RESEARCH ARTICLE

Clinical and pathological factors in pathologic stage IA lung adenocarcinoma: relevance of the micropapillary pattern and tumor necrosis

Authors
Karol De Aguiar-Quevedo[a], Julia Cruz Mojarrieta[b], José Cerón Navarro[c], Nuria MancheñoFranch[d], Carlos Jordá Aragón[c], Miguel Arrarás Martínez[a], Encarnación Martínez Pérez[e], Francisco Vera Sempere[f], Amparo Ruiz Saurí[g], Juan Carlos Peñalver Cuesta[h]

Affiliations
[a] Thoracic surgery department, Valencian Institute of Oncology, Valencia – Spain.
[b] Pathology department, Valencian Institute of Oncology, Valencia – Spain.
[c] Thoracic surgery department, La Fe university and polytechnic hospital, Valencia - Spain.
[d] Pathology department, La Fe university and polytechnic hospital, Valencia - Spain.
[e] Pneumology department, Valencian Institute of Oncology, Valencia – Spain.
[f] Chief of the Department of Pathology, La Fe university and polytechnic hospital, Valencia - Spain.
[g] Department of Pathology, University of Valencia, Valencia - Spain
[h] Chief of the Thoracic surgery department, Valencian Institute of Oncology, Valencia – Spain.

Correspondence
Karol De Aguiar Quevedo
Address: Calle Profesor Beltrán Báguena, nº 8
46009 Valencia - Spain
Phone. +34 96 1114655
e-mail: karol.deaguiar@gmail.com

Abstract

Introduction: The objective was to investigate and correlate clinical and morphological lung adenocarcinoma factors with clinical results in patients treated with surgery in pathological stage IA.

Methodology: An observational, analytical and retrospective study of patients diagnosed with pathological stage IA pulmonary adenocarcinoma was performed and the clinical and pathological variables were analyzed using disease-free survival (DFS) and cancer-specific survival (CSS), in a univariate and multivariate study.

Results: 77.3% of men were found, with an average age of 61.6 (SD 8.5) and 83.2% of smokers and ex-smokers. 93.3% of the patients underwent lobectomy with lymphadenectomy. The mean total tumor size was 21.9mm (SD 6.4) and the size of the invasion was 16.9mm (SD 9.4). The most frequent histological pattern was acinar and the least frequent was micropapillary. A total of 28.6% of patients with tumor recurrence were found, which were distant metastases in 70.6%. The DFS and CSS at 5 years was 74.9% and 82.7% respectively. Clinical-pathological variables were analyzed as prognostic factors in a multivariable model for DFS and CSS. The micropapillary component obtained an HR of 3.9 for DFS and 3.4 for CSS, while the tumor necrosis obtained an HR of 3.4 and 3, respectively.

Conclusions: Presence of the micropapillary component and tumor necrosis were independent factors with poor prognosis in the clinical outcome.

Keywords: lung adenocarcinoma, prognosis, micropapillary, tumor necrosis, lung cancer

1. Introduction

According to the most recently available world data, lung cancer (LC) is the most frequently diagnosed neoplasm and that which causes the most deaths worldwide[1,2]. Despite advances in diagnosis and treatment, LC remains a very poor-prognosis disease with a survival rate below 15%[2–4]. In Spain, survival is reported for patients diagnosed between 2000 and 2007 at 11.7%[3].

Almost the entire survival of this disease is at the expense of surgery. In the last TNM classification (8th edition), T1a-b-cN0M0 tumors (Stage IA1, IA2 and IA3) have a 5-year survival of 90%, 85% and 80%, respectively[5]. However, we ought to forget that a percentage of these patients suffer recurrences and die by metastasis, hence the interest in looking for prognostic factors not contemplated in the current TNM system[6–9].

Currently adenocarcinoma (ADC) is the most frequent subtype among bronchogenic carcinomas, even in smokers[10–12].

The latest lung ADC classification adopted by the World Health Organization (WHO) has been challenging because it is a very heterogeneous neoplasm, in not only its histopathological complexity, but also its molecular, clinical, radiological and surgical terms[13,14]. One major deficiency of the previous WHO 2004 classification was that the mixed subtype comprised more than 90% of all ADCs, with a wide variety of clinical outcomes[15,16]. This new classification has impacted precisely the prognostic value of ADC subtypes according to the presence and proportion of five different histological patterns (lepidic, acinar, papillary, micropapillary and solid), which emphasizes the predominant pattern[5,13,14]. The lepidic pattern, the acinar and papillary invasive patterns and the micropapillary and solid patterns seems to have a better, intermediate and poor prognosis, respectively[13,14]. Even when micropapillary and solid patterns are not predominant, some studies claim that they are associated with a poorer prognosis, a low survival rate and occult nodal involvement[17,18].

Therefore, this work was designed to investigate and correlate clinical and morphological factors of lung ADC with clinical outcomes in patients resected and classified as T1a-b-cN0M0 according to the 8th edition of the TNM classification.

2. Methodology

A retrospective clinico-analytical and observational research project was carried out with cases using medical records from two hospitals.

The included patients had been diagnosed with pathologic stage IA lung ADC according to the 8th edition of the TNM classification and had undergone surgical resection in two institutions, the reference hospital University and Polytechnic "Hospital La Fe" (H. La Fe) between January 1, 1990 and December 31, 2007, and in the monographic cancer centers Valencia Institute of Oncology Foundation (FIVO) during the period from November 1, 2008 to January 31, 2012.

After revising samples, the patients whose pathological anatomy differed from ADC, those on some form of neoadjuvant therapy and any patient with previous malignant neoplasias were excluded from the study. The final study cohort included 119 patients.

The follow-up period went from surgery to relapse or death, and those who survived continued until the study ended on January 1, 2018. Follow-up was carried out during the external consultations held in both hospitals by means of anamnesis and an image scan.

The study was conducted in accordance with the principles of the Declaration of Helsinki and national legal regulations. The protocol was approved by our Hospital's Ethical Research Committee (EC).

Initially 104 patients of the H.U. La Fe were included, and just five patients were ruled out due to different ADC histological pattern (three neuroendocrine tumors and two adenosquamous carcinomas). Four patients were also ruled out due to the absence of a tumor in the paraffin blocks. In the FIVO

institution, 29 patients were selected, and three were excluded due to lack of sample in the paraffin preserved histological blocks and other two patients were excluded due to a distinct malignant neoplasm. Thus, the final cohort of the study was 119 patients

2.1. Pathological study

Tumor, peritumoral and healthy lung tissue samples, fixed in 10% buffered formaldehyde and preserved were included in paraffin blocks, processed samples using an automatic Leica® tissue processor (Germany). Tumor tissue samples were analyzed by ruling out those areas morphologically altered by atelectasis or lung emphysema. Sections3 micron- (μ) thick sections were prepared using a Minot Leica® microtome (Germany) to be mounted on slides with poly-L-lysine. Conventional histological techniques were used for the morphological study. Eosin (Dako®, Denmark) and hematoxylin (Dako®, Denmark) (H-E) were applied. Each sample was submitted to a staining procedure, which was done in a CoverStainer® automatic dyer following the Dako protocol. A Dako Omnis® automatic immunotherapeutic device was employed for the immunohistochemistry (IHC) stains for each primary antibody following the manufacturer's instructions.

When a morphological assessment was poorly defined and doubtful as no clear signs of glandular or squamous differentiation appeared, different IHC techniques were followed: e.g., synaptophysin (monoclonal antibody, DAKO®, Glostrup, Denmark); p63 (monoclonal antibody, Abcam®, Cambridge, UK); TTF1 (monoclonal antibody, DAKO®, Glostrup, Denmark); chromogranin (polyclonal antibody, DAKO®, Glostrup, Denmark); CK 5/6 (monoclonal antibody, DAKO®, Glostrup, Denmark).

Samples were analyzed by an expert pathologist.

2.2. Variables

The study variables were age, gender, smoking, surgical excision extension, and morphological classification according to WHO 2015[13]. This study does not include ADC variants, such as Mucinous tumors. tumor of differentiation according to the predominant growth pattern, grade I for ADC type *in situ* (AIS) and ADC minimally invasive (MIA), grade II for invasive ADC predominantly lepidic non mucinous, papillary or acinar, and grade III for invasive ADC, predominantly mucinous, colloid, solid or micropapillary. The presence (from 5%) or absence of each histological (lepidic, acinar, papillary, micropapillary and solid) component was assessed. Tumor size, invasion size and pathologic TNM were also evaluated according to the 8th edition. Microscopic vascular and lymphatic invasion was defined as being absent or present. If vascular invasion (VI) or lymphatic invasion (LI) with H-E was non conclusive, then CD34 was used for the vascular endothelium evaluation and D2-40 (monoclonal antibody, DAKO®, Glostrup, Denmark) was utilized to define lymphatic endothelium. Tumoral necrosis cataloged as absent or present was evaluated. Therefore, it was included as minimal necrosis until a large amount of necrosis. Consequently, the number of mitoses was also evaluated. Finally, the nuclear grade was analyzed according to the criteria of Barletta et al.[19], identified as: G1 for the nuclei with a uniform size and morphology with no evidence for visible nucleolus; G2 for the nuclei of an intermediate size with discrete irregularity of morphology and an evident nucleolus; G3 for the nuclei of increased size and irregular contours with enlarged nucleoli.

With the patients' outcomes, tumor recurrence was evaluated. Its location was recorded as local-regional recurrence, this being the presence of tumoral recurrence in the primary tumor location, which can even be taken as mediastinal adenopathies being present; distant relapse, presence of systemic metastases; a second primary tumor; and all this in accordance with Martini´s criteria[7].

The patient's condition was designated as living, exitus by LC and exitus due to another cause than LC.

2.3. Statistical analysis

The statistical analysis was performed with the SPSS Package for Windows®, version 22. With the acquired information, descriptive and analytical statistical analyses were run.

The statistics parameters were calculated to describe the continuous quantitative variables, centralization and dispersion. The description of the categorical, ordinal or nominal, variables, and the percentage and absolute frequency measurements, were also calculated.

To calculate both the DFS and CSS, the Kaplan-Meier method was used. The variables were established as independent prognostic factors by means of the Cox multivariate method for the clinical-pathological characteristics, using those variables whose level of significance was $p \leq 0.25$ in the univariate analysis. The level of significance was established at $p \leq 0.05$. To compare the survival curves according to the different prognostic factors, the log-rank test was performed.

3. Results

The clinical and pathological characteristics of the series are summarized in table 1.

In the sample, the mean total tumor size was 21.9 mm (SD 6.4). However, according to the 8th edition of the TNM, the size of tumor invasion is the parameter used to classify the descriptor T of this classification and, consequently, the final staging. It was found that invasion size was 16.9 mm (SD 9.4) in this series.

Table 2 shows the frequency of the different cellular components or the histological patterns found in the ADCs analyzed based on the five histological described types (figure 1).

The median follow-up time was 7.2 years, with a range from 0.8 to 25.5 years. During these patients' evolution, it 28.6% had tumor recurrence (table 3). Of these, 70.6% (24/34) were metastases at distances, which were cerebrally located in 11 patients (45.8%). The time of appearance of metastases was a median of 2 years, with a minimum of 0.3 and a maximum of 10 years. Of the entire series, 25.2% of patients died due to lung ADC (Table 3), with a median of 4 years and a range between 1 and 13 years. A second primary tumor appeared in 11 patients (9.2%), with a mortality of 9/11 patients and a median time of 9.4 years [5.7-23.2].

The survival analysis was performed for the DFS, CSS and OS of the series. The results are shown in table 4 and figures 2-4.

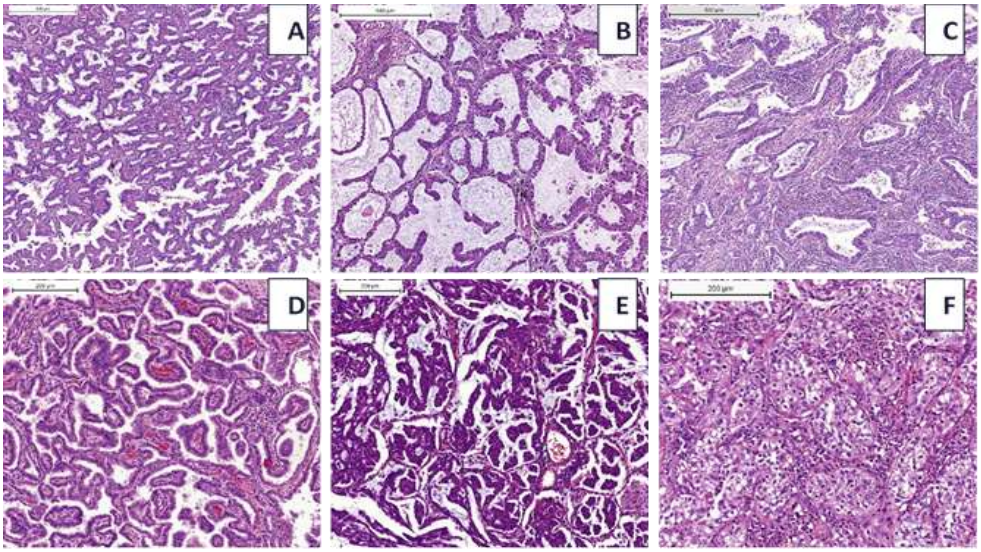

Figure 1: Histopathological images of the different tumor patterns: lepidic pattern, HE 4x (A), mucinous lepidic pattern, HE 4x (B), acinar pattern, HE 4x (C), papillary pattern, HE 10x (D), micropapillary pattern, HE 10x (E), solid pattern, HE 10x (F).

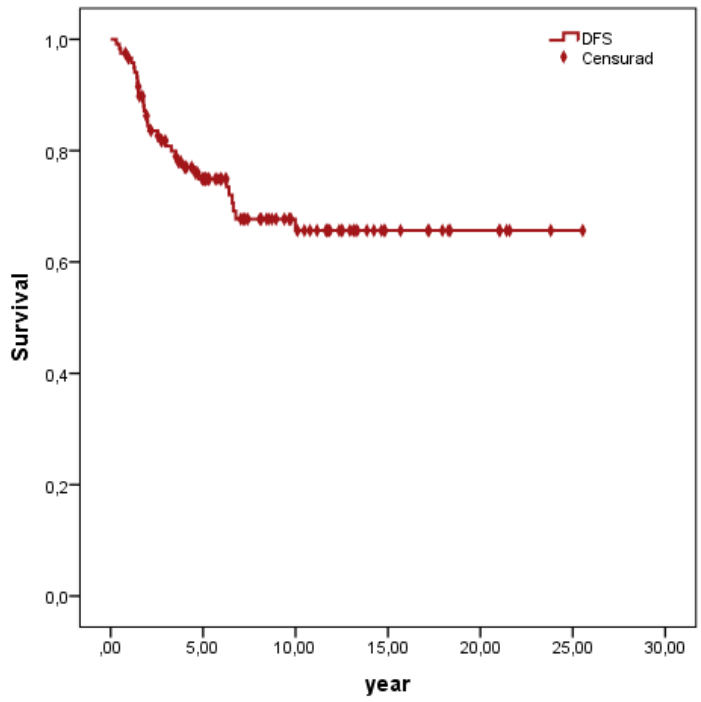

Figure 2: Disease free survival curve

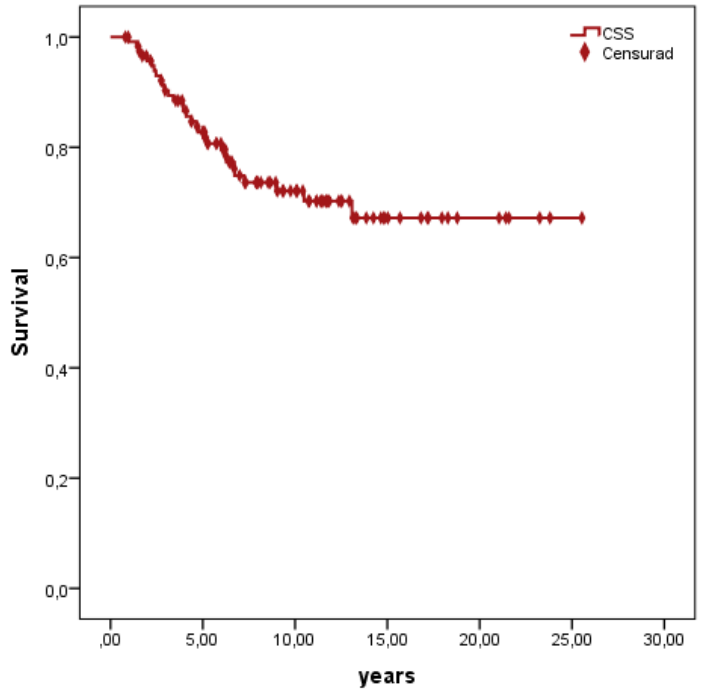

Figure 3: Cancer specific survival curve

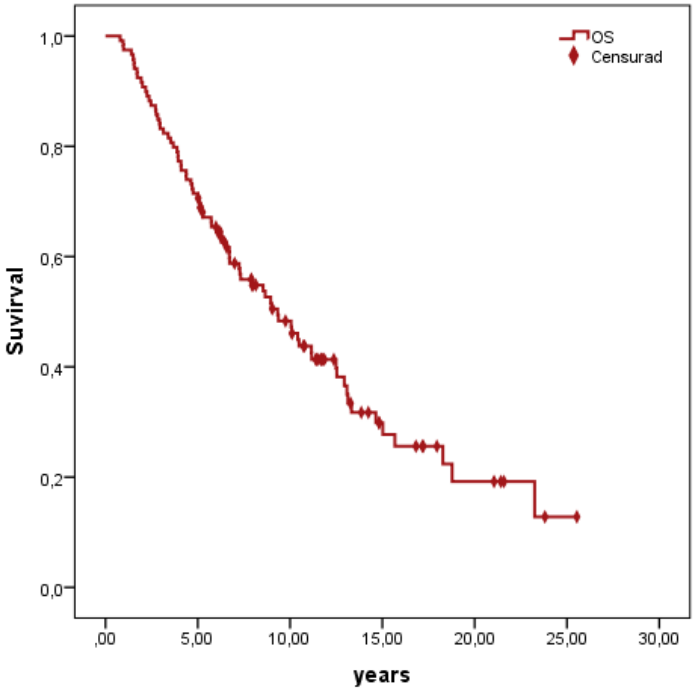

Figure 4: Overall survival curve

The clinical and pathological variables were analyzed as prognostic factors in a univariate Cox regression model for DFS and CSS (table 5).

The TNM was dichotomized into two variables by grouping Tis and T1a compared to T1b and T1c. The presence and absence of each histological component was assessed. The ADC subtypes were not included individually but were grouped into differentiation degrees according to their architecture. These, in turn, were grouped as grades I and II in relation to the most aggressive types (solid and micropapillary) associated with grade III. Finally, in the nuclear grade, grades 2 and 3 were combined to compare it to the less aggressive degree, grade 1. The number of mitosis was dichotomized with the cut-off point in the median.

To assess the variables that may be independent prognostic factors, the multivariate Cox analysis was performed with those variables with $p \leq 0.25$. The variables, namely TNM, the acinar component and the micropapillary, the degree of differentiation, the nuclear grade, the IL and necrosis, were introduced into the multivariable Cox model related to DFS. For the relation with the CSS, the variables used in the model were the acinar and micropapillary component, the degree of differentiation, the nuclear grade and necrosis. The results are shown in table 6, with the variables that regressed the micropapillary component and necrosis. Figures 5-8 show the survival curve of the variables that went into regression.

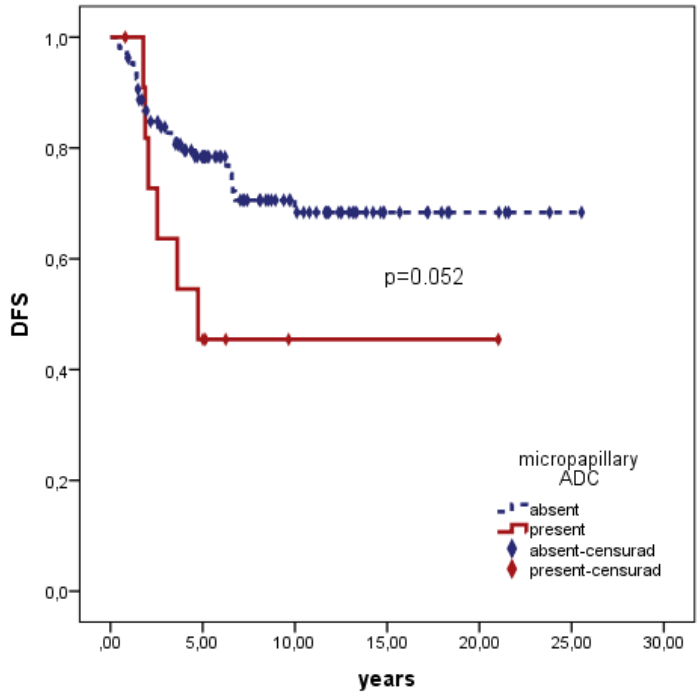

Figure 5: Disease free survival curve and micropapillary ADC

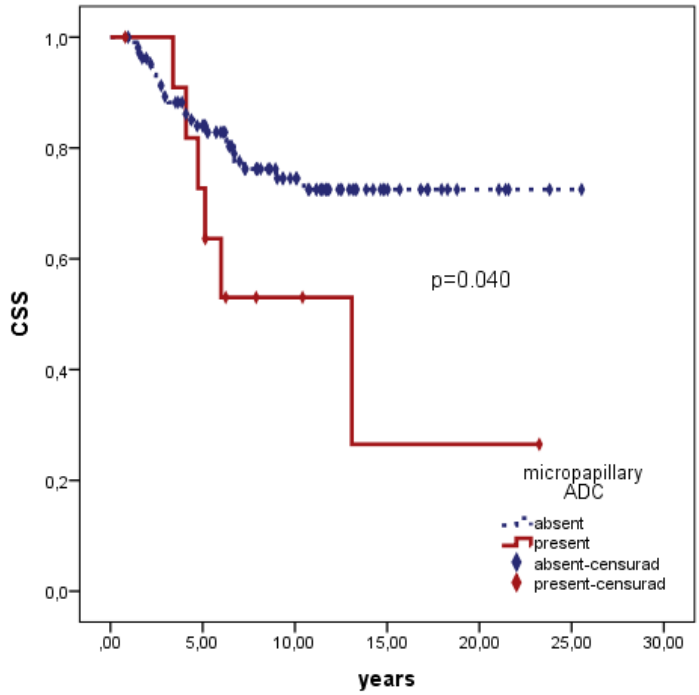

Figure 6: Cancer specific survival curve and micropapillary ADC

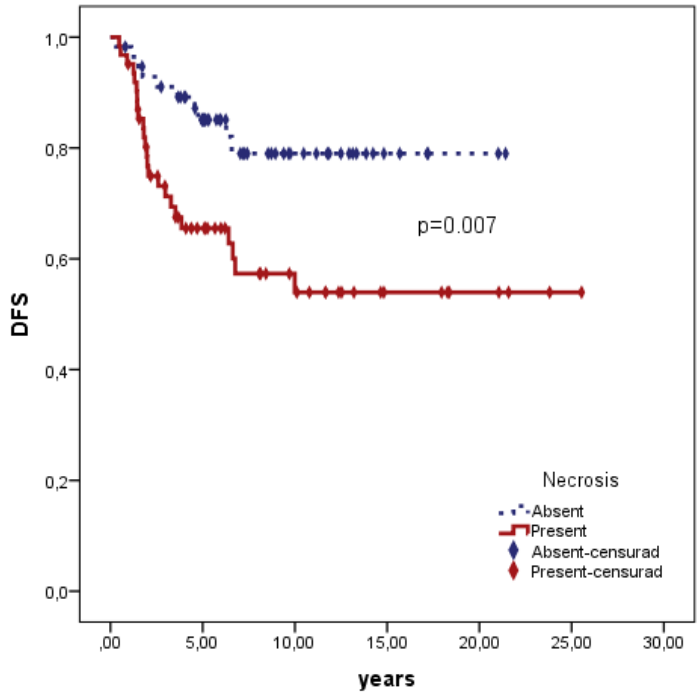

Figure 7: Disease free survival curve and tumor necrosis

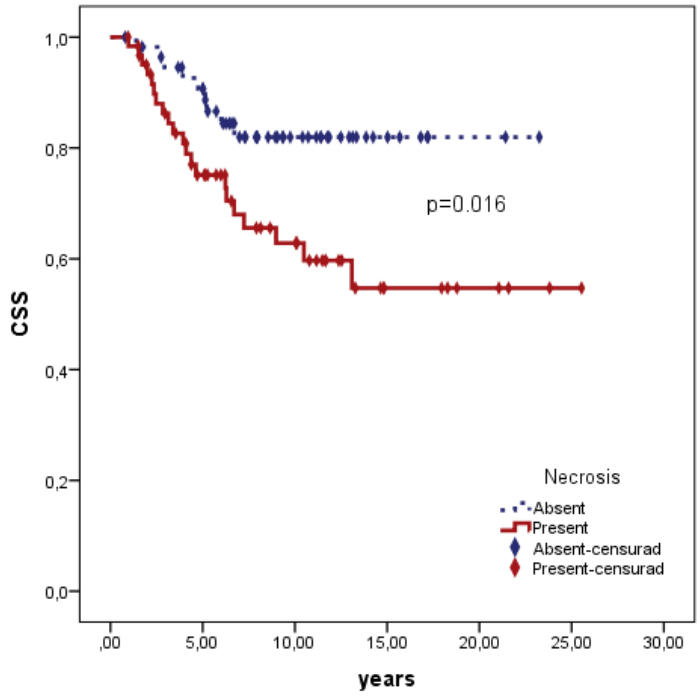

Figure 8: Cancer specific survival curve and tumor necrosis

Table 1: Clinical-pathological characteristics

		N (119)	%
Gender	Women	27	22.7
	Men	92	77.3
Age	Mean (SD)	61.6 (8.5)	
Smoking	No Smoker	20	16.8
	Smoker	66	55.5
	Former smoker	33	27.7
Type of surgery	Anatomical segmentectomy	7	5.9
	Lobectomy	111	93.3
	Pneumonectomy	1	0.8
TNM (Stage)	Tis (0)	7	5.9
	T1a (IA1)	23	19.3
	T1b (IA2)	47	39.5
	T1c (IA3)	42	35.3
ADC subtype	AIS	7	5.9
	MIA	8	6.7
	ADC invasive	104	87.4
	Lepidic	*18*	*15.1*
	Acinar	*49*	*41.2*
	Papillary	*5*	*4.2*
	Micropapillary	*3*	*2.5*
	Solid	*29*	*24.4*
Tumor differentiation	GI	14	11.8
	GII	68	57.1
	GIII	37	31.1
Nuclear grade	1	27	22.7
	2	76	63.9
	3	16	13.4
Lymphatic invasion		29	24.4
Vascular invasion		30	25.2
Tumoral necrosis		62	52.1
Recurrence		34	28.6
	Local-Regional	7	5.9
	Systemic metastases	27	22.6
	Brain	*11*	*9.2*
	Pulmonary	*4*	*3.3*
	Others	*12*	10.1
Status	Live	43	36.1
	Exitus due ADC	30	25.2
	Exitus others causes	37	31.1
	Second primary tumors	9	7.6

SD: Standard Deviation
ADC: Adenocarcinoma
AIS: Adenocarcinoma In Situ
MIA: Minimally Invasive Adenocarcinoma

Table 2: Absolute and relative frequency of the histological patterns

Histological patterns	**N**	**%**
Lepidic	53	44.5
Acinar	101	84.9
Papillary	18	15.1
Micropapillary	12	10.1
Solid	69	58

Table 3: Absolute and relative frequency of recurrences and status

	N (119)	**%**
Recurrence	**34**	**28.6 (100)**
Loco-regional	7	5.9 (20.6)
Distance	24	20.2 (70.6)
Both	3	2.5 (8.8)
Status		
Live	43	36.1
Mortality by ADC	30	25.2
Mortality by others cause	37	31.1
Mortality by second primary tumor	9	7.6

ADC: Adenocarcinoma

Table 4: Survival for the DFS, CSS y OS

	DFS	**CSS**	**OS**
1 year	95.8%	99.1%	97.5%
2 years	83.6%	95.7%	90.8%
5 years	74.9%	82.7%	69.7%
10 years	65.7%	72.1%	47.2%

DFS: Disease free survival
CSS: Cancer-specific suvival
OS: Overallsurvival

Table 5: Univariate analysis of the clinical and pathological features with the DFS and the CSS

Variable	**DFS**			**CSS**		
	HR	**IC95%**	**Valor p**	**HR**	**IC95%**	**Valor p**
Gender	0.770	0.335-1.769	**0.537**	0.720	0.294-1.764	**0.473**
Age *(≤62 vs >62)*	1.111	0.567-2.178	**0.759**	1.048	0.511-2.147	**0.898**
Smoking	1.069	0.441-2.592	**0.882**	1.177	0.449-3.086	**0.740**
TNM *(Tis/1a VS T1b/c)*	1.794	0.742-4.334	**0.194**	1.521	0.621-3.723	**0.358**
Histological patterns						

Lepidico	0.671	0.336-1.341	**0.258**	0.756	0.364-1.570	**0.453**
Acinar	3.356	0.804-14.00	**0.097**	2.631	0.627-11.05	**0.186**
Papillary	0.568	0.173-1.864	**0.351**	0.712	0.215-2.353	**0.577**
Micropapilary	2.352	0.968-5.715	**0.059**	2.488	1.013-6.108	**0.047**
Solid	1.260	0.640-2.482	**0.505**	1.399	0.682-2.870	**0.360**
Differentiation degree *(grade 1 y 2/grade 3)*	1.015	0.495-2.082	**0.968**	1.094	0.512-2.339	**0.816**
Nuclear grade *(grade 1 /grade 2 y 3)*	3.634	1.111-11.89	**0.033**	3.017	0.915-9.948	**0.070**
Mitosis *(≤6 vs >6)*	0.841	0.427-1.656	**0.617**	0.658	0.317-1.368	**0.263**
LI	1.297	0.620-2.714	**0.490**	1.286	0.588-2.812	**0.529**
VI	1.666	0.813-3.349	**0.166**	1.393	0.651-2.981	**0.384**
Necrosis	2.645	1.263-5.540	**0.010**	2.520	1.153-5.506	**0.020**

DFS: Disease free survival
CSS: Cancer specific survival
LI: Lymphatics invasion
VI: Vascular invasion

Table 6: Multivariate analysis of clinical and pathological features with DFS and CSS.

Variable	**DFS**			**CSS**		
	HR	**IC95%**	**Valor p**	**HR**	**IC95%**	**Valor p**
Micropapillary patterns	3.890	1.495-10.12	**0.005**	3.370	1.339-8.484	**0.010**
Necrosis	3.433	1.557-7.568	**0.002**	2.995	1.340-6.694	**0.008**

4. Discussion

LC is a serious health problem with a huge impact both from the health and socio-economic points of view. In particular, pulmonary ADC is recognized as a highly variable and heterogeneous neoplasm in its pathogenesis, its histology and prognosis[15,20,21], hence interest in the impact that the different histological subtypes of pulmonary ADC have on survival, even in tumors classified in the same stage according to the TNM classification[13,14,22,23].

Accordingly, survival due to this tumor is still disappointing and recurrences are very high as approximately 50% of patients undergoing surgery with curative intent[24,25]. In our series limited to stage IA, with a long follow-up period, 28.6% of the patients suffered recurrence, of whom 70.6% were distant metastases, mostly cerebral, which are similar figures to those informed in other publications[24,25]. The median time during which relapses appeared in these patients was 2 years, a finding that coincides with Villar et al.[25], who describe three peaks of incidence (at 9 months, 2 years and 4 years). The median time during which the death of the patients included in our study occurred was 4 years, which are similar to the results described in other Spanish series[26]. The disease caused 25.2% of the patients to die, with CSS described as 99.1% at 1 year, 82.7% at 5 years and 72.1% at 10 years, according to other publications[5,9,26]. However, the OS of our series was established at 97.5%

per year and fell to 69.7% and 47.2% at 5 and 10 years, respectively. This could be explained by the greater probability of dying from other causes apart from the primary tumor after 6 years, that is, competitive events as explained by Jordá et al.[27].

It is interesting to note that a change in the presentation of LC has also been emphasized. In their comparative study of LC over two decades, Leiro et al.[28] verified a higher percentage of patients with LC diagnosis in localized stages, which is attributed to more access for the population to medical specialties, the implementation of rapid diagnostic circuits and new imaging techniques and better access to them, which all evidence a significant increase in LC as an incidental finding. Another factor to take into account is the introduction of early diagnosis programs. In a recent study carried out by our group, 104 LC were diagnosed in which ADC was the most frequent histological diagnosis, with 79.5%. Of these 104 cases, 79.8% had undergone surgical resection, with 63.8% of tumors classified in stage IA[29]. Similar data have been observed in other screening studies [30–32].

For this reason, our fundamental objective could provide valuable information on the prognostic value of the clinical and pathological features in resected lung ADC in a very homogeneous group of patients and in its earliest stage.

The descriptive analysis of this research somewhat reflects the news in lung ADC. The average age, 61.6 years, fell within the range that was previously described in the Spanish literature[10,26,28]. In this study, and coinciding with other authors[33–35], the patient's age did not condition survival[36,37].

The male/female ratio was established at 10/1 in the 1990s [26,28], and has progressively lowered. In this series, the observed ratio was 3.4/1, which is comparable to other publications [26,28]. These data are the result of increased female smoking and, consequently, their diagnosis has increased by 5.1%[4,28]. As a prognostic factor, gender had no impact on patients' evolution, as other studies have pointed out[38–40].

In the present work, only 16.8% of the patients had no history of smoking. Recent studies published in our country show similar incidence rates[28,41]. Coinciding with both Subramanian et al.[37] and Meguid et al.[36] in studies conducted to assess the impact of smoking on LC survival, no prognostic influence was observed in this series.

Regarding surgical resection, recent studies have begun proposing anatomical segmental resections for ADCs under 2 cm peripheral [42,43]. However, lobectomy remains the standard treatment in this tumor type[42,44].

This resection type of was the most frequently found one in the patients herein included and represented 93.3% of the practiced resection.

Currently, staging based on the TNM is the way to stratify patients for therapeutic management and prognosis predictions. The latest edition of the TNM presents major changes, especially for early-stage ADC, where it includes stage 0 for Tis tumors. Stage IA, formed by tumors with a maximum diameter of 3 cm, is subdivided into three separate subgroups, centimeter in centimeter: stage IA1, IA2 and IA3. Tumor diameter is determined by tumor invasion size instead of total tumor size[45–48]. In the present work, an average total tumor size of 21.9 mm was obtained, while invasion size was 16.9 mm, which are comparable results to those reported by Tsutani et al. [49].

In line with the results collected in the new TNM classification[5], population stage IA2 was the most frequent in this study with 39.5%, followed by stage IA3 with 35.3%. However, and unlike what other authors have verified [26,45,50,51], the stage IA subtypes proposed in the 8th classification of the TNM[5] were not found to influence either DFS or CSS.

As in other publications[52,53], the acinar component was the most frequent of the observed morphological patterns with 84.9%,and was also the most frequent subtype as the predominant pattern with 41.1%. In

contrast, the micropapillary subtype was the least common predominant pattern with 2.5%. The presence of this last pattern is described to lie between 9.5% and 60.4% of all cases[54,55]. In the present research, this pattern was found in 10.1% of ADCs. The presence of the micropapillary component favored the appearance of metastasis, but with limited statistical significance, p = 0.052, and it significantly conditioned CSS, p = 0.047. When the Cox multivariate analysis was performed, it was the first variable that went into regression with an HR of 3.9 for DFS and one of 3.4 for CSS. Similar data have been published by Tsubokawa et al.[56] in the same tumor stage.

This histological pattern was described for the first time in lung ADC by Amin et al.[57] in 2002, when they reported a worse prognosis of ADCs with this pattern. Later Miyosi et al.[55] and Maeda et al.[58] confirmed these findings by finding an association between this component and angiolymphatic invasion, pleural carcinomatosis, distant metastases and, consequently, they reported worse survival. In the current classification, this histological pattern has been introduced, and has been cataloged by several authors as a poor-prognosis component[13,14,21,53]. More recent studies confirm the poor prognosis of this histological component, even by the presence of minimal proportions, but not by the mere fact of being the predominant component. In this study, the simple presence of the micropapillary component (from 5 to 100%) was analyzed, as in the above-cited studies[17,18,54,56,59].

Other histopathological characteristics are less standardized in the different consulted publications. The degree of differentiation is still not well-established in ADC[15]. However, in recent studies it has been related to the predominant architectural pattern, as described above. The moderately differentiated group, grade II, outlined by predominant lepidic, acinar or papillary tumors, was the most frequently found one in this work, which coincides with other authors[14,60]. Contrarily to the statement made by Yoshizawa et al.[14,61], no relation was found between the degree of differentiation and patients' evolution.

Regarding the nuclear grade, the available objective criteria do not suffice to establish a standardized grading system[62,63]. That which has shown a relation as a prognostic factor is the method of Barletta et al.[19] which was herein used. Like the degree of differentiation, the commonest nuclear grade was grade 2. In this study, the tumors classified as G1 *versus* G2/3 had a significantly lower probability of developing metastasis (p = 0.033) with limited the statistical significance (p = 0.070) compared to CSS. Regarding the number of mitoses, with a median of six in this series, no impact was observed in patients' evolution, which coincides with Barleta et al.[19] and Kadota et al.[62].

MVI is very variable in its assessment. Some studies analyze it together, e.g., both VI and LI, while others analyze it separately. In this study, VI and LI were separately assessed with IV present in 25.2% of cases, and LI in 24.4%. In the broad meta-analysis by Wang et al.[64], the presence of VI in LC ranged between 6% and 77%, and behaved as an independent prognostic factor. In LI terms, the incidence ranged between 15% and 36%[65,66]. These authors also carried out a meta-analysis in which the presence of IL was found to lie between 3% and 71% of the analyzed tumors, and it also proved to bea determining prognostic factor[67]. In the present study, neither of the two factors conditioned survival.

Finally, the presence of tumor necrosis in the present study was 52.1%, which is high compared to other research works carried out in the same stage, with a range between 12% and 27%[68,69]. It is noteworthy that the presence of necrosis represented worse prognosis, with an HR of 3.4 for DFS and one of 3 for CSS. This observation coincides with those reported by Park et al.[69], Fan et al.[68] and Swinson et al.[70] in early NSCLC stages.

A limitation of the present study is that its sample size was small, and its study design was partly retrospective. Therefore, a selection

bias may exist. Nonetheless, this selection was made to obtain very homogeneous LC group.

In conclusion, according to the latest classification of ADCs, the micropapillary component is a poor-prognosis factor, even in the presence of small proportions without them being the main lesion component. Likewise, the presence of tumor necrosis was an independent poor-prognosis factor, regardless of whether it is coming in minimal or significant amounts.

Acknowledgements

This project is supported by the Spanish Thoracic Surgery Society (SECT) grant, prize awarded to first place of research project 2016.

We thank Dr. José Padilla Alarcón and Dr. Cristóbal Aguilar for their collaboration in the project.

Conflict of interests

"The authors declare that there is no conflict of interest regarding the publication of this article"

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86.
2. Bray F, Felay J, Soerjomataram I, Siegel R, Torre L, Jermal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
3. López-Abente G, Núñez O, Pérez-Gómez B, Aragonés N, Pollán M. La situación del cáncer en España: Informe 2015. I CN de EI de SC, editor. Madrid: Centro Nacional de Epidemiología Instituto de Salud Carlos III; 2015. 154 p.
4. Sociedad Española de Oncología Médica (SEOM). Las cifras del cáncer en españa 2018 [Internet]. Las cifras del cáncer en España 2017. 2018. Available from: www.seom.org
5. Goldstraw P, Chansky K, Crowley J, Rami-Porta R, Asamura H, Eberhardt WEE, et al. The IASLC lung cancer staging project: Proposals for revision of the TNM stage groupings in the forthcoming (eighth) edition of the TNM classification for lung cancer. J Thorac Oncol. 2016;11(1):39–51.
6. Goldstraw P. The 7th Edition of TNM in Lung Cancer: what now? J Thorac Oncol [Internet]. 2009;4(6):671–3. Available from: http://dx.doi.org/10.1097/JTO.0b013e31819e7814
7. Martini N, Bains MS, Burt ME, Zakowski MF, McCormack P, Rusch VW, et al. Incidence of local recurrence and second primary tumors in resected stage I lung cancer. J Thorac Cardiovasc Surg [Internet]. 1995;109(1):120–9. Available from: http://www.jtcvsonline.org/article/S0022522395704272/fulltext
8. Maeda R, Yoshida J, Ishii G, Hishida T, Nishimura M, Nagai K. Risk factors for tumor recurrence in patients with early-stage (stage I and II) non-small cell lung cancer: Patient selection criteria for adjuvant chemotherapy according to the seventh edition TNM classification. Chest [Internet]. 2011;140(6):1494–502. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21622548
9. Yang Y, Mao Y, Yang L, He J, Gao S, Mu J, et al. Prognostic factors in curatively resected pathological stage I lung adenocarcinoma. J Thorac Dis. 2017;9(12):5267–77.
10. Sánchez de Cos Escuín J, Serra Mitjans M, Hernández Hernández J, Hernández Rodríguez H. Registro del Grupo Cooperativo de Cáncer de Pulmón-II de la Sociedad Española de Neumología y Cirugía Torácica . Estudio descriptivo. Arch Bronconeumol. 2013;49(11):462–7.
11. Devesa SS, Bray F, Vizcaino AP, Parkin DM. International lung cancer trends by histologic type: Male:female differences diminishing and adenocarcinoma rates rising. Int J Cancer. 2005;117(2):294–9.
12. Janssen-Heijnen MLG, Coebergh JWW. The changing epidemiology of lung cancer in Europe. Lung Cancer. 2003;41(3):245–58.
13. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. WHO Classification of tumours of ther Lung, Pleura, Thymus and Heart. 4th ed. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG, editors. Lyon: International Agency for Research on Cancer (IARC); 2015.
14. Yoshizawa A, Motoi N, Riely GJ, Sima CS, Gerald WL, Kris MG, et al. Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma:

prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases. Mod Pathol [Internet]. 2011;24(5):653–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21252858

15. Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JHM, Beasley MB, et al. The 2015 World Health Organization classification of lung tumors. Impact of genetic, clinical and radiologic advances since the 2004 classification. J Thorac Oncol [Internet]. 2015;10(9):1243–60. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=01243894-900000000-98927
16. Chilosi M, Murer B. Mixed adenocarcinomas of the lung: place in new proposals in classification, mandatory for target therapy. Arch Pathol Lab Med [Internet]. 2010;134(1):55–65. Available from: papers3://publication/uuid/6FEA3AF7-824A-4A40-A290-3FF8A5BBD65E
17. Zombori T, Nyári T, Tiszlavicz L, Pálföldi R, Csada E, Géczi T, et al. The more the micropapillary pattern in stage I lung adenocarcinoma, the worse the prognosis—a retrospective study on digitalized slides. Virchows Arch. 2018;472(6):949–58.
18. Yanagawa N, Shiono S, Abiko M, Katahira M, Osakabe M, Ogata SY. The clinical impact of solid and micropapillary patterns in resected lung adenocarcinoma. J Thorac Oncol [Internet]. 2016;11(11):1976–83. Available from: http://dx.doi.org/10.1016/j.jtho.2016.06.014
19. Barletta JA, Yeap BY, Chirieac LR. The Prognostic Significance of Grading in Lung Adenocarcinoma. Cancer. 2010;116(3):659–69.
20. Kerr KM. Pulmonary adenocarcinomas: Classification and reporting. Histopathology. 2009;54(1):12–27.
21. Van Schil PE, Sihoe ADL, Travis WD. Pathologic classification of adenocarcinoma of lung. J Surg Oncol. 2013;108(5):320–6.
22. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger K, Yatabe Y, et al. Diagnosis of lung cancer in small biopsies and cytology: Implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European respiratory society classification. Arch Pathol Lab Med. 2013;137(5):668–84.
23. Brierley JD, Gospodarowicz MK, Wittekind C. TNM classification of malignant tumours. Eighth. O'Sullivan B, Mason M, Asamura H, Lee A, Van Eycken E, Denny L, et al., editors. Union for International Cancer Control (UICC). Union for International Cancer Control (UICC); 2017. 241 p.
24. Subotic D, Mandaric D, Radosavljevic G, Stojsic J, Gajic M, Ercegovac M. Relapse in resected lung cancer revisited: Does intensified follow up really matter? A prospective study. World J Surg Oncol. 2009;7:1–11.
25. Villar Álvarez F, Muguruza Trueba I, Vicente Antunes SI. Apuntes sobre recidivas y segundos tumores en el cáncer de pulmón. Arch Bronconeumol. 2016;52(11):545–6.
26. Pérez-Martínez O, Vidal-García I, Montero-Martínez C, Provencio M, Ruano-Ravina A. Características al diagnóstico y supervivencia de estadios I y II de cáncer de pulmón. Arch Bronconeumol [Internet]. 2018;54(8):420–6. Available from: https://www.sciencedirect.com/science/article/pii/S0300289618300619
27. Jordá Aragón C, Peñalver Cuesta JC, Mancheño Franch N, De Aguiar Quevedo K, Vera Sempere F, Padilla Alarcón J. Mortalidad en carcinoma pulmonar no microcítico resecado, con tamaño máximo de 3 cm y sin afectación

ganglionar: análisis de riesgos competitivos. Med Clin (Barc). 2015;145(5):185–91.
28. Leiro-Fernández V, Mouronte-Roibás C, Ramos-Hernández C, Botana-Rial M, González-Piñeiro A, García-Rodríguez E, et al. Cambios en el estadio y presentación clínica del cáncer de pulmón a lo largo de dos décadas. Arch Bronconeumol. 2014;50(10):417–21.
29. De Aguiar Quevedo K, Arrarás Martínez M, Martínez Pérez E, Arana E, Mojarrieta JC, González Añó M, et al. Lung resections in the lung screening of International Early Lung Cancer Action Program (IELCAP): 6 years of experience. Med Res Arch. 2016;4(I):1–17.
30. Crestanello J a, Allen MS, Jett JR, Cassivi SD, Nichols FC, Swensen SJ, et al. Thoracic surgical operations in patients enrolled in a computed tomographic screening trial. J Thorac Cardiovasc Surg. 2004;128(2):254–9.
31. Aberle DR, Abtin F, Brown K. Computed tomography screening for lung cancer: Has it finally arrived? implications of the national lung screening trial. J Clin Oncol. 2013;31(8):1002–8.
32. The National Lung Screening Trial Research Team. Reduced Lung-Cancer Mortality with Low-Dose Computed Tomographic Screening. N Engl J Med. 2011;365(5):395–409.
33. Yamamoto K, Padilla Alarcón J, Calvo Medina V, García Zarza A, Pastor Guillen J, Blasco Armengod E, et al. Surgical results of stage I non-small cell lung cancer: Comparison between elderly and younger patients. Eur J Cardio-thoracic Surg [Internet]. 2003;23(1):21–5. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed6&NEWS=N&AN=2002460179
34. Goodgame B, Viswanathan A, Zoole J, Gao F, Miller CR, Subramanian J, et al. Risk of recurrence of resected stage i non-small cell lung cancer in elderly patients as compared with younger patients. J Thorac Oncol [Internet]. 2009;4(11):1370–4. Available from: http://dx.doi.org/10.1097/JTO.0b013e3181b6bc1b
35. Palma DA, Tyldesley S, Sheehan F, Mohamed IG, Smith S, Wai E, et al. Stage I non-small cell lung cancer (NSCLC) in patients aged 75 years and older: Does age determine survival after radical treatment? J Thorac Oncol [Internet]. 2010;5(6):818–24. Available from: http://dx.doi.org/10.1097/JTO.0b013e3181d6e052
36. Meguid RA, Hooker CM, Harris J, Xu L, Westra WH, Sherwood JT, et al. Long-term survival outcomes by smoking status in surgical and nonsurgical patients with non-small cell lung cancer: Comparing never smokers and current smokers. Chest. 2010;138(3):500–9.
37. Subramanian J, Govindan R. Lung cancer in never smokers: A review. J Clin Oncol [Internet]. 2007;25(5):561–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17290066
38. Campione A, Ligabue T, Luzzi L, Chiribelli C, Paladini P, Voltolini L, et al. Impact of size, histology, and gender on stage IA non-small cell lung cancer. Asian Cardiovasc Thorac Ann [Internet]. 2004;12(2):149–53. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L39010977
39. Asamura H, Goya T, Koshiishi Y, Sohara Y, Eguchi K, Mori M, et al. A Japanese lung cancer registry study: Prognosis of 13,010 resected lung cancers. J Thorac Oncol [Internet]. 2008;3(1):46–52. Available from: http://dx.doi.org/10.1097/JTO.0b013e31815e8577
40. Sakao Y, Sakuragi T, Natsuaki M, Itoh

T. Clinicopathological analysis of prognostic factors in clinical IA peripheral adenocarcinoma of the lung. Ann Thorac Surg. 2003;75(4):1113–7.

41. Peñalver Cuesta JC, Jordá Aragón C, Cerón Navarro JA, Mancheño Franch N, de Aguiar Quevedo K, Arrarás Martínez M, et al. Factores pronóstico en el carcinoma bronquial no microcítico menor de 3 centímetros (análisis actuarial, incidencia acumulativa y grupos de riesgo). Arch Bronconeumol. 2015;51(9):431–9.
42. Liu S, Wang R, Zhang Y, Li Y, Cheng C, Pan Y, et al. Precise diagnosis of intraoperative frozen section is an effective method to guide resection strategy for peripheral small-sized lung adenocarcinoma. J Clin Oncol. 2016;34(4):307–13.
43. Nakamura K, Saji H, Nakajima R, Okada M, Asamura H, Shibata T, et al. A Phase III randomized trial of lobectomy versus limited resection for small-sized peripheral non-small cell lung cancer (JCOG0802/WJOG4607L). Jpn J Clin Oncol. 2009;40(3):271–4.
44. Ginsberg RJ, Rubinstein L V. Sublobectomy versus Lobectomy for stage IA (T1a) non-small-cell lung cancer: A meta-analysis study. Ann Thorac Surg. 1995;60:615–23.
45. Travis WD, Asamura H, Bankier AA, Beasley MB, Detterbeck F, Flieder DB, et al. The IASLC lung cancer staging project: Proposals for coding T categories for subsolid nodules and assessment of tumor size in part-solid tumors in the forthcoming eighth edition of the TNM classification of lung cancer. Vol. 11, Journal of Thoracic Oncology. 2016. p. 1204–23.
46. Aokage K, Miyoshi T, Ishii G, Kusumoto M, Nomura S, Katsumata S, et al. Clinical and Pathological Staging Validation in the Eighth Edition of the TNM Classification for Lung Cancer: Correlation between Solid Size on Thin-Section Computed Tomography and Invasive Size in Pathological Findings in the New T Classification. J Thorac Oncol [Internet]. 2017;12(9):1403–12. Available from: http://dx.doi.org/10.1016/j.jtho.2017.06.003
47. Nicholson AG, Tsao MS, Travis WD, Patil DT, Galateau-Salle F, Marino M, et al. Eighth Edition Staging of Thoracic Malignancies Implications for the Reporting Pathologist for the Members of the International Association for the Study of Lung Cancer Staging and Prognostic Factors and Pathology Committees. Arch Pathol Lab Med. 2018;142:645–61.
48. Wang L, Anraku M, Sato M, Nitadori JI, Nagayama K, Kitano K, et al. Impact of the 8th Edition of the UICC-TNM Classification on Clinical Stage 0-IA Lung Adenocarcinoma: Does the New Classification Predict Postoperative Prognosis More Precisely than the Previous One? Ann Thorac Cardiovasc Surg. 2018;24(5):223–9.
49. Tsutani Y, Miyata Y, Mimae T, Kushitani K, Takeshima Y, Yoshimura M, et al. The prognostic role of pathologic invasive component size, excluding lepidic growth, in stage I lung adenocarcinoma. J Thorac Cardiovasc Surg [Internet]. 2013 [cited 2018 May 9];146:580–5. Available from: https://www.jtcvs.org/article/S0022-5223(13)00497-2/pdf
50. Rami-Porta R, Bolejack V, Crowley J, Ball D, Kim J, Lyons G, et al. The IASLC Lung Cancer Staging Project: Proposals for the Revisions of the T Descriptors in the Forthcoming Eighth Edition of the TNM Classification for Lung Cancer. TL - 10. J Thorac Oncol [Internet]. 2015;10(7):990–1003. Available from: http://dx.doi.org/10.1097/JTO.0000000000000559
51. Rami Porta R, Perez Ochoa F, González

Pont G. Propuestas para la nueva clasificación TNM del cáncer de pulmón y clasificación anatomopatológica. In: Matilla González JM, editor. Monografia 4: Cáncer de pulmón. Barcelona: RESPIRA. FUNDACION ESPAÑOLA DEL PULMON. SEPAR; 2016. p. 25–38.

52. Hung JJ, Yeh YC, Jeng WJ, Wu KJ, Huang BS, Wu YC, et al. Predictive value of the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification of lung adenocarcinoma in tumor recurrence and patient survival. J Clin Oncol. 2014;32(22):2357–64.
53. Kadota K, Villena-Vargas J, Yoshizawa A, Motoi N, Sima C, Riely GJ, et al. Prognostic Significance of Adenocarcinoma in situ, Minimally Invasive Adenocarcinoma, and Nonmucinous Lepidic Predominant invasive Adenocarcinoma of the lung in patients with stage I disease. Am J Surg Pathol. 2014;38(4):448–60.
54. Zhao ZR, To KF, Mok TSK, Ng CSH. Is there significance in identification of non-predominant micropapillary or solid components in early-stage lung adenocarcinoma? Interact Cardiovasc Thorac Surg. 2017;24(1):121–5.
55. Miyoshi T, Satoh Y, Okumura S, Nakagawa K, Shirakusa T, Tsuchiya E, et al. Early-stage lung adenocarcinomas with a micropapillary pattern, a distinct pathologic marker for a significantly poor prognosis. Am J Surg Pathol. 2003;27(1):101–9.
56. Tsubokawa N, Mimae T, Sasada S, Yoshiya T, Mimura T, Murakami S, et al. Negative prognostic influence of micropapillary pattern in stage IA lung adenocarcinoma. Eur J Cardio-Thoracic Surg. 2015;49(1):293–9.
57. Amin MB, Tamboli P, Merchant SH, Ordóñez NG, Ro J, Ayala AG, et al. Micropapillary component in lung adenocarcinoma: a distinctive histologic feature with possible prognostic significance. Am J Surg Pathol [Internet]. 2002;26(3):358–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11859208
58. Maeda R, Isowa N, Onuma H, Miura H, Harada T, Touge H, et al. Lung adenocarcinomas with micropapillary components. Gen Thorac Cardiovasc Surg. 2009;57(10):534–9.
59. Yuan Y, Ma G, Zhang YQ, Chen H. Presence of micropapillary and solid patterns are associated with nodal upstaging and unfavorable prognosis among patient with cT1N0M0 lung adenocarcinoma: a large-scale analysis. J Cancer Res Clin Oncol [Internet]. 2018;144(4):743–9. Available from: http://dx.doi.org/10.1007/s00432-017-2571-7
60. Travis WD, Brambilla E, Riely GJ. New pathologic classification of lung cancer: Relevance for clinical practice and clinical trials. J Clin Oncol. 2013;31(8):992–1001.
61. Yoshizawa A, Sumiyoshi S, Sonobe M, Kobayashi M, Fujimoto M, Kawakami F, et al. Validation of the IASLC/ATS/ERS lung adenocarcinoma classification for prognosis and association with EGFR and KRAS gene mutations: Analysis of 440 Japanese patients. J Thorac Oncol [Internet]. 2013;8(1):52–61. Available from: http://dx.doi.org/10.1097/JTO.0b013e3182769aa8
62. Kadota K, Suzuki K, Kachala SS, Zabor EC, Sima CS, Moreira AL, et al. A grading system combining architectural features and mitotic count predicts recurrence in stage I lung adenocarcinoma. Mod Pathol [Internet]. 2012;25(8):1117–27. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4382749&tool=pmcentrez&rendertype=abstract

63. Nakazato Y, Miyagi Maeshima A, Ishikawa Y, Yatabe Y, Fukuoka J, Yokose T, et al. Interobserver Agreement in the Nuclear Grading of Primary Pulmonary Adenocarcinoma. J Thorac Oncol [Internet]. 2013 [cited 2018 May 9];8(6):736–43. Available from: https://www.jto.org/article/S1556-0864(15)32848-3/pdf
64. Wang J, Chen J, Chen X, Wang B, Li K, Bi J, et al. Blood Vessel Invasion as a Strong Independent Prognostic Indicator in Non-Small Cell Lung Cancer: A Systematic Review and Meta-Analysis. PLoS One [Internet]. 2011 [cited 2018 May 9];6(12). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3237541/pdf/pone.0028844.pdf
65. Funai K, Sugimura H, Morita T, Shundo Y, Shimizu K, Shiiya N. Lymphatic vessel invasion is a significant prognostic indicator in stage IA lung adenocarcinoma. Ann Surg Oncol. 2011;18(10):2968–72.
66. Rigau V, Molina TJ, Chaffaud C, Huchon G, Audouin J, Chevret S, et al. Blood vessel invasion in resected non small cell lung carcinomas is predictive of metastatic occurrence. Lung Cancer. 2002;38(2):169–76.
67. Wang J, Wang B, Zhao W, Guo Y, Chen H, Chu H, et al. Clinical Significance and Role of Lymphatic Vessel Invasion as a Major Prognostic Implication in Non-Small Cell Lung Cancer: A Meta-Analysis. PLoS One. 2012;7(12).
68. Fan X, Zhang X, Wang H, Jin B. Reevaluation of survival and prognostic factors in pathologic stage I lung adenocarcinoma by the new 2009 TNM classification. Tumor Biol. 2014;35(6):5905–10.
69. Park SY, Lee HS, Jang HJ, Lee GK, Chung KY, Zo JI. Tumor necrosis as a prognostic factor for stage IA non-small cell lung cancer. Ann Thorac Surg. 2011;91(6):1668–73.
70. Swinson DE., Jones JL, Richardson D, Cox G, Edwards JG, O'Byrne KJ. Tumour necrosis is an independent prognostic marker in non-small cell lung cancer: correlation with biological variables. Lung Cancer [Internet]. 2002;37(3):235–40. Available from: http://www.sciencedirect.com/science/article/pii/S0169500202001721

