Original paper

Prognostic impact of spread through air spaces and its association with *KRAS* mutation and histopathologic factors in resected colorectal lung metastases

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Spread through air spaces (STAS) represents an independent prognostic factor for poor overall survival (OS) in patients with colorectal carcinoma who underwent pulmonary metastasectomy. This study aims to evaluate the prognostic impact of STAS and explore its associations with histopathologic features and *KRAS* mutation status/subtypes. We retrospectively analyzed 61 consecutive patients with colorectal cancer (CRC) who underwent pulmonary metastasectomy at a single tertiary center (May 2016 – July 2024). Histopathologic review assessed STAS and other histopathologic features. *KRAS* mutations were tested using a real-time polymerase chain reaction assay detecting 19 variants (codons 12, 13, 59, 61, 117, 146).
Spread through air spaces was present in 37/61 cases (60.7%). Median OS was 1702 days (95% CI: 1495–NA) in STAS-negative and 1288 days (95% CI: 523–NA) in STAS-positive patients (log-rank $p = 0.041$). In univariable analysis, STAS remained an independent predictor of poorer OS in multivariable modeling (hazard ratio: 2.37; 95% CI: 1.17–4.80; $p = 0.017$). *KRAS* mutations (present in 44.3% of tested cases; common subtypes G12V and G12D) showed no significant association with STAS ($p = 0.34$) or with OS.
Spread through air spaces is an independent poor prognostic factor in resected CRC pulmonary metastases, whereas *KRAS* mutation status and subtypes were not prognostic in this cohort.

Key words: prognostic factors, pathology, *KRAS*, pulmonary metastasectomy, colorectal cancer, spread through air spaces (STAS).

Introduction

Colorectal cancer (CRC) is one of the most prevalent malignancies worldwide [1]. The lung represents the second most frequent metastatic site after the liver, with approximately 10–15% of CRC developing pulmonary metastases [2]. The time of initial diagnosis, nearly 10% of patients present with synchronous pulmonary metastases, while approximately 5% experience disease recurrence due to pulmonary metastases within five years following primary CRC treatment [3]. Surgical resection combined with systemic chemotherapy constitutes the cornerstone of management for pulmonary metastases arising from CRC. Numerous studies have demonstrated that pulmonary metastasectomy in CRC is associated

with improved long-term survival and is influenced by various prognostic factors. However, precise criteria defining which patient subsets derive the greatest benefit from metastasectomy remain inadequately established [4]. Advances in imaging technologies such as computed tomography (CT) have enhanced the detection of suspicious pulmonary nodules, leading to an increased number of patients undergoing pulmonary metastasectomy [5].

Spread through air spaces (STAS) was first described in 2015 by Kadota *et al*. [6] as a pattern of tumour spread in lung carcinoma. In lung cancer, the recognized criteria for invasion include: all growth patterns except lepidic pattern, myofibroblastic proliferation associated with desmoplasia, vascular and pleural invasion. The 2015 World Health Organization (WHO) Classification of Lung Tumours introduced STAS as an additional invasive growth pattern. Spread through air spaces is defined as the presence of micropapillary clusters, solid nests, or single tumour cells found within alveolar spaces, detached from the primary tumour mass. Although a unique morphologic feature of lung cancers, STAS has been categorized alongside invasive growth patterns such as lymphovascular and pleural invasion [7, 8].

The *KRAS* gene, located on chromosome 12, encodes a membrane-associated small GTPase belonging to the RAS family, which functions as a molecular switch cycling between active and inactive conformations. *KRAS* plays an important role in cell proliferation, differentiation, and survival. Mutations in *KRAS* stabilize the protein in its active "on" state, leading to constitutive activation of downstream signaling pathways and enhanced tumourigenesis. Exon 2 of *KRAS* is particularly mutation-prone, with the most common alterations involving glycine substitutions at codons 12 (G12) and 13 (G13). *KRAS* mutations, detected in approximately 42–52% of CRC cases, represent one of the most prevalent oncogenic driver mutations [9–11]. Beyond conventional clinicopathological features, molecular and genetic biomarkers are increasingly recognized as critical determinants of prognosis and treatment stratification in CRC patients undergoing pulmonary metastasectomy. Among these, *KRAS*, *BRAF*, and *TP53* mutations are most frequently studied. *KRAS* mutations, present in ~ 40–50% of CRC patients, have been consistently associated with worse overall survival (OS) and recurrence-free survival following pulmonary metastasectomy. A meta-analysis by Kang *et al*. [12] involving more than 15,000 patients confirmed that *KRAS* mutations were significantly correlated with lower OS.

Despite substantial evidence highlighting the benefits of pulmonary metastasectomy in CRC, limited data exist regarding the prognostic relevance of histopathologic features of metastatic lesions, particularly STAS. Furthermore, no previous study has comprehensively investigated the relationship between STAS, other histopathologic factors and *KRAS* mutation status (including mutation subtypes) in CRC pulmonary metastases. Thus, it remains unclear whether the presence of STAS correlates statistically with other adverse histopathologic features, such as vascular invasion. In this study, we aimed to characterize the prognostic and morphological features of surgically resected pulmonary metastases from CRC. Specifically, we evaluated the prognostic significance of STAS, its association with established histopathologic parameters and its relationship with *KRAS* mutation status and subtypes. Additionally, we examined the impact of *KRAS* mutation status and its subtypes on survival outcomes in patients undergoing pulmonary metastasectomy.

Material and methods

Between May 2016 and July 2024, a total of 61 patients with primary CRC who underwent pulmonary metastasectomy were retrospectively included in this study. All patients underwent whole-body CT prior to thoracotomy. Only patients with well-controlled primary CRC and adequate cardiopulmonary reserve to tolerate lung resection were considered eligible for pulmonary metastasectomy.

Surgically resected specimens were fixed in 10% neutral-buffered formalin. Following fixation, 5–10 mm tissue slices were prepared, and representative sections including tumour and adjacent lung parenchyma were embedded in paraffin blocks. From these blocks, 5-μm-thick sections were cut and stained with hematoxylin and eosin (HE) for routine histopathologic evaluation (Figure 1).

To confirm tumour type, immunohistochemical staining with CK7, CK20, CDX2, TTF-1, Napsin A and SATB2 was performed (Figure 2).

The histopathologic evaluation of STAS was performed on all available hematoxylin and eosin stained slides for each case. On average, 6 slides per case (range 4–10) were reviewed. Two experienced pathologists (with 16 and 10 years of practice in thoracic pathology) independently assessed the presence of STAS. In case of disagreement, a joint review was conducted using a multi-headed microscope and a consensus diagnosis was reached. For the purpose of this study, STAS was defined as detached single cells or clusters spreading within air spaces beyond the edge of the main tumour, in accordance with the current WHO criteria. This operational definition was applied consistently across all cases. For STAS evaluation, the diagnostic criterion was defined as the presence of tumour cell clusters located at least 0.5 mm away from the main metastatic lesion within alveolar spaces (Figure 3). Histopathologic assessment included determination of tumour subtype, presence of STAS, vascular invasion, perineural invasion, lymphatic invasion, pleural invasion and lymph node

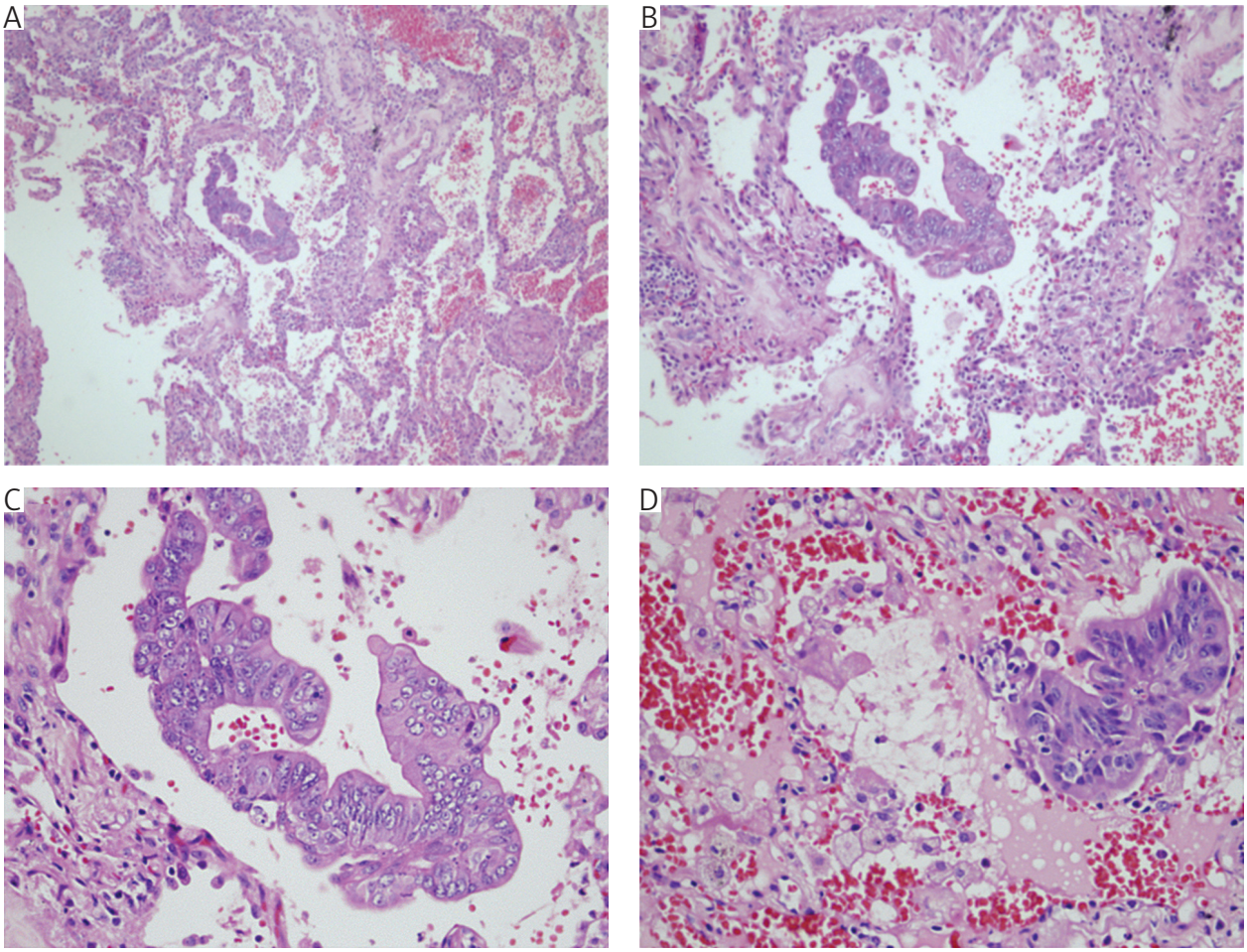

Figure 1. A) The tumour cluster in the alveolar space (HE 10×). **B**) Spread through air spaces (STAS) with increased lymphoplasmacytic infiltration in the interalveolar septa (HE 20×). **C**) STAS with reactive type 2 pneumocyte hyperplasia (HE 40×). **D**) STAS with intra-alveolar foamy histiocytes (HE 40×)

metastases in cases where lymph node dissection was performed.

The AmoxyDx *KRAS* Mutation Detection Kit was used for this study. The *KRAS* Mutation Detection Kit was intended to assess *KRAS* mutation status in CRC patients. It was a real-time polymerase chain reaction (PCR) assay for qualitative detection of 19 somatic mutations in codons 12, 13, 59, 61, 117 and 146 of the *KRAS* gene in human genomic DNA extracted from formalin-fixed paraffin-embedded tumour tissue. The kit was for *in vitro* diagnostic use, it was trained laboratory environment. It adopted amplification refractory mutation system technology which comprises specific primers and fluorescent probes to detect gene mutations in real-time PCR assay. During the nucleic acid amplification, the targeted mutant DNA was matched with the bases at the 3' end of the primer, amplified selectively and efficiently, then the mutant amplicon was detected by fluorescent probes labeled. When the wild type DNA could not be matched with specific primers, there was no amplification occurring. The human genomic DNA was extracted from formalin-fixed paraffin-embedded tumour tissue. Before DNA extraction, it was essential to use a standard pathology methodology to ensure tumour sample quality. The tumour samples were not homogeneous, they contained non-tumour tissue. DNA from non-tumour tissue might not contain detectable *KRAS* mutations. In our study, we used tumour tissue samples with more than 30% of tumour cells.

Statistical analysis

The primary objective of this study was to evaluate pathological factors associated with long-term survival following pulmonary metastasectomy. Survival analyses were conducted using the Kaplan-Meier method, log rank testing, Cox proportional hazards (PH) regression modeling and formal assessment of the PH assumption.

Kaplan-Meier survival curves were constructed according to the presence or absence of STAS. Median survival times and their corresponding 95% confidence intervals (CI) were calculated for each group and intergroup differences were compared

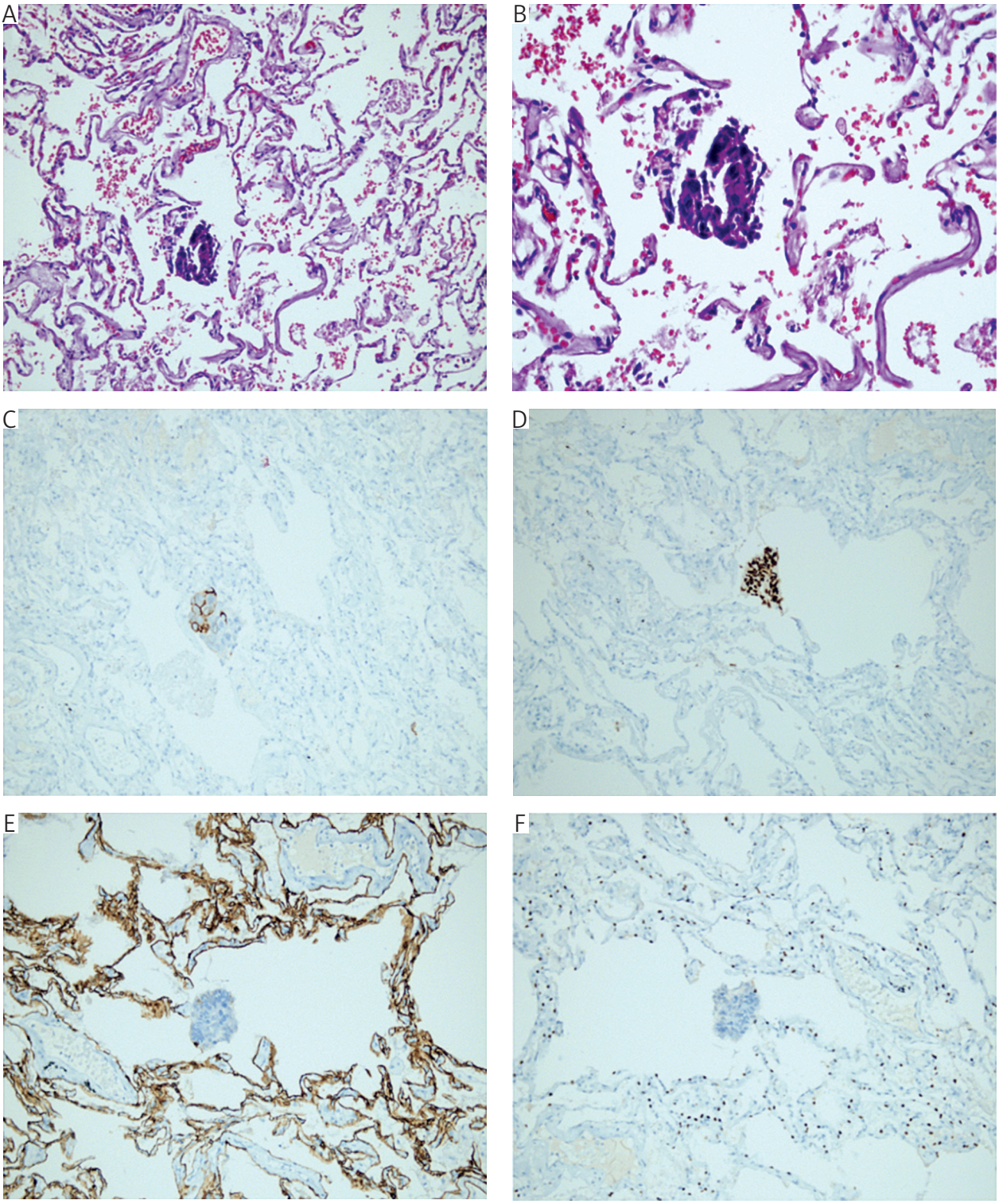

Figure 2. A) The tumour clusters floating in the alveolar space (HE 20×). B) The tumour clusters in the alveolar space (HE 40×). C) Tumour cells show diffuse CK20 membranous positivity (IHC stain, 20×). **D**) Tumour cells show strong diffuse CDX2 nuclear positivity (IHC 20×). **E**) Tumour cells are negative for CK7 expression (IHC stain 20×). **F**) Tumour cells are negative for TTF1 expression (IHC stain 20×)

using the log-rank test. To identify prognostic variables influencing OS, univariable Cox regression analyses were performed for each clinicopathological factor. Variables with a p-value < 0.10 in univariable analysis were subsequently incorporated into the multivariable Cox regression model. For both univariable and multivariable models, hazard ratios (HR), 95% CI, and p-values were reported.

To detect all-cause mortality predictors, univariable and multivariable Cox PH were used. Effects of predictors were given as HR with 95% CI. Candidate predictors of the multivariable regression model were

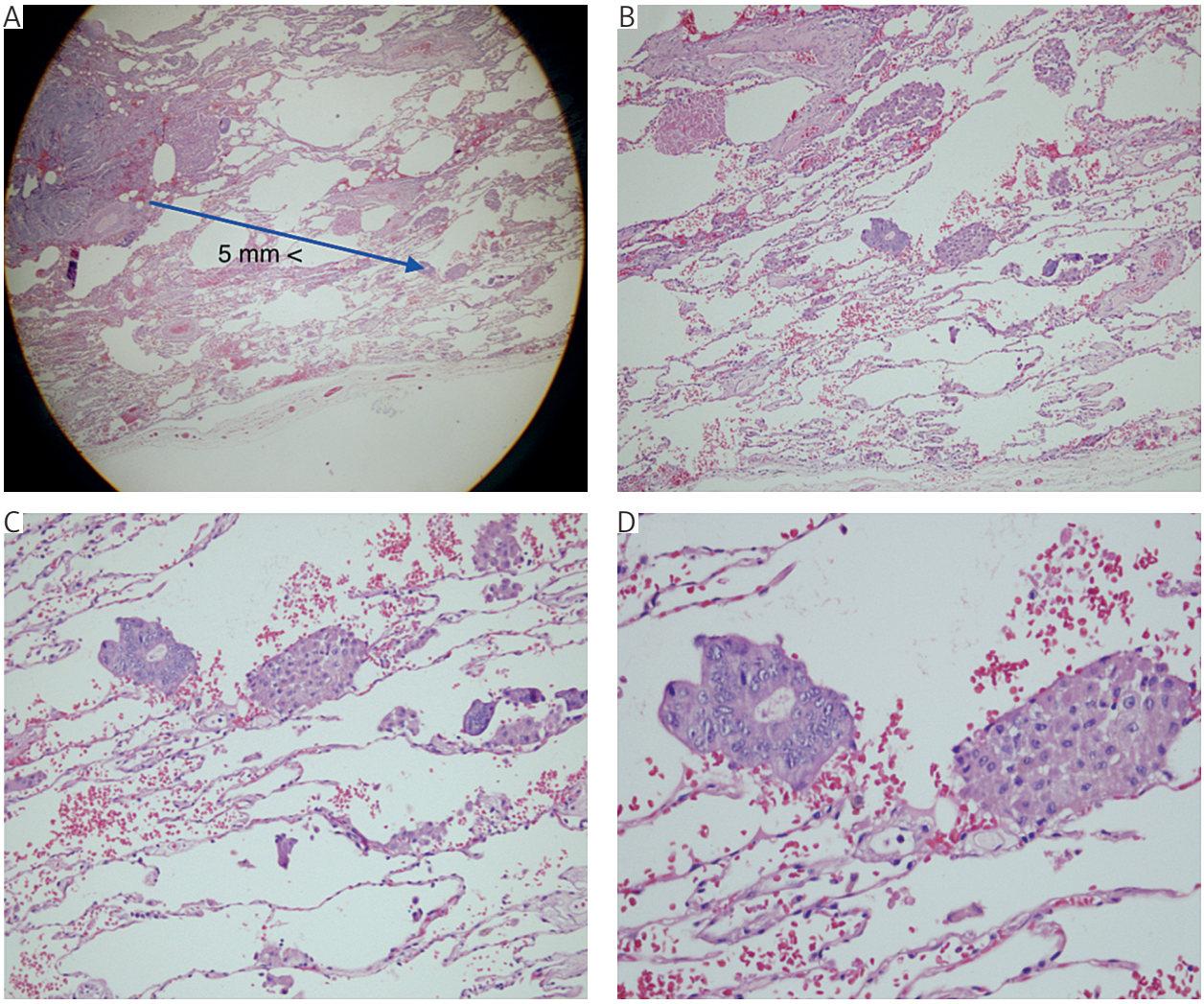

Figure 3. A) The spread through air spaces criterion was the presence of tumour clusters floating in the alveolar space and tumour clusters separated from the main metastatic lesion by at least 0.5 mm (HE 40×). **B**) The tumour clusters and macrophages in the alveolar space (HE 10×). **C**) The tumour clusters and macrophages in the alveolar space HE 20×). **D**) The tumour clusters and macrophages in the alveolar space (HE 40×)

age, vascular invasion, gender, lymphatic invasion, tumour size and *KRAS* mutation selected according to clinically and biologically plausible association with established prognostic assessment. In addition, visual depiction of mortality between STAS presence or not made by Kaplan-Meier curve, long-rank test was used for comparison.

Model discrimination and overall performance were evaluated using the concordance index (C-index), likelihood ratio test, and coefficient of determination (R^2). The proportional hazards assumption was tested globally and for individual covariates using Schoenfeld residual-based tests. A p-value > 0.05 was considered indicative of no violation of the PH assumption.

Results

The baseline clinicopathological characteristics of the 61 patients who underwent pulmonary metastasectomy are summarized in Table I. The mean age of the study cohort was 62.0 ±9.2 years. Among these, STAS was identified in 37 patients (60.7%), whereas 24 patients (39.3%) were STAS-negative. Thus, the number of STAS-positive cases exceeded that of STAS-negative cases.

The mean age of STAS-positive patients was 61.8 ±8.8 years compared with 62.0 ±9.2 years in STAS-negative patients, with no statistically significant difference between the groups ($p = 0.81$). Of the 61 patients, 32 (52.5%) were male and 29 (47.5%) were female. Although the proportion of male patients was higher in the STAS-negative group (66.7% vs. 33.3%), no significant association between sex and STAS was detected ($p = 0.07$).

With respect to the type of surgical resection, wedge resection was the most commonly performed procedure overall (82.0%), with comparable distributions between STAS-negative (79.2%) and STAS-pos-

Table I. Baseline demographic and clinical characteristics of patients according to spread through air spaces status

Parameters	STAS absent (N = 24)	STAS present (N = 37)	Total (N = 61)	*p*-value
Age				0.81
Mean ± SD	62.3 ±9.9	61.8 ±8.8%	62.0 ±9.2	
Range	36.0–79.0	44.0–79.0	36.0–79.0	
Sex, *n* (%)				0.07
Male	16.0 (66.7)	16 (43.2)	32 (52.5)	
Female	8.0 (33.3)	21 (56.8)	29 (47.5)	
Type of resection, *n* (%)				0.19
Wedge resection	19.0 (79.2)	31 (83.8)	50 (82.0)	
Lobectomy	3.0 (12.5)	6 (16.2)	9 (14.8)	
Trisegmentectomy	2.0 (8.3)	0 (0.0)	2 (3.3)	
Laterality, *n* (%)				0.17
Left	14.0 (58.3)	15 (40.5)	29 (47.5)	
Right	10.0 (41.7)	22 (59.5)	32 (52.5)	
Tumour location, *n* (%)				0.04
Lower lobe	8.0 (33.3)	12 (32.4)	20 (32.8)	
Upper lobe	14.0 (58.3)	14 (37.8)	28 (45.9)	
Upper and lower lobes	0 (0.0)	6 (16.2)	6 (9.8)	
Middle lobe	0 (0.0)	5 (13.5)	5 (8.2)	
Middle and lower lobes	1 (4.2)	0 (0.0)	1 (1.6)	
Upper and middle lobes	1 (4.2)	0 (0.0)	1 (1.6)	

STAS – spread through air spaces

itive patients (83.8%). Lobectomy was performed in 16.2% of STAS-positive patients and 12.5% of STAS-negative patients, whereas trisegmentectomy was performed in two patients (8.3%), both of whom were STAS-negative. No statistically significant association was observed between the type of resection and STAS ($p = 0.22$).

Laterality analysis revealed metastases in the right lung in 32 patients (52.5%) and in the left lung in 29 patients (47.5%). Spread through air spaces was identified in 22 patients (59.5%) with right-sided metastases and 15 patients (40.5%) with left-sided metastases; however, this difference was not statistically significant ($p = 0.174$). Regarding lobar distribution, 28 patients (45.9%) had metastases in the upper lobes, followed by 20 patients (32.8%) with metastases in the lower lobes. A statistically significant association was not observed between metastasis location and STAS ($p = 0.041$).

Vascular invasion was detected in 6 patients (9.8%), whereas 55 patients (90.2%) showed no evidence of vascular invasion. Among patients without vascular invasion, STAS was present in 33 (89.2%) and absent in 22 (91.7%). Of the six patients with vascular invasion, STAS was identified in four (10.8%) and absent in two (8.3%). No statistically significant association was found between STAS and vascular invasion ($p = 0.751$), indicating that tumour cell groups with vascular invasion were not more likely to demonstrate STAS. Similarly, no significant associations were detected between STAS and either perineural invasion or lymphatic invasion ($p = 0.09$).

The mean metastatic tumour diameter was 2.0 cm. Tumour size range was 0.5–6.0 cm in STAS-negative patients and 0.5–5.5 cm in STAS-positive patients. No significant correlation was observed between tumour diameter and STAS ($p = 0.8661$). Parenchymal margin positivity was identified in 3 patients (4.9%), with no significant association with STAS ($p = 0.8272$). Pleural invasion was observed exclusively in 7 patients (11.5%), all of whom were STAS-positive, yet the association did not reach statistical significance ($p = 0.02$) (Table II).

With respect to molecular findings, *KRAS* mutation analysis revealed exon 2 codon 12 G12V mutations in 10 patients (16.4%), followed by G12D mutations in 9 patients (14.8%), G12C mutations in 3 patients (4.9%), G13D mutations in 2 patients (3.3%), and G12A mutations in 2 patients (3.3%). No *KRAS* mutation was detected in 17 patients (27.9%), while

Table II. Pathological characteristics according to spread through air spaces status

Parameters	STAS absent ($N = 24$)	STAS present ($N = 37$)	Total ($N = 61$)	*p*-value
Vascular invasion, *n* (%)				0.75
Absent	22 (91.7)	33 (89.2)	55 (90.2)	
Present	2 (8.3)	4 (10.8)	6 (9.8)	
Perineural invasion, *n* (%)				0.08
Absent	24 (100.0)	35 (94.6)	59 (96.7)	
Present	0 (0.0)	2 (5.4)	2 (3.3)	
Lymphatic invasion, *n* (%)				0.09
Absent	24 (100.0)	33 (89.2)	57 (93.4)	
Present	0 (0.0)	4 (10.8)	4.0 (6.6)	
Tumour diameter [cm]				0.86
Mean ± SD	2.0 ±1.2	2.0 ±1.3	2.0 ±1.2	
Range	0.5–6.0	0.5–5.5	0.5–6.0	
Parenchymal surgical margin positivity, *n* (%)				0.82
Absent	23 (95.8)	35 (94.6)	58 (95.1)	
Present	1 (4.2)	2 (5.4)	3 (4.9)	
Pleural invasion, *n* (%)				0.02
Absent	24 (100.0)	30 (81.1)	54 (88.5)	
Present	0 (0.0)	7 (18.9)	7 (11.5)	
Mortality, *n* (%)				0.90
Absent	14 (58.3)	21 (56.8)	35 (57.4)	
Present	10 (41.7)	16 (43.2)	26 (42.6)	

STAS – spread through air spaces

mutational analysis was not performed in 18 patients (29.5%). Neither the presence of *KRAS* mutations nor their specific subtypes showed a statistically significant association with STAS ($p = 0.34$) (Table III).

Kaplan-Meier survival analysis demonstrated a significant difference in OS between STAS-positive and STAS-negative patients. Patients without STAS (STAS = 0) had a median survival time of 1702 days (95% CI: 1495–NA), whereas patients with STAS (STAS = 1) had a shorter median survival of 1288 days (95% CI: 523–NA). The survival difference between the two groups was statistically significant (log- rank $p = 0.041$). Survival curves clearly demonstrated inferior outcomes in STAS-positive patients (Figure 4).

In univariable Cox regression analysis, STAS was significantly associated with increased mortality risk (HR: 2.36, 95% CI: 1.02–5.48; $p = 0.045$). In the multivariable model, STAS remained an independent predictor of poorer survival (HR: 2.37, 95% CI: 1.17–4.80; $p = 0.017$), after adjusting for other pathological covariates. None of the other factors, including age, tumour size, vascular invasion, lymphatic invasion demonstrated statistical significance in the multivariable analysis. Perineural invasion was excluded from regression modeling due to the small number of events ($n = 2$).

In univariable Cox regression analysis, while STAS was demonstrated to be associated with all-cause mortality [2.37 (1.17–4.80) p:0.017] during follow-up lymphatic invasion, vascular invasion, *KRAS* mutation was not associated with all-cause mortality. In addition, multivariable Cox regression analysis demonstrated that STAS was associated with all-cause mortality [2.340 (1.04–5.45) *p*: 0.040] however tumour size was not associated with all-cause mortality (Table IV).

Model performance was acceptable, with a C-index of 0.637, $R^2 = 0.066$, and a likelihood ratio test *p*-value of 0.041. The global Schoenfeld residual test for the PH assumption yielded a borderline non-significant result ($p = 0.08067$), indicating that the PH assumption was not violated.

Discussion

In this study, we demonstrated that STAS represents an independent prognostic factor for poor OS in patients with CRC who underwent pulmonary metastasectomy. To our knowledge, this is one

Table III. KRAS mutation status and survival according to spread through air spaces

Parameters	STAS absent (N = 24)	STAS present (N = 37)	Total (N = 61)	*p*-value
KRAS mutation status, *n* (%)				0.34
Not tested	6 (25.0)	12 (32.4)	18 (29.5)	
Wild-type (not detected)	8 (33.3)	9 (24.3)	17 (27.9)	
Exon 2 codon 12 G12V	5 (20.8)	5 (13.5)	10 (16.4)	
Exon 2 codon 12 G12D	2 (8.3)	7 (18.9)	9 (14.8)	
Exon 2 codon 12 G12C	1 (4.2)	2 (5.4)	3 (4.9)	
Exon 2 codon 13 G13D	0 (0.0)	2 (5.4)	2 (3.3)	
Exon 2 codon 12 G12A	2 (8.3)	0 (0.0)	2 (3.3)	
Metastasis-free survival (days)				< 0.001
Mean ± SD	1220.9 ±559.5	635.2 ±460.7	865.6 ±575.0	
Range	224–2100	126–2134	126–2134	

STAS – spread through air spaces

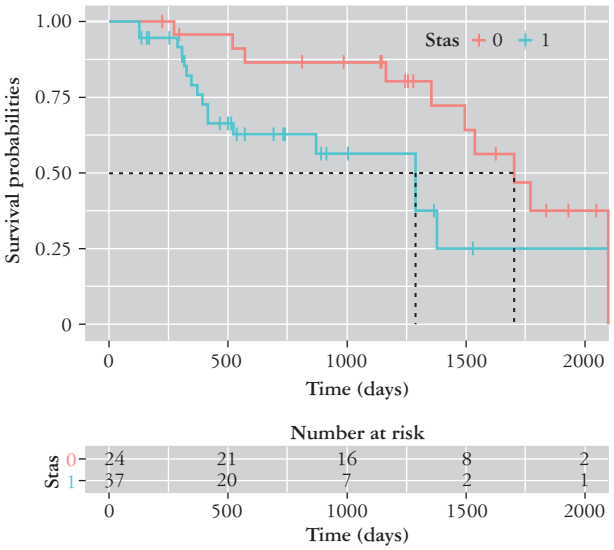

Figure 4. Kaplan-Meier survival analysis demonstrated a significant difference in overall survival between spread through air spaces (STAS)-positive and STAS-negative patients

of the few studies investigating histopathologic prognostic factors that may reflect the biological aggressiveness of metastatic CRC in the lung. Our findings should be validated by larger multicenter studies, thus we recommend that STAS should be reported as a prognostic marker in the pathology reports of CRC pulmonary metastases.

We systematically evaluated the relationship between STAS and various clinicopathological variables, including patient age, sex, tumour laterality, tumour location, tumour size, vascular invasion, perineural invasion, pleural invasion, parenchymal margin status, *KRAS* mutation status, and *KRAS* mutation subtypes. Our analysis revealed no statistically significant associations between STAS and any of these parameters. Nonetheless, we hypothesize that larger patient cohorts may eventually demonstrate meaningful correlations, potentially identifying patient subgroups more likely to exhibit STAS. Importantly, while STAS emerged as an independent predictor of adverse survival, none of the other pathological covariates, including *KRAS* mutation and its subtypes, significantly influenced prognosis.

Surgical advances in recent years have substantially improved outcomes for patients with CRC, with pulmonary metastasectomy being performed at an increasing rate. In our own institution, 61 patients underwent pulmonary metastasectomy between 2016 and 2024, with a marked increase in surgical cases after 2020. This rise likely reflects both improvements in surgical safety and accumulating evidence that patients undergoing resection demonstrated better prognostic outcomes compared to those managed only with systemic chemotherapy. Consistent with previous reports, pulmonary metastasectomy continues to represent an essential component of the multimodal management of CRC [13–15].

Minimally invasive surgical approaches, particularly video-assisted thoracic surgery, have gained widespread popularity in recent years and are increasingly employed in pulmonary metastasectomy. Video-assisted thoracic surgery wedge resection, in particular, is frequently favored because it minimizes parenchymal loss while ensuring oncologic control. In a series by Cho *et al.* [14], wedge resection was performed in 82.1% of cases, segmentectomy in 6.0%, and lobectomy in 11.9%. In line with these findings, wedge resection was also the most frequently performed procedure in our study cohort. Whereas younger age (< 54 years) has been reported as an adverse prognostic factor in previous studies [14]. We did not observe a statistically significant association between age and survival in our analysis.

Table IV. Cox proportional hazards regression analysis of pathological variables associated with long-term mortality after pulmonary metastasectomy

Parameters	Univariable HR (95% CI)	*p*-value	Multivariable HR (95% CI)	*p*-value
Age [years]	1.01 (0.98–1.05)	0.443	–	–
Vascular invasion (yes vs. no)	1.68 (0.69–4.12)	0.254	–	–
STAS (yes vs. no)	2.37 (1.17–4.80)	0.017	2.32 (1.04–5.45)	0.040
Sex (female vs. male)	1.07 (0.48–2.35)	0.875	–	–
Lymphatic invasion (yes vs. no)	3.06(0.67–13.97)	0.151	–	–
Tumour size [cm]	1.19 (0.88–1.60)	0.269	1.20 (0.88–1.59)	0.225
KRAS mutation (mutant vs. wild-type)	1.23 (0.51–3.00)	0.641	–	–

HR – hazard ratio, CI – confidence interval, STAS – spread through air spaces
Due to a low number of events for perineural invasion (n=2), this variable was not included in the Cox regression analysis. KRAS mutation analysis was available for 43 patients. Except for KRAS, all analyses were performed using complete data from 61 patients, with a total of 26 death events.

Several large-scale studies have further elucidated prognostic determinants in pulmonary metastasectomy. Tsitsias *et al*. [16] reported a 5-year survival rate of 52.5% and identified tumour diameter, number of metastases, intrathoracic lymph node involvement, pre-thoracotomy carcinoembryonic antigen (CEA) levels and response to induction chemotherapy as key prognostic factors. Similarly, the Metastatic Lung Tumour Study Group of Japan, analyzing 1030 patients in 2013, found a 5-year survival rate of 53.5%, with independent prognostic factors including tumour size, tumour number, CEA level, lymph node involvement, and completeness of resection [4].

Our study showed the prognostic impact of STAS in CRC pulmonary metastases. Consistent with reports, we found that STAS independently predicted poor survival outcomes. Notably, the OS rate in STAS-negative patients was 72.3%, further supporting its value as a histopathologic marker of tumour aggressiveness. Collectively, these findings suggest that STAS should be recognized as a clinically relevant factor in both pathological evaluation and therapeutic decision-making for patients with resected CRC lung metastases.

In a recent study by Shalabi *et al*. [17], local recurrence was observed even in cases where complete resection was histopathologically confirmed. Although only partially understood, this phenomenon is particularly recognized in metastatic nodules of colorectal origin. STAS has been suggested as a plausible explanation for such recurrences, especially in pulmonary metastases of CRC [18]. Shalabi *et al*. [17] specifically reported that local recurrence despite complete resection may be attributed to STAS, underlining its clinical importance in colorectal metastasectomy. In our study, STAS emerged as an independent adverse prognostic factor for survival. We investigated its associations with age, sex, tumour location, tumour laterality, tumour size, pleural invasion, vascular invasion, perineural invasion, parenchymal margin positivity, and *KRAS* mutation status. However, no statistically significant associations were identified between STAS and these parameters. Thus, while STAS clearly predicts poorer survival, our data did not permit the identification of clinicopathological or molecular markers that could reliably predict its presence. The paucity of studies investigating the relationship between STAS and other histopathologic features highlights the need for further research with larger patient cohorts.

Supporting our findings, Shiono *et al*. [19] demonstrated that STAS, together with vascular, lymphatic, and pleural invasion, was associated with poor outcomes in patients with pulmonary metastases of CRC. Importantly, their study identified STAS and vascular invasion as independent prognostic factors, and showed that patients positive for both features had significantly worse survival, while those negative for both had better outcomes. They further suggested that STAS and vascular invasion may represent histopathologic hallmarks of tumour aggressiveness. In contrast, although we also explored the relationship between STAS and molecular markers as potential indicators of aggressiveness, no significant associations were observed. Larger studies may help clarify these interactions.

In our cohort, three patients demonstrated parenchymal surgical margin positivity, of whom two were STAS-positive. However, we did not identify a significant association between STAS and margin positivity. Previous studies, such as one reporting positive margins in 5 of 89 patients, likewise failed to demonstrate prognostic significance of margin status [19]. It is possible that studies with larger numbers of patients with positive margins may provide more definitive conclusions.

Historically, most investigations have focused on the morphological features of primary tumours, whereas fewer studies have assessed histopathologic characteristics of metastatic lesions and their resection mar-

gins. Shirakusa *et al*. [20] categorized patients into infiltrative and non-infiltrative types based on the histologic appearance of the metastatic tumour border. Infiltrative-type metastases were associated with worse 5-year survival and higher rates of lymph node metastasis. Karjula *et al*. [21] evaluated the prognostic effect of tumour budding and tumour-stroma ratio in resected CRC pulmonary metastases but concluded that these features lacked prognostic value.

In our analysis, hematoxylin-eosin-stained sections of CRC pulmonary metastases were reviewed, and STAS was defined as tumour clusters present at least 0.5 mm away from the main metastatic lesion within alveolar spaces. Consistent with previous reports, STAS was associated with poor survival outcomes. However, despite exploring its associations with other histopathologic parameters, including vascular invasion, we were unable to identify significant correlations.

Experimental evidence suggests that alterations in cell-to-cell adhesion may underlie the pathogenesis of STAS. Cadherins have been shown to play an essential role in tumour cell invasiveness [22]. Shiono *et al*. [23] further reported that reduced E-cadherin expression served as an independent poor prognostic factor, reflecting malignant behavior in metastatic lesions. Moreover, loss of intercellular adhesion has been proposed as a biological mechanism facilitating STAS [19]. In our study, we specifically investigated whether STAS was associated with other invasive patterns – vascular, perineural, pleural, or lymphatic invasion – but found no statistically significant relationships. Nevertheless, our findings suggest that the biological mechanisms leading to STAS, such as cell adhesion loss, may also drive other invasive patterns, warranting further investigation.

In contrast to the findings of Kang *et al*. [12], who demonstrated that *KRAS* mutations were significantly associated with poorer OS, our study did not identify a statistically significant association between *KRAS* mutational status and survival outcomes in patients undergoing pulmonary metastasectomy for CRC. In another study, unlike our own work, it was found that, compared with *KRAS* wild-type patients, patients with the *KRAS* gene mutation had a trend of poor prognosis in patients with stage II colon cancer [24]. Although external evidence consistently demonstrates that *KRAS* mutations are associated with an adverse prognosis in patients with CRC metastasizing to the lung, our study did not observe a statistically significant effect of *KRAS* status on survival outcomes. This lack of significance is most likely related to the limited sample size and the incomplete availability of molecular data, both of which reduce the statistical power to detect modest associations.

In the study by Takeda-Miyata *et al*. [25], STAS was shown to be related to a poor prognosis and surgical margin relapse. In another study, Haj Khalaf *et al.* [26] found a statistically positive relationship between the number of alveoli invaded with STAS and locoregional recurrence of metastases. Additionally, Castro *et al*. [27] found that locoregional recurrence and mortality were higher in patients with STAS (40% vs. 22.5% and 40% vs. 20%, respectively). In our study, similar to these works, we found that STAS is an adverse prognostic factor.

It remains possible, however, that studies with larger sample sizes may reveal clinically meaningful correlations. Immunohistochemical evaluation of metastatic lesions was performed using CK7, CK20, CDX2, TTF-1, and Napsin A. While CK7, TTF-1, and Napsin A were uniformly negative in all tumours, CK20 and CDX2 were consistently positive, confirming the colorectal origin of these metastases and supporting the reliability of the immunohistochemical panel employed.

Limitations

This study has several limitations. First, its retrospective design may have introduced bias, particularly due to heterogeneity in treatment protocols before and after resection. Additionally, response to adjuvant therapies was not incorporated into the survival analysis, which may have contributed to residual confounding or selection bias. Finally, as a single-center study, the relatively small cohort size limited the statistical power and generalizability of our findings. Larger, prospective, multicenter studies are necessary to validate these results and to further explore the clinicopathological and molecular determinants of STAS. Due to our small patient number, our findings need to be validated by other studies.

Conclusions

In this study, STAS emerged as an independent adverse prognostic factor in patients undergoing pulmonary resection for CRC metastases. The identification of STAS may therefore contribute to improved risk stratification, surgical planning and follow-up strategies. To better elucidate the biological mechanisms underlying STAS and to identify clinicopathological or molecular parameters predictive of its occurrence, future investigations with larger and multicenter cohorts are warranted to validate these findings. Importantly, we advocate for the inclusion of STAS status in pathology reports to guide clinicians in this patient population.

Disclosures

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2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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