의학 박사 학위논문

# Clinicopathologic significance of tumor spread through air spaces (STAS) in resected lung cancers

**- Special reference on STAS grading –**

폐암에서 폐포 내 종양 세포 전파 (STAS)의

임상 및 병리학적 의미 고찰

– STAS 등급 분류에 대한 제안 –

2021 년 2월


**서울대학교 대학원**

**의학과 병리학 전공**

**한 연 비**

A thesis of the degree of Doctor of Medical science

# 폐암에서 폐포 내 종양 세포 전파 (STAS)의 임상 및 병리학적 의미 고찰

## – STAS 등급 분류에 대한 제안 –

# Clinicopathologic significance of tumor spread through air spaces (STAS) in resected lung cancers

## - Special reference on STAS grading –

**February 2021**

**Seoul National University College of Medicine**

**Department of Pathology**

**Yeon Bi Han**

# 폐암에서 폐포 내 종양 세포 전파 (STAS)의 임상 및 병리학적 의미 고찰

## - STAS 등급 분류에 대한 제안 -

지도교수 정 진 행

이 논문을 병리학 박사 학위논문으로 제출함

2020 년 10월

서울대학교 대학원

의학과 병리학 전공

한 연 비

한연비의 박사 학위논문을 인준함

2021 년 1월

위 원 장 이 춘 택 (인)

부 위 원 장 정 진 행 (인)

위 원 김 지 은 (인)

위 원 조 석 기 (인)

위 원 장 기 택 (인)

# ABSTRACT

# Clinicopathologic significance of tumor spread through air spaces (STAS) in resected lung cancers

## - Special reference on STAS grading -

**Yeon Bi Han**

**Department of Pathology, College of Medicine**

**The Graduate School**

**Seoul National University**

**Introduction:** Tumor spread through air spaces (STAS) is an invasive pattern of lung cancer that was recently described. However, its definition and clinical impact are still under debate. In this study, the investigator investigated the association

between the extent of STAS and clinicopathological characteristics and patient outcomes in resected lung cancers.

**Materials and methods:** STAS has been prospectively described from 2008 and graded its extent with a two-tiered system (STAS I: < 2500 μm [one field of x10 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. The investigator retrospectively analyzed the correlations between the extent of STAS and clinicopathologic characteristics and prognostic significance in 2000 resected lung cancers.

**Results:** Histologic subtypes of the 2000 cases were as follows; 1544 cases (77.2%) with adenocarcinoma (ADC), 325 cases (16.3%) with squamous cell carcinoma, 41 cases (2.1%) with neuroendocrine carcinoma (NEC), 16 cases (0.8%) with carcinoid tumors, and 74 cases (3.7%) with others. STAS was observed in 830 cases (41.5%) with 472 STAS I (23.6%) and 358 STAS II (17.9%). STAS was frequently found in patients with NEC (85.4%), pleural invasion, lymphovascular invasion, and 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 sublobar and lobar resection groups ($p=0.001$ and $p=0.023$, respectively).

**Conclusions:** Presence of STAS II was an independent poor prognostic factor in stage IA non-mucinous ADC regardless of the extent of resection. Thus, including the STAS status and grade in the pathology report would be helpful for treatment decision making, regardless of the extent of resection.

…….....

**Keywords: Tumor spread through air spaces (STAS), Lung cancer, Adenocarcinoma, Extent of resection, Grading**

***Student number:*** **2017-26693**

# CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF ABBREVIATION

ADC: adenocarcinoma

AJCC: American joint committee on cancer

EMT: epithelial to mesenchymal transition

HR: hazard ratio

L inv: lymphatic invasion

LCNEC: large cell neuroendocrine carcinoma

LCSS: lung cancer specific survival

MP: micropapillary

N inv: perineural invasion

NEC: neuroendocrine carcinoma

NET: neuroendocrine tumor

OS: overall survival

RFS: recurrence free survival

RM: resection margin

SCLC: small cell lung carcinoma

SqCC: squamous cell carcinoma

STAS: tumor spread through air spaces

V inv: vascular invasion

VATS: video-assisted thoracic surgery

# INTRODUCTION

The concept of spread through air spaces (STAS) was introduced for pulmonary adenocarcinomas (ADC) in the 2015 World Health Organization (WHO) 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 (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 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.

The investigator 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. The investigator 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 lung cancers.

# MATERIALS AND METHODS

## 1. Patient cohorts

This study was approved by our institutional review board (B-2003-600-105) and the need for informed consent was waived. The investigator 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. According to these criteria, the investigator identified a total of 2000 lung cancer cases. The pathologic stage was reclassified according to the 8$^{th}$ edition of the *American joint committee on cancer (AJCC) 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).

## 2. Pathologic examination of Resected Lung cancer specimens

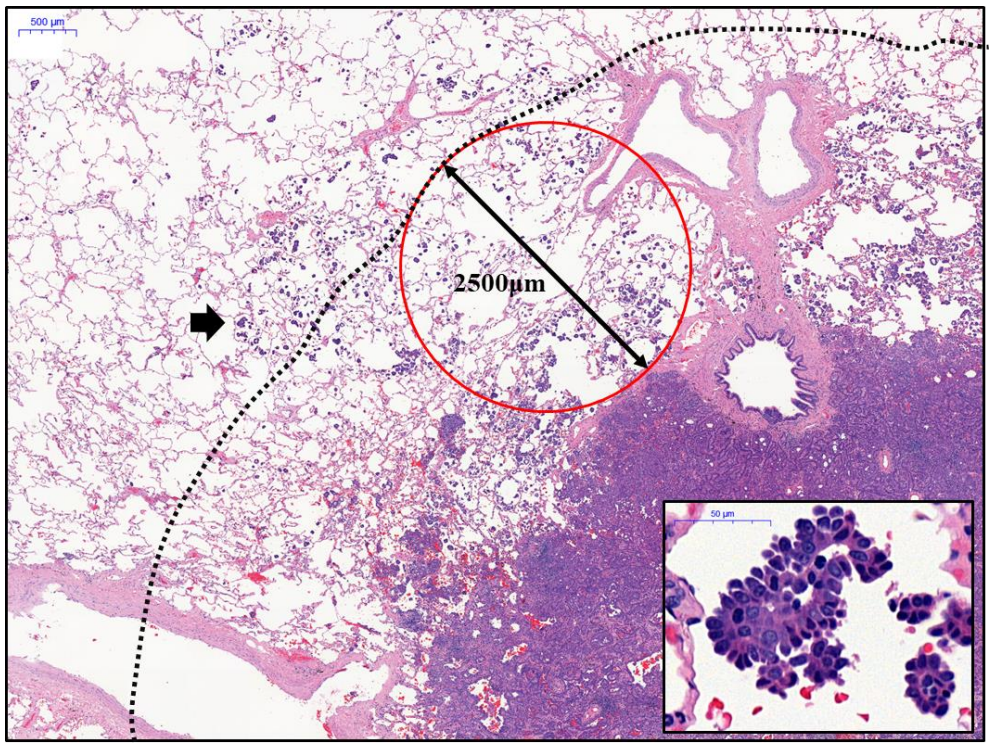
In our institution, since 2004, all resected lung cancer specimens have been delivered to the pathology ward as quickly as possible to reduce a cold ischemia time. After gentle injection of diluted OCT media for frozen section or neutral buffered 10% formalin through the pleural surface or lobar bronchus using a syringe, the specimen was fixed for about 24 hours. After fixation, the specimen was serially cut in 5-mm thick sections (20-22). The investigator 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 non-neoplastic lung parenchyma was completely submitted with mapping, and all the sampled tissue blocks were annotated on the photographs.

## 3. Definition of STAS (Aerogenous spread) and Grading system

The investigator 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 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 x10 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 2500μm away from the edge of tumor (Figure 1). Of note, the investigator 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.

**Figure 1. Definition of extent of STAS grading in histologic examination**




Definition of STAS grading; when tumor clusters existed within one field of x10 objective lens (2500μm 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 (x20 magnification). This case was STAS II in adenocarcinoma (black arrow; x400 magnification).

## 4. 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).

# RESULTS

## 1. Clinicopathologic characteristics and STAS

The clinicopathologic characteristics of patients are shown in Table 1. Histologically, 1544 patients (77.2%) were diagnosed with ADC, 325 patients (16.3%) with SqCC, 41 patients (2.1%) with neuroendocrine carcinoma (NEC) (32 patients (1.6%) with small cell lung carcinoma (SCLC) and nine patients (0.5%) with large cell neuroendocrine carcinoma (LCNEC)), and 16 patients (0.8%) with carcinoid tumor (nine patients (0.5%) with atypical carcinoid and seven patients (0.4%) with typical carcinoid). STAS was observed in 830 cases (41.5%), and 472 cases (23.6%) showed STAS I, whereas 358 cases (17.9%) showed STAS II. Presence of STAS was significantly associated with 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 lobar 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) were not associated with STAS (Table 1).

**Table 1. Association of STAS with clinicopathologic characteristics**

| Characteristics | n (%) | Presence of STAS (n=2000) | | p value | Grade of STAS (n=830) | | p value |
|---|---|---|---|---|---|---|---|
| | | Absent | Present | | STAS I | STAS II | |
| | | n (%) | n (%) | | n (%) | n (%) | |
| **Age** | | | | | | | |
| **median (range)** | 65 (13-93) | | | 0.338 | | | 0.229 |
| ≤65 years | | 602 (59.5) | 409 (40.5) | | 224 (54.8) | 185 (45.2) | |
| >65 years | | 568 (57.4) | 421 (42.6) | | 248 (58.9) | 173 (41.1) | |
| **Sex** | | | | 0.298 | | | 0.442 |
| Male | 1131 (56.6) | 673 (59.5) | 458 (40.5) | | 255 (55.7) | 203 (44.3) | |
| Female | 869 (43.5) | 497 (57.2) | 372 (42.8) | | 217 (58.3) | 155 (41.7) | |
| **Smoking status*** | | | | 0.842 | | | 0.157 |
| Never | 933 (46.7) | 548 (58.7) | 385 (41.3) | | 229 (59.5) | 156 (40.5) | |
| Former or current | 1077 (53.3) | 622 (58.3) | 445 (41.7) | | 243 (54.6) | 202 (45.4) | |
| **Histologic subtypes** | | | | <0.001 | | | <0.001‡ |
| ADC | 1544 (77.2) | 860 (55.7) | 684 (44.3) | | 393 (57.5) | 291 (42.5) | |
| SqCC | 325 (16.3) | 244 (75.1) | 81 (24.9) | | 63 (77.8) | 18 (22.2) | |
| NEC | 41 (2.1) | 6 (14.6) | 35 (85.4) | | 3 (8.6) | 32 (91.4) | |
| Carcinoid tumor | 16 (0.8) | 9 (56.3) | 7 (43.8) | | 5 (71.4) | 2 (28.6) | |
| Others† | 74 (3.7) | 51 (68.9) | 23 (31.1) | | 8 (34.8) | 15 (65.2) | |
| **Pleural invasion** | | | | <0.001 | | | 0.006 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Absent | 1546 (77.3) | 1002 (64.8) | 544 (35.2) | | 328 (60.3) | 216 (39.7) | |
| Present | 454 (22.7) | 168 (37.0) | 286 (63.0) | | 144 (50.3) | 142 (49.7) | |
| **Vascular invasion** | | | | <0.001 | | | <0.001 |
| Absent | 1520 (76.0) | 1013 (66.6) | 507 (33.4) | | 342 (67.5) | 165 (32.5) | |
| Present | 480 (24.0) | 157 (32.7) | 323 (67.3) | | 130 (40.2) | 193 (59.8) | |
| **Lymphatic invasion** | | | | <0.001 | | | <0.001 |
| Absent | 1315 (65.8) | 944 (71.8) | 371 (28.2) | | 252 (67.9) | 119 (32.1) | |
| Present | 685 (34.3) | 226 (33.0) | 459 (67.0) | | 220 (47.9) | 239 (52.1) | |
| **Perineural invasion** | | | | 0.055 | | | 0.968 |
| Absent | 1895 (94.8) | 1118 (59.0) | 777 (41.0) | | 442 (56.9) | 335 (43.1) | |
| Present | 105 (5.3) | 52 (49.5) | 53 (50.5) | | 30 (56.6) | 23 (43.4) | |
| **Necrosis** | | | | <0.001 | | | 0.001 |
| Absent | 1326 (66.3) | 838 (63.2) | 488 (36.8) | | 300 (61.5) | 188 (38.5) | |
| Present | 674 (33.7) | 332 (49.3) | 342 (50.7) | | 172 (50.3) | 170 (49.7) | |
| **Pathologic stage (AJCC 8th)** | | | | <0.001 | | | <0.001 |
| I | 1345 (67.3) | 910 (67.7) | 435 (32.3) | | 287 (66.0) | 148 (34.0) | |
| IA1 | 303 (15.2) | 278 (91.7) | 25 (8.3) | | 19 (76.0) | 6 (24.0) | |
| IA2 | 446 (22.3) | 310 (69.5) | 136 (30.5) | | 92 (67.6) | 44 (32.4) | |
| IA3 | 298 (14.9) | 174 (58.4) | 124 (41.6) | | 84 (67.7) | 40 (32.3) | |
| IB | 298 (14.9) | 148 (49.7) | 150 (50.3) | | 92 (61.3) | 58 (38.7) | |
| II | 337 (16.9) | 146 (43.3) | 191 (56.7) | | 106 (55.5) | 85 (44.5) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| IIA | 76 (3.8) | 34 (44.7) | 42 (55.3) | | 28 (66.7) | 14 (33.3) |
| IIB | 261 (13.1) | 112 (42.9) | 149 (57.1) | | 78 (52.3) | 71 (47.7) |
| III | 258 (12.9) | 97 (37.6) | 161 (62.4) | | 63 (38.9) | 99 (61.1) |
| IIIA | 212 (10.6) | 81 (38.2) | 131 (61.8) | | 53 (40.5) | 78 (59.5) |
| IIIB | 46 (2.3) | 15 (32.6) | 31 (67.4) | | 10 (32.3) | 21 (67.7) |
| IIIC | 0 (0.0) | 0 (0.0) | 0 (0.0) | | 0 (0.0) | 0 (0.0) |
| IV | 60 (3.0) | 18 (30.0) | 42 (70.0) | | 16 (38.1) | 26 (61.9) |
| IVA | 57 (2.9) | 15 (26.3) | 42 (73.7) | | 16 (38.1) | 26 (61.9) |
| IVB | 3 (0.2) | 3 (100.0) | 0 (0.0) | | 0 (0.0) | 0 (0.0) |
| **Extent of resection** | | | | <0.001 | | 0.053 |
| Sublobar resection | 285 (14.3) | 219 (76.8) | 66 (23.2) | | 45 (68.2) | 21 (31.8) |
| Wedge resection | 157 (7.9) | 120 (76.4) | 37 (23.6) | | 25 (67.6) | 12 (32.4) |
| Segmentectomy | 128 (6.4) | 99 (77.3) | 29 (22.7) | | 20 (69.0) | 9 (31.0) |
| Lobar resection | 1715 (85.8) | 951 (55.5) | 764 (44.5) | | 427 (55.9) | 337 (44.1) |
| Lobectomy | 1650 (82.5) | 906 (54.9) | 744 (45.1) | | 418 (56.2) | 326 (43.8) |
| Bilobectomy | 33 (1.7) | 22 (66.7) | 11 (33.3) | | 4 (36.4) | 7 (63.6) |
| Pneumonectomy | 32 (1.6) | 23 (71.9) | 9 (28.1) | | 5 (55.6) | 4 (44.4) |
| **Surgical approach** | | | | 0.850 | | 0.560 |
| VATS | 1804 (90.2) | 1054 (58.4) | 750 (41.6) | | 429 (57.2) | 321 (42.8) |
| Open | 196 (9.8) | 116 (59.2) | 80 (40.8) | | 43 (53.8) | 37 (46.3) |
| Thoracotomy | 134 (6.7) | 77 (57.5) | 57 (42.5) | | 31 (54.4) | 26 (45.6) |

| | | | | | |
|---|---|---|---|---|---|
| Conversion to open | 57 (2.9) | 36 (63.2) | 21 (36.8) | 10 (47.6) | 11 (52.4) |
| Sternotomy | 5 (0.3) | 3 (60.0) | 2 (40.0) | 2 (100.0) | 0 (0.0) |

*Smoking status was defined as follows: never smoker (< 100 cigarettes per lifetime); former 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)

†Others included adenosquamous carcinoma, sarcomatoid carcinoma, salivary gland type carcinoma and lymphoepithelioma-like carcinoma

‡p-value was obtained by Fisher's exact test.

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).

## Table 2. Association of STAS with clinicopathologic characteristics in ADC

| Characteristics | n (%) | Presence of STAS (n=1544) | | | Grade of STAS (n=684) | | |
|---|---|---|---|---|---|---|---|
| | | Absent | Present | p value | Gr I | Gr II | p value |
| | | 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*** | | | | 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†** | | | | 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¶ |
| 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‡ | 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) | |
| **Pathologic N stage (AJCC 8th)#** | | | | <0.001¶ | | | <0.001¶ |
| 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 |
| Sublobar 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) |
| Lobar 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) |

*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)

† EGFR mutation status was evaluated for 1219 patients.

‡ Others included mucinous, colloid and enteric adenocarcinomas.

#Pathologic N staging was available in 1418 patients.

¶ p-value was obtained by Fisher's exact test.

In SqCC, STAS was observed in 81 cases (24.6%), 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 (Table 3).

In NEC, STAS was observed in 35 cases (85.4%), and only three cases (7.3%) showed STAS I, whereas 32 cases (78.0%) showed STAS II. In carcinoid tumor, STAS was observed in seven cases (43.8%), and five cases (31.3%) showed STAS I, whereas two cases showed STAS II. STAS was observed frequently in NEC, especially in SCLC (93.8%) (Figure 2). Of clinicopathologic features, lymphatic invasion and vascular invasion were significantly associated with STAS in NEC, while vascular invasion was the only significant factor associated with STAS in carcinoid tumor (Table 4).

## Table 3. Association of STAS with clinicopathologic characteristics in SqCC

| Characteristics | n (%) | Presence of STAS (n=325) | | p value | Grade of STAS (n=81) | | p value |
|---|---|---|---|---|---|---|---|
| | | Absent | Present | | Gr I | Gr II | |
| | | n (%) | n (%) | | n (%) | n (%) | |
| **Age** | | | | | | | |
| **median (range)** | 70 (28-90) | | | 0.429 | | | 1.000 |
| ≤65 years | 97 (29.8) | 70 (72.2) | 27 (27.8) | | 21 (77.8) | 6 (22.2) | |
| >65 years | 228 (70.2) | 174 (76.3) | 54 (23.7) | | 42 (77.8) | 12 (22.2) | |
| **Sex** | | | | 0.476† | | | 0.212† |
| Male | 314 (96.6) | 237 (75.5) | 77 (24.5) | | 61 (79.2) | 16 (20.8) | |
| Female | 11 (3.4) | 7 (63.6) | 4 (36.4) | | 2 (50.0) | 2 (50.0) | |
| **Smoking status*** | | | | 0.502† | | | 0.212† |
| Never | 12 (3.7) | 8 (66.7) | 4 (33.3) | | 2 (50.0) | 2 (50.0) | |
| Former or current | 313 (96.3) | 236 (75.4) | 77 (24.6) | | 61 (79.2) | 16 (20.8) | |
| **Pleural invasion** | | | | 0.181 | | | 0.216† |
| Absent | 265 (81.5) | 203 (76.6) | 62 (23.4) | | 46 (74.2) | 16 (25.8) | |
| Present | 60 (18.5) | 41 (68.3) | 19 (31.7) | | 17 (89.5) | 2 (10.5) | |
| **Vascular invasion** | | | | 0.019 | | | 0.952 |
| Absent | 230 (70.8) | 181 (78.7) | 49 (21.3) | | 38 (77.6) | 11 (22.4) | |
| Present | 95 (29.2) | 63 (66.3) | 32 (33.7) | | 25 (78.1) | 7 (21.9) | |
| **Lymphatic invasion** | | | | 0.001 | | | 0.635 |
| Absent | 209 (64.3) | 169 (80.9) | 40 (19.1) | | 32 (80.0) | 8 (20.0) | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Present | 116 (35.7) | 75 (64.7) | 41 (35.3) | | 31 (75.6) | 10 (24.4) | |
| **Perineural invasion** | | | | 0.839 | | | 0.441† |
| Absent | 283 (87.1) | 213 (75.3) | 70 (24.7) | | 53 (75.7) | 17 (24.3) | |
| Present | 42 (12.9) | 31 (73.8) | 11 (26.2) | | 10 (90.1) | 1 (9.1) | |
| **Necrosis** | | | | 0.905 | | | 0.367† |
| Absent | 31 (9.5) | 23 (74.2) | 8 (25.8) | | 5 (62.5) | 3 (37.5) | |
| Present | 294 (90.5) | 221 (75.2) | 73 (24.8) | | 58 (79.5) | 15 (20.5) | |
| **Pathologic T stage (AJCC 8th)** | | | | 0.461 | | | 0.260† |
| T1 | 114 (35.1) | 89 (78.1) | 25 (21.9) | | 17 (68.0) | 8 (32.0) | |
| T1a | 5 (1.5) | 4 (80.0) | 1 (20.0) | | 1 (100.0) | 0 (0.0) | |
| T1b | 41 (12.6) | 37 (90.2) | 4 (9.8) | | 2 (50.0) | 2 (50.0) | |
| T1c | 68 (20.9) | 48 (70.6) | 20 (29.4) | | 14 (70.0) | 6 (30.0) | |
| T2 | 122 (37.5) | 89 (73.0) | 33 (27.0) | | 27 (81.8) | 6 (18.2) | |
| T2a | 80 (24.6) | 62 (77.5) | 18 (22.5) | | 15 (83.3) | 3 (16.7) | |
| T2b | 42 (12.9) | 27 (64.3) | 15 (35.7) | | 12 (80.0) | 3 (20.0) | |
| T3 | 60 (18.5) | 42 (70.0) | 18 (30.0) | | 16 (88.9) | 2 (11.1) | |
| T4 | 29 (8.9) | 24 (82.8) | 5 (17.2) | | 3 (60.0) | 2 (40.0) | |
| **Pathologic N stage (AJCC 8th)‡** | | | | 0.636 | | | 0.926† |
| N0 | 209 (65.9) | 159 (76.1) | 50 (23.9) | | 39 (78.0) | 11 (22.0) | |
| N1 | 73 (23.0) | 55 (75.3) | 18 (24.7) | | 14 (77.8) | 4 (22.2) | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| N2 | 35 (11.0) | 24 (68.6) | 11 (31.4) | | 8 (72.7) | 3 (27.3) | |
| **Pathologic M stage (AJCC 8th)** | | | | 0.685† | | | 0.222† |
| M0 | 317 (97.5) | 237 (74.8) | 80 (25.2) | | 63 (78.8) | 17 (21.3) | |
| M1 | 8 (2.5) | 7 (87.5) | 1 (12.5) | | 0 (0.0) | 1 (100.0) | |
| M1a | 4 (1.2) | 3 (75.0) | 1 (25.0) | | 0 (0.0) | 1 (100.0) | |
| M1b | 3 (0.9) | 3 (100.0) | 0 (0.0) | | . | . | |
| M1c | 1 (0.3) | 1 (100.0) | 0 (0.0) | | . | . | |
| **Pathologic stage (AJCC 8th)** | | | | 0.200† | | | 0.231† |
| I | 139 (42.8) | 111 (79.9) | 28 (20.1) | | 21 (75.0) | 7 (25.0) | |
| IA1 | 5 (1.5) | 4 (80.0) | 1 (20.0) | | 1 (100.0) | 0 (0.0) | |
| IA2 | 35 (10.8) | 32 (91.4) | 3 (8.6) | | 2 (66.7) | 1 (33.3) | |
| IA3 | 50 (15.4) | 37 (74.0) | 13 (26.0) | | 9 (69.2) | 4 (30.8) | |
| IB | 49 (15.1) | 38 (77.6) | 11 (22.4) | | 9 (81.8) | 2 (18.2) | |
| II | 105 (32.3) | 72 (68.6) | 33 (31.4) | | 28 (84.8) | 5 (15.2) | |
| IIA | 23 (7.1) | 13 (56.5) | 10 (43.5) | | 8 (80.0) | 2 (20.0) | |
| IIB | 82 (25.2) | 59 (72.0) | 23 (28.0) | | 20 (87.0) | 3 (13.0) | |
| III | 73 (22.5) | 54 (74.0) | 19 (26.0) | | 14 (73.7) | 5 (26.3) | |
| IIIA | 61 (18.8) | 45 (73.8) | 16 (26.2) | | 11 (68.8) | 5 (31.3) | |
| IIIB | 12 (3.7) | 9 (75.0) | 3 (25.0) | | 3 (100.0) | 0 (0.0) | |
| IV | 8 (2.5) | 7 (87.5) | 1 (12.5) | | 0 (0.0) | 1 (100.0) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| IVA | 7 (2.2) | 6 (85.7) | 1 (14.3) | | 0 (0.0) | 1 (100.0) | |
| IVB | 1 (0.3) | 1 (100.0) | 0 (0.0) | | . | . | |
| **Extent of resection** | | | | 0.584† | | | 0.329† |
| Sublobar resection | 19 (5.8) | 13 (68.4) | 6 (31.6) | | 6 (100.0) | 0 (0.0) | |
| Wedge resection | 12 (3.7) | 9 (75.0) | 3 (25.0) | | 3 (100.0) | 0 (0.0) | |
| Segmentectomy | 7 (2.2) | 4 (57.1) | 3 (42.9) | | 3 (100.0) | 0 (0.0) | |
| Lobar resection | 306 (94.2) | 231 (75.5) | 75 (24.5) | | 57 (76.0) | 18 (24.0) | |
| Lobectomy | 264 (81.2) | 195 (73.9) | 69 (26.1) | | 52 (75.4) | 17 (24.6) | |
| Bilobectomy | 20 (6.2) | 16 (80.0) | 4 (20.0) | | 3 (75.0) | 1 (25.0) | |
| Pneumonectomy | 22 (6.8) | 20 (90.9) | 2 (9.1) | | 2 (100.0) | 0 (0.0) | |
| **Surgical approach** | | | | 0.241 | | | 1.000† |
| VATS | 245 (75.4) | 180 (73.5) | 65 (26.5) | | 50 (76.9) | 15 (23.1) | |
| Open | 80 (24.6) | 64 (80.0) | 16 (20.0) | | 13 (81.3) | 3 (18.8) | |
| Thoracotomy | 63 (19.4) | 48 (76.2) | 15 (23.8) | | 12 (80.0) | 3 (20.0) | |
| Conversion to open | 17 (5.2) | 16 (94.1) | 1 (5.9) | | 1 (100.0) | 0 (0.0) | |

*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)

†p-value was obtained by Fisher's exact test.

‡Pathologic N staging was available in 317 patients.

**Figure 2. Typical case of STAS in SCLC (x50 magnification)**

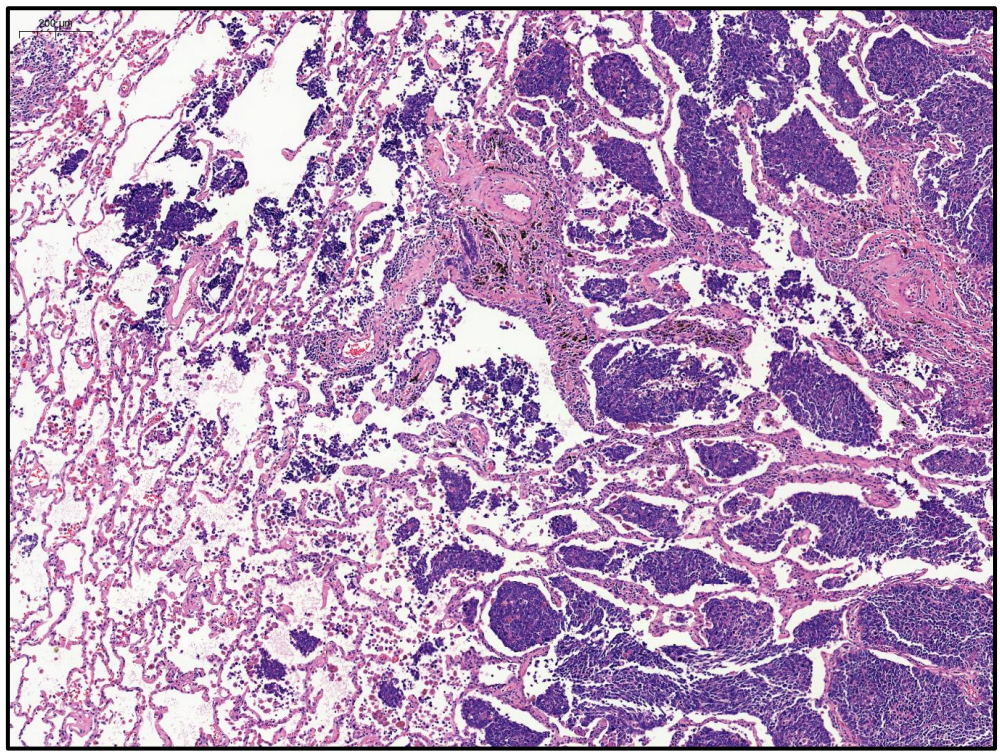


**Table 4. Association of extent of STAS with clinicopathologic characteristics in NETs**

| Characteristics | NEC (n=41) | | | | Carcinoid tumor (n=16) | | | |
|---|---|---|---|---|---|---|---|---|
| | Extent of STAS | | | p value | Extent of STAS | | | p value |
| | Absent | Gr I | Gr II | | Absent | Gr I | Gr II | |
| | n (%) | n (%) | n (%) | | n (%) | n (%) | n (%) | |
| **Age** | | | | 0.086* | | | | 1.000* |
| ≤65 years | 0 (0.0) | 2 (13.3) | 13 (86.7) | | 6 (54.5) | 3 (27.3) | 2 (18.2) | |
| >65 years | 6 (23.1) | 1 (3.8) | 19 (73.1) | | 3 (60.0) | 2 (40.0) | 0 (0.0) | |
| **Sex** | | | | 1.000* | | | | 0.608* |
| Male | 6 (15.8) | 1 (7.9) | 29 (76.3) | | 5 (62.5) | 3 (37.5) | 0 (0.0) | |
| Female | 0 (0.0) | 0 (0.0) | 3 (100.0) | | 4 (50.0) | 2 (25.0) | 2 (25.0) | |
| **Smoking status†** | | | | 0.443* | | | | 1.000* |
| Never | 0 (0.0) | 1 (20.0) | 4 (80.0) | | 6 (54.5) | 3 (27.3) | 2 (18.2) | |
| Former or current | 6 (16.7) | 2 (5.6) | 28 (77.8) | | 3 (60.0) | 2 (40.0) | 0 (0.0) | |
| **Histologic classification‡** | | | | 0.014* | | | | 0.633* |
| SCLC | 2 (6.3) | 2 (6.3) | 28 (87.5) | | | | | |
| LCNEC | 4 (44.4) | 1 (11.1) | 4 (44.4) | | | | | |
| Atypical carcinoid | | | | | 4 (44.4) | 3 (33.3) | 2 (22.2) | |
| Typical carcinoid | | | | | 5 (71.4) | 2 (28.6) | 0 (0.0) | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Pleural invasion** | | | | 0.840* | | | | 0.325* |
| Absent | 5 (17.2) | 2 (6.9) | 22 (75.9) | | 8 (57.1) | 5 (35.7) | 1 (7.1) | |
| Present | 1 (8.3) | 1 (8.3) | 10 (83.3) | | 1 (50.0) | 0 (0.0) | 1 (50.0) | |
| **Vascular invasion** | | | | 0.013* | | | | 0.035* |
| Absent | 5 (33.3) | 2 (13.3) | 8 (53.3) | | 8 (80.0) | 1 (10.0) | 1 (10.0) | |
| Present | 1 (3.8) | 1 (3.8) | 24 (92.3) | | 1 (16.7) | 4 (66.7) | 1 (16.7) | |
| **Lymphatic invasion** | | | | 0.034* | | | | 0.113* |
| Absent | 4 (36.4) | 1 (9.1) | 6 (54.5) | | 8 (66.7) | 2 (16.7) | 2 (16.7) | |
| Present | 2 (6.7) | 2 (6.7) | 26 (86.7) | | 1 (25.0) | 3 (75.0) | 0 (0.0) | |
| **Perineural invasion** | | | | 0.712* | | | | NA |
| Absent | 6 (16.7) | 3 (8.3) | 27 (75.0) | | 9 (56.3) | 5 (31.3) | 2 (12.5) | |
| Present | 0 (0.0) | 0 (0.0) | 5 (100.0) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| **Necrosis** | | | | 0.476* | | | | 0.079* |
| Absent | 0 (0.0) | 0 (0.0) | 8 (100.0) | | 9 (69.2) | 3 (23.1) | 1 (7.7) | |
| Present | 6 (18.2) | 3 (9.1) | 24 (72.7) | | 0 (0.0) | 2 (66.7) | 1 (33.3) | |
| **Pathologic stage (AJCC 8th)** | | | | 0.485* | | | | 0.149* |
| I | 4 (25.0) | 1 (6.3) | 11 (68.8) | | 8 (72.7) | 2 (18.2) | 1 (9.1) | |
| II | 2 (11.8) | 2 (11.8) | 13 (76.5) | | 1 (25.0) | 2 (50.0) | 1 (25.0) | |
| III | 0 (0.0) | 0 (0.0) | 8 (100.0) | | 0 (0.0) | 1 (100.0) | 0 (0.0) | |
| IV | 0 (0.0) | 0 (0.0) | 0 (0.0) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Extent of resection** | | | | 1.000* | | | | 1.000* |
| Sublobar resection | 0 (0.0) | 0 (0.0) | 4 (100.0) | | 3 (50.0) | 2 (33.3) | 1 (16.7) | |
| Lobar resection | 6 (16.2) | 3 (8.1) | 28 (75.7) | | 6 (60.0) | 3 (30.0) | 1 (10.0) | |
| **Surgical approach** | | | | 1.000* | | | | 0.625* |
| VATS | 5 (14.7) | 3 (8.8) | 26 (76.5) | | 7 (50.0) | 5 (35.7) | 2 (14.3) | |
| Open | 1 (14.3) | 0 (0.0) | 6 (85.7) | | 2 (100.0) | 0 (0.0) | 0 (0.0) | |

*p-value was obtained by Fisher's exact test.

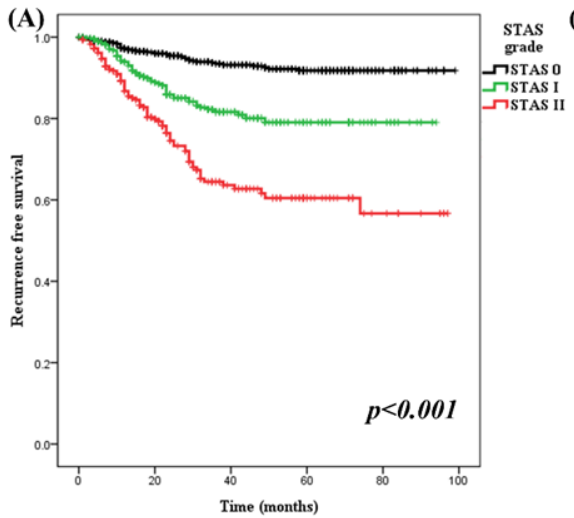
†Smoking status was defined as follows: never smoker (< 100 cigarettes per lifetime); former smoker (100 cigarettes per lifetime and quit > 1 year prior to the diagnosis); current smoker (100 cigarettes per lifetime and smoked at the time of lung cancer diagnosis or quit 1 year prior to the diagnosis)

## 2. Survival analysis

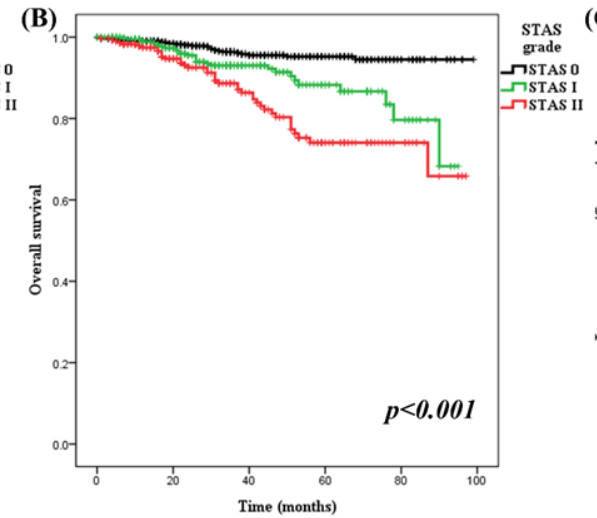
### ADC cohort

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 lung cancer specific survival (LCSS) according to the extent of STAS ($p<0.001$, respectively) (Figure 3). 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.

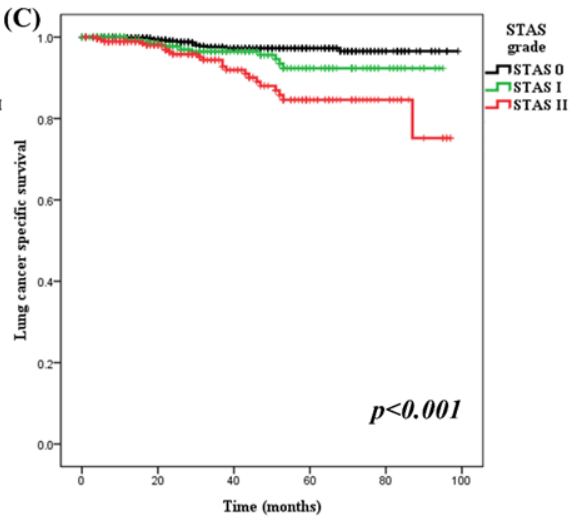
**Figure 3. RFS, OS and LCSS stratified by STAS grade in ADC**




| | 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 |

(A) recurrence free survival according to STAS grade (B) overall survival according to STAS grade (C) lung cancer specific survival according to STAS grade. Hazard ratios obtained by Cox proportional hazards regression modeling.

***Subgroup analysis in Stage IA non-mucinous ADC***

The investigator 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 (5 with lung cancer specific death).

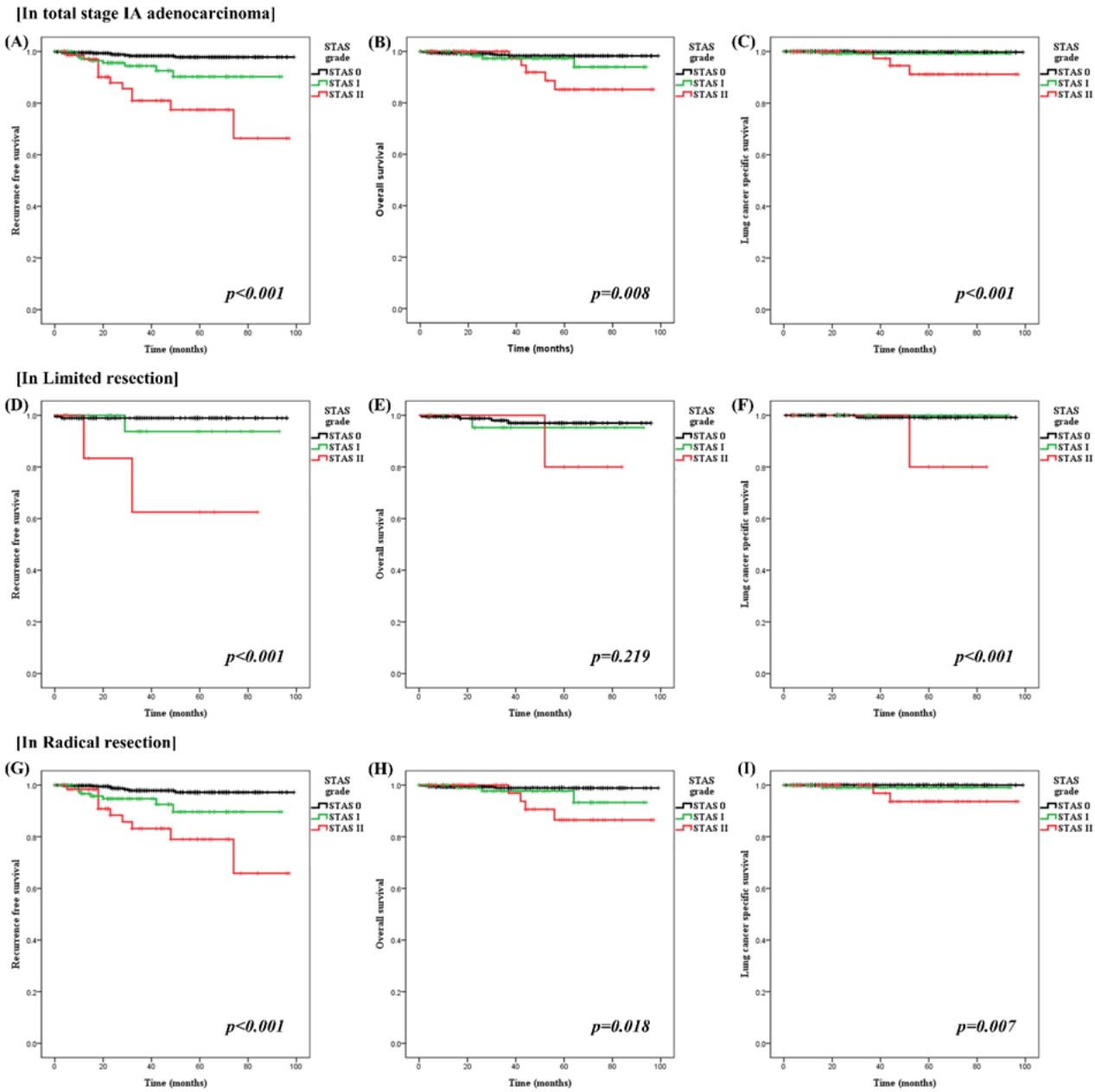
In stage IA non-mucinous ADC, STAS was observed in 237 (27.2%) cases including 164 (18.9%) with STAS I and 73 (8.4%) with STAS II. In this group, 222 (25.5%) patients underwent sublobar resection (including wedge resection and segmentectomy) and 648 (74.5%) patients underwent lobar resection (including lobectomy, bilobectomy and pneumonectomy). In the sublobar resection group, STAS was observed in 33 (14.9%) cases with 25 (11.3%) STAS I and eight (3.6%) STAS II. In the lobar 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 ($p<0.001$, $p=0.008$ and $p<0.001$, respectively). When stratified by the extent of resection, there were significant differences in RFS and LCSS in sublobar resection group, but not in OS ($p<0.001$, $p<0.001$ and $p=0.219$, respectively). In lobar resection group, there were significant differences in RFS, OS and LCSS according to the STAS grade ($p<0.001$, $p=0.018$ and $p=0.007$, respectively) (Figure 4). 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 sublobar resection and $p=0.023$ for lobar resection) (Table 5). 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 6). 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 (Table 7).

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), the investigator 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 (Figure 5). 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) (Table 8).

**Figure 4. RFS, OS and LCSS stratified by STAS grade in stage IA non-mucinous ADC 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) sublobar 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) lobar 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%)

**Table 5. Multivariate analysis for RFS in stage IA non-mucinous ADC according to the extent of resection**

| Variables | | Sublobar resection (n=222) | | | | Lobar 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** | >65years vs. ≤65years | 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*** | < Tumor max diameter vs. ≥ Tumor max diameter | 1.553 (0.259-9.298) | 0.630 | | | NA | | | |
| **Pathologic stage** | IA3 vs. IA1 & 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†** | 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 |

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

†Presence of STAS was analyzed separately from the grade of STAS.

**Table 6. Multivariate analysis for RFS, OS and LCSS in stage IA non-mucinous ADC (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** | >65years vs. ≤65years | 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 |
| **Pathologic stage** | IA3 vs. IA1 & 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*** | 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 |
| **STAS grade** | STAS II vs. STAS I vs. STAS 0 | | <0.001 | | <0.001 | | 0.018 | | 0.738 | | 0.018 |

| | | | |
|---|---|---|---|
| **Vascular invasion** | Present vs. Absent | 3.530 (0.520-23.957) | 0.197 |
| **Lymphatic invasion** | Present vs. Absent | | |
| **Necrosis** | Present vs. Absent | | |
| **Pathologic stage** | IA3 vs. IA1 & IA2 | | |
| **STAS*** | Present vs. Absent | 11.516 (1.286-103.101) | 0.029 |
| **STAS grade** | STAS II vs. STAS I vs. STAS 0 | | 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 |

*Presence of STAS was analyzed separately from the grade of STAS.

**Table 7. Multivariate analysis for RFS in stage IA non-mucinous ADC using additional MP cut-offs (n=870)**

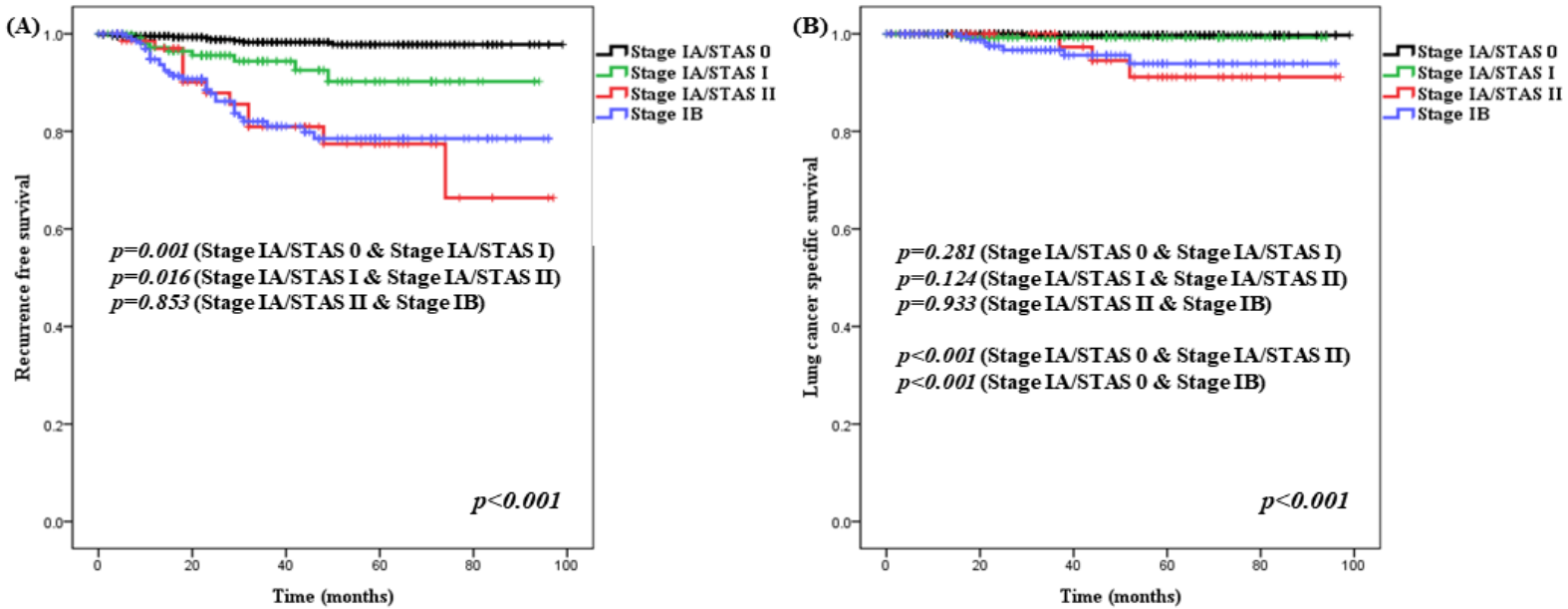
| Variables | | RFS | | | |
|---|---|---|---|---|---|
| | | Univariate | | Multivariate | |
| | | HR (95% CI) | p value | HR (95% CI) | p value |
| **Age** | >65years vs. ≤65years | 1.988 (0.963-4.102) | 0.063 | | |
| **Sex** | Male vs. Female | 1.172 (0.573-2.397) | 0.665 | | |
| **Smoking status** | Ever vs. Never | 1.951 (0.948-4.018) | 0.07 | | |
| **MP pattern*** | ≥5% vs. <5% | 1.962 (0.359-10.726) | 0.437 | 0.519 (0.065-4.120) | 0.535 |
| | ≥10% vs. <10% | 1.491 (0.174-12.785) | 0.716 | 0.253 (0.020-3.236) | 0.291 |
| | ≥20% vs. <20% | 2.579 (0.300-22.179) | 0.388 | 0.488 (0.035-6.874) | 0.595 |
| **Vascular invasion** | Present vs. Absent | 5.018 (2.291-10.990) | <0.001 | 1.411 (0.144-13.803) | 0.767 |
| **Lymphatic invasion** | Present vs. Absent | 4.748 (2.301-9.794) | <0.001 | 3.017 (0.561-16.234) | 0.198 |
| **Necrosis** | Present vs. Absent | 4.163 (1.701-10.188) | 0.002 | 1.283 (0.098-16.744) | 0.849 |

| | | | | | |
|---|---|---|---|---|---|
| **Pathologic stage** | IA3 vs. IA1 & IA2 | 3.564 (1.740-7.303) | 0.001 | 6.290 (1.152-34.347) | 0.034 |
| **STAS†** | Present vs. Absent | 6.756 (3.091-14.764) | <0.001 | 1.339 (0.247-7.264) | 0.735 |
| **STAS grade** | STAS II vs. STAS I vs. STAS 0 | | <0.001 | | 0.426 |
| | STAS I vs. STAS 0 | 4.266 (1.692-10.760) | 0.002 | 0.676 (0.067-6.836) | 0.74 |
| | STAS II vs. STAS 0 | 11.973 (5.042-28.433) | <0.001 | 2.866 (0.406-20.223) | 0.291 |

*Percentage of MP pattern was available in 504 cases.

†Presence of STAS was analyzed separately from the grade of STAS.

**Figure 5. RFS and LCSS according to STAS grade in stage I non-mucinous ADC (Stage IA with STAS grade vs. Stage IB)**




(A) recurrence free survival according to stage IA without STAS (STAS 0), stage IA with STAS I, stage IA with STAS II and stage IB (B) lung cancer specific survival according to stage IA without STAS (STAS 0), stage IA with STAS I, stage IA with STAS II and stage IB

**Table 8. Multivariate analysis for RFS and LCSS in stage I non-mucinous ADC (stage IA with STAS status and stage IB) (n=1089)**

| Variables | | RFS | | | | LCSS | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 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** | >65years vs. ≤65years | 1.366 (0.826-2.258) | 0.224 | | | 2.134 (0.675-6.747) | 0.197 | | |
| **Sex** | Male vs. Female | 1.489 (0.899-2.469) | 0.122 | | | 2.337 (0.704-7.762) | 0.166 | | |
| **Smoking status** | Ever vs. Never | 2.296 (1.374-3.839) | 0.002 | 2.210 (0.833-5.863) | 0.003 | 7.336 (1.607-33.482) | 0.01 | 7.580 (1.653-34.749) | 0.009 |
| **MP pattern** | Present vs. Absent | 5.611 (3.088-10.195) | <0.001 | 2.207 (1.133-4.298) | 0.02 | 18.125 (2.339-140.475) | 0.006 | 12.418 (1.571-98.176) | 0.017 |
| **MP predominant** | MP predominant vs. Others | 2.646 (0.364-19.206) | 0.336 | | | NA | | | |
| **Pleural invasion** | Present vs. Absent | 4.870 (2.899-8.180) | <0.001 | 1.944 (0.860-4.397) | 0.11 | 8.017 (2.578-24.931) | <0.001 | 5.484 (1.717-17.509) | 0.004 |
| **Vascular invasion** | Present vs. Absent | 6.305 (3.808-10.442) | <0.001 | 2.447 (1.400-4.276) | 0.002 | 3.322 (1.000-11.037) | 0.05 | 0.853 (0.241-3.026) | 0.806 |
| **Lymphatic invasion** | Present vs. Absent | 4.509 (2.726-7.457) | <0.001 | 1.811 (1.048-3.128) | 0.033 | 4.217 (1.359-13.091) | 0.013 | 1.876 (0.570-6.171) | 0.301 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Perineural invasion** | Present vs. Absent | 6.145 (2.226-16.965) | <0.001 | 2.392 (0.822-6.960) | 0.11 | 7.736 (0.997-60.023) | 0.05 | 5.543 (0.670-45.875) | 0.112 |
| **Necrosis** | Present vs. Absent | 4.133 (2.383-7.167) | <0.001 | 1.273 (0.706-2.296) | 0.422 | 6.575 (2.085-20.733) | 0.001 | 2.234 (0.686-7.277) | 0.182 |
| **Pathologic stage** | IA3 & IB vs. IA1 & IA2 | 5.403 (2.975-9.815) | <0.001 | 1.723 (0.822-3.614) | 0.150 | 7.865 (1.723-35.906) | 0.008 | 1.772 (0.308-10.195) | 0.522 |
| **Stage/STAS group*** | Group 3 vs. Group 2 vs. Group 1 vs. Group 0 | | <0.001 | | 0.031 | | 0.014 | | 0.422 |
| | Group 1 vs. Group 0 | 4.238 (1.681-10.680) | 0.002 | 2.307 (0.869-6.123) | 0.093 | 4.307 (0.269-68.925) | 0.302 | 1.334 (0.077-23.034) | 0.843 |
| | Group 2 vs. Group 0 | 12.029 (5.067-28.557) | <0.001 | 4.358 (1.645-11.544) | 0.003 | 23.767 (2.472-228.516) | 0.006 | 4.286 (0.402-45.736) | 0.228 |
| | Group 3 vs. Group 0 | 11.255 (5.356-23.651) | <0.001 | 2.884 (1.018-8.169) | 0.046 | 22.169 (2.725-180.354) | 0.004 | 0.696 (0.027-17.760) | 0.826 |

*Stage/STAS group was categorized as follows; Group 0: Stage IA without STAS (STAS 0), Group 1: Stage IA with STAS I, Group 2: Stage IA with STAS II, Group 3: Stage IB

## 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 patients 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).

## NET cohort

At the time of analysis, in NEC, the median RFS was 17.0 months and the median OS was 22.0 months. During this time, 11 patients (26.8%) experienced recurrence (three with locoregional recurrence; seven with distant recurrence; one with both) and 10 patients (24.4%) deceased (six patients with lung cancer specific death).

Those with STAS II tended to show worse RFS and OS, but were not statistically significant (RFS: STAS I vs. STAS II; p=0.114, STAS 0 vs. STAS II; p=0.078, OS: STAS I vs. STAS II; p=0.127, STAS 0 vs. STAS II; p=0.151).

For carcinoid tumors, only the one patient with atypical carcinoid tumor experienced ipsilateral lung recurrence and other patient with typical carcinoid tumor deceased due to other medical condition.

# DISCUSSION

In this study, the investigator 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 x10 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 more than 5 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 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). Although there were only limited studies on NETs, the increasing prevalence of STAS from typical carcinoid through atypical carcinoid to LCNEC and SCLC was consistent with other studies (6, 7). While the investigator 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 and NETs. 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. As described previously, high frequency of STAS II in NEC, especially in SCLC, might be partly explained by EMT phenomenon. Since only a limited number of groups studied on STAS in SqCC (3-5) and NETs (6-8), 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 sublobar 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 sublobar resection but also in the lobar 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 (Tables 9 and 10). Both locoregional recurrence and distant recurrence were associated with the presence of STAS. Not only in sublobar resection, but also in lobar resection, cases with any recurrence showed a higher incidence of STAS compared to those without recurrence ($p=0.024$ and $p<0.001$, respectively). Furthermore, the association with recurrence was more significant with STAS II than STAS I in both the sublobar and lobar resection groups ($p=0.008$ and $p=0.312$ in the sublobar resection group and $<0.001$ and 0.012 in the lobar 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 sublobar resection group but also in the lobar resection group. The clinical significance of STAS could be extended from a R factor for sublobar resection to a feature representing aggressive biology in ADC in general independent of the surgical extent.

**Table 9. Recurrence pattern according to STAS grade and resection margin status of sublobar resection group in ADC (n=230)***

| | STAS 0 (n=183) | | | STAS I (n=33) | | | STAT II (n=14) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Recurrence | | | Recurrence | | | Recurrence | | |
| | - | Loco-regional† | Distant | - | Loco-regional† | Distant | - | Loco-regional† | Distant |
| **RM < tumor size‡** | 82 | 0 | 1 | 21 | 2 | 2 | 7 | 2 | 1 |
| **RM ≥ tumor size‡** | 99 | 0 | 1 | 8 | 0 | 0 | 3 | 1 | 0 |
| **Total** | 181 | 0 | 2 | 29 | 2 | 2 | 10 | 3 | 1 |

*Margin distance was available in 230 out of 252 adenocarcinoma cases.

†All cases only developed ipsilateral lung recurrence.

‡RM: At our institution, if the length of margin is thought to be less than the diameter of the tumor in a sublobar resection and the patient condition allows, thoracic surgeons typically remove additional lung parenchyma or proceed with completion lobectomy. Therefore, the investigator classified the margin distance into two groups: ≥ vs. < the maximal diameter of tumor.

**Table 10. Recurrence pattern according to the extent of resection in stage IA non-mucinous ADC (n=870)**

| Extent of resection | n (%) | Recurrence | n (%) | STAS (+) n (%) | STAS I n (%) | STAS II n (%) | MP (+) n (%) | V inv (+) n (%) | L inv (+) n (%) | N inv (+) n (%) | RM<tumor size‡ n (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sublobar resection | 222 (25.5) | **Recurrence (+)** | **5 (2.3)** | 3 (60.0) | 1 (20.0) | 2 (40.0) | 2 (40.0) | 1 (20.0) | 1 (20.0) | 0 (0.0) | 3 (60.0) |
| | | Locoregional* | 2 (0.9) | 2 (100.0) | 0 (0.0) | 2 (100.0) | 2 (100.0) | 1 (50.0) | 1 (50.0) | 0 (0.0) | 1 (50.0) |
| | | Distant† | 3 (1.4) | 1 (33.3) | 1 (33.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (66.7) |
| | | **Recurrence (-)** | 217 (97.7) | 30 (13.8) | 24 (11.1) | 6 (2.8) | 45 (20.7) | 5 (2.3) | 11 (5.1) | 0 (0.0) | 95 (48.0)‡ |
| Lobar resection | 648 (74.5) | **Recurrence (+)** | 25 (3.9) | 18 (72.0) | 8 (32.0) | 10 (40.0) | 20 (80.0) | 8 (32.0) | 12 (48.0) | 0 (0.0) | |
| | | Locoregional* | 10 (1.5) | 7 (70.0) | 2 (20.0) | 5 (50.0) | 9 (90.0) | 2 (20.0) | 3 (30.0) | 0 (0.0) | |
| | | ipsilateral lung | 7 (1.1) | 4 (57.1) | 1 (14.3) | 3 (42.9) | 6 (85.7) | 2 (28.6) | 2 (28.6) | 0 (0.0) | |
| | | ipsilateral LN | 3 (0.5) | 3 (100.0) | 1 (33.3) | 2 (66.7) | 3 (100.0) | 0 (0.0) | 1 (33.3) | 0 (0.0) | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Distant† | 15 (2.3) | 11 (73.3) | 6 (40.0) | 5 (33.3) | 11 (73.3) | 6 (40.0) | 9 (60.0) | 0 (0.0) |
| **Recur (-)** | 623 (96.1) | 186 (29.9) | 131 (21.0) | 55 (8.8) | 229 (36.8) | 44 (7.1) | 106 (17.0) | 5 (0.8) |

*All cases only developed ipsilateral lung recurrence.

†Distant recurrence +/- locoregional recurrence.

‡RM<tumor size: the distance of resection margin from the tumor less than the maximal diameter of tumor. Of note, the margin status was evaluable in all five patients with recurrence and 198 patients without recurrence.

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, the investigator only evaluated distance other than amount or volume of STAS. Uruga et al. showed that high STAS ($\geq$ 5 single cells or clusters of STAS by using a 20x objective and a 10x 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 the investigator 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. Finally, the investigator included small numbers of histologic subtypes other than ADC. Thus, additional studies on SqCC and NETs are needed to confirm the clinical significance of STAS in those tumors.

# CONCLUSIONS

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 sublobar resections but also in lobar resections, including the STAS status and grade in the pathology report would be helpful for treatment decision making, regardless of the extent of resection.

# REFERENCES

1. Warth A, Muley T, Kossakowski CA, Goeppert B, Schirmacher P, Dienemann H, et al. Prognostic impact of intra-alveolar tumor spread in pulmonary adenocarcinoma. Am J Surg Pathol. 2015; 39: 793-801.

2. Kadota K, Nitadori J, Sima CS, Ujiie H, Rizk NP, Jones DR, et al. 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. 2015; 10: 806-14.

3. Kadota K, Kushida Y, Katsuki N, Ishikawa R, Ibuki E, Motoyama M, et al. 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. 2017; 41: 1077-86.

4. Lu S, Tan KS, Kadota K, Eguchi T, Bains S, Rekhtman N, et al. Spread through air spaces (STAS) is an independent predictor of recurrence and lung cancer-specific death in squamous cell carcinoma. J Thorac Oncol. 2017; 12: 223-34.

5. Yanagawa N, Shiono S, Endo M, Ogata SY. 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. 2018; 120: 14-21.

6. Aly RG, Rekhtman N, Li X, Takahashi Y, Eguchi T, Tan KS, et al. Spread through air spaces (STAS) is prognostic in atypical carcinoid, large cell neuroendocrine carcinoma, and small cell carcinoma of the lung. J Thorac Oncol. 2019; 14: 1583-93.

7. Altinay S, Metovic J, Massa F, Gatti G, Cassoni P, Scagliotti GV, et al. Spread through air spaces (STAS) is a predictor of poor outcome in atypical carcinoids of the lung. Virchows Arch. 2019; 475: 325-34.

8. Toyokawa G, Yamada Y, Tagawa T, Kinoshita F, Kozuma Y, Matsubara T, et al. High frequency of spread through air spaces in resected small cell lung cancer. Anticancer Res. 2018; 38: 1821-5.

9. Chen D, Mao Y, Wen J, She Y, Zhu E, Zhu F, et al. Tumor spread through air spaces in non-small cell lung cancer: A systematic review and meta-Analysis. Ann Thorac Surg. 2019; 108: 945-54.

10. Wang S, Hao J, Qian C, Wang H. Tumor spread through air spaces ss a survival predictor in non-small-cell lung cancer. Clin Lung Cancer. 2019; 20: e584-e91.

11. Liu H, Yin Q, Yang G, Qie P. Prognostic impact of tumor spread through air spaces in non-small cell lung cancers: a meta-analysis including 3564 patients. Pathol Oncol Res. 2019; 25: 1303-10.

12. Blaauwgeers H, Flieder D, Warth A, Harms A, Monkhorst K, Witte B, et al. 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. 2017; 41: 1226-30.

13. Blaauwgeers H, Russell PA, Jones KD, Radonic T, Thunnissen E. Pulmonary loose tumor tissue fragments and spread through air spaces (STAS): Invasive pattern or artifact? A critical review. Lung Cancer. 2018; 123: 107-11.

14. Shiono S, Endo M, Suzuki K, Yarimizu K, Hayasaka K, Yanagawa N. Spread through air spaces is a prognostic factor in sublobar resection of non-small cell lung cancer. Ann Thorac Surg. 2018; 106: 354-60.

15. Masai K, Sakurai H, Sukeda A, Suzuki S, Asakura K, Nakagawa K, et al. Prognostic impact of margin distance and tumor spread through air spaces in limited resection for primary lung cancer. J Thorac Oncol. 2017; 12: 1788-97.

16. Eguchi T, Kameda K, Lu S, Bott MJ, Tan KS, Montecalvo J, et al. Lobectomy is associated with better outcomes than sublobar resection in spread through air spaces (STAS)-positive T1 lung adenocarcinoma: A propensity score-matched analysis. J Thorac Oncol. 2019; 14: 87-98.

17. Toyokawa G, Yamada Y, Tagawa T, Kozuma Y, Matsubara T, Haratake N, et al. Significance of spread through air spaces in resected pathological stage I lung adenocarcinoma. Ann Thorac Surg. 2018; 105: 1655-63.

18. Uruga H, Fujii T, Fujimori S, Kohno T, Kishi K. Semiquantitative assessment of tumor spread through air spaces (STAS) in early-stage lung adenocarcinomas. J Thorac Oncol. 2017; 12: 1046-51.

19. Detterbeck FC, Boffa DJ, Kim AW, Tanoue LT. The eighth edition lung cancer stage classification. 2017; 151: 193-203.

20. Radonic T, Dickhoff C, Mino-Kenudson M, Lely R, Paul R, Thunnissen E. Gross handling of pulmonary resection specimen: maintaining the 3-dimensional orientation. J Thorac Dis. 2019; 11(Suppl 1): S37-S44.

21. Myung, J. K., Choe G., Chung D. H., Seo J. W., Jheon S., Lee C.-T., et al. A simple inflation method for frozen section diagnosis of minute precancerous lesions of the lung. Lung Cancer, 2008; 59: 198-202.

22. Xu, X., Chung J.-H., Jheon S., Sung S. W., Lee C.-T., Lee J. H., et al. The accuracy of frozen section diagnosis of pulmonary nodules: evaluation of inflation method during intraoperative pathology consultation with cryosection. J Thorac Oncol. 2010; 5: 39-44.

23. Onozato ML, Klepeis VE, Yagi Y, Mino-Kenudson M. A role of three-dimensional (3D)-reconstruction in the classification of lung adenocarcinoma. Anal Cell Pathol (Amst). 2012; 35: 79-84.

24. Onozato ML, Kovach AE, Yeap BY, Morales-Oyarvide V, Klepeis VE, Tammireddy S, et al. Tumor islands in resected early-stage lung adenocarcinomas are associated with unique clinicopathologic and molecular characteristics and worse prognosis. Am J Surg Pathol. 2013; 37: 287-94.

25. Saladin Kenneth S. Human anatomy. 3rd ed. New York: McGraw-Hill company; 2011.

26. Liu Y, Chen D, Qiu X, Duan S, Zhang Y, Li F, et al. Relationship between MTA1 and spread through air space and their joint influence on prognosis of patients with stage I-III lung adenocarcinoma. Lung Cancer. 2018; 124: 211-8.

27. Kozuma Y, Toyokawa G, Yamada Y, Shoji F, Yamazaki K, Oda Y, et al. Spread through air spaces in non-small cell lung cancer. Journal of thoracic disease. 2019; 11(Suppl 15): S1881-S4.

28. Dai C, Xie H, Su H, She Y, Zhu E, Fan Z, et al. Tumor spread through air spaces affects the recurrence and overall survival in patients with lung adenocarcinoma >2 to 3 cm. J Thorac Oncol. 2017; 12: 1052-60.

29. Yi E, Bae MK, Cho S, Chung JH, Jheon S, Kim K. Pathological prognostic factors of recurrence in early stage lung adenocarcinoma. ANZ J Surg. 2018; 88: 327-31.

30. Qiu X, Chen D, Liu Y, Duan S, Zhang F, Zhang Y, et al. Relationship between stromal cells and tumor spread through air spaces in lung adenocarcinoma. Thorac Cancer. 2019; 10: 256-67.

31. Boelens MC, van den Berg A, Vogelzang I, Wesseling J, Postma DS, Timens W, et al. Differential expression and distribution of epithelial adhesion molecules in non-small cell lung cancer and normal bronchus. J Clin Pathol. 2007; 60: 608-14.

32. Shiono S, Yanagawa N. Spread through air spaces is a predictive factor of recurrence and a prognostic factor in stage I lung adenocarcinoma. Interact Cardiovasc Thorac Surg. 2016; 23: 567-72.

33. Thunnissen E, Blaauwgeers HJLG, Cuba EMVd, Yick CY, Flieder DB. Ex vivo artifacts and histopathologic pitfalls in the lung. Arch Pathol Lab Med. 2016; 140: 212-20.

34. Warth A. Spread through air spaces (STAS): a comprehensive update. Transl Lung Cancer Res. 2017; 6: 501-7

# 국문 초록

# 폐암에서 폐포 내 종양 세포 전파 (STAS)의 임상 및 병리학적 의미 고찰

## – STAS 등급 분류에 대한 제안 –

**한연비**

**의학과 병리학 전공**

**서울대학교 대학원**

**서론:** 폐포 내 종양세포 전파 (STAS)는 최근 폐암, 특히 폐선암종에서 침윤 형태의 하나로 최근 새롭게 정의된 개념이다. 하지만, 폐포 내 종양세포 전파 (STAS)의 정확한 정의 및 임상적인 중요성에 대해서는 여전히 논란이 존재한다. 본 연구에서는 수술적 절제를 시행한

폐암 환자를 대상으로, 폐포 내 종양세포 전파 (STAS)의 분포 정도를 거리 기준으로 분류한 등급과 임상, 병리학적 특성 및 환자의 재발 및 생존의 차이를 분석하여 폐포 내 종양세포 전파 및 그 등급의 임상적 의미에 대하여 알아보고자 하였다.

**방법:** 분당서울대학교 병원에서는 2008년부터 폐포 내 종양세포 전파 (STAS)의 유무를 병리 보고서에 기록해 왔으며, 2011년부터는 폐포 내 종양세포 전파 (STAS)의 분포 정도를 종양 경계면의 가장자리로부터의 거리에 따라 2계층 시스템으로 분류해왔다. 주 종양의 경계면으로부터 2500 μm (10배 대물렌즈 한 필드) 거리 이내에만 종양 군집이 존재하는 경우 등급 I로 평가하였으며, 등급 I 영역을 벗어나 종양 군집이 존재하는 경우 등급 II로 평가하였다. 2000례의 수술적 절제를 시행한 폐암을 대상으로, 전향적으로 수집된 폐포 내 종양세포 전파 (STAS)의 등급과 임상, 병리학적 특성 및 재발 과 생존의 차이 여부를 후향적으로 평가하였다.

**결과:** 2000례의 수술적 절제를 시행한 폐암의 조직학적 분류는 다음과

같다. 1544례 (77.2%)는 선암종으로 분류되었으며, 325례 (16.3%)는 편평세포암종, 41례 (2.1%)는 신경내분비암종 (Neuroendocrine carcinoma), 16례 (0.8%)는 카르시노이드 종양 (carcinoid tumors), 그리고 74례 (3.7%)는 위의 분류에 포함되지 않는 기타 암종으로 분류되었다. 전체 2000례 중 폐포 내 종양세포 전파 (STAS)는 830례 (41.5%)에서 관찰되었으며, 그 중 472례 (23.6%)는 등급 I 로 평가되었고, 358례 (17.9%)는 등급 II로 평가되었다. 폐포 내 종양세포 전파 (STAS)는 조직학적 분류가 신경내분비암종인 경우 높은 빈도로 관찰되었으며 (85.4%), 흉막, 림프관 및 혈관 침습이 있는 경우와 병리학적 병기가 높은 경우에 유의미하게 높은 빈도로 관찰되었다. 선암종의 경우 폐포 내 종양세포 전파 (STAS)의 등급에 따라서 무재발 생존율 (recurrence free survival), 전체 생존율 (overall survival) 및 폐암 특이 생존율 (lung cancer specific survival)이 통계학적으로 유의미한 차이를 보였다. IA기 비점액성 선암종에서는 폐포 내 종양세포 전파 (STAS) 등급 II가 짧은 무재발 생존기간 ($p<0.001$) 및 폐암

특이 생존기간 (p=0.006)을 보인다는 것이 다변량 분석을 통하여 확인되었다. 또한, 해당 그룹 내에서 폐포 내 종양세포 전파 등급 II는 분엽 절제술 (sublobar resection) (p=0.001)을 받은 환자군 뿐만 아니라 폐엽 절제술 (lobar resection) (p=0.023)을 받은 환자군에서도 독립적인 나쁜 예후인자임이 확인되었다.

결론: IA기 비점액성 선암종에서, 폐포 내 종양세포 전파 (STAS) 등급 II의 존재는 수술적 절제 범위에 관계없이 독립적인 불량한 예후인자임을 확인하였다. 이는 분엽 절제술 뿐만 아니라 폐엽 절제술을 받은 폐 선암종 환자의 병리 보고서에 폐포 내 종양세포 전파 (STAS) 및 등급을 표기하는 것의 임상적인 유효성을 제기할 수 있다.

.....

**주요어: 폐포 내 종양세포 전파, 폐암, 선암종, 수술적 절제 범위, 등급 분류**

**학번: 2017-26693**