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Predictive value of a prognostic model based on pathologic features in lung invasive adenocarcinoma

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ABSTRACT

Objectives: Tumor spread through air spaces (STAS) was recently reported as a novel risk factor for the prognosis of patients with resected lung adenocarcinoma that indicates invasive tumor behavior. The purpose of this study was to build a prognostic model consisting of STAS and other pathologic features including visceral pleural invasion (VPI), vascular invasion (VI) and histological subtype (HS) in lung invasive adenocarcinoma.

Materials and methods: A total of 289 patients with resected lung invasive adenocarcinomas ≤4 cm were analyzed retrospectively to evaluate the potential prognostic value of STAS, VPI, VI and HS for recurrence-free survival (RFS) and overall survival (OS).

Results: STAS was observed in 143 patients (49.5%). Univariate and multivariate analysis showed that STAS, VPI, VI and HS were significant prognostic factors for poorer RFS and OS. Thus, a prognostic model including STAS, VPI, VI and HS was built using the results of the multivariate analysis. Nomograms were developed to predict the 5-year RFS and OS. The concordance index (C-index) of the prognostic model was 0.8122 for predicting 5-year RFS and 0.8539 for predicting 5-year OS in the internal validation. Moreover, the calibration curves for the 5-year RFS and OS showed that the nomograms were calibrated well. In addition, a similar predicted capability of the prognostic model was observed in the validation cohort.

Conclusion: STAS, VPI, VI and HS were significant prognostic factors for poorer RFS and OS. The prognostic model including STAS, VPI, VI and HS could effectively predict prognosis.

1. Introduction

The 2015 World Health Organization (WHO) classification introduced tumor spread through air spaces (STAS) officially as a new tumor invasion characteristic and presented it as a new exclusion criterion for minimally invasive adenocarcinoma [1]. Tumor STAS is defined as tumor cells within the air spaces in the lung parenchyma beyond the edge of the main tumor and is composed of three morphological patterns: single cells, micropapillary structures, and solid nests or tumor islands [2]. Although it remains controversial whether tumor STAS is caused by an in vivo effect or an artifact of cutting through a tumor with a knife [3,4], tumor STAS has recently been described as a novel pattern of tumor invasion that differs from traditional invasion characteristics, such as the presence of nonlepidic patterns and the infiltration of stroma and blood vessels into structures such as the visceral pleura [5]. Regarding the association between tumor STAS and prognosis, tumor STAS was reported as a significant independent risk factor associated with reduced recurrence-free survival (RFS) and overall survival (OS) in patients with lung adenocarcinoma of any stage following surgical resection [2,6–9]. Furthermore, an unfavorable prognostic impact of STAS has also been reported in squamous cell carcinoma [10–12] and lung pleomorphic carcinoma [13].

Therefore, we retrospectively characterized the clinicopathologic findings of patients with resected lung adenocarcinoma and sought to investigate whether a prognostic model including tumor STAS combined with visceral pleural invasion (VPI), vascular invasion (VI) and histological subtype (HS) could significantly predict RFS and OS.

Abbreviations: STAS, spread through air spaces; VPI, visceral pleura invasion; VI, vascular invasion; HS, histological subtype; RFS, recurrence-free survival; OS, overall survival; STAKS, spreading through a knife surface; RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe; SD, standard deviation; HR, hazard ratio; CI, confidence interval; NSCLC, non-small cell lung cancer

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2. Material and methods

2.1. Patient cohorts

We reviewed patients with lung adenocarcinoma ≤4 cm who had undergone surgical resection and were diagnosed between April 2013 and September 2013 at our institution. This study was performed in line with the Helsinki Declaration. The Institutional Review Board of Affiliated Hospital of Qingdao University approved this retrospective study (IRB#QYFYKY 2018-10-11-2). Informed consent was waived by the institutional review board because this was a retrospective study. Patients were excluded from the study cohort if one of the following criteria was met: (1) presence of multiple nodules; (2) presence of variants of adenocarcinoma including invasive mucinous, fetal, or colloid adenocarcinoma; (3) presence of other components, such as squamous or neuroendocrine differentiation; or (4) loss to follow-up. Ultimately, 289 patients with resected lung invasive adenocarcinoma were reviewed in the study cohort. The demographic information of the patients and the data regarding the surgical procedures performed, including gender, age, surgical approach, length of hospital stay, and smoking history, were retrospectively collected and sorted from the electronic medical records. Outpatient clinic revisit records and telephone follow-up were used to collect survival information. The end date of follow-up for the present study was October 18, 2018. Furthermore, we also included a validation cohort that was composed of 91 patients with the same exclusion criterion as above between December 2012 and February 2013.

2.2. Histopathology examination

Hematoxylin and eosin (HE)-stained tumor slides of resected tumor specimens acquired from formalin-fixed paraffin-embedded tissues were microscopically reviewed and evaluated by two pathologists who were blinded to the clinical characteristics and survival outcomes of patients. Any disagreements were resolved by consensus.

The pathological features of the tumor were recorded, including tumor size, HS, VPI, VI, and STAS. VI was observed by performing CD-31 and D2-40 staining for each patient. VPI was observed by HE and elastic fibers staining. The stage of the tumor was based on the pathologic tumor-node-metastasis (TNM) stage (International Union against Cancer staging system, 8th edition) [14]. The histological subtype that was the predominant pattern of main lung adenocarcinoma was evaluated according to the 2015 WHO classification [1].

The definition of tumor STAS was based on the original report [2]. Tumor STAS in lung adenocarcinoma has been defined as tumor cells within air spaces in the surrounding lung parenchyma beyond the edge of the main tumor. Tumor STAS was identified by the presence of tumor cells within air spaces and included three morphological subtypes: (1) micropapillary or papillary structures; (2) solid nests or tumor islands; and (3) single cells. Micropapillary structures consisted of papillary structures without central fibrovascular cores in the surrounding lung parenchyma; solid nests or tumor islands consisted of solid collections of tumor cells filling the air spaces; and single cells consisted of scattered discohesive single tumor cells in the air spaces. The edge of the main tumor was defined as the smooth surface of the tumor that was easily recognizable grossly or with a low-power field examination [2]. In addition, it was controversial whether tumor STAS was considered to be one type of tumor invasion or a reproducible artifact secondary to mechanical forces [3,15,16], which was recognized as “spreading through a knife surface”(STAKS). Some previously described discriminative methods were used to distinguish STAS from artificially detached tumor cells arising from tumor dissection [2,3,10,15]. Tumor STAS was mostly contiguous but was sometimes randomly scattered throughout the lung tissue. Tissue fragments with and without jagged edges were carefully identified and deemed to be STAKS. Tumor STAS was also distinguished from alveolar macrophages according to morphological features. The former were characterized by a high nuclear-to-cytoplasmic ratio and nuclear atypia, whereas the latter exhibited small nuclei and foamy cytoplasm that sometimes contained faint pigment [2]. Two pathologists diagnosed STAS when any of the morphological subtypes were present. In the event of disagreement, a consensus was reached after discussion.

2.3. Statistical analysis

The associations between tumor STAS and clinicopathologic parameters were evaluated using Fisher’s exact test for categorical variables and Student’s test for numerical variables. RFS was defined as the interval between the date of surgical resection and that of detection of recurrence. Recurrences were categorized as locoregional or distant [2,17]. OS was defined as the time from surgical resection to the date of death or the last follow-up visit. Cox proportional hazards regression models were used to assess the prognostic value of each factor found to be significant in the univariate analysis. Furthermore, multivariate survival analysis was performed using a Cox proportional hazards regression model to adjust for potential confounders of the association between pathologic factors with RFS/OS independently. Multivariate models including factors that were significant in the univariate analysis were built. The RFS and OS analyses were performed using the Kaplan-Meier estimator, and nonparametric group comparisons were performed using the log-rank test. Then, a prognostic model was built using the results of the multivariate analysis. Further, nomograms [18,19] were constructed to develop a statistical prognostic model that generates the predictive probability of 5-year RFS and OS. Internal validation was evaluated by the concordance index (C-index) and calibration curves. Bootstrap resampling (1000 resamples) was used for the calibration curves. Moreover, external validation of the prognostic model was assessed in a validation cohort.

Statistical analyses were performed with IBM SPSS Statistics version 25.0 (IBM Corp. New York, USA) and R (version 3.5.1, R Development Core Team), including the “survival” and “rms” packages. All p values were two-sided, and a p value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Patient characteristics

We identified a total of 289 patients with a tumor diameter ≤4 cm in the study cohort. The detailed clinicopathological characteristics of the study cohort and validation cohort are summarized in Table 1. In the study cohort, the mean age at the time of diagnosis was 60.36 years. For the surgical approach, the majority of the reviewed patients underwent lobectomy (89.3% for lobectomy versus 10.7% for limited resection). Among 289 patients with a tumor size ≤4 cm in the study cohort, 141 (48.8%) patients had T1 stage disease (T1a: 11.8%, T1b: 25.3%, T1c: 11.8%), while 148 (51.2%) patients had T2a stage disease, of which 116 (78.4%) patients had a tumor size ≤3 cm with VPI. All patients had M0 stage disease. During follow-up, 38 (13.1%) patients died, and 44 (15.2%) patients had recurrence. The mean follow-up time of the patients alive at the endpoint of the analysis was 58.58 months.

Tumor STAS was observed in 143 patients (49.5%) in the study cohort and 50 patients (54.9%) in the validation cohort. Representative microphotographs of tumor STAS in adenocarcinoma are shown in Fig. 1. Table 1 also shows the distribution of clinicopathologic factors according to the presence of tumor STAS in the study and validation cohort. There were significant correlations between the presence of tumor STAS and several pathologic features, including larger tumor size (mean, 2.471 cm versus 1.841 cm, $p < 0.001$), higher pathologic T stage ($p < 0.001$), higher pathologic N stage ($p < 0.001$), higher pathologic stage ($p < 0.001$), more invasive histological subtype ($p < 0.001$), the presence of VPI ($p < 0.001$), and the presence of VI

Table 1
Clinicopathologic characteristics according to the presence of STAS in the study and validation cohorts.

Characteristic	Study cohort (n = 289)				Validation cohort(n = 91)			
	All Patients (n = 289) Number (%)	STAS Absence (n = 146) Number (%)	STAS Presence (n = 143) Number (%)	P Value	All Patients (n = 91) Number (%)	STAS Absence (n = 41) Number (%)	STAS Presence (n = 50) Number (%)	P Value
Gender				0.053				0.085
Male	125 (43.3)	55 (44.0)	70 (56.0)		49 (53.8)	18 (36.7)	31 (63.3)	
Female	164 (56.7)	91 (55.5)	73 (44.5)		42 (46.2)	23 (54.8)	19 (45.2)	
Age				0.502				0.797
≤65	207 (71.6)	102 (49.3)	105 (50.7)		59 (64.8)	26 (44.1)	33 (55.9)	
>65	82 (28.4)	44 (53.7)	38 (46.3)		32 (35.2)	15 (46.9)	17 (53.1)	
Smoking history				0.812				0.103
Never	218 (75.4)	111 (50.9)	107 (49.1)		68 (74.7)	34 (50.0)	34 (50.0)	
Former/current	71 (24.6)	35 (49.3)	36 (50.7)		23 (25.3)	7 (30.4)	16 (69.6)	
Postoperative hospitalization days				0.021				0.085
Mean ± SD	7.14 ± 2.280	6.84 ± 1.969	7.45 ± 2.528		8.67 ± 2.508	8.17 ± 2.155	9.08 ± 2.717	
Range	4-14	4-13	4-14		4-15	5-13	4-15	
Surgery				0.042				0.037
Lobectomy	258 (89.3)	125 (48.4)	133 (51.6)		86 (94.5)	41 (47.7)	45 (52.3)	
Limited resection	31 (10.7)	21 (67.7)	10 (32.3)		5 (5.5)	0 (0.0)	5 (100.0)	
Tumor location				0.483				0.451
RUL	98 (33.9)	54 (55.1)	44 (44.9)		30 (33.0)	14 (46.7)	16 (53.3)	
RML	23 (8.0)	12 (52.2)	11 (47.8)		5 (5.5)	1 (20.0)	4 (80.0)	
RLL	61 (21.1)	32 (52.5)	29 (47.5)		20 (22.0)	11 (55.0)	9 (45.0)	
LUL	63 (21.8)	31 (49.2)	32 (50.8)		21 (23.1)	7 (33.3)	14 (66.7)	
LLL	44 (15.2)	17 (38.6)	27 (61.4)		15 (16.5)	8 (53.3)	7 (46.7)	
Tumor size(cm)				< 0.001				< 0.001
0-1	43 (14.9)	38 (88.4)	5 (11.6)		9 (9.9)	9 (100.0)	1 (10)	
1-2	116 (40.1)	65 (56.0)	51 (44.0)		32 (35.2)	18 (56.2)	14 (43.8)	
2-3	97 (33.6)	33 (34.0)	64 (66.0)		34 (37.4)	10 (29.4)	24 (70.6)	
3-4	32 (11.1)	10 (31.3)	22 (68.8)		16 (17.6)	4 (25.0)	12 (75.0)	
Mean ± SD	2.15 ± 0.862	1.841 ± 0.844	2.471 ± 0.760		2.39 ± 0.839	2.02 ± 0.903	2.68 ± 0.654	
Pathologic T stage				< 0.001				0.001
T1a	34 (11.8)	32 (94.1)	2 (5.9)		8 (8.8)	8 (100.0)	0 (0.0)	
T1b	73 (25.3)	49 (67.1)	24 (32.9)		16 (17.6)	10 (62.5)	6 (37.5)	
T1c	34 (11.8)	12 (35.3)	22 (64.7)		8 (8.8)	1 (12.5)	7 (87.5)	
T2a	148 (51.2)	53 (35.8)	95 (64.2)		59 (64.8)	22 (37.3)	37 (62.7)	
Pathologic N stage				< 0.001				0.012
N0	230 (79.6)	138 (60.0)	92 (40)		66 (72.5)	36 (54.5)	30 (45.5)	
N1	18 (6.2)	3 (16.7)	15 (83.3)		7 (7.7)	1 (14.3)	6 (85.7)	
N2	41 (14.2)	5 (12.2)	36 (87.8)		18 (19.8)	4 (22.2)	14 (77.8)	
Pathologic stage				< 0.001				< 0.001
Stage IA1	33 (11.4)	32 (97.0)	1 (3.0)		8 (8.8)	8 (100.0)	0 (0.0)	
Stage IA2	65 (22.5)	47 (72.3)	18 (27.7)		15 (16.5)	10 (66.7)	5 (33.3)	
Stage IA3	22 (7.6)	10 (45.5)	12 (54.5)		4 (4.4)	0 (0.0)	4 (100.0)	
Stage IB	110 (38.1)	49 (44.5)	61 (55.5)		39 (42.9)	18 (46.2)	21 (53.8)	
Stage IIB	18 (6.2)	3 (16.7)	15 (83.3)		7 (7.7)	1 (14.3)	6 (85.7)	
Stage IIIA	41 (14.2)	5 (12.2)	36 (87.8)		18 (19.8)	4 (22.2)	14 (77.8)	
HS				< 0.001				< 0.001
Lepidic	71 (24.6)	53 (74.6)	18 (25.4)		7 (7.7)	6 (85.7)	1 (14.3)	
Acinar	116 (40.1)	62 (53.4)	54 (46.6)		36 (39.6)	26 (72.2)	10 (27.8)	
Papillary	54 (18.7)	25 (46.3)	29 (53.7)		15 (16.5)	5 (33.3)	10 (66.7)	
Micropapillary	21 (7.3)	3 (14.3)	18 (85.7)		18 (19.8)	3 (16.7)	15 (83.3)	
Solid	27 (9.3)	3 (11.1)	24 (88.9)		15 (16.5)	1 (6.7)	14 (93.3)	
VPI				< 0.001				0.025
Absence	147 (50.9)	97 (66.0)	50 (34.0)		33 (36.3)	20 (60.6)	13 (39.4)	
Presence	142 (49.1)	49 (34.5)	93 (65.5)		58 (63.7)	21 (36.2)	37 (63.8)	
VI				< 0.001				0.011
Absence	236 (81.7)	136 (57.6)	100 (42.4)		71 (78.0)	37 (52.1)	34 (47.9)	
Presence	53 (18.3)	10 (18.9)	43 (81.1)		20 (22.0)	4 (20.0)	16 (80.0)	

RUL = Right upper lobe; RML = Right middle lobe; RLL = Right lower lobe; LUL = Left upper lobe; LLL = Left lower lobe; SD = Standard deviation; STAS = Spread through air spaces; VPI = Visceral pleura invasion; VI = Vascular invasion; HS = Histological subtype.

($p < 0.001$).

3.2. Survival analysis in the study cohort

In the univariate analysis of all cases, a Cox proportional hazards regression model was used to calculate the prognostic significance of clinicopathologic risk factors for RFS and OS in the present study cohort (Table 2). Larger tumor size ($p < 0.001$, respectively), pathologic T stage ($p < 0.001$ versus $p = 0.001$, respectively), pathologic stage ($p < 0.001$, respectively), HS ($p < 0.001$, respectively), and the presence of VPI ($p < 0.001$, respectively), VI ($p < 0.001$, respectively) and tumor STAS ($p < 0.001$, respectively) were identified as significant predictors of both RFS and OS. Furthermore, the univariate analysis showed that patients with tumor STAS had a worse RFS and OS than those without tumor STAS (hazard ratio (HR), 9.401; 95% confidence interval (CI), 3.704–23.862; $p < 0.001$) versus (HR, 13.473; 95% CI, 4.143–43.817; $p < 0.001$).

Additional multivariate models were then applied to identify

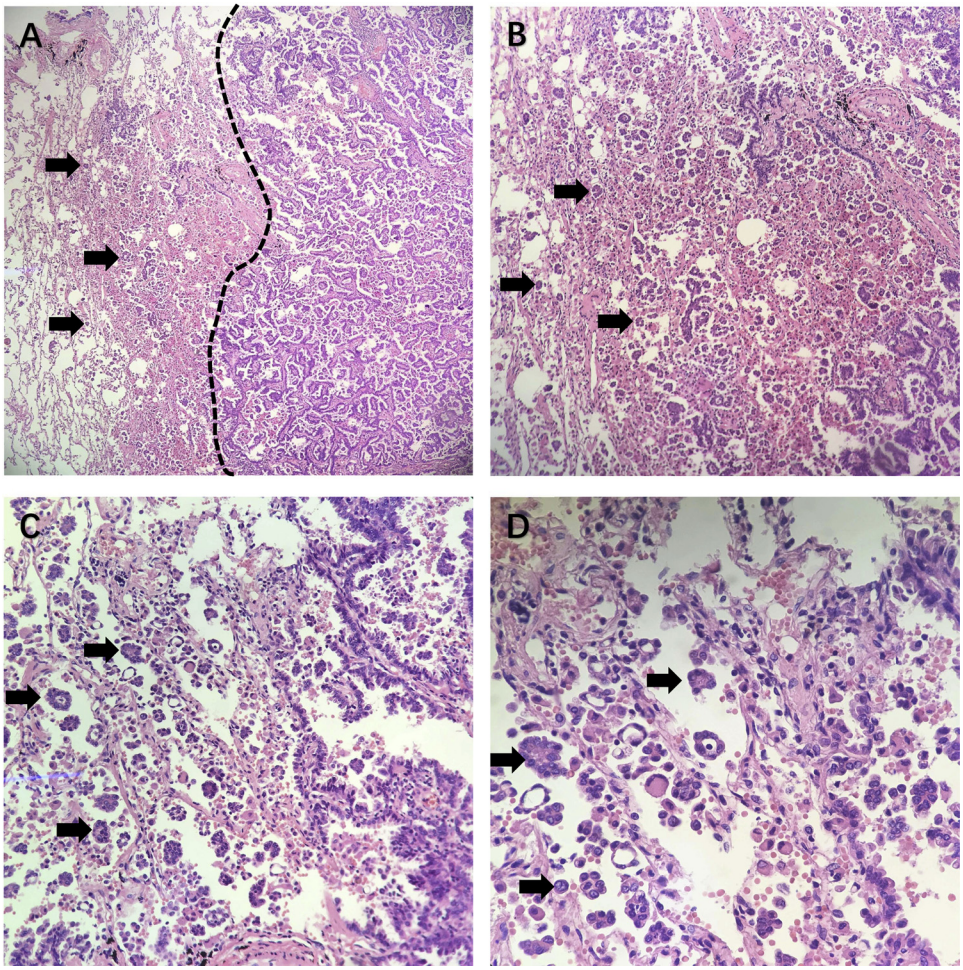

Fig. 1. Representative histopathologic images of tumor STAS in lung adenocarcinomas ≤4 cm, showing micropapillary pattern STAS (arrows) and several single-cell STAS (arrows). A, magnification X100 field of a tumor section with micropapillary pattern STAS and adenocarcinoma components. The dotted line highlights the edge of the main tumor. B, magnification X100 field of a tumor section. C, magnification X200 field of a tumor section. D, magnification X400 field of a tumor section.

independent prognostic factors (Table 2). In the multivariate analysis that contained the pathologic stage, HS, VPI, VI and STAS. HS (HR, 1.294; 95% CI, 1.026–1.634; p = 0.030), the presence of VPI (HR, 2.914; 95% CI, 1.276–6.654; p = 0.011), VI (HR, 2.837; 95% CI, 1.345–5.983; p = 0.006) and STAS (HR, 4.318; 95% CI, 1.593–11.701; p = 0.004) were independent prognostic factors for poor RFS. This outcome was also observed in multivariate models of OS: HS (HR, 1.404; 95% CI, 1.087–1.814; p = 0.009), VPI presence (HR, 2.957; 95% CI, 1.174–7.446; p = 0.021), VI presence (HR, 2.808; 95% CI, 1.219–6.472; p = 0.015) and STAS presence (HR, 4.421; 95% CI, 1.273–15.354; p = 0.019). Therefore, STAS, VPI, VI and HS remained significant predictors of higher recurrence rates and poorer survival.

Kaplan-Meier analysis was conducted to compare survival rates according to the presence of STAS, VPI, VI and HS (Fig. 2). STAS-presence patients had significantly worse RFS and OS than STAS-absence patients. The 5-year RFS rates in STAS-presence and STAS-absence patients were 72.0% and 96.6%, respectively, and the 5-year OS rates were 75.5% and 97.9%, respectively. Furthermore, patients with VPI also had significantly worse RFS and OS than patients without VPI (p < 0.001, respectively). VI-presence patients had poorer RFS and OS than VI-absence patients (p < 0.001, respectively). Patients with invasive histological subtypes including micropapillary and solid patterns had poorer RFS and OS than patients with other histological subtypes (p < 0.001, respectively).

3.3. Nomograms of the prognostic model

A prognostic model was built using the results of the multivariate analysis. To explore the predictive value of STAS, VPI, VI and HS, nomograms were used to predict the 5-year RFS and OS. The predictive accuracy for RFS and OS is shown by the calibration curves in the internal validation. The calibration plots for the predictive probability showed a great correlation between the nomogram-estimated prediction and the actual observed result. The discrimination and the prognostic accuracy of the prognostic model for the 5-year RFS and OS was calculated by the C-index in the internal validation (5-year RFS, C-index, 0.8539 versus 5-year OS, C-index, 0.8122).

3.4. Predictive accuracy of the prognostic model in the validation cohort

We also evaluated the predictive accuracy of the prognostic model including STAS, VPI, VI and HS in a validation cohort. The C-index were used to assess the predictive accuracy of the prognostic model for the 5-year RFS and OS in the external validation. In the validation cohort, the C-index of the prognostic model was 0.7928 for predicting 5-year RFS and 0.8249 for predicting 5-year OS. The C-index demonstrated that the model showed excellent discriminative ability in the validation cohort. Furthermore, the nomograms of the prognostic model were externally validated by the calibration curves for 5-year RFS and OS, which showed that the nomograms were calibrated well.

Table 2
Univariate and Multivariate analysis for RFS and OS in the study cohort.

Characteristic,Factor	Univariate Analysis						Multivariate Analysis					
	RFS			OS			RFS			OS		
	HR	95% CI	P Value	HR	95% CI	P Value	HR	95% CI	P Value	HR	95% CI	P Value
Clinical Factors												
Gender,Male	1.339	0.709-2.530	0.368	1.011	0.557-1.836	0.972	–	–	–	–	–	–
Age,Older, > 65	0.716	0.354-1.449	0.353	0.773	0.366-1.633	0.5	–	–	–	–	–	–
Smoking history,Former/Current	1.030	0.521-2.038	0.932	1.448	0.731-2.870	0.289	–	–	–	–	–	–
Surgery,Limited resection	1.608	0.421-2.711	0.889	1.289	0.503-3.301	0.597	–	–	–	–	–	–
Tumor location,Lower lobe	0.907	0.486-1.692	0.759	0.705	0.350-1.422	0.329	–	–	–	–	–	–
Pathologic Factors												
Tumor size (cm),Larger (per cm)	2.306	1.638-3.246	< 0.001	2.216	1.535-3.199	< 0.001	–	–	–	–	–	–
Pathologic T stage (per stage)	2.388	1.550-3.680	< 0.001	2.723	1.618-4.585	< 0.001	–	–	–	–	–	–
Pathologic T stage,T2 vs T1	4.206	2.021-8.753	< 0.001	5.664	2.368-13.548	< 0.001	–	–	–	–	–	–
Pathologic N stage (per stage)	2.265	1.652-3.104	< 0.001	2.981	2.134-4.165	< 0.001	–	–	–	–	–	–
Pathologic N stage,(N1 & N2)vsN0	4.224	2.329-7.658	< 0.001	7.606	3.963-14.599	< 0.001	–	–	–	–	–	–
Pathologic stage (per stage)	1.810	1.449-2.263	< 0.001	2.237	1.716-2.916	< 0.001	1.022	0.741-1.410	0.895	1.243	0.856-1.806	0.253
Pathologic stage,II vs I	2.575	0.893-7.429	0.080	5.025	1.825-13.841	0.002	–	–	–	–	–	–
Pathologic stage,III (vs I & II)	4.669	2.521-8.645	< 0.001	7.174	3.787-13.590	< 0.001	–	–	–	–	–	–
HS	1.762	1.419-2.186	< 0.001	2.001	1.583-2.531	< 0.001	1.294	1.026-1.634	0.030	1.404	1.087-1.814	0.009
HS,(Micropapillary & Solid) vs Others	4.696	2.583-8.540	< 0.001	6.094	3.222-11.527	< 0.001	–	–	–	–	–	–
VPI,Presence	4.610	2.215-9.594	< 0.001	6.193	2.589-14.814	< 0.001	2.914	1.276-6.654	0.011	2.957	1.174-7.446	0.021
VI,Presence	4.828	2.663-8.753	< 0.001	6.858	3.614-13.016	< 0.001	2.837	1.345-5.983	0.006	2.808	1.219-6.472	0.015
STAS,Presence	9.401	3.704-23.862	< 0.001	13.473	4.143-43.817	< 0.001	4.318	1.593-11.701	0.004	4.421	1.273-15.354	0.019

STAS = Spread through air spaces; VPI = Visceral pleura invasion; VI = Vascular invasion; HS = Histological subtype; HR = Hazard ratio; CI = Confidence interval.

4. Discussion

In the present study, a new prognostic model that was composed of STAS, VPI, VI and HS was created to provide an indication of prognosis. We confirmed that the prognostic model was significant.

Regarding the definition of STAS, the 2015 WHO classification introduced tumor STAS officially as a new tumor invasion characteristic [1]. STAS was defined by Kadota et al. as tumor cells within air spaces in the lung parenchyma beyond the edge of the main tumor and was composed of three morphological patterns: single cells, micropapillary structures, and solid nests or tumor islands [2]. In the report, the presence of STAS was a significant risk factor for recurrence in patients with small stage I adenocarcinomas treated with limited resection but not in those who underwent lobectomy [2]. Kadota et al. speculated that undetected STAS in the alveolar space beyond the surgical margin may cause recurrence [2]. Before that, an early report indicated that the pathologic features of aerogenous spread with floating cancer cells (ASFC) was a significant prognostic factor in patients with metastatic lung cancer of colorectal origin [20,21]. Subsequently, tumor islands in the alveolar spaces were discovered by three-dimensional reconstruction analysis that was connected to the main tumor [22]. In a further study, Onozato et al. reported that the prognosis of stage I-II lung adenocarcinomas with tumor islands was significantly worse than that of those without [23]. Therefore, tumor islands could be one type of morphological pattern of STAS, although it is controversial whether tumor islands should be included in STAS or not [24]. Free tumor clusters (FTC) have been reported as distinctive morphological features in patients with lung adenocarcinoma harboring a micropapillary component and influenced the decision regarding the pathologic T stage [25]. FTC could be one type of STAS. Warth et al. subsequently defined STAS as a small solid cell nest including at least five tumor cells. In the cohort, STAS was reported as a significant risk factor for reducing OS and disease-free survival in patients with completely resected stage I–IV lung adenocarcinoma, and there was no significant difference in patients with extensive STAS and those with limited STAS [6].

In our study, we used a comprehensive definition of STAS. When we evaluated the presence of STAS, which was observed in 49.5% of patients in the study cohort with stage I-III lung adenocarcinomas ≤4 cm, 54.9% of patients in the validation cohort were included. Kadota et al. reported that STAS was observed in 38% of patients with lung adenocarcinomas ≤2 cm [2]. By contrast, Warth et al. reported that 50.6% of patients with stage I–IV lung adenocarcinoma showed STAS [6]. Consequent research showed similar findings of the presence of STAS in patients with other NSCLCs: 47.6% of patients with stage I lung adenocarcinoma [9], 50.6% of patients with stage I-III lung adenocarcinoma [26], 58.4% of patients with stage I–IV lung adenocarcinoma [27], and 73% of patients with lung adenocarcinoma with lymph node metastasis [28]. In particular, we found that there were significant correlations between STAS and several pathologic features including tumor size, pathologic stage, HS, VPI and VI. Similarly, STAS was reported to be associated with tumor size [9,29], pathologic stage [6,7,26,27,30], lymphatic or vascular invasion, [2,7,9,26,27,29,31] pleural invasion [7,9,29–31] and more invasive histological subtypes, such as micropapillary and solid patterns [2,6,8,9,25,26,31]. Therefore, it was inferred that STAS could be affected by the pathologic features of the main tumor itself and by invasive tumor behavior. Thus, the positive rate for STAS can be diverse in any cohort because of the pathologic features of the main tumor itself and its invasive pattern, even lymph node metastasis. Additionally, we focused on VPI, VI and HS. Among them, VI included VI in the lymphatics or vasculature.

Univariate and multivariate Cox proportional hazards regression analysis in the study cohort showed that STAS, VPI, VI and HS were significant unfavorable risk factors for RFS and OS in patients with resected lung invasive adenocarcinomas ≤4 cm, which was consistent with studies in patients with lung adenocarcinoma [7,27,31–33]. Recently, STAS was designated as a novel invasive pattern in lung

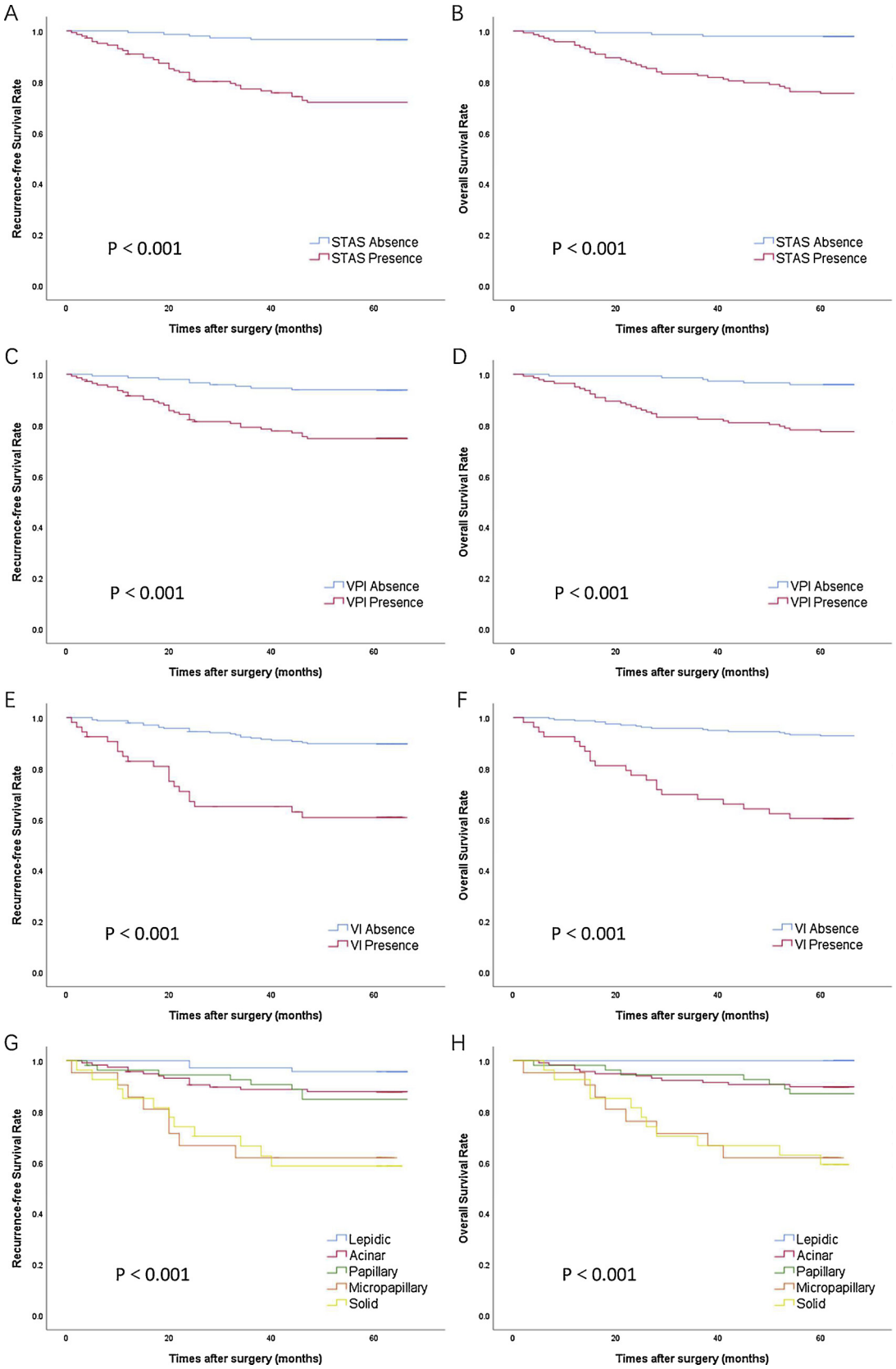

Fig. 2. Kaplan-Meier survival analysis of RFS and OS according to the presence of STAS, VPI, VI and HS in the study cohort. A, RFS in patients with STAS versus without STAS ($P < 0.001$). B, OS in patients with STAS versus without STAS ($P < 0.001$). C, RFS in patients with VPI versus without VPI ($P < 0.001$). D, OS in patients with VPI versus without VPI ($P < 0.001$). E, RFS in patients with VI versus without VI ($P < 0.001$). F, OS in patients with VI versus without VI ($P < 0.001$).G, RFS in patients with different histological subtype ($P < 0.001$). H, OS in patients with different histological subtype ($P < 0.001$).

adenocarcinoma. Additionally, STAS was associated with poorer OS and RFS in squamous cell carcinomas [10–12] and lung pleomorphic carcinoma [13] but not small cell lung cancer [34]. Although it should be emphasized that STAS-presence tumors occurred concomitantly with tumors exhibiting VPI and VI more frequently than STAS-absence tumors, multivariate analysis showed that STAS, VPI, VI and HS were significantly independent prognostic factors of RFS and OS. Therefore, it can be inferred that STAS, VPI and VI may be different pathologic characteristics of invasive tumor behavior. Additionally, there was no sufficient evidence to clearly prove any causality between them.

Thus, we attempted to build a prognostic model that combined STAS, VPI, VI and HS. Nomograms [18,19] have been validated as reliable tools to predict recurrence or survival and were used statistical prognostic models to generate the predictive probability of 5-year RFS and OS in this study. Our nomogram consisted of four factors: STAS, VPI, VI and HS that were pathologically significant factors in the multivariate analysis of our study. The effects of several separate pathologic features were integrated into the nomogram to provide an individualized risk assessment for 5-year RFS and OS of each patient. Based on previous findings [2,6–9,23–26,29–33], STAS may be viewed as a new type of invasive tumor behavior for lung adenocarcinoma, and its incorporation into the novel prognostic model can contribute to improvements in prognostic stratification.

Consider a patient with resected lung invasive adenocarcinoma ≤4 cm whose histological subtype is papillary pattern. The pathologic features of the tumor show the presence of STAS, VPI and VI. This patient`s nomogram calculated that the 5-year risk of recurrence is 55% (Fig. 3A; nomogram calculations are as follows: STAS = Presence, which corresponds to 100 points; VPI = Presence, which corresponds to 77 points; VI = Presence, which corresponds to 73 points; HS = papillary, which corresponds to 28 points; this equals 278 total points, corresponding to a 5-year RFS of 45%, recurrence of 55%). Similarly, the patient`s nomogram calculated that the 5-year risk of mortality is 52% (Fig. 3B; nomogram calculations are as follows: STAS = Presence, which corresponds to 67 points; VPI = Presence, which corresponds to 59 points; VI = Presence, which corresponds to 56 points; HS = papillary, which corresponds to 68 points; this equals 250 total points, corresponding to a 5-year OS of 48%, mortality of 52%). Therefore at 5 years, the patient has a 55% risk of recurrence, calculated by a nomogram that can identify a recurrence 81.22% of the time, the patient has a 52% risk of mortality, calculated by a nomogram that can identify a mortality 85.39% of the time. Discrimination is the ability to distinguish patients who experience recurrence and mortality from those who do not [18]. The C-index of the nomogram for predicting the 5-year RFS was 0.8122 in the study cohort and 0.7928 the validation cohort. Similarly, the C-index of the nomogram for predicting the 5-year OS was 0.8539 in the study cohort and 0.8249 in the validation cohort. This showed that the nomogram can discern a patient with the event from a patient without the event approximately 80% of the RFS time and approximately 84% of the OS time. Thus, the prognostic model that included STAS, VPI, VI and HS could significantly predict the 5-year RFS and OS (Fig. 4).

In both the study cohort and validation cohort, the calibration curves of the nomograms showed a good correlation between the nomogram-estimated risk and the actual observed risk in the 5-year RFS and OS. Furthermore, a substantially worse nomogram performance in external cohort does not necessarily render the nomogram invalid [18]. Although the calibration curves for the predictive probability did not show a better correlation between the nomogram-estimated prediction and the actual observed result in the validation cohort, we still considered that the performance metrics were acceptable. Thus, it would be appropriate to recommend the nomogram for use. In other words, the nomogram of the prognostic model was almost accurate for predicting the 5-year RFS and OS.

It is controversial whether STAS can be considered to be associated with one type of tumor invasion or a reproducible artifact secondary to mechanical forces [3,15,16]. A multicenter prospective study showed that loose tissue fragments could be caused by mechanical forces during specimen handling, which was recognized as “STAKS [3,15]. Thus, it is important to accurately distinguish STAS from STAKS. There were no disagreements among the observers regarding the assessment of STAS in our study.

The present study has several limitations. First, it was a retrospective study conducted in a single institution. For the prognostic model, it is necessary to assess whether the model actually improves predictive power over other prognostic tools, such as the pathologic TNM stage, in additional multicenter prospective studies. Second, the related expressed proteins or driver gene mutations remain unknown. According to previous studies, STAS has been reported to be associated with higher rates of BRAF mutations, more ALK rearrangement, and the wild-type EGFR gene. However, the association with KRAS mutations was not significant [6,7,26]. As for protein expression, STAS presence

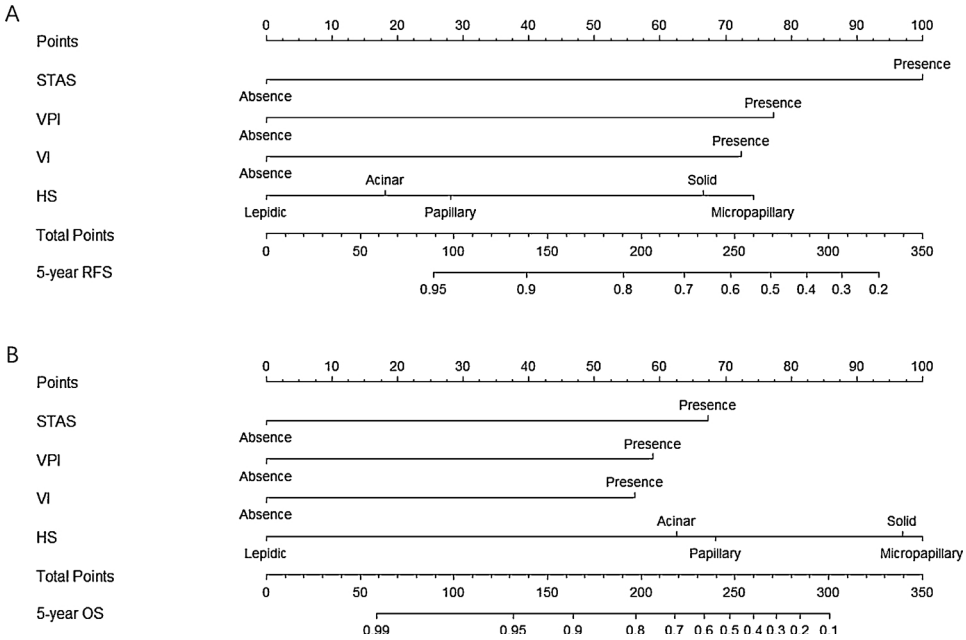

Fig. 3. Nomograms for 5-year RFS and OS in the study cohort. A, nomogram of 5-year RFS. B, nomogram of 5-year OS. In the nomogram, a line is drawn upwards to determine the number of points received for each risk factor. The sum of these numbers is located on the total points axis, and a line is then drawn downwards to the survival axis to predict the 5-year RFS and OS attributing an individual patient.

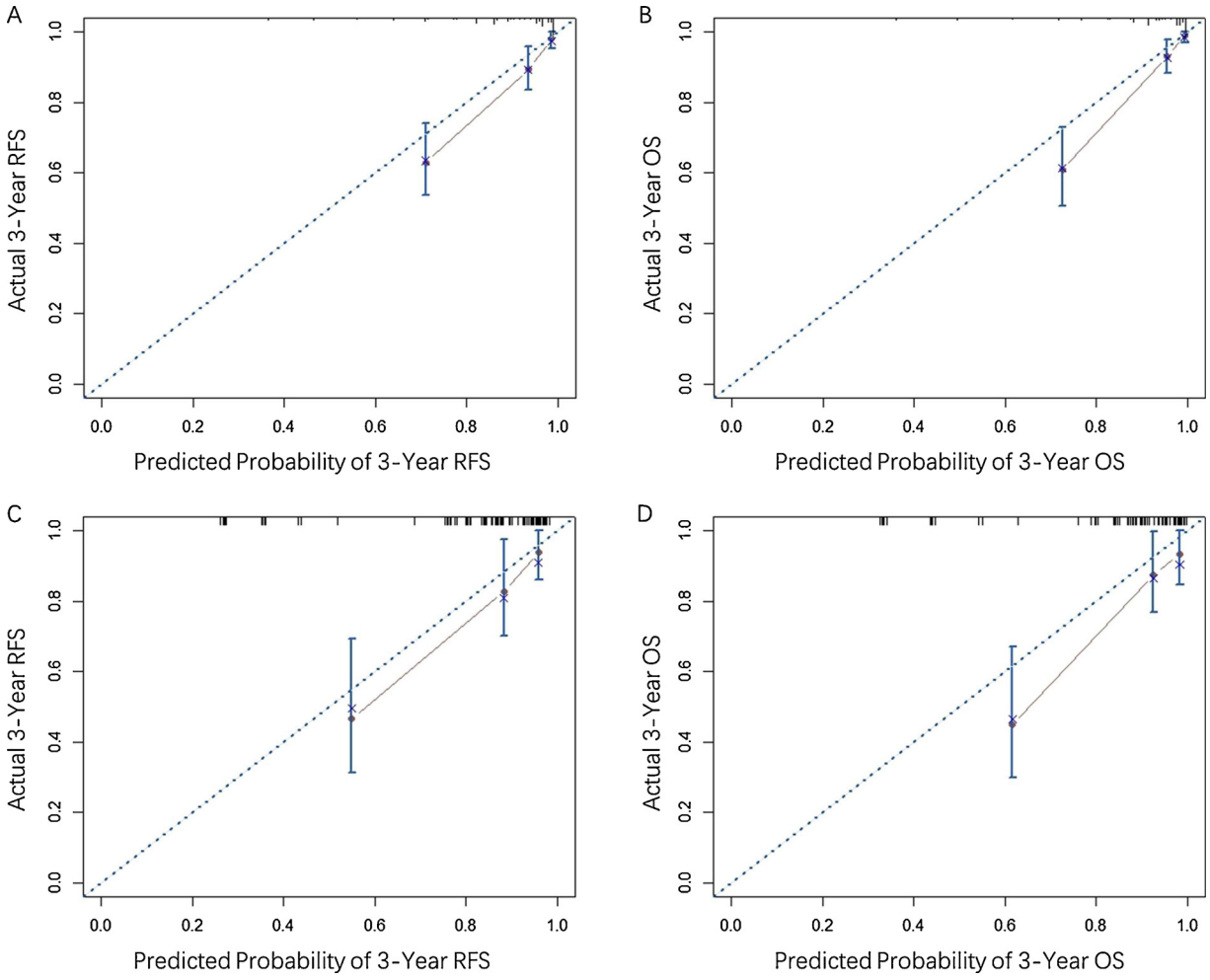

Fig. 4. Calibration curves of the nomograms for 5-year RFS and OS in the study and validation cohorts. A/B, the calibration curve in the study cohort. C/D, the calibration curve in the validation cohort. A/C, the calibration curve for the prediction of 5-year RFS; the predicted probability of 5-year RFS is plotted on the X axis; the actual 5-year RFS is plotted on the Y axis. B/D, the calibration curve for the prediction of 5-year OS; the predicted probability of 5-year OS is plotted on the X axis; the actual 5-year OS is plotted on the Y axis.

was not associated with the expression of TTF1, napsin, CK7 [6], or even the expression of PD-L1, which is a key molecule for immunotherapy in patients [35–37].

5. Conclusions

In conclusion, STAS, VPI, VI and HS were significant prognostic factors for poorer RFS and OS. A prognostic model that combined STAS with VPI, VI and HS could effectively predict recurrence and mortality.

Conflict of interest

The authors have no conflicts of interest to declare.

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References

[1] E. Brambilla, W.D. Travis, A.P. Burke, A. Marx, A.G. Nicholson, WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. International Agency for Research on Cancer, Lyon, 2015.
[2] K. Kadota, J.-i. Nitadori, C.S. Sima, H. Ujiie, N.P. Rizk, D.R. Jones, P.S. Adusumilli, W.D. Travis, Tumor spread through air spaces is an important pattern of invasion and impacts the frequency and location of recurrences after limited resection for small stage I lung adenocarcinomas, J. Thorac. Oncol. 10 (5) (2015) 806–814.
[3] E. Thunnissen, H.J. Blaauwgeers, E.M. de Cuba, C.Y. Yick, D.B. Flieder, Ex vivo artifacts and histopathologic pitfalls in the lung, Arch. Pathol. Lab. Med. 140 (3) (2016) 212–220.
[4] A. Warth, M.B. Beasley, M. Mino-Kenudson, Breaking new ground: the evolving concept of spread through air spaces (STAS), J. Thorac. Oncol. 12 (2) (2017) 176–178.
[5] W.D. Travis, E. Brambilla, M. Noguchi, A.G. Nicholson, K.R. Geisinger, Y. Yatabe, D.G. Beer, C.A. Powell, G.J. Riely, P.E. Van Schil, K. Garg, J.H. Austin, H. Asamura, V.W. Rusch, F.R. Hirsch, G. Scagliotti, T. Mitsudomi, R.M. Huber, Y. Ishikawa, J. Jett, M. Sanchez-Cespedes, J.P. Sculier, T. Takahashi, M. Tsuboi, J. Vansteenkiste, I. Wistuba, P.C. Yang, D. Aberle, C. Brambilla, D. Flieder, W. Franklin, A. Gazdar, M. Gould, P. Hasleton, D. Henderson, B. Johnson, D. Johnson, K. Kerr, K. Kuriyama, J.S. Lee, V.A. Miller, I. Petersen, V. Roggli, R. Rosell, N. Saijo, E. Thunnissen, M. Tsao, D. Yankelewitz, International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma, J. Thorac. Oncol. 6 (2) (2011) 244–285.
[6] A. Warth, T. Muley, C.A. Kossakowski, B. Goeppert, P. Schirmacher, H. Dienemann, W. Weichert, Prognostic impact of intra-alveolar tumor spread in pulmonary adenocarcinoma, Am. J. Surg. Pathol. 39 (6) (2015) 793–801.
[7] S. Shiono, N. Yanagawa, Spread through air spaces is a predictive factor of recurrence and a prognostic factor in stage I lung adenocarcinoma, Interact. Cardiovasc. Thorac. Surg. 23 (4) (2016) 567–572.

[8] C. Dai, H. Xie, H. Su, Y. She, E. Zhu, Z. Fan, F. Zhou, Y. Ren, D. Xie, H. Zheng, X. Kadeer, D. Chen, L. Zhang, G. Jiang, C. Wu, C. Chen, Tumor spread through air spaces affects the recurrence and overall survival in patients with lung adenocarcinoma &2 to 3 cm, J. Thorac. Oncol. 12 (7) (2017) 1052–1060.
[9] H. Uruga, T. Fujii, S. Fujimori, T. Kohno, K. Kishi, Semiquantitative assessment of tumor spread through air spaces (STAS) in early-stage lung adenocarcinomas, J. Thorac. Oncol. 12 (7) (2017) 1046–1051.
[10] S. Lu, K.S. Tan, K. Kadota, T. Eguchi, S. Bains, N. Rekhtman, P.S. Adusumilli, W.D. Travis, Spread through air spaces (STAS) is an independent predictor of recurrence and lung cancer-specific death in squamous cell carcinoma, J. Thorac. Oncol. 12 (2) (2017) 223–234.
[11] K. Kadota, Y. Kushida, N. Katsuki, R. Ishikawa, E. Ibuki, M. Motoyama, K. Nii, H. Yokomise, S. Bandoh, R. Haba, Tumor spread through air spaces is an independent predictor of recurrence-free survival in patients with resected lung squamous cell carcinoma, Am. J. Surg. Pathol. 41 (8) (2017) 1077–1086.
[12] N. Yanagawa, S. Shiono, M. Endo, S.Y. Ogata, Tumor spread through air spaces is a useful predictor of recurrence and prognosis in stage I lung squamous cell carcinoma, but not in stage II and III, Lung Cancer 120 (2018) 14–21.
[13] S. Yokoyama, T. Murakami, H. Tao, H. Onoda, A. Hara, R. Miyazaki, M. Furukawa, M. Hayashi, H. Inokawa, K. Okabe, Y. Akagi, Tumor spread through air spaces identifies a distinct subgroup with poor prognosis in surgically resected lung pleomorphic carcinoma, Chest (2018).
[14] P. Goldstraw, K. Chansky, J. Crowley, R. Rami-Porta, H. Asamura, W.E. Eberhardt, A.G. Nicholson, P. Groome, A. Mitchell, V. Bolejack, S. International Association for the Study of Lung Cancer, A.B. Prognostic Factors Committee, I. Participating, S. International Association for the Study of Lung Cancer, B. Prognostic Factors Committee Advisory, I. Participating, The IASLC Lung Cancer Staging Project: Proposals for Revision of the TNM Stage Groupings in the Forthcoming (Eighth) Edition of the TNM Classification for Lung Cancer, J. Thorac. Oncol. 11 (1) (2016) 39–51.
[15] H. Blaauwgeers, D. Flieder, A. Warth, A. Harms, K. Monkhorst, B. Witte, E. Thunnissen, A prospective study of loose tissue fragments in non-small cell lung Cancer resection specimens: an alternative view to "Spread through air spaces, Am. J. Surg. Pathol. 41 (9) (2017) 1226–1230.
[16] H. Blaauwgeers, P.A. Russell, K.D. Jones, T. Radonic, E. Thunnissen, Pulmonary loose tumor tissue fragments and spread through air spaces (STAS): invasive pattern or artifact? A critical review, Lung Cancer 123 (2018) 107–111.
[17] J. Nitadori, A.J. Bograd, K. Kadota, C.S. Sima, N.P. Rizk, E.A. Morales, V.W. Rusch, W.D. Travis, P.S. Adusumilli, Impact of micropapillary histologic subtype in selecting limited resection vs lobectomy for lung adenocarcinoma of 2cm or smaller, J. Natl. Cancer Inst. 105 (16) (2013) 1212–1220.
[18] V. Balachandran, M. Gonen, J. Smith, R. DeMatteo, Nomograms in oncology: more than meets the eye, Lancet Oncol. 16 (4) (2015) e173–80.
[19] A. Iasonos, D. Schrag, G. Raj, K. Panageas, How to build and interpret a nomogram for cancer prognosis, J. Clin. Oncol. 26 (8) (2008) 1364–1370.
[20] S. Shiono, G. Ishii, K. Nagai, J. Yoshida, M. Nishimura, Y. Murata, K. Tsuta, Y.H. Kim, Y. Nishiwaki, T. Kodama, M. Iwasaki, A. Ochiai, Predictive factors for local recurrence of resected colorectal lung metastases, Ann. Thorac. Surg. 80 (3) (2005) 1040–1045.
[21] S. Shiono, G. Ishii, K. Nagai, J. Yoshida, M. Nishimura, Y. Murata, K. Tsuta, Y. Nishiwaki, T. Kodama, A. Ochiai, Histopathologic prognostic factors in resected colorectal lung metastases, Ann. Thorac. Surg. 79 (1) (2005) 278–282 discussion 283.
[22] M.L. Onozato, V.E. Klepeis, Y. Yagi, M. Mino-Kenudson, A role of three-dimensional (3D)-reconstruction in the classification of lung adenocarcinoma, Anal. Cell. Pathol. 35 (2) (2012) 79–84.
[23] M.L. Onozato, A.E. Kovach, B.Y. Yeap, V. Morales-Oyarvide, V.E. Klepeis, S. Tammireddy, R.S. Heist, E.J. Mark, D. Dias-Santagata, A.J. Iafrate, Y. Yagi, M. Mino-Kenudson, Tumor islands in resected early-stage lung adenocarcinomas are associated with unique clinicopathologic and molecular characteristics and worse prognosis, Am. J. Surg. Pathol. 37 (2) (2013) 287–294.
[24] V. Morales-Oyarvide, M. Mino-Kenudson, Tumor islands and spread through air spaces: distinct patterns of invasion in lung adenocarcinoma, Pathol. Int. 66 (1) (2016) 1–7.
[25] J. Morimoto, T. Nakajima, H. Suzuki, K. Nagato, T. Iwata, S. Yoshida, M. Fukuyo, S. Ota, Y. Nakatani, I. Yoshino, Impact of free tumor clusters on prognosis after resection of pulmonary adenocarcinoma, J. Thorac. Cardiovasc. Surg. 152 (1) (2016) 64–72 e1.
[26] J.S. Lee, E.K. Kim, M. Kim, H.S. Shim, Genetic and clinicopathologic characteristics of lung adenocarcinoma with tumor spread through air spaces, Lung Cancer 123 (2018) 121–126.
[27] G. Toyokawa, Y. Yamada, T. Tagawa, T. Kamitani, Y. Yamasaki, M. Shimokawa, Y. Oda, Y. Maehara, Computed tomography features of resected lung adenocarcinomas with spread through air spaces, J. Thorac. Cardiovasc. Surg. 156 (4) (2018) 1670–1676 e4.
[28] G. Toyokawa, Y. Yamada, T. Tagawa, F. Kinoshita, Y. Kozuma, T. Matsubara, N. Haratake, S. Takamori, T. Akamine, F. Hirai, Y. Oda, Y. Maehara, Significance of spread through air spaces in resected lung adenocarcinomas with lymph node metastasis, Clin. Lung Cancer 19 (5) (2018) 395–400 e1.
[29] S. Shiono, M. Endo, K. Suzuki, K. Yarimizu, K. Hayasaka, N. Yanagawa, Spread through air spaces is a prognostic factor in sublobar resection of non-small cell lung Cancer, Ann. Thorac. Surg. (2018).
[30] G. Toyokawa, Y. Yamada, T. Tagawa, Y. Oda, Significance of spread through air spaces in early-stage lung adenocarcinomas undergoing limited resection, Thorac. Cancer (2018).
[31] K. Masai, H. Sakurai, A. Sukeda, S. Suzuki, K. Asakura, K. Nakagawa, H. Asamura, S.I. Watanabe, N. Motoi, N. Hiraoka, Prognostic impact of margin distance and tumor spread through air spaces in limited resection for primary lung Cancer, J. Thorac. Oncol. 12 (12) (2017) 1788–1797.
[32] G. Toyokawa, Y. Yamada, T. Tagawa, Y. Kozuma, T. Matsubara, N. Haratake, S. Takamori, T. Akamine, Y. Oda, Y. Maehara, Significance of spread through air spaces in resected pathological stage I lung adenocarcinoma, Ann. Thorac. Surg. (2018).
[33] Y.K. Kang, Y.S. Song, S. Cho, S. Jheon, W.W. Lee, K. Kim, S.E. Kim, Prognostic stratification model for patients with stage I non-small cell lung cancer adenocarcinoma treated with surgical resection without adjuvant therapies using metabolic features measured on F-18 FDG PET and postoperative pathologic factors, Lung Cancer 119 (2018) 1–6.
[34] G. Toyokawa, Y. Yamada, T. Tagawa, F. Kinoshita, Y. Kozuma, T. Matsubara, N. Haratake, S. Takamori, T. Akamine, F. Hirai, Y. Oda, Y. Maehara, High frequency of spread through air spaces in resected small cell lung Cancer, Anticancer Res. 38 (3) (2018) 1821–1825.
[35] D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy, Nat. Rev. Cancer 12 (4) (2012) 252–264.
[36] S.L. Topalian, J.M. Taube, R.A. Anders, D.M. Pardoll, Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy, Nat. Rev. Cancer 16 (5) (2016) 275–287.
[37] C.G. Drake, E.J. Lipson, J.R. Brahmer, Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer, Nat. Rev. Clin. Oncol. 11 (1) (2014) 24–37.