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ARTICLE

Tumor spread through air spaces (STAS): prognostic significance of grading in non-small cell lung cancer

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Abstract

Tumor spread through air spaces (STAS) is an invasive pattern of lung cancer that was recently described. In this study, we investigated the association between the extent of STAS and clinicopathological characteristics and patient outcomes in resected non-small cell lung cancers (NSCLCs). STAS has been prospectively described from 2008 and graded its extent with a two-tiered system (STAS I: <2500 μm [one field of ×10 objective lens] from the edge of tumor and STAS II: ≥2500 μm from the edge of tumor) from 2011 in Seoul National University Bundang Hospital. We retrospectively analyzed the correlations between the extent of STAS and clinicopathologic characteristics and prognostic significance in 1869 resected NSCLCs. STAS was observed in 765 cases (40.9%) with 456 STAS I (24.4%) and 309 STAS II (16.5%). STAS was more frequently found in patients with adenocarcinoma (ADC) (than squamous cell carcinoma), pleural invasion, lymphovascular invasion, and/or higher pathologic stage. In ADC, there were significant differences in recurrence free survival (RFS), overall survival (OS), and lung cancer specific survival (LCSS) according to the extent of STAS. In stage IA non-mucinous ADC, multivariate analysis revealed that STAS II was significantly associated with shorter RFS and LCSS ($p < 0.001$ and $p = 0.006$, respectively). In addition, STAS II was an independent poor prognostic factor for recurrence in both limited and radical resection groups ($p = 0.001$ and $p = 0.023$, respectively). In conclusion, presence of STAS II was an independent poor prognostic factor in stage IA non-mucinous ADC regardless of the extent of resection.

Introduction

The concept of spread through air spaces (STAS) was introduced for pulmonary adenocarcinomas (ADC) in the 2015 World Health Organization Classification based on two large independent cohort studies [1, 2] where STAS is defined as micropapillary (MP) clusters, solid nests, or single cells spreading within air spaces beyond the edge of the main tumor. STAS is now established as an invasion pattern of ADC.

After its introduction in 2015, many studies have validated the significance of STAS, in particular in ADC, while few studies evaluated STAS in squamous cell carcinoma (SqCC) [3–5] and neuroendocrine tumors (NETs) [6–8]. Recent meta-analyses have revealed that STAS is a potentially significant prognostic factor for patients with surgically resected non-small cell lung cancers (NSCLCs) [9–11]. However, it is still controversial whether STAS is an in vivo phenomenon or potentially an ex vivo artifact [12, 13], and whether it carries a prognostic significance only in limited resection cases. Kadota

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et al. reported that STAS was a significant risk factor of recurrence in small-sized ADCs treated with limited resection but not in those who underwent lobectomy [2], and Shiono et al. and Masai et al. have confirmed the results [14, 15]. Eguchi et al. also reported that lobectomy was associated with better outcomes than sublobar resection in patients with STAS-positive T1 lung ADC [16]. As most studies did not specify the extent of surgery, however, the significance of STAS needs to be further validated according to surgical extent.

There have been several attempts to grade STAS according to the distance from tumor edge [1, 3–5] or the number of tumor clusters [17, 18]. Although Uruga et al. reported that larger numbers of tumor clusters of STAS predicted worse recurrence free survival (RFS) [18], neither the standard method nor the significance of STAS grading has been established.

We recognized this phenomenon in resected lung cancer specimens in 2008 and have reported STAS with the term of "aerogenous spread" in the pathology report since then. We started grading the extent of STAS according to its distance from the edge of tumor border with a two-tiered system from 2011. The objective of this study was to investigate the association of the extent of STAS with clinicopathologic features and patient outcomes in the prospectively collected database of surgically resected NSCLCs.

Materials and methods

Patient cohorts

This study was approved by our institutional review board (B-2003-600-105) and the need for informed consent was waived. We reviewed 2775 pathology reports with lung cancers that had been surgically resected between 2011 and 2018. Patients with other malignancy, neoadjuvant therapy, other surgical, or systemic treatment history and other disease progression were excluded from the study cohort. Patients who diagnosed as NETs or other rare entities were excluded from the study cohort. According to these criteria, we identified a total of 1869 NSCLC cases. The pathologic stage was reclassified according to the 8th edition of the *American joint committee on cancer staging manual* [19].

Recurrences were confirmed by clinical, radiological, and/or pathological assessments, including locoregional and distant recurrences. Locoregional recurrence was defined as evidence of a tumor in the ipsilateral lung, ipsilateral hilar lymph nodes, and/or ipsilateral mediastinal lymph nodes. Distant recurrence was defined by evidence of a tumor in the contralateral lung, contralateral mediastinal lymph nodes, ipsilateral supraclavicular lymph nodes, and/or outside the hemithorax [2].

Pathologic examination of resected lung cancer specimens

In our institution, since 2004, all resected lung cancer specimens have been delivered to the pathology ward as quickly as possible to reduce a cold ischemia time. After gentle injection of diluted OCT media for frozen section or neutral buffered 10% formalin through the pleural surface or lobar bronchus using a syringe, the specimen was fixed for about 24 h. After fixation, the specimen was serially cut in 5 mm thick sections [20–22]. We sectioned and submitted the entire tumor for microscopic examination when the tumor was 3 cm or smaller. In addition, the slab that represented the largest dimension of the tumor and surrounding nonneoplastic lung parenchyma was completely submitted with mapping, and all the sampled tissue blocks were annotated on the photographs.

Definition of STAS (aerogenous spread) and grading system

We defined STAS as MP or solid clusters of or single tumor cells free floating within air spaces beyond the edge of the tumor and, it has been recorded as "aerogenous spread" in the pathology report by an experienced pulmonary pathologist (JHC) since 2008. From 2011, the extent of STAS was graded according to the distance from the edge of tumor with a two-tiered system. When all tumor clusters were present within 2500 μm (equivalent to one field of ×10 objective lens) from the edge of the tumor, STAS was graded as I, while it was graded as II when any of tumor clusters were seen equal or greater than 2500 μm away from the edge of tumor (Fig. 1). Of note, we have paid special attention to differentiating STAS from artifacts. Artifacts were defined as; (1) tumor cell clusters with jagged edges owing to tumor fragmentation or knife cuts during specimen processing; (2) linear strips of cells that were lifted off the alveolar walls; (3) rare isolated tumor clusters found at a distance rather than spreading in a continuous manner.

Statistical analysis

The chi-square test (or Fisher exact test when appropriate) was used to assess the significance of the association of STAS grade with clinicopathological parameters. A Kaplan–Meier analysis was performed to construct survival curves and statistical significance was assessed using the log-rank test. Univariate and multivariate analyses were performed by Cox proportional hazards regression modeling. All statistical tests were two sided and p value < 0.05 was used to establish statistical significance. All statistical analysis was performed using Statistical Package for the Social Sciences ver. 21 (IBM Corp., Armonk, NY, USA).

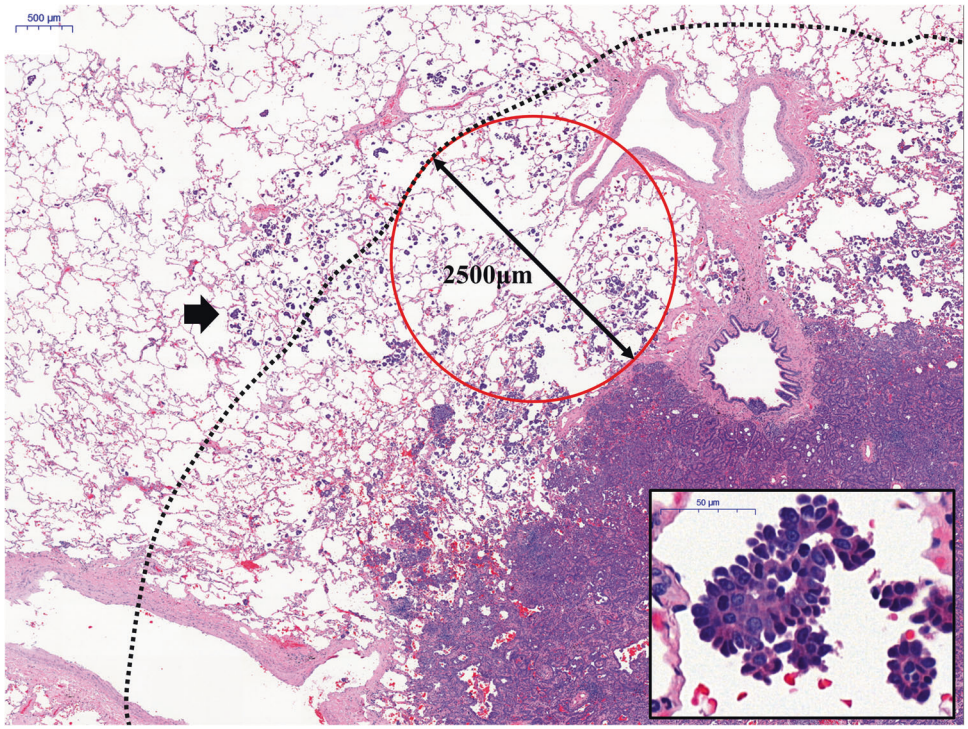


Fig. 1 Definition of extent of STAS grading in histologic examination. Definition of STAS grading; when tumor clusters existed within one field of ×10 objective lens (2500 μm diameter: red circle) away from edge of the main tumor, inside the dotted line, it was graded I, and tumor clusters existing beyond the STAS I area, graded II (×20 magnification). This case was STAS II in adenocarcinoma (black arrow; ×400 magnification).

Results

Clinicopathologic characteristics and STAS

The clinicopathologic characteristics of patients are shown in Table 1. Histologically, 1544 patients (82.6%) were diagnosed with ADC and 325 patients (17.4%) with SqCC. STAS was observed in 765 cases (40.9%), and 456 cases (24.4%) showed STAS I, whereas 309 cases (16.5%) showed STAS II. Presence of STAS was significantly associated with ADC ($p < 0.001$), pleural invasion ($p < 0.001$), vascular invasion ($p < 0.001$), lymphatic invasion ($p < 0.001$), presence of necrosis ($p < 0.001$), higher pathologic stage ($p < 0.001$), and radical resection ($p < 0.001$). In a subgroup analysis of STAS-positive tumors, those with STAS II were more likely to show these aggressive features than those with STAS I. Sex, smoking status and method of surgical approach (video-assisted thoracic surgery (VATS) vs. open) was not associated with STAS (Table 1).

In ADC, STAS was observed in 684 cases (44.3%), and 393 cases (25.5%) showed STAS I, whereas 291 cases (18.8%) showed STAS II. The presence and grade of STAS was significantly associated with the predominant growth pattern ($p < 0.001$). STAS was observed in an ascending frequency from lepidic-predominant tumors to acinar, papillary, solid, and MP-predominant tumors, and the proportion of STAS II showed the same trend. MP-predominant tumors showed the highest prevalence of STAS, which was predominantly grade II. Of note, the presence of MP pattern irrespective of its amount (even if <5%) also associated with STAS status ($p < 0.001$). STAS was more frequently found in *EGFR* wild-type tumors ($p = 0.001$), but there was no association between STAS grade and the *EGFR* mutation status ($p = 0.775$). Interestingly, STAS, irrespective of its extent, was more frequently found in open surgical approach than VATS ($p = 0.004$). Other clinicopathologic factors including lymphovascular invasion, necrosis and higher stage were significantly associated with STAS grade (Table 2).

In SqCC, STAS was observed in 81 cases (24.9%), and 63 cases (19.4%) showed STAS I, whereas 18 cases (5.5%) showed STAS II. Vascular invasion ($p = 0.019$) and lymphatic invasion ($p = 0.001$) were significantly correlated with the presence of STAS, but other factors were not (Supplementary Table 1).

Survival analysis

ADC cohort

At the time of analysis, the median RFS was 27.0 months and the median OS was 32.0 months in the entire ADC cohort. During this time, 184 patients (11.9%) suffered recurrence (46 with locoregional recurrence; 101 with distant recurrence; 37 with both) and 96 patients (6.2%) deceased (51 with lung cancer specific death). There were significant differences in RFS, overall survival (OS) and

Table 1 Association of STAS with clinicopathologic characteristics.

Characteristics		Presence of STAS ($n = 1869$)		p value	Grade of STAS ($n = 765$)		p value
		Absent	Present		Gr I	Gr II	
	n (%)	n (%)	n (%)		n (%)	n (%)	
Age							
Median (range)	65 (20–93)			0.305			0.090
≤65 years	955 (51.1)	575 (60.2)	380 (39.8)		215 (56.6)	165 (43.4)	
>65 years	914 (48.9)	529 (57.9)	385 (42.1)		241 (62.6)	144 (37.4)	
Sex				0.136			0.702
Male	1028 (55.0)	623 (60.6)	405 (39.4)		244 (60.2)	161 (39.8)	
Female	841 (45.0)	481 (57.2)	360 (42.8)		212 (58.9)	148 (41.1)	
Smoking status[a]				0.717			0.900
Never	897 (48.0)	526 (58.6)	371 (41.4)		222 (59.8)	149 (40.2)	
Former or current	972 (52.0)	578 (59.5)	394 (40.5)		234 (59.4)	160 (40.6)	
Histologic subtypes				<0.001			<0.001
Adenocarcinoma	1544 (82.6)	860 (55.7)	684 (44.3)		393 (57.5)	291 (42.5)	
Squamous cell carcinoma	325 (17.4)	244 (75.1)	81 (24.9)		63 (77.8)	18 (22.2)	
Pleural invasion				<0.001			0.009
Absent	1460 (78.1)	958 (65.6)	502 (34.4)		316 (62.9)	186 (37.1)	
Present	409 (21.9)	146 (35.7)	263 (64.3)		140 (53.2)	123 (46.8)	
Vascular invasion				<0.001			<0.001
Absent	1456 (77.9)	970 (66.6)	486 (33.4)		335 (68.9)	151 (31.1)	
Present	413 (22.1)	134 (32.4)	279 (67.6)		121 (43.4)	158 (56.6)	
Lymphatic invasion				<0.001			<0.001
Absent	1250 (66.9)	898 (71.8)	352 (28.2)		245 (69.6)	107 (30.4)	
Present	619 (33.1)	206 (33.3)	413 (66.7)		211 (51.1)	202 (48.9)	
Perineural invasion				0.094			0.380
Absent	1780 (95.2)	1059 (59.5)	721 (40.5)		427 (59.2)	294 (40.8)	
Present	89 (4.8)	45 (50.6)	44 (49.4)		29 (65.9)	15 (34.1)	
Necrosis				<0.001			0.028
Absent	1287 (68.9)	813 (63.2)	474 (36.8)		297 (62.7)	177 (37.3)	
Present	582 (31.1)	291 (50.0)	291 (50.0)		159 (54.6)	132 (45.4)	
Pathologic T stage (AJCC 8th)				<0.001			<0.001
T1	1103 (59.0)	772 (70.0)	331 (30.0)		219 (66.2)	112 (33.8)	
T1mi	119 (6.4)	119 (100.0)	0 (0.0)		–	–	
T1a	185 (9.9)	159 (85.9)	26 (14.1)		20 (76.9)	6 (23.1)	
T1b	455 (24.3)	309 (67.9)	146 (32.1)		97 (66.4)	49 (33.6)	
T1c	344 (18.4)	185 (53.8)	159 (46.2)		102 (64.2)	57 (35.8)	
T2	546 (29.2)	239 (43.8)	307 (56.2)		180 (58.6)	127 (41.4)	
T2a	433 (23.2)	194 (44.8)	239 (55.2)		139 (58.2)	100 (41.8)	
T2b	113 (6.0)	45 (39.8)	68 (60.2)		41 (60.3)	27 (39.7)	
T3	161 (8.6)	62 (38.5)	99 (61.5)		46 (46.5)	53 (53.5)	
T4	59 (3.2)	31 (52.5)	28 (47.5)		11 (39.3)	17 (60.7)	
Pathologic N stage (AJCC 8th)[b]				<0.001[c]			<0.001[c]
N0	1363 (78.6)	867 (63.6)	496 (36.4)		325 (65.5)	171 (34.5)	
N1	193 (11.1)	81 (42.0)	112 (58.0)		57 (50.9)	55 (49.1)	
N2	178 (10.3)	50 (28.1)	128 (71.9)		51 (39.8)	77 (60.2)	
N3	1 (0.1)	0 (0.0)	1 (100.0)		0 (0.0)	1 (100.0)	
Pathologic M stage (AJCC 8th)				<0.001			0.006
M0	1811 (96.9)	1087 (60.0)	724 (40.0)		440 (60.8)	284 (39.2)	
M1	58 (3.1)	17 (29.3)	41 (70.7)		16 (39.0)	25 (61.0)	
M1a	34 (1.8)	8 (23.5)	26 (76.5)		11 (42.3)	15 (57.7)	
M1b	21 (1.1)	6 (28.6)	15 (71.4)		5 (33.3)	10 (66.7)	
M1c	3 (0.2)	3 (100.0)	0 (0.0)		–	–	
Pathologic stage (AJCC 8th)				<0.001			<0.001
I	1294 (69.2)	881 (68.1)	413 (31.9)		279 (67.6)	134 (32.4)	
IA1	303 (16.2)	278 (91.7)	25 (8.3)		19 (76.0)	6 (24.0)	
IA2	424 (22.7)	298 (70.3)	126 (29.7)		88 (69.8)	38 (30.2)	

Table 1 (continued)

Characteristics		Presence of STAS ($n = 1869$)		p value	Grade of STAS ($n = 765$)		p value
		Absent	Present		Gr I	Gr II	
	n (%)	n (%)	n (%)		n (%)	n (%)	
IA3	284 (15.2)	165 (58.1)	119 (41.9)		81 (68.1)	38 (31.9)	
IB	283 (15.1)	140 (49.5)	143 (50.0)		91 (63.6)	52 (36.4)	
II	285 (15.2)	121 (42.5)	164 (57.5)		101 (61.6)	63 (38.4)	
IIA	66 (3.5)	27 (40.9)	39 (59.1)		27 (69.2)	12 (30.8)	
IIB	219 (11.7)	94 (42.9)	125 (57.1)		74 (59.2)	51 (40.8)	
III	232 (12.4)	85 (36.6)	147 (63.4)		60 (40.8)	87 (59.2)	
IIIA	192 (10.3)	73 (38.0)	119 (62.0)		50 (42.0)	69 (58.0)	
IIIB	40 (2.1)	12 (30.0)	28 (70.0)		10 (35.7)	18 (64.3)	
IIIC	0 (0.0)	–	–		–	–	
IV	58 (3.1)	17 (29.3)	41 (70.7)		16 (39.0)	25 (61.0)	
IVA	55 (2.9)	14 (25.5)	41 (74.5)		16 (39.0)	25 (61.0)	
IVB	3 (0.2)	3 (100.0)	0 (0.0)		–	–	
Extent of resection				<0.001			0.031
Limited resection	271 (14.5)	212 (78.2)	59 (21.8)		43 (72.9)	16 (27.1)	
Wedge resection	150 (8.0)	117 (78.0)	33 (22.0)		24 (72.7)	9 (27.3)	
Segmentectomy	121 (6.5)	95 (78.5)	26 (21.5)		19 (73.1)	7 (26.9)	
Radical resection	1598 (85.5)	892 (55.8)	706 (44.2)		413 (58.5)	293 (41.5)	
Lobectomy	1541 (82.5)	850 (55.2)	691 (44.8)		404 (58.5)	287 (41.5)	
Bilobectomy	29 (1.6)	21 (72.4)	8 (27.6)		4 (50.0)	4 (50.0)	
Pneumonectomy	28 (1.5)	21 (75.0)	7 (25.0)		5 (71.4)	2 (28.6)	
Surgical approach				0.972			0.863
VATS	1695 (90.7)	1001 (59.1)	694 (40.9)		413 (59.5)	281 (40.5)	
Open	174 (9.3)	103 (59.2)	71 (40.8)		43 (60.6)	28 (39.4)	
Thoracotomy	118 (6.3)	67 (56.8)	51 (43.2)		31 (60.8)	20 (39.2)	
Conversion to open	51 (2.7)	33 (64.7)	18 (35.3)		10 (55.6)	8 (44.4)	
Sternotomy	5 (0.3)	3 (60.0)	2 (40.0)		2 (100.0)	0 (0.0)	

VATS video-assisted thoracic surgery.

[a]Smoking status was defined as follows: never smoker (<100 cigarettes per lifetime); ex-smoker (100 cigarettes per lifetime and quit > 1 year prior to the diagnosis); current smoker (100 cigarettes per lifetime and smoked at the time of lung cancer diagnosis or quit 1 year prior to the diagnosis).

[b]Pathologic N staging was available in 1735 patients.

[c]p value was obtained by Fisher's exact test.

lung cancer specific survival (LCSS) according to the extent of STAS ($p < 0.001$, respectively) (Fig. 2). The 5-year RFS of patients with no STAS, that with STAS I and that with STAS II were 91.8%, 79.0%, and 60.5%, respectively, ($p < 0.001$) and the 5-year OS were 95.2%, 88.3%, and 74.1%, respectively, ($p < 0.001$). The 5-year LCSS of patients with no STAS, that with STAS I and that with STAS II were 97.3%, 92.3%, and 84.6%, respectively.

Subgroup analysis in stage IA non-mucinous ADC We performed a subgroup analysis on stage IA non-mucinous ADC ($n = 870$) consisting of 292 (33.6%) stage IA1, 366 (42.1%) stage IA2, and 212 (24.4%) stage IA3 cases. The median RFS and OS were 34.0 and 35.0 months. During this time, 30 (3.4%) patients experienced recurrence (12 with locoregional recurrence, 16 with distant recurrence, and 2 with both) and 17 (2.0%) patients deceased (five with lung cancer specific death).

In stage IA non-mucinous ADC, STAS was observed in 237 (27.2%) cases including 164 (18.9%) with STAS I and 73 (8.4%) with STAS II. In this group, 222 (25.5%) patients underwent limited resection (including wedge resection and segmentectomy) and 648 (74.5%) patients underwent radical resection (including lobectomy, bilobectomy and pneumonectomy). In the limited resection group, STAS was observed in 33 (14.9%) cases with 25 (11.3%) STAS I and eight (3.6%) STAS II. In the radical resection group, STAS was observed in 204 (31.5%) cases with 139 (21.5%) STAS I and 65 (10.0%) STAS II.

There were significant differences in RFS, OS and LCSS according to the extent of STAS in stage IA non-mucinous ADC ($p < 0.001$; $p = 0.008$; $p < 0.001$, respectively). When stratified by the extent of resection, there were significant differences in RFS and LCSS in limited resection group, but not in OS ($p < 0.001$; $p < 0.001$; $p = 0.219$, respectively). In radical resection group, there were significant differences in RFS, OS and LCSS according to

Table 2 Association of STAS with clinicopathologic characteristics in adenocarcinoma.

Characteristics		Presence of STAS ($n = 1544$)		p value	Grade of STAS ($n = 684$)		p value
		Absent	Present		Gr I	Gr II	
	n (%)	n (%)	n (%)		n (%)	n (%)	
Age							
Median (range)	64 (20–93)			0.005			0.172
≤65 years	858 (55.6)	505 (58.9)	353 (41.1)		194 (55.0)	159 (45.0)	
>65 years	686 (44.4)	355 (51.7)	331 (48.3)		199 (60.1)	132 (39.9)	
Sex				0.229			0.398
Male	714 (46.2)	386 (54.1)	328 (45.9)		183 (55.8)	145 (44.2)	
Female	830 (53.8)	474 (57.1)	356 (42.9)		210 (59.0)	146 (41.0)	
Smoking status[a]				0.009			0.157
Never	885 (57.3)	518 (58.5)	367 (41.5)		220 (59.9)	147 (40.1)	
Former or current	659 (42.7)	342 (51.9)	317 (48.1)		173 (54.6)	144 (45.4)	
EGFR mutation status[b]				0.001			0.775
Wild type	617 (50.6)	294 (47.6)	323 (52.4)		177 (54.8)	146 (45.2)	
Mutant	602 (49.4)	343 (57.0)	259 (43.0)		145 (56.0)	114 (44.0)	
Predominant growth pattern				<0.001			<0.001[c]
Lepidic	201 (13.0)	191 (95.0)	10 (5.0)		9 (90.0)	1 (10.0)	
Acinar	600 (38.9)	370 (61.7)	230 (38.3)		158 (68.7)	72 (31.3)	
Papillary	402 (26.0)	191 (47.5)	211 (52.5)		119 (56.4)	92 (43.6)	
Solid	202 (13.1)	56 (27.7)	146 (72.3)		73 (50.0)	73 (50.0)	
Micropapillary	45 (2.9)	2 (4.4)	43 (95.6)		7 (16.3)	36 (83.7)	
Others[d]	94 (6.1)	50 (53.2)	44 (46.8)		27 (61.4)	17 (38.6)	
Presence of MP pattern				<0.001			<0.001
Absent	786 (50.9)	655 (83.3)	131 (16.7)		100 (76.3)	31 (23.7)	
Present	758 (49.1)	205 (27.0)	553 (73.0)		293 (53.0)	260 (47.0)	
Pleural invasion				<0.001			0.006
Absent	1195 (77.4)	755 (63.2)	440 (36.8)		270 (61.4)	170 (38.6)	
Present	349 (22.6)	105 (30.1)	244 (69.9)		123 (50.4)	121 (49.6)	
Vascular invasion				<0.001			<0.001
Absent	1226 (79.4)	789 (64.4)	437 (35.6)		297 (68.0)	140 (32.0)	
Present	318 (20.6)	71 (22.3)	247 (77.7)		96 (38.9)	151 (61.1)	
Lymphatic invasion				<0.001			<0.001
Absent	1041 (67.4)	729 (70.0)	312 (30.0)		213 (68.3)	99 (31.7)	
Present	503 (32.6)	131 (26.0)	372 (74.0)		180 (48.4)	192 (51.6)	
Perineural invasion				<0.001			0.989
Absent	1497 (97.0)	846 (56.5)	651 (43.5)		374 (57.5)	277 (42.5)	
Present	47 (3.0)	14 (29.8)	33(70.2)		19 (57.6)	14 (42.4)	
Necrosis				<0.001			<0.001
Absent	1256 (81.3)	790 (62.9)	466 (37.1)		292 (62.7)	174 (37.3)	
Present	288 (18.7)	70 (24.3)	218 (75.7)		101 (46.3)	117 (53.7)	
Pathologic T stage (AJCC 8th)				<0.001			<0.001
T1	989 (64.1)	683 (69.1)	306 (30.9)		202 (66.0)	104 (34.0)	
T1mi	119 (7.7)	119 (100.0)	0 (0.0)		–	–	
T1a	180 (11.7)	155 (86.1)	25 (13.9)		19 (76.0)	6 (24.0)	
T1b	414 (26.8)	272 (65.7)	142 (34.3)		95 (66.9)	47 (33.1)	
T1c	276 (17.9)	137 (49.6)	139 (50.4)		88 (63.3)	51 (36.7)	
T2	424 (27.5)	150 (35.4)	274 (64.6)		153 (55.8)	121 (44.2)	
T2a	353 (22.9)	132 (37.4)	221 (62.6)		124 (56.1)	97 (43.9)	
T2b	71 (4.6)	18 (25.4)	53 (74.6)		29 (54.7)	24 (45.3)	
T3	101 (6.5)	20 (19.8)	81 (80.2)		30 (37.0)	51 (63.0)	
T4	30 (1.9)	7 (23.3)	23 (76.7)		8 (34.8)	15 (65.2)	

Table 2 (continued)

Characteristics		Presence of STAS ($n = 1544$)		p value	Grade of STAS ($n = 684$)		p value
		Absent	Present		Gr I	Gr II	
	n (%)	n (%)	n (%)		n (%)	n (%)	
Pathologic N stage (AJCC 8th)[e]				<0.001[c]			<0.001[c]
N0	1154 (81.4)	708 (61.4)	446 (38.6)		286 (64.1)	160 (35.9)	
N1	120 (8.5)	26 (21.7)	94 (78.3)		43 (45.7)	51 (54.3)	
N2	143 (10.1)	26 (18.2)	117 (81.8)		43 (36.8)	74 (63.2)	
N3	1 (0.1)	0 (0.0)	1 (100.0)		0 (0.0)	1 (100.0)	
Pathologic M stage (AJCC 8th)				<0.001			0.021
M0	1494 (96.8)	850 (56.9)	644 (43.1)		377 (58.5)	267 (41.5)	
M1	50 (3.2)	10 (20.0)	40 (80.0)		16 (40.0)	24 (60.0)	
M1a	30 (1.9)	5 (16.7)	25 (83.3)		11 (44.0)	14 (56.0)	
M1b	18 (1.2)	3 (16.7)	15 (83.3)		5 (33.3)	10 (66.7)	
M1c	2 (0.1)	2 (100.0)	0 (0.0)		–	–	
Pathologic stage (AJCC 8th)				<0.001			<0.001
I	1155 (74.8)	770 (66.7)	385 (33.3)		258 (67.0)	127 (33.0)	
IA1	298 (19.3)	274 (91.9)	24 (8.1)		18 (75.0)	6 (25.0)	
IA2	389 (25.2)	266 (68.4)	123 (31.6)		86 (69.9)	37 (30.1)	
IA3	234 (15.2)	128 (54.7)	106 (45.3)		72 (67.9)	34 (32.1)	
IB	234 (15.2)	102 (43.6)	132 (56.4)		82 (62.1)	50 (37.9)	
II	180 (11.7)	49 (27.2)	131 (72.8)		73 (55.7)	58 (44.3)	
IIA	43 (2.8)	14 (32.6)	29 (67.4)		19 (65.5)	10 (34.5)	
IIB	137 (8.9)	35 (25.5)	102 (74.5)		54 (52.9)	48 (47.1)	
III	159 (10.3)	31 (19.5)	128 (80.5)		46 (35.9)	82 (64.1)	
IIIA	131 (8.5)	28 (21.4)	103 (78.6)		39 (37.9)	64 (62.1)	
IIIB	28 (1.8)	3 (10.7)	25 (89.3)		7 (28.0)	18 (72.0)	
IV	50 (3.2)	10 (20.0)	40 (80.0)		16 (40.0)	24 (60.0)	
IVA	48 (3.1)	8 (16.7)	40 (83.3)		16 (40.0)	24 (60.0)	
IVB	2 (0.1)	2 (100.0)	0 (0.0)		–	–	
Extent of resection				<0.001			0.058
Limited resection	252 (16.3)	199 (79.0)	53 (21.0)		37 (69.8)	16 (30.2)	
Wedge resection	138 (8.9)	108 (78.3)	30 (21.7)		21 (70.0)	9 (30.0)	
Segmentectomy	114 (7.4)	91 (79.8)	23 (20.2)		16 (69.6)	7 (30.4)	
Radical resection	1292 (83.7)	661 (51.2)	631 (48.8)		356 (56.4)	275 (43.6)	
Lobectomy	1277 (82.7)	655 (51.3)	622 (48.7)		352 (56.6)	270 (43.4)	
Bilobectomy	9 (0.6)	5 (55.6)	4 (44.4)		1 (25.0)	3 (75.0)	
Pneumonectomy	6 (0.4)	1 (16.7)	5 (83.3)		3 (60.0)	2 (40.0)	
Surgical approach				0.004			0.649
VATS	1450 (93.9)	821 (56.6)	629 (43.4)		363 (57.7)	266 (42.3)	
Open	94 (6.1)	39 (41.5)	55 (58.5)		30 (54.5)	25 (45.5)	
Thoracotomy	55 (3.6)	19 (34.5)	36 (65.5)		19 (52.8)	17 (47.2)	
Conversion to open	34 (2.2)	17 (50.0)	17 (50.0)		9 (52.9)	8 (47.1)	
Sternotomy	5 (0.3)	3 (60.0)	2 (40.0)		2 (100.0)	0 (0.0)	

MP micropapillary, *VATS* video-assisted thoracic surgery.

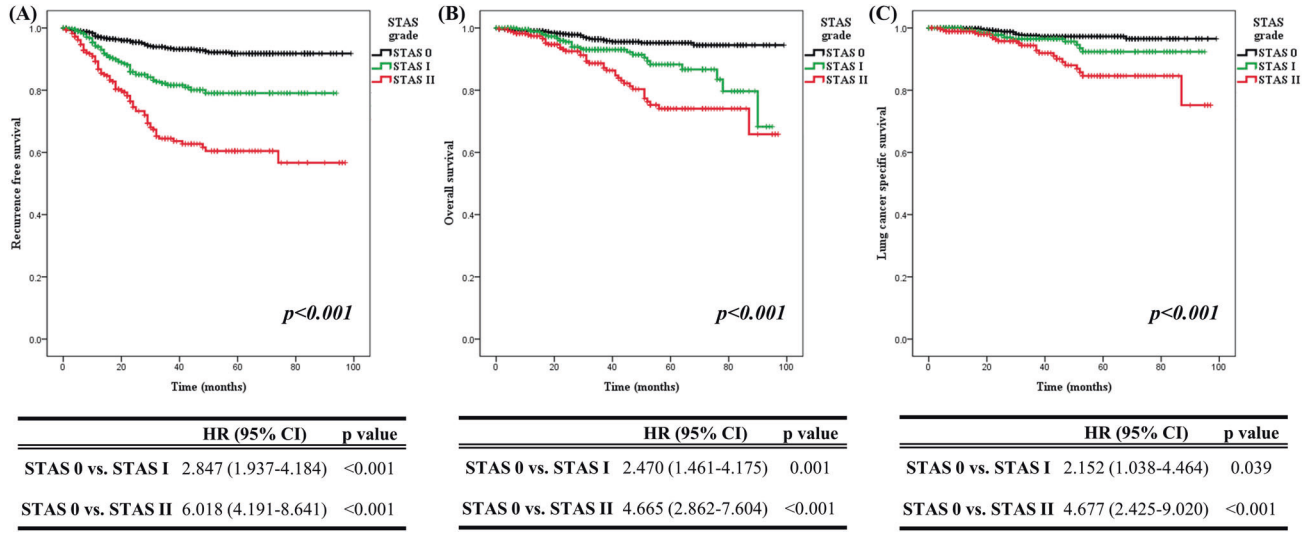
[a]Smoking status was defined as follows: never smoker (<100 cigarettes per lifetime); ex-smoker (100 cigarettes per lifetime and quit > 1 year prior to the diagnosis); current smoker (100 cigarettes per lifetime and smoked at the time of lung cancer diagnosis or quit 1 year prior to the diagnosis).

[b]EGFR mutation status was evaluated for 1219 patients.

[c]p value was obtained by Fisher's exact test.

[d]Others included mucinous, colloid and enteric adenocarcinomas.

[e]Pathologic N staging was available in 1418 patients.



	HR (95% CI)	p value
STAS 0 vs. STAS I	2.847 (1.937-4.184)	<0.001
STAS 0 vs. STAS II	6.018 (4.191-8.641)	<0.001

	HR (95% CI)	p value
STAS 0 vs. STAS I	2.470 (1.461-4.175)	0.001
STAS 0 vs. STAS II	4.665 (2.862-7.604)	<0.001

	HR (95% CI)	p value
STAS 0 vs. STAS I	2.152 (1.038-4.464)	0.039
STAS 0 vs. STAS II	4.677 (2.425-9.020)	<0.001

Fig. 2 Recurrence free survival, overall survival, and lung cancer specific survival stratified by STAS grade in adenocarcinoma. (**A**) recurrence free survival according to STAS grade, (**B**) overall survival according to STAS grade, and (**C**) lung cancer specific survival according to STAS grade. Hazard ratios obtained by Cox proportional hazards regression modeling.

the STAS grade ($p < 0.001$; $p = 0.018$; $p = 0.007$, respectively) (Fig. 3). In multivariate analysis, the presence of STAS was an independent poor prognostic factor for recurrence in stage IA non-mucinous ADC, regardless of the extent of resection. When STAS was stratified by the grade, only the STAS II remained as an independent risk factor for recurrence regardless of the extent of resection ($p = 0.001$ for limited resection and $p = 0.023$ for radical resection) (Table 3). Further, multivariate analysis revealed that STAS II was an independent poor prognostic factor for RFS and LCSS in stage IA non-mucinous ADC ($p < 0.001$; $p = 0.006$, respectively) (Table 4). In this model, vascular invasion was also an independent poor prognostic factor for RFS, but the presence of MP pattern had no bearing on prognosis in stage IA non-mucinous ADC even when a cut-off of 5, 10 or 20% for the presence was applied (Supplementary Table 2).

As STAS grade was an independent prognostic factor for RFS and LCSS in stage IA non-mucinous ADC and not in stage IB ($n = 219$; $p = 0.314$ for RFS, $p = 0.359$ for LCSS), we further classified stage IA cases according to STAS grade and compared RFS and LCSS between three stage IA and stage IB groups. Interestingly, RFS and LCSS of patients with stage IA with STAS II were similar to those of patients with stage IB (Supplementary Fig. 1). Furthermore, multivariate analysis for RFS revealed that the risk of recurrence (compared to stage IA without STAS) was higher in stage IA tumors with STAS II than in stage IB ($p = 0.003$, hazard ratio (HR) [95% confidence interval (CI)]: 4.358 [1.645–11.544]; $p = 0.046$, HR [95% CI]: 2.884 [1.018–8.169]; respectively) (Supplementary Table 3).

SqCC cohort

At the time of analysis, the median RFS was 24.0 months and the median OS was 30.0 months. During this time, 48 patients (14.8%) experienced recurrence (15 with locoregional recurrence; 26 with distant recurrence; 7 with both) and 51 patients (15.7%) deceased (22 with lung cancer specific death). There were no significant differences in RFS, OS and LCSS according to the presence and extent of STAS in total SqCC. Among patients with stage I, those with higher STAS grade tended to show worse RFS but were not statistically significant (STAS 0 vs. STAS I, $p = 0.409$; STAS 0 vs. STAS II, $p = 0.679$).

Discussion

In this study, we found that STAS II was an important prognostic factor in stage IA non-mucinous ADC. Notably the extent of STAS according to how far the tumor cells had spread from the edge of the tumor was evaluated in a relatively objective and practical manner using the ×10 objective lens field (2500 μm diameter) as a cut-off for high-grade (extensive) STAS. Importantly, although the presence of STAS was an independent poor prognostic factor for recurrence in stage IA non-mucinous ADC, regardless of the extent of resection, when the presence of STAS was stratified by the grade, STAS I had no bearing on recurrence in multivariate analysis. It is possible that some of the STAS I may have been equivalent to “tumor islands” (connected to the main mass in deeper sections) that would carry distinct biology and a different prognostic impact from “free floating” clusters [23, 24]. Since tumor clusters were at least

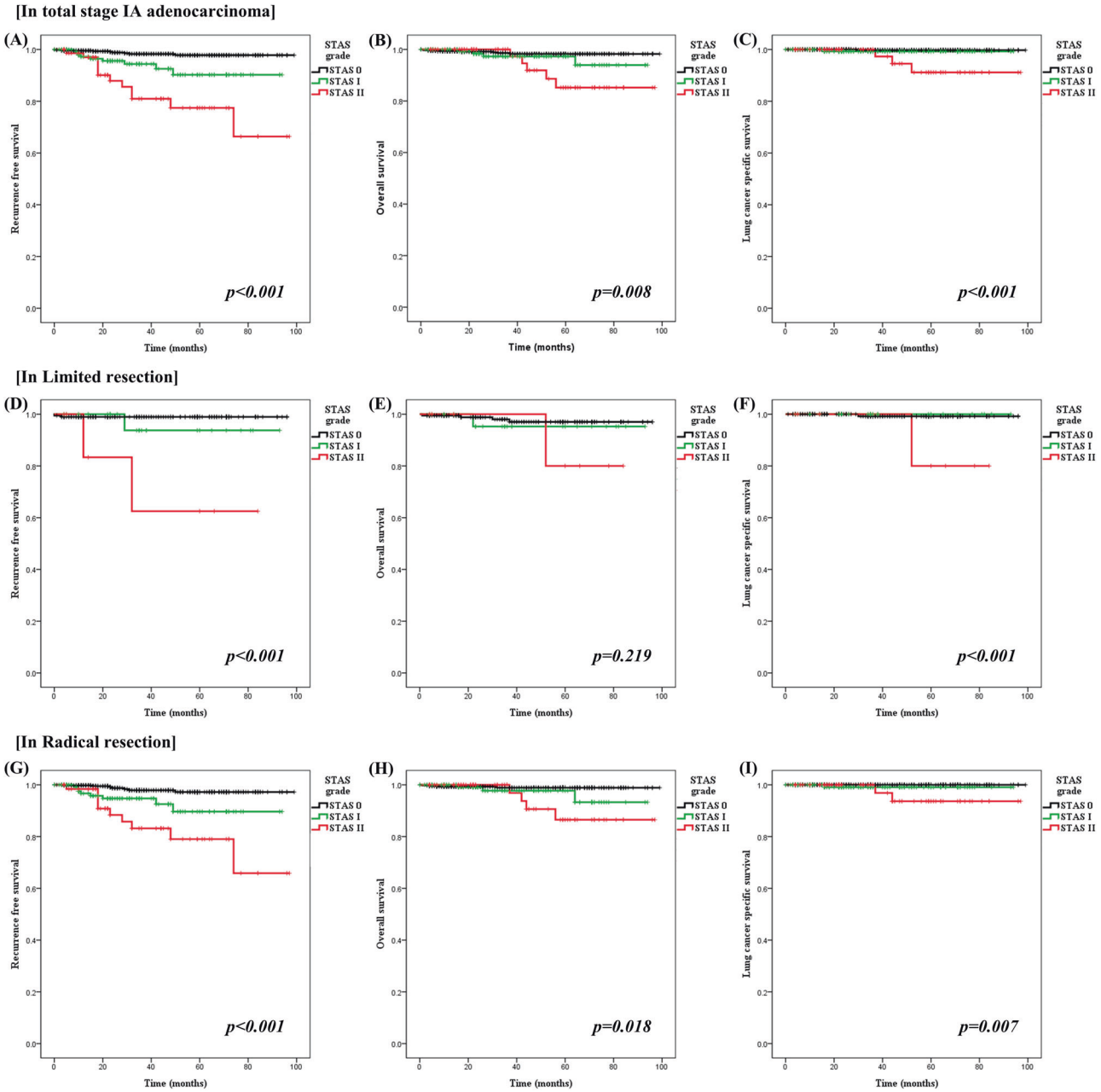


Fig. 3 Recurrence free survival, overall survival, and lung cancer specific survival stratified by STAS grade in stage IA non-mucinous adenocarcinoma according to the extent of resection. (**A**)–(**C**) Total stage IA non-mucinous adenocarcinoma ($n = 870$); (**A**) Recurrence free survival (RFS) according to STAS grade (5-year RFS; STAS 0, STAS I, and STAS II; 97.8, 90.2, and 77.4%), (**B**) overall survival (OS) according to STAS grade (5-year OS; STAS 0, STAS I, and STAS II; 98.2, 97.3, and 85.2%), (**C**) lung cancer specific survival (LCSS) according to STAS grade (5-year LCSS; STAS 0, STAS I, and STAS II; 99.7, 99.2, and 91.1%). (**D**)–(**F**) Limited resection ($n = 222$); (**D**) RFS according to STAS grade (5-year RFS; STAS 0, STAS I, and STAS II; 98.9, 93.8, and 62.5%), (**E**) OS according to STAS grade (5-year OS; STAS 0, STAS I, and STAS II; 97.0, 95.2, and 80.0%), (**F**) LCSS according to STAS grade (5-year LCSS; STAS 0, STAS I, and STAS II; 99.2, 100.0, and 80.0%). (**G**)–(**I**) radical resection ($n = 648$); (**G**) RFS according to STAS grade (5-year RFS; STAS 0, STAS I, and STAS II; 97.2, 89.6, and 79.0%), (**H**) OS according to STAS grade (5-year OS; STAS 0, STAS I, and STAS II; 98.9, 97.7, and 86.5%), (**I**) LCSS according to STAS grade (5-year LCSS; STAS 0, STAS I, and STAS II; 100.0, 99.1, and 93.6%).

more than five alveolar spaces from edge of the main tumor in the STAS II of our study [25], it is less likely to have "tumor islands" in this group.

Toyokaya et al. reported that the difference in frequency of STAS between small cell lung cancer and other histologic types, such as ADC and SqCC, might be explained by an epithelial to mesenchymal transition (EMT) phenomenon [8]. Several attempts have been made to examine the biological significance of STAS in association with the EMT phenomenon [26, 27]. Although more studies

Table 3 Multivariate analysis for recurrence free survival in stage IA non-mucinous adenocarcinoma according to the extent of resection.

Variables		Limited resection ($n = 222$)				Radical resection ($n = 648$)			
		Univariate		Multivariate		Univariate		Multivariate	
		HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Age	>65 years vs. ≤65 years	2.673 (0.445–16.052)	0.282			1.723 (0.781–3.799)	0.178		
Sex	Male vs. female	1.583 (0.265–9.477)	0.615			1.118 (0.510–2.450)	0.781		
Smoking status	Ever vs. never	2.350 (0.392–14.067)	0.350			1.845 (0.838–4.064)	0.129		
MP pattern	Present vs. absent	2.367 (0.395–14.170)	0.345			7.240 (2.716–19.302)	<0.001	3.308 (1.020–10.727)	0.046
Vascular invasion	Present vs. absent	8.442 (0.943–75.591)	0.056	3.113 (0.266–36.490)	0.366	4.279 (1.838–9.966)	0.001	2.811 (1.188–6.646)	0.019
Lymphatic invasion	Present vs. absent	4.609 (0.515–41.252)	0.172			4.409 (2.008–9.682)	<0.001	1.692 (0.722–3.963)	0.226
Necrosis	Present vs. absent	6.132 (0.685–54.933)	0.105			3.686 (1.382–9.828)	0.009	1.487 (0.532–4.159)	0.449
Resection margin[a]	<Tumor max diameter vs. ≥tumor max diameter	1.553 (0.259–9.298)	0.630			NA			
Pathologic stage	IA3 vs. IA1 and IA2	8.774 (0.981–78.516)	0.052	13.067 (0.956–178.587)	0.054	3.088 (1.387–6.875)	0.006	1.722 (0.738–4.019)	0.209
STAS[b]	Present vs. absent	8.799 (1.470–52.678)	0.017	8.799 (1.470–52.678)	0.017	6.032 (2.517–14.456)	<0.001	2.765 (0.998–7.663)	0.050
STAS grade	STAS II vs. STAS I vs. STAS 0		0.005		0.003		<0.001		0.073
	STAS I vs. STAS 0	3.771 (0.342–41.593)	0.279	2.075 (0.152–28.396)	0.585	4.091 (1.481–11.299)	0.007	2.178 (0.697–6.802)	0.181
	STAS II vs. STAS 0	26.483 (3.722–188.451)	0.001	32.472 (4.262–247.395)	0.001	9.678 (3.681–25.442)	<0.001	3.783 (1.205–11.879)	0.023

MP micropapillary.

[a]The resection margin status was available in 198 patients who had undergone limited resection and the margin distance from the main tumor was classified into <the maximal diameter of tumor vs. ≥the maximal diameter of tumor.

[b]Presence of STAS was analyzed separately from the grade of STAS.

are warranted, it could be hypothesized that tumors with distally located tumor cell clusters (extensive STAS) are more likely to exhibit the EMT phenomenon than those without STAS or only with tumor clusters located nearby (limited STAS). Both the association with several aggressive features such as lymphovascular invasion and MP pattern and the poor prognosis of tumors with STAS II could be explained in part by EMT.

It is not certain, however, whether the longer distance as the cut-off used in our study better stratified low- and high-grade STAS. Warth et al. reported that OS and disease-free survival were similar between extensive and limited STAS with the distance of three alveoli as the cut-off [1], and Dai et al. also used the same cut-off (three alveoli) for extensive STAS and failed to identify a more aggressive behavior of extensive STAS compared to limited STAS [28]. Therefore, large-scale studies are warranted to establish the universal standard for grading the extent of STAS. In order to use "distance from the tumor edge" as criteria for STAS grading (such as our definition), specimen handling and histologic preparation also need to be standardized.

The prevalence of STAS according to histologic subtypes in this study was similar to those reported in the previous studies [1, 2, 5, 28–30]. While we also confirmed the association of STAS with well-known risk factors for recurrence after lung cancer surgery, the association was only evident in ADC, but not in SqCC. In SqCC, STAS was less frequently observed and neither the presence nor grade of STAS was an independent risk factor for recurrence or death. Interestingly, less frequent and a late pattern of metastasis in SqCC as compared with ADC has been attributed in part to desmosomal molecules rich in SqCC [31] that also explains an adhesive nature and less frequent STAS in SqCC. Since only a limited number of groups studied on STAS in SqCC [3–5], however, additional large-cohort studies on this issue are warranted.

Several studies evaluating the significance of STAS stratified by the extent of resection reported that STAS was a significant risk factor of recurrence for patients with small-sized ADCs treated with limited resection but not in those who had undergone lobectomy [2, 14, 15]. In the current study, however, multivariate analysis revealed that STAS II was a significant prognostic factor not only in the limited resection but also in the radical resection groups. To confirm the implication of STAS according to the extent of resection, recurrence patterns in association with the extent of resection were also analyzed in stage IA non-mucinous ADC, including resection margin status (Supplementary Tables 4, 5). Both locoregional recurrence and distant recurrence were associated with the presence of STAS. Not only in limited resection, but also in radical resection, cases with any recurrence showed a higher incidence of STAS compared to those without recurrence ($p = 0.024$ and $p <$

Table 4 Multivariate analysis for recurrence free survival, overall survival and lung cancer specific survival in stage IA non-mucinous adenocarcinoma ($n = 870$).

Variables		RFS				OS				LCSS			
		Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
		HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Age	>65 years vs. ≤65 years	1.988 (0.963–4.102)	0.063			26.669 (3.527–201.638)	0.001	20.759 (2.719–158.501)	0.003	6.869 (0.764–61.778)	0.086		
Sex	Male vs. female	1.172 (0.573–2.397)	0.665			2.129 (0.787–5.758)	0.137			0.774 (0.129–4.635)	0.779		
Smoking status	Ever vs. never	1.951 (0.948–4.018)	0.07			2.698 (0.998–7.296)	0.051	2.019 (0.725–5.622)	0.179	2.221 (0.371–13.295)	0.382		
MP pattern	Present vs. absent	5.874 (2.614–13.200)	<0.001	1.921 (0.689–5.359)	0.212	2.974 (1.132–7.815)	0.027	1.679 (0.566–4.982)	0.350	8.517 (0.952–76.217)	0.055	2.000 (0.123–32.513)	0.626
Vascular invasion	Present vs. absent	5.018 (2.291–10.990)	<0.001	2.546 (1.123–5.772)	0.025	3.284 (1.067–10.107)	0.038	1.765 (0.554–5.621)	0.336	2.526 (0.282–22.661)	0.408		
Lymphatic invasion	Present vs. absent	4.748 (2.301–9.794)	<0.001	1.606 (0.718–3.594)	0.249	3.314 (1.225–8.966)	0.018	1.517 (0.503–4.577)	0.459	4.217 (0.704–25.266)	0.115		
Necrosis	Present vs. absent	4.163 (1.701–10.188)	0.002	1.400 (0.546–3.587)	0.483	10.442 (3.968–27.479)	<0.001	6.570 (2.481–17.402)	<0.001	9.827 (1.640–58.873)	0.012	3.530 (0.520–23.957)	0.197
Pathologic stage	IA3 vs. IA1 and IA2	3.564 (1.740–7.303)	0.001	2.109 (1.002–4.440)	0.050	3.438 (1.326–8.911)	0.011	1.984 (0.752–5.236)	0.166	4.563 (0.762–27.314)	0.096		
STAS[a]	Present vs. absent	6.756 (3.091–14.764)	<0.001	3.462 (1.384–8.656)	0.008	3.172 (1.223–8.225)	0.018	1.218 (0.412–3.602)	0.721	11.516 (1.286–103.101)	0.029	11.516 (1.286–103.101)	0.029
STAS grade	STAS II vs. STAS I vs. STAS 0		<0.001		<0.001		0.018		0.738		0.018		0.018
	STAS I vs. STAS 0	4.266 (1.692–10.760)	0.002	3.445 (1.350–8.792)	0.01	2.172 (0.653–7.222)	0.206	0.989 (0.272–3.597)	0.987	4.566 (0.285–73.114)	0.283	4.566 (0.285–73.114)	0.283
	STAS II vs. STAS 0	11.973 (5.042–28.433)	<0.001	8.426 (3.441–20.632)	<0.001	5.007 (1.637–15.310)	0.005	1.597 (0.429–5.949)	0.486	23.238 (2.417–223.439)	0.006	23.238 (2.417–223.439)	0.006

MP micropapillary.

[a]Presence of STAS was analyzed separately from the grade of STAS.

0.001, respectively). Furthermore, the association with recurrence was more significant with STAS II than STAS I in both the limited and radical resection groups ($p = 0.008$ and $p = 0.312$ in the limited resection group and <0.001 and 0.012 in the radical resection group, respectively). Along with several other studies demonstrating the negative impact of STAS in patients who underwent lobectomy [1, 28, 32], the results of our study support the significance of STAS not only in the limited resection group but also in the radical resection group. The clinical significance of STAS could be extended from a R factor for limited resection to a feature representing aggressive biology in ADC in general independent of the surgical extent.

It is still controversial whether STAS is an in vivo phenomenon or an ex vivo artifact induced by cutting though a tumor with a knife [33]. One may argue that in procedures like VATS lobectomy, the entire resection specimens including tumors of various sizes are squeezed through small-caliber holes in the rigid thoracic wall, which might result in the detachment of tumor cells at the tumor periphery [34]. However, in our study, the VATS approach was not associated with the presence of STAS in the entire cohort. Interestingly, in ADC, the prevalence of STAS was higher in the open approach than in the VATS. However, upon stratified by pathologic stage, there was no difference in the frequency of STAS according to the surgical approach. Thus, the type of surgical approach was not associated with occurrence of STAS in our study speaking against STAS being an ex vivo artifact secondary to VATS lobectomy.

There are some limitations in this study. First, we only evaluated distance other than amount or volume of STAS. Uruga et al. showed that high STAS (≥5 single cells or clusters of STAS by using a ×20 objective and a ×10 ocular lens) was associated with worse RFS [18]. It is reasonable to think that STAS II has more clusters than STAS I, but the association between the distance from the tumor edge and the number of clusters have not been studied. As we only used the distance from the main tumor to evaluate the extent of STAS, combinations of the quantity and distance of STAS need to be evaluated in future large-cohort studies to refine the extent of STAS. Secondly, this study was carried out in a single institution and cross validation was not performed. Therefore, multicenter studies involving several pulmonary pathologists are needed to verify our results and examine the feasibility, reproducibility and prognostic performance of the STAS grading.

In conclusion, the presence of STAS II was an independent poor prognostic factor in stage IA non-mucinous ADC. To establish globally accepted grading criteria for STAS, specimen handling needs to be standardized and the reproducibility and prognostic performance of the grading system needs to be evaluated in a multi-institutional manner. In addition, as STAS II was a poor prognostic factor not only in limited resections but also in radical resections, including the STAS status and grade in the pathology report would be helpful for treatment decision making, regardless of the extent of resection.

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Compliance with ethical standards

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

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