, quality of life, and healthcare costs. Combining the two main searches for endobronchial therapy and surgical therapy yielded 3111 records. Finally, 43 studies concerning surgical treatment and 9 studies related to endobronchial treatment for pulmonary carcinoid were included.

Results

Assessment of included studies showed that lymph node involvement, histological grade, tumor location and tumor diameter were identified as poor prognostic factors and seem to be important for patients with pulmonary carcinoid. For patients with a more favorable prognosis, tumor location and tumor diameter are important factors that can help decide on the optimal treatment strategy.

Conclusion

Centrally located small intraluminal pulmonary carcinoids, without signs of metastasis can be treated with minimally invasive alternatives such as endobronchial treatment or parenchyma sparing surgical resection. Patients with parenchyma sparing resections should be followed with long term follow up to exclude recurrence of disease. In a multidisciplinary setting, it should be determined whether individual patients are eligible for parenchyma sparing procedures or anatomical resection. Overall evidence is of low quality and future studies should focus on prospective trials in the treatment of pulmonary carcinoid.

Introduction

Pulmonary carcinoid tumors are family of the neuro-endocrine tumors (NET), originating from the neuro-endocrine Kulchitsky cells, and comprise around 2% of all pulmonary cancers [1]. By morphological analysis, carcinoid tumors can be classified as typical carcinoid (TC) and atypical carcinoid (AC), depending on mitotic cell count (TC 0-2 and AC 2-10 per 2-mm^2) and on the presence of necrosis (AC) [2]. AC's exhibit a slightly more aggressive behaviour with a higher rate of recurrences and tendency to metastasize when compared to TC [1]. Carcinoids are often centrally located, predominantly intraluminal tumors without invasion of adjacent tissues [3]. These characteristics make them particularly suitable for parenchyma sparing surgical treatment options such as sleeve lobectomy or bronchoplastic procedures. Although surgery still is the standard treatment to date, endobronchial treatment modalities (e.g. laser treatment, cryotherapy) are gaining popularity. For selected patients with TC and AC, good results have been reported after endobronchial treatment (EBT) [4-14] and the low-grade nature of carcinoids, makes that incomplete endobronchial resection can still be completed by radical surgical resection, with good results [6,12]. To justify its use, the outcomes of (initial) endobronchial treatment should not be inferior to surgical resection. Against this background this review of available literature was performed to identify the optimal curative treatment strategy for patients with pulmonary carcinoid, with a particular focus on the feasibility and outcome of endobronchial treatment and surgical techniques.

Materials and Methods

Search strategy

A literature search was performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)-statement [15]. Studies reporting on endobronchial treatment, surgical treatment, or both, for pulmonary carcinoid tumors, were identified using the online databases MEDLINE (PubMed), Embase (Ovid), and the Cochrane Database of Systematic Reviews and Central Register of Controlled Trials (Wiley). Studies were searched by a clinical librarian and investigator (ER) up to July 2018. Search terms included controlled terms (MeSH in PubMed and Emtree in Embase) as well as free text terms. Free text terms only were used in The Cochrane Library. Only English language papers were included in the review.

A first search was based on bronchoscopic treatment for pulmonary carcinoid. The following keywords and medical subject heading (MeSH) terms were used: "Carcinoid Tumor"[Mesh] OR carcinoid*[tiab] OR neuroendocrin*[tiab]) AND

("Lung"[Mesh] OR "Lung Diseases"[Mesh] OR lung[tiab] OR lungs[tiab] OR pulmonar*[tiab] OR bronchop*[tiab] OR bronchu*[tiab] OR bronchi*[tiab] OR trache*[tiab]. 'Carcinoid'/de OR 'neuroendocrine tumor'/de OR carcinoid*:ab,ti OR neuroendocrin*:ab,ti. The first combination, carcinoid* or neuroendocrin*) and (lung or lungs or pulmonar* or bronch* or trache*)):ti,ab,kw was combined with Bronchoscop* or endoscop* or endobronch*:ti,ab,kw, and ibt or laser* or (initial near/3 treatment*):ti,ab,kw .

A second search was based on surgery for pulmonary carcinoid. The following search terms were used: "Carcinoid Tumor"[Mesh] OR carcinoid*[tiab] OR neuroendocrin*[tiab]) AND ("Lung"[Mesh] OR "Lung Diseases"[Mesh] OR lung[tiab] OR lungs[tiab] OR pulmonar*[tiab] OR bronchop*[tiab] OR bronchu*[tiab] OR bronchi*[tiab] OR trache*[tiab])), (("Carcinoid Tumor"[Mesh] OR carcinoid*[ti] OR neuroendocrin*[ti]) AND ("Lung"[Mesh] OR "Lung Diseases"[Mesh] OR lung[ti] OR lungs[ti] OR pulmonar*[ti] OR bronchop*[ti] OR bronchu*[ti] OR bronchi*[ti] OR trache*[ti]. "Thoracic Surgery, Video-Assisted"[Mesh] OR "Thoracotomy"[Mesh] OR "Pneumonectomy"[Mesh] OR "surgery" [Subheading] OR "Surgical Procedures, Operative"[Mesh] OR "Perioperative Care"[Mesh] OR surger*[tiab] OR surgical*[tiab] OR operation*[tiab] OR operative*[tiab] OR perioperati*[tiab] OR bronchoplast*[tiab] OR sleeve resect*[tiab] OR lobectom*[tiab] OR pneumonectom*[tiab] OR thoracotom*[tiab] OR vats[tiab]. Word variations have been searched.

Study selection

Inclusion criteria were 1) human studies with patients of 18 years and older 2) endobronchial or 3) surgical treatment of 4) pulmonary carcinoid. Exclusion criteria were as follows: 1) case studies <10 patients 2) non-English language studies, posters or meeting abstracts, 3) publication before 1990 and 4) population based studies. The records were first screened for title or abstract by two independent reviewers (ER and CD), and subsequently screened for full text. A standardized form was used for each study. Following the removal of duplicates, articles were initially screened by title and abstract to exclude non-relevant reports. The remaining articles were accessed in full text and further screened to identify those meeting the inclusion criteria. Finally, the reference lists of relevant articles were searched. Debate over article selection was resolved with consensus.

Outcome Measures

The evaluated outcomes were overall survival, disease free survival, recurrence rate, complications, quality of life, and healthcare costs. This review defined major complications as re-admission, re-operation and peri-or postoperative death. Others were registered as minor complications. Assessment of included studies showed that lymph node involvement, histological grade, tumor location and tumor diameter were identified as poor prognostic factors and were consequently further analyzed.

Data extraction and analysis

Meta-analysis was not feasible because the included studies were heterogeneous in terms of outcome measures. Overall and disease free survival and recurrence is influenced by different prognostic factors such as lymph node involvement, histology, tumor diameter and location. Prognostic factors with $p<0.05$ in the multivariate analysis of the included studies, were further analyzed.

Results

The study selection flow-chart is presented in Figure 1. Combining the two main searches for endobronchial therapy and surgical therapy yielded 3111 records. Subsequently, 693 duplicates were removed. After abstract and title screening, 2981 records were excluded, leaving 130 studies for full text screening. Finally, 9 studies for endobronchial treatment (Appendix A), and 43 studies for surgical treatment (Appendix B) meeting the inclusion criteria, were included and analyzed.

Description of studies

Endobronchial treatment

Patient and study characteristics of included reports on EBT are presented in Appendix A. All studies were non-randomized cohort studies (7 retrospective and 2 prospective), performed in Europe and published in the 21th century. Six studies included patients with TC only [4,5,7,9-11] , whereas 3 included patients with both TC and AC [6,8,12]. For resection of the carcinoid tumor, 7 studies used laser therapy [4-7,9,11,12], of which 5 also used cryotherapy [4-6,8,12], 1 study described diode laser or argon plasma coagulation [8] and 1 study used a forceps [10]. Pre-treatment evaluation of intrabronchial and/or extrabronchial tumor localization was assessed with both CT scan and bronchoscopy in 7 studies [4-6,8-10,12], and not reported in 2 studies [7,11]. Completing surgery for residual or recurrence of disease was common practice in all selected studies.

Surgical treatment

Studies related to surgical treatment were predominantly retrospective cohort studies [3,13,16-41] and performed in Europe [3,13,17-19,21-29,31,36,38,40-50]. Both anatomical pulmonary resections (lobectomy, bilobectomy and pneumonectomy) and parenchyma sparing surgical techniques (segmentectomy, sleeve lobectomy, bronchial sleeve, wedge or enucleation of the tumor) were described. Three studies only performed parenchyma sparing resections [27,42,46], 1 study described a bronchotomy procedure in polypoid growing TC [3], 1 study presenting a bronchoplastic resection without lung resection [36], 2 studies also combined EBT with

surgery [13,14]. Studies who performed a parenchyma sparing resection did not show more recurrences compared to studies with only anatomical resections. None of the studies reported on surgical or parenchyma sparing resection via VATS procedure. Studies were heterogeneous in defining parenchymal sparing, limited or bronchoplastic resection.

Outcome measures

Overall survival & disease free survival

Several studies reported on disease free survival (DFS), but the majority reported overall survival (OS) (Table 1 & 2). Five and ten year OS for TC ranged from 82-100% and 60-100%, and for AC 50-95% and 38-75% respectively [13,17,18,20,22-31,34,35,38,39,41-44,46-49,51-53]. Five-and ten year DFS for TC ranged from 83-100% and 73-95% for TC, and 44-87% and 24-71% in AC, respectively [3,16,18,20,30,49-51,53]. No studies reported DFS in endobronchial treated pulmonary carcinoid.

Recurrence rate

Recurrence of disease was defined as loco-regional (bronchial or hilar/mediastinal lymph nodes) or distant recurrence (distant metastasis) (Table 3). Recurrence in surgery ranged loco-regionally from 0-8% [3,14,27,32,36,42,46,52 41,54] and distantly from 0-23% [14,27,36,42,46,49] respectively. The loco-regional and distant recurrence rate was lower in EBT treated patients, ranging from 0-5% [5,7-9,11] to 0-4% [4,5,7-9,11] respectively. Atypical carcinoid had a higher incidence of recurrence, especially in higher N+ stage [20,22,24,31,45,51]. From studies included in this review, there was a lower recurrence rate in parenchyma sparing surgery (loco-regional 0% and distant 0%) [3,14,27,36,42,46] and EBT (loco-regional 0-11% and 0-4%) [4-9,11], compared to studies with also anatomical resection.

Complications

Complications were commonly reported, with an overall complication rate for surgery and EBT ranging from 1-63% and 0-30%, respectively (Table 4). However, no clear definition for complications was defined, and the number of patients included varied from 11 to >250 patients [3-14,18,20,21,23-25,27-33,35-39,41,42,44,46,48-50]. Table 4 presents all documented complications. Frequently reported complications after surgery were prolonged air leak, respiratory tract infections, cardiac arrhythmias and empyema. Three studies described stenosis after lung preserving surgery, treated with revision surgery or bronchoscopic dilatation [14,35,42]. The most important complication resulting from EBT was bleeding which was usually stopped using endobronchial interventions such as cryotherapy, adrenaline injection, Xylomethazolin or compression. Only one study reported a massive bleeding during EBT, which needed emergency surgery [6]. Three studies reported 2 stenosis after EBT, which were treated non surgically [5,7,11].

Quality of life

For pulmonary carcinoid, there were no studies identified reporting on quality of life.

Healthcare costs

There are no studies assessing healthcare costs in patients with pulmonary carcinoid.

Prognostic factors

Lymph node involvement

Lymph node involvement impacts on survival. Overall survival in N1 and N2 disease was 60-93% [20,22,28,34,35,38] and 50-55% [20,22,34,35] after 5 and 10 years respectively, compared to 90-100% [22,28,34,35,38] and 74-93% [22,28,31,34,35] in stage 1 disease. Lymph node involvement in AC showed a much poorer overall survival (5 year: stage I 85-100%, stage II 62-100% and stage III 0-28%, 10 year: stage I 72-100%, stage II 66% and stage III 0%) compared to TC (5 year: stage I 99-100%, stage II 75-100% and stage III 33-50%, 10 year: stage I 92-100%, stage II 75%, stage III 0%) [24,25,31,45]. Survival assessed in AC consisted small groups of patients compared to TC.

Histology

AC was found in 10-35% of all included patients [22,31] and is known to be poor a prognostic factor due to a more aggressive character than TC [16,20,22,24,28-32,34,35,38,44,45,47-51,53-55]. AC is associated with higher N status and recurrence rate when compared with TC. Some studies suppose that AC originates in older patients, which negatively affects survival in these patients. [19,31,35,45] . Survival is also negatively influenced by AC. Five year overall survival ranged from 50-95% and declined to 38-75% after 10 years [13,17,20-31,34,35,38,39,41,43,44,47,48,51,53]. Five and ten year disease free survival was 44-81% and 24-71% respectively [16,20,30,38,49-51].

Tumor diameter & location

Tumor location [28,45] and diameter [12,39,45,54] are important factors to predict successful treatment. Seventy percent of all carcinoids are centrally located [29,31]. Although central localization is not precisely defined, it usually means that the tumor can be visualized during bronchoscopy before the level of the subsegmental bronchi. Central tumors seem to have a better prognosis than peripheral tumors [16], which might be explained by the fact that peripheral tumors are often larger, and are associated with a higher incidence of AC [31,45]. Central tumors usually present with symptoms related to bronchial obstruction (pneumonia, cough, wheezing) and therefore it is assumed that they are diagnosed earlier than peripheral tumors, which are more asymptomatic. One study described that purely intraluminal growing carcinoid tumors of ≤2 cm are candidates for successful EBT [12]. Two studies described a diameter of >3 cm is related to a worse outcome and higher N stage [31,39]. A median tumor diameter of 22.5 mm was seen in the N0 group compared to a tumor diameter

of 33.5 mm and 38 mm in the N1 and N2 group. The 10-year survival rate was 93% for the N0 group and declined to < 30% for the N1 group and N2 group [31]. Another study showed that 50% of the AC tumors had a tumor diameter of >3 cm [39].

Discussion

This is the first systematic review evaluating both surgical -and endobronchial treatment for patients with pulmonary carcinoid tumors. Studies describing new techniques such as EBT and parenchyma sparing resection, are limited. However for selected patients, at least comparable survival, recurrence rate and complication rate can be achieved. Because of the low-grade nature of carcinoids, even incomplete endobronchial resection followed by radical surgical resection can result in good outcome [6,12]. In patients with non-small cell lung carcinoma (NSCLC), parenchyma sparing resections are associated with better QOL compared to pneumonectomy [56]. Although pulmonary carcinoid tumors have a different morphology compared to other NSCLC, it is likely that parenchyma sparing resection or EBT for patients with pulmonary carcinoid also result in better QOL when compared with anatomical resections, although we could not conclude this from the included studies.

Not all patients with carcinoid located in the central airways are candidates for endobronchial treatment. Pulmonary carcinoids with extraluminal growth, larger tumor diameter, or with suspected loco-regional or distant metastasis, are generally considered not suitable for EBT [12]. If curation cannot be achieved, other advantages of EBT arise, including desobstruction of the involved bronchus with ensuing resolution of post-obstructive pneumonia and limiting the extension of the subsequent surgical resection [13].

Survival is negatively affected by several prognostic factors such as tumor diameter, atypical carcinoid histology, and lymph node involvement. These prognostic factors have to be taken into account when selecting the appropriate treatment. Regarding tumor diameter, Aydin et al. described a median tumor diameter of 22.5 mm to be associated with N0 and a median tumor diameter of 33.5 mm and of 38 mm with N1 and N2, respectively [31]. Yang et al. showed that a tumor size of ≥3 cm outcome was significantly worse than for patients with a tumor size <3 cm [39]. So, when EBT is selected, it is likely that patients with small tumors will benefit the most. A recent study, assessing prognostic factors for EBT in pulmonary carcinoid, showed that small intraluminal tumors of ≤2 cm are suitable for endobronchial resection. All other tumors should be referred for surgery [12]. Histological classification and tumor localization have shown prognostic significance in many studies. AC is associated with other poor prognostic factors such as larger diameter, peripheral location and increased incidence of lymph node involvement. Tumor localization is also important

for selecting treatment, as only central carcinoids will be suitable for EBT. Although AC behaves more aggressively than TC, patients with small intraluminal AC and TC tumors and no signs of lymph node involvement might be appropriate candidates for lung parenchyma sparing procedures [6,8,12,13,27,42]. Lymph node status is also an important prognostic factor. Presence of lymph node involvement does not exclude surgical treatment because long-term survival is still possible for TC and AC treated with resection. About 90% of lymph node negative pulmonary carcinoid patients are still alive after 10 years, compared to around 50% in patients with lymph node involvement. Although lymph node involvement is rare in pulmonary carcinoid, compared to NSCLC, lymph node dissection is recommended. De Leyn et al. published the revised ESTS guidelines for preoperative mediastinal lymph node staging for non-small-cell lung cancer [57]. Whether these guidelines for pulmonary carcinoid should be applied in the diagnostic work-up for carcinoid tumors is unclear. Hilar (N1) or mediastinal (N2) lymph node involvement in pulmonary carcinoid will in most patients not change treatment, because neo-adjuvant treatment in pulmonary carcinoid has never been shown effective in increasing resectability or survival [58,59]. In addition, lymph node metastasis is rare, like it is in patients with T1 NSCLC. A growing number of T1N0 NSCLC patients are nowadays treated with stereotactic body radiation (SBRT) [60,61]. In these patients surgical lymph node staging, like in EBT for carcinoid, is lacking. The results of SBRT however, seem comparable. Therefore, small intraluminal typical and atypical tumors, without signs of extraluminal growth are good candidates for EBT, which is a less invasive treatment.

There's no consensus in duration of follow up after parenchyma saving procedures. However a recent study showed only a recurrence of 8% after a 10 year follow up in patients with pulmonary carcinoid treated with EBT [12]. Concurrent surgical salvage was uncompromised. This might suggest that annual close surveillance after 10 years might be omitted, or at least decreased in frequency. Because determination of pTNM is not possible for those patients with carcinoid tumors treated with EBT and the predominantly slow growing nature of this tumor, a close long term follow up of at least 10 years with bronchoscopy for intraluminal and CT scan with coronal and sagittal reconstruction, is advised. This to exclude recurrence and/or metastatic disease [6,12].

TC and AC have a more indolent tumor characteristic when compared with NSCLC, inclusion of pulmonary carcinoids in the TNM for NSCLC is under debate [40,54,62]. This issue was discussed by Cattoni et al [54]. They regrouped tumor stage creating a unique neuroendocrine prognostic system which might better predict survival. To date, however, pulmonary carcinoids are staged according the TNM-system for non-small cell lung cancer (NSCLC) [63]. The staging of pulmonary carcinoids may benefit from separating carcinoid tumors from other NSCLC cases because their pathologic and clinical outcomes are highly variable among different subtypes when compared with NSCLC [62].

To evaluate location, extent of the tumor and local or distant metastasis, both CT and bronchoscopy were used in included studies. Imaging techniques with FDG-PET/CT for TC are unreliable for diagnosis of carcinoid, due to low uptake of FDG [64-66]. Newer techniques using 68Gallium (68Ga) Dotatate, which is a somatostatin analogue and used as a tracer in PET-CT scanning, show promising results in diagnosing pulmonary carcinoid, with a reported diagnostic accuracy of >90% [67-73]. However, differentiation between TC and AC is not possible with these imaging techniques, and therefore histologic biopsy remains mandatory. For central tumors, bronchoscopic biopsy results in the diagnosis in the majority of patients. In patients with a more peripheral localization, not reachable during bronchoscopy, transthoracic puncture of the lesion might help for definite diagnosis. Optimal histopathological analysis is based on morphology (mitotic index) and additional parameters such as Ki-67 labeling index, and can be challenging. Pathologists can sometimes only hint towards the diagnosis of a neuroendocrine tumor or typical and atypical carcinoid. It is important not to compromise outcome, by ruling out regional and distant microscopic spread, especially for patients who are possible candidates for EBT. With a reported negative predictive value and sensitivity of >80% for the assessment of nodal involvement in patients with pulmonary carcinoids, a preoperative CT scan seems to be a reliable tool for excluding lymph node involvement, and is therefore mandatory in the workup for patients with pulmonary carcinoid tumors [16,43]. Endobronchial ultrasound (EBUS) is standard in the work up for centrally located NSCLC, or NSCLC with clinical suspected lymph node involvement. However, the role of EBUS in pulmonary carcinoid is unclear. Even large series examining the efficacy of EBUS in pulmonary carcinoid report no or only very rare diagnosis of carcinoid tumor [74,75].

The most important limitation of this systematic review is that no randomized trials have been performed at present, and most studies were retrospective. Therefore, results have to be interpreted with caution. For example, this review showed a lower loco-regional (0-5%) and distant recurrence (0-4%) rate in the EBT and parenchyma sparing surgery group compared to anatomical resections (0-8% and 0-23% respectively). Patients that are selected for parenchyma sparing procedures are probably "better" patients, with small carcinoid tumors and thus a lower probability of lymph node involvement. This systematic review performed a search from studies published from 1990 onward. Studies performed in the nineties were often of poor quality with a low patient number inclusion. All included studies are classified among low or very low evidence, regarding the Grades of Recommendation, Assessment, Development and Evaluation Working Group (GRADE criteria) [76]. The low incidence of pulmonary carcinoid presents a great challenge for performing randomized controlled trials. International multicenter collaboration is necessary in order to acquire more robust data about the available treatment options and their effects on important endpoints such as overall survival, disease free survival, quality of life and cost-effectiveness. Unfortunately, EBT is currently limited to a low number of centers

with sufficient expertise in interventional pulmonology. Nonetheless, it should be feasible to perform such studies within an international collaborative.

It is important to underscore that patient selection for parenchyma sparing procedures and anatomical resection should be done during multidisciplinary discussion in centralized referral centers with experienced radiologists for accurate and systematic CT scan evaluation, interventional pulmonologists familiar with endobronchial treatment, and pulmonary surgeons with experience in bronchoplastic procedures.

Conclusion

Tumor histology, tumor diameter and nodal status seem to be important prognostic factors for survival in patients with pulmonary carcinoid. For patients with a more favorable prognosis, tumor location and tumor diameter are important factors that can help decide on the optimal treatment strategy. The factors can help physicians to determine the optimal curative treatment strategy for their patients, while also keeping the treatment burden in mind. Centrally located small intraluminal pulmonary carcinoids, without signs of metastasis can be treated with minimally invasive alternatives such as endobronchial treatment or parenchyma sparing surgical resection. Patients with parenchyma sparing resections should be followed with a long term close clinical and radiological follow up to exclude recurrence of disease. Multidisciplinary tumor board discussion is essential in patient selection and should precede all treatments. Unfortunately, the quality of the available evidence is low and future studies should focus on prospective trials in the treatment of pulmonary carcinoid.

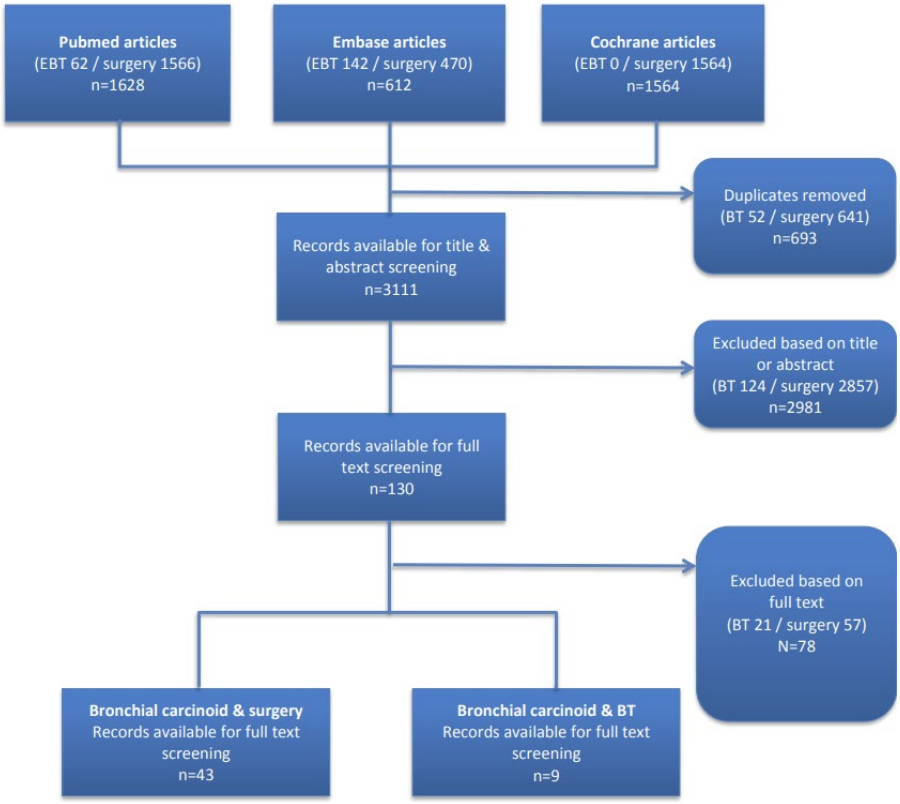

Figure 1: Study selection process for records in endobronchial treatment (EBT) and surgery in pulmonary carcinoid.

	Endobronchial therapy (%)				Surgical resection (%)				Remark
	TC		AC		TC		AC		
	5 yrs	10 yrs	5 yrs	10yrs	5 yrs	10 yrs	5 yrs	10 yrs	
Shah et al. [17]	-	-	-	-	100	-	78	-	
Ducroq et al. [18]	-	-	-	-	92	88	-	-	
Ferguson et al. [20]	-	-	-	-	90	-	70	-	
Ruggieri et al. [21]	-	-	-	-	-	77	-	40	
Filosso '02 et al. [22]	-	-	-	-	97	93	77	52	
Fiala et al. [23]	-	-	-	-	99	87	95	74	
Kaplan et al. [51]	-	-	-	-	82	60	65	40	
Mezetti et al. [24]	-	-	-	-	92	90	71	60	
Cardillo et al. [25]	-	-	-	-	99	-	70	-	
Daddi et al. [26]	-	-	-	-	96	-	88	-	
Terzi et al. [27]	-	-	-	-	98	96	68	50	Only 3 AC included
Divisi et al. [43]	-	-	-	-	96	-	68	-	
Kyriss et al. [44]	-	-	-	-	94	82	92	62	
Rea et al. [28]	-	-	-	-	98	92	78	65	
Bini et al. [29]	-	-	-	-	91	91	88	44	
Rizzardi et al. [46]	-	-	-	-	100	100			
Ferolla et al. [47]	-	-	-	-	97	90	76	67	
Machuca et al. [30]	-	-	-	-	91	89	56	47	
Aydin et al. [31]	-	-	-	-	92	83	73	46	
Wei et al. [34]	-	-	-	-	100	92	90	75	
Zhong et al. [35]	-	-	-	-	90	74	74	58	
Filosso '13 et al. [48]	-	-	-	-	91	86	69	43	
Ichiki et al. [52]	-	-	-	-	91	-	91	-	Only 11 patients included
Filosso '14 et al. [49]	-	-	-	-	89	83	58	38	
Maurizi et al. [38]	-	-	-	-	100	-	88	-	
Herde et al. [53]	-	-	-	-	86	86	83	47	
Neuberger et al. [13]	-	-	-	-		89	-	68	
Yang et al. [39]	-	-	-	-	88	-	50	-	
Kasprzyk et al. [41]	-	-	-	-	96	-	83	-	
Lukrasz et al. [10]	89	84	-	-	-	-	-	-	
Neyman et al. [11]	94	-	-	-	-	-	-	-	Maximum 3 years follow up

Table 1: Overall survival (OS), -: no results in article, TC: typical carcinoid, AC: atypical carcinoid, EBT: endobronchial therapy.

	Endobronchial therapy (%)				Surgical resection (%)			
	TC		AC		TC		AC	
	5 yrs	10 yrs	5 yrs	10yrs	5 yrs	10 yrs	5 yrs	10 yrs
Chugtai et al. [16]					97	95	44	44
Ducroq et al. [18]	-	-	-	-	100	91	-	-
Tastepe et al. [3]	-	-	-	-	100	-	-	-
Ferguson et al. [20]	-	-	-	-	95	-	73	-
Kaplan et al. [51]	-	-	-	-	87	82	67	45
Machuca et al. [30]	-	-	-	-	94	-	74	-
Filosso '14 et al. [49]	-	-	-	-	83	73	49	24
Maurizi et al. [38]	-	-	-	-	93	-	44	-
Cusamano et al. [50]	-	-	-	-	99	90	87	71

Table 2: Disease free survival (DFS), -: no results in article, TC: typical carcinoid, AC: atypical carcinoid.

	Endobronchial therapy				Surgical resection		Remarks
	Total n/total population (%)	Loco-regional[1] n (%)	distant[2] n (%)	**Total n/total population (%)**	Loco-regional[1] n (%)	distant[2] n (%)	
Chugtai et al. [16]	-	-	-	**9/84 (11)**	4 (5)	5 (6)	
Shah et al. [17]	-	-	-	**2/29 (6)**	1 (3)	1 (3)	
Ducroq et al. [18]	-	-	-	**4/139 (3)**	1 (1)	3 (2)	Only TC; discrepancy in text, study described 2 local recurrences and 3 distant metastasis, but stated only 4 recurrences
Tastepe et al. [3]	-	-	-	**0/16 (0)**	0	ND	Only bronchotomy procedures.
El Jamal et al. [19]	-	-	-	**7/95 (7)**	4 (4)	3 (3)	
Ferguson et al. [20]	-	-	-	**8/139 (6)**	ND	ND	
Fadel et al. [42]	-	-	-	**0/30 (0)**	0	0	Only 16 patients included, performed only sleeve lobectomy.
Filosso '02 et al. [22]	-	-	-	**12/126 (10)**	4 (3)	8 (7)	
Fiala et al. [23]	-	-	-	**2/96 (21)**	ND	ND	
Kaplan et al. [51]	-	-	-	**4/206 (2)**	2 (1)	2 (1)	
Terzi et al. [27]	-	-	-	**0/25 (0)**	0	0	Only bronchosplastic procedures
Kyriss et al. [44]	-	-	-	**11/111 (10)**	1 (1)	8 (9)	2 recurrences unknown
Garcia-Yuste et al. [45]	-	-	-	**32/661 (5)**	8 (1)	24 (4)	
Rea et al. [28]	-	-	-	**20/252 (8)**	2 (1)	18 (7)	
Bini et al. [29]	-	-	-	**ND**	ND	ND	Recurrence not clearly defined
Rizzardi et al. [46]	-	-	-	**0/70 (0)**	0	0	Sleeve & bronchoplastic procedures in TC
Ferolla et al. [47]	-	-	-	**ND**	ND	12	
Machuca et al. [30]	-	-	-	**8/126 (6)**	5 (4)	3 (2)	
Aydin et al. [31]	-	-	-	**15/104 (14)**	ND	ND	
Bagheri et al. [32]	-	-	-	**1/40 (3)**	0	1 (3)	
Dewan et al. [33]	-	-	-	**1/31 (3)**	ND	ND	
Wei et al. [34]	-	-	-	**9/82 (11)**	ND	ND	
Zhong et al. [35]	-	-	-	**9/131**	3	6	

				(7)	(2)	(5)	
Filosso '13 et al. [48]	-	-	-	**36/126 (28)**	8 (6)	28 (22)	
Ichiki et al. [52]	-	-	-	**2/11 (18)**	0	2 (18)	
Nowak et al. [36]	-	-	-	**0/13 (0)**	0	0	Bronchoplastic procedure without lung resection
Filosso '14 et al. [49]	-	-	-	**30/106 (28)**	5 (5)	25 (23)	
Jethava et al. [37]	-	-	-	**3/30 (10)**	2 (7)	1 (3)	
Maurizi et al. [38]	-	-	-	**8/65 (12)**	2 (3)	6 (9)	
Herde et al. [53]	-	-	-	**4/59 (7)**	1 (2)	3 (5)	
Cusumano et al. [50]	-	-	-	**13/195 (7)**	5 (3)	8 (4)	
Kasprzyk et al. [41]	-	-	-	**15/90 (17)**	7 (8)	8 (9)	
Pikin et al. [14]	-	-	-	**0/25 (0)**	0	0	Combined EBT & parenchyma sparing surgery
Cattoni '18 et al [54]	-	-	-	**83/409 (20)**	33 (8)	50 (12)	
Cavaliere et al. [7]	**0/35 (0)**	0	0	-	-	-	3 operated after surgical resection and no signs of recurrence
Bertoletti et al. [4]	**2/18 (11)**	2 (11)	0	-	-	-	Only TC inlcuded
Lukrasz et al. [10]	**1/28 (4)**	ND	ND				Only TC included, 1 recurrence which was successful operated.
Fruchter et al. [9]	**0/10 (0)**	0	0	-	-	-	
Neyman et al. [11]	**0/25 (0)**	0	0	**0/48 (0)**	0	0	Patients received EBT or (complementary) surgery
Brokx et al. [6]	**10/112 (9)**	6 (5)	4 (4)	-	-	-	Patients not suitable for EBT were referred for surgery
Dalar et al. [8]	**0/29 (0)**	0	0	-	-	-	8 patients were operated after EBT
Boyaci et al. [5]	**0/14 (0)**	0	0	-	-	-	Only TC included
Reuling et al. [12]	**10/125 (8)**	ND	ND	**3/125 (2)**	ND	ND	Patients not suitable for EBT were referred for surgery

Table 3: Recurrence rate; loco-regional[1]: recurrence local or hilar/mediastinal, distant[2]: distant metastasis, ND: not definable; - : no results in article, EBT: endobronchial therapy, TC: typical carcinoid.

	EBT			Surgery			Remarks
	Total n/total population (%)	Minor[1] n (%)	Major[2] n (%)	Total n/total population (%)	Minor[1] n (%)	Major[2] n (%)	
Ducroq et al. [18]	-	-	-	**19/139 (14)**	19 (14)	0	9x PAL, 1x ChT, 1x cardiac arrhythmia, 6x pleural space disease & 1x empyema for which thoracostomy, 1x unknown fever,
Tastepe et al. [3]	-	-	-	**6/16 (38)**	6 (38)	0	Only 16 patients included, 3x sputum retention, 1x BPF, 2x granulation tissue for which bronchoscopic removal
Ferguson et al. [20]	-	-	-	**ND**	ND	ND	7% CV, 15% pulm, 12% other, 1 perioperative death
Ruggieri et al. [21]	-	-	-	**3/18 (17)**	3 (17)	0	2x PAL, 1x atelectasis
Fadel et al. [42]	-	-	-	**3/30 (10)**	2 (7)	1 (3)	1x bronchial stenosis for which re-operation, 1x empyema, 1x HTX
Fiala et al. [23]	-	-	-	**11/96 (11)**	10 (10)	1 (1)	5x atelectasis, 5x PAL, 1x BPF for which surgical intervention.
Mezzetti et al. [24]	-	-	-	**9/98 (9)**	9 (9)	0	4x cardiac arrhythmia & 5x LRTI
Cardillo et al. [25]				**24/163 (15)**	ND	ND	7x pulm, 5x CV, 8x other, 4x mixed
Terzi et al. [27]	-	-	-	**0 (0)**	0	0	Only bronchoplastic procedures
Kyriss et al. [44]	-	-	-	**18/111 (16)**	ND	ND	4x bleeding, 3x PTX, 3x HF, 1x bronchial stomp insufficiency, 7x unknown
Rea et al. [28]	-	-	-	**17/252 (7)**	15 (6)	2 (1)	4 PTX after chest tube removal, 4 AF, 3 PAL, 2 HTX for which blood transfusion, 2x empyema, 2x re-operations for stenosis
Bini et al. [29]	-	-	-	**ND**	ND	ND	
Rizzardi et al. [46]	-	-	-	**1/70 (1)**	ND	ND	Only bronchoplastic procedures; 1x empyema
Machuca et al. [30]	-	-	-	**34/126 (27)**	28 (22)	6 (5)	12x LRTI, 4 PAL, 4x WI, 4 pleural effusion (conservative treatment), 4x other minor clinical complications, 3x HTX & 1x empyema for which re-operation, 2x per-operative deaths
Aydin et al. [31]	-	-	-	**17/104 (16)**	ND	ND	5x LRTI, 4x PAL, 2x PTX, 2x empyema, 2x ChT, 1 arrhythmia, 1 postoperative bleeding
Bagheri et al. [32]	-	-	-	**6/38 (16)**	6 (16)	0	4x PAL, 2x WI; 2 patients not operated
Dewan et al. [33]	-	-	-	**4/31 (13)**	4 (13)	0	4x atelectasis with prolonged air leak
Zhong et al. [35]	-	-	-	**13/131 (10)**	10 (8)	3 (2)	4x AF, 3x PAL, 2x WI, 1 vocal cord paralysis, reoperation for 1x HTX, 1x BPF and 1x bronchial stenosis
Filosso '13 et al. [48]	-	-	-	**9/126 (7)**	ND	ND	1x cardiac arrhythmia, 2x LRTI, 4x RI, 1x CVA, 1x pleural effusion
Nowak et al. [36]	-	-	-	**0 (0)**	0	0	Only bronchoplastic procedures without lung resection
Filosso '14 et al. [49]	-	-	-	**ND**	ND	ND	Also included LCNEC & SCLC
Jethava et al. [37]	-	-	-	**19/30 (63)**	18 (60)	1 (3)	9x PAL, 4x cardiac arrhythmia, 3x PTX, 1x HF, 1x ARF, 1x bleeding for which re-operation.
Maurizi et al. [38]	-	-	-	**10/65**	9	1	4x PAL, 3x cardiac arrhythmia, 1x atelectasis, 1x pneumonitis, 1x bleeding for which re-operation.

				(15)	(14)	(1)	
Yang et al. [39]	-	-	-	**6/44 (14)**	6 (14)	0	2x PAL, 2x LRTI, 1x arrhythmia, 1x WI
Kasprzyk et al. [41]	-	-	-	**ND**	ND	ND	5x AF, 3x PAL, 3x PTX, 2x atelectasis, 2x LRTI, 1x pleural hematoma, 1x WI, 1x psychosis, 1x bleeding for which re-operation (discrepancy among complications in Table (n=20) and text (n=13)
Pikin et al. [14]	-	-	-	**7/25 (28)**	6 (24)	1 (4)	Combined approach of EBT and complementary surgery; 4x LRTI, 1x cardiac arrhythmia, 1x WI, 1x bleeding after EBT for which operation.
Cavaliere et al. [7]	**7/35 (20)**	ND	ND	-	-	-	7x hemorrhage which treated conservatively, 2x stenosis
Bertoletti et al. [4]	**0/18 (0)**	0	0				
Lukrasz et al. [10]	**1/28 (4)**	1 (4)	0	-	-	-	1x bleeding
Fruchter et al. [9]	**ND**	ND	ND	-	-	-	Only 10 patients included; only mentioned that no major complications occurred.
Neyman et al. [11]	**3/25 (12)**	2 (8)	1 (4)	**0/48 (0)**	0	0	1x not fatal fire in bronchoscope, 2x bronchial stenosis for which dilatation in EBT group.
Dalar et al. [8]	**6/29 (21)**	6 (21)	0	-	-	-	6x bleeding which was treated conservatively
Boyaci et al. [5]	**6/14 (43)**	ND	ND	-	-	-	2 arrhythmias, 1x bleeding for which local application, 1x hypoxia, 2 stenosis for which cryo-and balloon dilatation, 1x granulation tissue for which cryotherapy (discrepancy among complications in Table (n=6) and text (n=7)
Reuling et al. [12]	**17/125 (14)**	16 (13)	1 (1)				Patients not suitable for EBT were referred for surgery, 11x bleeding for which conservative treatment, 1x bronchospasm, 1x broken tooth, 1x vocal cord paralysis, 2x stricture, 1x major bleeding for which emergency operation and stricture for which balloon dilatation.

Table 4 : Complications; minor[1]: non-major, major[2]: re-admission, re-operation, peri-or postoperative death. ND; not definable, - ; no results in article, ARF: acute renal failure, BPF: bronchopulmonary fistula, ChT: chylothorax, CV: cardiovascular, CVA: cerebral vascular accident, EBT: endobronchial treatment, HF: heart failure, HTX: hematothorax, LCNEC: large cell neuro endocrine tumor, LRTI: lower respiratory tract infection, PAL: prolonged air leak, PTX: pneumothorax, Pulm: pulmonic, RI: respiratory insufficiency, SCLC: small cell lung carcinoma WI: wound infection.

Author	Study design	Year	Patients number	Histology TC/AC	Therapy	Follow up	Remarks
Cavaliere et al. [7]	Retrospective cohort	2002	35	35/0	YAG laser	Max 198 mths	
Bertolleti et al. [4]	Prospective cohort	2006	18	18/0	Yag laser/Cryotherapy	44.5 mths*	
Luckraz et al. [10]	Retrospective cohort	2006	28	28/0	Biopsy forceps	8.8 yrs~	
Fruchter et al. [9]	Retrospective cohort	2009	10	10/0	Laser therapy	29 mths*	
Neyman et al. [11]	Retrospective cohort	2012	25	25/0	YAG laser	3 years *	Total population; 25 EBT & 48 surgical resection
Brokx et al. [6]	Prospective cohort	2015	112	83/29	YAG laser/cryotherapy	112 mths*	
Dalar et al. [8]	Retrospective cohort	2016	29	24/5	Diode laser/APC/cryotherapy	49 mths*	
Boyaci et al. [5]	Retrospective cohort	2017	14	14/0	APC©/YAG laser/electrosurgery/mechanical excision/cryotherapy	32 mths*	
Reuling et al. [12]	Retrospective cohort	2017	125	106/19	YAG laser, cryo- or electrosurgery, mechanical debulking	82 mths*	

*Appendix A: patient and study characteristics of included selected research for bronchoscopic treatment for pulmonary carcinoid; APC: Argon plasma-coagulation, AC: atypical carcinoid, EBT: endobronchial therapy, *: mean, ~: median, TC: typical carcinoid.*

Author	Study design	Publication year	Patients number	Histology TC/AC	Surgery conservative/radical resection	Follow up	Remarks
Chugtai et al. [16]	Retrospective cohort	1997	84	72/12	15/69	6.8 yrs*	
Shah et al. [17]	Retrospective cohort	1997	29	24/5	5/22	Max. 5 yrs	
Ducrocq et al. [18]	Retrospective cohort	1998	139	139/0	33/106	87 mths^	
Tastepe et al. [3]	Retrospective cohort	1998	16	-	16/0	Max 23 yrs	Only bronchotomy procedures
El Jamal et al. [19]	Retrospective cohort	2000	95	81/14	28/43	3.9 yrs*	
Ferguson et al. [20]	Retrospective cohort	2000	139	109/26	32/107	53.1 mths*	4 histology results were unknown
Ruggieri et al. [20]	Retrospective cohort	2000	18	13/5	3/15	Max. 10 yrs	
Fadel et al. [42]	Retrospective cohort	2002	30	25/5	30/0	64 mths*	Only sleeve resections
Filosso et al. [22]	Retrospective cohort	2002	126	82/44	26/96	99 mths*	4 surgical procedures were unknown
Fiala et al. [23]	Retrospective cohort	2003	96	77/19	40/55 (A-B)	77.8 mths*	1 patient had tracheal carcinoid for which operation could not be performed.
Kaplan et al. [51]	Retrospective Cohort	2003	206	144/62	?	75.2 mths*	
Mezzetti et al. [24]	Retrospective cohort	2003	98	88/10	23/65	-	
Cardillo et al. [25]	Retrospective cohort	2004	163	121/42	10/153	58 mths*	
Daddi et al. [26]	Retrospective cohort	2004	87	79/8	19/68	-	
Terzi et al. [27]	Retrospective cohort	2004	25	22/3	25/0	137 mths*	Only bronchopastic procedures
Divisi et al. [43]	Prospective cohort	2005	42	26/16	6/36	Max 5 yrs	

Kyriss et al. [44]	Retrospective cohort	2006	111	97/14	7/103	73.4 mths*	1 exploratory thoracotomy
Garcia et al. [45]	Retrospective & prospective cohort	2007	661	569/92	141/494	Max 5 years	Operations less than inclusion, unclear.
Rea et al. [28]	Retrospective cohort	2007	252	174/78	103/149	121 mths*	
Bini et al. [29]	Retrospective cohort	2008	54	45/9	12/42	67 mths*	
Rizzardi et al. [46]	Retrospective cohort	2008	70	70/0	70/0	14 yrs~	Sleeve & bronchoplastic procedures in TC
Ferolla et al. [47]	Retrospective cohort	2009	123	100/23	24/99	73.8 mths (TC) 69.3 mths* (AC)	
Machuca et al. [30]	Retrospective cohort	2010	126	110/16	30/98	Max 10 years	1 patient had 3 wedge resections in 1 operation
Aydin et al. [31]	Retrospective cohort	2011	104	84/20	56/72	72 mths*	
Bagheri et al. [32]	Retrospective cohort	2011	40	36/4	8/30	9.5 yrs*	
Cao et al. [55]	Retrospective cohort	2011	186	164/22	25/173	8.0 yrs*	12 patients underwent 2 separate surgical resections
Dewan et al. [33]	Retrospective cohort	2012	31	28/3	5/26	Max 3 yrs	
Wei et al. [34]	Retrospective cohort	2011	82	60/22	33/9	135 mths*	
Zhong et al. [35]	Retrospective cohort	2012	131	106/25	42/89	87 mths*	
Filosso et al. [48]	Retrospective cohort	2013	126	83/43	18/108	60 mths~	
Ichiki et al. [52]	Retrospective cohort	2013	11	6/5	0/11	-	
Nowak et al. [36]	Retrospective cohort	2013	13	12/1	13/0	6.3 yrs~	Bronchoplastic procedure without lung resection
Filosso et al. [49]	Retrospective cohort	2014	106	71/35	7/99	6.5 yrs~	
Jethava et al. [37]	Retrospective cohort	2014	30	28/2	5/25	-	

Maurizi et al. [38]	Retrospective cohort	2014	65	55/10	22/43	58 mths~	
Herde et al. [53]	Retrospective cohort	2015	59	47/12	19/36	4.4 yrs~	4 not operated
Neuberger et al. [13]	Retrospective cohort	2016	208	119/13	61/49	70 mths~	Compared EBT & surgery with only surgery
Yang et al. [39]	Retrospective cohort	2017	44	32/12	11/33	59 mths*	
Cattoni et al. [40]	Retrospective cohort	2017	240	240/0	73/167	42 mths~	
Cusumano et al. [50]	Retrospective cohort	2017	195	159/36	36 /159	75 mths~	
Kasprzyk et al. [41]	Retrospective cohort	2017	90	69/21	9 /81	Max. 10 yrs	
Pikin et al. [14]	Retrospective cohort	2018	25	23/2	25/0	84 mths~	Combined EBT & parenchyma sparing surgery
Cattoni et al. [54]	Retrospective cohort	2018	409	341/68	ND	51 mths	Total included patients was 510 (409 TC/AC & 101 LCNEC)

*Appendix B: Patient and study characteristics of included selected research for surgical treatment for pulmonary carcinoid. AC: atypical carcinoid, LCNEC: large cell neuro endocrine carcinoma. *: mean, ~: median, mths: months, -: no results in article, TC: typical carcinoid, yrs: years.*

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Chapter 7

Morbidity and extent of surgical resection of carcinoid tumors after endobronchial treatment

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Abstract

Objectives

This study assessed whether endobronchial therapy (EBT) for bronchial carcinoid, if not curative, reduces the extent of the surgical resection and whether EBT is associated with increased surgical morbidity.

Material and Methods

Analysis was performed in a cohort of patients with bronchial carcinoid who have undergone surgical resection. A group that underwent EBT before the surgery (S+EBT) was compared with a group where no EBT was performed (S-EBT). Postoperative complications were also compared between both groups.

Results

A total of 254 patients treated for a bronchial carcinoid tumor between 2003 and 2019 were screened for inclusion. A total of 65 surgically treated patients were included, of whom 41 (63%) underwent EBT prior to surgery. In 5 out of 41 patients (12%) from the S+EBT group, less parenchyma was resected versus 2 out of 24 (8%) from the S-EBT group (OR 1.528, 95% CI 0.273-8.562, p=1.000). Two patients from the S+EBT group (5%) underwent lobectomy instead of sleeve lobectomy versus 0 from the S-EBT group (OR 1.051, 95% CI 0.981-1.127, p=0.527). Comparing complications between the S+EBT and S-EBT group did not result in increased postoperative surgical morbidity (15% S+EBT, 24% S-EBT).

Conclusion

EBT, if not curative, does not reduce the extent of the subsequent surgical resection. Therefore, if curative EBT is not anticipated, patients should directly be referred for surgery. If curative EBT seems feasible, it should be attempted not only because surgical resection can be prevented, but also because failure of EBT is not associated with excess surgical morbidity.

Introduction

Carcinoid tumors in the lung are pulmonary neoplasms that are characterized by neuroendocrine differentiation and comprise approximately 2% of all pulmonary malignancies [1]. Carcinoid tumors are frequently located in the central airways, and are predominantly located intraluminally without invading adjacent tissues [2]. Morphological analysis allows these tumors to be classified as typical carcinoid (TC) and atypical carcinoid (AC), depending on mitotic cell count (TC 0-2 and AC 2-10 per 2 mm^2) and on the presence of necrosis (AC) [1]. Although the treatment of pulmonary carcinoids has evolved, surgery is considered the cornerstone of treatment for early stage disease ever since. Nevertheless, minimally invasive endobronchial treatment (EBT) has emerged as a potential alternative for intraluminal located bronchial carcinoid tumors. Recent studies report at least comparable survival, recurrence and complication rates for EBT when compared to surgery in selected patients *with small (<2cm), intraluminal carcinoid tumors* [3-11].

Where EBT can be curative for patients with small intraluminal carcinoid tumors, tumor debulking prior to surgery may potentially result in less lung parenchyma that has to be removed during surgery to achieve complete resection of the tumor [12]. In addition, EBT may reduce the need for sleeve resection in order to achieve radical margins when bulky tumors are removed and facilitates pre-operative bronchial imaging to assess tumor margin in the bronchial wall. However, pre-operative EBT might impair subsequent surgery by inducing inflammation and airway scarring [13].

Against this background, we aimed to identify whether EBT prior to surgery reduces the volume of resected lung parenchyma and diminishes the need for sleeve lobectomies in patients with bronchial carcinoid tumors. Also, the impact on surgical morbidity of this strategy was evaluated.

Material and Methods

A cohort of patients from 2 University Medical Centers (Amsterdam University Medical Center and Radboud University Medical Center, IRB IRB00002991) was screened for eligibility after approval of the institutional Medical Ethical Committees. Detailed information regarding patient characteristics of the cohort and EBT technique were previously described [11]. For this study, patients diagnosed with bronchial carcinoid, who had surgical resection between 2003 and 2019, were screened for inclusion. Two groups of patients were defined: patients who had prior EBT before surgery (S+EBT group), and those not preoperatively treated with EBT (S-EBT group). Baseline patient and tumor characteristics, peri- and postoperative complications, and follow-up data were collected from an existing database.

Three surgeons with at least 10 years of experience in thoracic surgery, from 3 different centers, were asked to analyze the study cohort (KH, AV, CD). Blinded for previous treatment with EBT and actual performed surgical procedure, they reviewed anonymized baseline ≤5mm sliced Computed Tomography (CT) images together with CT- and bronchoscopy reports of patients with pathology proven pulmonary carcinoid tumors. Based on these reports and imaging, the experts were asked to make a surgical resection plan with curative intent of the carcinoid tumor. A structured form was used to capture the proposed surgical approach (open/video assisted thoracoscopic surgery (VATS)), the type of resection (wedge, segmentectomy, bilobectomy, lobectomy, sleeve lobectomy, bronchial sleeve resection, bronchotomy, pneumonectomy), nodal resection (node sampling or radical lymphadenectomy), and which lymph node station according the IASLC node map [14].

For the analysis of the impact of EBT on surgical extent, we compared the type of resection that was performed with the proposed procedure by the surgical panel. We defined parenchyma saving as 1) the resected amount of parenchyma was less than proposed by the panel, e.g. segmentectomy instead of a proposed lobectomy, lobectomy instead of a proposed bilobectomy, lobectomy instead of a proposed pneumonectomy, or bronchotomy instead of a proposed parenchyma resection, or as 2) a less extended resection than proposed, e.g. lobectomy instead of a proposed sleeve lobectomy. For a numeric expression of the preserved amount of parenchyma, the number of resected lung segments was compared with the number of segments proposed by the panel. For interobserver agreement in proposed type of surgery, 3 categories were defined: 1) full consensus; all 3 surgeons proposed the same surgical procedure, 2) near consensus; 2 out of the 3 surgeons proposed the same surgical procedure, and 3) no consensus; none of the surgeons proposed the same surgical procedure. Full and near consensus were determined as consensus and included for analysis.

Analyses

The statistical analyses and calculations were performed with SPSS 26.0 (SPSS Inc., Chicago, Illinois, USA). Data are presented as frequency and percentage for categorical variables and as mean and range for continuous variables. The $\chi 2$ and Fisher exact tests were used to compare categorical variables. To assess normal distribution, the Two-Sample Kolmogorov-Smirnov Test was used. Differences in mean values of continuous variables were analysed by the independent samples T-test. A *p* value less than 0.050 indicated statistical significance.

Results

Demographics

A total of 254 patients diagnosed with bronchial carcinoid tumor between 2003 and 2019 were screened for inclusion. Excluded were patients who were not operated (successfully performed EBT (*n*=71)), unfit for surgical resection (*n*=5), lost to follow-up (*n*=1), suffering metastatic disease (*n*=1), refusing surgery (*n*=2) or whose CT imaging, radiological or bronchoscopy reports were missing (*n*=109) (Figure 1). A total of 65 patients operated for carcinoid tumors located in the central airway or proximal segmental bronchi were selected. Forty-one patients received pre-operative EBT (S+EBT, 63%), and 24 patients were directly referred for surgery (S-EBT, 37%). Demographics and tumor characteristics are presented in Table 1. Patient and tumor characteristics were equally distributed in both groups, except for tumor histology based on pre-operative tumor sampling, with typical carcinoid accounting for 76% of patients in S+EBT compared with 38% in S-EBT group (*p*=0.002). The surgical procedures performed in the S+EBT group included 16 (39%) lobectomies, 10 (25%) bilobectomies, 11 (27%) sleeve lobectomies, 2 (5%) bronchial sleeve and 1 (2%) segmental resections, and 1 (2%) sleeve with segmental resection. In the S-EBT group there were 10 (43%) lobectomies, 3 (13%) bilobectomies, 6 (25%) sleeve lobectomies, 1 (4%) bronchial sleeve and 4 (17%) segmental resections. In both S+EBT and S-EBT group, resection was predominantly performed via thoracotomy (85% and 71% respectively). After surgery, pathological examination revealed TC in 22 (54%) and AC in 17 (41%) patients in the S+EBT group, and 14 (58%) TC and 10 (42%) in the S-EBT group. In 2 patients (5%) with S+EBT, no residual tumor was found in the resected specimen; 1 patient (2%) was resected for atypical carcinoid, another patient was successfully treated with EBT but developed an airway stenosis on the site of EBT, for which surgical treatment was indicated. Radical resection was achieved in all but 1 patient (*n*=40, 98%) treated with S+EBT and 22 (92%) patients who were not preoperatively treated with EBT. However, pathology results were unavailable for 2 (8%) S-EBT patients.

Morbidity

Reported complications from the EBT procedure were bleeding (controlled during the same procedure) (*n*=5, 12%) and bronchial stricture (*n*=2, 5%) due to scar tissue formation on the site of EBT. Bronchial strictures were all resolved with a surgical intervention. Furthermore, mild bronchospasm (*n*=1, 2%), bradycardia (*n*=1, 2%) and temporary vocal cord paralysis (*n*=1, 2%) (Table 2) were documented. The perioperative course in the S+EBT group remained uneventful in 85% (*n*=34) of the patients, compared with 76% (*n*=18) in the S-EBT group. After surgery, complications were mainly self-limiting, except for a lingular torsion after tri-segmentectomy of the left upper lobe, urging a completion upper lobe resection and a surgical plication of the diaphragm due to phrenic nerve damage in the S-EBT group. Pre-operative EBT was not associated with a higher peri-and postoperative complication rate on univariate analysis (odds ratio [OR], 0.618; 95% confidence interval [CI], 0.180 to 2.115; *p*=0.526).

Extent of surgery

Overall, based on pretreatment CT images and reports obtained from CT-scan and bronchoscopy, the panelists nearly or fully agreed on type of resection needed to achieve radical margins in 60 patients (92%). No consensus was found in 5 patients (8%). The panelists reached predominantly full consensus in proposed lobectomies (24/35, 71%) and sleeve lobectomies (9/11, 82%) (Figure 2). We found 7 patients (11%) in whom less parenchyma was resected than proposed by the panel: 5 out of 41 patients (12%) from the S+EBT group and 2 out of 24 (8%) from the S-EBT group (OR 1.528, 95% CI 0.273-8.562, *p*=1.000) (Table 3). Two patients from the S+EBT group (5%) underwent lobectomy instead of a proposed sleeve lobectomy (OR 1.051, 95% CI 0.981-1.127, *p*=0.527). The actual procedure was in 16 cases (25%) more extensive than the procedure proposed by the panelists, with equal percentages in the S+EBT (*n*=10, 24%) and S-EBT group (*n*=6, 25%).

Discussion

To the best of our knowledge, this is the first study that evaluated the effect of EBT on the extent of subsequent surgery and on surgical outcome in patients with bronchial carcinoid tumors. No significant impact on the amount of resected lung parenchyma was found when S+EBT was compared with S-EBT, although it reduced the amount of resected lung segments and sleeve lobectomies needed to achieve radical resection in some patients. In addition, we found surgical morbidity equally distributed in both the S+EBT and S-EBT group, and complications were mostly self-limiting.

After adding the current findings to the existing literature, it can be postulated that the parenchyma sparing effects of EBT are mainly achieved by preventing surgical resection. Apparently, if EBT is not curative, its effects on ensuing surgical resection with regard to the volume of resected lung parenchyma are modest, considering that in only a few patients less parenchyma was resected than predicted prior to EBT by the panel of surgeons. Although not supported by the data from this study, a possible explanation for this observation is the relation between de base and shape of the carcinoid and its position in the bronchial tree. Especially a carcinoid tumor with a polypoid shape and long stalk, that extends significantly beyond an important bifurcation proximal from its base, can be a good candidate for debulking. Reduction of the tumor back into the originating bronchus can help to facilitate lobectomy instead of pneumonectomy or sleeve lobectomy. Detailed anatomical information based on CT-scan and bronchoscopy images helps to select optimal treatment. Bronchoscopy offers a clear impression of the intraluminal extension of the tumor and where it is attached to the bronchial wall, guiding the extent of resection needed to achieve radical bronchial margins [15]. Additionally, a diagnostic CT can support these findings, visualizes extra-luminal involvement, and is a reliable tool for excluding lymph node involvement [16,17]. Finally, although there is no clear evidence to support this, bronchoscopic debulking may improve patients' pre-operative physical condition by resolving post-obstruction pneumonia, resulting in less perioperative morbidity. Therefore, a multidisciplinary setting with surgeons, radiologists and interventional pulmonary physicians is strongly advised for accurate treatment planning in patients with carcinoid tumors.

The incidence of complications from EBT can be reduced in well trained hands of experienced interventional pulmonologists who are familiar with endobronchial treatment. Nevertheless, EBT might lead to potentially serious complications such as airway wall perforation or vascular injury [13,18,19]. We found 5 (12%) minor bleedings which could all be treated bronchoscopically with suction, topical adrenaline, xylomethazolin or a bronchial blocker. Furthermore, no airway wall perforation was seen. Clinicians might argue that EBT potentially induces strictures or stenosis of the involved bronchus and impairs subsequent surgical resection or has its impact on perioperative course. In this study, strictures (n=2) did not negatively influence the extent or outcome of the subsequent surgical resection and the extra step of EBT in the S+EBT group compared with the S-EBT group did not result in increased perioperative surgical morbidity (17% S+EBT, 25% S-EBT).

The use of minimally invasive surgical techniques has increased in the last decade [20-22]. The patients in our study predominantly underwent resection via thoracotomy. This could be explained by the fact that a significant proportion of the patients were treated more than a decade ago. All VATS procedures in our study group were performed after 2008. Another explanation for the high incidence of open surgical approach is the fact that bronchial carcinoids are often located in the central airways,

which makes the surgery more complex and probably less suitable for a video-assisted approach. Finally, the location of the tumor, which is sometimes situated directly after the airway junction between two lobes, complicates the use of stapling devices in this area because of the width of the stapling rows. In these patients, an open approach facilitates intraluminal inspection of the airway, reassuring radical resection, e.g. through bronchoplasty or sleeve lobectomy.

The findings of the present study must be interpreted in the context of several potential limitations. First, the surgical panel was not exposed to bronchoscopy images (only bronchoscopy reports and CT images and reports). Second, complications where retrospectively assessed, which might induce recall bias as complications might be under-documented in medical files. Third, we found 24% (10/41) discrepancy between preoperative biopsy diagnosis and definitive postoperative histology. This is in line with the results of a recent study which reported that classification of carcinoids based on pre-operative biopsies is imprecise [23]. Hypothetically, atypical carcinoid is unfavorable for EBT, as they have a poorer prognosis and a higher tendency to disseminate, and should be referred for surgery without prior EBT. However, good results have been reported for patients with small, intraluminal previous atypical carcinoid treated with EBT [11]. Finally, the lung sparing effect of EBT was not significant, however the sample size of the current study is limited. The incidence of bronchial carcinoid tumors is low, and EBT is currently limited to a small number of medical centers with sufficient expertise in interventional pulmonology. A large study, within an international collaborative, would allow analysis of the impact of EBT with more power.

Conclusion

EBT, if not curative, does not reduce the extent of the subsequent surgical resection. Therefore, if curative EBT is not anticipated, patients should directly be referred for surgery. If curative EBT seems feasible (small intraluminal lesions) it should be attempted not only because surgical resection can be prevented, but also because failure of EBT is not associated with excess surgical morbidity.

Demographics	S + EBT ($n = 41$)	Percentage (%)	S-EBT ($n = 24$)	Percentage (%)	p-value
Patients					
- Female	28	68	17	71	**0.830**
- Male	13	32	7	29	**0.830**
Age at surgery, median in years [IQR]	48 [31-59]		56 [42-64]		**0.317**
Comorbidity					
- ASA1	23	56	8	33	**0.076**
- ASA2	13	32	12	50	**0.143**
- ASA3	5	12	4	17	**0.715**
Pre-operative tumor sampling					
- Typical	31	76	9	38	**0.002**
- Atypical	6	15	1	4	**0.246**
- No tumor	0	0	1*	4	**0.369**
- Undifferentiated	1	2	5	21	**0.023**
- Not performed	3	8	8	33	**0.014**
Number of EBT, median [IQR]	1 (1-1.5)		NA		–
Time between EBT and Surgery, median in months [IQR]	2 (0.50-4.5)		NA		–
Surgical approach					
- Open	35	85	17	71	**0.204**
- VATS	6	15	7	29	**0.204**
Surgical procedure					
- Lobectomy	16	39	10	43	**0.834**
- Bilobectomy	10	25	3	13	**0.342**
- Sleeve lobectomy	11	27	6	25	**0.871**
- Bronchial sleeve resection	2	5	1	4	**1.000**
- Sleeve with segmental resection	1	2	0	0	**1.000**
- Segmental resection	1	2	4	17	**0.058**
Pathology result after surgery					
- Typical	22	54	14	58	**0.714**
- Atypical	17	41	10	42	**0.987**
- No tumor	2	5	0	0	**0.527**
Tumor diameter					
- ≤0.5 cm	6	15	0	0	**0.077**
- >0.5-≤1 cm	7	17	3	13	**0.733**
- >1-≤2 cm	11	27	10	42	**0.217**
- >2 cm	17	41	11	45	**0.731**
Lymph node status					
- N0	36	88	22	92	**1.000**
- N1	5^	12	0	0	**0.149**
- N2	0	0	1	4	**0.369**
- Unknown	0	0	1	4	**0.369**
Resection margin					
- R0	40	98	22	92	**0.549**
- R1	1	2	0	0	**1.000**
- Unknown	0	0	2	8	**0.133**

*Table 1: Demographics - ASA: American Society of Anesthesiologists; EBT: endobronchial treatment; VATS: video-assisted thoracoscopic surgery; S+EBT: patients pre-operatively treated with EBT; S-EBT: patients not pre-operatively treated with EBT; * clinically high suspicious for carcinoid due to positive Octreotide scan.*

Complications after EBT	EBT (*n*=41)	No EBT (*n*=24)	OR [95% CI], *p*-value
No complications	31 (76%)	NA	
Any complication	10 (24%)	NA	
Bleeding*	5 (12%)	NA	
Stricture	2 (5%)	NA	
Bronchospasm	1 (2%)	NA	
Bradycardia	1 (2%)	NA	
Vocal cord paralysis	1 (2%)	NA	
Complications after surgery			
No complications	34 (83%)	18 (75%)	**1.619 [0.473, 5.545], 0.526**
Any complication	7 (17%)	6 (25%)	**0.618 [0.180, 2.115], 0.526**
Atrial fibrillation	2 (5%)	1 (4%)	
Atelectasis	1 (2%)	1 (4%)	
Pneumonia	1 (2%)	1 (4%)	
Persistent wheezing	1 (2%)	0 (0%)	
Empyema~	0 (0%)	1 (4%)	
Parapneumonic effusion	1 (2%)	0 (0%)	
Extended air leak	1 (2%)	0 (0%)	
Phrenic nerve damage	0 (0%)	1 (4%)	
Torsion of the lingula∞	0 (0%)	1 (4%)	

*Table 2: Complications after EBT and surgery in the S+EBT and S-EBT group - * All conservatively treated, ~ conservatively treated with drainage, ∞ re-operation with lingula resection.*

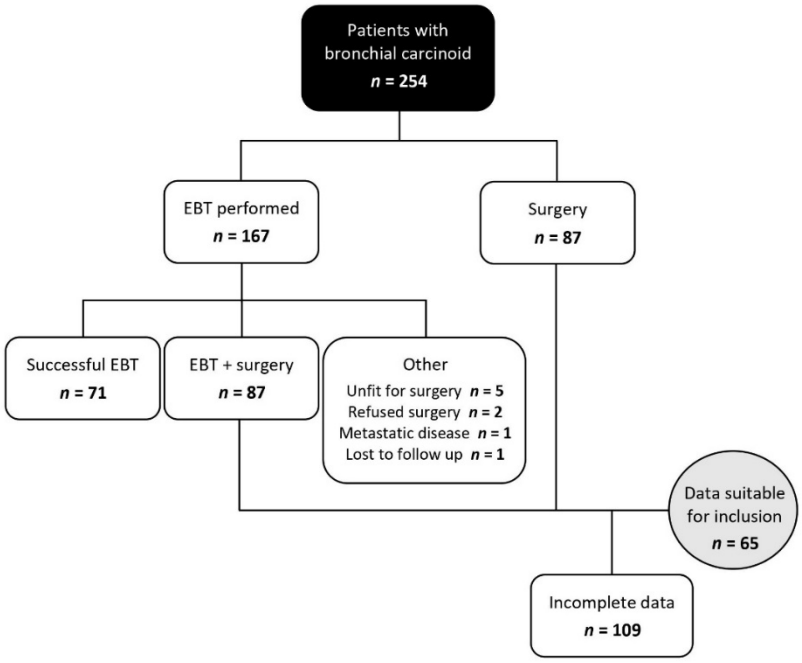

Figure 1: Flowchart for patients surgically and non-surgically (EBT) treated for bronchial carcinoid.

	S+EBT (*n*)	Lung segments saved	S-EBT (*n*)	Lung segments saved	OR (95% CI)	*p*-value~
Less lung parenchyma resected than proposed					1.528 (0.273-8.562)	1.000
- Segmentectomy instead of lobectomy	1	4	2	2 1		
- Lobectomy instead of bilobectomy	2	2 2	0	0		
- Lobectomy instead of pneumonectomy	0	0	0	0		
- Bronchotomy instead of parenchyma resection	0	0	0	0		
- Sleeve lobectomy instead of bilobectomy	1	5	0	0		
- Bronchial sleeve instead of sleeve lobectomy	1	3	0	0		
Reduced extent of surgery					1.051 (0.981-1.127)	0.527
- Lobectomy instead of sleeve lobectomy	2	0	0	0		
Total	7	16	2	3	2.265 (0.430-11.916)	0.466

Table 3: Differences between the S+EBT (n=41) and S-EBT (n=24) groups with regard to parenchyma sparing surgery

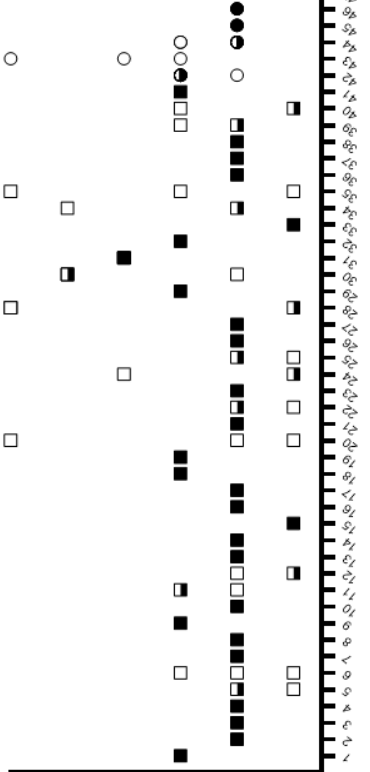

Figure 2: Graphic representation of consensus among all experts in the S+EBT (squares) and S-EBT group (circles). One black symbol per patient corresponds to full consensus (all surgeons agreed) and three white symbols per patient indicate no consensus among surgeons. The combination of one lower half black and one white symbol per patient represents near consensus (2 out of 3 surgeons agreed).

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Chapter 8

Chapter 8.1

Treatment of Bronchial Carcinoid Tumours: Is Surgery Really Necessary?

Respond to publication of Hendifar et al; "Neuroendocrine Tumors of the Lung: Current Challenges and Advances in the Diagnosis and Management of Well-Differentiated Disease"

E.M.B.P. Reuling
C. Dickhoff
J.M.A. Daniels

Journal of Thoracic Oncology 2021

To the Editor

With great interest we have read the recent article of Hendifar et al.[1], in which they discuss the current challenges in the diagnosis and treatment of pulmonary neuro-endocrine tumors, with a special focus on typical and atypical carcinoid tumors (TC/AC). For localized TC/AC, the authors state that surgical resection is the only curative treatment option for resectable lung carcinoids.

Although traditionally surgery is the treatment of choice, several studies have shown that both bronchial TC and AC can be safely treated with bronchoscopic treatment (BT) [2–5]. Bronchoscopy facilitates the local application of ablative techniques such as electrocautery, cryotherapy and laser. Brokx et al. treated 112 patients with TC (n= 83) and AC (n=29) in this fashion. Bronchoscopic treatment was curative in 47 patients (42%), with excellent long term outcome. Patients who underwent surgical resection after unsuccessful BT also had excellent long term outcome [3]. Dalar et al. reported an even higher success rate of BT (21/29, 72%) [4]. These observations show that BT is a parenchyma sparing alternative to surgical resection. In a comprehensive review about the treatment of pulmonary carcinoid, it is at least worth mentioning that for carcinoids located in the central airways, BT is an alternative to surgical resection.

Nowadays not many centers offer BT to patients with bronchial carcinoids, which is a pity because not all of these patients require surgery. In order to optimize the effectiveness and implementation of BT, several issues have to be addressed. First, BT can be challenging, mainly due to risk of bleeding. Therefore BT should be performed by dedicated interventional pulmonologists who are trained in carcinoid removal and skilled at hemorrhage management. In addition, thoracic surgeons should be available to perform emergency thoracotomy in the rare occurrence of uncontrollable bleeding. Second, it would be favorable if the success rate of BT can be increased. Improved patient selection could be a key factor. In our cohort of 125 patients who received BT for bronchial carcinoid we have identified baseline predictors of successful BT (manuscript under review). Ideally, a randomized controlled trial comparing BT to surgical resection would be required to show non-inferiority of BT. However such a trial would be difficult to perform as bronchial carcinoid is a relatively rare disease. A large sample size would be required since the number of expected recurrences would be very low and follow-up would have to be long because of the indolent nature of TC/AC.

In conclusion, we advocate the use of BT in selected cases of bronchial carcinoid in specialized and well-equipped centers. We encourage other physicians to take note of this minimally invasive and parenchyma sparing alternative to surgical resection and to join us in further investigating and improving this technique.

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Chapter 8.2

Author's reply

Respond to comments of Petrello et al. on our publication 'endobronchial treatment of bronchial carcinoid: patient selection and predictors of outcome'

E.M.B.P. Reuling
C. Dickhoff
J.M.A. Daniels

Respiration 2018

Letter to the Editor: Authors' response

Dear Editor,

Thank you for the opportunity to respond to the comments of Petrello et al., whom we kindly thank for reading and commenting on our paper.

In our article entitled ''endobronchial treatment for bronchial carcinoid: patient selection and predictors of outcome'', we have described how patients can be selected for endobronchial treatment as an initial treatment modality for patients with bronchial carcinoid [1]. Because radical treatment is vital, the endobronchial treatment, if unsuccessful, is followed by radical surgical resection. This strategy is performed in close cooperation with the thoracic surgeons and all patients are discussed in the multidisciplinary thoracic tumour board prior to treatment.

Petrello et al. find it doubtful that we propose endobronchial treatment as a first line treatment because the success rate for intraluminal treatment is only 72% and no survival data are presented. We would like to point out that endobronchial treatment cannot be compared with surgical resection as a single treatment because unsuccessful endobronchial treatment is always followed by radical surgical resection. In a previous publication, the long-term survival data of our cohort were presented [2]. The 5-year overall survival and disease-specific survival were 97% and 100% respectively and the 10-year overall and disease-specific survival were 80% and 97% respectively for this combined treatment strategy [2]. Bearing these reassuring survival data in mind, we sought to further improve the selection of patients, which resulted in the current paper. Mortality was reported in the online data supplement. After successful endobronchial treatment, 8 patients died, all of other causes (8/61, 13%). After unsuccessful endobronchial treatment and subsequent surgical resection, five patients died, 1 of postoperative complications, 2 of metastatic disease and 2 of another cause (5/64, 8%). With a median follow-up of 82 months (interquartile range (IQR) 98 months), the total disease-specific mortality was 2,4% (3/125).

A second issue that was raised by Petrello et al. is the fact that not all bronchial carcinoids are purely intraluminal. We fully acknowledge this, which is why we perform careful and long term follow-up after endobronchial treatment. In addition, we apply cryotherapy to the base of the tumour to improve the penetration depth of the treatment while sparing the cartilaginous structures, as has been reported by another group with favourable long-term results [3]. If we observe significant involvement of the bronchial wall, either on CT images or during bronchoscopy, we refer the patient for surgery. In our study, with a median follow-up of 82 months (IQR 98 months), we have reported 4 patients with a local recurrence within 2 years and 6

patients with a local recurrence >2 years after endobronchial treatment. Seven patients who subsequently underwent surgical resection are still alive without disease (median follow-up 173 months, IQR 168 months) and three patients who refused surgery are also still alive with local disease (follow-up time 98, 135 and 184 months). These data can be found in the online data supplement.

In conclusion, we believe that our recent data and previously supported survival data support our conclusion that endobronchial treatment for bronchial carcinoid is a safe, minimally invasive and tissue sparing first line alternative in a selected group of patients. Ideally, a randomized trial should be conducted to compare the initial endobronchial approach with the purely surgical approach for bronchial carcinoid. Such an effort however, would be very time consuming because of the its low incidence and indolent behaviour, which require both a long enrolment period and follow-up time.

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Chapter 8.3

Letter to the editor on 'sublobar resection of typical carcinoid tumours of the lung'

Respond to the publication of Cattoni et al. 'Sublobar resection in the treatment of peripheral typical carcinoid tumors of the lung'

E.M.B.P. Reuling
C. Dickhoff
J.M.A. Daniels

Annals of thoracic surgery 2020

To the editor

With interest we have read the paper on the optimal treatment of pulmonary carcinoid by Cattoni et al. in Annals of Thoracic Surgery, in which they conclude that for selected patients with typical carcinoid, sublobar resection is not associated with worse short-term and long-term survival when compared with lobectomy [1]. Although we encourage the efforts of the authors to add more scientific knowledge on the treatment of this rare tumor, we do have some concerns.

First, in our opinion, the conclusion of the authors is not supported by the data presented in the paper. The follow-up of patients is presented in terms of total person-years, which leaves the reader guessing about the distribution of the follow-up time in the investigated population. The follow-up of 301 person-years in the sublobar population and 508 person-years in 103 patients treated with lobectomy, results in a mean follow-up of 301/74 = 4.06 years and 508/103 = 4.93 years respectively. This suggests a shorter follow-up period in the sublobar group, which can lead to underestimation of the number of events in the sublobar group.

An issue underexposed, is the fact that sublobar resection consisted either segmentectomy (n=20) or wedge resection (n=54). This is remarkable, because from the non-small cell lung cancer literature, we know that wedge resections result in smaller parenchymal margins, lower yield of lymph nodes, and worse outcome when compared with segmentectomy [2]. It is therefore important to look at outcome for the 2 subtypes of sublobar resection separately.

Finally the lack of statistical significance does not prove that there is no difference between both groups [3]. Overall survival *is* better (5-year survival rate 97.4% vs. 91.7%) and the number of R1 resections lower (1% vs. 7%) in the lobectomy group. These findings might be genuine, but may dismissed by only looking at the p-value. Because the follow-up in this study was too short for such an indolent disease with a low incidence of recurrences, even a small signal should be taken seriously. To conclude that sublobar resection is not associated with worse short-term and long-term survival would require a study with adequate power and sufficient follow-up for all patients.

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Chapter 9

Summary
Discussion
Future perspectives

Summary of thesis

1. Patients with purely intraluminal carcinoid tumors with a diameter <20 mm on CT scan are good candidates for endobronchial treatment (EBT). Operable patients with a tumor diameter ≥20 mm should be directly referred for surgery (chapter 2).

2. Histological classification in central carcinoid tumors is frequently discordant in small biopsies. A cumulative biopsy surface of at least 4 mm^2 tumor increases diagnostic accuracy (chapter 3).

3. Synaptophysin is the most reliable diagnostic neuro-endocrine marker, especially in small biopsies (chapter 4).

4. CD44 is the most reliable prognostic neuro-endocrine marker, especially in small biopsies (chapter 4).

5.. Adding CD44, OTP and Ki-67 to the widely used TC/AC classification, provides a multimodal biomarker that accurately stratifies patients in favorable and unfavorable prognostic categories (chapter 5).

6. Centrally located small intraluminal pulmonary carcinoids, without signs of metastasis can be treated with minimally invasive alternatives such as endobronchial treatment or parenchyma sparing surgical resection (chapter 6).

7. EBT, if not curative, does not reduce the extent of the subsequent surgical resection. If curative EBT seems feasible, it should be attempted not only because surgical resection can be prevented, but also because failure of EBT is not associated with excess surgical morbidity (chapter 7).

Discussion

In this thesis we describe the treatment of centrally located bronchial carcinoid tumors. While guidelines recommend surgical resection as the preferred curative treatment, we have shown that endobronchial therapy (EBT) is an effective, minimally invasive and parenchyma sparing alternative [1,2]. Nevertheless, currently only a few patients are offered EBT, due to several concerns. First, safety is a concern, because carcinoid tumors are highly vascularized tumors and airway hemorrhage after local treatment can potentially compromise the patient during a bronchoscopic procedure [3,4]. Some have even used bronchial artery embolization before EBT in an attempt to prevent airway hemorrhage [5,6]. However, as described in chapter 6, airway haemorrhage resulting from EBT can be effectively stopped using endobronchial interventions such as local instillation of adrenaline, xylometazoline or balloon blockers. Besides, there is only one study reporting massive bleeding in a patient treated with EBT requiring emergency surgery [7]. A second concern is the curative potential of EBT. Some argue that EBT is not a treatment with curative intent because resection margins cannot be assessed and a pathological lymph node status cannot be determined. However, EBT is a combination of resection of the tumor, and subsequent ablative treatment where tumorous tissue is treated with heat or cold to achieve destruction of tumor cells. Where the intraluminal tumor tissue can be directly resected during EBT, remaining carcinoid tissue in the bronchial wall can be treated with ablative techniques such as electrocautery or cryotherapy [8,9]. In this sense, EBT can be compared to stereotactic body radiation or radiofrequency ablation, which are established ablative oncological treatments in which there are also no resection margins and lymph node dissection.

Instead of comparing features that are inherent to the different treatments, i.e. surgical resection and EBT, it is more interesting to focus on patient outcome. The results of the systematic review presented in chapter 6 showed that no randomized trials have been performed in patients with bronchial carcinoid comparing EBT with surgery. The available series that were analyzed showed a lower loco-regional (0–5%) and distant recurrence (0–4%) rate in the EBT and parenchyma sparing surgery group compared with anatomical pulmonary resections (0–8% and 0–23% respectively). This difference may, at least in part, be explained by selection, where patients with small intraluminal tumors were more likely to be assigned to parenchyma sparing techniques and patients with larger tumors and/or lymph node involvement to anatomical resections. It seems therefore that outcomes can be favourable after both EBT and surgical resection, but assignment to the most appropriate treatment for each individual treatment is crucial.

The central issue of the thesis is how we can establish the optimal selection process for patients with bronchial carcinoid. In order to establish this process, several questions require thought.

Can computed tomography help to assign patients with bronchial carcinoid to the optimal treatment?

All patients with (suspected) bronchial carcinoid undergo a CT scan during the diagnostic process. It provides valuable information about the size of the lesion, its location and its relation to adjacent structures. In chapter 2 we analyzed whether radiological features can predict successful endobronchial treatment in patients with (mainly typical) bronchial carcinoid. Experienced radiologists assessed the tumor diameter, the presence of intra- and/or extraluminal disease and lymph node status. We found that, in patients with an intraluminal carcinoid <20 mm and no signs of lymph node involvement, EBT had high success rate of 72%.

Based on these data, CT scan has an important role in directing patients towards the most suitable treatment modality. While patients with a bronchial carcinoid tumor of ≥20 mm in diameter should be referred for surgery, purely intraluminal carcinoids with a diameter <20 mm can be treated with EBT with a high success rate. Although it seems feasible, the low number of included patients with AC limits solid recommendations regarding the use of EBT for treatment of AC [10,11].

Are there any biomarkers helpful to assign patients with bronchial carcinoid to the optimal treatment?

In the current era of precision medicine, we aim to predict the optimal treatment at the individual level, accounting for an individual risk for harm and benefit outcomes. The corner stone of precision medicine is the establishment of heterogeneity of a treatment effect between subgroups, which allows for allocation of the appropriate patients to the appropriate treatments. To qualify for this approach, markers ideally have to show predictive properties in randomized controlled trials. However, bronchial carcinoid is rare and has a low tendency to metastasize, making an adequately designed RCT expensive and time consuming. A different strategy is to use biomarkers to predict tumor recurrence or dissemination. Currently, the best known biomarker in this context are mitotic index and presence of necrosis to differentiate between TC and AC.

Besides that the mitotic index is prone for interobserver variability [12], a prerequisite for accurate classification of bronchial carcinoids is a representative tissue sample of sufficient volume. Reliable differentiation between TC and AC is important as dissemination predominantly occurs in AC. In chapter 3 we compared tumor classification between bronchoscopic and surgical biopsies of central carcinoids and showed discordance in 45% of the biopsies. The discordance was caused by

misclassification of AC as TC. In biopsies measuring <4 mm^2, 15/15 AC (100%) were misclassified compared to 14/23 AC (61%) of biopsies ≥ 4 mm^2. Distinguishing carcinoid tumor into typical or atypical carcinoid on biopsies <4 mm^2 should therefore be discouraged and keep the diagnosis 'carcinoid NOS' for carcinoids with ≤ 1 mitosis per 2 mm^2. Based on these results physicians should aim for biopsies with a cumulative surface of at least 4 mm^2. Preferably this should be done in a controlled setting under propofol sedation or general anesthesia and adequate hemorrhage management.

Ki-67 is a widely accepted marker in the diagnostic pathology of gastrointestinal neuroendocrine tumors but currently not used for distinction between TC and AC in bronchial carcinoid. However, some literature and expert opinion in the current 2021 WHO classification suggest that a Ki-67 ≥5% might be suggestive for AC [14], a finding not confirmed by our study group. However, combined with other prognostic markers, Ki-67 does have a role in classifying bronchial carcinoid tumors into favorable and unfavorable prognosis.

In contrast to the mitotic count and Ki-67, but also Synaptophysin, OTP and CD44 are very reliable biomarkers in small biopsies, which is shown in chapter 4. Although they cannot classify carcinoid in TC or AC, they can help to designate tumors as favorable or unfavorable. Our study in chapter 5 described that loss of OTP or CD44, immunohistochemistry for Ki-67 (≥5%) and mitotic count (≥2 per 2mm^2) were independently associated with a higher risk of distant metastasis. Adding OTP, CD44 and Ki-67 to the classification of bronchial carcinoids facilitates selection of individuals with favorable and unfavorable prognosis, which may be helpful when selecting patients for EBT or surgery.

In summary, adding the radiological tumor stage (cT) to the results of the pathological prognostic immunohistochemical markers of the histological biopsies can improve accurate selection for EBT. Small intraluminal carcinoid tumors on CT scan, combined with favorable prognostic markers (mitotic count of <2 per 2 mm^2, Ki-67 <5% and positive OTP and/or CD44) can be safely treated with EBT. On the other hand, tumors of >2 cm with or without extraluminal growth and/or unfavorable biomarkers (mitotic count >2 per 2 mm^2, Ki-67 >5% and negative OTP and/or CD44) should be directly referred for surgery.

Does EBT influence subsequent surgical resection?

The use of minimally invasive surgical techniques such as video assisted thoracoscopic surgery (VATS) and parenchyma paring techniques such as bronchoplasty and sleeve resections has increased during recent decades [16-19]. Because bronchial carcinoids often originate from the central airways, the application of parenchyma sparing techniques is crucial to prevent large anatomical resections. The use of EBT prior to surgery has theoretical advantages. First, debulking of a

bronchial carcinoid tumor with reduction of the tumor back into the originating bronchus may facilitate lobectomy or sleeve lobectomy instead of bilobectomy or pneumonectomy. Second, debulking and recanalization may help relieve postobstructive pneumonia and thereby improve the patients' condition before surgery. On the contrary, EBT may also have negative effects on subsequent surgical resection. Airway scarring after EBT might increase complications of subsequent surgical resection. In chapter 7 we analyzed 65 patients who did or did not undergo EBT prior to surgery. We investigated whether EBT had impact on the volume of resected lung parenchyma and whether it changed the type of resection that was performed. The lung sparing effect of EBT was not significant, however the sample size of the study was limited. The incidence of bronchial carcinoid tumors is low, and EBT is currently limited to a small number of medical centers with sufficient expertise in interventional pulmonology. A large study, within an international collaborative, would allow analysis of the impact of EBT on subsequent resection with more power.

Opponents of EBT also state that EBT may result in increased morbidity like bleeding, airway wall perforation, strictures or stenosis [3,20]. Our study in chapter 7 showed that EBT did not result in a higher complication rate of surgical resection of carcinoid tumors compared to patient without EBT prior to surgery [21]. However, because airway hemorrhage may occur, it is advised to perform EBT in an experienced center for interventional pulmonology with an in-house thoracic surgery department.

Multidisciplinary meetings, treatment recommendation and follow up in patients with bronchial carcinoid.

The incidence of bronchial carcinoids in the Netherlands is around 200 per year. As there are 116 hospital locations in the Netherlands, the estimated amount of cases per hospital is only 1.5 per year. To optimize diagnosis, to minimize the risk of complications and to maximize the potential of EBT, we highly advocate that EBT is centralized to referral centers with experienced radiologists for accurate and systematic CT scan evaluation, with pathologists specialized in histological analysis of carcinoids and with interventional pulmonologists, and pulmonary surgeons, with skills for EBT and the management of complications such as airway hemorrhage.

In line with the search for less invasive techniques for accurate diagnosis, minimal invasive surgery and parenchyma sparing techniques such as EBT are increasingly adopted for the treatment of patients with bronchial carcinoid. Although potential morbidity, such as the risk of major bleeding necessitating urgent surgical intervention may occur, this is very rare, and should not prevent the implementation of EBT as interventional pulmonologists can be trained to manage such bleeding adequately.

There is very little evidence regarding the benefits of postoperative surveillance in bronchial carcinoid. Currently, guidelines suggest a follow up of at least 5-10 years

after radical surgery [34]. In one large series, scheduled imaging failed to detect most recurrences, and the rate of recurrence was very low (<3 percent) in patients with node-negative low-grade TC [35]. These data suggest that patients with node-negative low-grade lung neuroendocrine tumors are highly unlikely to benefit from any type of postoperative surveillance. For patients with node-positive TC or AC independent of N status, there is a higher risk of recurrence, and post-treatment surveillance is advised for at least 10 years [36]. However, these recommendations are based on surgical series, and follow-up for patients treated with EBT is therefore unclear. Based on our results and because local recurrence occurs in a small proportion of patients after EBT, yearly follow-up is obviously mandatory. However, after radical surgical resection of a tumor with a favorable biomarker profile (low mitotic count, low Ki-67 expression and normal OTP and CD44 expression), patients may be spared from unnecessary hospital visits and CT-imaging in the future.

Although evidence is not based on prospective data, Figure 1 presents a suggested treatment flowchart for patients with bronchial carcinoid based on the results of this thesis.

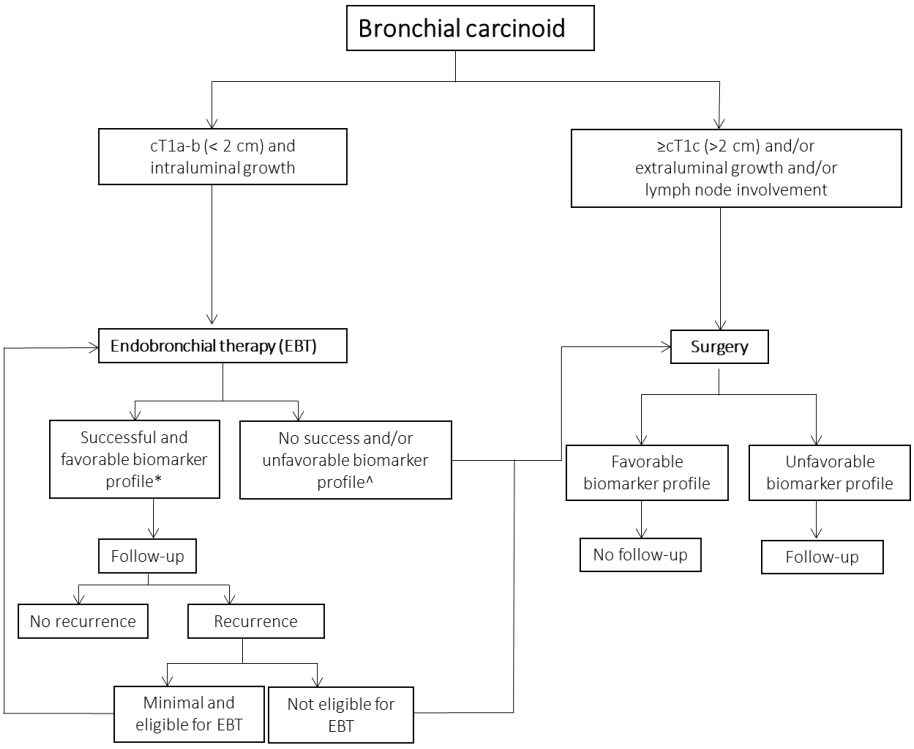

*Figure 1: Suggested flowchart for treatment of patients with bronchial carcinoid based on the results of this thesis. *Favorable profile: Ki-67 <5%, mitotic count <2 per 2 mm2, OTP or CD44 positivity; ^unfavorable profile: Ki-67 ≥5%, mitotic count ≥2 per 2 mm2, and loss of OTP or CD44 expression.*

Future perspectives

Currently, invasive tests such as bronchoscopy or needle biopsy and a histological/cytological diagnosis are required to define the type of tumor. Ideally, an accurate diagnosis is made with less invasive techniques. Unfortunately, conventional imaging modalities, such as CT-scan and PET-scan, are not able to discriminate specific neoplastic subtypes. However, nuclear medicine techniques have improved over the last decades with the aim of helping the physicians in the challenging management of bronchial carcinoids. Primarily positron emission tomography (PET) tracers have been proposed for the assessment of disease extent, re-staging, and therapy response. F-18-FDG (glucose analogue) is widely used in evaluating aggressive tumors such as high-grade neuro-endocrine tumors including LCNEC and SCLC. However, it seems of limited value for the evaluation of low grade NET such as TC and AC [29,30]. In the last years, novel tracers, in particular somatostatin analogues labeled with gallium-68 (Ga-68-DOTA-peptides), have allowed accurate imaging of typical bronchial carcinoids with a sensitivity and specificity of 96 and 100% respectively [31]. The uptake of these tracers is not dependent on the metabolic rate of the tumor cells. As pre-operative biopsies are unreliable in classifying TC and AC, 68Ga-dotatate PET/CT scan might improve the differentiation by variable tracer uptake. Typical carcinoids show significantly higher SUVmax than atypical tumors [32]. Although incidence is low, 68Ga-dotatate PET/CT also helps identifying the presence of tumour metastasis [32]. Future studies should focus on how accurately this new nuclear technique provides information on somatostatin receptor (SSTR) expression and with what therapeutical implications. For example, metastatic carcinoid with high SSTR expression could be suitable for peptide radioreceptor therapy [30,33].

Substantial data is currently available to support the idea that blood sampling can provide useful oncological information. A circulating neoplastic molecular signature could limit the need for invasive biopsies, define therapeutic targets and provide a real-time monitoring tool to evaluate disease status. Such strategies, or 'liquid biopsies', are currently being used in non-small cell lung cancer (NSCLC), e.g. for monitoring treatment responses to epidermal growth factor receptor (EGFR) inhibitors through identification of mutation T790M in circulating tumor DNA [22]. Recent studies analyzed a blood test to detect neuroendocrine transcriptome activation (NETest transcripts) and compared its accuracy to that of chromogranin A. The NETest accurately differentiated bronchopulmonary carcinoids from controls in 93%, compared to 19% in chromogranin [23,24].

Next-generation sequencing (NGS) technologies and the use of proteomics has enabled rapid genome-wide surveys of oncogenic- and tumor suppressive signaling molecules and proteins in various cancers. NGS accelerated understanding of cancer biology and the development of novel diagnostics and therapeutics. Large-scale

sequencing studies have revealed that bronchial carcinoid has a large variety of genetic abnormalities but it is still unclear which genetic alterations or pathways are key players in the development and progression of disease [25-28]. RNA sequencing will increasingly become important, e.g. to identify risk factors for lymph node- and distant metastasis.

The low incidence of pulmonary carcinoid also presents a great challenge for performing randomized controlled trials. This lack of evidence understandably impedes the implementation of EBT. Unfortunately, as EBT is currently limited to a low number of centers with sufficient experience, centralization would make patient inclusion for a adequately powered RCT much easier and more efficient. However, preferably international multicenter collaboration is necessary in order to acquire more robust data about the available treatment options and their effects on important endpoints such as overall survival, disease free survival, quality of life and cost-effectiveness and for implementation of evidence based guidelines.

Conclusion

Radiological and pathological parameters can improve stratification of patients with bronchial carcinoid tumors suitable for endobronchial resection. Patients are candidates for EBT in case of intraluminal growing tumors of <2cm. Sufficient biopsies can be used to classify bronchial carcinoid tumors with favorable and unfavorable characteristics based on OTP, CD44 and Ki-67. This enables risk stratification in order to provide a tailored approach with regard to treatment and follow-up of patients with bronchial carcinoid. To optimize patient selection, this should preferably be performed in specialized centers with an experienced multidisciplinary team.

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Chapter 10

Nederlandse samenvatting
Discussie
Toekomst perspectief

Nederlandse samenvatting

Dit proefschrift beschrijft een relatief nieuwe behandeling voor bronchiaal carcinoïd tumoren. Bronchiaal carcinoïd is een neuro endocriene tumor uitgaande van met name de centrale luchtwegen. Neuro-endocriene tumoren van de long kunnen worden opgedeeld in goed gedifferentieerde laaggradige tumoren zoals typisch (TC) en atypische (AC) carcinoïden en slecht gedifferentieerde hooggradige neuro-endocriene tumoren zoals grootcellige neuro-endocriene tumoren en kleincellige long carcinomen. Dit proefschrift richt zich op de laaggradige tumoren zoals TC en AC.

De incidentie van bronchiaal carcinoïd van de long is laag met gemiddeld 0.2 per 100.000 inwoners. Dit komt overeen met ongeveer 125 TC en 45 AC's per jaar in Nederland. Door de polipeuze groei en het indolent groeiende karakter zijn TC en AC geschikt voor een parenchymsparende behandeling. Wereldwijd wordt er voor een bronchiaal carcinoïd tumor nog een (parenchymsparende) longresectie gedaan. Echter heeft in de laatste decennia de endobronchiale therapie (EBT) een opmars gemaakt. EBT is een minimaal invasieve parenchymsparende behandeling waarbij middels liscoagulatie de tumor wordt verwijderd. Vervolgens wordt er cryotherapie op de basis van de verwijderde laesie toegepast om de kans op een lokaal recidief te verkleinen. De behandeling gebeurt middels een starre scopie onder algehele anesthesie en wordt door de interventie longarts uitgevoerd. Na de behandeling wordt de patiënt jaarlijks poliklinisch met een computed tomography (CT) scan en bronchoscopie vervolgd. Indien er sprake is van een niet succesvolle endobronchiale behandeling, bijvoorbeeld bij een incomplete endobronchiale resectie of recidief van de tumor, wordt de patiënt opnieuw behandeld met EBT of verwezen voor chirurgische resectie.

EBT is een invasieve behandelmethode. Dit kan gepaard gaan met complicaties (anesthesie, bloeding) en een eventuele operatie onnodig uitstellen. Om dit te voorkomen is het essentieel om de juiste patiënten te selecteren. Dit proefschrift richt zich met name op het optimaliseren van de selectie van patiënten voor EBT en is opgedeeld in 2 delen.

Deel 1 (hoofdstuk 2 t/m 5) beschrijft nieuwe histologische inzichten en prognostische biomarkers voor patiënten met bronchiaal carcinoïd.

In **hoofdstuk 2** gaat dit proefschrift in op radiologische selectiecriteria voor EBT voor patiënten met bronchiaal carcinoïd tumoren. Deze studie toont een analyse van 125 patiënten die in de afgelopen 30 jaar behandeld zijn voor bronchiaal carcinoïd. De resultaten laten zien dat 72% van alle intraluminale tumoren met een diameter <20 mm op de CT scan, geschikt zijn voor endobronchiale behandeling en dat

extraluminaal groeiende tumoren van ≥20 mm moeten worden verwezen voor chirurgische resectie.

De histologische differentiatie van bronchiaal carcinoïden wordt in de huidige WHO classificatie gebaseerd op het aantal mitosen en de aanwezigheid van necrose. TC wordt gedefinieerd als neuro-endocriene tumor met 0-1 mitosen pet 2 mm^2 zonder necrose en AC als 2-10 mitosen per 2 mm^2 en/of necrose. Deze TC/AC classificatie, welke onbetrouwbaar is op flexibele biopten, bepaalt maar deels het disseminatie karakter van een bronchiaal carcinoïd tumor. Met name TC hebben een laag metastatisch karakter (8%), echter 23% van de AC metastaseren. Deze agressievere tumoren wil je bij voorkeur chirurgisch en niet endobronchiaal behandelen.

In **hoofdstuk 3** laten we zien dat de diameter van het biopt van essentieel belang is voor de betrouwbaarheid van de histologische uitkomst. In biopten met een diameter van <4 mm^2 werd AC voor 59% ondergeclassificeerd in TC in vergelijking met 30% in biopten ≥4 mm^2. Longartsen moeten daarom bij voorkeur streven naar grotere biopten, bijvoorbeeld 4 biopten van 1 mm^2 of 2 biopten van 2 mm^2. Toevoeging van Ki-67 liet geen verbeterde differentiatie zien tussen TC en AC.

In **hoofdstuk 4** vergelijken we verschillende diagnostische (Chromogranine, Synaptofysine, CD56 en INSM-1) en prognostische (Ki-67 (proliferatie index), Rb, P16, OTP en CD44) biomarkers, tussen het histologisch biopt en de chirurgische resectie. Chromogranine toont zich als de meest stabiele marker in vergelijking met andere neuro-endocriene markers. Als prognostische marker toont CD44 zich het meest stabiel en ook het meest betrouwbaar in flexibele biopten vergeleken met OTP, Rb en P16.

In **hoofdstuk 5** worden morfologische en histologische karakteristieken bepaald om de prognostische classificatie van bronchiaal carcinoïd tumoren te verbeteren. Een mitose index van ≥2 per 2 mm^2 (AC), een Ki-67 van >5% en uitval van CD44 of OTP, zijn voorspellende biomarkers op het ontwikkelen van afstandsmetastasen. Patiënten met een AC, een Ki-67 van ≥5% en uitval van OTP en/of CD44 moeten een chirurgische resectie ondergaan. Anderzijds, patiënten met een TC, een Ki-67 <5% en een positieve OTP en/of CD44, hebben een zeer lage kans op gemetastaseerde ziekte en kunnen parenchymsparende technieken worden overwogen.

Deel 2 (hoofdstuk 6 t/m 8) beschrijft de behandelstrategieën voor bronchiaal carcinoïden.

In **hoofdstuk 6** wordt een systematische literatuur analyse vanaf 1990 beschreven. Hierin analyseren we de beschikbare literatuur ten aanzien van de endobronchiale en chirurgische behandeling van bronchiaal carcinoïd. In dit artikel komt naar voren dat tumor diameter, lymfeklier status en histologische uitkomst (AC) van de tumor belangrijke voorspellers zijn voor overleving. Bij centraal groeiende carcinoïd

tumoren zonder tekenen van lymfeklier of afstandsmetastasen kunnen behandeling middels minimaal invasieve technieken zoals EBT of parenchymsparende chirurgie worden overwogen.

Tegenstanders van EBT beargumenteren dat een longresectie na EBT gepaard kan gaan met meer complicaties (bijvoorbeeld bloeding of stenose). Hoofdstuk 7 analyseert deze bewering en vergelijkt chirurgisch behandelde patiënten met en zonder EBT op de uitgebreidheid van de longresectie. Theoretisch zou EBT de tumor, uitpuilend in de centrale hoofdbronchus, kunnen debulken waardoor er enkel een lobectomie hoeft te worden verricht in plaats van een sleeve lobectomie of pneumonectonie. Tijdens deze laatste 2 procedures wordt meer parenchym gereseceerd ten opzichte van een lobectomie. Onze studie heeft niet kunnen aantonen dat EBT een hogere kans geeft op een parenchymsparende operatie. Van de in totaal 62 patiënten waren er 7 patiënten in de chirurgie met EBT groep en 2 patiënten in de chirurgie zonder EBT groep die een kleinere longresectie hadden ondergaan. Echter belangrijk te vermelden is dat er ook geen toename van postoperatieve complicaties werd gezien na behandeling met EBT.

Als reactie op gepubliceerde artikelen omtrent EBT bij bronchiaal carcinoïd zijn nog enkele zogenoemde "letters to editor" toegevoegd. Deze zijn ondergebracht in hoofdstuk 8.

Hoofdpunten van dit proefschrift

1. Patiënten met een intraluminaal groeiend carcinoïd tumor en een diameter van <20 mm op CT scan zijn goede kandidaten voor endobronchiale therapie (EBT). Alle patiënten met een tumor diameter ≥20 mm moeten worden verwezen voor een chirurgische resectie (hoofdstuk 2).

2. Histologische classificatie in centraal groeiende carcinoïd tumoren is frequent discordant in kleine biopten. Een biopsie oppervlak van minstens 4 mm^2 tumor verhoogd de diagnostische betrouwbaarheid (hoofdstuk 3).

3. Synaptofysine is de meest betrouwbare diagnostische neuro-endocriene marker, met name ook in kleine biopten (hoofdstuk 4).

4. CD44 is de meest betrouwbare prognostische neuro-endocriene marker, met name ook in kleine biopten (hoofdstuk 4).

5. Toevoeging van CD44, OTP en Ki-67 aan de veelgebruikte TC/AC classificatie, zorgt voor een multimodale biomarker die de classificatie in prognostisch gunstige en ongunstige tumoren verbetert (hoofdstuk 5).

6. Centraal groeiende intraluminale bronchiaal carcinoïd tumoren, zonder aanwijzingen op metastasen, kunnen worden behandeld middels minimaal invasieve alternatieven zoals endobronchiale behandeling (EBT) of parenchymsparende chirurgische resecties (hoofdstuk 6).

7. Een niet curatieve behandeling met EBT vermindert niet de uitgebreidheid van de aanvullende chirurgische resectie. Indien curatieve EBT haalbaar lijkt, wordt deze behandeling geadviseerd, omdat een chirurgische resectie kan worden voorkomen en EBT niet zorgt voor aanvullende chirurgische morbiditeit (hoofdstuk 7).

Discussie

In dit proefschrift wordt de behandeling van centraal gelokaliseerde bronchiaal carcinoïd tumoren beschreven. In de huidige richtlijnen wordt als curatieve behandeling de voorkeur gegeven aan een chirurgische resectie [1,2]. Echter wint EBT aan populariteit en is het een in, toenemende mate, effectieve, minimaal invasief en parenchym sparend alternatief. Helaas wordt EBT door verschillende redenen nog niet wereldwijd toegepast. Allereerst betwijfelen specialisten de veiligheid en curativiteit van EBT. Carcinoïd tumoren zijn sterk gevascularisseerde tumoren en een bloeding in de luchtweg na lokale behandeling kan een potentiele bedreiging zijn voor de ademweg [3,4]. Er zijn studies die, alvorens over te gaan op EBT, een embolisatie van de bronchiaal arterie verrichten [5,6]. Echter, zoals beschreven in hoofdstuk 6, kan een bloeding door EBT effectief worden verholpen door endobronchiale interventies zoals adrenaline injecties, xylomethozoline spray of ballon blokkers. Daarnaast is er maar 1 studie die een massale bloeding heeft beschreven waarbij een spoedoperatie noodzakelijk was [7]. Ten tweede, door het verwerpen van de oncologische resectiestrategie, waarbij een radicale resectie met lymfadenectomie wordt verricht, wordt door artsen de curativiteit van EBT in twijfel getrokken. Echter met EBT wordt een ablatieve therapie geïnitieerd door een combinatie van diathermische destructie van de tumorcellen en het gebruik van cryotherapie. Een intraluminale tumor kan direct gereseceerd worden met EBT waarbij vervolgens het resterende weefsel in de bronchuswand nogmaals behandeld wordt met electrocoagulatie en cryotherapie [8,9]. EBT kan worden vergeleken met stereotactische lichaamsradiatie of radiofrequente ablatie (RFA). Dit zijn gevestigde ablatieve oncologische behandelingen zonder tumorvrije resectieranden en lymfeklier dissecties.

In plaats van kenmerken te vergelijken die inherent zijn aan de verschillende behandelingen, bijvoorbeeld EBT en chirurgische resectie, is het veel interessanter om te kijken naar de uitkomsten van de diverse behandelingen. De systematische review in hoofdstuk 6 benadrukt dat er geen gerandomiseerde onderzoeken zijn waarbij EBT met chirurgie in patiënten met bronchiaal carcinoïd worden vergeleken. De beschikbare geanalyseerde studies lieten wel zien dat loco-regionale (0-5%) en afstand (0-4%) metastase in de EBT en parenchymsparende chirurgie groep minder werden gezien in vergelijking met de anatomische longresecties (0–8% and 0–23% respectievelijk). Dit verschil kan, in ieder geval deels, verklaard worden door selectie, waarbij kleine intraluminaal groeiende tumoren waarschijnlijk meer geselecteerd worden voor parenchymsparende technieken en patiënten met grotere tumoren en/of lymfeklier betrokkenheid voor anatomische resecties. Deze selectie kan natuurlijk er voor zorgen dat uitkomstmaten positief kunnen uitvallen na EBT of een chirurgische resectie. Echter is het cruciaal om de meest geschikte behandeling individueel toe te wijzen. De centrale vraag van het proefschrift is daarom hoe we de

optimale behandeling voor patiënten met bronchiaal carcinoïd kunnen bepalen. Om dit proces tot stand te brengen, moeten verschillende vragen worden bediscussieerd.

Kan een computed tomography (CT scan) bijdragen in het bepalen van de behandelstrategie voor een patiënt met bronchiaal carcinoïd?

Patiënten met een (verdenking op) bronchiaal carcinoïd krijgen een CT scan bij diagnosestelling. Hierbij zal informatie over de grootte van de tumor, de locatie en zijn relatie met omgevende structuren worden geïdentificeerd. In hoofdstuk 2 laten we zien hoe radiologische kenmerken een succesvolle endobronchiale behandeling van (met name typische) carcinoïd tumoren kunnen voorspellen. Twee ervaren radiologen analyseerden de tumor diameter, intra- of extraluminale ziekte en lymfeklier status. Hierin vonden we een EBT slagingspercentage van 72% in patiënten met een intraluminaal groeiende tumor van <20 mm en geen lymfeklier betrokkenheid.

Op basis van deze gegevens speelt de CT-scan daarom een belangrijke rol in de selectie voor de behandelingsmodaliteit. Patiënten met een bronchiale carcinoïd tumor ≥20 mm moeten worden verwezen voor een operatie. Anderzijds hebben puur intraluminale carcinoïden met een diameter <20 mm een hoog slagingspercentage met EBT. Ondanks dat onze studie aantoonde dat EBT in AC haalbaar lijkt, blijft het bewijs om AC met alleen EBT te behandelen beperkt [10,11].

Zijn er biomarkers die bijdragen in het bepalen van de behandelstrategie voor een patient met bronchiaal carcinoïd?

In het huidige tijdperk streven we ernaar om een voorspelling te doen over de optimale behandeling per individu. Hierbij houden we rekening met het individuele risico. In de precisie geneeskunde wint het tot stand brengen van heterogeniteit van een behandeleffect tussen subgroepen aan populariteit. Hierdoor kunnen de juiste patiënten aan de juiste behandelingen worden toegewezen. Idealiter moeten gerandomiseerde onderzoeken (RCT) met biomarkers voorspellende eigenschappen aantonen om voor deze benadering in aanmerking te komen. Echter wordt het vaststellen van dergelijke voorspellende biomarkers bemoeilijkt door verschillende factoren. Ten eerste is bronchiaal carcinoïd een zeldzame ziekte waarbij een internationale multicenterstudie vereist is voor het verkrijgen van adequate patiënten aantallen. Ten tweede heeft bronchiaal carcinoïd een laaggradig en niet metastaserend karakter. Om negatieve "events" te monitoren zullen een lange follow up duur en grote patiënten aantallen nodig zijn. Deze problemen zouden een RCT tijdrovend maken. Een andere strategie is het gebruik van biomarkers die recidief ziekte of metastasering zouden kunnen voorspellen. De meest gebruikte huidige biomarker is de mitose index en maakt alleen onderscheid tussen TC en AC op basis van het aantal mitosen en aanwezigheid van necrose.

Naast het feit dat deze mitose telling zeer gevoelig is voor interobservervariabiliteit [12], is een representatief weefselmonster met voldoende weefselvolume een voorwaarde voor een nauwkeurige classificatie van bronchiale carcinoïden. Een betrouwbare differentiatie in TC en AC is belangrijk, omdat metastasering voornamelijk plaatsvindt in AC. In hoofdstuk 3 vergeleken we tumor classificatie tussen bronchoscopische en chirurgische biopten van centraal groeiende carcinoïd tumoren. Hierbij toonden wij aan dat de histologische classificatie in 45% van de biopten niet overeenkomt met de resectie. Deze discordantie werd veroorzaakt door misclassificatie van AC in TC. In biopten van <4 mm^2, werd 100% gemisclassificeerd vergeleken met 61% in biopten ≥4 mm^2. Het onderscheiden van carcinoïd tumor in typisch of atypisch carcinoïd op biopten <4 mm^2 moet daarom worden ontmoedigd en de diagnose 'carcinoïd NOS (not other specified)' worden behouden voor carcinoïden met ≤1 mitose per 2 mm^2. Gebaseerd op deze resultaten dienen artsen te streven naar biopten met een cumulatief oppervlak van minimaal 4 mm^2. Bij voorkeur gebeurt dit in een gecontroleerde setting met algehele anesthesie en de mogelijkheid tot adequate hemostase controle.

Ki-67 is een veel gebruikte pathologisch diagnostische marker voor gastro-intestinale neuro-endocriene tumoren, maar wordt niet gebruikt voor het onderscheid tussen TC en AC in bronchiale carcinoïden. In de huidige WHO-classificatie van 2021 wordt echter door beperkte literatuur gesuggereerd dat een Ki-67 ≥5% past bij een AC [13]. Onze studie toonde geen toegevoegde waarde van Ki-67 in discriminatie van TC en AC in de biopten en resectie. Echter, samen met andere prognostische markers levert het wel een bijdrage aan de classificatie in gunstige of ongunstige carcinoïd tumoren.

In tegenstelling tot de mitosetelling beschrijft hoodstuk 4 dat Ki-67, maar ook Synaptofysine, OTP en CD44 zeer betrouwbare biomarkers zijn in kleine biopten. Hoewel deze biomarkers de carcinoïden dus niet kunnen classificeren in TC of AC, initiëren zij wel een onderverdeling in gunstige en ongunstige tumoren en helpen zij dus bij het voorspellen van de uitkomst van de ziekte. Onze studie in hoofdstuk 5 beschreef daarnaast dat, naast verlies van OTP of CD44, immunohistochemie voor Ki-67 (≥5%) en mitose telling (≥2 per 2 mm^2) onafhankelijk geassocieerd waren met een hoger risico op afstandmetastasen. Het toevoegen van OTP, CD44 en Ki-67 aan de classificatie van bronchiale carcinoïden faciliteert de selectie tussen individuen met een gunstige en ongunstige prognose en beïnvloedt vervolgens de behandelstrategie tussen EBT en chirurgie.

Samenvattend zal de selectie voor EBT worden verbeterd door, naast het radiologische tumorstadium (cT), de resultaten van de pathologische prognostische immunohistochemische markers van de histologische biopten toe te voegen. Kleine intraluminale carcinoïd tumoren op de CT-scan gecombineerd met gunstige prognostische markers (mitose telling van <2 per 2 mm^2, Ki-67 <5% en positieve OTP

en/of CD44) kunnen veilig worden behandeld met EBT. Daarentegen moeten tumoren van >2 cm met of zonder extraluminale groei en/of ongunstige biomarkers (mitose telling >2 per 2 mm^2, Ki-67 >5% en negatieve OTP en/of CD44) worden doorverwezen voor chirurgie.

Beïnvloedt EBT een aanvullende chirurgische resectie?

In het afgelopen decennia is het gebruik van minimaal invasieve chirurgische technieken zoals video-ondersteunde thoracoscopische chirurgie (VATS) en parenchymparing-technieken zoals een bronchoplastiek en een sleeve-resectie toegenomen [14-17]. Bronchiale carcinoïden zijn vaak afkomstig uit de centrale luchtwegen. Door deze centrale ligging is de toepassing van parenchymsparende technieken cruciaal om grote anatomische resecties te voorkomen. Het gebruik van EBT voorafgaand aan de operatie heeft theoretische voordelen. Ten eerste, een carcinoïd tumor kan een polipeuze groei hebben richting een hoofdbronchus. Debulking tijdens EBT kan ervoor zorgen dat de tumor gereduceerd wordt richting de bronchus van origine. Dit kan een lobectomie of sleeve-lobectomie vergemakkelijken en een bilobectomie of pneumonectomie eventueel voorkomen. Ten tweede kunnen debulking en rekanalisatie helpen bij het verlichten van een postobstructie pneumonie. Daardoor kan de pre-operatieve gezondheid van een patiënt worden verbeterd. Echter, EBT kan ook negatieve effecten hebben op een aanvullende chirurgische resectie. Littekenweefsel na EBT zouden complicaties van een chirurgische resectie kunnen vergroten. In hoofdstuk 7 analyseerden we 65 geopereerden patiënten waarvan 24 zonder voorbehandeling en 41 met voorbehandeling van EBT. We onderzochten of EBT het volume van gereseceerd longparenchym verminderde en invloed uitoefende op het type resectie dat werd uitgevoerd (meer parenchymsparend). Het longsparende effect van EBT was niet significant, echter was de onderzoeksgroep van 65 patiënten beperkt. De incidentie van bronchiale carcinoïd tumoren is laag en EBT is momenteel beperkt tot een klein aantal medische centra met voldoende expertise in endobronchiale long interventies. Een grotere studie, binnen een internationaal samenwerkingsverband, zal een krachtigere analyse van de impact van EBT mogelijk maken.

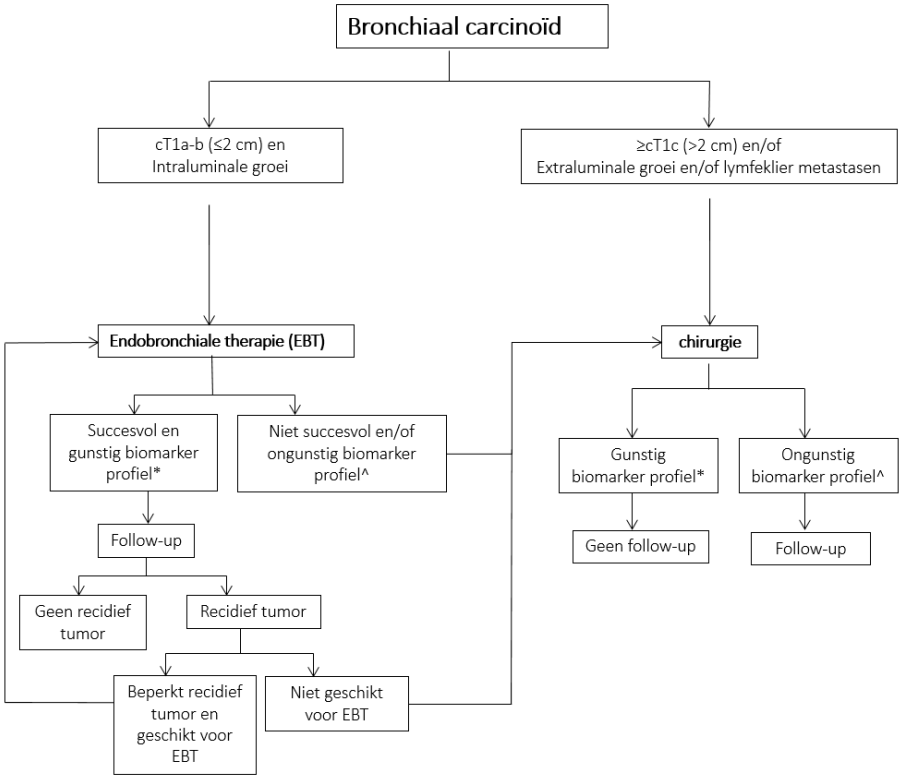
Tegenstanders van EBT bediscussiëren deze behandeling omdat het complicaties zoals bloedingen, wandperforatie, stricturen of stenose [3,18] kan veroorzaken. Onze studie in hoofdstuk 7 toonde echter aan dat EBT geen toename van complicaties bij chirurgisch verwijderde carcinoïd tumoren laat zien vergeleken met patiënten zonder EBT voorafgaand aan de operatie.

Multidisciplinaire besprekingen, behandelsuggesties en follow up in patiënten met bronchiaal carcinoïd

De incidentie van bronchiale carcinoïden in Nederland ligt rond de 200 per jaar. Aangezien er in Nederland 116 ziekenhuislocaties zijn, is het geschatte aantal patiënten per ziekenhuis slechts 1,5 per jaar. Om de diagnosestelling te optimaliseren, het risico op complicaties te minimaliseren en de potentie van EBT te maximaliseren, pleiten we er sterk voor dat EBT wordt gecentraliseerd in verwijzingscentra met ervaren radiologen voor een nauwkeurige en systematische evaluatie van CT-scans, een patholoog gespecialiseerd in histologische analyse van carcinoïden en interventie longartsen en longchirurgen, met EBT vaardigheden en de behandeling van complicaties zoals luchtwegbloedingen.

Er is maar weinig bekend over de voordelen van postoperatieve surveillance bij bronchiale carcinoïden. Momenteel adviseren richtlijnen na radicale chirurgie een follow-up van ten minste 5-10 jaar [19]. In één grote serie konden de meeste recidieven gedurende de follow-up niet met beeldvorming worden gedetecteerd. Daarnaast was het percentage recidieven (<3%) bij patiënten met lymfklier negatieve laaggradige TC erg laag [20]. Deze gegevens suggereren dat het zeer onwaarschijnlijk is dat patiënten met lymfeklier negatieve laaggradige long-NET's baat hebben bij enige vorm van postoperatief follow-up. Voor patiënten met lymfeklier-positieve TC of AC, onafhankelijk van de N-status, is het risico op recidief groter en wordt follow-up gedurende ten minste 10 jaar na behandeling geadviseerd [21]. Dit advies geldt voor chirurgisch behandelde patiënten. De follow-up voor patiënten die met EBT worden behandeld is nog onduidelijk. Op basis van onze resultaten, mede omdat bij een klein deel van de patiënten na EBT een lokaal recidief kan optreden, wordt een jaarlijkse follow-up geadviseerd. Jaarlijkse follow-up en CT-scans zouden kunnen worden verworpen na radicale chirurgische resectie van een tumor met een gunstig biomarker profiel (laag mitose aantal, lage Ki-67-expressie en normale OTP en CD44 expressie).

Op basis van de resultaten van dit proefschrift toont Figuur 1 een voorgesteld behandelschema voor patiënten met een bronchiaal carcinoïd tumor.



*Figuur 1: Voorgestelde flowchart voor de behandeling van patiënten met bronchiaal carcinoïd gebaseerd op de resultaten van dit proefschrift. *Gunstig biomarker profiel: Ki-67 <5%, mitose telling <2 per 2 mm2, OTP of CD44 positiviteit; ^Ongunstig biomarker profiel: Ki-67 ≥5%, mitose telling ≥2 per 2 mm2, en uitval van OTP of CD44 expressie.*

Toekomst perspectief

In lijn met de zoektocht naar minder invasieve technieken voor nauwkeurige diagnosestelling, worden minimaal invasieve chirurgie en parenchymsparende technieken zoals EBT steeds meer aangepast voor de behandeling van patiënten met bronchiale carcinoïden. Hoewel potentiële morbiditeit, zoals het risico op ernstige bloedingen die een spoedchirurgische ingreep noodzakelijk maken, zeer zeldzaam is zou dit de implementatie van EBT niet moeten verhinderen. Interventie longartsen moeten ook in de toekomst kunnen worden opgeleid om dergelijke bloedingen te behandelen.

Momenteel zijn invasieve bronchoscopie of naaldbiopten en een histologische/cytologische diagnose vereist om het type tumor te bepalen. Idealiter wordt een nauwkeurige diagnose gesteld met minder invasieve technieken. Helaas zijn conventionele beeldvormende modaliteiten, zoals CT-scan en PET-scan, niet bruikbaar om specifiek het neoplastisch type te differentiëren. De technieken voor nucleaire geneeskunde zijn de afgelopen decennia echter verbeterd om de artsen te ondersteunen bij de uitdagende behandeling van bronchiale carcinoïden. Voornamelijk positron emissie tomografie (PET) tracers zijn voorgesteld voor de beoordeling van de omvang van de ziekte, herstadiëring en therapierespons. F-18-FDG (glucose-analoog) wordt veel gebruikt bij het evalueren van agressieve tumoren zoals hoogwaardige bronchiale neuro-endocriene tumoren als LCNEC en SCLC. Het lijkt echter van beperkte waarde voor de evaluatie van indolent groeiende TC en AC met een laag glucosemetabolisme [22,23]. In de afgelopen jaren hebben nieuwe tracers, in het bijzonder somatostatine-analogen gelabeld met gallium-68 (Ga-68-DOTA-peptiden), de diagnostisering en follow up verbeterd. Deze Ga-68-Dotataat scan heeft een sensitiviteit en specificiteit van respectievelijk 96 en 100% [24]. De opname van deze tracer is niet afhankelijk van het cel metabolisme zoals bij de F-18-FDG scan. Aangezien preoperatieve biopten onbetrouwbaar kunnen zijn bij het classificeren van TC en AC, zou een aanvullend 68Ga-dotataat PET/CT-scan met variabele traceropname de differentiatie kunnen verbeteren. Typische carcinoïden vertonen significant hogere SUVmax dan atypische tumoren. Ondanks de lage incidentie, kan een 68Ga-dotataat PET/CT ook aanwezigheid van afstandsmetastasen identificeren [25]. Toekomstige studies zouden zich moeten focussen op de accuraatheid van deze nieuwe nucleaire techniek. Niet-invasief onderzoek zou informatie kunnen verschaffen over SSTR-expressie en eventuele therapeutische implicaties kunnen bieden. Gemetastaseerd carcinoïd met hoge SSTR-expressie zou bijvoorbeeld geschikt kunnen zijn voor peptide-radioreceptortherapie [26].

Er is toenemend wetenschappelijk bewijs dat bloedafname nuttige oncologische informatie kan opleveren. Een circulerende neoplastische moleculaire marker zou invasieve biopten kunnen gaan beperken, therapeutische doelen kunnen definiëren

en een 'realtime' monitoringstool kunnen bieden om de ziektestatus te evalueren. Dergelijke strategieën, of 'vloeibare biopten', hebben al waarde bij long tumoren. Bijvoorbeeld voor het volgen van de behandelingsrespons op epidermale groeifactorreceptor (EGFR)-remmers door identificatie van de mutatie T790M in circulerend tumor-DNA [27]. Recente studies vergeleken een bloedtest om neuro-endocriene transcriptoom activatie te detecteren (NETest-transcripts). Zij vergeleken de nauwkeurigheid ervan met die van chromogranine A. De NETest onderscheidde in 93% nauwkeurigheid de bronchiale carcinoïden van de controle groep vergeleken met 19% bij het gebruik van Chromogranine [28,29].

Next-generation sequencing (NGS)-technologieën en proteomix hebben snelle genoombrede onderzoeken mogelijk gemaakt omtrent oncogenetische en tumorsuppressor signaalmoleculen bij verschillende vormen van kanker. Dit versnelde het begrip in de kanker biologie en de ontwikkeling van nieuwe diagnostiek en therapieën. Grootschalige sequencing-onderzoeken hebben aangetoond dat bronchiale carcinoïden een grote verscheidenheid aan genetische afwijkingen hebben. Echter is het nog steeds onduidelijk welke genetische veranderingen of routes de belangrijkste rol spelen bij de ontwikkeling en progressie van de ziekte [30-33]. RNA-sequencing zal steeds belangrijker worden. Bijvoorbeeld om risicofactoren voor lymfeklier-en afstandsmetastasen te identificeren.

De lage incidentie van bronchiaal carcinoïd vormt ook een grote uitdaging voor het uitvoeren van gerandomiseerde studies. Het gebrek aan betrouwbare studies belemmert de implementatie van EBT. Aangezien EBT momenteel beperkt is tot een klein aantal centra met voldoende ervaring, zou centralisatie de inclusie van patiënten voor een adequaat aangedreven RCT veel gemakkelijker en efficiënter maken. Internationale multicenter-samenwerking is noodzakelijk voor implementatie van evidence-based richtlijnen en het beantwoorden van vragen op belangrijke eindpunten zoals algehele overleving, ziektevrije overleving, kwaliteit van leven en kosteneffectiviteit.

Conclusie

Radiologische en pathologische tumorprofielen verbeteren de stratificatie van patiënten met bronchiaal carcinoïd tumoren die geschikt zijn voor endobronchiale resectie. Patiënten met een intraluminaal groeiende tumor van <2cm kunnen worden geselecteerd voor EBT. Aanvullende biopten zullen bronchiale carcinoïd tumoren met gunstige en ongunstige tumorkenmerken classificeren op basis van OTP, CD44 en Ki-67. Dit verbetert risicostratificatie waardoor selectievere behandel-en follow-up strategieën per patiënt kunnen worden gemaakt. Om de patiënten selectie te optimaliseren, gebeurt dit bij voorkeur in gespecialiseerde centra met een ervaren multidisciplinair team.

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Chapter 11

Acknowledgements / Dankwoord
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List of publications

Dankwoord

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About the author

Ellen Marie Brigitte Paulien Reuling was born on August 11, 1982. She grew up in Wehl (Gelderland) and attended secondary school at "het Ludgercollege" in Doetinchem. At an age of 17 she moved out of the "Dutch outback" and started studying physical therapy in Utrecht and graduated Cum Laude in 2002.

Her medical journey continued as she started her medicine study at het University of Amsterdam which she finished in 2009. After a 2 year residency in surgery and gynecology she completed her registration as Tropical doctor and moved to South Africa. She worked as a medical doctor on the trauma department in Tygerberg hospital in Cape Town and in Mseleni Mission hospital in the rural region of the department Kwazulunatal near the Mozambican border.

In 2012 she got accepted into the training for surgery in the Netherlands. Her residency took place in the Albert Schweitzer Hospital in Dordrecht (under dr. P.W. Plaisier) and in the Academical Medical Center in Amsterdam (under Prof. Dr. E. Nieveen-van Dijkum). In 2014, during her surgical training, she got interested in thoracic surgery and started a PhD at the department of thoracic surgery in the Vrije Universiteit (VU) Medical Center supervised by dr. Hans Daniels and dr. Chris Dickhoff. She completed this research along with her clinical activities and presented results of her research at national and international congresses. This eventually led to the completion of this thesis.

In 2019 she completed her differentiation in oncological surgery. As pediatric surgery always kept her interest, she was accepted for a fellowship in pediatric surgery in the Wilhelmina Children's Hospital in Utrecht (under Dr. S. Tytgat) and became staff member in 2022. She is currently working as a pediatric surgeon specialized in thoracoscopic repair in children born with an esophageal atresia.

She is board member of the Netherlands Society for International Surgery (NSIS), which advocate for safe surgical care in low-and middle income countries and supports the training program in the Netherlands to become Medical Doctor in Global Health and Tropical Medicine (Tropical doctor).

Ellen Reuling likes all kind of sports, especially running, tennis and volleyball. She loves cheerleading her sporty children and spending time with her family and friends. She's living in Amsterdam with her boyfriend and has a son and a daughter.

List of publications

This thesis

1. **Reuling EMBP**, Dickhoff C, Daniels JMA. Treatment of Bronchial Carcinoid Tumors: Is Surgery Really Necessary? *J Thorac Oncol. 2017;12(5):e57-e8.*

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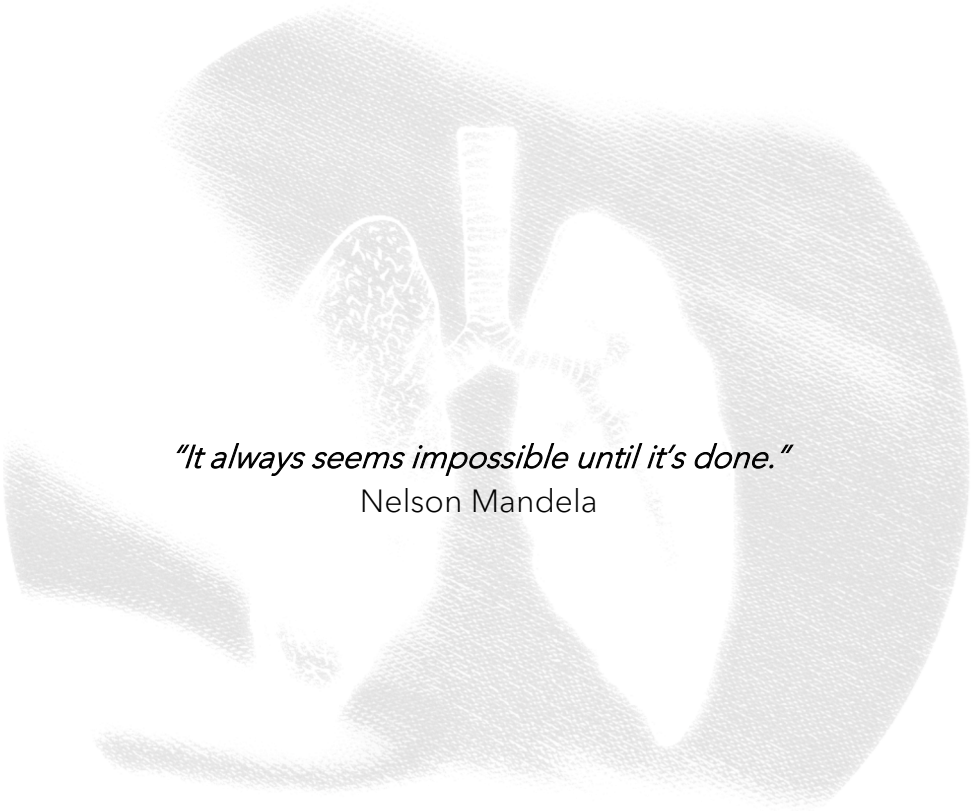

"It always seems impossible until it's done."
Nelson Mandela