Lung Cancer 132 (2019) 141–149

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Characteristics of 252 patients with bronchopulmonary neuroendocrine tumours treated at the Copenhagen NET Centre of Excellence

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ARTICLE INFO

Keywords:
Neuroendocrine tumour
Neuroendocrine neoplasm
Carcinoid tumour
Typical carcinoid
Atypical carcinoid
Bronchial neuroendocrine tumour
Bronchopulmonale tumour
Large cell neuroendocrine tumour
Ki67 index
Mitotic count
FDG-PET

ABSTRACT

Background: Bronchopulmonary neuroendocrine tumours are divided into typical carcinoid (TC), atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC), and small cell lung cancer (SCLC).
Aim: To thoroughly describe a cohort of 252 patients with TC, AC and LCNEC (SCLC excluded).
Material and methods: Collection of data from 252 patients referred to and treated at Rigshospitalet 2008-2016. Data was collected from electronic patient files and our prospective NET database. Statistics were performed in SPSS.
Results: 162 (64%) had TC, 29 (12%) had AC and 61 (24%) had LCNEC. Median age at diagnosis was 69 years (range: 19–89) with no difference between genders. Thoraco-abdominal CT was performed in all patients at diagnosis. FDG-PET/CT was performed in 207 (82%) at diagnosis and was positive in 95% of the entire cohort, with no difference between tumour types. Synaptophysin was positive in 98%, chromogranin A in 92% and CD56 in 97%. Mean Ki67 index was 5% in TC, 16% in AC and 69% in LCNEC ($p < 0.001$). Metastatic disease was found in 4% of TC, 27% of AC and 58% of LCNEC at time of initial diagnosis ($p < 0.001$). In total 179 patients (71%) underwent surgical resection; TC: 87%, AC: 72% and LCNEC: 28% ($p < 0.001$). Of the resected patients, 11 (6%) had recurrence. Five-year survival rate was 88% for TC, 63% for AC and 20% for LCNEC.
Conclusion: In this comprehensive study of a cohort of 252 patients, one of the largest until date, with TC, AC and LCNEC, the gender distribution showed female predominance with 68%. FDG-PET/CT was positive in 95% of the patients independent of tumour type, which confirms that FDG-PET/CT should be a part of the pre-operative work-up for TC, AC and LCNEC. Tumour type was the single most potent independent prognostic factor.

1. Introduction

Bronchopulmonary neuroendocrine tumours (BP-NETs) represent a group of rare neoplasms with increasing incidence. [1–4] In the 2015 WHO classification the group included low-grade typical carcinoid (TC), intermediate-grade atypical carcinoid (AC), high-grade large cell neuroendocrine carcinoma (LCNEC) and small cell lung carcinoma (SCLC) [5]. TC includes tumours with a mitotic count of $< 2/2mm^2$ and no necrosis, AC a mitotic count of 2–10/2 mm^2 and/or tumour necrosis and LCNEC and SCLC a mitotic count $> 10/2mm^2$ and abundant necrosis. The SCLC comprises 15% of all lung malignancies, but will not be further addressed in this paper. LCNEC represents 3% whereas bronchopulmonary carcinoids (TC and AC) represent 1%–2% of all new lung cancers. The diagnostic work-up of these tumours has evolved over the past decades with analysis of histological differentiation and new improved methods such as immune staining for neuroendocrine features as diagnostic corner stones [6]. The gold standard of treatment for localized TC and AC is surgical resection, whereas the treatment regimen in advanced disease still remains a field of controversy. Management and treatment of LCNEC is also associated with controversy,

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https://doi.org/10.1016/j.lungcan.2019.03.013
Received 13 November 2018; Received in revised form 20 February 2019; Accepted 14 March 2019

which is probably partly due to the heterogeneity of the disease, but the majority of studies have shown that the treatment of LCNEC with chemotherapy mimicking SCLC treatment regime is associated with better survival than treatments used in non-small cell lung cancer [7–9]. The highest mortality is found in high-grade tumours, however advanced disease in low-grade tumours similarly is associated with poor prognosis. Few studies have compared lung carcinoids and LCNEC despite their common histological neuroendocrine features. This study aims to illustrate the similarities and differences amongst TC, AC and LCNEC through a comprehensive evaluation of diagnostic procedures, prognostic factors and overall survival in 252 patients from a single institution.

2. Materials & methods

2.1. Patient cohort and preoperative work-up

The study included all patients with TC, AC and LCNEC referred to and treated at the departments of thoracic surgery, oncology, endocrinology and gastrointestinal surgery at the Neuroendocrine Tumour (NET) Centre of Excellence at Rigshospitalet, University of Copenhagen through collection of data from November 2008 to December 2016. All patients with neuroendocrine tumours as well as all patients in need of thoracic surgery from Eastern Denmark (population 2.4 mill.) are referred to and treated at Rigshospitalet. Information on 252 patients was obtained from patient files and the prospective electronic NET database at Rigshospitalet (approved by The Danish Data Protection Agency, ref.no. 2007-58-0015) and the Danish National Pathology Database.

All patients were followed until death or until the end of the follow-up registration period (December 31, 2016).

An extensive preoperative work-up was performed including lung function tests, fiber-optic bronchoscopy, endobronchial ultrasound (EBUS) with fine needle aspiration (FNA) and thoraco-abdominal CT-scanning with determination of tumour stage. The tumours were classified as “central” if visualized through bronchoscopy and “peripheral” if not visible by endoscopy. A 2-deoxy-2-^{18F}fluoro-D-glucose (FDG) positron emission tomography/computer tomography (PET/CT) was performed in 82% of patients preoperatively or initially before medical treatment in non-operated patients. ^{68}Ga-DOTATOC-PET/CT was performed preoperatively in 12% of patients, but was performed in all TC and AC postoperatively or before medical treatment in non-operated patients. All PET/CT readings were performed as part of the clinical routine following the European Association of Nuclear Medicine (EANM) guidelines [10]. PET scans were read by a board-certified Nuclear Medicine physician in consensus with a board-certified radiologist reading the CT part of the scan.

All patients were discussed at a multidisciplinary tumour board (MTB) at the time of diagnosis for evaluation of physical operability (severe co-morbidities, lung function and age) and surgical resectability. If not considered operable or resectable, choice of medical treatment was decided. At a postoperative MTB conference, resected patients were evaluated for further observation or medical treatment. In radically resected patients adjuvant medical therapy was only considered for LCNECs. Standard treatment at this institution is a three port VATS lobectomy as previously described [11]. Patients with tumours larger than 7–8 cm and/or centrally located tumours, visible by bronchoscopy, might require a sleeve resection or pneumonectomy with a muscle sparing anterior or postero-lateral thoracotomy. In patients with a compromised lung function a VATS segmentectomy or a VATS wedge resection was performed. All patients underwent a systematic lymph node dissection.

2.2. Pathology and immunohistochemistry

Pathological work up and classification were performed in all patients by expert pulmonary pathologists at the Department of Pathology, Rigshospitalet. All patients with a diagnosis prior to the new WHO classification in 2015 were re-classified accordingly [12]. Tumour staging was evaluated according to the seventh edition of the TNM classification for lung cancer [13]. Immunohistochemistry for chromogranin A (CgA), synaptophysin and CD56 was performed at diagnosis as a minimum of immunohistochemical stains in this NET-centre.

Polyclonal Rabbit Anti-Human CgA was used for the demonstration of chromogranin in the tumour tissue. Synaptophysin was demonstrated using monoclonal mouse anti human synaptophysin (Dako DM828). Monoclonal Mouse Anti-human Serotonin 5HT-H209 was used for demonstration of serotonin in tumour tissue. In demonstration of CD56 expression, the Ready-to-use Monoclonal Mouse Anti-Human CD56 Clone 123C3 was used. Anti-Thyroid Transcription Factor-1 (SP141) Rabbit Monoclonal Primary Antibody was used for the demonstration of TTF1 expression in tumour tissue.

The Ki-67 index was determined immunohistochemically by applying a monoclonal mouse anti-human Ki-67 antigen (Ki67 clone 30-9). Twenty hot spot areas were estimated, and the mean percentage of Ki-67 labelled nuclei calculated. In this study the Ki67 index was examined using the WHO guidelines from 2015, that stratifies the Ki67 index into 0–5 % for TCs, > 5-20% for ACs and above 20% for LCNECs. The presence of necrosis was evaluated during pathological work up and classified as either “none”, “focal” or “widespread”.

2.3. Charlson comorbidity score

The Charlson comorbidity score (CCS) was calculated for every patient at diagnosis [14]. (Table 1.)

2.4. Follow-up protocol

Patients were followed according to ENETs guidelines [2] with CT 3 and 6 months after surgery and then every 6–12 months thereafter depending on tumor type. Plasma CgA was measured at the same frequency as the CT scans. Somatostatin receptor imaging with ^{68}Ga-DOTATOC or ^{64}Cu-DOTATATE [15] was performed postoperatively and then every 6–12 months depending on tumor type.

2.5. Statistical analysis

All statistical tests were performed in IBM SPSS statistics version 24. Unpaired *t*-test or one-way ANOVA were used for continuous parameters to detect differences between groups, whereas the Chi square test was used for discrete parameters. Overall survival analyses were estimated according to Kaplan Meier, and the log-rank test was used for comparisons. Survival analyses, using the Kaplan-Meier and Cox proportional hazards methods, were performed analysing for the following variables: age, gender, prior cancers, pulmonary symptoms, weight loss, imaging modalities, bronchoscopy, Ki67, mitotic count, necrosis, immunohistochemistry, metastasis at diagnosis, treatment modalities incl. surgery and medical treatment. The prognostic significance of the selected variables was calculated for all three histologic subtypes. ($p < 0.05$) was considered significant.

2.6. Data sources

Data was extracted from patient files, the Danish National Pathology database and the prospective NET database, Rigshospitalet.

3. Results

Demographic and clinical features are listed in Table 1. From the prospective NET database we consecutively included 252 patients: 162 patients with TC, 29 patients with AC and 61 patients with LCNEC. The median follow-up time was 48 months (42–54 months) calculated

Table 1
Distribution of baseline characteristics, tumour staging, imaging and initial treatment regime for 252 patients with typical carcinoid (TC), atypical carcinoid (AC) and large cell neuroendocrine carcinoma (LCNEC). For age and Charlson score (CCS), median values and range are shown. Differences in age and CCS between the groups were tested non-parametrically. For categorical variables, the number of cases is shown in parentheses. Differences in the distribution of categorical variables were tested by chi square statistics. * For each decade > 40 a score of 1 was added to the total CCS.

N (n = 252)	TC 162	AC 29	LCNEC 61	P-value
Median age at diagnosis (range), years	68 (19-89)	72 (25-85)	69 (40-87)	*0.046*
Male	69 (25-85)	73 (25-83)	69 (40-87)	
Female	66 (19-89)	72 (44-85)	69 (45-85)	
Sex male/female %	27 / 73	28 / 72	46 / 54	*0.024*
Charlsson score	0.7 (0-3)	0.8 (0-3)	0.8 (0-3)	*0.594*
Charlsson score ajusted for age*	3.2	3.7	3.7	*0.089*
T-classification, n (%)				
T1	107 (66)	10 (34)	15 (24)	*< 0.001*
T2	30 (19)	5 (17)	14 (23)	
T3	8 (5)	6 (21)	6 (10)	
T4	4 (2)	4 (14)	14 (23)	
Tx	13 (8)	4 (14)	12 (20)	
N-classification, n (%)				
N0	126 (85)	13 (52)	12 (23)	*< 0.001*
N1	11 (7)	6 (24)	11 (22)	
N2	11 (7)	4 (16)	22 (43)	
N3	1 (1)	2 (8)	6 (12)	
M-classification, n (%)				
M0	143 (96)	19 (73)	23 (42)	*< 0.001*
M1	6 (4)	7 (27)	32 (58)	
Coughing symptoms	41 % (67/162)	59 % (17/29)	31 % (19/61)	*0.045*
No pulmonary symptoms	38 % (61/162)	34 % (10/29)	36 % (22/61)	*0.938*
Prior cancer diagnosis	28 % (45/162)	38 % (11/29)	30 % (19/61)	*0.546*
Central tumor location	58 % (69/119)	48 % (11/23)	36 % (9/25)	*0.121*
Surgery	87 % (141/162)	72 % (21/29)	28 % (17/61)	*< 0.001*
Smoking				
Never	44%	38%	12%	*< 0.001*
Prior	50%	48%	62%	
Current	6%	14%	26%	
Mitotic count (%)				
< 2%	100%	14%	0%	*< 0.001*
2-10%	0%	86%	4%	*< 0.001*
> 10%	0%	0%	96%	*< 0.001*
Ki67 proliferation index (mean)	4.7 %	16%	69.5%	*< 0.001*
Pre-OP FDG-PET scan performed	82 % (133/162)	90 % (26/29)	77 % (47/61)	
Positive FDG-PET scan	93 % (124/133)	96.2 % (25/26)	100 % (47/47)	*0.142*
Pre-OP Ga-PET scan performed	11 % (18/162)	24% (7/29)	10% (6/61)	
Positive Ga-PET scan	100 % (18/18)	100% (7/7)	100% (6/6)	

according to the reverse Kaplan Meier method [16]. Median age at diagnosis was 69 (19–89) years. Median age was higher in AC and LCNEC compared to TC *(p = 0.046)*. Gender distribution varied amongst the subtypes with TC and AC showing a significant female predominance with 73% and 72%, respectively and LCNEC with 46% males and 54% females *(p = 0.024)*.

3.1. Symptoms and smoking history

Prior to diagnosis 29% of the patients presented with weight loss, 40% with a cough and 19% of patients had suffered from pneumonia in the months prior to diagnosis. Thirty-seven patients (37%) had no symptoms prior to diagnosis and the tumour was diagnosed incidentally. One patient presented with Cushing's syndrome at the time of diagnosis and one patient developed the carcinoid syndrome during follow-up.

Of the TC patients 56% were prior or current smokers. The corresponding percentages for AC and LCNEC were 66% and 87%, respectively *(p = 0.001)*. The number of patients with a smoking history did not differ significantly in the TC and AC groups; however only 5.6% of patients in the TC group were current smokers.

3.2. Location of the primary tumour and tumour stage

All patients were diagnosed with a primary tumour located in the lungs or bronchia. There was no significant difference between TC, AC and LCNEC considering the localization of the primary tumour (left or right lung or centrally or peripherally located).

Table 1 shows the distribution of T, N and M-category for the three subgroups. There was a significant difference in the N-category and M-category distribution between the three groups *(p = 0.001)*. Lymph node involvement (N1-N3) was found in 15% of TC, in 48% of AC and in 77% of LCNEC patients. LCNEC patients presented with the highest rate of metastatic disease (M1a/M1b) with 58%, whereas 27% of patients in the AC group and 4% of patients in the TC group presented with metastatic disease at time of diagnosis.

3.3. Mitotic count, Ki67 proliferation index and necrosis

Patients in the TC group presented with a mean Ki67 proliferation index of 5% (range 1–14%), the AC group with a mean Ki67 index of 16% (range 2–40%) and the LCNEC with a mean Ki67 of 69% (range 2–100%) as listed in Fig. 1. Of TC patients, 74% presented with Ki67 up to 5% (0–5%) whereas 20% presented with Ki67 between 5 and 10%. All patients in the TC group had tumours with a mitotic count between 0–1/2mm^2. In the AC group 86% presented with a mitotic count

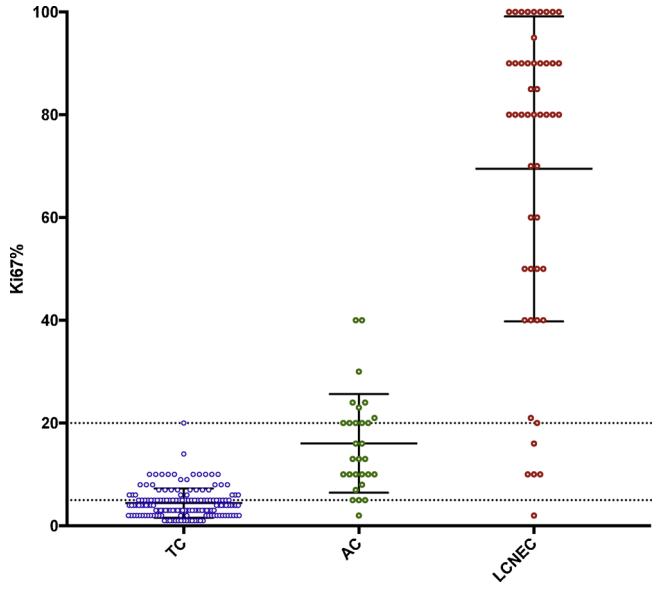


Fig. 1. Ki67 proliferation index according to the three subtypes, typical carcinoid (TC), atypical carcinoid (AC) and large cell neuroendocrine carcinoma (LCNEC). Dotted lines represent cut off values according to WHO classification 2015 with TC Ki67 values 0–5%, AC values > 5% and < 20% and LCNEC values above 40%.

Table 2
Distribution of Immunohistochemical markers stratified for the thee subgroups typical carcinoid (TC), atypical carcinoid (AC) and large cell neuroendocrine carcinomas (LCNEC).

Survival (n)	TC (162)	AC (29)	LCNEC (61)
1-year-survival (%)	**96**	**89**	**59**
3-year-survival(%)	**91**	**78**	**30**
5-year-survival (%)	**87**	**63**	**22**
5-year-survival (%) (Study = 12.300)*	**87**	**44**	**15**

between 2–10/2mm^2, whereas 14% had a mitotic count between 0–1/2mm^2, but the tumours had foci of necrosis. LCNEC tumours presented with a mitotic count above 10/2mm^2 in all patients.

3.4. Immunohistochemistry

In surgical specimens or biopsies, synaptophysin was positive in 98%, chromogranin A (CgA) in 92% and CD56 in 97%. Synaptophysin showed an almost 100% positivity in TC and AC tumours whereas 8% of LCNEC tumours were synaptophysin negative. Positive TTF-1 expression was found in 69% in TC, 84% in AC and 78% in LCNEC tumours. Further details on the distribution of the different immunohistochemical markers in TC, AC and LCNEC are listed in Table 2. None of the evaluated immune markers showed any significant prognostic value when looking at overall survival.

3.5. Imaging modalities

In total, 207 (82%) patients had FDG-PET/CT at diagnosis and before surgery. TC and AC presented with positive FDG-PET scans in 93% and 96% of cases, respectively. LCNEC showed positive FDG-PET scans in 100% of patients. Thirty-one patients (12%) had a ^{68}Ga-DOTATOC-PET/CT performed and twenty-three patients (9%) had ^{111}In-DTPA-octreotide scintigraphy performed at diagnosis or preoperatively, all were positive. Twenty-one patients were scanned with both FDG-PET and ^{68}Ga-DOTATOC. Of these, 2 patients (1 TC and 1 AC) were FDG-negative, while the remaining 19 cases were congruent positive on FDG-PET and ^{68}Ga-DOTATOC.

3.6. Surgical and medical management

A total of 179 patients (71%) underwent surgical resection, 26% by thoracotomy and 74% by video assisted thoracoscopic surgery (VATS). The majority of patients in the TC and AC group had surgery, 87% and 72% respectively, whereas only 28% of patients with LCNEC underwent surgery *($p < 0.0001$)*.

In the non-operated group (n = 73), 55% of patients with TC, 50% with AC and 4% of patients with LCNEC received treatment with somatostatin analogues. Seven patients received streptozotocin and 5-fluorouracil (STZ + 5-FU). Etoposide and carboplatin was administered to 39 patients, of which 35 (90%) had LCNEC. Fifteen patients were treated with temozolomide, 12 patients with LCNEC, two with AC and one patient with TC.

Of the 179 patients, who underwent surgery, 11 (6%) had recurrence, one patient with TC, six patients with AC and four patients with LCNEC. The mean time to recurrence was 72.2 months in the TC group, 32.7 months in the AC and 5.1 months in the LCNEC group. Of the eleven patients with recurrence seven patients received chemotherapy, three underwent surgery again and three were treated with somatostatin analogues. At the end of follow-up, 4 of the 11 patients (1 of 6 AC's and 3 of 4 LCNECs) had died.

3.7. Overall survival and prognostic factors

Sixty-five patients (26%) died during follow-up. Twenty nine (36%) of the men died compared with 36 (30%) of the women. However, gender did not show any prognostic value in any of the three subgroups. One, 3 and 5-year survival rates for the whole cohort were 86%, 75% and 69%, respectively.

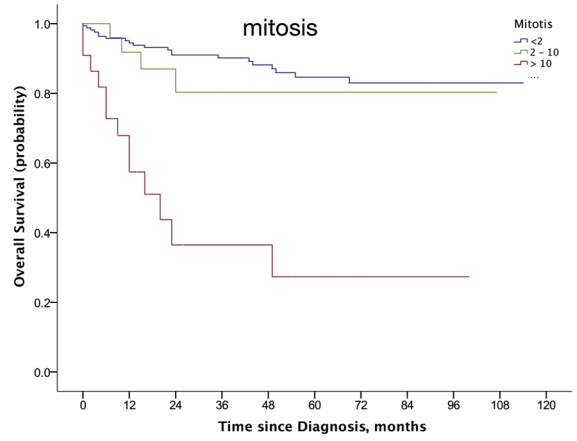
Fig. 2 shows survival functions stratified according to the diagnostic criteria for BP-NETs with mitotic count (A), necrosis (B) and Ki67 index (C). Individually, none of these three parameters could discriminate low-risk from intermediate-risk patients. Identification of low-risk and intermediate-risk patients was more prominent when combining the diagnostic criteria as in the WHO 2015 classification (Fig. 2,D) (Hazard Ratio (HR) of 2.5 (95% Confidence Interval (CI): 0.99–6.4; *$p = 0.053$)*. The 1, 3 and 5-year survival rates were highest in TC patients with 96%, 91% and 87%, respectively, compared to 89%, 78% and 63% for the AC patients. The lowest 1, 3 and 5-year survival rates were found in the LCNEC group with 59%, 30% and 22%, respectively. Median survival time was only reached for the LCNEC group (16 months, (CI): 8.2–23.8). By multivariate Cox regression analysis entering mitosis, necrosis and Ki67 index in the model, none of the predictors had independent prognostic value, underscoring the value of the current multifactor assessment for tumour type determination.

The Kaplan Meier curves in Fig. 3 shows the survival profile of operated vs non-operated patients. Regardless of tumour type, patients who underwent surgical resection had a better overall survival than patients who were not operated (A, B and C). For the whole cohort the operated patients also had a better prognosis (HR 11.8, CI: 6.8–20.6; *$p < 0.001$)* and 5-year survival rates for operated patients versus non-operated patients were 87% and 26%, respectively (Fig. 4,D). If taking into account, in a multivariate Cox regression analysis, N stage and M stage, operational status remained an independent predictor of OS (HR 6.0, CI: 2.4–15.0; *$p < 0.001$)*.

The impact of N stage on survival for the TC, AC and LCNECs is shown in Fig. 4a, b and c, respectively. Few patients with TCs had N1 or N2 stage disease (n = 11 for both groups), but a significantly longer OS was found for TC patients with N0 stage compared to patients with N2 stage (HR 7.2, CI: 2.2–15.0; *$p < 0.001$)* and the 5 year survival rates were 92%, 72% and 63% for N0, N1 and N2 stage, respectively. Although the same trend was seen for the AC and LCNEC patients, there

A

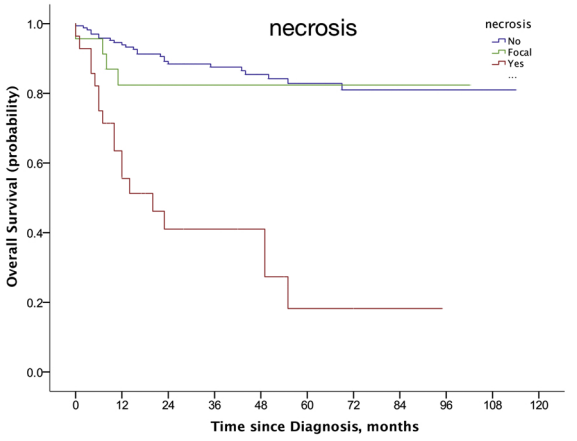
	Median Survival, mths	HR	p	1-yr survival	3-yr survival	5-yr survival
<2	NR	1.7 (0.6-4.7)[1]	0.345	95%	90%	85%
2-10	NR	5.2 (1.7-16.2)[2]	0.004	92%	79%	79%
> 10	20 (6.4-33.6)	2.8 (2.0-4.1)[3]	<0.001	67%	38%	27%



<2%	166	145	127	104	82	63	45	21	9	1
2-10	25	21	13	8	3	3	3	3	1	
> 10%	22	13	5	4	4	2	1	1	1	

B

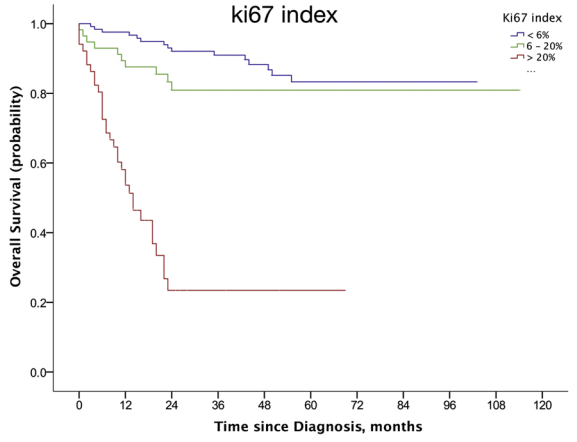
	Median Survival, mths	HR	p	1-yr survival	3-yr survival	5-yr survival
No	NR	1.4 (0.5-4.0)[1]	0.544	94%	87%	83%
Focal	NR	5.0 (1.7-14.9)[2]	0.004	82%	82%	82%
Yes	20 (5.0-35.0)	2.7 (2.0-3.7)[3]	<0.001	63%	41%	19%



No	168	145	123	96	73	57	39	18	8	1
Focal	23	18	12	11	9	6	6	5	2	
Yes	28	16	8	7	6	2	2	1		

C

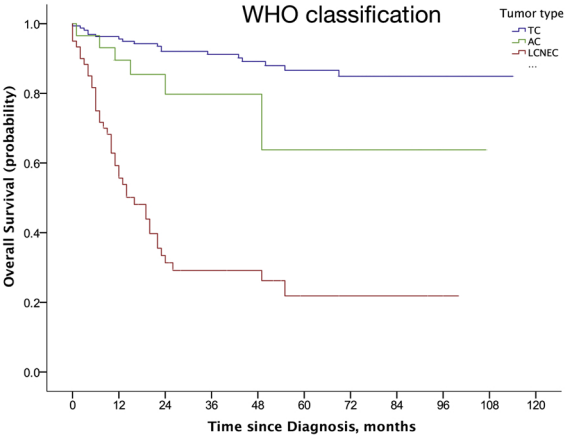
	Median Survival, mths	HR	p	1-yr survival	3-yr survival	5-yr survival
< 6%	NR	1.7 (0.8-3.7)[1]	0.209	97%	91%	83%
6 - 20%	NR	6.4 (3.1-13.1)[2]	<0.001	89%	80%	80%
> 20%	14 (9.1-18.9)	3.7 (2.6-5.1)[3]	<0.001	57%	27%	27%



< 6%	125	110	99	81	60	45	35	15	6	
6 - 20%	57	49	35	25	19	14	8	7	2	1
> 20%	51	26	7	3	2	1				

D

	Median Survival, mths	HR	p	1-yr survival	3-yr survival	5-yr survival
TC	NR	2.5 (0.99-6.4)[1]	0.053	96%	91%	87%
AC	NR	4.6 (2.0-10.9)[2]	<0.001	89%	78%	63%
LCNEC	16 (8.2-23.8)	3.3 (2.5-4.4)[3]	<0.001	59%	30%	22%



TC	162	143	125	102	80	62	44	21	9	1
AC	29	24	15	10	5	4	4	3	1	
LCNEC	60	33	16	11	10	4	3	3	2	

Fig. 2. Survival functions stratified according to diagnostic criteria for BP-NETs A) according to number of mitosis pr. 2mm2, B) according to degree of necrosis, C) according to Ki67 proliferation index, D) all 3 criteria considered and stratified for typical carcinoid (TC), atypical carcinoid (AC) and large cell neuroendocrine carcinoma (LCNEC).

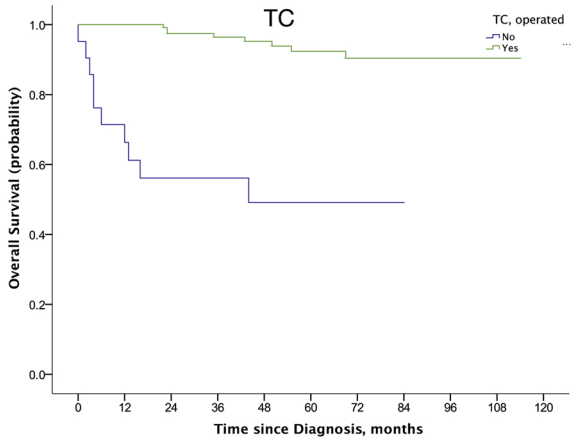
was no significant difference in OS for patients of different stages of disease. Patients with TC and N2 stage that were operated had a higher 5-year survival rate of 85% compared to 25% for non-operated patients (Fig. 4d).

4. Discussion

In this study of 252 patients the distribution of tumour subtype consisted of 64% with TC, 12% AC and 24% LCNEC. This correlates well with prior studies describing a 10:1 relationship between frequencies of TC and AC and the fact that TC represent approximately 70–80% of neuroendocrine lung tumours [17]. TC presented with

A

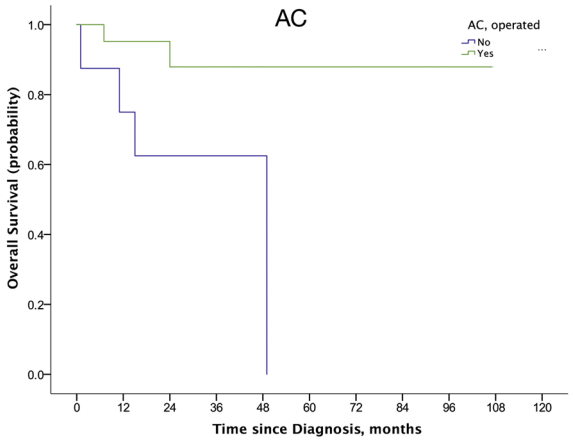
	Median Survival, mths	HR	p	1-yr survival	3-yr survival	5-yr survival
Yes	NR	12.1 (4.8-30.9)	< 0.001	100%	97%	93%
No	NR			71%	56%	49%



Yes	141	129	114	93	73	58	41	20	9	1
No	21	14	11	9	7	4	3	1		

B

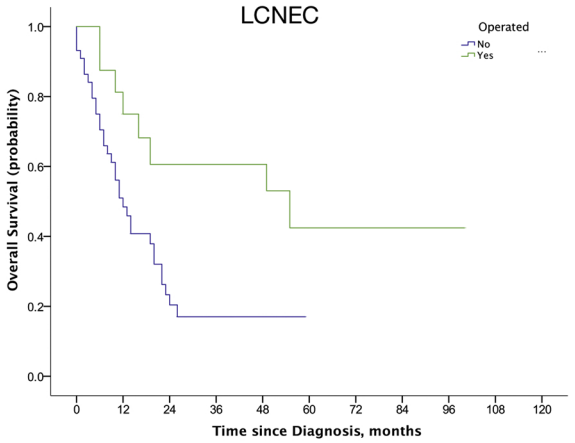
	Median Survival, mths	HR	p	1-yr survival	3-yr survival	5-yr survival
Yes	NR	6.9 (1.2-38.3)	0.028	95%	87%	87%
No	NR			75%	58%	-



Yes	21	18	13	9	4	4	4	3	1	
No	8	6	2	1	1	0				

C

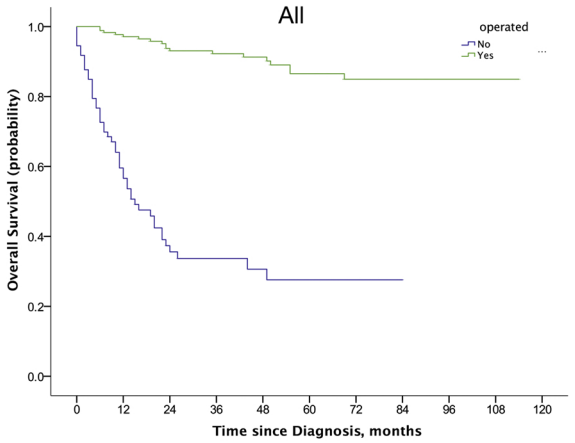
	Median Survival, mths	HR	p	1-yr survival	3-yr survival	5-yr survival
Yes	55 (0.0 - 110)	2.7 (1.2-6.0)	0.015	81%	61%	44%
No	12 (8.0 - 16.0)			51%	17%	17%



Yes	16	13	8	8	8	4	3	3	2	
No	44	20	8	3	2					

D

	Median Survival, mths	HR	p	1-yr survival	3-yr survival	5-yr survival
Yes	NR	11.8 (6.8-20.6)	< 0.001	98%	92%	87%
No	15 (8.4-21.6)			59%	33%	26%



Yes	178	160	135	110	85	66	48	26	12	1
No	73	40	21	13	10	4	3	1		

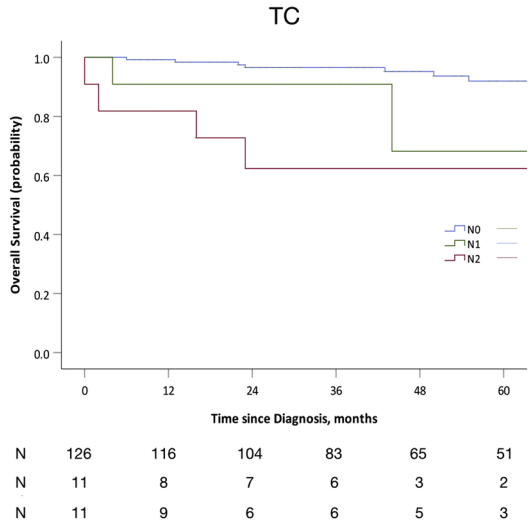
Fig. 3. Survival functions for operated vs. non-operated patients stratified for A) typical carcinoid (TC), B) atypical carcinoid (AC), C) large cell neuroendocrine carcinoma (LCNEC) and D) all three subtypes.

centrally located tumours in 58% of patients whereas both AC and LCNEC presented with peripherally located tumours in the majority of patients, 52% and 64% respectively. This finding correlates well with prior studies describing TC as often centrally located and AC and LCNEC as often peripherally located [18].

Prior studies have found median age at diagnosis in TC as low as 45 years whereas our study found a median age at diagnosis for TCs of 68 years [19]. In the AC group we found a median age of 72 years compared to prior studies describing a median age of 55 for patients with AC [20]. Median age in LCNEC patients was 69 years; this corresponds to previously described assumptions that LCNEC often occur in the seventh decade [17,21]. This study describes a female predominant distribution in both TC and AC with female percentages of 73% and 72%, respectively. Of LCNEC patients, 54% were female in contrast to prior studies that found a male predominance of these tumours [21]. Moreover, the low number of patients with functioning tumours (only

A

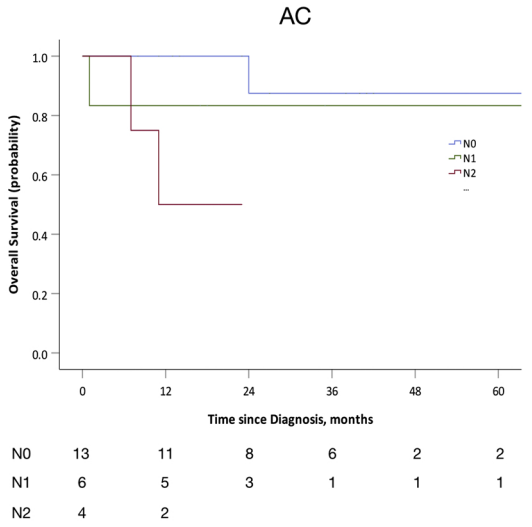
	Median Survival, mths	HR	p	1-yr survival	3-yr survival	5-yr survival
N0	NR	4.3 (0.9-20.8)[1]	0.070	99%	97%	92%
N1	NR	8.0 (2.3-27.3)[2]	0.001	90%	90%	72%
N2	NR	2.0 (0.4-10.9)[3]	0.427	82%	63%	63%



N	126	116	104	83	65	51
N	11	8	7	6	3	2
N	11	9	6	6	5	3

B

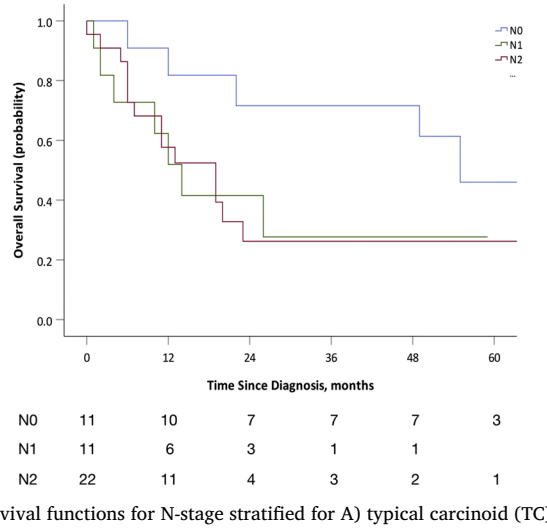
	Median Survival, mths	HR	p	1-yr survival	3-yr survival	5-yr survival
N0	NR	2.5 (0.2-40.3)[1]	0.514	100%	87%	87%
N1	NR	14.4 (0.9-235)[2]	0.062	83%	83%	83%
N2	11 (no CI)	2.9 (0.3-32.4)[3]	0.363	50%	-	-



N0	13	11	8	6	2	2
N1	6	5	3	1	1	1
N2	4	2				

C

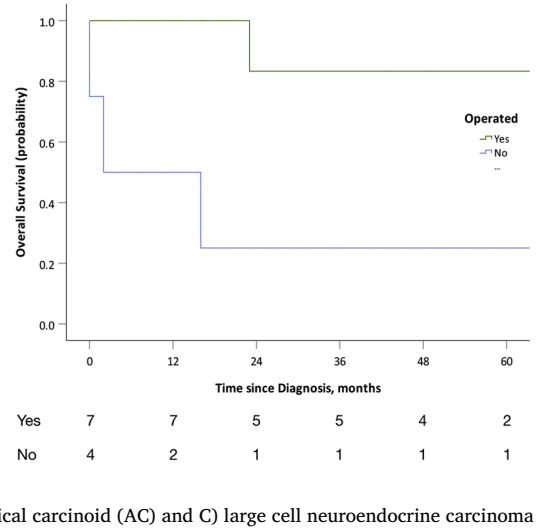
	Median Survival, mths	HR	p	1-yr survival	3-yr survival	5-yr survival
N0	55 (no CI)	2.6 (0.8-8.5)[1]	0.103	91%	72%	48%
N1	14 (8.1-19.9)	2.6 (0.9-7.4)[2]	0.075	62%	24%	24%
N2	19 (9.1-28.9)	0.96 (0.4-2.4)[3]	0.927	57%	29%	29%



N0	11	10	7	7	7	3
N1	11	6	3	1	1	
N2	22	11	4	3	2	1

D

	Median Survival, mths	HR	p	1-yr survival	3-yr survival	5-yr survival
Surgery	NR	9.6 (0.95-97)	0.055	100%	85%	85%
No Surgery	2 (0.0 - 17.7)			50%	25%	25%



Yes	7	7	5	5	4	2
No	4	2	1	1	1	1

Fig. 4. Survival functions for N-stage stratified for A) typical carcinoid (TC), B) atypical carcinoid (AC) and C) large cell neuroendocrine carcinoma (LCNEC). In D) operated and non-operated patients with TC and N2 disease are compared.

2) is surprisingly low compared to previous findings in other studies [2].

The role of Ki67 in BP-NETs has been debated and still remains a field of controversy. Some studies have investigated Ki67 as a prognostic factor and its role in overall survival, but final data to support this theory is still lacking. Several studies suggest that Ki67, because of its qualities in distinguishing low-grade and high-grade tumours in small biopsies or in cases with crush artefacts, merely should be seen as an adjunct diagnostic marker in cooperation with the mitotic count and necrosis in the initial workup. Other studies have suggested that Ki67 may potentially function as an independent meaningful prognostic marker in BP-NETs [22–24]. We found that 20% in the TC group presented with Ki67 proliferation index between 5 and 10%. This may suggest that the current WHO classification should be changed for TC to include Ki67 up to 10%.

Although we were not able to find an independent prognostic value

of mitotic count, it still remains the most important feature for classification of lung NETs. A previous study by Beasley et al. showed a significantly worse overall survival in patients with AC with a mitotic count between 6–10 mitoses/2mm^2 compared to those with 2–5 mitoses/2mm^2 [25]. We performed a similar test for AC patients comparing mitotic count with 2–5 and 6–10 mitoses/2mm^2, however we did not find any significant difference in overall survival between the two groups, which could be due to the relatively low number of patients with AC compared to the study by Beasley et al (29 vs. 106 patients with AC).

Individually, neither mitotic count, necrosis or Ki67 index were found to have prognostic value for discriminating TC and AC. However, when the WHO 2015 classification is used, taking into consideration all three pathological parameters it's possible to accurately risk stratify patients into low risk (TC) and intermediate risk (AC) groups. This underlines the power of combining diagnostic parameters and furthermore the importance of continuous development of new guidelines as well as developing new diagnostic tools.

The WHO diagnostic criteria from 2015 recommend analysis of immunohistochemical markers (CgA, synaptophysin and CD56) to confirm neuroendocrine differentiation. CgA and synaptophysin are considered the two most valuable markers in the diagnostic process. Zahel et al demonstrated a highly sensitive but not perfect role of synaptophysin and CgA in diagnosis of BP-NETs [26]. None of these markers are suitable for distinguishing between TC and AC, nor has it been proven that any specific immune marker holds prognostic value when it comes to BP-NETs. In line with this, we did not find any significant prognostic value in any of the investigated immunohistochemical markers in this study. However, we did find that the expression of TTF-1 in TC and AC were positive in 68% and 84% respectively. This finding challenges the conception in the WHO guidelines applicable for BP-NETs, where TTF-1 is described as "mostly negative" in TC and AC [12].

Prior studies describe FDG-PET scans as best suitable, i.e. FDG positive, in tumours exceeding Ki67 index of 10 and 20% [27]. The hitherto unseen high portion of positive FDG-PET scans in patients with TC and AC found in this study challenges this conception and should be taken into account in future imaging guidelines for these types of tumours. New imaging modalities using radiolabeled somatostatin analogues such as the ^{68}Ga-DOTATOC are gaining impact in the imaging of BP-NETs due to high resolution and sensitivity [28,29]. However, the FDG-PET scan still has great value as first-line clinical imaging in visualizing location and activity of tumours and metastasis. Some studies suggest that SUV_{max} can be used as predictor of death and recurrence though this needs to be further confirmed before final conclusions can be made [30]. Due to Danish national guidelines concerning guaranty of treatment after a certain period after diagnosis, only 12% of our patients had somatostatin imaging done before treatment. However, all patients with TC and AC had a postoperative ^{68}Ga-DOTATOC scan performed to verify that no residual disease was present.

Survival analysis showed a correlation between tumour type and overall survival. Patients with TC had the best prognosis with 5-year survival rates of 87% compared to 63% for the AC group and 22% for the LCNECs. These results correlate well with analysis of overall survival from prior studies [31,32]. Survival analysis showed a significant difference in survival rates between patients who underwent surgery and patients who did not. Survival rates in the operated group mimic those found in previous studies and in the literature with 5-year survival rates of 97% in patients with TC and 78% in patients with AC [30] compared with findings in this paper with 5-year survival in operated TC and AC patients of 93% and 87%, respectively. It could be argued that stage explains the observed difference in OS between operated and non-operated patients. However, when performing multivariate analysis, taking into account N and M stage, operation still remained an independent factor for OS in the current study. Although only a small proportion of patients with TC had N2 stage disease in the current cohort, we were still able to show a difference in survival between operated and non-operated patients, indicating that it should be consided to operate TC patients with N2 stage disease if otherwise acceptable from a clinical point of view.

This study is a retrospective study hence some limitations are relevant to mention. As in all retrospective studies there will be a risk of bias when analysing a group of patients treated throughout a timeframe of 9 years due to development of new treatments and new clinical guidelines over time. This study is however a single institution study which minimizes the risk of biases and allows for comparison of survival parameters. Another limitation in this study is the relative small number of events especially in the low grade tumour groups TC and AC due to the rather indolent nature of these tumours and the small number of patients with AC compared to TC and LCNEC. In addition, some treatments such as adjuvant chemotherapy administrated for TC and AC patients were rarely given and therefore no assessment can be drawn regarding the effect of these treatments.

5. Conclusion

To our knowledge this study is the largest study to analyse and compare diagnostic and prognostic factors in a group of 252 BP-NET patients treated at a single institution.

Our study supports the assumption that the Ki67 index may play an important role as both a diagnostic as well as a prognostic marker together with the mitotic count and amount of necrosis in BP-NETs. Our study suggests that the WHO classification regarding Ki67 in TC tumours may be changed to include proliferation index up to 10% because of the significant portion of TC tumours presenting with Ki67 between 5–10%.

Investigation of imaging modalities showed a high portion of positive FDG-PET scans in TC and AC patients, which has not previously been shown. Surgical resection remains the preferred treatment of BP-NETs in patients with adequate pulmonary reserve and this paper, as prior studies, demonstrates increased survival rates in these patients. Even TC patients with N2 stage disease who underwent surgery had a longer survival than non-operated TC patients with N2 stage disease, underscoring the value of surgical resection at advanged stage.

Tumour type remains the single most potent and independent prognostic factor among BP-NETs. This underlines the importance of precise and efficient diagnostic work-up early in the medical course and further emphasizes the importance of more comprehensive studies in order to update and align international clinical guidelines.

Conflict of interest

No author of this work had any conflicts of interest. Co-author R.H. Petersen has received speeker fees from Medtronic.

Funding

Danish Cancer Society, Denmark.

References

[1] D. Carter, M. Vazquez, D.B. Flieder, E. Brambilla, A. Gazdar, M. Noguchi, et al., Comparison of pathologic findings of baseline and annual repeat cancers diagnosed on CT screening, Lung Cancer 56 (2) (2007) 193–199.
[2] M.E. Caplin, E. Baudin, P. Ferolla, P. Filosso, M. Garcia-Yuste, E. Lim, et al., Pulmonary neuroendocrine (carcinoid) tumors: european Neuroendocrine Tumor Society expert consensus and recommendations for best practice for typical and atypical pulmonary carcinoids, Ann. Oncol. 26 (8) (2015) 1604–1620.
[3] F.C. Detterbeck, Management of carcinoid tumors, Ann. Thorac. Surg. 89 (3) (2010) 998–1005.
[4] E.M. Tabaksblat, S.W. Langer, U. Knigge, H. Gronbaek, J. Mortensen, R.H. Petersen, et al., Diagnosis and treatment of bronchopulmonary neuroendocrine tumours: state of the art, Acta Oncol. (Madr) 55 (1) (2016) 3–14.
[5] A. Marx, J.K. Chan, J.M. Coindre, F. Detterbeck, N. Girard, N.L. Harris, et al., The 2015 World Health Organization Classification of Tumors of the Thymus:

Continuity and Changes, J. Thorac. Oncol. 10 (10) (2015) 1383–1395.
[6] P.A. Schnabel, K. Junker, [Pulmonary neuroendocrine tumors in the new WHO 2015 classification: Start of breaking new grounds?], Pathologe 36 (3) (2015) 283–292.
[7] N. Rekhtman, M.C. Pietanza, M.D. Hellmann, J. Naidoo, A. Arora, H. Won, et al., Next-generation sequencing of pulmonary large cell neuroendocrine carcinoma reveals small cell carcinoma-like and non-small cell carcinoma-like subsets, Clin. Cancer Res. 22 (14) (2016) 3618–3629.
[8] M. Fasano, C.M. Della Corte, F. Papaccio, F. Ciardiello, F. Morgillo, Pulmonary large-cell neuroendocrine carcinoma: from epidemiology to therapy, J. Thorac. Oncol. 10 (8) (2015) 1133–1141.
[9] J.L. Derks, N. Leblay, E. Thunnissen, R.J. van Suylen, M. den Bakker, H.J.M. Groen, et al., Molecular subtypes of pulmonary large-cell neuroendocrine carcinoma predict chemotherapy treatment outcome, Clin. Cancer Res. 24 (1) (2018) 33–42.
[10] R. Boellaard, R. Delgado-Bolton, W.J. Oyen, F. Giammarile, K. Tatsch, W. Eschner, et al., FDG PET/CT: EANM procedure guidelines for tumour imaging: version 2.0, Eur. J. Nucl. Med. Mol. Imaging 42 (2) (2015) 328–354.
[11] H.J. Hansen, R.H. Petersen, Video-assisted thoracoscopic lobectomy using a standardized three-port anterior approach - the Copenhagen experience, Ann. Cardiothorac. Surg. 1 (1) (2012) 70–76.
[12] W.D. Travis, E. Brambilla, A.P. Burke, A. Marx, A.G. Nicholson, Introduction to the 2015 world health organization classification of tumors of the lung, pleura, Thymus, and heart, J. Thorac. Oncol. 10 (9) (2015) 1240–1242.
[13] P.E. Postmus, E. Brambilla, K. Chansky, J. Crowley, P. Goldstraw, E.F. Patz Jret al., The IASLC Lung Cancer Staging Project: proposals for revision of the M descriptors in the forthcoming (seventh) edition of the TNM classification of lung cancer, J. Thorac. Oncol. 2 (8) (2007) 686–693.
[14] M.E. Charlson, P. Pompei, K.L. Ales, C.R. MacKenzie, A new method of classifying prognostic comorbidity in longitudinal studies: development and validation, J. Chronic Dis. 40 (5) (1987) 373–383.
[15] A. Pfeifer, U. Knigge, T. Binderup, J. Mortensen, P. Oturai, A. Loft, et al., 64Cu-DOTATATE PET for neuroendocrine tumors: a prospective head-to-Head comparison with 111In-DTPA-Octreotide in 112 patients, J. Nucl. Med. 56 (6) (2015) 847–854.
[16] M. Schemper, T.L. Smith, A note on quantifying follow-up in studies of failure time, Control. Clin. Trials 17 (4) (1996) 343–346.
[17] B.I. Gustafsson, M. Kidd, A. Chan, M.V. Malfertheiner, I.M. Modlin, Bronchopulmonary neuroendocrine tumors, Cancer. 113 (1) (2008) 5–21.
[18] W.D. Travis, Advances in neuroendocrine lung tumors, Ann. Oncol. 21 (Suppl 7) (2010) vii65–71.
[19] G. Cardillo, F. Sera, M. Di Martino, P. Graziano, R. Giunti, L. Carbone, et al., Bronchial carcinoid tumors: nodal status and long-term survival after resection, Ann. Thorac. Surg. 77 (5) (2004) 1781–1785.
[20] H. Skuladottir, F.R. Hirsch, H.H. Hansen, J.H. Olsen, Pulmonary neuroendocrine tumors: incidence and prognosis of histological subtypes, A population-based study in Denmark. Lung Cancer. 37 (2) (2002) 127–135.
[21] H. Takei, H. Asamura, A. Maeshima, K. Suzuki, H. Kondo, T. Niki, et al., Large cell neuroendocrine carcinoma of the lung: a clinicopathologic study of eighty-seven cases, J. Thorac. Cardiovasc. Surg. 124 (2) (2002) 285–292.
[22] G. Pelosi, G. Rindi, W.D. Travis, M. Papotti, Ki-67 antigen in lung neuroendocrine tumors: unraveling a role in clinical practice, J. Thorac. Oncol. 9 (3) (2014) 273–284.
[23] G. Rindi, C. Klersy, F. Inzani, G. Fellegara, L. Ampollini, A. Ardizzoni, et al., Grading the neuroendocrine tumors of the lung: an evidence-based proposal, Endocr. Relat. Cancer 21 (1) (2014) 1–16.
[24] A.E. Walts, D. Ines, A.M. Marchevsky, Limited role of Ki-67 proliferative index in predicting overall short-term survival in patients with typical and atypical pulmonary carcinoid tumors, Mod. Pathol. 25 (9) (2012) 1258–1264.
[25] M.B. Beasley, F.B. Thunnissen, E. Brambilla, P. Hasleton, R. Steele, S.P. Hammar, et al., Pulmonary atypical carcinoid: predictors of survival in 106 cases, Hum. Pathol. 31 (10) (2000) 1255–1265.
[26] T. Zahel, S. Krysa, E. Herpel, A. Stenzinger, B. Goeppert, P. Schirmacher, et al., Phenotyping of pulmonary carcinoids and a Ki-67-based grading approach, Virchows Arch. 460 (3) (2012) 299–308.
[27] D. Horsch, K.W. Schmid, M. Anlauf, K. Darwiche, T. Denecke, R.P. Baum, et al., Neuroendocrine tumors of the bronchopulmonary system (typical and atypical carcinoid tumors): current strategies in diagnosis and treatment. Conclusions of an expert meeting February 2011 in Weimar, Germany, Oncol. Res. Treat. 37 (5) (2014) 266–276.
[28] I. Kayani, B.G. Conry, A.M. Groves, T. Win, J. Dickson, M. Caplin, et al., A comparison of 68Ga-DOTATATE and 18F-FDG PET/CT in pulmonary neuroendocrine tumors, J. Nucl. Med. 50 (12) (2009) 1927–1932.
[29] A.R. Haug, R. Cindea-Drimus, C.J. Auernhammer, M. Reincke, B. Wangler, C. Uebleis, et al., The role of 68Ga-DOTATATE PET/CT in suspected neuroendocrine tumors, J. Nucl. Med. 53 (11) (2012) 1686–1692.
[30] G. Cusumano, L. Fournel, S. Strano, D. Damotte, M.C. Charpentier, A. Galia, et al., Surgical resection for pulmonary carcinoid: long-term results of multicentric study-the importance of pathological N status, more than we thought, Lung 195 (6) (2017) 789–798.
[31] J.C. Yao, M. Hassan, A. Phan, C. Dagohoy, C. Leary, J.E. Mares, et al., One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States, J. Clin. Oncol. 26 (18) (2008) 3063–3072.
[32] J.M. Cameselle-Teijeiro, J.A. Mato Mato, O. Fernandez Calvo, J. Garcia Mata, Neuroendocrine pulmonary tumors of low, intermediate and high grade: anatomopathological diagnosis-prognostic and predictive factors, Mol. Diagn. Ther. 22 (2) (2018) 169–177.