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ORIGINAL ARTICLE

The more the micropapillary pattern in stage I lung adenocarcinoma, the worse the prognosis—a retrospective study on digitalized slides

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Abstract

Although the majority of lung adenocarcinomas show mixed pattern, only the predominant component is taken into account according to the novel classification. We evaluated the proportion of different patterns and their impact on overall survival (OS) and disease-free survival (DFS). Patterns were recorded according to predominance and their proportions were rated and calculated by objective area measuring on digitalized, annotated slides of resected stage I lung adenocarcinomas. Spearman's rank correlation, Kaplan-Meier models and the log rank test were used for statistical evaluation. Two hundred forty-three stage I adenocarcinoma were included. Lepidic pattern is more frequent in tumours without recurrence (20 vs. 8%), and lepidic predominant tumours have favourable prognosis (OS 90.5%, DFS 89.4%), but proportions above 25% are not associated with improving outcome. Solid and micropapillary patterns are more frequent in patients with recurrence (48 vs. 5% and 13 vs. 4%) and predominance of each one is associated with unfavourable prognosis (OS 64.1%, DFS 56.3% and OS 28.1%, DFS 28.1%, respectively). Above 25%, a growing proportion of solid or micropapillary pattern is not associated with worsening prognosis. In contrast, tumours having micropapillary pattern as secondly predominant form a different intermediate group (OS 51.1%, DFS 57.8%). Our study was based on measured area of each growth pattern on all available slides digitalized. This is the most precise way of determining the size of each component from the material available. We propose using predominant and secondly predominant patterns for prognostic purposes, particularly in tumours having solid or micropapillary patterns.

Keywords Lung adenocarcinoma · Growth pattern · Predominant · Second predominant component · Survival

Introduction

The new classification of lung cancers was introduced by the World Health Organization (WHO) in 2015 [1]. Adenocarcinoma is the most frequent histological subtype among non-small cell carcinomas. Most cases of adenocarcinoma are neoplasms with mixed architecture. They should be subclassified according to the predominant growth pattern, after identification and quantification of all histological patterns in the tumour in 5% increments [1, 2]. Examples of the different growth patterns are demonstrated in Supplementary Fig. 1.

Recent studies have focused on predominant growth patterns and their impact on survival. It is well documented that lepidic predominant carcinomas have better outcome [3], while solid and micropapillary predominant carcinomas have an unfavourable prognosis [3–7]. These results have validated the novel classification of lung adenocarcinomas. However,

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many lung adenocarcinomas show mixed-subtype patterns with two or more different growth patterns, and the impact of non-predominant growth patterns on survival is controversial.

Three growth patterns of invasive adenocarcinomas have been investigated recently, namely the lepidic, the solid and micropapillary patterns. Higher proportion of lepidic component is usually associated with better prognosis [8]. Pure lepidic carcinoma is defined as in situ adenocarcinoma with 100% overall survival (OS) and disease-free survival (DFS). The rates of OS and DFS are similarly excellent in case of minimally invasive adenocarcinoma which is a lepidic predominant tumour with invasive focus less than 5 mm [3]. Invasive, lepidic predominant carcinomas belong to low-grade tumours according to the architectural grade, due to their favourable prognosis [7].

A higher proportion of solid or micropapillary pattern refers to worse prognosis. Local recurrence, nodal involvement and distant metastases are more frequent among these neoplasms [9]. Some authors have found that even 1% [10–13] or 5% [5, 14–18] of these components may cause an unfavourable outcome. In contrast, Roh and coworkers [19] could not confirm that 5% of micropapillary component results in worse OS. Similarly, Sumiyoshi et al. found that the mean percentages of micropapillary pattern showed no significant differences in the recurrent (20.4%) and non-recurrent (18.3%) groups [20].

Beneath the evaluation of proportions of components, predominant and secondary predominant growth patterns can be investigated, as well. Zhao and coworkers have found that acinar/papillary carcinomas having secondary predominant solid or micropapillary patterns show worse prognosis than acinary/papillary carcinomas without secondary solid or micropapillary components [21].

Our aim was to analyse the predominant and the secondary predominant components and the proportions of different growth patterns, namely lepidic, acinar, papillary, solid, micropapillary and cribriform, in stage I lung adenocarcinoma and their influence on OS and DFS. To reach this aim, we decided to use an objective measurement, i.e. the best approximation of areas involved by each pattern available for the cases.

Materials and methods

Haematoxylin-eosin slides of consecutive patients having pulmonary adenocarcinoma in stage I according the 8th Edition of TNM Classification [22] were analysed in our retrospective cohort study. The patients were operated on between 2004 and 2013 at the Division of Thoracic Surgery, Department of Surgery, University of Szeged. The following cases were excluded: patients having multicentric, metachronous or metastatic tumours, or variants of adenocarcinoma, namely invasive mucinous, mixed invasive mucinous/non-mucinous, colloid, foetal, enteric and pleomorphic/sarcomatoid, and those having lung cancer surgery in the preceding 2 years, positive surgical margins, perioperative death, vascular invasion and lack of immunohistochemical phenotyping. For all cases included, mucin staining and immunohistochemistry were applied in the routine diagnostic process. Mucin production and TTF-1 positivity were considered as evidence in support of a primary pulmonary adenocarcinoma for non-small cell lung cancer cases. For TTF-1-negative tumours, further immunohistochemical results have been considered to rule out squamous cell carcinoma (p40 negativity) and metastatic carcinoma (different markers for different primaries).

Clinical data, including gender, age, tumour localization, type of surgery, smoking habits, KRAS and EGFR mutation status and site of recurrence, and follow-up data were obtained from medical charts. Stage I was defined by the combination of tumour size and nodal status in addition to clinical data about the lack of distant metastasis. In all cases, lymphadenectomy was part of the operation and the lymph nodes were examined histologically. The follow-up of patients consisted of three-monthly physical examination, chest x-ray examination and abdominal ultrasonography evaluation in the first 2 years, then six monthly until the fifth year. Chest computer tomography (CT) was performed every 6 months for the first 2 years, then 6 or 12 monthly depending on the patient, until the fifth year. In case of any suspicion of progression, chest CT and abdominal ultrasonography were included. The follow-up period ended on the 31th August 2017.

All available tumour containing slides were digitalized by a Pannoramic 250 scanner (3DHistec, Budapest). As one section was taken from each centimetre of the largest tumour dimension, the number of slides digitalized was influenced by tumour size. The Case Viewer software (3DHistec, Budapest) was utilised for evaluating the cases.

A previous study [7] was based on the evaluation of glass slides; the proportions of growth patterns and the predominant component were estimated by two pathologists (TL, ZT), and consensus was always reached. We also used these results for the assessment of reproducibility between glass slide-based estimation of areas and digital slide-based estimation of the same areas. In the present study, we used digitalized slides. In the first step, the proportions of growth patterns were estimated in 5% increments, and the predominant, secondly and thirdly predominant components were determined with naked eye evaluation. In the second step, the different patterns of the entire tumour were annotated and their areas were measured in square millimetre (Fig. 1). The proportions of each component were calculated from the measured areas. In the third step, the predominant and secondly predominant patterns were re-evaluated in one third of the cases after a time period of minimum 2 weeks. The patterns were re-annotated for assessing

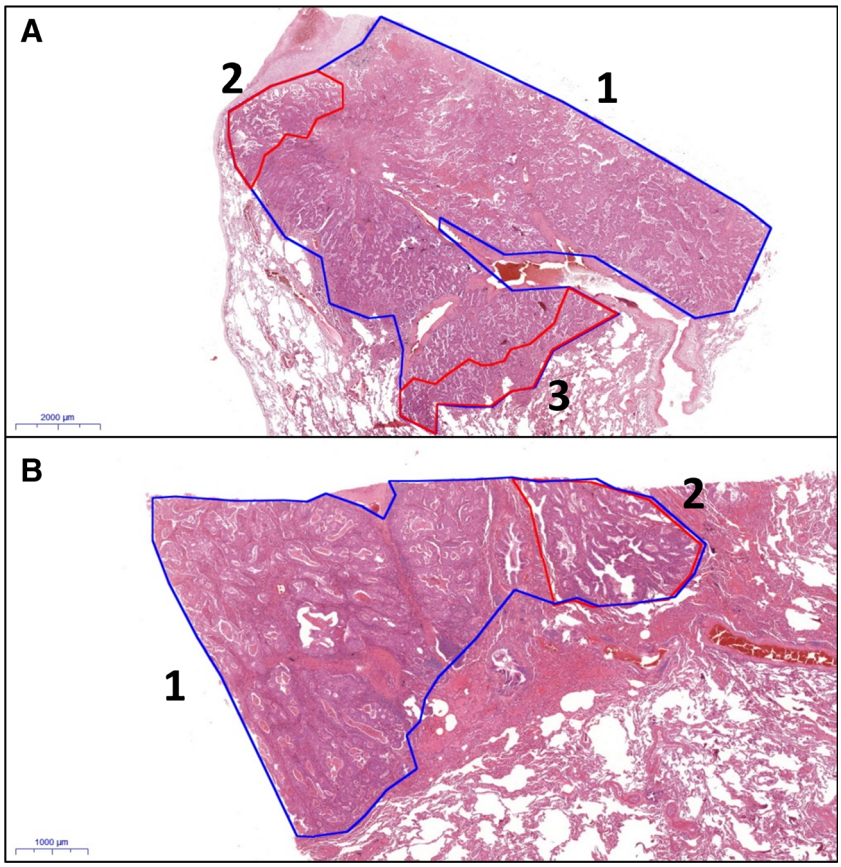

Fig. 1 Examples of annotations of digitalized slides (A: HE, 0.5×; B: HE, 1.5×; 1 (blue): whole tumour, 2 and 3 (red): minor components of tumour)

intra-observer (ZT) variability. All available tumour slides were used for all the listed evaluations.

Statistical methods

Statistical models were based on the calculated proportions of the components (second step evaluation mentioned above). Spearman's rank correlation was used to investigate intra-observer variability. Five-year OS and DFS estimates and mean survival times with their 95% confidence intervals (95% CI) for OS and DFS were assessed using Kaplan-Meier estimates. The log rank test was used for pairwise comparisons. All statistical tests were two-sided, and $p < 0.05$ values were considered statistically significant. We utilised the SPSS Statistics software (IBM, SSPS 22.0, Armonk, NY, USA).

This retrospective study was approved by the institutional ethical committee of the Albert Szent-Györgyi Clinical Centre of the University of Szeged (ethical approval: 168/2016-SZTE).

Results

Altogether, 327 patients matched the inclusion criteria described in the "Materials and methods" section. Clinical follow-up data were missing in 35 cases of surviving and in 31 cases of deceased patients. Slides were not available in 18 cases. After exclusion of patients with missing data or slides, 243 cases remained for this retrospective analysis, with 141 cases in stage IA1–3 and 102 cases in stage IB.

Median age of the patients was 62.3 years (range 33–85). No gender predominance was observed (female 50.7% vs. male 49.3%) in stage I. Most patients had complete lobectomy (lobectomy 90.1% vs. sublobar resection 9.9%). Supplementary Table 1 displays the most important clinicopathological data.

With all available haematoxylin-eosin-stained slides digitalized and evaluated, the median number of slides per patient was 3 (range 2–5). Although the statistical results described below were based on the calculated proportions of the components, the results, not displayed in this study, were similar if the proportions were determined with naked eye (see also below and Supplementary Table 2).

In Table 1, the different growth patterns were recorded in all cases as predominant, secondly predominant, thirdly predominant or absent and associated with 5-year OS and DFS estimates. The median follow-up was 61.5 months (range 1.5–175.3 months). As listed under the table, significant differences in survival rates were found in association with proportions of lepidic pattern (better survival) and proportions of micropapillary or solid patterns (worse survival).

Table 1 Kaplan-Meier estimates of 5-year overall survival (OS) and disease-free survival (DFS) rates, and mean values of OS and DFS associated with predominant, secondly and thirdly predominant or absent growth patterns and the log rank *p* values found significant are displayed

Growth pattern	Predominant (1)			Secondly predominant (2)			Thirdly predominant (3)			Absent (0)			
	n (%)	OS (%)	DFS (%)	*n* (%)	OS (%)	DFS (%)	*n* (%)	OS (%)	DFS (%)	*n* (%)	OS (%)	DFS (%)	*p* (log rank test)
Lepidic	48 (20)	90.5	89.4	25 (10)	67.2	54	34 (14)	75	75	136 (56)	69.4	60.3	p1–0 = 0.005 (OS)
													p1–0 = 0.004 (DFS)
													p1–2 = 0.019 (OS)
													p1–2 < 0.001 (DFS)
Acinar	36 (15)	83.3	61.,5	45 (19)	84.4	76.3	17 (7)	58.4	63.5	145 (59)	70.9	66.7	
Papillary	35 (15)	85.2	72.4	44 (19)	72.4	73.9	31 (13)	91.4	82.,5	133 (53)	66.,8	56.1	
Solid	100 (41)	64.1	56.3	10 (4)	69.5	60	26 (11)	73.1	72.1	107 (44)	82.9	73.2	p1–0 < 0.001 (OS)
													p1–0 = 0.023 (DFS)
Micropapillary	16 (7)	28.1	28.1	20 (8)	51.1	57.8	26 (11)	65.7	63.4	181 (73)	74.9	67.8	p1–0 < 0.001 (OS)
													p1–0 = 0.004 (DFS)
													p2–0 = 0.002 (OS)
													p2–0 = 0.05 (DFS)
													p1–2 = 0.02 (OS)
													p1–2 = 0.004 (DFS)
Cribriform	8 (4)	75	75	9 (4)	88	88	7 (3)	68.6	71.4	219 (89)	73.3	64.2	
	Mean OS	95%CI		Mean OS	95%CI		Mean OS	95%CI		Mean OS	95%CI		
		lower	upper		lower	upper		lower	upper		lower	upper	
Lepidic	127.8	115.4	140.3	101.6	64.3	118.8	97.7	87.5	120.4	82.0	64.4	109.0	
Acinar	105.7	93.2	118.4	108.8	91.7	123.4	98	89.6	114.8	102	90.0	115.8	
Papillary	112.4	94.9	130.7	103.0	87.2	112.8	115.7	102.3	128.4	93.4	85.5	108.8	
Solid	89.4	77.0	101.8	98.4	65.6	131.7	104.1	84.7	123.6	119.2	109.1	129.2	
Micropapillary	53.9	32.8	73.7	95.5	75.6	109.4	101.5	68.2	115.8	117.7	111.5	134.4	
Cribriform	98.2	65.2	131.5	126.4	97.6	155.2	79.6	55.9	98.4	104.5	96.7	112.5	

95CI 95% confidence interval

Table 2 demonstrates the OS and DFS estimates of growth patterns grouped into five groups, namely 0, ≤ 25, 26–50, 51–75 and ≥ 75%. Significant differences were observed between various subgroups of lepidic, solid and micropapillary patterns (see Table 3).

The OS and DFS rates of growth patterns classified as ≥ 5 and < 5% and ≥ 1 and < 1% are displayed in Table 4. With the 5% cut-off point, significant differences in survival were observed in lepidic, solid and micropapillary patterns, and with the 1% cut-off point in lepidic and solid patterns.

There was no recurrence in 151 cases (62.1%). Among these cases, the predominant patterns were the following: lepidic ($n = 40$), acinar ($n = 22$), papillary ($n = 25$), solid ($n = 54$), micropapillary ($n = 5$) and cribriform ($n = 5$). Recurrence was diagnosed in 92 cases including lepidic ($n = 8$), acinar ($n = 14$), papillary ($n = 10$), solid ($n = 46$), micropapillary ($n = 11$) and cribriform ($n = 3$) carcinomas. The rate of recurrence was low in lepidic carcinoma (16.6%); intermediate in acinar (38.8%), papillary (28.5%) and cribriform carcinomas (37.5%); and high in solid (46%) and micropapillary carcinomas (68.7%). Systemic dissemination was detected in 59 patients including lepidic ($n = 5$), acinar ($n = 8$), papillary ($n = 6$), solid ($n = 30$), micropapillary ($n = 9$) and cribriform ($n = 1$) carcinomas. In the non-recurrent group, the average proportion of lepidic, solid and micropapillary patterns were 20, 4 and 5%, respectively, whereas in the recurrent group, these rates were 8, 48 and 13%, respectively. The proportions of other patterns were close to equal in the recurrent and non-recurrent groups.

Besides the morphological evaluation, KRAS and EGFR mutation profiles were analysed in cases with available data (Supplementary Table 3). Sixty-seven percent of KRAS mutations were found in high-grade tumours, especially in solid neoplasms, while most EGFR activating mutations (88.8%) were in low–intermediate-grade tumours, like lepidic, acinar and papillary carcinomas.

In this study, we used naked eye estimation and area measuring for determining the proportions of patterns. As concerns the variability of these methods for the determination of the area of different growth patterns, the opinion agreement was the highest in lepidic, acinar and solid patterns, while papillary, micropapillary and cribriform patterns showed higher variability. In all but one case, the concordance was significant, i.e. there were no relevant differences between the estimated and the calculated proportions of growth patterns (Supplementary Table 2).

Discussion

The new WHO classification of lung adenocarcinomas follows the recommendations of the International Association for the Study of Lung Cancer (IASLC), the American

Table 2 Kaplan-Meier estimates of 5-year overall survival (OS) and disease-free survival (DFS) and mean values of OS and DFS associated with different proportions of growth patterns

Growth pattern	> 75%			51–75%			25–50%			< 25%			0%		
	n (%)	OS (%)	DFS (%)	*n* (%)	OS (%)	DFS (%)	*n* (%)	OS (%)	DFS (%)	*n* (%)	OS (%)	DFS (%)	*n* (%)	OS (%)	DFS (%)
Lepidic	25 (10)	88	91.4	14 (6)	70.3	76.2	4 (2)	75	75	21 (9)	75.2	60	179 (73)	70.5	61
Acinar	20 (8)	90	68.7	15 (6)	93.3	79.4	21 (9)	72.5	66.3	51 (21)	78.3	72.8	136 (56)	67.8	60
Papillary	24 (10)	82.9	70	14 (6)	92.9	77.9	13 (5)	84.6	76.9	63 (26)	78.3	74.9	129 (53)	65.1	56.8
Solid	81 (34)	58.2	52.2	15 (6)	68	65.2	13 (5)	50	53.8	29 (12)	80	78.1	105 (43)	82.5	72.7
Micropapillary	15 (6)	25	25	18 (7)	38.9	37	12 (5)	55	57.80	37 (15)	70.6	68.4	161 (67)	74.1	68
Cribriform	6 (2)	66.7	66.7	7 (3)	100	100	5 (2)	100	100	18 (7)	82.5	63.2	207 (86)	72.4	73.1
	Mean OS	95%CI		Mean OS	95%CI		Mean OS	95%CI		Mean OS	95%CI		Mean OS	95%CI*	
		Lower	Upper		Lower	Upper		Lower	Upper		Lower	Upper		Lower	Upper
Lepidic	131.6	114.9	148.4	114.1	89.1	125.7	106.7	87.4	121.2	105.3	85.3	110.4	98.2	88.8	105.1
Acinar	110.4	97.6	124.4	113.4	101.5	124.7	107.4	82.7	131.5	105.6	90.1	120.6	97.3	86.0	108.5
Papillary	112.4	94.9	130.7	103.0	87.2	112.8	100.2	82.8	123.4	115.7	102.3	128.4	92.5	82.4	106.4
Solid	76.3	49.3	96.9	83.5	70.2	106.3	94.7	78.8	101.2	111.8	98.5	125.1	118.7	108.5	128.9
Micropapillary	66.1	35.4	95.2	66.0	40.6	86.7	78.1	50.6	106.3	112.1	99.4	120.3	119.4	104.4	135.7
Cribriform	101.6	91.4	122.7	105.7	95.3	126.8	110.2	92.2	125.1	103.2	92.6	118.9	102.4	89.1	110.3

95CI 95% confidence interval

Table 3 Significant differences of proportions displayed in Table 2

Growth pattern	Categories	Log rank test	
		p (OS)	*p* (DFS)
Lepidic	0 and > 75%	0.024	0.004
Lepidic	< 25 and > 75%	0.05	0.04
Solid	0 and 25–50%	0.038	0.16
Solid	0 and > 75%	0.001	0.015
Solid	< 25 and 25–50%	0.15	0.045
Solid	< 25 and > 75%	0.032	0.028
Micropapillary	0 and 25–50%	0.034	0.039
Micropapillary	0 and 51–75%	0.002	0.006
Micropapillary	0 and > 75%	0.023	0.05
Micropapillary	< 25 and 25–50%	0.22	0.035
Micropapillary	< 25 and 51–75%	0.001	0.012
Micropapillary	< 25 and > 75%	0.045	0.117

Thoracic Society (ATS) and the European Respiratory Society (ERS). Although the majority of these carcinomas are mixed, the classification takes only the predominant pattern into consideration. Recent studies have shown that secondly predominant patterns or even a small proportion of some patterns can alter prognosis.

Lepidic growth pattern is defined by neoplastic cells growing along the pre-existing alveolar walls. This pattern is the first morphologic sign of carcinogenesis, and if the tumour shows only this pattern, it is called in situ carcinoma and has an excellent prognosis [3]. The lepidic pattern may be associated with other patterns in mixed tumours. Lepidic carcinoma is an invasive tumour with lepidic predominant component and is associated with favourable outcome; therefore, it may be proposed that the more lepidic pattern, the better the prognosis. Mäkinen and coworkers have proven in their series that carcinomas with non-predominant lepidic pattern have more favourable outcome [8]. Although a significant difference was seen between OS and DFS of adenocarcinomas without lepidic component and tumours having ≥1 or ≥ 5% lepidic component, there was no difference in OS and DFS between tumours without lepidic component and tumours having ≤ 25, 25–50 or 51–75% lepidic areas. Our results confirm the evidence that lepidic predominant carcinoma has a favourable prognosis, but there was no difference in OS or DFS between tumours with secondly predominant lepidic component and tumours without lepidic component. A difference was found between the mean proportion of lepidic component of tumours with recurrence (8%) and those without recurrence (20%).

Solid pattern lacking glandular differentiation is a feature of high-grade lung adenocarcinomas. Recent studies have shown that solid predominant adenocarcinoma has a poor outcome [3, 23] and secondary predominant solid pattern or even a small amount of solid component (≥ 5 or ≥ 1%) may worsen the prognosis [12, 18, 21]. A significant difference was observed between OS and DFS of tumours having ≥ 5 or ≥ 1% solid component and those having less (Table 4). Similarly, significant differences were found in OS and DFS between various comparisons of tumours with solid component of 0, ≤ 25, 26–50, 51–75 and > 75% (Table 3). Concerning a solid predominant component, significant differences were found between the OS and DFS estimates of solid predominant tumours and neoplasms without solid features. Despite the worsening tendency of OS and DFS with growing proportion of the solid pattern (Table 1), there were no differences between tumours having secondly or thirdly predominant solid component and tumours without solid pattern. The mean proportion of solid pattern in tumours with recurrence was 48%, contrasting with the 5% in adenocarcinomas without recurrence.

The micropapillary pattern has been incorporated in the adenocarcinoma classification since 2015. Although, according to the classification based on predominant pattern, it would seems that only the greatest proportion of this pattern matters, some studies [5, 10–12, 14–18, 20, 21, 24, 25] indicated that even a minimal amount of micropapillary area is associated with poor prognosis (Table 5).

Kamiya and coworkers have divided their patients into four groups according to the proportion of micropapillary pattern in the tumour: none (0%), focal (≤ 10%), moderate (≤ 50%) and extensive (> 50%). They found that both OS and DFS estimates were worse with the increase in the proportion of the micropapillary component. The latter three groups had significantly less favourable outcomes than tumours without micropapillary pattern, but comparisons among the three latter groups were not done [10]. Zhang et al. [26] have divided their patients into four groups according to the extent of micropapillary component, namely < 1, 1–5, 6–50 and > 51%. Their conclusion was similar to that of Kamiya et al. Our results also parallel these two cited studies. The tumours having more than 25% of micropapillary component formed a uniform group according to OS and DFS estimates and differed from tumours having 0–25% micropapillary area. When using the 5% cut-off for micropapillary component, a significant difference was observed in survival in comparison with tumours with no micropapillary component, while at 1% cut-off point, such a difference was not found. In contrast with Sumiyoshi and coworkers [20], a difference was observed between the groups of patients with and without recurrence: the mean proportions of micropapillary pattern were 13 and 4%, respectively. In the present study, a significant difference in survival was observed between tumours without micropapillary pattern and micropapillary predominant tumours. Similarly to Zhao and coworkers [21], tumours having secondly predominant micropapillary pattern in our series constituted a different group with prognosis between micropapillary predominant carcinomas and tumours having

Table 4 Five-year overall survival (OS) and disease-free survival estimates and mean values of ≥ 5% or less and ≥ 1% or less component with log rank model results

Growth pattern	≥ 5%			< 5%			Log rank	
	n (%)	OS (%)	DFS (%)	*n* (%)	OS (%)	DFS (%)	*p* (OS)	*p* (DFS)
Lepidic	61 (25)	81.4	77.8	182 (75)	72.1	61.6	0.04	0.021
Acinar	100 (42)	72.6	61.2	143 (58)	68.5	59.8	0.23	0.28
Papillary	104 (43)	78.6	71.4	139 (57)	73.4	67.4	0.54	0.12
Solid	127 (52)	67.5	58.7	116 (48)	80.2	71.7	0.012	0.005
Micropapillary	56 (23)	61.2	53	187 (77)	75.6	68.7	0.045	0.041
Cribriform	24 (10)	86.3	72	219 (90)	82	64.3	0.31	0.56
	Mean OS	95%CI		Mean OS	95%CI			
		Lower	Upper		Lower	Upper		
Lepidic	121.5	105.0	131.4	99.4	90.6	106.2		
Acinar	105.2	91.1	112.2	101.2	93.7	115.6		
Papillary	110.7	102.8	118.6	105.6	95.5	113.5		
Solid	95.1	84.1	106.7	116.7	107.5	126.5		
Micropapillary	94.5	81.5	105.1	114.5	105.2	124.4		
Cribriform	102.6	89.5	110.0	101.1	91.8	118.7		
Growth pattern	≥ 1%			< 1%			Log rank	
	n (%)	OS (%)	DFS (%)	*n* (%)	OS (%)	DFS (%)	*p* (OS)	*p* (DFS)
Lepidic	64 (27)	82.2	78.9	179 (73)	71.7	61	0.037	0.008
Acinar	107 (44)	75.8	71	167 (56)	67.8	60	0.33	0.58
Papillary	114 (47)	80.3	74.1	129 (53)	71.5	65.2	0.27	0.078
Solid	138 (57)	66.7	60.2	105 (43)	82.5	72.7	0.045	0.005
Micropapillary	72 (30)	69.4	56.6	171 (70)	74.1	68.6	0.95	0.11
Cribriform	31 (13)	82.5	71.9	212 (87)	72.4	64	0.42	0.49
	Mean OS	95%CI		Mean OS	95%CI			
		Lower	Upper		Lower	Upper		
Lepidic	119.8	106.5	131.6	98.7	88.8	107.7		
Acinar	104.9	92.4	116.6	95.4	82.2	106.5		
Papillary	115.8	106.2	125.4	108.7	95.4	115.7		
Solid	89.4	75.6	101.2	107.6	98.6	116.9		
Micropapillary	105.4	91.1	119.0	105.8	95.6	114.3		
Cribriform	103.5	82.4	125.8	96.6	87.4	108.2		

95CI 95% confidence interval

thirdly predominant micropapillary component or lacking this morphology.

By using 1 or 5% cut-off, significant differences were seen between OS and DFS of tumours having lepidic, solid or micropapillary component and tumours lacking them. After division of mixed adenocarcinomas into four groups, namely 0, ≤ 25, 26–50, 51–75 and ≥ 75%, significant differences were found between OS and DFS of tumours lacking solid or micropapillary component and tumours having > 25% solid or micropapillary pattern. The latter three groups (26–50, 51–75 and ≥ 75%) showed no difference in OS or DFS.

As our study showed, there is a broad spectrum of morphological intra-tumour heterogeneity in lung adenocarcinomas. Interestingly, the invasive tumours having only one component had a more unfavourable prognosis, than neoplasms having mixed pattern (Supplementary Table 4). This finding may be explained by the fact that most tumours having one component were solid tumours with poor outcome. Several series have concluded that this morphological heterogeneity is paralleled by a more complex genetic heterogeneity, as well. Instead of the traditional single-gene approaches, a huge number of genes can be analysed by next-generation sequencing. This new method revealed that there are trunk mutations (e.g. EGFR or KRAS), branching mutations (e.g. EZH2, PIK3CA and p53) and private mutations (ABL1, ALK, BRAF, HER2, etc.) according to the phylogenetic tree model of tumorigenesis. In keeping with earlier published results [27–31], we have found that most EGFR activating mutations are present in the low- or intermediate-grade tumours (lepidic, acinar and papillary), while two thirds of KRAS mutations are associated with high-grade morphology (solid, micropapillary and cribriform). Pelosi and coworkers have demonstrated that one or more branching mutations predicted poor overall survival, independent from age, gender and stage. Finally, additional

Table 5 Recent studies on micropapillary (M) and solid (S) patterns as non-predominant components (*OS* overall survival, *DFS* disease-free survival, *CIR* cumulative incidence of recurrence)

Author	Journal	Year	Number	Stage	Follow-up	Pattern	Cut off
Miyoshi et al. [14]	Am J Surg Pathol	2003	154	I	OS	M	> 5%
Makimoto et al. [24]	Histopathology	2004	85	I	OS	M	> 10%
Mora- Sanchez et al. [15]	Hum Pathol	2008	92	I	OS	M	> 5%
Kamiya et al. [10]	Mod Pathol	2008	197	IA	OS	M	> 1%
Nagano et al. [11]	Lung Cancer	2010	156	I	OS	M	> 1%
Yeh et al. [16]	J Clin Pathol	2012	176	I	DFS	M	> 5%
Sumiyoshi et al. [20]	Lung Cancer	2013	256	IA	OS/DFS	M	> 5%
Nitadori et al. [5]	J Natl Cancer Inst	2013	734	IA (< 2 cm)	CIR	M	> 5%
Koga et al. [28]	Lung Cancer	2013	99	IA	OS	M	> 10%
Cha et al. [12]	J Thorac Cardiovasc Surg	2014	511	IA (< 3 cm)	OS/DFS	M/S	1 %
Zhao et al. [21]	Lung Cancer	2015	201	IA (< 3 cm)	OS/DFS	M/S	Second predominant
Tsubokawa et al. [17]	Eur J Cardiothorac Surg	2016	347	IA	DFS	M	> 5%
Yanagawa et al. [18]	J Thorac Oncol	2016	420	I	OS/DFS	M/S	> 5%

mutations were especially clustered in poorly differentiated regions with KRAS mutations or ALK translocation [27]. The gene mutation landscape is a new dimension with a lot of potential prognostic markers. These factors may explain that the growing proportion of favourable or unfavourable histological component is not necessarily followed by better or poorer prognosis. The gene mutations cannot be predicted by using only morphological parameters; therefore, the molecular studies have inevitable role for clinical management and therapy. Parallel phylogenetic studies of different morphological patterns of the same tumour could help clarifying how these patterns could correspond to genetic alterations.

The prognostic impact of predominant growth pattern was proven on the basis of measuring the area occupied by each pattern on all available (and digitalized) slides, i.e. the most precise way of determining the size of each component from the material available. We think that the study is unique in this respect. Tumours with a secondary predominant micropapillary component demonstrated significant differences in OS and DFS from micropapillary predominant tumours and non-micropapillary tumours. Therefore, we suggest using predominant and secondly predominant patterns particularly in tumours having solid (as suggested by others [12, 18, 21]) or micropapillary (as proven by our data) patterns.

As concerns reproducibility, the reproducibility of the novel WHO classification was proven by several studies [32, 33], but to our knowledge, this is the first study where all tumour-containing slides were digitalized, annotated (Fig. 1), and the area involved by each pattern was objectively calculated to be compared with the proportions gained by naked eye estimation. The statistical analysis revealed that there was no significant difference between these two methods; therefore, naked eye estimation of proportions can be viewed as a useful, time-sparing method of evaluation instead of the objective, but time-consuming, area measurement. The statistical analysis pointed out that the differences in intra-observer variability were low in most patterns evaluated. The strongest congruency was seen in cases of lepidic and solid patterns, while the weakest congruency was observed in papillary and micropapillary components.

Concerning the limitations of the study, elastic staining has been proposed to better delineate the alveolar structures, but was not performed in this study. Lepidic, acinar and papillary growth may have low interobserver reproducibility without this adjunct method. In a previous work, growth patterns of these cases were estimated on glass slides with elastic staining if necessary [7], and the concordance between the estimation results of naked eye and digitalized slide analysis was high. Another limitation is that only stage I adenocarcinomas have been included. In higher stages, the impact of growth patterns is controversial [34, 35], and this is why we have limited our study to early-stage tumours.

In summary, by using the best approximation of the areas occupied by different morphological patterns, we have confirmed that lepidic predominant stage I adenocarcinomas have a good prognosis and solid or micropapillary predominant ones have the worst prognosis. A secondly predominant component of the bad prognostic patterns also worsen prognosis; therefore, the reporting of all patterns observed beyond the predominant component is recommended. Naked eye estimation of the proportions of each pattern does not seem to be worse than objective measurement on digitised slides and can be used instead in routine practice.

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More specifically, author contribution is listed as follows:

TZ: Concept, case selection, application and refinement of the methods, evaluation of all cases, analysis of data, preparation of the manuscript, finalisation and approval of the manuscript

TN: Statistical analysis, preparation of the manuscript, finalisation and approval of the manuscript

LT: Case selection, application and refinement of the methods, evaluation of histology slides, finalisation and approval of the manuscript

GC: Development of the methods, supervision, preparation of the manuscript, finalisation and approval of the manuscript

RP: Data collection, finalisation and approval of the manuscript

EC: Data collection, finalisation and approval of the manuscript

TG: Resources, finalisation and approval of the manuscript

AO: Resources, finalisation and approval of the manuscript

BP: Resources, finalisation and approval of the manuscript

JF: Concept, case selection, finalisation and approval of the manuscript

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Compliance with ethical standards

The authors have consulted the journal policy regarding compliance with ethical standards and state that accepted principles of ethical and professional conduct have been followed. The authors include information regarding sources of funding (previous section) and potential conflicts of interest (financial or non-financial) (next section). This retrospective study was approved by the institutional ethical committee of the Albert Szent-Györgyi Clinical Centre of the University of Szeged. The study did not include animals; therefore, issues relating to animal welfare do not apply.

Conflict of interest The authors declare that they have no conflict of interest.

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