Significance of tumor spread through air spaces (STAS) in lung cancer from the pathologist perspective

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Abstract: Airspace invasion in lung cancer has been known over the last 30 years, but it was only recently that WHO 2015 formally recognized it as a mechanism of invasion with the terminology of tumor spread through air spaces (STAS). Multiple studies have shown the association of STAS with lower survival and suggest that STAS is an independent prognostic factor across lung adenocarcinoma of all stages and in other histologic subtypes of lung cancer as well. Consequently, STAS is designated as an exclusion criterion of adenocarcinoma in situ and minimally invasive adenocarcinoma; thus, the presence of STAS impacts the diagnosis and staging of lung adenocarcinoma. Further, wedge resection and segmentectomy have been increasingly applied for small node negative tumors and the presence of STAS in those specimens may indicate the requirement of completion lobectomy. Given these significant clinical implications, we, pathologists, need to recognize and appropriately report STAS (possibly including at the time of intraoperative consultation). However, emerging data suggests that more work should be done to improve consensus and identification of STAS, including at frozen section. In this review, the evolution of our understanding of airspace invasion over the past decade, the clinical significance of STAS, and controversies and practical issues associated with the diagnosis of STAS are discussed.

Keywords: Lung cancer; spread through air spaces (STAS); airspace invasion; sublobar resection; frozen section

Introduction

Invasion in lung cancer is traditionally defined as: (I) a non-lepidic histologic pattern of growth; (II) myofibroblastic proliferation with desmoplasia; and (III) vascular or pleural invasion. The 2015 World Health Organization (WHO) Classification of Lung Tumors fascicle has introduced a new concept of invasion, spread through air spaces (STAS) that is defined as "spread of micropapillary clusters, solid nests, and/or single cancer cells into airspaces in the lung parenchyma beyond the edge of the main tumor." (1,2). It is assumed that STAS represents airspace invasion that is unique to the lung, and may be considered equal to other more established patterns of invasive growth, such as lymphovascular or pleural invasion.

The significance of this concept is accentuated given the updated lung adenocarcinoma classification system outlining criteria for in-situ, minimally invasive, and invasive adenocarcinoma categories as proposed by multidisciplinary panels from the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS), and the European Respiratory Society (ERS) and adopted by the 8th edition of the American Joint Committee on Cancer (AJCC) (3,4). Now, the presence of STAS excludes a diagnosis of adenocarcinoma in-situ or minimally invasive adenocarcinoma in small tumors.

Further, sublobar resections (wedge resection and segmentectomy) have been increasingly applied for not only poor-risk patients but also good-risk patients with clinical stage IA non-small cell lung cancer (NSCLC), given the increase in detection of small peripheral tumors secondary to the advancement of imaging techniques (5-8). The

presence of STAS in sublobar resection specimens is now considered an indication for completion lobectomy by some surgeons. Thus, it is important for pathologists to recognize and report STAS.

In this review, I will discuss the evolution of our understanding of airspace invasion over the past decade and the current concept, controversies and practical issues associated with STAS from the pathologist perspective.

Concept of airspace invasion

The concept of airspace invasion is not new. Kodama and colleagues reported in 1980 a case with multifocal "aerogenous" spread that appeared to be equivalent to STAS based on its description (9). They showed using electron microscopy that the tumor cells in airspaces were rather poorly differentiated but somewhat resembled the hyperplastic cuboidal alveolar cells seen in the damaged lung and that they proliferated in airways, representing "aerogenous metastases." Further, the authors concluded that biologic behavior of the tumor cells might be partly explained by their dyshesive nature (9). Subsequently, Chung and colleagues at Seoul National University Bundang Hospital implemented aerogenous spread in their lung cancer synoptic report in 2008 (personal communication). Yi and colleagues also reported the presence of aerogenous spread as an independent pathological risk factor of recurrence in stage I lung adenocarcinoma by multivariate analysis (10).

Similarly, Onozato and colleagues found unique island-like structures in lung adenocarcinomas by 3D reconstruction and named them as "tumor islands" (11). Tumor island is a detached, large cluster of tumor cells in an alveolar space separated from the main tumor mass, but has been confirmed to be connected to the main tumor in different tissue levels by the 3D reconstruction. Tumor islands appear to share characteristic features with some of the solid nest type of STAS, in particular large ones (see below). The subsequent clinicopathologic study revealed their association with aggressive pathologic features, *KRAS* mutations and unfavourable prognosis in 261 stage I-II lung adenocarcinomas (12).

Subsequently, Kadota and colleagues proposed the current form of airspace invasion "tumor spread through airspaces" (STAS) and designated the possibility of airspace invasion as a new pattern of invasion in 2015 (13). They defined STAS as the spread of tumor cells (as micropapillary structures, solid nests, and/or single cells) within airspaces

in the lung parenchyma beyond the edge of the main tumor, even if it existed only in the first alveolar layer from the tumor edge (*Figure 1*), and found STAS in 38% of 144 resected, small stage I lung adenocarcinomas. Upon correlating STAS with clinicopathologic features, STAS-positive tumors were more likely to show, lymphovascular invasion, vascular invasion, micropapillary pattern, solid pattern and less lepidic pattern in the main tumor, and the presence of STAS was associated with an increased risk of any types of recurrence in sublobar resections. Further, STAS was an independent and the only risk factor of any recurrence (HR, 3.08; P=0.014) in multivariate analysis in the sublobar resection cohort, while STAS was not associated with an increased risk of any recurrence [5-year cumulative incidence of recurrence (CIR), 12.7% *vs.* 9.5%; P=0.50] in the lobectomy cohort. The overall findings suggest that the presence of STAS indicates a higher risk of recurrence in small lung adenocarcinomas and that limited resection may not be sufficient for those with STAS (13).

STAS and its prognostic implications in lung cancer

Since the original report of STAS, there have been multiple studies that reported the prevalence and prognostic significance of STAS and its equivalent mostly in lung adenocarcinoma (*Table 1*) (8,10,13-26). Although the majority of reported studies have focused on early stage lung adenocarcinoma, similar findings have also been demonstrated in late stage adenocarcinoma. For instance, Warth and colleagues showed that the presence of STAS had significantly reduced recurrence free survival (RFS) and overall survival (OS) at any stage, and is more commonly seen in high stage, node positive adenocarcinomas with distant metastases (14). Another study by Terada and colleagues showed that STAS was a significant risk factor for recurrence even in stage III adenocarcinoma (27). Further, Dai and colleagues showed that stage IA adenocarcinoma with STAS had RFS and OS comparable to stage IB adenocarcinoma, suggesting not only the aggressive biology but also the implications of STAS on the future of T staging (18).

The reported frequency of STAS in NSCLC ranges from 15–56% depending on tumor stages included in the cohort (*Table 1*), and the vast majority of the studies showed association of STAS with micropapillary and/or solid patterns and less lepidic pattern in the main tumor and other adverse pathologic features. Several studies also reported higher rates of *KRAS* or *BRAF* mutations, *ALK* or *ROS1* rearrangements and wild-type *EGFR* in lung
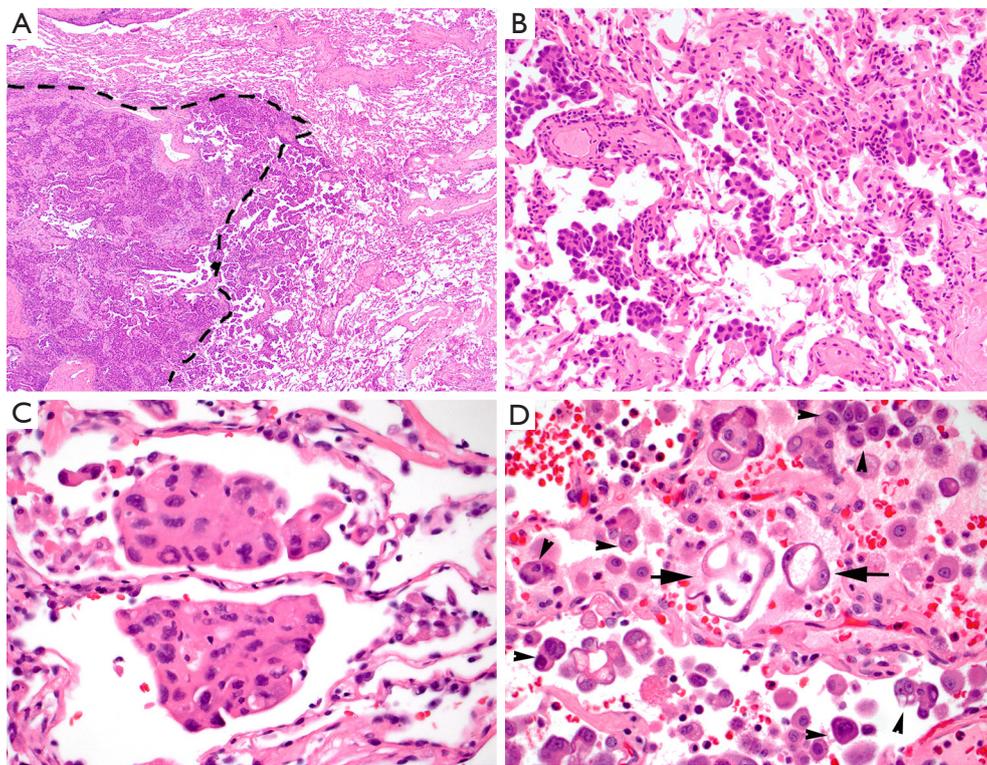
Figure 1 Histology of tumor spread through air spaces (STAS). STAS is classified into micropapillary, solid nest and single cell patterns. More than one pattern of STAS is often seen in one case, and micropapillary STAS is most common in lung adenocarcinoma. The micropapillary pattern (A,B) is characterized by small free-floating clusters of tumor cells with no fibrovascular core beyond the border of the tumor (dotted line in A). The solid nest pattern (C) shows large nests of tumor cells that are free-floating in alveolar spaces, in a pattern reminiscent of tumor islands. The single cell pattern (D) shows scattered highly atypical single cells (arrowheads) admixed with intra-alveolar macrophages with low-grade cytology. Of note, ring-like structures (arrows in D) are classified into the micropapillary pattern. Hematoxylin and eosin stain, original magnification: (A) ×20; (B) ×200; (C&D) ×400.

adenocarcinomas with STAS (12,14,21,26,28). Importantly, almost all studies have confirmed the association of STAS with shorter RFS and/or shorter OS. Consequently, STAS was included as an exclusion criterion along with lymphovascular and pleural invasions for adenocarcinoma in situ (AIS) and minimally invasive adenocarcinoma (MIA) in WHO 2015 (1).

STAS has also been described in other primary lung malignancies, including squamous cell carcinoma (SCC), neuroendocrine tumors and pleomorphic carcinoma (*Figure 2*). STAS in primary lung SCC was first characterized by Lu and colleagues in a study of 445 patients, in which the presence of STAS was associated with significantly higher cumulative incidence of relapse (CIR). Interestingly, however, there was no difference in overall survival (29). An additional study by Kadota and colleagues confirmed that STAS was an independent predictor of RFS in all stages of

SCC (30); however, another study has suggested that it is a useful predictor only in stage I tumors (31). There is also recent literature on primary lung neuroendocrine neoplasms (32-34). Of those, Aly and colleagues retrospectively studied 487 neuroendocrine tumors, including typical carcinoid, atypical carcinoid, large cell neuroendocrine carcinoma (LCNEC), and small cell carcinoma (SCLC) (33). Histologic review showed that 26% of these patients had evidence of STAS, which was associated with distant metastasis and a higher CIR and lung cancer-specific cumulative incidence of death (LC-CID) overall (independent of histologic subtype). Further, they found STAS in LCNEC and SCNEC to be an independent poor prognostic factor, while Toyokawa and colleagues reported high (83%) frequency of STAS identified in SCLC but no significant differences in RFS and OS between patients with no/low STAS and those with high STAS (32).

Table 1 Selected studies on lung adenocarcinoma *vs.* STAS (or its equivalent)

First author	Year of publication	Number of subjects and histology	Tumor stage	Prevalence	RFS[#]	OS[#]
Kadota (13)	2015	411 ADC	I (≤2 cm)	38%	Increased CIR	N/A
Warth (14)	2015	569 ADC	I–IV	Limited STAS: 21.6%, extensive STAS: 29.0%	Decreased irrespective of etent, but not significant by MVA	Decreased irrespective of extent, but not significant by MVA
Morimoto (15)*	2016	444 ADC	I–IV	46.3% of tumors w/mPAP pattern	Decreased: HR 2.13 in tumors with mPAP pattern	N/A
Shiono (16)**	2016	318 ADC	I	14.8%	Decreased: HR 2.24	Decreased: HR 2.40
Uruga (17)	2017	208 ADC	I (≤2 cm)	Low: 18.3%, high: 29.3%	Decreased: HR 7.35 for high vs. no, decreased: HR 4.45 for high *vs.* low	Not significant by MVA
Dai (18)	2017	383 ADC	IA	30.3%	Decreased: HR 2.36 for tumors 2–3 cm	Decreased: HR 3.94 for tumors 2–3 cm
Masai (8)	2017	508 NSCLC treated w/sublobar resection	IA	15.0%	Decreased: HR 11.24 for local recurrence	N/A
Yi (10)***	2018	368 ADC	0–IB	N/A	Decreased: HR 3.20	N/A
Toyokawa (19)	2018	276 ADC	I	Low: 17.4%, high: 38.0%	Decreased: HR: 3.27 for any STAS vs. no STAS	Decreased: HR: 5.67 for any STAS vs. no STAS
Shiono (20)	2018	514 NSCLC	IA	20.2%	Decreased: HR 3.11 for sublo-bar resection only	Decreased: HR 3.17 for sublobar resection only
Lee (21)	2018	316 ADC	I–III	50.6%	Decreased	Not significant by MVA
Yang (22)	2018	242 ADC w/ radical lobectomy	IA–IB	33.5%	Decreased: HR 2.80 for tumors 2–4 cm	Decreased: HR 4.50 for tumors 2–4 cm
Qiu (23)	2019	208 ADC w/ lobectomy	I–IIIA	51.4%	Decreased: HR 1.76	Decreased: HR 3.39
Shiono (24)	2019	848 NSCLC including 638 ADC	I	16.4%	Decreased: HR 1.48	N/A
Ren (25)	2019	752 ADC	I	29.9%	Decreased: HR 3.53 for sublo-bar resection	Decreased: HR 4.55 for sublobar resection
Kadota (26)	2019	735 ADC	I–IV	34.0%	Decreased: HR 5.33 for all, decreased: HR 6.87 for stage I	Decreased: HR 2.32 for all, decreased: HR 2.85 for stage I

STAS, tumor spread through air spaces; ADC, adenocarcinoma; NSCLC, non-small cell lung cancer; mPAP, micropapillary; N/A, not available; RFS, recurrence free survival; CIR, cumulative incidence of recurrence; HR, hazard ratio; OS, overall survival; MVA, multivariate analysis. *Free tumor clusters; **Aerogenous spread with floating cancer cell clusters (ASFC)/STAS; ***Aerogenous spread; [#]Results are based on MVA.

Recently, the first systematic reviews and meta-analyses of STAS were published, and both confirmed that the presence of STAS was associated with shorter RFS and OS with an absent to moderate heterogeneity between studies (35,36). Subgroup analysis by tumor histology revealed that STAS associated with adenocarcinoma had shorter OS, but interestingly, STAS associated with SCC or pleomorphic carcinoma did not demonstrate a shorter OS, attributed in part to the older age of the patients (29,35). Nevertheless, it has become clear that STAS is a very important prognostic
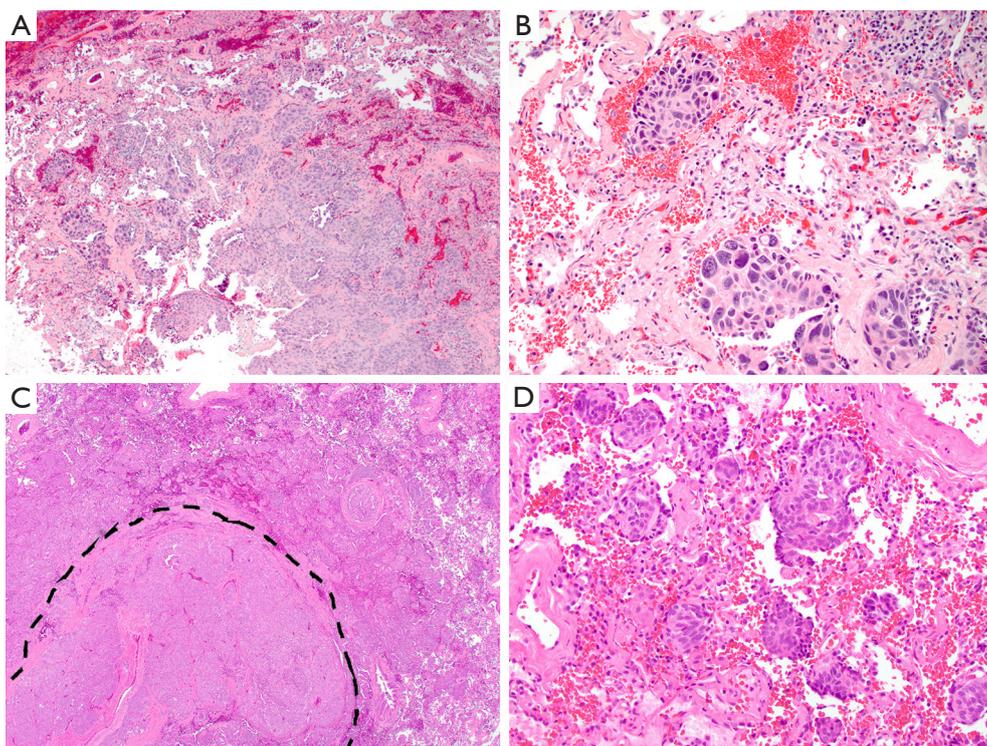
Figure 2 Tumor spread through air spaces (STAS) in other histologic subtypes. An example of STAS seen in squamous cell carcinoma (A,B) and that in carcinoid tumor (C,D) are shown. The periphery of the carcinoid tumor is outlined in black (C). Of note, the solid nest pattern of STAS is typically seen in squamous cell carcinoma and neuroendocrine tumors. Hematoxylin and eosin stain, original magnification: (A) ×40; (B) ×200; (C) ×20; (D) ×200.

factor across lung adenocarcinoma of all stages (*Table 1*), and is likely important in other histologic subtypes of lung carcinoma as well.

Clinical implications of STAS in stage IA NSCLC

Lobectomy has been the standard surgical procedure for clinical stage IA NSCLC (37); however, sublobar resection, including wedge resection and segmentectomy, is considered an acceptable alternate for comorbid patients to preserve pulmonary function (38). Further, the recent advancement in imaging studies has resulted in increased detection of early stage lung cancers, either incidental or thorough low-dose CT lung screening (38-40). Subsequently, although the evidence is still insufficient, limited resection has increasingly been applied for good-risk patients with early stage NSCLC as well as for poor-risk patients (5-7).

Importantly, a recent large cohort study by Eguchi and colleagues has shown that sublobar resection is suboptimal for T1 lung adenocarcinoma with STAS from the oncology perspective (41). They evaluated multiple clinicopathologic variables including STAS in propensity score-matched lobectomy (n=349) and sublobar resection (n=349) cohorts and reported that the presence of STAS was associated with higher rates of recurrence and cancer-specific death in the sublobar resection cohort but not in the lobectomy cohort, irrespective of surgical margin clearance in patients with STAS. The findings are in line with those of the initial study by Kadota and colleagues (13) and more recent literature in early stage tumors (13,20,41). However, other studies have not found a survival difference between sublobar resections and lobectomies (15,19,20,42).

Nevertheless, some surgeons recommend that the patient with small T1N0M0 adenocarcinoma undergo subsequent completion lobectomy when STAS is found in a sublobar resection specimen (personal experience and communication with thoracic surgeons at a few US academic centers). This clinical scenario is not extremely uncommon given that sublobar resection has been increasingly applied for patients with small, node negative lung adenocarcinoma, and tumor

size (≤2 *vs*. >2 cm) plays a significant role in preoperative decision making on the type of resection (sublobar resection *vs*. lobectomy) to perform (6).

In the aforementioned study, Eguchi and colleagues reported an optimal performance of frozen section in identifying STAS with the overall sensitivity of 71%, specificity of 92%, overall agreement of 75% and substantial interobserver concordance (41). The findings imply that FS analysis may be useful to detect STAS and aid intraoperative decision making on the most appropriate type of resection for a patient with early-stage lung adenocarcinoma. Thus, thoracic surgeons in many academic institutions (at least in the US) now request the pathologist to evaluate the presence or absence of STAS (along with histology, resection margin status, etc.) at the time of intraoperative consultation. This has made the pathologist realize significant clinical implications of the STAS diagnosis including that on frozen section. However, to implement STAS in routine diagnosis/lung cancer synoptic report as well as frozen section diagnosis, there are multiple issues to address. First, there have been controversies as to whether STAS is a real biological phenomenon or merely represents *ex vivo* artifacts. In fact, some pathologists do not believe in the biological relevance of STAS; thus, they do not diagnose it. Second, the definition of STAS has not been established, while it is important to have the universally accepted gold standard to achieve adequate interobserver concordance. Third, only limited and conflicting data are available to assess the performance of FS on the diagnosis of STAS.

Controversies on STAS *vs. ex vivo* artifacts

There have been controversies as to whether any or all of the free-floating tumor cell clusters identified as STAS are actually *ex vivo* artifactual. There are multiple potential mechanisms that may lead to the development of free floating tumor cells *ex vivo* that complicate interpretation of STAS (43,44). Some of these are routine artifacts specific to lung specimens, including surgical collapse, but the most notable is the possibility that the tumor cell clusters can be spread through knife cuts made at the time of specimen processing. The concept of spread through a knife surface (STAKS) was first introduced by Thunnissen and colleagues, who showed that artifactual carryover after a knife cut is a real phenomenon that increases free-floating tumor clusters with each sequential cut, if the knife is not cleaned between the cuts (44). Subsequently, Blaauwgeers and colleagues have shown in their multi-institutional

study that the number of free-floating tumor cell clusters increased in sequential sections with the same prosecting blade and that the vast majority of the free-floating tumor cell clusters could be explained by mechanical forces associated with tissue handling (43).

However, there are multiple conditions, not only *ex vivo* but also *in vivo*, that could generate mechanical forces leading to the detachment of tumor cell clusters including palpation by the surgeon at the time of operation and retrieval of the specimen through a small caliber of VATS port as well as knife cuts during tissue processing. Even high flow velocities inherent to the physics of breathing may lend themselves to the detachment of tumor cell clusters, and once the clusters appear in airspaces beyond the border of the tumor, they are considered free-floating tumor clusters (STAS or "artifacts"). Interestingly, Isaka and colleagues detected tumor cell clusters in one fifth of airway secretion cytology specimens collected from the segmental or lobar bronchus of resected lung adenocarcinomas and squamous cell carcinomas. Further, the positive airway secretion cytology was only seen in tumors with histologically confirmed STAS, and the morphology of STAS was similar to that of the tumor cell clusters in the airway secretion (45). Ren and colleagues also identified isolated tumor cell lusters in the residual lung parenchyma in 9% of simulated segmentectomy specimens that were made from lobectomies for stage 1A adenocarcinoma (25). These findings support the notion that free-floating tumor cell clusters could be generated by palpation by the surgeon at the time of operation.

Given the fact that air spaces are surrounded by a capillary network in the alveolar interstitium in the normal lung, the free-floating clusters, if they remain in the residual lung parenchyma, could survive and may become a new nidus for neoplastic proliferation/tumor recurrence. In general, STAS may be considered akin to the findings in invasive mucinous adenocarcinoma in which the presence of abundant extracellular mucin serves as a fluid conduit for the spread of neoplastic cells to more distant locales (1). Although the tumor cells of STAS do not produce the medium for spread by themselves, it is conceivable that these free-floating tumor clusters can also remain viable in the alveolar space for extended periods of time, given that alveolar macrophages can survive within air spaces for up to 40 days (46). Further, a recent study using multiplex immunofluorescence has shown tumor clusters attached to alveolar walls away from the main tumor and close proximity to an alveolar capillary (vessel co-option) (47). We
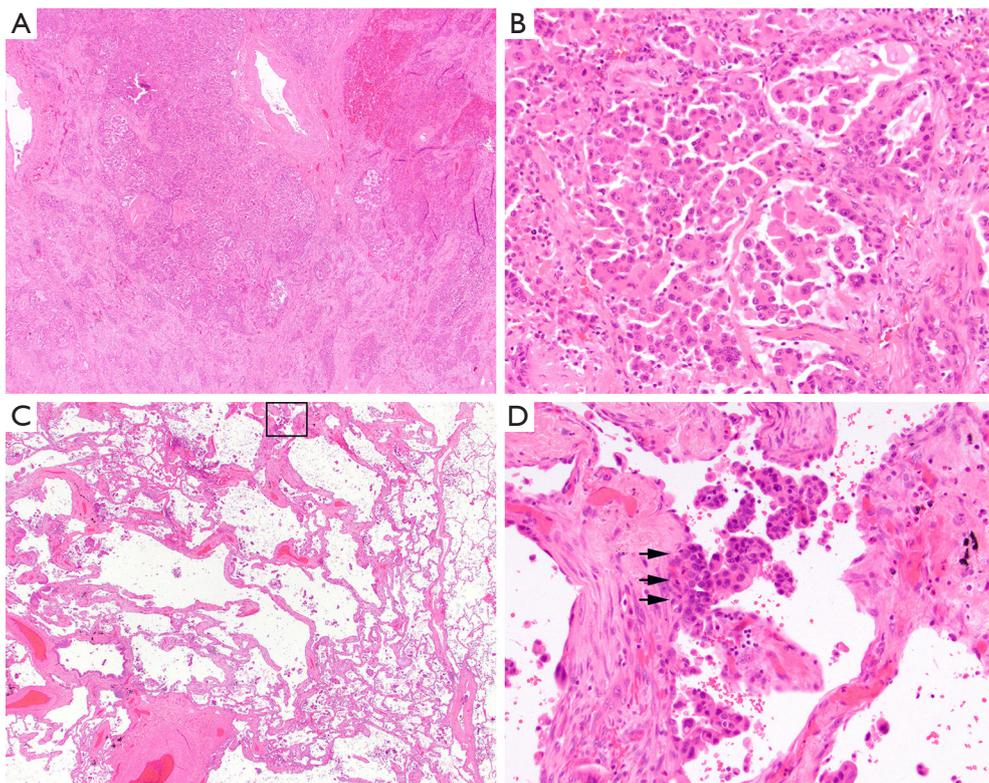
Figure 3 Re-implantation of STAS with stromal response. An example of micropapillary adenocarcinoma with abundant STAS is shown. (A,B) The main mass exhibits predominant micropapillary pattern. (C) In the section away from the main tumor, there are scattered STAS with micropapillary pattern or small solid nests in the background of emphysematous changes with alveolar wall thickening. (D) A higher magnification of the boxed area in C reveals a focus with micropapillary pattern or small nests of tumor cells in close proximity to and focally attached to the alveolar wall (arrows) associated with organizing fibrosis suggestive of stromal reaction to re-implantation of STAS. Hematoxylin and eosin stain, original magnification: (A) ×20; (B); ×200; (C) ×20; (D) ×200.

have also experienced cases demonstrating re-attachment of tumor clusters to normal alveolar walls away from the main tumor with associated stromal reaction (*Figure 3*).

Now, it is reasonable to think that the differentiation of free-floating tumor cell clusters that developed before resection of the tumor (*in vivo*) and those generated after resection (*ex vivo*) would have significant clinical implications. However, in the opinion of the author, the question of how much of this morphology might represent artifact and when it is generated is a somewhat academic one. The fact remains that multiple studies (that made reasonable efforts to differentiate STAS from obvious processing artifacts) have shown that the presence of STAS can be used at least as a surrogate for aggressive behavior and has often been identified as an independent prognostic factor (*Table 1*). The prognostic significance would be difficult, if not impossible, to explain by artifact alone.

Further, given the association of STAS and micropapillary or high-grade patterns in the main tumor, the possibility that the processing artifacts may be related to the inherent discohesiveness ("invasiveness") of the tumor cells.

Differentiation from *ex vivo* artifacts

Now one of the most important issues associated with STAS for the pathologist is to differentiate STAS from "real" artifacts made by strenuous mechanical forces generated by knife cuts during tissue processing. In order to help the differentiation, Kadota (13) and Aly (33) have described several features of artifacts as exclusion criteria of STAS: (I) mechanically dissociated tumor floaters (clusters of tumor cells with a ragged-edge, located randomly and/or located at the edge of the tumor section) (*Figure 4A,B*); (II) normal benign pneumocytes or bronchial cells (*Figure*
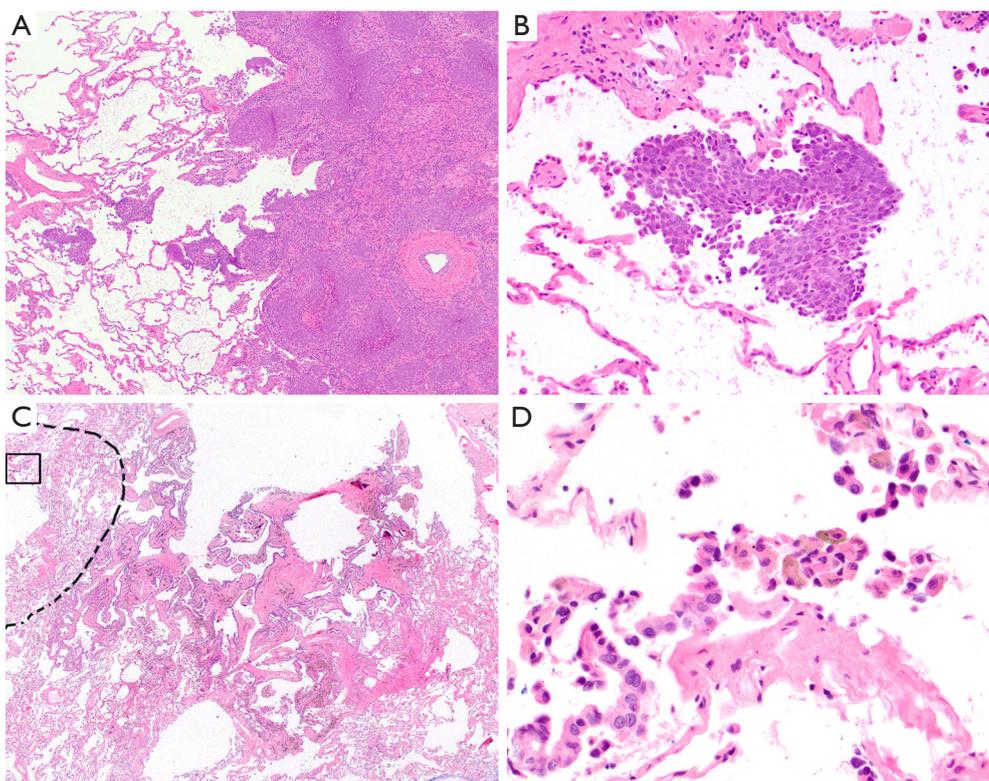
Figure 4 Histology of mechanical artifacts. (A) An example of non-keratinizing squamous cell carcinoma demonstrating large tumor nests away from the tumor border. (B) A high-power magnification of A reveals jagged edges of the nest consistent with a mechanical artifact. (C) Adenocarcinoma with predominant lepidic and focal acinar patterns in the background of emphysema. There are multiple small clusters of epithelial cells away from the tumor border (dotted circle). (D) A higher magnification of the boxed area in C reveals strips of benign respiratory epithelial cells admixed with alveolar macrophages. Some epithelial cells exhibit reactive changes that may be confused with tumor cells, in particular, at the time of intraoperative consultation. The linear configuration and association with blue ink are useful features for differentiating artifacts from STAS. Hematoxylin and eosin stain, original magnification: (A) ×20; (B); ×200; (C) ×40; (D) ×400.

4C,D); (III) strips of tumor cells detached from alveolar walls or stroma due to poor preservation; (IV) isolated tumor clusters distantly situated away from the tumor not in a continuous manner. They also considered the presence of a single focus of STAS in the entire tumor as an artifact. Additionally, Warth and colleagues emphasized the presence of loose small groups and distribution consistent with the overall configuration of the circumferential tumor edge in differentiating STAS from artifacts (14). STAS may occasionally be somewhat difficult to distinguish from intra-alveolar macrophages, and the distinction should be made on the bases of nuclear-to-cytoplasmic ratio and degree of nuclear atypia (*Figure 1D*). In difficult cases, an immunohistochemical stain for cytokeratin and/or that for CD68/CD163 may be helpful to make the distinction. Similarly, detached fragments of benign respiratory epithelium may exhibit reactive atypia and mimic tumor cells. They may be confused with STAS, in particular, at the time of frozen section (*Figure 4C,D*). The linear configuration is a useful feature for differentiation of an artifact from STAS in this context.

Definition of STAS

The evaluation of STAS in the literature has been somewhat limited by some variability in definitions that have been applied. While the majority of studies have adopted the original description by Kadota and colleagues that defined STAS as micropapillary clusters, solid nests, and/or single cells within airspaces in the lung parenchyma beyond the edge of the main tumor (13), minimum number of tumor cells and/or clusters and the necessary distance from the

Table 2 Various definitions of STAS applied in selected studies

First author	Publication year	Terminology used	Number of clusters/cells	Distance from the main tumor
Kadota (13)	2015	STAS	N/A	Beyond the edge of the main tumor
Warth (14)	2015	STAS	Small solid cell nests (at least 5 tumor cells)	Limited: ≤3 alveolae away, extensive: >3 alveolae away
Jin (28)	2015	Aerogenous spread	N/A	At least one 40× field away
Morimoto (15)	2016	Free tumor clusters	More than 3 clusters containing <20 nonintegrated micropapillary tumor cells	>3 mm apart from the main tumor
Shiono (16)	2016	Aerogenous spread with floating cancer cell clusters (ASFC)/STAS	N/A	At least 0.5 mm from the main tumor
Uruga (17)	2017	STAS	Low: 1–4 single cells or clusters, high: ≥5 single cells or clusters	Beyond the edge of the main tumor
Dai (18)	2017	STAS	N/A	Beyond the edge of the main tumor
Masai (8)	2017	STAS	N/A	At least one alveolus away from the main tumor
Yi (10)	2018	Aerogenous spread	N/A	At least one 40× field away
Toyokawa (19)	2018	STAS	Low: 1–4 single cells or clusters, high: ≥5 single cells or clusters	Beyond the edge of the main tumor
Shiono (20)	2018	STAS	More than a few clusters	At least 0.5 mm from the main tumor
Lee (21)	2018	STAS	N/A	Beyond the edge of the main tumor
Yang (22)	2018	STAS	N/A	Beyond the edge of the main tumor
Shiono (24)	2019	STAS	More than a few clusters	At least 0.5 mm from the main tumor
Ren (25)	2019	STAS	N/A	Beyond the edge of the main tumor
Kadota (26)	2019	STAS	N/A	Beyond the edge of the main tumor

STAS, tumor spread through air spaces; N/A, not available.

border of the main tumor have not been so well-established. For instance, various studies have used distances from the first alveolar layer beyond the tumor edge, to a few alveolar spaces, to at least 3 mm from the main tumor (*Table 2*) (8,10,13-22,24-26,28). Importantly, however, the variation in definition has not hampered agreement across multiple studies that there is an association between STAS and decreased RFS and/or OS (*Table 1*).

In contrast, the amount of STAS appears to be significant. Recent studies by Uruga and colleagues and Toyokawa and colleagues used a semi-quantitative approach to assess the amount of STAS in the average ×200 microscopic field as no STAS; low STAS (1–4 tumor cell clusters in tumors with micropapillary or solid STAS, or 1–4 tumor cells in single-cell predominant STAS); and high

STAS (≥5 tumor cell clusters in tumors with micropapillary or solid STAS, or ≥5 tumor cells in single-cell predominant STAS) (17,19). Both studies found low STAS in 17–18% and high STAS in 29–38% of stage 1 lung adenocarcinomas. Uruga and colleagues also reported the association of high STAS with pleural invasion, lymphovascular invasion, and a solid-predominant pattern of growth, while both showed a significant association between increased STAS and shorter RFS by univariate analysis, and STAS being a significant predictor of RFS by multivariate analysis (17,19).

There is only limited data on interobserver concordance for the diagnosis of STAS; however, the globally accepted definition of STAS, if established, would improve interobserver agreement on STAS and facilitate the implementation of STAS diagnosis in routine pathology practice.
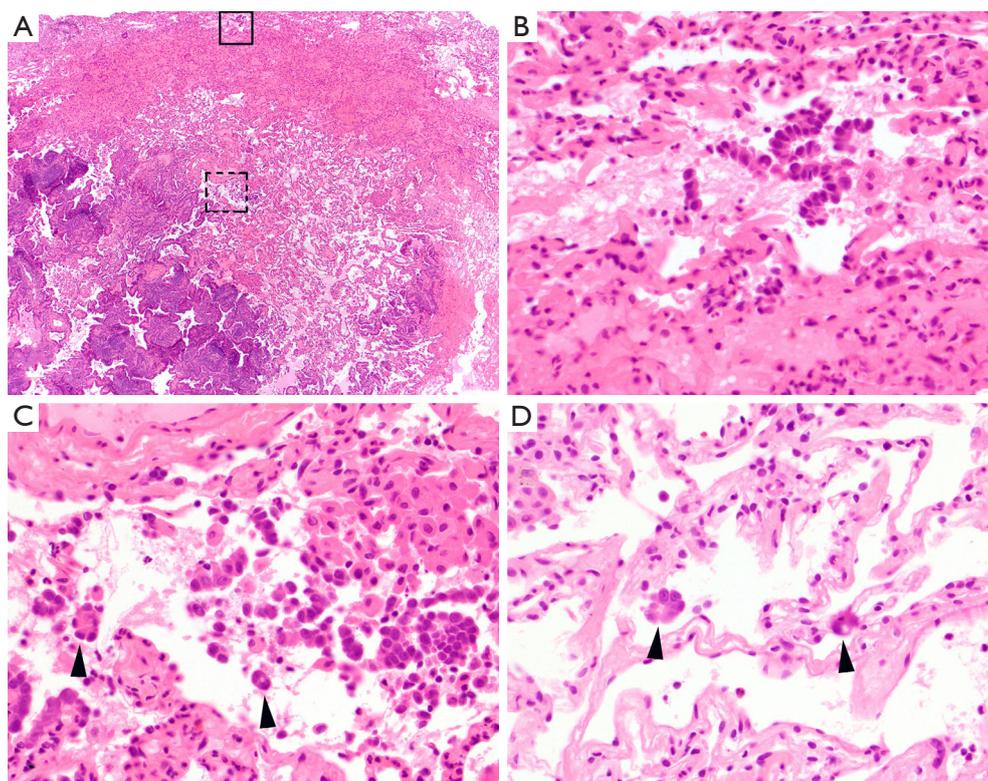
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Figure 5 Abundant artifacts in frozen section and frozen permanent section slides. (A) A frozen permanent slide of adenocarcinoma with predominant lepidic and papillary patterns exhibiting multiple free-floating clusters in the airspaces away from the tumor border. (B) A higher magnification of the boxed area of A at the edge of the section shows a few strips of tumor cells consistent with an artifact. (C) A higher magnification of the dotted box area of A reveals micropapillary cluster of or single tumor cells (arrowheads) admixed with strips of tumor cells and alveolar macrophages. (D) Additionally, rare isolated tumor cells with micropapillary pattern (arrowheads) are identified in the separate area away from the tumor border. The presence of abundant artifacts, in particular in the setting of frozen section, may hamper an optimal assessment for STAS and interobserver agreement. Hematoxylin and eosin stain, original magnification: (A) ×20; (B); ×200; (C) ×400; (D) ×400.

Performance of frozen section on the diagnosis of STAS

While the aforementioned study by Eguchi indicates the potential utility of frozen section for the intraoperative assessment of STAS, given its high sensitivity, excellent specificity and substantial interobserver concordance on STAS diagnosis (41), Walts and colleagues reported rather discouraging results. In their study with 48 stage I-II lung adenocarcinoma resections, they found 50% sensitivity and only 8% negative predictive value of frozen section in identifying STAS, while its positive predictive value was 100% (48). They suggest that the selection of samples including a larger portion of nonneoplastic tissue adjacent to the tumor and/or microscopic examination of additional deeper levels prepared from the frozen

tissue block are important in a confident designation of STAS. These processes are not typically feasible at the time of frozen section, however. Further, Morimoto and colleagues clearly state that the diagnosis of STAS may only be possible on permanent section since, in frozen section, the lung is not sufficiently inflated to optimally assess detached tumor cells/clusters in airspaces (15). In our experience, frozen section is highly specific but not sensitive for the diagnosis of STAS in early stage lung adenocarcinoma, with only fair interobserver agreement at frozen section. We also found frequent processing artifacts in frozen section (and frozen permanent) slides with some along with STAS, possibly leading to the low sensitivity and fair interobserver agreement of the STAS diagnosis (*Figure 5*) (unpublished data). Disagreement in the findings in current literature regarding the ability of pathologists

to confidently identify STAS suggests interinstitutional variation in its interpretation. While it appears premature to globally implement the assessment of STAS at frozen section, pathologists may elect to comment on the presence of unequivocal STAS when seen at frozen section. It is important, however; thoracic surgeons should be advised that there has been no prospective data to confirm that this information is useful to help stratify patients for lobectomy versus sublobar resections intraoperatively (48).

Conclusion

Irrespective of how the detached tumor cells/clusters are generated, STAS is biologically important and is associated with unfavorable patient outcomes in not only lung adenocarcinomas but also in other types of lung cancer. STAS has significant clinical implications; thus, we, pathologists, need to recognize and appropriately report STAS. Therefore, establishment of the globally accepted definition, confident differentiation from processing artifacts and standardization in reporting of STAS including at the time of intraoperative consultation are warranted.

Acknowledgments

Footnote

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