Ex Vivo Artifacts and Histopathologic Pitfalls in the Lung

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• ***Context.***—**Surgical and pathologic handling of lung physically affects lung tissue. This leads to artifacts that alter the morphologic appearance of pulmonary parenchyma.**

Objective.—**To describe and illustrate mechanisms of ex vivo artifacts that may lead to diagnostic pitfalls.**

Design.—**In this study 4 mechanisms of ex vivo artifacts and corresponding diagnostic pitfalls are described and illustrated.**

Results.—**The 4 patterns of artifacts are: (1) surgical collapse, due to the removal of air and blood from pulmonary resections; (2) ex vivo contraction of bronchial and bronchiolar smooth muscle; (3) clamping edema of open lung biopsies; and (4) spreading of tissue fragments and individual cells through a knife surface. Morphologic pitfalls include diagnostic patterns of adenocarcinoma, asthma, constrictive bronchiolitis, and lymphedema.**

Conclusion.—**Four patterns of pulmonary ex vivo artifacts are important to recognize in order to avoid morphologic misinterpretations.**

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Normal lung histology including usual tissue artifacts is well described.[1] Unfortunately, virtually all writings and images are concerned with "static" morphologic findings. Recently, attention has been focused on dynamic changes in the lung that cause a pitfall in the diagnosis of pulmonary papillary adenocarcinoma.[2] It is certain that when one concentrates on a specific problem, one may not notice other, perhaps peripheral, issues. This "inattentional blindness" can occur in the interpretation of lung biopsies, especially when the pathologist focuses only on a particular aspect of a tissue sample and in doing so neglects other findings or even the possibility that the morphology has been altered by artifact(s).[3] Ex vivo artifacts in lung samples have not been systematically investigated and it is useful to describe in detail possible mechanisms associated with several obvious changes. During the dynamic process of pulmonary resection and tissue handling several ex vivo artifacts occur, which have an impact on the histologic appearance of the lung tissue. The aim of this study is to describe and illustrate 4 mechanisms of ex vivo artifacts, namely, "surgical collapse," "ex vivo contraction," "clamping edema," and "spreading through a knife surface." These 4 mechanisms individually or in combination manifest with morphologic patterns that may lead to diagnostic pitfalls.

MECHANISMS OF EX VIVO ARTIFACTS

Surgical Collapse

The lung is usually deflated during segmentectomy, lobectomy, and pneumonectomy, causing so-called surgical atelectasis or collapse (Figure 1, A). Deflation of the surgical lung is achieved by double-lumen endotracheal intubation, allowing selective ventilation of the nonsurgical lung.[4,5] Surgical collapse also affects the pulmonary vasculature with emptying of the blood and lymph vessels. During removal of the lung, the physiologic negative pressure between the visceral and parietal pleura is replaced by atmospheric pressure, leading to additional compression of the removed lung tissue. Manual pressure applied by the surgeon(s) may also contribute. Thus, surgical collapse encompasses 3 components. In the time frame of inspiration and expiration, approximately 3 to 4 seconds, 250 ml of air flows in and out of each lung. Interestingly, the deflation process during surgery takes longer. This is in part due to the "columns" of lymph in the superficial and deep lymphatic vessels. Emptying of the lymph into the draining lymph nodes is a slower process (minutes) compared to inspiration and expiration.

Importantly, during this process of surgical collapse not all lung parts may collapse in a similar manner. If, in addition to the abovementioned 3 aspects, adenocarcinoma in situ or atypical adenomatous hyperplasia is present, then the alveolar walls are more rigid. This reduction in compliance causes a slight reduction in lung collapse, compared with adjacent uninvolved areas. Inspection of the lung during collapse may reveal a focally elevated pleural surface. This finding may help the surgeon identify the location of the underlying lesion and guide clamp and staple gun place-

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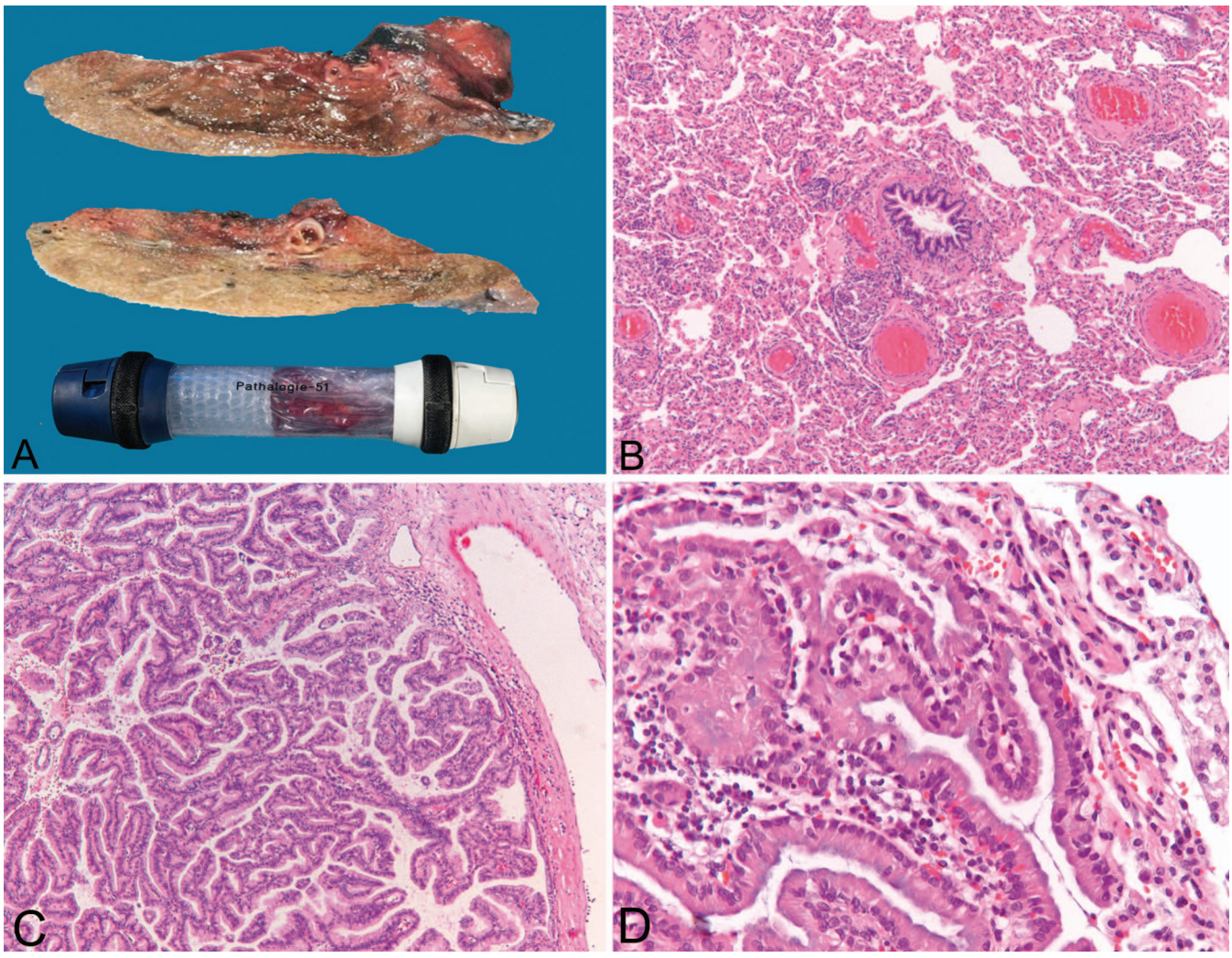

Figure 1. *Images supporting the hypothesis of the collapse artifact. A, Lower panel: lung resection specimen in vacuum transport cassette (diameter 5 cm); upper panel: 2 gross slices of collapsed lung. The width of each slice is approximately one-third the width of the thoracic cavity. B, Overview of collapsed peripheral lung tissue. C and D, Adenocarcinoma in situ in collapsed lung, mimicking papillary carcinoma (hematoxylin-eosin, original magnifications ×2.5 [B], ×5 [C, center], and ×20 [D, periphery]).*

ment. This observation may be especially useful in cases of small nonpalpable lesions.

Surgical collapse of peripheral lung leads to approximation of the alveolar walls, possibly providing the impression of increased cellularity and more extracellular matrix. This pitfall should not be confused with interstitial fibrosis.[1] This artifact can be highlighted with immunohistochemical staining for CK (cytokeratin) (eg, CK 7) and endothelium markers (eg, CD31), or with an elastic stain.

Peripheral lung collapse may give rise to 3 pitfalls in the diagnosis of adenocarcinoma. First, depending on the amount of diminished air, the collapsed alveoli may show a pseudopapillary pattern (Figure 1, B). If the alveolar walls are covered with tumor cells (ie, lepidic pattern), this may be mistaken for a papillary carcinoma[2,6] (Figure 1, C and D). However, in 3 dimensions, these "papillae" are connected with other fragments above or beneath the plane of section, indicating that the stromal components of these "pseudopapillae" are actually the stromal components of preexisting alveolar walls. The elastin stain is helpful in this instance, since the identification of elastin in these thin alveolar walls points to the underlying architecture of peripheral lung tissue.[2] Conceptually, in statistical terms the probability of finding a straight papilla in a section of peripheral lung is low, probably as close as the probability of finding a completely straight hair in a histologic section of the skin. The probability of finding 15 papillae in 1 microscopic field of view is much smaller, providing an additional argument that the proliferation represents cross-sections of lepidic pattern as opposed to true papillae. In our opinion in case of thin "papillae" the presence of (frequently discontinuous) elastin fibers is diagnostic of lepidic pattern and supports in the distinction of a papillary carcinoma.[2,7]

A second pitfall may occur if preexisting fibrosis is present adjacent to lepidic patterns of adenocarcinoma. This fibrosis may be collagenous or of the infarction type with abundant fibroelastosis. Owing to the partial collapsed irregular alveolar walls, structures looking like irregular acini may mimic acinar adenocarcinoma, also seen in an earlier reproducibility study among 28 pathologists[6] (Figure 2, A through F; images not published with previous study). This may introduce the possibility of an erroneous diagnosis of invasive adenocarcinoma adjacent to a fibrotic area.[6] Moreover, in lung cancer resection specimens with smok-

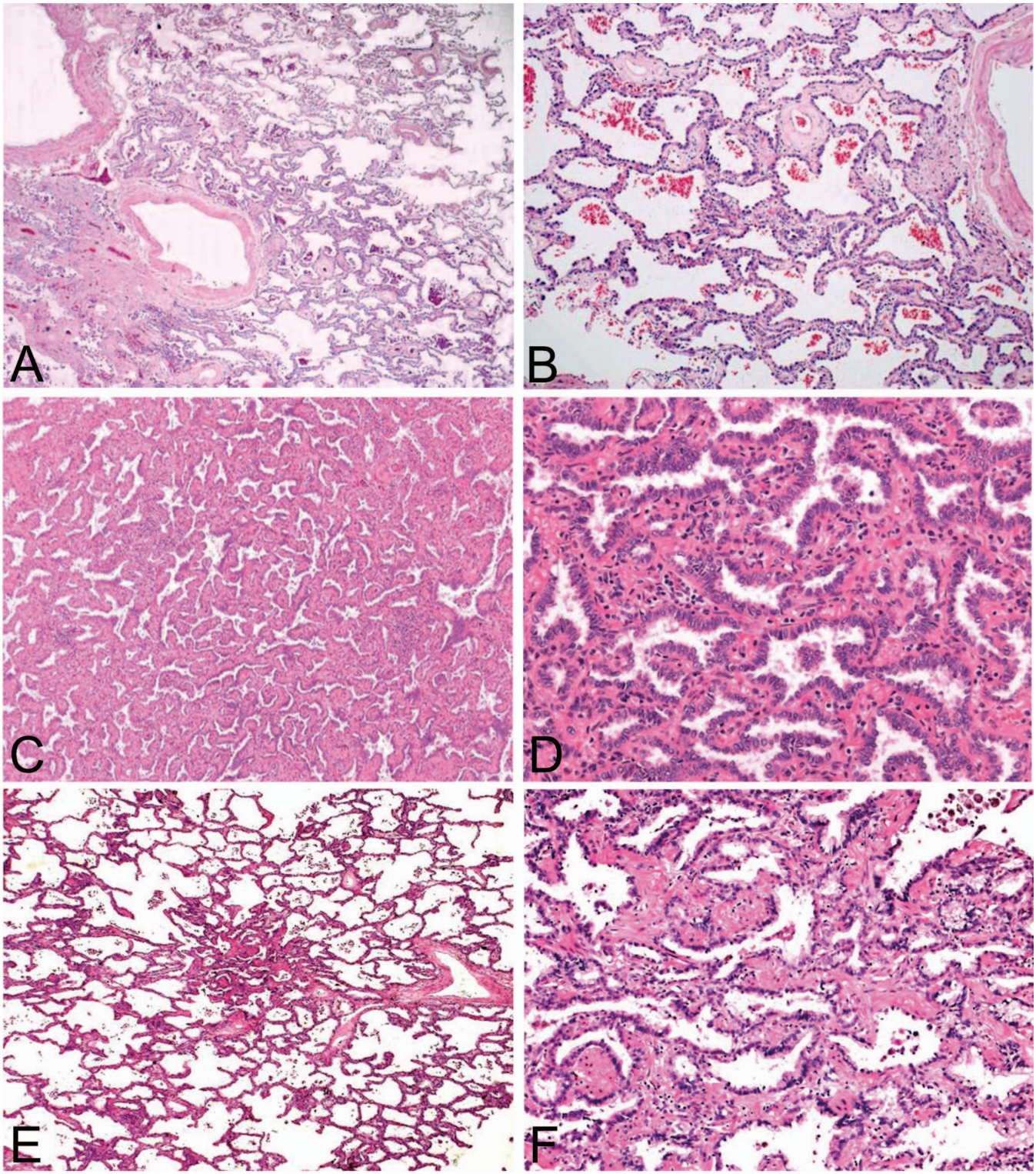

Figure 2. *An increase in surgical collapse parallels reduction in the amount of air, which is associated with greater doubt about presence or absence of invasion. Note the irregular shape of the epithelial structures and lack of desmoplastic stroma. A and B, Example 1: 7% of the scores, invasion; 7%, doubt; 86%, no invasion. C and D, Example 2: 36% of the scores, invasion; 49%, doubt; 25%, no invasion. E and F, Example 3: 32% of the scores, invasion; 36%, doubt; 32%, no invasion. Note also the presence of alveolar macrophages in compressed alveolar lumen (hematoxylin-eosin, original magnifications ×5 [A, C, and E; overview of case to reveal context]; and ×20 [B, D and F; detailed image used for scoring[6]; images not published with previous study]).*

ing-related lung disease, the stromal changes in remodeled lung should not be a priori attributed to lung cancer, leading one to consider any epithelial atypia as neoplastic. Preexisting scarring should be taken into account as well. Usually this fibrosis consists of dense collagen fibers with scarce fibroblasts.

The third pitfall occurs in partial collapse giving an appearance of "tufting," that is, the piling of the cells in airspaces without fibrovascular core formation. Sometimes this may give the impression of loose cells floating freely in alveolar spaces in close association with a lepidic growth pattern. As tufting is the morphologic hallmark of micropapillary carcinoma, the occasional appearance of this pattern should not be diagnosed as micropapillary carcinoma. Although the minimal amount of micropapillae required for a diagnosis of papillary carcinoma is not established, practical considerations suggest that it should be extensive.[8]

Ex Vivo Smooth Muscle Contraction

The collapse of peripheral lung tissue due to surgical atelectasis has additional morphologic consequences. While the bronchial lumens are essentially unaffected secondary to the presence of cartilaginous rings and plates, bronchiolar luminal patency is greatly affected. In vivo the bronchioles remain under physiologic conditions, expanded by the negative pressure between the parietal and visceral pleura. This "outward" force is transferred by the elastin meshwork between the visceral pleura, alveolar walls, and airways and results in circumferential traction to keep the bronchiolar lumens open. In vivo the actual bronchiolar diameter is a balance between the "outward" tension provided by the elastic fibers and "inward" contraction tension of the smooth muscle cells. At the end of expiration a slightly lower lumen diameter is noted as compared to the end of inspiration.

In the ex vivo situation the outward tension of elastin fibers is abolished, resulting in unopposed smooth muscle forces. Examples of ex vivo smooth muscle contraction are well appreciated in other organs such as uterus (Figure 3, A) and intestinal wall, where the muscle layers of the resected organs contract or shorten as compared to overlying mucosa (not shown). Smooth muscle contraction in the lung leads to a reduction in bronchiolar luminal diameter, with secondary infolding of the mucosa, including the basement membrane and elastin fibers (Figure 3, B and C). The outer diameter of the epithelium, that is, the circumference at the side of the basement membrane, should theoretically be unchanged. It is not clear whether the surface area of underlying connective tissue is unchanged. However, in the presence of a preexisting luminal mucous plug or fibromyxoid connective tissue polyp, the airway lumen is unchanged or only minimally narrowed (Figure 3, D and E). Thus, the bronchiolar epithelium will demonstrate only partial invaginations around the intraluminal material. The smooth muscle contraction was reversible by perfusion fixation (Figure 3, F).

The ex vivo bronchiolar smooth muscle contraction causes prominent narrowing of the bronchiolar lumen, which may give a false impression of constrictive bronchiolitis or other small airway diseases, including asthma.[9–12] This should not be called asthma because of a lack of goblet cell hyperplasia, eosinophilic granulocytes, and thickened basement membrane. In constrictive or obliterative bronchiolitis the airway has luminal narrowing due to subepithelial scarring (ie, between epithelium and smooth muscle cells, which are absent in the ex vivo contraction artifact).[12–16]

The smooth muscle cells in pulmonary blood vessels will also shrink. However, the architecture in the tunica media is different, as it is able to cope in the pulmonary artery with pressure changes between 4 and 30 mm Hg. Ex vivo during the collapse the pulmonary blood vessels will shrink in the longitudinal direction. In the cross-sectional direction the pulmonary artery will shrink ex vivo owing to the difference in structure, looking more like a rubber band without tension: there will be evidence of a (flexible) remaining lumen. This shrinking may be diminished by intimal fibrosis (Figure 3, G).

Clamping Edema

During lobectomies and pneumonectomies, lymph fluid in the lung drains into local and regional lymph nodes. However, during video-assisted thoracoscopic procedures the lymph vessels are not emptied before clamping. The staple gun compresses the lung tissue before adding the 2 rows of staples. This rapid compression squeezes the lymph in the lymph vessel columns both centrally and peripherally. The centrally directed lymph remains in vivo, but the peripherally pressed lymph dilates lymph vessels, leading to interstitial overflow. This is usually most prominent in segmental interstitium, as shown in Figure 4, A and B. This clamping artifact is only seen in video-assisted thoracoscopic specimens. One should not mistake this artifact for either septal edema or lymphatic dilatation.[1]

Spreading Through a Knife Surface

An extraneous tissue contaminant on a slide is called a *floater*. This potential source of diagnostic confusion occurs in approximately 1.2% of prepared slides.[17] Floaters occur at the slide level in 73% and at the paraffin-block level in 16% of cases.[18] Approximately half of these contaminations are derived from the same patient sample.[18] When the origin of the tissue floater is uncertain, DNA analysis can be helpful.[19,20] Although contamination may occur outside the pathology laboratory, it mainly happens inside the laboratory during gross handling, cutting, or slide processing.[17,21] The location of the contamination may be from the specimen surface or from a cut surface.[17]

During lung resection specimen prosection, tumor cells may be displaced by the knife along the plane of sectioning: spreading through a knife surface (STAKS). The contaminants may either be displaced within the specimen or left on the surface of the tissue. Depending on the size of the loose tissue or cellular fragment, the floater may end up in airways, blood vessels, alveolar ducts, or sacs. Microscopic examples of STAKS are shown in Figure 5, A through H. STAKS likely occurs during gross handling, as the lung contains many spaces, allowing displaced tumor fragments to settle distant from their origin. An artificial model of an apple with ink clearly shows displaced ink in the direction of cutting (Figure 6, A through D). Since lung spaces are filled with paraffin during processing, the knife carryover within tissue holes is less likely to occur during microtome cutting, but may occur at the edge of the paraffin section (Figure 7, A). It is of paramount importance that the tissue floats are recognized as such and not considered separate foci of carcinoma in a lung tumor resection case. This may be challenging given the propensity for lung cancer cells to spread through vascular spaces or "presumed aerogenous spread." Possibly, at least a part of the STAKS effect can be

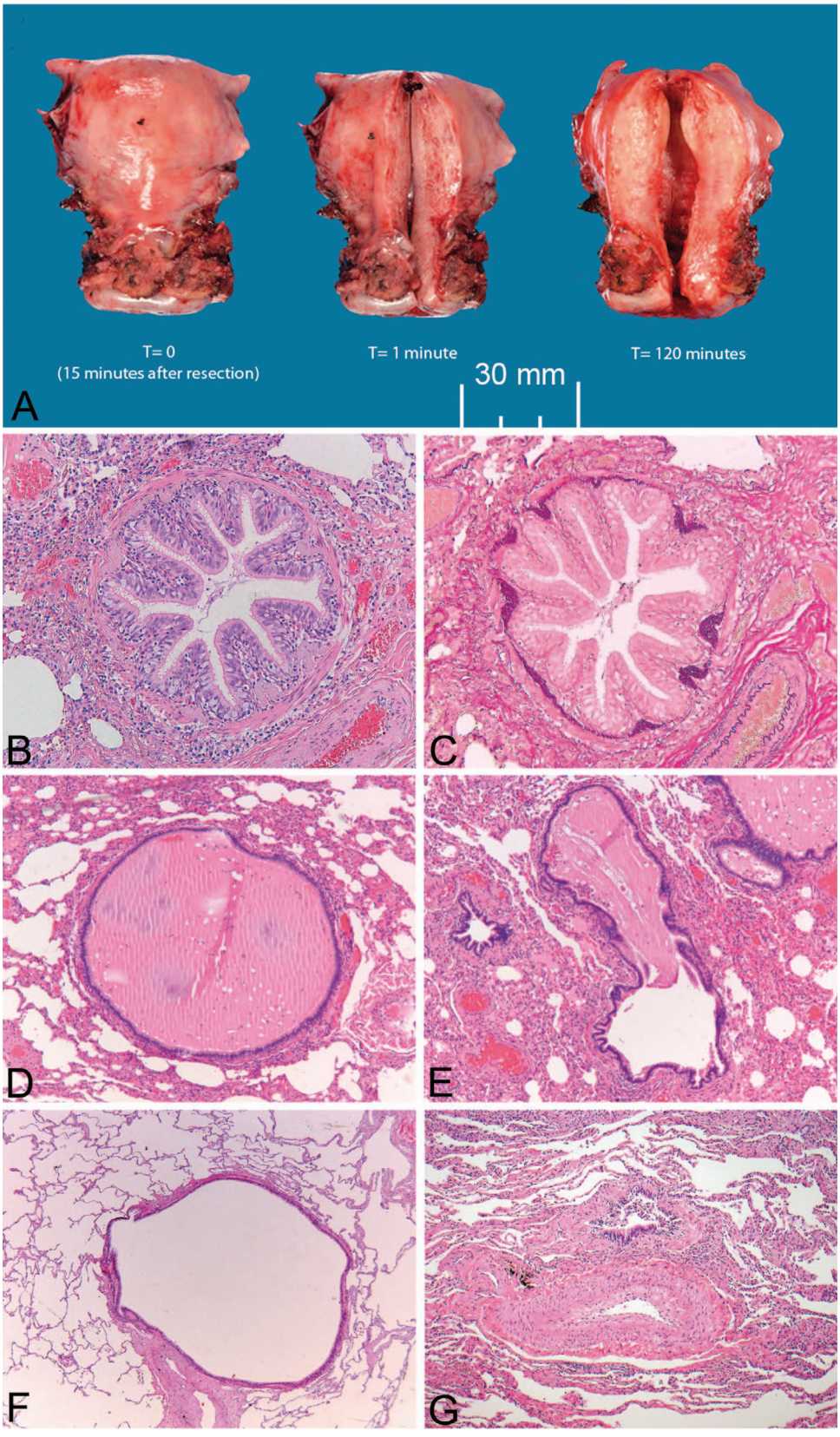

Figure 3. *Images supporting the hypothesis of the contraction artifact. A, Uterus resection specimen before and after sagittal cut till cavum uteri. Note the difference in shape after 1 minute and 120 minutes at room temperature, owing to ex vivo smooth muscle contraction. B and C, Cross-section through bronchiole of collapsed lung resected for lung cancer, showing ex vivo smooth muscle contraction with folding of respiratory epithelium and underlying basement membrane and focal ex vivo aggregation of the elastin. Note that these histologic components are physiologic, except for occasional lymphocytes and surrounding edema. The pathologic diagnosis for this image may be mild chronic bronchiolitis. D and E, Images from 1 case with the same magnification of bronchioles with in vivo existing intraluminal mucus plug prohibiting the ex vivo smooth muscle contraction. Note in*

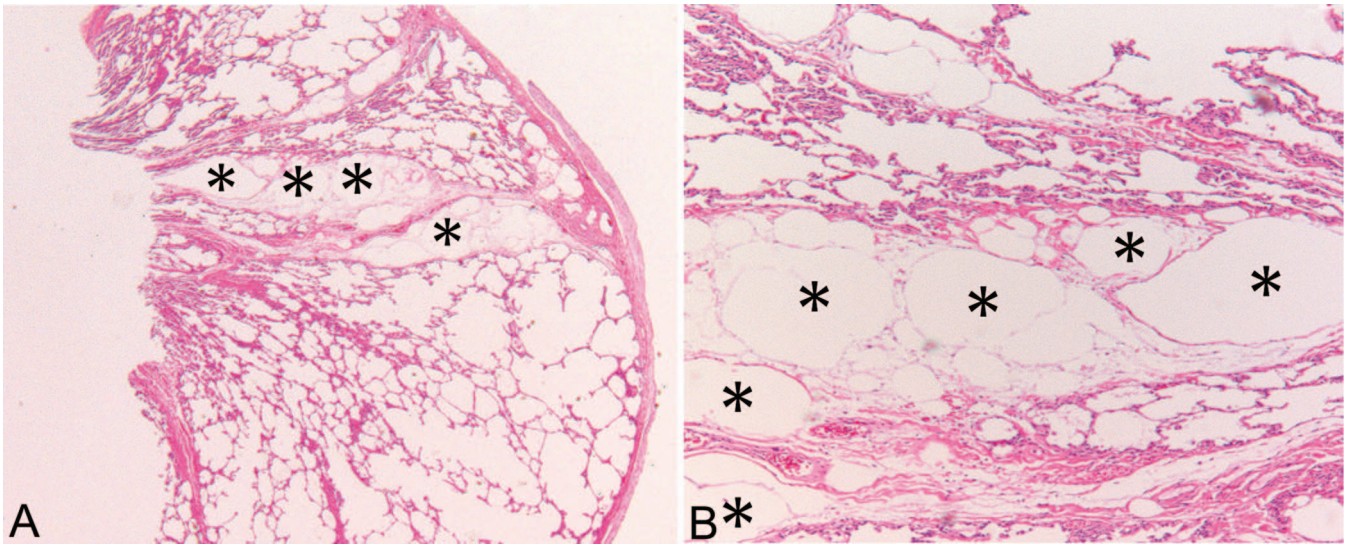

Figure 4. *Images supporting the hypothesis of the clamping artifact. Spaces with asterisks (*) denote dilation by lymph fluid, which is most prominent in the septum and only minorly present in the pleura, emphasizing that the lymph vessels running toward the hilum are compressed during clamping (hematoxylin-eosin, original magnifications ×1.25 [A] and ×5 [B]).*

avoided by quickly cleaning the knife blade after each slice with a wet sponge or tissue paper.

COMMENT

This study describes the effect of 4 patterns of ex vivo artifacts occurring in the lung. Three of the artifact patterns have to some extent been described before. Pseudolipoid change is another artifact representing air bubbles after bronchial biopsy as described before[1] (Figure 7, B). In the same textbook,[1] crinkling and telescoping of epithelium are ascribed to airway compression. In our opinion these findings are a combination of the ex vivo artifacts "collapse" and "contraction."

Recognizing "contraction" artifact may affect our perception of morphologic lung changes in asthma. Increased smooth muscle mass is consistently seen and a feature of airway remodeling; perhaps a degree of this is secondary to ex vivo artifact.[22,23] Intuitively, the morphology of contraction artifact is associated with functional airway obstruction. However, in most patients with folded bronchiolar epithelium there is no airway obstruction, except in airway-related diseases such as asthma and constrictive bronchiolitis, where other characteristics (increased luminal mucus, inflammation, thickened basement membrane, and/or fibrosis) explain the obstruction. The question arises as to what extent this artifact has been taken into account in previous studies measuring basement membrane thickness.[24] In many asthma studies smooth muscle area is measured relative to basement membrane length, giving reproducible results for larger airways (but not always for smaller airways with perimeter of basement membrane < 4 mm).[25] The contraction artifact is specifically present at the bronchiolar level, possibly being another confounder in this study, beside variation between centers in inflation procedures, presence or absence of mucus in bronchiolar lumen, selection differences in airway sizes, and differences in calibration of measurement instruments. In a reproducibility study on the morphologic interpretation of transbronchial lung biopsies, the observer agreement was low for bronchiolitis obliterans.[26] Perhaps the contraction effect played a role here too.

Artifactual knife carryover is a well-recognized phenomenon. In fact, sometimes entire strips of mucosa are displaced into bronchiolar and alveolar spaces.[1] Interestingly, the 2015 World Health Organization classification of lung adenocarcinoma describes a pattern of lung cancer "spreading through the airways" (STAS).[27] This STAS-like pattern had also been described previously under different names.[28–31] Subsequently, the association of STAS and poor prognosis has been reported for larger studies.[32,33] Warth and colleagues[33] reported that the presence of STAS was tightly linked to specific growth patterns. In resected surgical specimens, STAS has been associated with reduced overall and disease-free survival, which was growth pattern, but not stage, independent. The study of Kadota and colleagues[32] reports a higher risk of recurrence in patients with STAS-positive tumors than in patients with STAS-negative tumors in a limited resection group. In contrast to the study of Warth and colleagues,[33] in the lobectomy group the presence of STAS was not associated with recurrence. In STAS the underlying biologic assumption is that in an adenocarcinoma with a micropapillary, acinar, or solid component with dissociated tumor cells, tumor fragments can spread aerogenously well beyond the mass lesion and

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(Figure 3, E) also the contracted bronchiole (on the left side lumen without mucus). F, Image of another case where one part of the lobe is perfusion fixed; the nonperfused part had a similar appearance as the bronchiole left side in (E). Note that the smooth muscle contraction was reversible at the moment of perfusion fixation. G, Image from yet another case where artery and bronchiole were captured in 1 slide. Notice the difference in diameter. The intimal fibrosis prevents contraction of smooth muscle in the pulmonary artery, while the ex vivo contraction of the bronchiole remains unimpeded. Image courtesy of Anja C. Roden, MD, Mayo Clinic, Rochester, Minnesota (original magnification ×0.5 [A, see ruler in picture]; hematoxylin-eosin, original magnifications ×10 [B], ×5 [D and E], ×2.5 [F], and ×10 [G]; Elastica van Gieson, original magnification ×10 [C]).

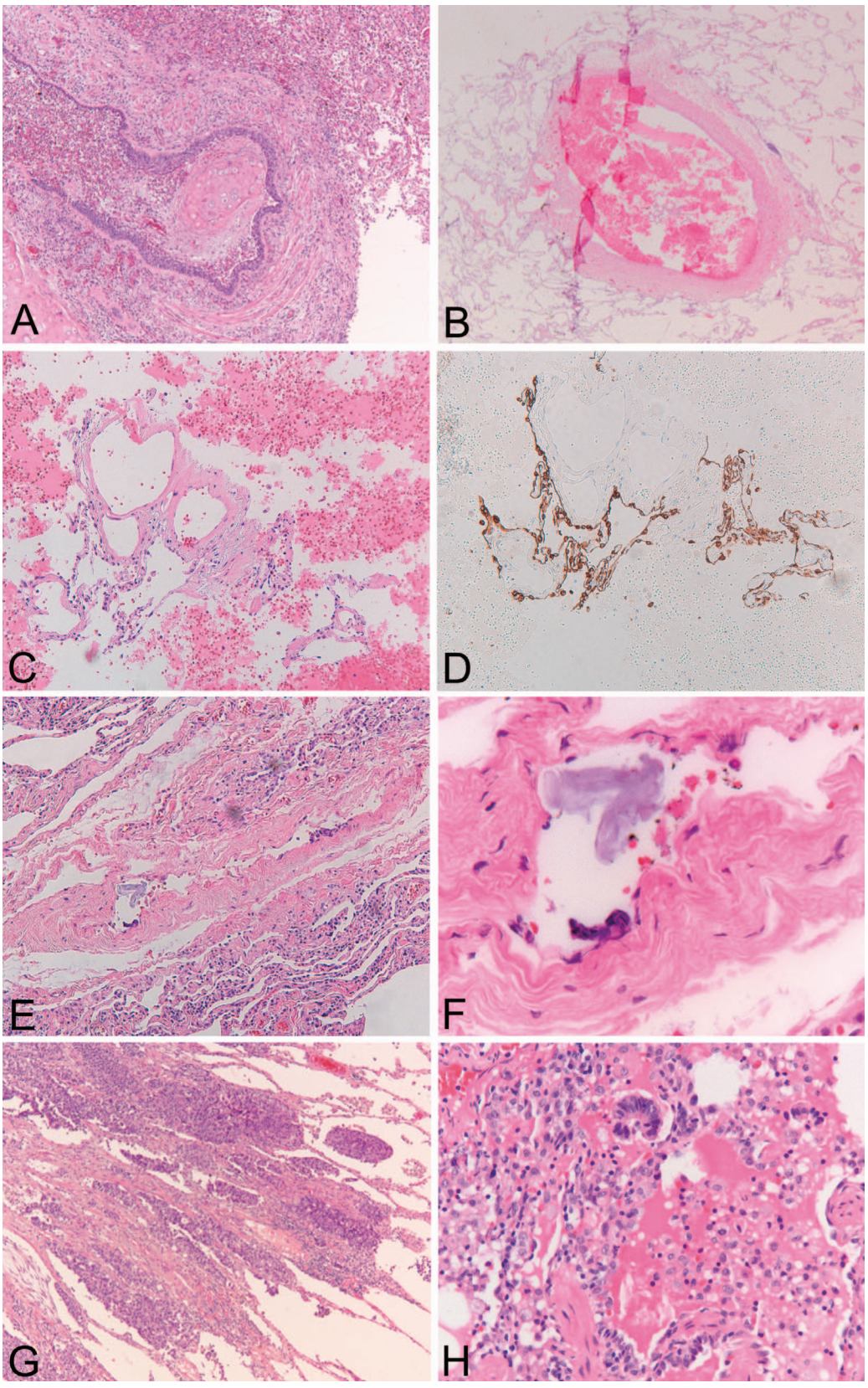

Figure 5. *Images supporting the hypothesis of STAKS artifact. A, Autopsy lung showing bronchiolus with intraluminal fragment of cartilage. B through D, Pulmonary artery with intraluminal fragment of peripheral lung tissue and erythrocytes. E and F, Largely collapsed pulmonary vein with intraluminal foreign body material. G, Lung resected for adenocarcinoma with focally loose intraalveolar parts. H, Open lung biopsy for interstitial lung disease with intraalveolar fragment of loose benign bronchiolar epithelium (hematoxylin-eosin, original magnifications ×2.5 [A], ×1.25 [B], ×10 [C, E, and G], ×40 [F], and ×20 [H]; cytokeratin 7, original magnification ×10 [D]).*

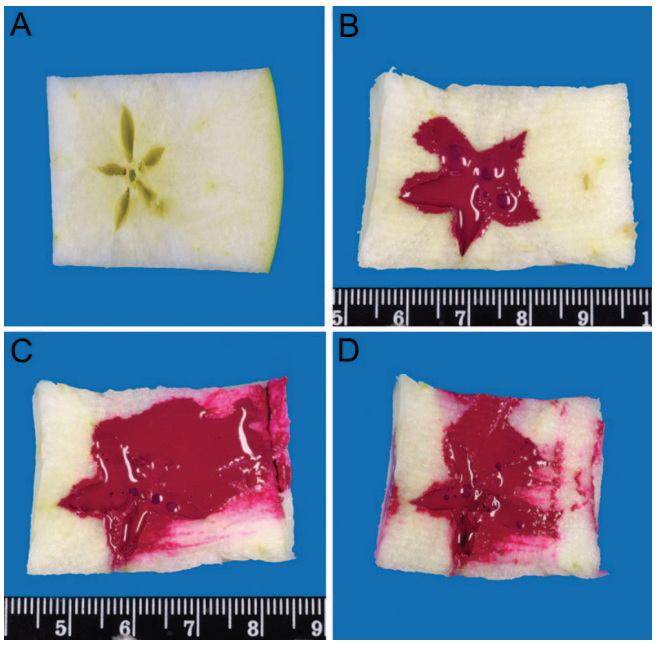

Figure 6. *Trimmed apple model demonstrating displacement of ink during fresh cutting (original magnification ×1.7 [A through D]). A, Pits are removed from the core. B, Ink is filled in core. C, A section is cut with clean knife to the right side. D, Same knife is used for second cut in same direction (without cleaning between [C] and [D]). Note that in (D) ink is also present at the beginning of the slice (left side).*

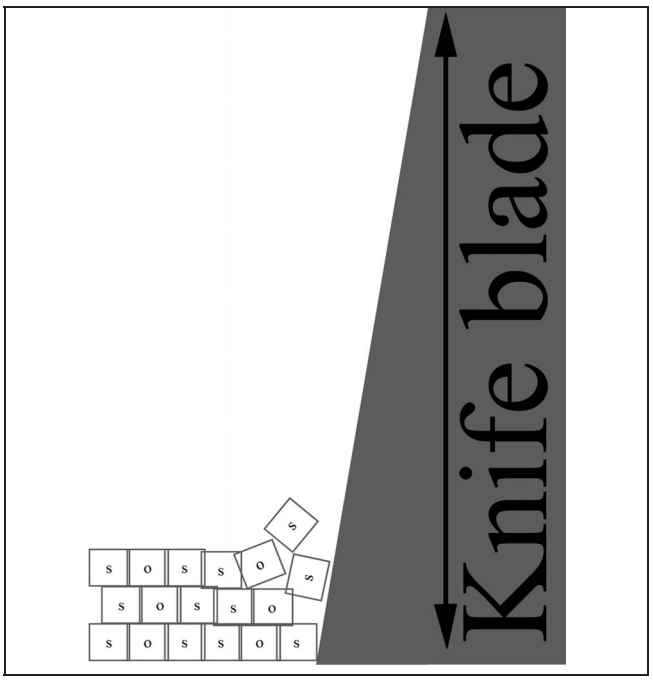

Figure 8. *The relative size of epithelial tumor cells (SOS), compared to thickness of the knife blade used for cutting.*

endanger the prospect of a complete resection if less than a lobectomy is performed.[27] The STAKS explanation for association between STAS and histologic patterns with poor prognosis lies in the fact that tumor cells have a tendency for dissociation. In the micropapillary pattern, the seemingly loose alveolar cells (partly dissociated tumor cells) in one section are connected to other tumor cells or to the basement membrane in the section above or below, but they are disconnected in another plane of sectioning. Moreover, the big (shovel-like) force applied during gross cutting (see Figure 8 for relative sizes) disconnects the cells or clusters of cells with a tendency to dissociate from their fixed connection, freeing them and allowing displacement, similar to the apple/ink phenomenon shown in Figure 6.

In our opinion, the reported frequency of STAS in roughly half of all resected adenocarcinomas is an indication of the frequency of STAKS. STAKS is a frequent finding in the lung, not only in adenocarcinomas but also in squamous cell carcinomas and benign lung tissue. Further validation and consideration of STAS for implementation in routine diagnostic evaluation and reporting,[33] or recognizing it as a pattern of invasion in lung adenocarcinoma,[32] is not appropriate for an artifact (STAKS). The prognostic associ-

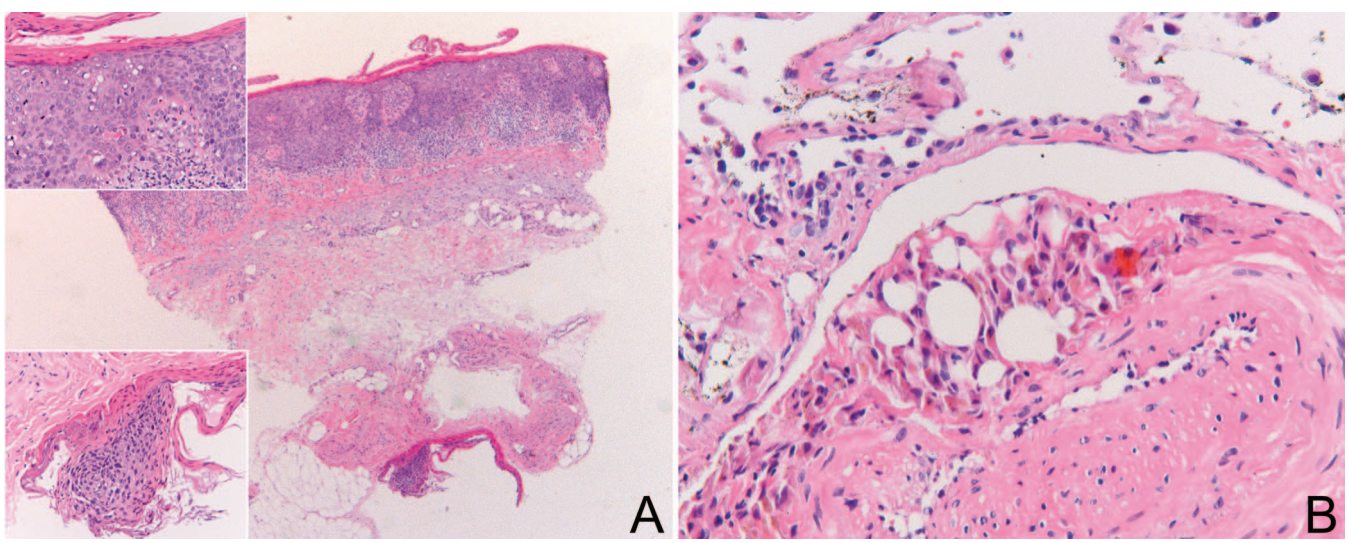

Figure 7. *A, Section of skin biopsy showing part of surface displaced to deeper part of biopsy specimen. Insets show details of squamous cell carcinoma in situ. This displacement may have been caused by cutting on microtome. B, Pseudolipoid change[1] present (central air bubbles after bronchial biopsy (hematoxylin-eosin, original magnifications ×2.5 [A] and ×20 [insets A, and B]).*

ation of STAS is in line with that of more than 100 other prognostic factors associated with poorly differentiated lung adenocarcinoma, which are not used in routine diagnostic reporting. Moreover, the radiation oncologist defines radiotherapy target volumes on the basis of microscopic tumor extent.[29,34] In this context the STAKS pattern is likely to be a confounder, and we suggest that the target area for radiotherapy in lung cancer should not be based on displaced tumor cells during gross examination.

Diaz and colleagues[34] described the displacement of tumor cells in breast samples. Tumor cell displacement was observed in 32% of patients who had undergone large-gauge needle core biopsies. Remarkably, the incidence and amount of tumor displacement was inversely related to the time interval between core biopsy and excision. The authors suggested that tumor cells do not survive displacement. The wall thickness of a gauge steel needle is approximately 0.2 mm. The size of the displaced cells, as reported in the article by Diaz et al,[34] is smaller than the wall thickness of the needle. The thickness of the gross knife may vary, but ranges from 0.4 to 1 mm, which is 20 to 50 times larger than normal epithelial cells (see Figure 8), implying a tremendous force. In the context of breast fine-needle aspirate cytology, the force applied during smearing of the aspirate may cause separation of tumor cells from the main epithelial cluster. This loss of cohesion, also called "dissociation," is used as a feature of malignancy.

In summary, the 4 patterns of ex vivo artifacts are important to recognize so as not to compromise morphologic diagnostic accuracy.

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