ANTICANCER RESEARCH *38*: 1821-1825 (2018)
doi:10.21873/anticanres.12421

High Frequency of Spread Through Air Spaces in Resected Small Cell Lung Cancer

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Abstract. *Background/Aim: Spread through air spaces (STAS) is a novel invasive pattern of lung cancer, especially adenocarcinoma and squamous cell carcinoma. However, its frequency and significance in patients with resected small cell lung cancer (SCLC) remains unclear. Patients and Methods: A total of 30 patients with resected SCLC were analyzed for STAS. STAS was classified as either no STAS, low STAS (1-4 single cells or clusters of STAS), or high STAS (≥5 single cells or clusters of STAS). We evaluated the association between STAS and clinicopathological characteristics and postoperative survivals. Results: Among 30 patients, 5 (17%), 6 (20%) and 19 (63%) were classified as having no, low and high STAS, respectively. Fisher's exact test demonstrated no significant associations between the positivity for STAS and clinicopathological characteristics. No significant differences were observed in recurrence-free and overall survival between STAS-negative/low and STAS-high patients. Conclusion: STAS was frequently observed in patients with resected SCLC.*

Lung cancer is the leading cause of cancer-related death in many countries, and its prognosis still remains unsatisfactory. Especially, small cell lung cancer (SCLC) is extremely devastating and most patients are treated with chemotherapy combined with or without radiotherapy (1). However, several reports have shown the possible benefit of surgical resection for patients with SCLC and surgical resection is considered as one of the standard treatment options for early-stage SCLC (2-5). For instance, Takenaka *et al*. reported that patients treated with surgical resection exhibited the possible longer survivals compared to those without (4). Thus, surgery appears to be the possible efficacious therapy for the selected patients with SCLC, especially early-stage SCLC; however, most patients inevitably encounter recurrence and eventual death. Therefore, convenient and inexpensive indicators that affect the prognosis after surgical resection should be sought. For instance, we previously demonstrated the prognostic impact of the programmed death-ligand 1 expression in the resected SCLC patients (6). Further prognostic indicators are sought to stratify patients with SCLC more accurately and to determine optimum surgical procedures and postoperative follow-up.

Spread through air spaces (STAS) is a novel invasive pattern of lung cancer defined as follows: 'STAS consists of micropapillary clusters, solid nests or single cells beyond the edge of the tumor into air spaces in the surrounding lung parenchyma' (7). Importantly, STAS differs from conventional invasiveness, such as the presence of non-lepidic patterns, and the infiltration of stroma and blood vessels or structures such as the visceral pleura (8). Regarding the possible cause of STAS, whether it is caused by an *in vivo* effect or is just an artifact of cutting through a tumor with a knife remains controversial (9, 10). Clinicopathologically, STAS was reported to be associated with male gender, a history of smoking, the presence of lymphovascular invasion, and more invasive subtypes such as micropapillary and solid patterns in patients with resected lung adenocarcinoma (11-13). Furthermore, an unfavorable prognostic impact was reported in both adenocarcinoma and squamous cell carcinoma (11-15). However, the clinicopathological features of SCLC with STAS and its prognostic impact have yet to be investigated.

In the present study, we evaluated the relationship between STAS and the clinicopathological characteristics of patients with SCLC and its prognostic influence on their postoperative survival.

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Key Words: Small cell lung cancer, spread through air spaces, surgery.

Patients and Methods

Study patients. Among 62 patients with SCLC who underwent surgery at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University between April 1974 and August 2015, 30 patients, whose resected specimens could be analyzed of STAS, were included in this translational study. Pathological staging was performed using the 7th edition of the TNM Classification of Malignant Tumors. In addition to the pathological stage, the patients' age, gender, performance status, smoking history, pathological tumor and pathological lymph nodal factors, pleural invasion (pl), lymphatic invasion (ly), vascular invasion (v) and surgical procedure were examined. The present study was approved by our institutional review board.

STAS. STAS was defined as tumor cells within air spaces in the lung parenchyma beyond the edge of the main tumor (12, 16). In addition, STAS was also classified into the following three categories: "no STAS" for cases without definite STAS, "low STAS" for cases with 1 to 4 single cells or clusters of STAS, and "high STAS" for cases with five or more single cells or clusters of STAS (11-13). All tumors were evaluated at a magnification of ×200 using an optical microscope (BX40; Olympus, Tokyo, Japan).

Statistical analyses. The associations between STAS and patient characteristics were analysed using Fisher's exact test. The overall survival (OS) was defined as the time from the initial surgery until death from any cause, while the recurrence-free survival (RFS) was defined as the time from the initial surgery until recurrence. The Kaplan-Meier method was used to estimate the survival probabilities, and the curves of the two or three groups were statistically compared using the log-rank test. All of the statistical analyses were conducted using the JMP version 12 software program (SAS Institute, Cary, NC, USA). *p*-Values of <0.05 were considered to indicate statistically significant differences.

Table I. *Patient characteristics*.

Factors	N
Age (years)	
<70	15
≥70	15
Gender	
Female	5
Male	25
Smoking history*	
Never-smoker	3
Smoker	25
T	
T1	12
≥T2	18
N	
Negative	17
Positive	13
Pathological stage	
I	16
≥II	14
pl*	
Negative	16
Positive	12
ly*	
Negative	21
Positive	8
v*	
Negative	13
Positive	16
Surgical procedure*	
≥Lobectomy	22
Sublobar resection	4
Adjuvant chemotherapy*	
None	11
Administered	10

*Cases whose data are available. pl: Pleural invasion; ly: lymphatic invasion; v: vascular invasion.

Results

Clinicopathological characteristics of the patients. Table I shows the characteristics of the patients included in this translational study. The median age of the patients was 70 years (range=48-84 years), and the number of female and male patients was five (17%) and 25 (83%), respectively. Twenty-five patients had a history of smoking. Twelve (40%) and 18 (60%) patients had T1 and ≥T2 tumor, respectively, and 13 (43%) patients were positive for lymph node metastasis. Sixteen (53%) and 14 (47%) patients were diagnosed with pathological stage I and II or III, respectively. Pathological examinations revealed pl, ly, and v in 12 (40%), eight (27%), and 16 (53%) patients, respectively. Twenty-two (73%) patients underwent surgical resection of more than one lobe, while four (27%) received sublobar resection. Ten (33%) patients received adjuvant chemotherapy.

Association between STAS and the clinicopathological characteristics. Representative images of STAS are shown in Figure 1. Among 30 patients, five (17%), six (20%) and 19 (63%) were classified as having no, low and high STAS, respectively (Figure 2). Fisher's exact test demonstrated that positivity for STAS was not significantly associated with any clinicopathological features (Table II).

Survival analyses according to the STAS. RFS and OS after surgical resection were not significantly different between STAS-negative/low and STAS-high patients (p=0.89 and p=0.75, respectively; Figures 3A and B). The five-year RFS in STAS-negative/low and STAS-positive patients were 33.3% and 28.9%, respectively. The five-year OS in STAS-negative/low and STAS-positive patients were 44.4% and 56.3%, respectively.

Discusion

In the current study, STAS was observed in 25 (83%) among 30 patients with SCLC, which was higher than those reported in adenocarcinoma and squamous cell carcinoma

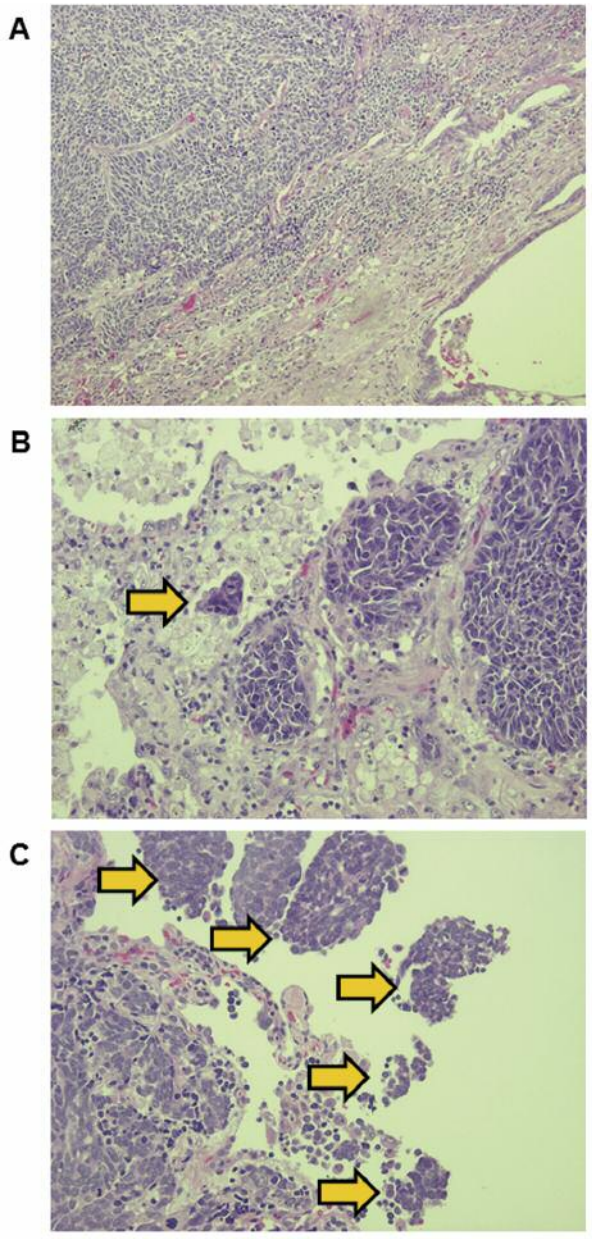

Figure 1. *Histological figures of spread through air spaces (STAS); the pictures show small cell lung cancer cases (SCLC; A) without STAS, (B) with a few floating clusters (arrows) of SCLC cells (low STAS), and (C) numerous clusters (arrows) of SCLC cells (high STAS) in the alveolar spaces near the margin of the tumors.*

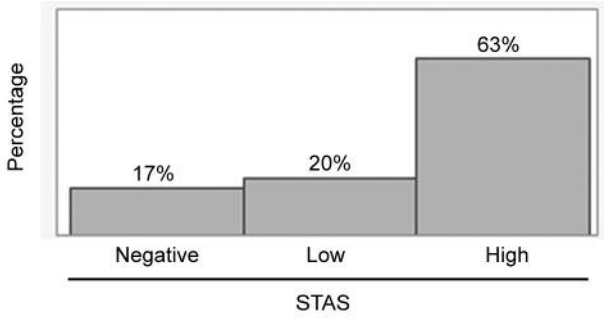

Figure 2. *Histogram which showing the frequency of spread through air spaces (STAS)-negative, low and high patients with the resected small cell lung cancer.*

Table II. *Association between STAS and clinicopathological factors in patients with resected small cell lung cancer.*

Factors	n	STAS Negative and low/High	*p*-Value
Age (years)			
<70	15	5/10	1.00
≥70	15	6/9	
Gender			
Female	5	0/5	0.13
Male	25	11/14	
Smoking history*			
Never-smoker	3	0/3	0.26
Smoker	25	11/14	
T			
T1	12	4/8	1.00
≥T2	18	7/11	
N			
Negative	17	6/11	1.00
Positive	13	5/8	
Pathological stage			
I	16	5/11	0.71
≥II	14	6/8	
pl*			
Negative	16	6/10	1.00
Positive	12	5/7	
ly*			
Negative	21	9/12	0.67
Positive	8	2/6	
v*			
Negative	13	3/10	0.25
Positive	16	8/8	
Surgical procedure*			
≥Lobectomy	22	8/14	0.28
Sublobar resection	4	3/1	
Adjuvant chemotherapy*			
None	11	5/6	0.36
Administered	10	2/8	

*Cases in which data were available. STAS: Spread through air spaces; pl: pleural invasion; ly: lymphatic invasion; v: vascular invasion.

(11-16). Although the presence of STAS was significantly associated with the clinicopathologically invasive features in lung adenocarcinoma and squamous cell carcinoma (11-13), the present study demonstrated no significant associations between STAS and clinicopathological features. Furthermore, survival analyses showed no significant

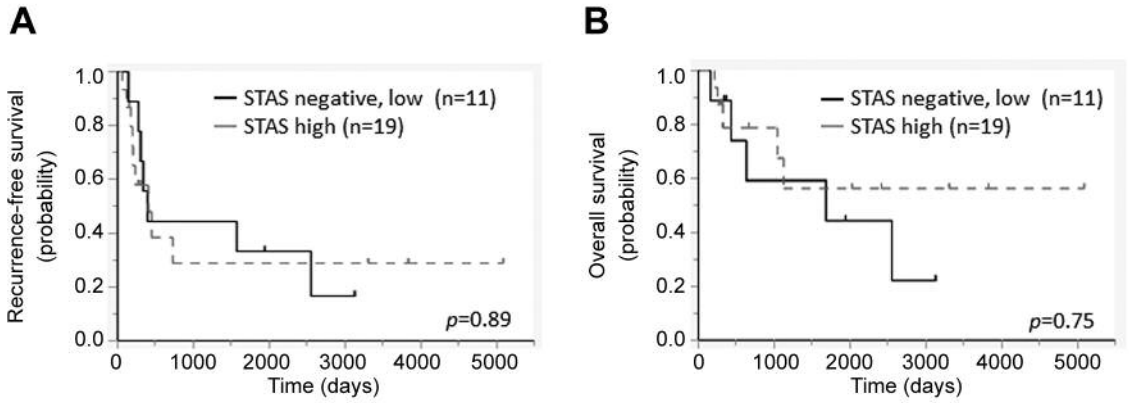

Figure 3. *The (A) recurrence-free and (B) overall survival according to spread through air spaces in patients with resected small cell lung cancer.*

differences in recurrence-free and overall survivals between STAS-negative/low and STAS-high patients, while the presence of STAS was reported to negatively affect postoperative survivals in lung adenocarcinoma and squamous cell carcinoma (11-16).

Although some reports suggested that STAS is caused simply by an artifact during the surgical procedure or is just an artifact of cutting through a tumor with a knife remains unclear (9, 10), others showed no significant differences in the formation of STAS among surgical procedures (11, 12). This discrepancy among reports might be due to differences in the surgical manipulation among surgeons and institutions, which cannot be standardized. The difference in the frequency of STAS between SCLC and other histologies, such as adenocarcinoma and squamous cell carcinoma, might be biologically explained. Previous reports showed that mutations in the *KRAS* and *BRAF* genes were significantly associated with STAS, and a wild-type *EGFR* gene was also found to be significantly associated with STAS (12, 16). These oncogenes are not typically identified in SCLC and other genes might be involved in STAS formation in the resected SCLC. Alternatively, STAS might be induced by epithelial to mesenchymal transition phenomenon, which should be analyzed by IHC for E-cadherin and vimentin.

The prognostic significance of STAS for patients with the resected lung cancer remains controversial. First, Kadota *et al*. reported that STAS was a significant risk factor of recurrence in small-sized adenocarcinomas treated with limited resection, but not in those who underwent lobectomy (11). However, according to the reports by Uruga *et al*. and Warth *et al*., STAS was significantly associated with a worse RFS and OS in patients with early-stage and stage I-IV adenocarcinoma that had been completely resected (13, 16). In addition, negative impact of STAS on RFS in patients with the resected lung squamous cell carcinoma was reported (14, 15). Our results showed that STAS did not impact the RFS and OS in patients with the resected SCLC and this is the first report which showed no possible survival effects in patients with SCLC.

There are several limitations associated with the present retrospective study, including that it examined a small cohort of patients with resected SCLC. Future studies are warranted to investigate the significance of STAS in a larger cohort of patients with the surgically resected SCLC.

In conclusion, STAS was very frequently observed in the resected SCLC and no impacts on postoperative survivals were identified.

Conflicts of Interest

All Authors declare no conflicts of interest in association with this study.

Acknowledgements

The Authors would like to thank Brian T. Quinn for his critical comments on the manuscript.

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Received January 4, 2018
Revised January 21, 2018
Accepted January 23, 2018