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The More Extensive the Spread through Air Spaces, the Worse the Prognosis Is: Semi-Quantitative Evaluation of Spread through Air Spaces in Pulmonary Adenocarcinomas

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Keywords
Pulmonary adenocarcinoma · Spread through air spaces · Free tumour cluster · Semi-quantitative analysis · Sublobar resection

Abstract
Introduction: The extent of spread through air spaces (STAS) is less investigated among patients with lung adenocarcinoma who underwent sublobar resection. Therefore, we aimed to evaluate the extent of STAS semi-quantitatively, to assess its prognostic impact on overall survival (OS) and recurrence-free survival (RFS), and to investigate the reproducibility of this assessment. ***Methods:*** The number of tumour cell clusters and single tumour cells within air spaces was recorded in three different most prominent areas (200x field of view). The extent of STAS was categorized into three groups, and the presence of free tumour cluster (FTC) was recorded. ***Results:*** Sixty-one patients were included. Recurrence was more frequent with higher grade ($p = 0.003$), presence of lymphovascular invasion ($p = 0.027$), and presence of STAS of any extent ($p = 0.007$). In multivariate analysis, presence of FTC (HR: 5.89; 95% CI: 1.63–21.26; $p = 0.005$) and more pronounced STAS (HR: 7.46; 95% CI: 1.60–34.6; $p = 0.01$) had adverse impact on OS and RFS, respectively. Concerning reproducibility, excellent agreement was found among STAS parameters (ICC range: 0.92–0.94). ***Discussion:*** More extensive STAS is an unfavourable prognostic factor in adenocarcinomas treated with sublobar resection. As the evaluation of extent of STAS is reproducible, further investigation is required to gather more evidence.

Introduction

Lung cancer remains the leading cause of cancer-related death worldwide [1]. Surgical resection is the standard therapy for lung cancer, particularly in stage I non-small cell lung cancer. Lung-sparing surgery may be performed

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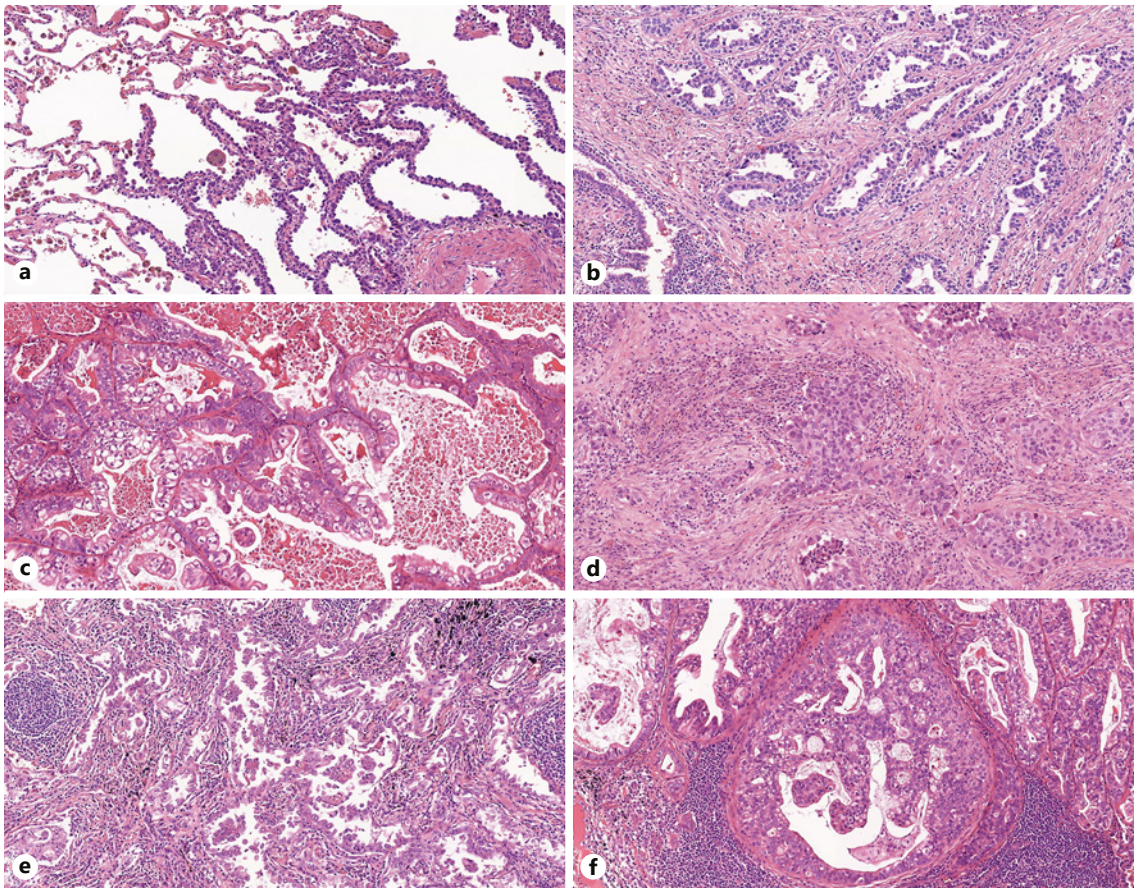

Fig. 1. Histological patterns of non-mucinous lung adenocarcinoma (haematoxylin-eosin, ×10). **a** Lepidic. **b** Acinar. **c** Papillary. **d** Solid. **e** Micropapillary. **f** Cribriform.

for small pulmonary nodules in selected patients [2–4]. Because of the high 5-year recurrence rate in nonsmall-cell lung cancer [5, 6], the prognostic factors of lung adenocarcinoma are currently the subject of extensive research. The most frequent patterns of nonmucinous adenocarcinoma are demonstrated in Figure 1.

Tumour spread through air spaces (STAS) as a form of invasive tumour spread was described by Kadota and associates. They found that the presence of STAS is a significant risk factor for recurrence of small stage I lung adenocarcinoma in patients who underwent limited resection [7]. Warth and co-workers have reported that STAS significantly reduced the recurrence-free survival (RFS), overall survival (OS), and disease-free survival in patients with resected adenocarcinomas of any stage in the categories of both extensive and limited STAS [8]. STAS was introduced in the 2015 World Health Organization (WHO) Classification of lung cancer [9], and the 2021 WHO Classification defines STAS as follows: "tumour cells within airspaces in the lung parenchyma beyond the edge of the main tumour" [10]. As Figure 2a and b displays, the extent of STAS ranges from scarce to extensive; however, the extent of the phenomenon has been barely investigated. Firstly, Uruga and co-workers [11] and Morimoto et al. [12] have focused on the semi-quantitative evaluation of STAS. The former research classified the extent of STAS semi-quantitatively and correlated the results with prognosis. The latter investigation has introduced the free tumour cluster (FTC) in pulmonary adenocarcinoma with micropapillary component (MPC), and the prognostic role of FTC has been evaluated [11, 12].

To our knowledge, the prognostic effect and the reproducibility of these semi-quantitative measurements of STAS have not been investigated yet. Therefore, the aim of this study was to evaluate semi-quantitatively the extent of STAS and the presence of FTC in lung adenocarcinomas resected by limited surgery (sublobar resec-

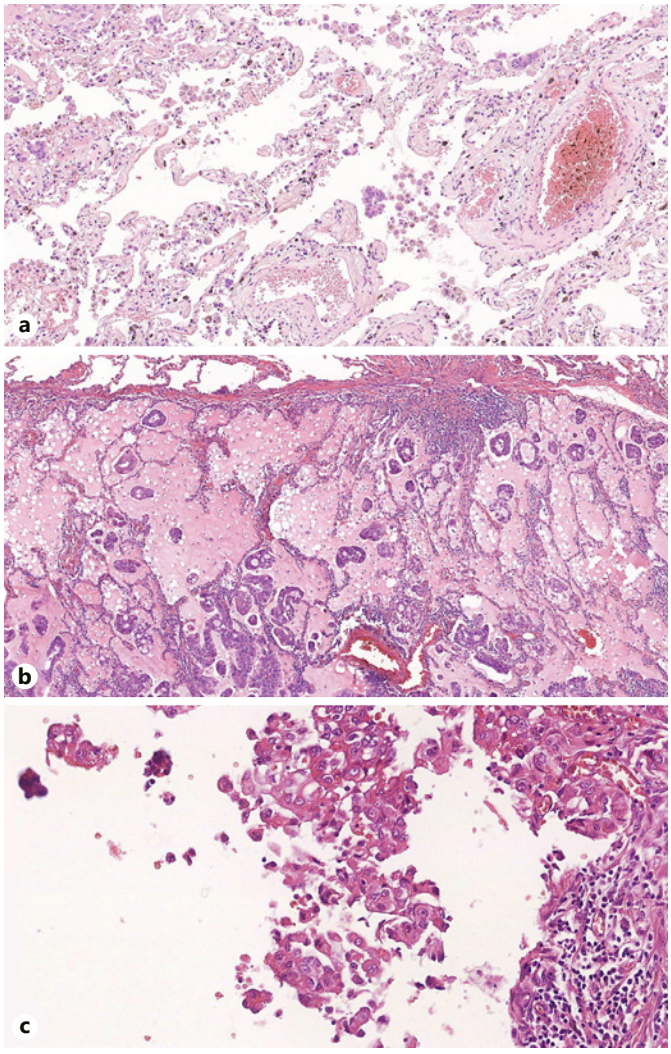

Fig. 2. Spectrum and mimickers of STAS. **a** Single neoplastic cell cluster in the peritumoral area. **b** Several neoplastic cell clusters in the peritumoral alveoli. **c** Example for artificial tumour disintegration (STAKS).

tion); to evaluate their prognostic impact on OS and RFS; and to analyse the reproducibility of assessing these features.

Materials and Methods

This study was conducted at Department of Pathology, Albert Szent-Györgyi Clinical Centre, University of Szeged, Hungary. Patients diagnosed with primary lung adenocarcinoma who underwent sublobar resection of the lung at the Department of Surgery, University of Szeged, between 1 January 2010 and 31 December 2019 were included. Exclusion criteria were positive surgical margins, perioperative death (within a month of surgery), lack of clinical data, and unavailable histological slides. The patients' demographic and clinicopathological parameters including age, gender, smoking history, type of surgery, Eastern Cooperative Oncology Group performance status, adjuvant therapy, histological subtype, International Association for Study of Lung Cancer (IASLC) grade [13], necrosis, tumour size, pT and pN status, stage [14], extranodal extension, distance from resection margin, lymphovascular, vascular, and pleural invasion were collected from medical records, while the presence of STAS (STAS1) was detected during the re-evaluation of all haematoxylin-eosin stained histological slides. The definition of STAS introduced by the WHO was utilized [10]. The ex vivo artefacts (shown in Fig. 2c) defined in our previous publication were excluded from evaluation [15]. All patients underwent follow-up that consisted of regular physical examination, chest X-ray, abdominal ultrasonography, and chest computer tomography. OS was defined from the date of resection to death from any cause, and RFS was measured from the date of resection to the development of clinical/radiologic recurrence of disease. Locoregional recurrence was interpreted as evidence of a tumour in an ipsilateral lobe (regardless of the original affected lobe(s)), in the ipsilateral hilar lymph nodes or in the ipsilateral mediastinal lymph nodes. The follow-up period ended on 1 June 2021.

Formalin-fixed, paraffin-embedded 4-μm-thick sections stained with haematoxylin-eosin were re-evaluated by two investigators independently (N.Z.T. and T.Z.). The number of tumour cell clusters and single tumour cells within the air spaces beyond the edge of the main tumour was recorded in three different fields of view in the most prominent area by using a multi-headed microscope (Olympus BX43, Tokyo, Japan) with ×20 objective and ×10 ocular lens (intermediate power field [IPF], 200x, visible area = 0.237 mm^2). Maximum, mean, and total number of tumour cell clusters and single tumour cells were recorded in each case. According to our preliminary results, the extent of STAS was defined as "no STAS," "low STAS" (1–10 tumour cell clusters or 1–4 single tumour cells per one 200x field of view), and "high STAS" (≥11 tumour cell clusters or ≥5 single tumour cells per one 200x field of view). Figure 2a and b demonstrate "low STAS" and "high STAS" categories. FTC was defined as >3 small clusters containing <20 nonintegrated micropapillary tumour cells with a distance >3 mm apart from the main tumour [12]. The distance of the farthest tumour cell cluster from the invasive front was also determined. Reproducibility of these categories was investigated on digitized slides. In each case, one histological slide with the most prominent areas of STAS was digitized with a Pannoramic MIDI II scanner (3DHistech, Budapest). Case Viewer software (3DHistech, Budapest) was utilized for the evaluation. All digitized slides were re-evaluated by four investigators (N.Z.T., S.A., A.S., and T.Z.) in order to examine inter-observer and inter-method variability.

The χ^2 and Mann-Whitney tests were utilized to identify association between variables and locoregional recurrence, and Spearman test was applied to evaluate the correlation between the variables and the STAS1. Receiver operating characteristic (ROC) curve analysis focusing on OS and RFS was utilized to compare sensitivity and specificity of STAS parameters including maximum, mean, and total number of tumour cell clusters and single tumour cells. Univariate Cox proportional hazard model was applied to detect morphological variables having impact on OS and RFS. Those found significant in the univariate analysis were entered into multivariate Cox proportional hazard model.

To decrease bias caused by adverse prognostic variables, two subgroup analyses were done. In the first subgroup, the cases with

Table 1. Patients' clinical characteristics

Variables	STAS0	STAS1	*p* value*
Age, mean (range), years	64.0 (54–76)	65.0 (52–76)	0.56
Gender			
Male	11	10	0.42
Female	16	24	
Type of surgery			
Open	14	14	0.41
VATS	13	20	
Smoking			
Never	7	4	
Former	17	26	0.15
No data	3	4	
ECOG performance status			
0	10	14	
1	11	16	
2	4	2	0.376
3	1	0	
No data	1	2	
Adjuvant chemotherapy			
No	15	11	
Yes	10	19	0.08
No data	2	4	

VATS, video-assisted thoracoscopic surgery; ECOG, Eastern Cooperative Oncology Group. * Spearman correlation.

Table 2. Morphologic features of patients

	STAS0	STAS1	*p* value*
Histological subtype			
Lepidic	2	0	
Acinar	2	7	
Papillary	8	15	0.35
Solid	14	8	
Micropapillary	0	3	
Cribriform	1	1	
IASLC grade			
Low grade	2	0	
Intermediate grade	9	8	0.11
High grade	16	26	
Necrosis			
Absent	18	24	0.78
Present	9	10	
Tumour size, mm			
Mean	17	23.5	**0.011**
Range	4–29	7–75	
T			
1	22	21	
2	1	9	0.20
3	1	3	
4	3	1	
N			
0	25	25	
1	1	2	**0.031**
2	1	7	
No data		1	
Stage			
I	21	19	
II	1	7	0.133
III	5	8	
Vascular invasion			
Absent	24	28	0.48
Present	3	6	
Lymphovascular invasion			
Absent	23	20	**0.025**
Present	4	14	
Pleural invasion (PL)			
PL0	22	22	
PL1	4	8	0.134
PL2	0	0	
PL3	1	4	

IASLC, International Association for the Study of Lung Cancer; STAS0, absence of STAS; STAS1, presence of STAS. * Spearman correlation.

lymph node metastasis, lymphatic spread, stage III, and pleural invasion were excluded. In the second subgroup, in addition to the former criteria, patients with vascular invasion were omitted, as well. Univariate and multivariate Cox proportional hazard models were utilized as described above.

Kaplan-Meier method was applied to evaluate the impact of "low STAS," "high STAS," and FTC on OS and RFS. Log-rank test was utilized for pairwise comparisons. Interclass correlation coefficient (ICC: two-way mixed effects, absolute agreement, single rater) was utilized to measure inter-method and inter-observer variability of STAS parameters. The ICC inter-rater agreement measures defined by Koo and Li [16] were applied. All statistical tests were two-sided, and $p < 0.05$ values were considered statistically significant. Statistical analysis of the data was carried out using SPSS Statistics software (IBM, SPSS 22.0, Armonk, NY, USA).

Results

Altogether, 89 patients were operated on at the Department of Surgery, University of Szeged, between 2010 and 2019. Adenocarcinoma was diagnosed in 68 cases, squamous cell carcinoma was verified in 14 patients, and neuroendocrine tumours were found in 4 cases. Rare tumours, namely, epithelioid hemangioendothelioma and malignant Langerhans cell histiocytosis, were seen in 2 and 1 patients, respectively. Of 68 patients diagnosed with adenocarcinoma, 7 cases were excluded due to the lack of follow-up data. Overall 61 patients who underwent sublobar resection were included. The clinicopathological characteristics of patients are summarized in Tables 1 and 2. The median age was 64 years, and the majority of pa-

Table 3. Main characteristics of semi-quantitative evaluation of STAS

FTC	*n* (%)
Absent	49 (80.3)
Present	12 (19.7)
Tumour cell cluster (3x IPF)	
Maximum (range)	1–85
Mean (range)	0.33–45
Total (range)	1–135
Single tumour cell (3x IPF)	
Maximum (range)	1–15
Mean (range)	0.33–11.33
Total (range)	1–34
Distance (tumour cell cluster), mm	
Mean	1.37
Range	0.46–7.1

FTC, free tumour cluster; IPF, intermediate power field (200x).

tients were females. Half of the patients have received adjuvant therapy (platinum-based chemotherapy with taxane or vinorelbine). Two-thirds of cases were either solid or papillary adenocarcinomas and high-grade tumours. The most frequent category was pT1pN0. The median follow-up time was 32.9 months (range: 5–131 months). In 25 patients, 12 locoregional and 13 distant recurrences were diagnosed. Altogether, 19 patients died from either progression of lung adenocarcinoma or other causes. The incidence of STAS is displayed in Table 2. The STAS1 was the highest in the high grade (76.4%) followed by the intermediate grade tumours (23.5%).

There were statistically significant associations between locoregional recurrence and higher IASLC grade (p = 0.003), presence of lymphovascular invasion (p = 0.027), and STAS1 of any extent (p = 0.007). STAS was correlated with greater tumour size (p = 0.015), higher N (p = 0.031) category, and presence of lymphatic spread (p = 0.046). STAS of any extent was found in 55.7% of cases. The main characteristics of semi-quantitative evaluation of STAS are presented in Table 3. The ROC curve analysis focusing on OS and RFS has identified that the maximum number of tumour cell clusters per 200x field of view and the maximum number of single tumour cells per 200x field of view have the highest area under the curve (AUC) values ($AUC_{tumour\ cluster}$: 0.734; $AUC_{single\ tumour\ cell}$: 0.813), respectively. Based on these parameters, STAS was categorized into three groups, namely, "no STAS," "low STAS," and "high STAS." The Kaplan-Meier analysis demonstrated significant differences between OS and RFS estimates of the different STAS categories (shown in Fig. 3a, b).

FTC was present in 19.7% of patients and had a significant impact on OS. Based on the Kaplan-Meier analysis, there were significant differences of OS estimates of tumours with STAS+/FTC+ and STAS+/FTC– and STAS– tumours (shown in Fig. 3c).

Table 4 demonstrates the pathological variables having significant impact on OS or RFS according to the univariate Cox proportional hazard model. In univariate analysis, higher T and N status, higher clinical stage, presence of lymphovascular and pleural invasion, presence of FTC, and "high STAS" were associated with unfavourable OS and RFS estimates. In multivariate analysis of OS estimates, higher T category, presence of lymphovascular invasion, and FTC were associated with adverse prognosis. Concerning the multivariate analysis of RFS estimates, the presence of lymphovascular invasion and "high STAS" category had unfavourable impact on prognosis.

The findings of subgroup analyses are presented in online supplementary Tables 1 and 2 (for all online suppl. material, see www.karger.com/doi/10.1159/000525456). The exclusion of adverse prognostic factors emphasized the prognostic role of STAS. The STAS1 had an unfavourable impact on RFS; furthermore, the data suggest that the higher the degree of STAS, the less favourable the prognosis.

The ICC values reflecting the consistency of the semi-quantitative STAS assessments are displayed in online supplementary Table 3. Concerning the inter-method variability (histological slide vs. digitized slide), the ICC revealed excellent agreement among STAS parameters (ICC range: 0.92–0.94), while concerning inter-observer variability on digitized slides, the inter-rater agreement was better in tumour cell cluster counting than in single tumour cell detection (ICC: 0.97 vs. 0.83).

Discussion

Aerogenic spread, first described by Cain [17], is present in primary and secondary neoplasms of the lung; however, this form of tumour spread is more frequent among primary pulmonary adenocarcinomas and thereby has been extensively studied. Clinicopathological parameters related to the STAS1 have been reported in several adenocarcinoma cohorts [18–22]. Kadota and coworkers [7] have examined 411 resected stage I lung adenocarcinomas. Lymphovascular invasion, micropapillary, and solid histological subtype have been associated with the STAS1 [7]. Warth and associates have analysed

569 patients with completely resected lung adenocarcinomas and found that micropapillary predominant high-stage, node-positive, and metastasized adenocarcinomas were associated with the STAS1 [8]. Shiono and Yanagawa [23] have investigated 318 patients with stage I lung adenocarcinomas and found that male gender, smoking, solid nodules, stage IB, lymphovascular and pleural invasion were associated with the STAS1. Similarly, in a previous study, we found that the STAS1 is an independent adverse prognostic factor in lobectomy specimens [15].

On the contrary, there are some series, where the prognostic role of STAS could not be supported [24, 25]. Furthermore, according to Blaauwgeers and co-workers [26], if a tumour is dissected cut by cut with the same blade without cleaning, the number of free tumour cell clusters within air spaces can be increased. The latter phenomenon is called spread through a knife surface (STAKS). Therefore, the pathogenesis and the significance of STAS are still debated. However, three meta-analyses have been published recently, in which the STAS1 was associated with adverse RFS and OS outcome [27–29].

The investigation conducted by Morimoto and associates was the first study focusing on the quantification of STAS and introduced FTC in a rather restrictive morphological definition of the phenomenon. They examined 67 patients with lung adenocarcinomas containing at least 5% MPC, in contrast to our study, where presence of FTC was identified in all types of adenocarcinomas regardless of the growth patterns. They found that the distance of the FTC never exceeded the diameter of the main tumour. Based on the survival analysis, RFS was significantly reduced in the MPC+/FTC+ group compared to the MPC+/FTC– group [12]. In contrast to the results of Morimoto, we found that FTC is a prognostic factor for OS in univariate (HR: 3.03; 95% CI: 1.09–8.38; $p = 0.034$) and multivariate analysis (HR: 5.909; 95% CI: 1.72–20.25; $p = 0.005$). Furthermore, we found that FTC+ adenocarcino-

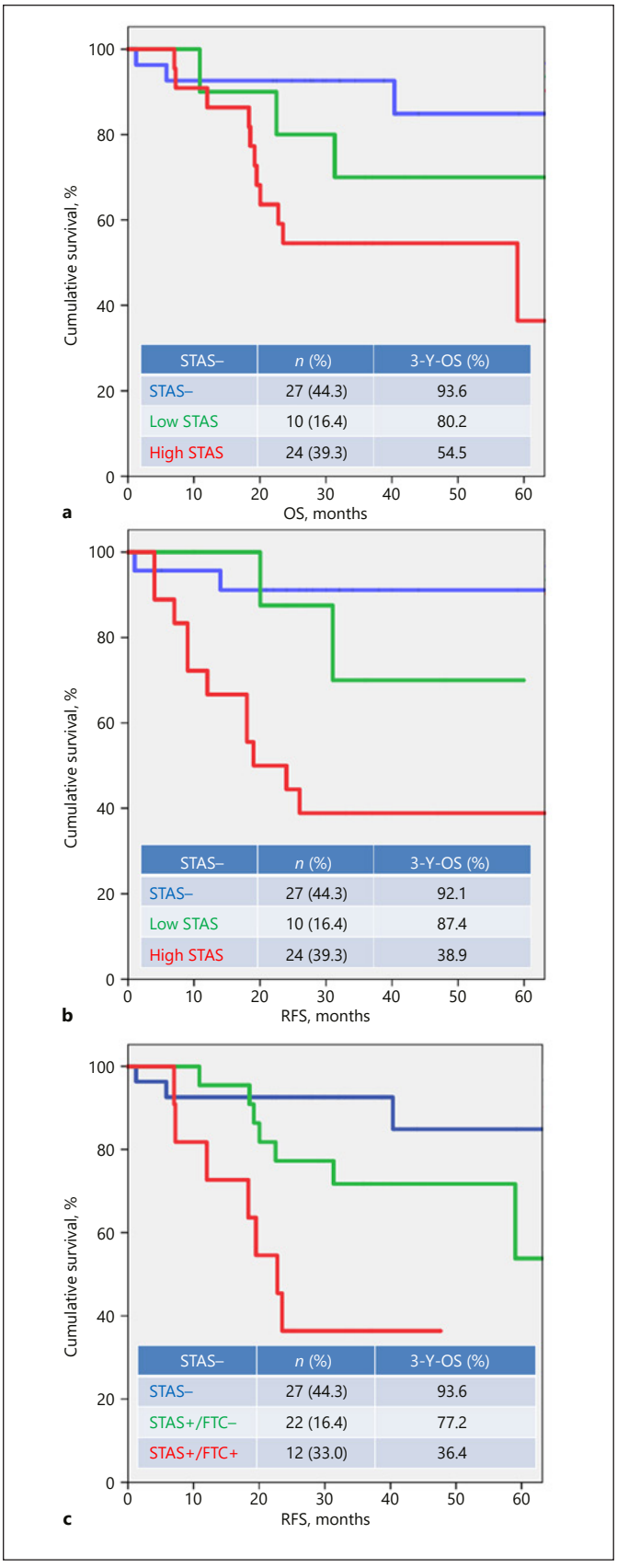

Fig. 3. a Kaplan-Meier analysis of "low STAS" and "high STAS" scheme. Significant differences were found between the OS estimates of STAS– versus "high STAS" tumours ($p < 0.001$) and between of STAS– versus "low STAS" tumours ($p = 0.04$). **b** Significant differences were detected between the RFS estimates of STAS– versus "high STAS" tumours ($p < 0.001$) and "high STAS" versus "low STAS" tumours ($p = 0.031$). **c** Kaplan-Meier analysis of STAS with or without FTC. Significant differences were found between the OS estimates of STAS– versus STAS+/FTC+ tumours ($p < 0.001$), between of STAS– versus STAS+/FTC– tumours ($p < 0.001$) and between of STAS+/FTC– versus STAS+/FTC+ tumours ($p = 0.025$). 3-Y-OS, 3-year OS; 3-Y-RFS, 3-year RFS.

Table 4. Univariate and multivariate Cox proportional hazard models with OS and RFS estimates

Variables	Univariate analysis						Multivariate analysis					
	Cox regression (OS)			Cox regression (RFS)			Cox regression (OS)			Cox regression (RFS)		
	HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value
T												
1	Reference			Reference			Reference					
2	1.57	0.42–5.82	0.49	**2.48**	**1.01–6.17**	**0.05**	0.73	0.27–4.31	0.66			
3	**8.23**	**2.46–27.5**	**0.001**	3.09	0.69–13.7	0.13	**7.69**	2.42–31.0	**0.002**			
4	1.86	0.39–8.67	0.432	1.78	0.38–7.63	0.47	2.02	0.35–8.15	0.39			
N												
0	Reference			Reference								
1	1.29	0.16–9.99	0.80	1.39	0.32–6.03	0.65						
2	**4.43**	**1.49–13.2**	**0.007**	**3.33**	**1.2–9.25**	**0.02**						
Stage												
I	Reference			Reference								
II	**4.49**	**1.26–16.0**	**0.02**	2.48	0.88–7.00	0.08						
III	**5.63**	**1.94–16.3**	**0.001**	**2.96**	**1.16–7.55**	**0.02**						
L (present)	**7.63**	**2.84–20.63**	**<0.001**	**3.34**	**1.48–7.59**	**0.004**	**11.33**	3.38–37.90	**<0.001**	**3.65**	**1.12–11.8**	**0.03**
Pleural invasion (present)	**2.14**	**1.45–3.16**	**0.001**	**1.52**	**1.01–2.29**	**0.044**						
STAS (present)	**5.66**	**1.61–19.8**	**0.001**	**5.16**	**1.89–14.11**	**0.001**						
"Low STAS"/"high STAS"												
Absent	Reference			Reference						Reference		
"Low STAS"	**4.19**	**1.02–18.9**	**0.045**	2.79	0.39–19.8	0.30				2.87	0.40–20.4	0.29
"High STAS"	**6.17**	**1.70–22.4**	**0.006**	**8.48**	**1.87–38.4**	**0.01**				**7.46**	**1.60–34.6**	**0.01**
FTC (presence)	**3.03**	**1.09–8.38**	**0.034**	2.28	0.93–5.58	0.071	**5.89**	1.63–21.26	**0.005**			

Only variables with at least one significant difference between a category and the reference category are presented. STAS, spread through air spaces; FTC, free tumour cluster.

mas have unfavourable OS and RFS estimates similar to that of pT3 adenocarcinomas (2-year OS rate: 48.2% vs. 46.4%; $p = 0.16$; 2-year RFS rate: 41.2% vs. 35.4%; $p = 0.18$). This finding supports the hypothesis that FTC might rather be an intrapulmonary micrometastasis, which might influence future definitions of the T categories.

Uruga and associates have used another method of quantifying STAS. They investigated 208 lung adenocarcinomas and classified the extent of STAS into three groups: "no STAS," "low STAS," and "high STAS." These groups take into consideration the number of tumour cell clusters and single tumour cells. They have found that solid subtype of invasive adenocarcinoma, lymphovascular and pleural invasion, and ≥10 mm tumour size were related to "high STAS." Based on univariate and multivariate analysis, "high STAS" was significantly associated with decreased RFS estimates [11]. In our series, the ROC curve analysis has revealed that the maximum number of tumour cell clusters and single tumour cells per 200x field of view has the most acceptable specificity and sensitivity to identify the risk of tumour-specific death and recurrence. The "low STAS" and "high STAS" categories were defined on the basis of the ROC curves. Only the tumour cell cluster cut-off values were different from that of Uruga and co-workers [11] (1–10 and ≥11 vs. 1–4 and ≥5 tumour cell cluster per 200x field of view). Similar to their results, we found significant differences among OS and RFS estimates of different STAS categories (shown in Fig. 3a, b).

Based on the findings of univariate Cox proportional hazard model, higher T and N categories, higher clinical stage, presence of pleural and lymphovascular invasion, "high STAS" category, presence of FTC were associated with unfavourable OS and RFS estimates. These results are consistent with the findings of the abovementioned studies [21–24]. Concerning the multivariate analysis, higher T category, presence of lymphovascular invasion, and FTC were associated with adverse OS prognosis. According to the findings of multivariate analysis of RFS estimates, the presence of lymphovascular invasion and "high STAS" category had unfavourable impact on prognosis (Table 4). Our subgroup analysis excluding patients with stage III, lymph node metastasis, vascular, lymphatic, and pleural invasion demonstrated that "high STAS" category has an adverse impact on RFS estimates. "Low STAS" category was not statistically significantly different from the reference category; however, it showed a trend towards worse outcome (online suppl. Table 2). These results are keeping with those of Yang and co-workers [28]. They have presented in their recent meta-analysis that the more extensive the STAS, the more unfavourable the prognosis [28].

Additionally, inter-observer and inter-method variabilities were assessed. Both histological and digitized slides could be used for the evaluation of the maximum number of tumour cell clusters and single tumour cells, for detecting FTC and for measuring the distance of the farthest tumour cell cluster from the main tumour. On the basis of the ICC values, the inter-rater agreement was good to excellent for the parameters investigated [16].

Our study has several limitations. Firstly, it was a retrospective study conducted in a single centre with a small number of patients. The incidence of the solid subtype may be a further limitation, as it represents an unfavourable group of adenocarcinoma. Despite the small number, statistical analysis could be applied and our results could be compared to that of Uruga's and Morimoto's investigations including 45 and 125 patients who underwent sublobar resection, respectively. Secondly, patients received platinum-based adjuvant therapy, which was not assessed in detail in our analysis. Thirdly, although the identification of cut-off values in the definition of "low STAS" and "high STAS" categories was based on ROC curve analysis and was done to allow a segregation of the smaller and larger amount of STAS, this cut-off may turn to be different with a greater cohort of tumours. However, the aim of the present study was not to define and recommend an exact cut-off value for the "low STAS" and "high STAS" categories but to allow a quantitative distinction between tumours on the basis of STAS. Nevertheless, some concerns may be raised about the method used for the semi-quantitative evaluation of STAS. As an analogy, it can be mentioned that the quantification of the extent of tumour budding in colorectal cancer was developed with very similar methodology [30]: namely, separate tumour buds are counted in one IPF of view (200x). Large-scale cohort studies demonstrated that high-grade tumour budding is an unfavourable prognostic feature in these colorectal adenocarcinomas. Furthermore, there are publications utilizing the same methods in the evaluation of tumour budding in lung squamous cell carcinoma [31, 32]. We believe that an internationally accepted methodology for these phenomena [11, 30–34] and the recommendation by the WHO to report tumour budding in daily routine [35] is an acceptable support for the evaluation of a very similar phenomenon, namely, the STAS. Furthermore, Uruga and co-workers used only one field of view with ×20 magnification to investigate STAS. To decrease the sampling error resulting from selection of

the most prominent area, we detected the extent of STAS in three fields of intermediate power view in each case.

Concerning the strengths of our study, this is the first study aiming at the prognostic validation of the "low STAS" versus "high STAS" and the FTC schemes of STAS quantification. Secondly, we focused only on lung-sparing surgery specimens. Thirdly, in contrast to Morimoto and co-workers, we have demonstrated that FTC has a prognostic role in all types of adenocarcinoma investigated regardless of growth patterns. The evaluation of the extent of STAS is reproducible on both routine histological slides and digitized slides. Therefore, we suggest to use these methods to gather more data allowing a robust analysis of STAS quantity on prognosis. Fourthly, tumours were sliced with prosecting blades cleaned before each slice, aiming at the minimization of the STAKS effect. Finally, the definition of STAS and the exclusion of artefacts were rigorously applied as described in investigation of Thunissen and co-workers [36]. Namely, the intra-alveolar tumour clusters found distant from the tumour without alveolar interconnection to the tumour mass, random clusters of tumour cells especially at the edges of the sample, jagged edges of tumour clusters, linear strips of cells lifted off alveolar walls were identified as ex vivo artefacts and excluded from investigation.

In summary, STAS was investigated semi-quantitatively in this retrospective study of consecutive cases, and more extensive STAS was related to more unfavourable OS and RFS in adenocarcinomas treated with sublobar resection. Despite the limitations, our results prove that the extent of STAS deserves further investigation. In keeping with the WHO classification, the latest lung cancer reporting protocol introduced by the College of American Pathologists recommends the reporting of STAS, as well [37]. Based on the literature [27–29] and our findings, we propose that the extent of STAS is worth to be reported in routine practice to gather more evidence.

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Statement of Ethics

This study protocol was reviewed and approved by the Institutional Ethical Committee of the Albert Szent-Györgyi Clinical Centre of the University of Szeged, approval number [58/2020-SZTE]. Written informed consent was not required by the decision of the Institutional Ethical Committee of the Albert Szent-Györgyi Clinical Centre of the University of Szeged. In the case of noninterventional retrospective research, where personal information would be impossible or disproportionately costly, based on the Regulation 23/2002 (V. 9.) EüM. Regulation 20/Q. §, information of a patient and application of a written informed consent can be disregarded.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Noémi Zombori-Tóth, Dóra Paróczai, Judit Lantos, Szintia Almási, Anita Sejben, László Tiszlavicz, Gábor Cserni, József Furák, and Tamás Zombori contributed to the study conception and revision. Histological slides were evaluated by Noémi Zombori-Tóth, Szintia Almási, Anita Sejben, and Tamás Zombori. Survival data collection was performed by Dóra Paróczai. Statistical analysis was performed by Noémi Zombori-Tóth and Tamás Zombori. The manuscript was written by Noémi Zombori-Tóth and Tamás Zombori, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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