European Journal of Cardio-Thoracic Surgery 45 (2014) 677–686
doi:10.1093/ejcts/ezt470 Advance Access publication 6 October 2013

ORIGINAL ARTICLE

Prognostic factors in a multicentre study of 247 atypical pulmonary carcinoids[†]

Niccolò Daddi[a,*], Marco Schiavon[b], Pier Luigi Filosso[c], Giuseppe Cardillo[d], Marcello Carlo Ambrogi[e], Angela De Palma[f], Luca Luzzi[g], Alessandro Bandiera[h], Christian Casali[i], Alberto Ruffato[j], Verena De Angelis[k], Luigi Gaetano Andriolo[b], Francesco Guerrera[c], Francesco Carleo[d], Federico Davini[e], Moira Urbani[a], Sandro Mattioli[j], Uliano Morandi[i], Piero Zannini[h], Giuseppe Gotti[g], Michele Loizzi[e], Francesco Puma[a], Alfredo Mussi[e], Alberto Ricci[l], Alberto Oliaro[c] and Federico Rea[b] on behalf of the Multi-Institutional Italian Pathology Group

[a] Thoracic Surgery Unit, Perugia University School of Medicine, Perugia, Italy
[b] Cardiothoracic and Vascular Sciences, Division of Thoracic Surgery, University of Padova Medical School, Padua, Italy
[c] Department of Thoracic Surgery, University of Torino Medical San Giovanni Battista Hospital, Torino, Italy
[d] Thoracic Surgery Unit, Ospedale Carlo Forlanini, Azienda Ospedaliera San Camillo Forlanini, Roma, Italy
[e] Cardiovascular and Thoracic Department, University of Pisa Medical School, Pisa, Italy
[f] Section of Thoracic Surgery, University of Bari Medical School, Bari, Italy
[g] Thoracic Surgery Unit, University Hospital of Siena, Siena, Italy
[h] Department of Thoracic Surgery, Vita-Salute San Raffaele University, San Raffaele Scientific Institute, Milano, Italy
[i] Division of Thoracic Surgery, Department of General Surgery and Surgical Specialties, University of Modena and Reggio Emilia Medical School, Modena, Italy
[j] Division of Thoracic Surgery, Villa Maria Cecilia Hospital, University of Bologna, Bologna, Italy
[k] Medical Oncology, S.Maria della Misericordia Perugia Hospital, Perugia, Italy
[l] Department of Pneumology, S.Andrea Hospital, University of Rome La Sapienza, Roma, Italy

* Corresponding author. Thoracic Surgery Unit, Department of Surgical and Biomedical Sciences, University of Perugia, S.Maria della Misericordia Hospital, Via M. Alinda Brunamonti Brunacci 51, Perugia 06122, Italy. Tel: +39-0-755782267; fax: +39-0-755782600; e-mail: niccolo.daddi@unipg.it (N. Daddi).

Received 28 May 2013; received in revised form 9 August 2013; accepted 13 August 2013

THORACIC

Abstract

OBJECTIVES: To analyse clinical and biomolecular prognostic factors associated with the surgical approach and the outcome of 247 patients affected by primary atypical carcinoids (ACs) of the lung in a multi-institutional experience.

METHODS: We retrospectively evaluated clinical data and pathological tissue samples collected from 247 patients of 10 Thoracic Surgery Units from different geographical areas of our country. All patients were divided into four groups according to surgical procedure: sub-lobar resections (SURG1), lobar resections (SURG2), tracheobronchoplastic procedures (SURG3) and pneumonectomies (SURG4). Overall survival analysis was performed using the Kaplan–Meier method and log-rank test. Survival was calculated from the date of surgery to the last date of follow-up or death. The parameters evaluated included age, gender, smoking habits, laterality, type of surgery, 7th edition of TNM staging, mitosis Ki-67 (MIB1), multifocal forms, tumourlets, type of lymphadenectomy and neo/adjuvant therapy. For multivariate analysis, a Cox regression model was used with a forward stepwise selection of covariates.

RESULTS: Two hundred and forty-seven patients (124 females and 123 males; range 10–84, median 60 years) underwent surgical resection for AC in the last 30 years as follows: $n = 38$ patients in SURG1, 181 in SURG2, 15 in SURG3 and 14 in SURG4. A smoking history was present in 136 of 247 (55%) patients. The median follow-up period was 98.7 (range 11.2–369.9) months. The overall survival probability analysis of the AC was 86.7% at 5 years, 72.4% at 10 years, 64.4% at 15 years and 58.1% at 20 years. Neuroendocrine multicentric forms were detected in 12 of 247 patients (4.8%; 1 of 12 pts) during the follow-up (range 11.2–200.4, median 98.7 months) and 33.4% had recurrence of disease. There were no significant differences between gender, tumour location and type of surgery at the multivariate analysis. Age [$P < 0.001$, hazard ratio (HR) 0.60; confidence interval (CI) 0.32–1.12], smoking habits ($P = 0.002$; HR 0.43, 95% CI 0.23–0.80) and lymph nodal metastatic involvement ($P = 0.008$; HR 0.46, 95% CI 0.26–0.82) were all significant at multivariate analysis.

CONCLUSIONS: ACs of the lung are malignant neuroendocrine tumours with a worst outcome in patients over 70 years and in smokers. With the exception of pneumonectomy, the extent of resection does not seem to affect survival and should be accompanied preferably by lymphadenectomy. Pathological staging, along with a mitotic index more than Ki-67 (MIB1), appears to be the most significant prognostic factor at the univariate analysis.

Keywords: Atypical carcinoids • Tumourlets • Multicentric forms • Surgery • Chemotherapy • Adjuvant therapy

[†]Presented at the 21st European Conference on General Thoracic Surgery, Birmingham, UK, 26–29 May 2013.

INTRODUCTION

Although primary atypical carcinoid (AC) of the lung is a relatively uncommon neuroendocrine tumour (NET), accounting for <1% of all resected cancers [1–5], it represents one of the most challenging neuroendocrine diseases in terms of diagnosis and treatment. In the past, several studies were published to better categorize this tumour. Most of the papers try to put forth useful concepts with debatable results [5–9].

The current classification published by the World Health Organization (WHO) in 2004 [10] encompasses a broad spectrum of neuroendocrine histotypes that range from a low-grade typical carcinoid (TC), intermediate-grade AC, to two high-grade malignancies such as large-cell neuroendocrine carcinoma (LCNEC) and small-cell lung carcinoma (SCLC).

Compared with the TCs of the lung, which represent over 80% of the NETs of the lung, ACs have a worse prognosis, frequently metastasizing not only locally to the intraparenchymal and hilar lymph nodes, but also distally to other organs through the blood stream [11]. Therefore, not infrequently, the misdiagnosed and often unrecognized AC represents a grey zone for the pathologist [12]. Hence, questionable results after surgical treatment, along with possible adjuvant chemo with/without radiation therapies adopted in metastatic diseases, occur in the actual management of this disease [5, 13]. The recently published clinical and pathological TNM staging from the American Joint Committee on Cancer (AJCC) and the International Association for the Study of Lung Cancer (IASLC) tries to clarify most of the addressed problems [6, 14].

The aim of this paper was to retrospectively investigate the clinical and biomolecular prognostic factors associated with the surgical approaches and outcomes of 247 patients affected by lung primary AC in a multi-institutional experience. In particular, we considered the demographic, clinical data, surgical and pathological outcomes along with a subgroup of patients who received adjuvant and/or neoadjuvant therapy.

MATERIALS AND METHODS

Patients

We retrospectively evaluated 1458 patients with primary carcinoid tumours of the lung from March 1980 to December 2010 in a multi-institutional national data set created in 2010 after the approval of the University and Hospital Research Ethical Boards. Initially, 231 of 1458 (15.8%) patients were identified, in whom diagnosis of AC had been posed after surgery. The pathological reports along with the slides were then reviewed by the Multi-institutional Italian Pathology Group and 33 of 1458 (2.26%) specimens were converted from TC to AC. Seven patients out of 231 (3%) with AC changed to TC histotype, and 10 of 264 (4.32%) patients affected by AC were upgraded to high-grade neuroendocrine malignancies of the lung. Pathological diagnoses of mixed, combined or synchronous ACs with other neuroendocrine histotypes or with non-small-cell lung cancer (NSCLC) were not considered in this study. Overall, 247 of 1458 (16.8%, 124 female and 123 male) patients were identified as AC according to the 2004 WHO classification [10]. Medical records of all ACs were then reviewed. All patients underwent pre-treatment staging procedures, including clinical examination, radiological and invasive procedures, for diagnostic purposes.

Surgical procedures and adjuvant therapies

According to clinical staging, 247 patients were grouped as: sublobar resections (SURG1), lobar resections (SURG2), tracheobronchoplastic procedures (SURG3) and pneumonectomies (SURG4). Four groups were stratified according to the presence or absence of intraoperative nodal dissection.

The European Society of Thoracic Surgery (ESTS) guidelines for intraoperative lymph node staging for NSCLC [15] were adopted accordingly, except for a group of patients who were defined at the pathological staging as (pNX), because no lymph nodes were taken during surgery (NX).

Hence, we divided the lymphadenectomy procedures as: no lymph nodal dissection (NX), lymph nodal sampling (Nsmpl), systematic node dissection (Nsyst) and lobe-specific systematic node dissection (Nlobspec). Neither elective lymph node biopsy nor extended lymph node dissection were performed in any of the 247 patients.

The Oncologists and Radiotherapy Colleagues considered the option of adjuvant chemo/radiation therapy based on age, co-morbidities, subsequent carcinoid syndrome and extension of disease. Neoadjuvant chemotherapy was approved in patients with initial diagnosis of SCLC at fine needle aspiration biopsy.

Pathological study

The Multi-institutional Italian Pathology Group reviewed all available pathological materials pertaining to the selected 247 patients. Immunohistochemical analysis with chromogranin, synaptophysin and CD56 was performed in all cases to confirm the neuroendocrine differentiation. Labelling index with Ki-67 (clone KiMIB1, Dako, Glostrup, Denmark; 1:200 dilution with microwave antigen retrieval) was performed on 211 of 248 (85%) accessible paraffin-embedded blocks.

The indications for pre- and postoperative chemo-radiotherapy varied in the considered period and according to the centre experience.

Statistical methods

For univariate analysis, the χ^2 (frequency >5%) test or Fisher's exact test (frequency <5%) was used for the comparison of the categorical variable, and the Mann–Whitney *U*-test for the comparison of continuous variables. Overall survival analysis was performed using the Kaplan–Meier method and compared using the log-rank test. Survival was calculated from the date of surgery to the last follow-up date or death. The evaluated parameters included age, mitosis, Ki-67 (MIB1), gender, smoke, 7th edition of TNM staging, laterality, type of surgery and type of lymphadenectomy. Data is expressed as a median and range when the sample numbers expressed were those of a population or continuous data (i.e. months), or as average ± standard deviation when the sample analysed was measured in units.

A significant difference was predetermined to be a *P* -value <0.05. For multivariate analysis, a Cox regression model was used with a forward stepwise selection of covariates. These analyses were performed using SPSS version 15.0 (SPSS GmbH, Munich, Germany).

RESULTS

Demographics and clinical findings

Demographic and clinical data of 247 patients are described in Table 1. In our multicentric series, presenting symptoms were invariably respiratory-related in central forms. Signs and symptoms were rarely associated with peripheral forms. Over half of the patients (136 of 247, 55.06%) had a smoking history. Passive smoking was not reported in clinical charts. At multivariate analysis, age [$P < 0.001$, hazard ratio (HR) 0.60, CI 0.32–1.12] and smoking habits ($P = 0.002$; HR 0.43, 95% CI 0.23–0.80) were significant, while no noteworthy differences between gender, tumour size and location were identified. Prognostic factors of age and smoking history were significantly evident also at the survival probability curves (Fig. 1A and B).

Two patients presented with Cushing syndrome, while 1 had a pulmonary AC incidentally discovered during the diagnostic work-up for his acromegaly. Another AC lesion was identified during a screening for multiple endocrine neoplasia Type 1. Carcinoid syndrome was observed in 4 patients with a peripheral AC, lung lesion. This subgroup of 8 patients in whom endocrine patterns were associated with AC was not considered for univariate and multivariate statistical analyses due to the small size.

Survival analysis based on surgical treatment and lymph Nodal dissection

Two-hundred and forty-seven patients underwent surgery for AC from 1980 to 2010. The overall survival probability analysis of the AC was 86.7% at 5 years, 72.4% at 10 years, 64.4% at 15 years and 58.1% at 20 years.

All surgical procedures were divided into four groups: SURG1 ($n = 38$), SURG2 ($n = 181$), SURG3 ($n = 15$) and SURG4 ($n = 14$). The outcome of bronchoplastic procedures (SURG3) appears slightly better in the survival curve (Fig. 4A) than that of lobectomy and sub-lobar resection groups (SURG1 and SURG2), although the number of patients is relatively small. Conversely, the data obtained with SURG2 and SURG3 compared with pneumonectomy (SURG4) were significantly better at the univariate analysis. A better outcome was noted by comparing sub-lobar resection (SURG1) with lobar resection (SURG2) according to the type of lymphadenectomy (Table 2).

Table 1: Cox proportional hazard analysis of the demographics, clinical and diagnostic characteristics of 247 patients surgically treated for primary atypical carcinoid of the lung

		Univariate *P*-value	Multivariate *P*-value	HR (95% CI)
Gender				
Women	124	0.107	NS	
Men	123			
Age				
<50	70	<0.001	<0.001	
50–70	126			0.15 (0.06–0.40)
>70	51			0.60 (0.32–1.12)
Social history				
Tobacco use	136 (55%)	0.002	0.008	0.43 (0.23–0.80)
No smoking	112 (45%)			
Paraneoplastic syndromes				
MEN-1	1 (0.4%)			
Cushing	2 (0.8%)			
Carcinoid Syndrome	4 (1.6%)			
Acromegaly	1 (0.4%)			
Radiological findings				
Laterality	134R/113L	0.190	NS	
Central	119	0.703	NS	
1980–90	6			
1991–2000	35			
2001–10	78			
Peripheral	128		NS	
1980–90	6			
1991–2000	38			
2001–10	84			
Right/left upper lobe	47R/53L	0.157	NS	
Middle lobe	32			
Right/left lower lobe	49R/50L			
Main bronchus	7R/10L			

MEN-1: multiple endocrine neoplasia Type 1; NS: not significant; CI: confidence interval; HR: hazard ratio.

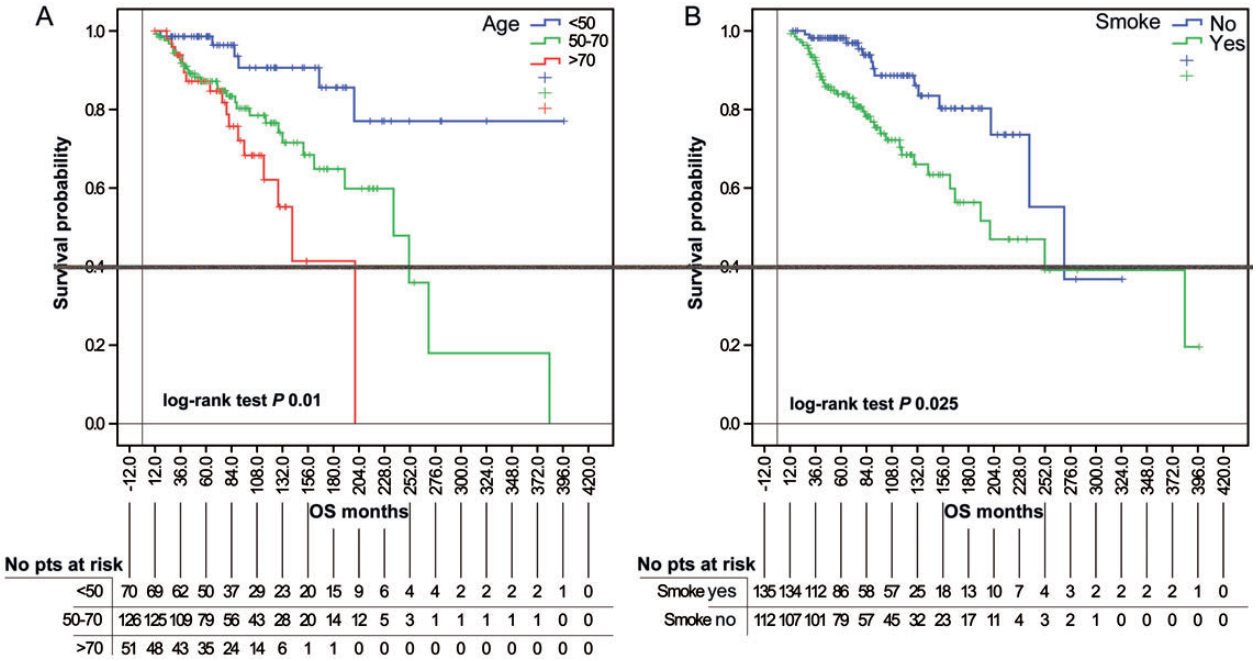

Figure 1: Kaplan–Meier survival probability estimates of age (**A**) and smoking habit (**B**) of 247 AC patients surgically treated. OS: overall survival; pts: patients.

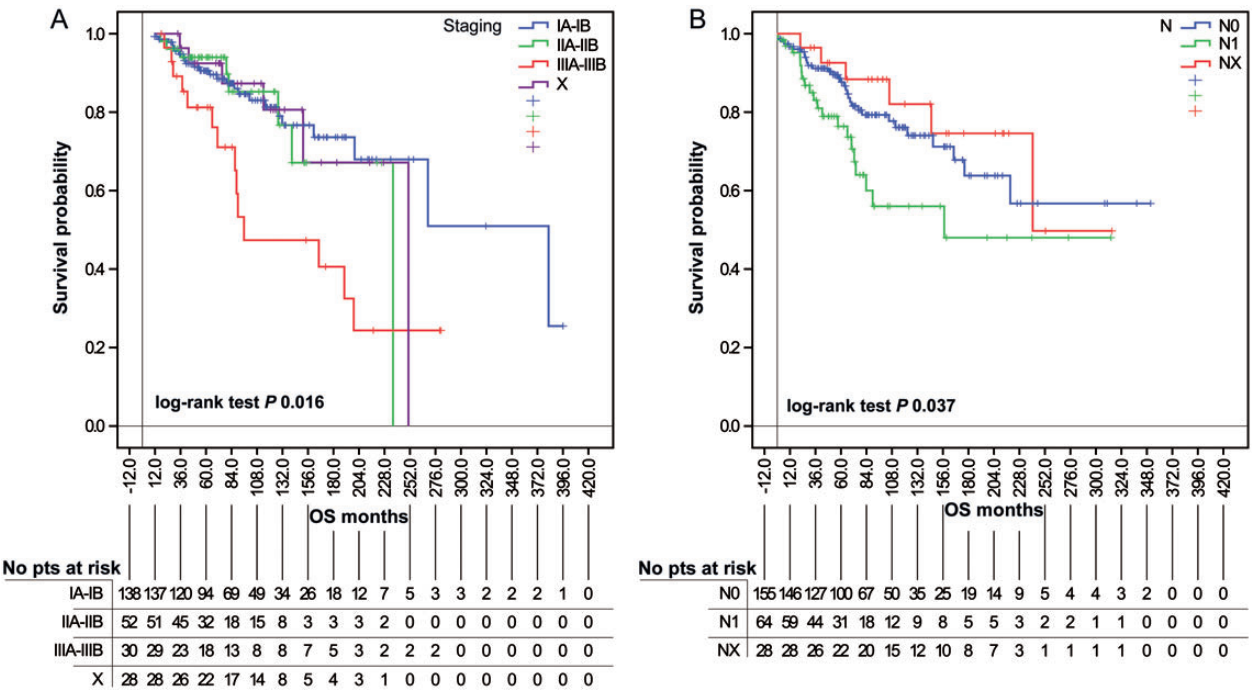

Figure 2: Kaplan–Meier survival probability estimates according to the 7th Staging System (**A**) and to the lymph nodal AC metastatic involvement (**B**). The group called X (**A**) and NX (**B**) are the undefined groups of patients that did not have any lymph nodes taken during surgery.

Lymphnodal dissection was performed in 220 of 248 (88.7%) surgical procedures and 64 of 220 (29%) patients were positive for metastatic cells (N1, *n* = 36; N2, *n* = 27; N3, *n* = 1) in the collected lymph nodes. Distant metastases occurred in 22 of 64 (34.3%) patients with pathological lymph nodal involvement (pN+). Higher percentage of pN+ was discovered when all the mediastinal tissue containing the lymph nodes was dissected and removed systematically within anatomical landmarks (Nsyst, 39.7%; Table 2) [16]. The distant metastatic disease in Nsyst was present in 8 of 35 pN+ (22.5%) patients at the follow-up and 12 of 35 pN+ (34.2%) died as a consequence of metastatic disease or from complicated adjuvant treatment.

In Table 2, the surgical procedures, divided by groups, are compared with the type of lymph nodal dissection. Nsyst or Nlobspec, matched with lobectomy or bilobectomy (SURG2), had a higher percentage of distant metastatic disease than loco-regional recurrence. Conversely, Nsmpl resulted in an increased risk of loco-regional recurrence. Although the percentages of the relapse

Table 2: Contingency table of the impact in the follow-up of 247 surgical resections for primary atypical carcinoids of the lung with or without lymph nodal dissection [15]

	Nx_	pN+ (%)	Nsmpl	pN+ (%)	Nsyst	pN+ (%)	Nlobspec	pN+ (%)	Total% N+/surgical procedures [% relapse]
SURG1	17	0	**14**	2	**4**	1	**3**	1	10.5
Relapse at follow-up (%)	3 (17.6%)	(–)	3 (21.4%)	(14.2)	0	(25)	0	(33.3)	[15.7]
L(ocal)/D(istant)/PL(eural)	2 AWED-D, 1 DDR-L/D		1 WED-PL, 1 WED-D, 1 DDR-D		–		–		
SURG2	10	0	**56**	12	**68**	26	**48**	7	19.3
Relapse at follow-up (%)	2 (20%)	(–)	15 (26.7%)	(21.4)	12 (17.9%)	(38.2)	17 (35.4%)	(14.5)	[25.4]
L(ocal)/D(istant)/PL(eural)	1 AWED-D, 1 DDR-L/D		4 AWED-L, 7 AWED-D, 4 DDR-D		4 AWED-D, 8 DDR		1 AWED-L,4 AWED-D, 12 DDR		
SURG3	1	0	**2**	1	**6**	3	**6**	3	46.6
Relapse at follow-up (%)	1 (100%)	(–)	0	(50)	2 (33.3%)	(50)	1 (16.6%)	(50)	[26.6]
L(ocal)/D(istant)/PL(eural)	1 DDR L/D				1 AWED-D, 1 DDR		1 AWED-L		
SURG4	0	0	**3**	1	**11**	7	–	–	57.1
Relapse at follow-up (%)		(–)	1 (33.3%)	(33.3)	6 (54.5%)				[50]
L(ocal)/D(istant)/PL(eural)			1 AWED-D		3 AWED-D, 3 DDR				
Total lymph nodal dissection [Relapse]/N+ (%)	28 [21.4%]	0 (–)	75 [25.3%]	16 (21.3)	88 [22.7%]	37 (42)	57 [31.5%]	11 (18.6)	Total **64/220**[a] (29%)

In the grey highlighted area are the surgical procedures with lymphnodal dissection.
HR: hazard ratio; SURG1: sub-lobar resection; SURG2: lobectomy/bilobectomy; SURG3: bronchial resection; SURG4: pneumonectomy; NX: no lymph nodal dissection; Nsmpl: lymph nodal sampling; Nsyst: systematic node dissection; Nlobspec: lobe-specific systematic node dissection; pN: pathological lymph nodal report; AWED: alive with evidence of disease; DDR: death disease-related.
[a]Total of the surgical procedures with lymph nodal dissection.

events are similar between lymph nodal techniques (total on the horizontal axis at the far lower side in Table 2), once we compare them with the surgical procedures (total on the vertical axis at the right-hand side in Table 2), the percentage of relapse appears to be increasingly higher between groups, due to the lymph nodal metastatic disease (pN+) on the surgical specimens.

No dissection of the lymph nodal stations (NX), in mostly T1a lesions (66.6%, 18 of 27 NX), showed distant metastatic disease or death-related disease in 21.4% at follow-up. The decision of not performing lymph nodal dissection concerned mainly peripheral lesions (mean 19 ± 0.9 mm) considered initially as typical carcinoid at the first diagnosis (77.7%, 14 of 18 patients) or solitary metastases from other distant primary cancer with the absence of nodal involvement at radiological work-up and intraoperative appearance (22.3%, 4 of 18 patients). This group consisted mainly of those with sub-lobar (60.7%) and lobar resections (35.7%) with a follow-up of 99.9 (range 33.4–251.2) months.

In the subgroup of patients who underwent Nsmpl, the local recurrence rate (Table 2) was similar between sub-lobar resection (SURG1, 3 of 14 patients, 21.4%) and lobectomy (SURG2, 15 of 56 N+ patients, 26.7%). Their outcome is described in Table 2.

Pathological staging, mitosis, Ki-67 (MIB1) clone and multicentric forms

All 247 patients affected by primary AC of the lung were macroscopically and microscopically with no residual tumour (R0) at the pathology report. The data analysis was initially evaluated in three time-frame periods such as 1980–98 ($n = 82$), 1999–2003 ($n = 61$) and 2004–2010 ($n = 104$). Although all three cohorts were similar in number of patients, the result did not show any significant differences among groups (log-rank test, $P = 0.627$). These periods of time were mainly assessed between the older classification based on Arrigoni (1980–98) [7] the period of transition between Travis and the Lyon Consensus Conference (1999–2003) [10] and the new WHO classification (2004–2010) [10]. Also, no differences were identified between surgical subgroups, when compared with the three time frames described above ($P = 0.723$).

Tumour-specific variables, like anatomical location, are described in Table 1. Pathological staging and lymph node involvement are illustrated, along with mitosis and the Ki-67 (MIB1) proliferative index, in Table 3. Their survival probability is described in Fig. 3A and B.

Tumour size, lymph nodal metastatic involvement, pathological staging, mitotic index and Ki-67 (MIB1) were all significant at the univariate analysis. Lymph nodal metastatic involvement was also significant at the multivariate analysis ($P = 0.008$; HR 0.46, CI 0.26–0.82, Fig. 2B). A limited group of patients with pNX ($n = 27$) at the pathological report had a low Ki-67 (MIB1) proliferative index (3.8 ± 2.5%) and relatively smaller size (21.8 ± 1.2 mm) of the primary resected AC lung lesion.

One patient was defined to be Stage IV, according to the 7th TNM staging, for his bilateral AC incidentally discovered during a follow-up for melanoma. One patient, who was operated on with a synchronous brain metastasis treated by radiation therapy, died

Table 3: Cox proportional hazard analysis of the pathological findings of the 247 patients affected by primary atypical carcinoid of the lung

		Univariate *P*-value	Multivariate *P*-value	HR (95% CI)
TNM pathological findings				
T size (mm)	30 ± 15	0.023	NS	
N0	156	0.016	0.008	0.46 (0.26–0.82)
Nx	28			
N+	64			
pStage 7th edition				
IA	84	0.049	NS	
IB	54			
IIA	41			
IIB	11			
IIIA	26			
IIIB	2			
IV	2			
X	27			
Ki-67 (MIB1)				
<5%	78	0.012	NS	
5–10%	61			
>10%	71			
Mitoses				
2–5	198	<0.001	NS	
6–10	50			
Multicentric forms		Percentage	Follow-up status	
Synchronous multicentric forms	12/247	4.8	8 ANED; 3 AWED; 2 DDR	
Multicentric carcinoids	4*/12	33.3	2 ANED; 1 AWED; 1 DDR	
Tumourlets	9*/12	75	6 ANED; 2 AWED; 1 DDR	

Stage X undefined group of patients that did not have any lymph nodes taken during surgery.
*One multicentric form had both histological findings of micro-carcinoids and tumourlets in the same specimen.
ANED: alive with no evidence of disease; AWED: alive with evidence of disease; DDR: death disease-related; NS: not significant; CI: confidence interval; HR: hazard ratio.

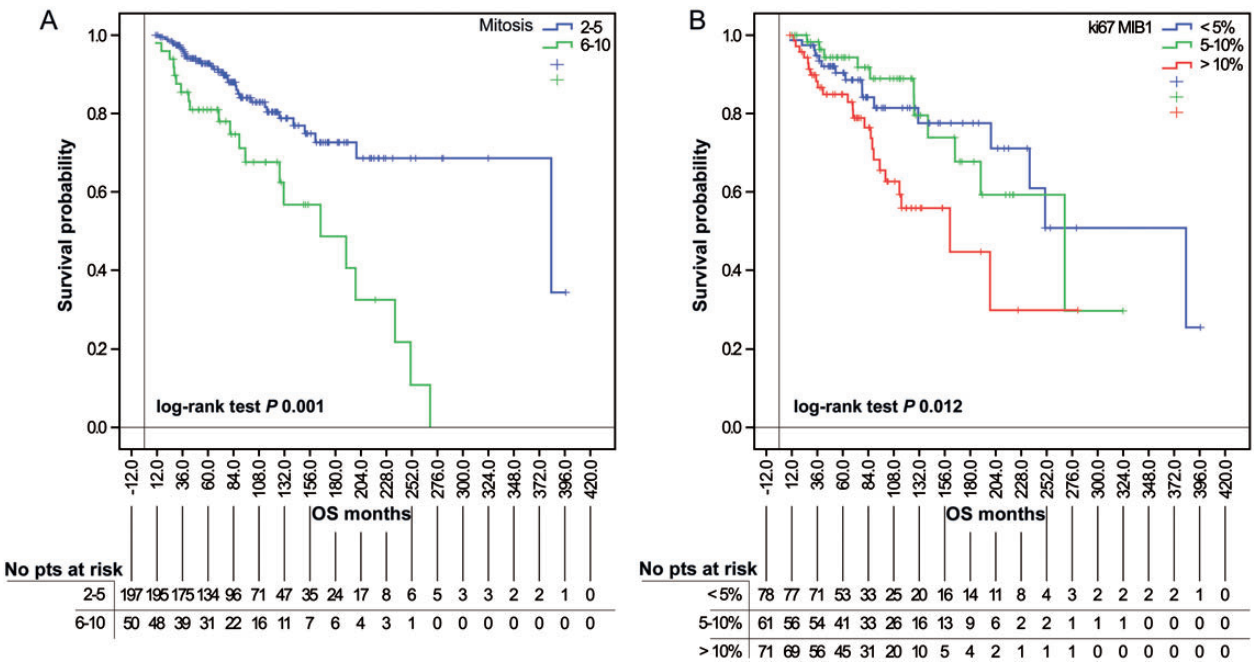

Figure 3: Kaplan–Meier survival probability estimates according to the mitosis (**A**) and Ki-67 (MIB1, **B**) index. OS: overall survival; pts: patients.

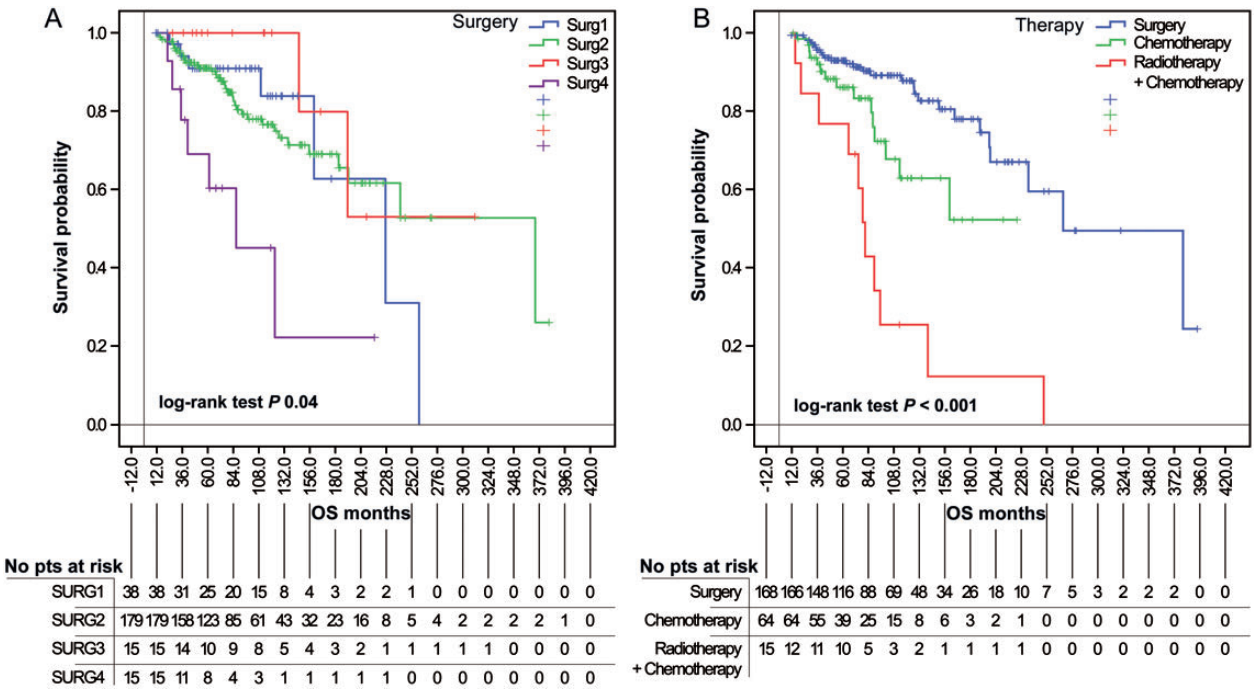

Figure 4: Kaplan–Meier survival probability estimates of the surgical approach (**A**) and the adjuvant therapy (**B**). SURG1: sub-lobar resection; SURG2: lobectomy/bilobectomy; SURG3: bronchial resection; SURG4: pneumonectomy; OS: overall survival; pts: patients.

of her disease 48 months after surgery. This aggressive trend was confirmed at the pathological staging Kaplan–Meier probability study, where the 2 patients with Stage IV grouped along with Stage IIIA and IIIB had significantly worse symptoms than other staging classes (Fig. 2A).

Multicentricity was identified in a small group of patients (12 of 247, 4.9%; range 11.2–200.4, median 98.7 months), not sufficient for the Kaplan–Meier statistical analysis, but it correlates, however, with a worse prognosis at follow-up. Two patients with disease-related death were with distinct multicentric forms in the same lobe and 3 with tumourlets are alive with evidence of distant metastatic disease, while 8 of 12 (66.6%) patients are alive without evidence of disease.

Adjuvant chemo with/without radiation therapies and survival analysis

Chemo with/without radiation therapies were adopted in 74 patients and their survival probability is described in (Fig. 4B). Neoadjuvant chemotherapy was chosen in 6 of 247 (2.8%) patients who had an initial diagnosis of SCLC at fine-needle aspiration biopsy. Adjuvant chemotherapy was adopted in 55 of 247 patients (22.2%) and another 6 of 247 (2.8%) received adjuvant radiation therapy alone, while another 7 of 247(%) received adjuvant radiation therapy together with chemotherapy. Initially, 21 of 74 (28.3%) patients were given adjuvant therapy for metastatic pathological N disease, while 30 of 74 (40.5%) underwent adjuvant therapy for distant metastatic disease discovered during their follow-up. The results of radiotherapy alone (6 of 74, 9.4%) or as synergistic treatment with chemotherapy (7 of 74, 6.7%) resulted in a worse prognosis in terms of morbidity and mortality during the follow-up (Fig. 4B).

DISCUSSION

Several clinical, pathological and therapeutical messages are derived from the analysis of our data and, due to the different features involved, it is more appropriate to analyse and discuss them in terms of: patient demographics and clinical outcome, therapeutic prognostic factors, pathological diagnosis and outcome.

Patient demographics and clinical outcome

No predominance of gender has been found in our cohort of patients affected by primary AC of the lung. The male/female ratio was near to 1 (0.99/1) in agreement with other reported series [1–4]. Furthermore, increasing age at diagnosis, which appears to have a worse prognosis in our series, was a negative predictive factor in another large series of 145 patients with AC collected from the SEER database [4].

The significant data on the prevalence of the smoking habit at multivariate analysis confirm what has been previously published in smaller single-institutional studies that evaluated pulmonary AC and their survival patterns [5]. The documentation collected in our study considers only patients with a past history of cigarette smoking or who were current smokers at surgery. Passive smokers were not included and their percentage might exponentially increase and consolidate this significant prognostic factor.

There is a slight but not statistically significant predominance of peripheral forms compared with central forms in our study, and this incidence has steadily increased during the past 30 years (Table 1). Detailed collected clinical and radiological data correlate with the improvement of the radiological techniques [5, 11].

Furthermore, since the advent of new cancer screening tools, in the past two decades, and the establishment of multidisciplinary teams, the frequency of incidental findings of AC of the lung has increased dramatically [1–4, 11, 12].

Paraneoplastic syndrome (PS) in atypical carcinoids of the lung is rare at initial clinical evaluation and usually associated with metastatic disease [4, 5]. In our cohort of patients, PS was present in 3.2% of all patients affected by primary AC of the lung at the time of preoperative work-up (Table 1). The most common PS in our series is the carcinoid syndrome (1.6%), which appears with higher incidence than the findings published in the literature [5, 11]. Among available studies with over 50 patients, carcinoid syndrome occurred at presentation in only 0.7%. All patients in our retrospective series who had PS with lymphnodal metastatic involvement at the pathological report appear to have also distant metastatic disease at follow-up (median 40.8 months, range 24.8–64). Two patients died, while the other 2 survive, free from clinical signs and on long-acting analogues of somatostatin.

The prevalence of Cushing syndrome (CS), which occurs in ∼1–6% of patients with pulmonary carcinoid tumours, appears to be fairly similar in our series (0.8%) if we consider that the incidence rate of atypical carcinoid usually is close to 20% of all described cases of CS lung carcinoids in the literature [5, 11]. All patients in our series were with N0 disease, which is a discordant finding compared with the published data, where nearly 60% of CS patients have lymph node involvement. Acromegaly, which has been rarely reported [5, 11], was present in 1 case (0.4%) in our series and resolved after surgical resection.

Therapeutic prognostic factors

Although adjuvant chemo with/without radiation therapies show some potential benefit, surgery still represents the gold standard for treating AC [5, 14, 16–18]. Several papers published in the past decade clearly outline the benefit of surgical treatment [5, 14, 16–18]. Since the advent of minimally invasive surgery in the early 1990s and the recent aid of robotic surgery, the best surgical treatment for primary lung carcinoid tumours represents a crucial test for the surgeon. Furthermore, the renaissance of anatomical sublobar resections as a possible alternative tool for the low-grade NETs of the lung has given more options to the surgeon, but also expanded the boundaries towards questionable new areas of interest [5, 16–18].

The role of limited resection is clearly stated in the literature as an alternative in patients with inadequate lung function due to a lobar resection and/or cardiovascular impairment [5, 16, 18]. In our retrospective analysis, the sub-lobar resections showed a similar outcome to the lobar resection in survival probability and the lesion resected was mostly a T1a or T1b nodule (29.9 ± 15.3 mm). Although the cohort of patients treated with these conservative approaches was slightly younger than other published experiences with a median age of 57.7 (range 25–82 years), the data are biased by the numerosity of the sample analysed and the shorter time of follow-up compared with the lobectomy group. Therefore, precaution should be taken before adopting this procedure as a standard of practice for clinical Stage 1A and 1B lesions. A prospective randomized study can clearly address most of the questions that are still pending before considering sublobar resections a valid alternative to the standard lobectomy in younger patients. From our multi-centre retrospective study, data emerge that the preferred treatment of choice for primary AC lesion of the lung was standard lobectomy with lymph nodal dissection. Bronchoplastic procedures with or without lung parenchyma resection (i.e. sleeve lobectomy) when feasible yield a better outcome in a limited number of patients treated for a centrally AC located lesion. The association of the two surgical procedure groups, lobar and bronchoplastic resections, with positive lymph nodal dissection was 73.4% of the total, and 93.4% of this subgroup of patients had a relapse distally in other organs at follow-up. Compared with bronchoplastic and lobar resection, pneumonectomy should be least preferred. Although lymph nodal dissection in this subgroup partially helps the outcome, the high rate (50%) of distant metastatic disease and disease-related death at follow-up clearly underline the worse prognosis. Our multicentre study supported by available data in the literature that concern N+ cases [5, 17] and clearly underline that lymph nodal dissection in lobar resections, as well as in pneumonectomies, plays a key role as a prognostic factor for the correct management of lung AC [5, 17, 18].

Hence, several terms were utilized in the past to define lymph nodal dissection and have often been the subject of dispute or created confusing caveats when associated with surgical resection. In our multi-institutional series, we mostly perform standard lobectomy with lymphadenectomy (Table 2) including at least three anatomical lymph nodal stations (but always sub-carinal). To clarify our approach, we categorized the lymph nodal dissection according to the ESTS guidelines for intraoperative lymph node staging for NSCLC. Atypical carcinoids have a greater tendency to metastasize distally (16–23% in large series) and to recur locally (3–23%) [4, 5, 17]. Five-year survival rates range widely from 30 to 95%; the corresponding rates at 10 years are 35–56% [4, 5, 11, 17]. The overall probability survival rates of the 247 AC patients at 5 and 10 years correspond, respectively, to 86.7 and 72.4%, while at 15 and 20 years these percentages are slightly reduced (to 64.4 and 58.1%, respectively).

In our series, the tendency to metastasize to lymph nodes was evident at surgery and at follow-up. While we had a 29.9% of N+ at the time of pathological diagnosis, the prevalence rates of developing metastatic disease at follow-up was 25.9% (64 of 247) (Fig. 5) and 10.9% (7 of 64), involving the remaining lymph nodes not resected at the time of surgery. These results strengthen the

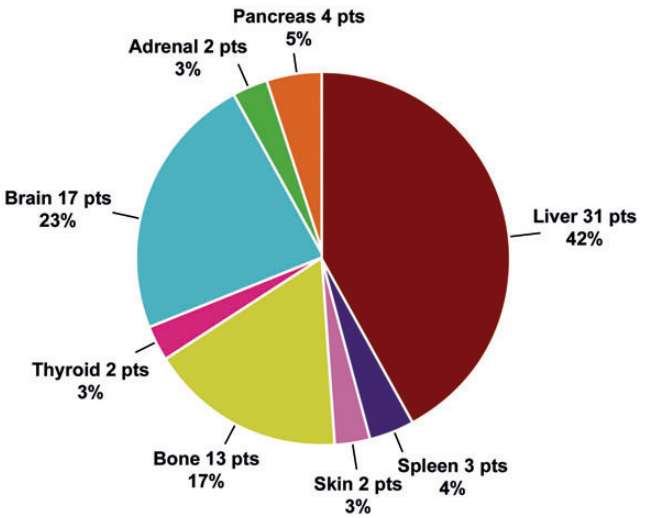

Figure 5: Sites of metastatic recurrence at the follow-up of primary pulmonary atypical carcinoid according to the 7th AJCC-TNM classification. Pts: patients.

statement that nodal positivity might be a prognostic factor also for vascular invasion by the AC, and therefore, a close follow-up is strictly advised. Positive Nsmpl in a sublobar resection usually required a completion lobectomy and nodal dissection.

Another important result was the percentage of metastatic cancer spreading through the blood stream to other organs that was dramatically higher (55 of 64, 85.9%) than locally to the intrapleural space or hilar structures such as the pericardium (16 of 64, 25%). Conversely, the pNX group behaves similarly to the pN0 group with regard to the survival probability in this study. These results can be explained primarily by the conversion of typical to atypical carcinoid at the pathology review (77.7%, 14 of 18 pT1a patients), the absence of N+ at the clinical work-up, the size of the tumour resected, that was usually <20 mm, and the low mitotic index of the primary AC lesion.

Lastly, adjuvant therapies to surgery such as chemotherapy and/or radiation therapy, as already published in the literature [5, 13, 19], did not clearly support the treatment in terms of complete regression of the metastatic disease aside from alleviating clinical signs and symptoms.

In our 74 patients treated by adjuvant therapy, we had a worse prognosis when radiation therapy was utilized alone or together with chemotherapy. Only in 29.5% of the patients with metastatic disease, chemotherapy alone appears to be a useful tool to reduce the progression rate of the disease.

Pathological diagnosis and outcome

After the first endobronchial resection of a histologically proven carcinoid tumour, done by Chevalier Jackson in 1914, the malignant behaviour of the lung carcinoid tumour was perceived or assumed by several authors in their study during the following three decades. Despite the first published reports, the higher grade and more aggressive type of carcinoid tumour were clearly described by Engelbreth-Holm in 1944. Hence, Arrigoni *et al.* [7] first defined the atypical carcinoid (AC) as a primary pulmonary neoplasm with an increased mitotic rate (five to ten mitoses per 10 high-power fields), necrosis, pleomorphism and disorganized hypercellularity. Since then, many studies were published with debatable data on prognostic factors and therapeutic treatments [7, 8]. Careful application of Arrigoni's criteria enables a proper separation of the AC not only from the typical carcinoid (TC), but also from the more aggressive forms of the NETs of the lung such as LCNEC and SCLC. In 1998, Travis *et al*. refined the above diagnostic criteria by including tumours of a lower mitotic index (between 2 and 10 per 2 mm^2 of viable tumour per 10 high-power fields) or coagulative necrosis [8, 9]. Hence, the modified NET 2004 WHO classification based on Travis *et al*. represents the cornerstone of actual pathological and clinical practice [10].

Multicentric forms are described in the literature more with typical than atypical carcinoids and in centrally located forms [5, 12]; our series shows 5.2% (13 of 247) of multicentric forms equally distributed among central and peripheral synchronous AC. Their presence might, in some way, be related to chronic lung damage and hypoxia, conditions known to elicit neuroendocrine cell hyperplasia [12, 20]. Since first being discovered in 1955 by Whitwell, and thereafter defined in the systematic study done by Cunningham *et al*. [21] in 1958, despite the WHO 2004 classification, the occurrence of multicentric forms is still a matter of debate [10, 12]. One of the major points of dispute is whether they can be called microcarcinoids (tumourlets) or if they represent metastatic disease rather than solitary, independent neuroendocrine foci [22, 23]. Several small reported series are published in the literature and none of them clearly answers these issues [11, 23]. A better in-depth analysis of this group in a large prospective series is strongly expected in the near future and possibly they would be better delineated in the new forthcoming neuroendocrine classification.

Although in the last decade we have witnessed tremendous improvement in new diagnostic technology tools [12, 23], a new neuroendocrine pathological classification is still currently under discussion. The ample panel of immunohistochemistry biomolecular markers and the recent ancillary technologies, such as proteomics, microarrays and molecular genetics, leave the problem unresolved [23]. Nowadays, it is still quite unclear whether the new forthcoming neuroendocrine pathological classification will be clarifying these pending problems. Recently, a few experts in the field have proposed to focus their attention to a classification similar to NET gastro-entero-pancreatic (NET-GEP) and to consider mostly Ki-67 (MIB1) along with the mitotic index [24].

The actual WHO 2004 classification of tumours of lung and thymus, unlike NETs arising from other sites such as gastrointestinal tract and pancreas, does not recognize the value of Ki-67 (MIB1). The role of the Ki-67 (MIB1) clone appears not to be the best prognostic factor in our series, particularly for patients with a Ki-67 (MIB1) lower than 5% in the first 5 years of follow-up. Discrepancies between the marker and the survival outcome have also been seen recently in the literature and in Consensus Conferences [11, 23]. One of the possible problems is how to collect the specimen immediately after surgery [23]. It is well known to our pathologists that the length of fixation with formalin affects the ability to detect antigens by immunohistochemistry and prolonged formalin fixation has a significant negative impact on the ability to detect Ki-67 (MIB1) [25].

It appears therefore necessary to create a larger prospective study with standardized pathological tissue procurement that provides better levels of evidence. These are fundamental before we definitely conclude that the Ki-67 (MIB1) index is not a useful test in the evaluation of pulmonary carcinoids.

Furthermore, creating an international tissue banking archive with a homogeneous protocol for specimen procurement might enhance not only a correct practice, but also clarify the standard of care between National and International Centres. Having a sizeable number of tissue samples archived as well as automated analysis protocols for tissue microarrays that support the pathologist's work can enhance a system of a proper panel of biomolecular markers that better defines not only the surgical management, but also the potential target of a tailored adjuvant therapy.

CONCLUSIONS

In conclusion, our data suggest that lung AC is a malignant NET with a worst outcome in patients over 70 years and in smokers. With the exception of pneumonectomy, the extent of resection does not seem to affect survival if associated with lymph nodal dissection.

Although sub-lobar resections appear to be, in our retrospective study, effective for AC lesions <20 mm, in the absence of clinical N+, prospective and multi-institutional studies are required to better understand the role of lung-parenchymal sparing resection compared with lobar and bronchoplastic procedures. Furthermore, the role of multicentric forms and their potential aggressive

behaviour along with the AC will need to be clearly defined in larger case studies and possibly outlined in the new forthcoming neuroendocrine classification. Pathological staging along with the mitotic index, more than Ki-67 (MIB1), appears to be the most significant prognostic factors. In our retrospective study, only standard adjuvant chemotherapy was found to be better compared with radiotherapy, as a support treatment for pathologically proven, surgically resected, primary lung AC with node positive and/or distant metastatic disease. Future tailored chemotherapeutic agent, along with surgery, might reduce dramatically the follow-up relapse of this dreadful disease.

ACKNOWLEDGEMENTS

We would like to greatly acknowledge Rosanna Capozzi, Valentina Tassi, Vincenzo Pagliarulo and Marco Ghisalberti for their invaluable help in collecting and constantly keeping updated the data during these past years.

Multi-institutional Italian Pathology Group: Fiorella Calabrese (Padova); Anna Sapino, Luisa Delsedime (Torino); Paolo Graziano (Roma); Greta Alì, Gabriella Fontanini (Pisa); Angelo Sidoni, Stefano Ascani (Perugia-Terni); Xenia Trabucco (Bari); Donatella Sina (Siena); Gianluigi Arrigoni (Milano); Giulio Rossi (Reggio Emilia); Claudio Agostinelli (Bologna).

Funding

Niccolò Daddi and Francesco Puma were supported by a grant from the Italian Minister of Research and University in Rome (no. 2008LFK7J5). Niccolò Daddi, Francesco Puma, Moira Urbani and Verena De Angelis are part of: (1) the Multidisciplinary Group for Diagnosis and Treatment of Neuroendocrine Tumors, Umbria Regional Cancer Network; (2) European Neuroendocrine Tumor Society (ENETS) Center of Excellence.

Conflict of interest: none declared.

REFERENCES

[1] Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE *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 2008;26:3063–72.

[2] Soga J, Yakuwa Y. Bronchopulmonary carcinoids: an analysis of 1,875 reported cases with special reference to a comparison between typical carcinoids and atypical varieties. Ann Thorac Cardiovasc Surg 1999;5: 211–9.

[3] García-Yuste M, Matilla JM, Cueto A, Paniagua JM, Ramos G, Cañizares MA *et al*. Spanish Multi-centric Study of Neuroendocrine Tumors of the Lung for the Spanish Society of Pneumonology and Thoracic Surgery (EMETNE-SEPAR). Typical and atypical carcinoid tumors: analysis of the experience of the Spanish Multi-centric Study of Neuroendocrine Tumors of the Lung. Eur J Cardiothorac Surg 2007;31:192–7.

[4] Bhatt JM, Young JN, Cooke DT. Comparison of patient survival after resection for pulmonary carcinoid tumors compared to other neuroendocrine tumors: A United States Population Study. Open J Thorac Surg 2012;2:99–103.

[5] Detterbeck FC. Management of carcinoid tumors. Ann Thorac Surg 2010; 89:998–1005.

[6] Travis WD, Giroux DJ, Chansky K, Crowley J, Asamura H, Brambilla E *et al*. International Staging Committee and Participating Institutions. The IASLC Lung Cancer Staging Project: proposals for the inclusion of broncho-pulmonary carcinoid tumors in the forthcoming (seventh) edition of the TNM Classification for Lung Cancer. J Thorac Oncol 2008;3: 1213–23.

[7] Arrigoni MG, Woolner LB, Bernatz PE. Atypical carcinoid tumors of the lung. J Thorac Cardiovasc Surg 1972;64:413–21.

[8] Travis WD, Rush W, Flieder DB, Falk R, Fleming MV, Gal AA *et al*. Survival analysis of 200 pulmonary neuroendocrine tumors with clarification of criteria for atypical carcinoid and its separation from typical carcinoid. Am J Surg Pathol 1998;22:934–44.

[9] Beasley MB, Thunnissen FB, Brambilla E, Hasleton P, Steele R, Hammar SP *et al*. Pulmonary atypical carcinoid: predictors of survival in 106 cases. Hum Pathol 2000;31:1255–65.

[10] Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC. (Eds) Pathology and Genetics of Tumors of the Lung, Pleura, Thymus and Heart. Lyon: IARC Press, 2004.

[11] Öberg K, Hellman P, Ferolla P, Papotti M. ESMO Guidelines Working Group. Neuroendocrine bronchial and thymic tumors: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012;23(7S):120–3.

[12] Righi L, Volante M, Rapa I, Scagliotti GV, Papotti M. Neuroendocrine tumors of the lung. A review of relevant pathological and molecular data. Virchows Arch 2007;451(Suppl 1):S51–9.

[13] Kaplan B, Stevens CW, Allen P, Liao Z, Komaki R. Outcomes and patterns of failure in bronchial carcinoid tumors. Int J Radiat Oncol Biol Phys 2003; 55:125–31.

[14] Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. American Joint Committee on Cancer: AJCC Cancer Staging Manual. 7th ed. New York: Springer, 2009.

[15] Lardinois D, De Leyn P, Van Schil P, Porta RR, Waller D, Passlick B *et al*. ESTS guidelines for intraoperative lymph node staging in non-small cell lung cancer. Eur J Cardiothorac Surg 2006;30:787–92.

[16] Fox M, Van Berkel V, Bousamra M II, Sloan S, Martin RC II. Surgical management of pulmonary carcinoid tumors: sublobar resection versus lobectomy. Am J Surg 2013;205:200–8.

[17] Wurtz A, Benhamed L, Conti M, Bouchindhomme B, Porte H. Results of systematic nodal dissection in typical and atypical carcinoid tumors of the lung. J Thorac Oncol 2009;4:388–94.

[18] El Jamal M, Nicholson AG, Goldstraw P. The feasibility of conservative resection for carcinoid tumors: is pneumonectomy ever necessary for uncomplicated cases? Eur J Cardiothorac Surg 2000;18:301–6.

[19] Wirth LJ, Carter MR, Jänne PA, Johnson BE. Outcome of patients with pulmonary carcinoid tumors receiving chemotherapy or chemoradiotherapy. Lung Cancer 2004;44:213–20.

[20] Adriaensen D, Brouns I, Pintelon I, De Proost I, Timmermans JP. Evidence for a role of neuroepithelial bodies as complex airway sensors: comparison with smooth muscle-associated airway receptors. J Appl Physiol 2006; 101:960–70.

[21] Cunningham GJ, Nassau E, Walter JB. The frequency of tumor-like formations in bronchiectatic lungs. Thorax 1958;13:64–8.

[22] Rizvi SM, Goodwill J, Lim E, Yap YK, Wells AU, Hansell DM *et al*. The frequency of neuroendocrine cell hyperplasia in patients with pulmonary neuroendocrine tumors and non-neuroendocrine cell carcinomas. Histopathology 2009;55:332–7.

[23] Klimstra DS, Modlin IR, Adsay NV, Chetty R, Deshpande V, Gönen M *et al*. Pathology reporting of neuroendocrine tumors: application of the Delphic consensus process to the development of a minimum pathology data set. Am J Surg Pathol 2010;34:300–13.

[24] Öberg K. Advances in pulmonary neuroendocrine tumor management. Future Med 2013:2–5.

[25] Walts AE, Ines D, Marchevsky AM. Limited role of Ki-67 (MIB1) proliferative index in predicting overall short-term survival in patients with typical and atypical pulmonary carcinoid tumors. Mod Pathol 2012;25: 1258–64.