ORIGINAL ARTICLE

Defining Morphologic Features of Invasion in Pulmonary Nonmucinous Adenocarcinoma With Lepidic Growth: A Proposal by the International Association for the Study of Lung Cancer Pathology Committee

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ABSTRACT

Introduction: Since the eight edition of the Union for International Cancer Control and American Joint Committee on Cancer TNM classification system, the primary tumor pT stage is determined on the basis of presence and size of the invasive components. The aim of this study was to identify histologic features in tumors with lepidic growth pattern which may be used to establish criteria for distinguishing invasive from noninvasive areas.

Methods: A Delphi approach was used with two rounds of blinded anonymized analysis of resected nonmucinous lung adenocarcinoma cases with presumed invasive and noninvasive components, followed by one round of reviewer deanonymized and unblinded review of cases with known outcomes. A digital pathology platform was used for measuring total tumor size and invasive tumor size.

Results: The mean coefficient of variation for measuring total tumor size and tumor invasive size was 6.9% (range: 1.7%–22.3%) and 54% (range: 14.7%–155%), respectively, with substantial variations in interpretation of the size and location of invasion among pathologists. Following the presentation of the results and further discussion among members at large of the International Association for the Study of Lung Cancer Pathology Committee, extensive epithelial proliferation (EEP) in areas of collapsed lepidic growth pattern is recognized as a feature likely to be associated with invasive growth. The EEP is characterized by multilayered luminal epithelial cell growth, usually with high-grade cytologic features in several alveolar spaces.

Conclusions: Collapsed alveoli and transition zones with EEP were identified by the Delphi process as morphologic features that were a source of interobserver variability. Definition criteria for collapse and EEP are proposed to improve reproducibility of invasion measurement.

Keywords: Adenocarcinoma; Invasion; Reproducibility; Extensive epithelial proliferation

Introduction

Primary lung adenocarcinomas have diverse histologic appearances and substantial intratumoral heterogeneity in growth pattern. According to the fourth (2015) and fifth (2021)[1] editions of WHO classification of nonmucinous lung adenocarcinoma, the predominant pattern is used for subtyping the tumors and is the basis for the proposed grading system for surgically resected adenocarcinomas.[2] Furthermore, it is suggested that the proportions of each pattern be recorded at 5% increments.[3] The concepts of adenocarcinoma in situ (AIS) and minimally invasive adenocarcinoma (MIA), first established in the 2015 WHO classification, are now recognized more frequently, particularly in patients diagnosed in lung cancer screening protocols.[4,5] Both AIS and MIA are associated with 100% 5-year survival after complete resection and essentially no metastatic risk. In contrast, tumors with at least 5 mm (effective diameter) of invasive pattern disease ($\geq$pT1a) have been associated with recurrence risk that increases with the extent of invasion.[6,7]

The importance of recognizing and distinguishing lepidic growth from other patterns regarded as "invasive" became highly relevant for pT staging in the eight edition of the Union for International Cancer Control and American Joint Committee on Cancer TNM classification system, which recommended that in nonmucinous lung adenocarcinoma the primary tumor size (pT) is determined by the invasive size excluding the lepidic component, which is considered noninvasive.[7,8] The International Association for the Study of Lung Cancer (IASLC) Staging and Prognostic Factor Committee also encouraged further research on what is the best method and reproducibility of measuring size of invasive versus lepidic components and how this could be improved.[7]

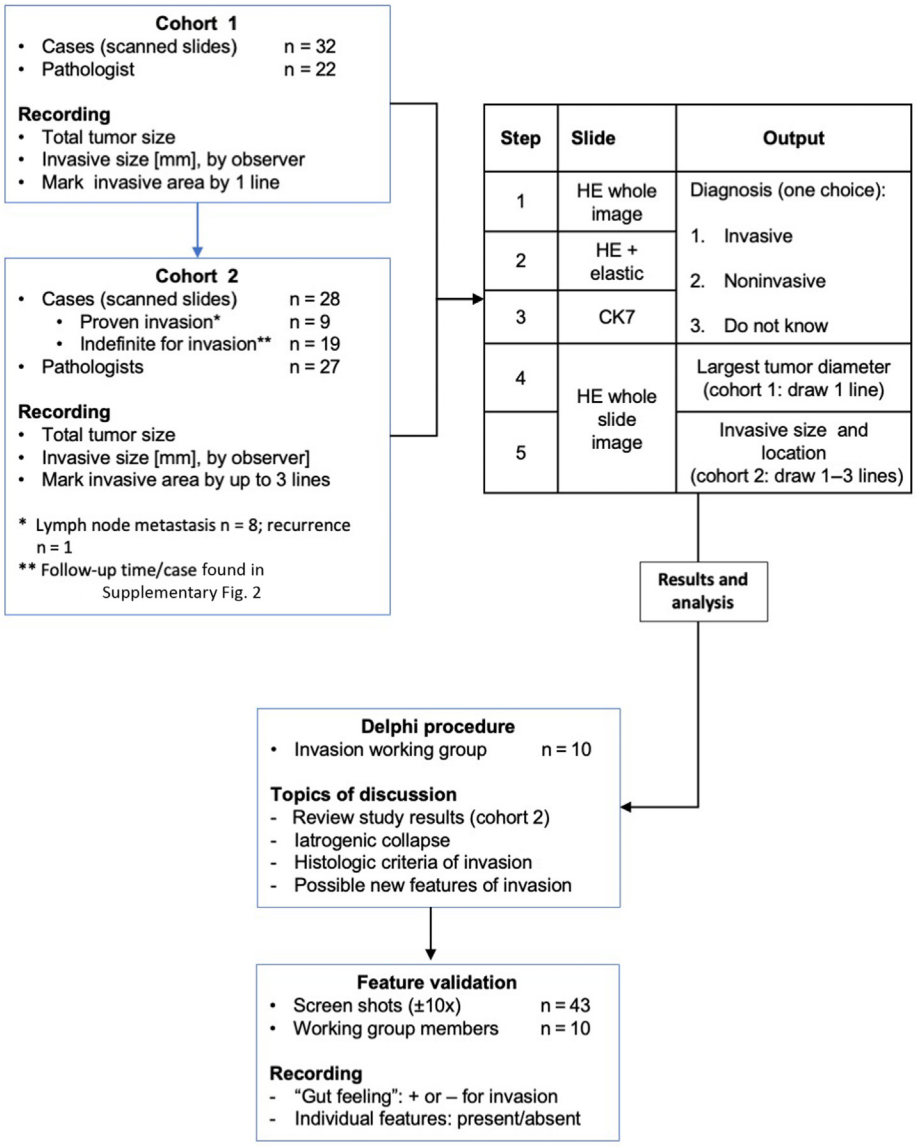

Figure 1. Graphical overview of the studies performed. CK7, cytokeratin 7; HE, hematoxylin and eosin.

Currently, pathologists are recommended to measure the maximum diameter of the invasive patterns or estimate the percentage of invasive patterns relative to overall size to calculate invasive size for pT staging.[1] Nevertheless, reproducibility in distinguishing different patterns of growth and recognition of invasion remain challenging in more than occasional cases of resected adenocarcinoma, especially when there is iatrogenic collapse of the alveolar framework, and particularly in distinguishing lepidic from papillary, acinar, or even micropapillary patterns.[9–12]

As a follow-up to the research questions posed in the IASLC lung cancer staging proposal on assessment of tumor size in part-solid tumors,[7] the IASLC Pathology Committee formed an Invasion Working Group to revisit the issue of recognizing areas of invasion in nonmucinous lung adenocarcinoma. The aims were to evaluate the reproducibility of invasive size measurement and to identify histologic features that may be used to establish criteria in distinguishing invasive from noninvasive patterns in resected lung nonmucinous adenocarcinomas having a lepidic component, especially in those cases at risk for iatrogenic collapse. To this end, the Delphi approach was used, which is a relevant source of evidence in health care research.[13–15]

Materials and Methods

An overview of the studies performed is found in Figure 1. Two different study sets, comprising resected lung adenocarcinoma cases, were used in this work. The first contained tumors regarded by the contributing

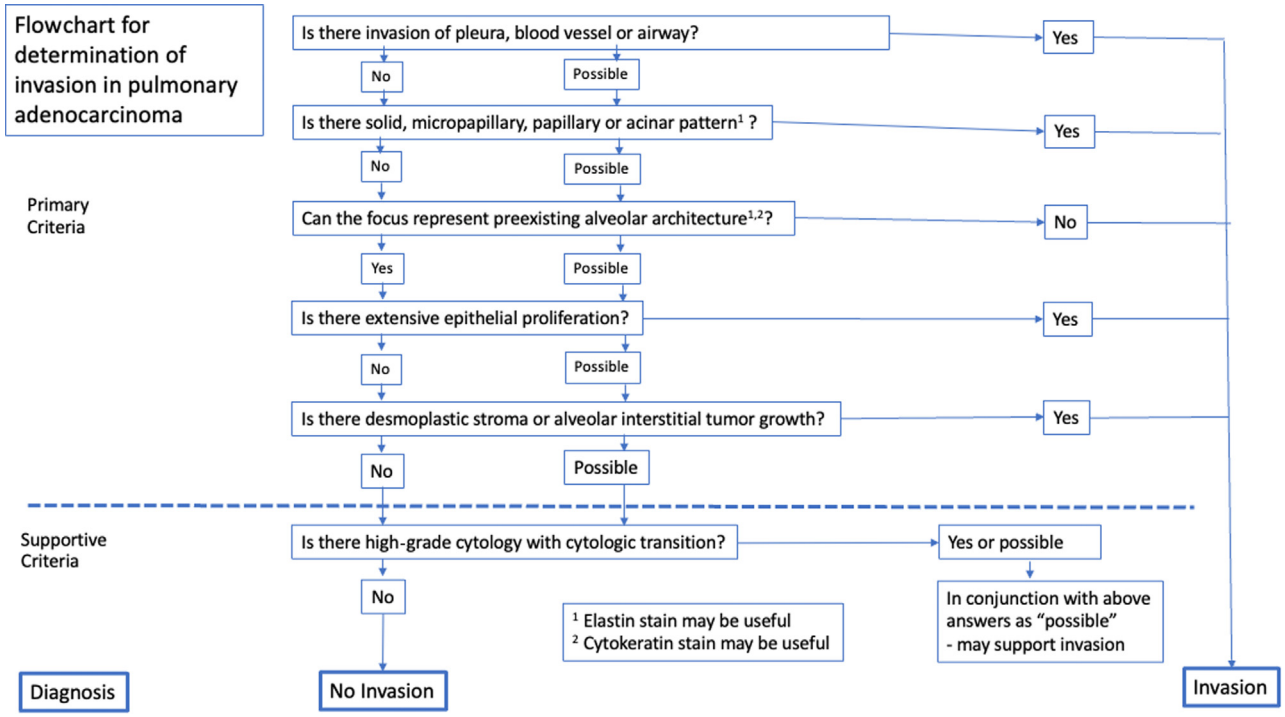

Figure 2. A flowchart for the thought processes for establishment of invasion in pulmonary adenocarcinoma.

pathologist to represent histologic invasive and noninvasive adenocarcinomas (total cases, n = 32; pathologists, n = 22). The second set (total cases, n = 28; pathologists, n = 27) included nine cases selected for the presence of lymph node metastases or recurrence as a proof of invasion and 19 cases thought by the contributing pathologist to have no evidence of invasion at diagnosis and found to have no clinical evidence of recurrence or metastases at follow-up. The reviewers were blinded to nodal status and outcome data. Institutional ethics approvals for the use of materials in this study were obtained by contributing pathologists at their respective hospitals.

Standard histologic slides were prepared from one representative formalin-fixed, paraffin-embedded block and stained with hematoxylin and eosin (HE), elastin stain, and cytokeratin 7 immunohistochemical stain. The slides were scanned and made for an online assessment available from a server at the University of Tsukuba, Japan, similarly to what previously described,[16] but for this study developed by Frontier System Co. Ltd. (Mito, Ibaraki, Japan). For each case in the first step of evaluation, only the whole slide HE image was available for reading. In the second step, the HE and elastin stains were available together for evaluation. In the third step, cytokeratin 7 stain was then added. After each step, the pathologist had to decide, on the basis of the 2015 WHO classification,[3] whether any invasive carcinoma was present and choose one of the following options: (1) invasive, (2) noninvasive, or (3) "do not know." Subsequently, the pathologists were asked to provide total tumor size and "invasive" tumor size measurement by using a digital ruler tool. For the second cohort, also blinded to outcome, the pathologists were also asked to draw a line to indicate the location of invasion. One measurement line was obligatory, but the viewer could choose to include up to two additional lines to locate the invasive areas. The line(s) were recorded for subsequent analysis. This set allowed identification of possible invasive and (non)invasive morphologic characteristics.

After the completion of slide review of both cohorts 1 and 2, tumor and invasive size measurements were revealed and the clinical outcomes were unblinded to a subset of pathologists in a study working group. This working group used this information to identify features that might potentially be useful to distinguish noninvasive lepidic pattern from other invasive patterns. In this phase, the lines drawn were deanonymized as to observer and the outcome unblinded. Importantly, the group focused on pT1 cases with a defined end point of nodal metastasis to deconstruct the criteria for invasion found in those cases using a Delphi procedure.[13–15] Expert diagnosis from a previous round and the reasons for their judgments were evaluated in a meeting of the invasion working group. It is believed that during this process the range of the answers will decrease and the group will converge toward the "correct" answer.

Table 1. Histologic Features Supporting the Distinction Between Invasive and Noninvasive Areas

	Criterion	Description	Invasion	Not Invasion
WHO	Invasive pattern	Acinar, papillary, micropapillary, solid	Present	Absent or uncertain
	Definitive lepidic pattern	Monolayer alveolar growth without collapse	Absent or uncertain	Present
IASLC major	EEP[a]	A possible lepidic pattern, but with luminal epithelial multilayered proliferation	Present	Absent
	Altered preexisting alveolar architecture	Owing to invasive tumor growth[b]	Present	Absent[c]
	Collapse[d]	Iatrogenic collapse with AIS parallel (streaming) pattern	Absent	Present
	Desmoplastic stroma[e]	Fibromyxoid stroma around tumor cells	Present[f]	Absent
	Interstitial growth[g]	Growth of malignant cells within the stroma of alveolar walls	Present	Absent
IASLC minor[h]	Cytology[i]	Nuclear grade[j]	High grade	Low grade
		Nuclear shape	Pleomorphic	Monotonous
	Cytologic transition[j]	Cells in putative invasive area have higher nuclear grade than those of adjacent lepidic pattern	Higher grade than adjacent lepidic pattern	NA
	Luminal alveolar macrophages	Macrophages in lumen of collapsed spaces	Absent[k]	Present

Note: Beside classic histologic criteria of invasion (pleural, vascular, bronchial invasion, lymph node metastases).
[a]Defined as a luminal epithelial proliferation beyond a monolayer that is two, three, or more layers in thickness. Optimal threshold needs to be defined in further studies. This may also be cribriform or solid (luminal filling).
[b]In the space beside alveoli: individual tumor cells, small acinar glands (smaller than alveolar size).
[c]Altered preexisting architecture by (preexisting) disease other than malignancy, for example, emphysema may also reveal in situ growth.
[d]Iatrogenic collapse may reveal parallel orientation, but it is not obligatory, as local circumstances determine the actual folding of the alveolar walls such as luminal filling by alveolar macrophages, fibrosis of the alveolar wall, and bronchovascular bundle in the vicinity. The presence of collapse does not exclude the presence of true invasion elsewhere (e.g., Fig. 5). In adenocarcinomas with tumor cell growth along the alveolar walls, this may also undergo iatrogenic collapse.
[e]Desmoplastic stroma is defined as fibromyxoid stroma with admixed tumor cells. Fibroblastic foci without tumor cells may be present in AIS.
[f]Not present in all patterns of invasion (e.g., micropapillary).
[g]Growth of malignant cells within the stroma of easily recognizable alveolar walls with lack of malignant cells on the alveolar wall lining is an uncommon sign of invasion. This pattern of invasion may also be found in metastases from other organs.[44]
[h]If major criteria are not present, the cytology may be helpful, but not decisive. More studies needed.
[i]In the context of preexisting architecture, EEP is usually accompanied with high-grade cytology. Without multilayering in the whole lesion, high-grade cytology alone in the context of preexisting architecture is not sufficient for invasion. A change of low-grade to high-grade cytology is frequently associated with other signs of invasion.
[j]Clear nuclear-grade difference (size/ shape/pleomorphism) between in situ and invasive components has been mentioned by Moore et al.[29] in invasive tumors.
[k]Alveolar macrophages are usually absent in EEP but may be present, for example, in areas with micropapillary growth.
AIS, adenocarcinoma in situ; EEP, extensive epithelial proliferation; IASLC, International Association for the Study of Lung Cancer; NA, not available.

The results were presented for discussion to members at large of the IASLC Pathology Committee on February 29, 2020. For this meeting, the participants received their own data, but not other pathologists' data. Further iterations of refinement took place virtually until July 2021. A flowchart was developed to encapsulate the deconstructed diagnostic process with elements in common among the group or which emerged from discussion (Fig. 2). The results of working group deliberation and a drafted manuscript were distributed to the Pathology Committee members at large for review and further discussion on September 22, 2021.

Criteria Testing

To assess the utility of the flowchart elements, a third, image-based, validation set of 43 images was sent to the 10 invasion working group members. Image magnification and size were comparable to the previous interobserver study.[9] In this validation phase, the participants recorded their initial morphologic impression regarding invasion and scored as present or absent those criteria identified after the Delphi meeting as supporting or refuting invasion: altered alveolar architecture (tumor-induced alteration versus iatrogenic collapse), extensive epithelial proliferation (EEP), desmoplasia, interstitial growth, high nuclear grade, nuclear shape from cuboidal to columnar or pleomorphic, visible transition in cytologic appearances, and (absence of) luminal alveolar macrophages between putative noninvasive and invasive areas (Table 1). The EEP is characterized by multilayered epithelial cells, usually having high-grade cytologic features (enlarged nuclei, increased nuclear-to-cytoplasmic ratio, nuclear pleomorphism) lining alveolar spaces, which would otherwise be considered as lepidic pattern disease (Fig. 3*A–H*). The panelists recorded their initial subjective or gestalt impression and coded each feature as present (1) or absent (0), where score of 1 favored

Table 2. Histologic Criteria That Are Not Informative for the Distinction Between Invasive and Noninvasive Areas

Criterion	Description
Angular "glands" without desmoplasia[a]	Tumor gland-like structures with sharp angles may also be present in noninvasive areas.
Round "glands" without desmoplasia	In collapsed alveolar structure with lepidic disease, spaces lined by epithelium may seem round without evidence of the streaming pattern. Usually, these are also small, with a proportionate increase in fibroelastosis.
Inflammation	Stromal lymphocytic, plasmacellular infiltrate.
	Neutrophilic granulocytes between tumor cells.
Alveolar wall thickness	Preexisting alveoli covered with tumor cells.

[a]Tumor glands with sharp angles may be observed in invasive adenocarcinomas of many organs. In collapsed lepidic (in situ) growth, angulated glands are frequently present.

invasion. Major criteria for invasion were the presence of EEP, desmoplasia, and altered alveolar architecture. The sum of the major criteria (up to a score of 3) was compared with the initial subjective impression. The maximum number of pathologists with the same score (categorized in 0–1 versus 2–3) was used to calculate the concordance percentage.

Statistical Analysis

For each case, the mean value, SD, and coefficient of variation (across pathologists) of total tumor size and invasive size were calculated by the study statistician (BLW).[17] Cases were ranked for the number of invasive diagnoses. For comparison of coefficient of variation, the modified signed likelihood-ratio test[18] in the R cvequality package was used. This test for comparison of coefficient of variations at case level was applied on total size and invasive size measurements and for comparison of coefficient of variations between one invasion size measurement and the sum of two or three lines. The criteria testing was evaluated with dichotomous McNemar test ($\geq$7 pathologists same score; invasion: gut feeling "yes" or 2–3 out of 3 criteria "present"). A p value less than 0.05 was considered to be significant.

Results

Tumor Size Measurements

For measuring total tumor size in the first cohort, the mean coefficient of variation among pathologists was 6.9% (range 1.7%–22.3%, see Supplementary Fig. 1*A*). In contrast, when measuring tumor invasive size, the mean coefficient of variation was 54% (range 14.7%–155%; Supplementary Fig. 1*B*). A casewise comparison of coefficient of variation between both measurements revealed a significant difference in almost all cases ($p < 0.001$), except for one case ($p = 0.34$). Most of the pathologists used one line for the designation of the invasive area. On average, two pathologists (range: 0–11) used two lines, and on average, two pathologists (range: 0–6) used three lines to designate invasive areas. The coefficient of variation was not significantly different for comparison between one or up to three lines for measurements of invasion.

Graphical displays of each case summarizing the areas of invasion, drawn by the 27 observers for cohort 2, revealed a frequent notable difference in interpretation of the size and location of invasion (Fig. 4*A* and *B*). The distribution of pathologists' scores for invasive versus noninvasive is found in Supplementary Figure 2. A pT category was assigned for this distribution if a category had three or more pathologists (>10%) of 27 pathologists' scores. The distribution of pathologic tumor (pT) categories in the second cohort is found in Supplementary Table 1. There was a marked difference in pT categorization in 26 of the 28 cases under assessment.

Following this analysis, an effort to identify morphologic features driving individual decisions to recognize areas as either "invasive" or "noninvasive" resulted in the following considerations, which are based on the cohort of cases with established invasive behavior (i.e., lymph node metastases) and those with long follow-up.

Morphologic Consideration in Defining Features of Invasion

Morphologic features that might lead to a more consistent designation of "lepidic (noninvasive)" versus "not lepidic" are proposed in Table 1.

The Effect of Iatrogenic Collapse

During the Delphi discussion, compression of the alveolar structure/lepidic pattern also called surgical collapse[19] or iatrogenic collapse[20] was recognized as a frequent phenomenon in pulmonary resection specimens (Supplementary Fig. 3). This pattern may affect the shape of normal alveoli and alveoli lined by tumor cells and can significantly modify the microscopic appearance of the tumor. It was acknowledged that collapse and compression of the alveolar structures, especially when

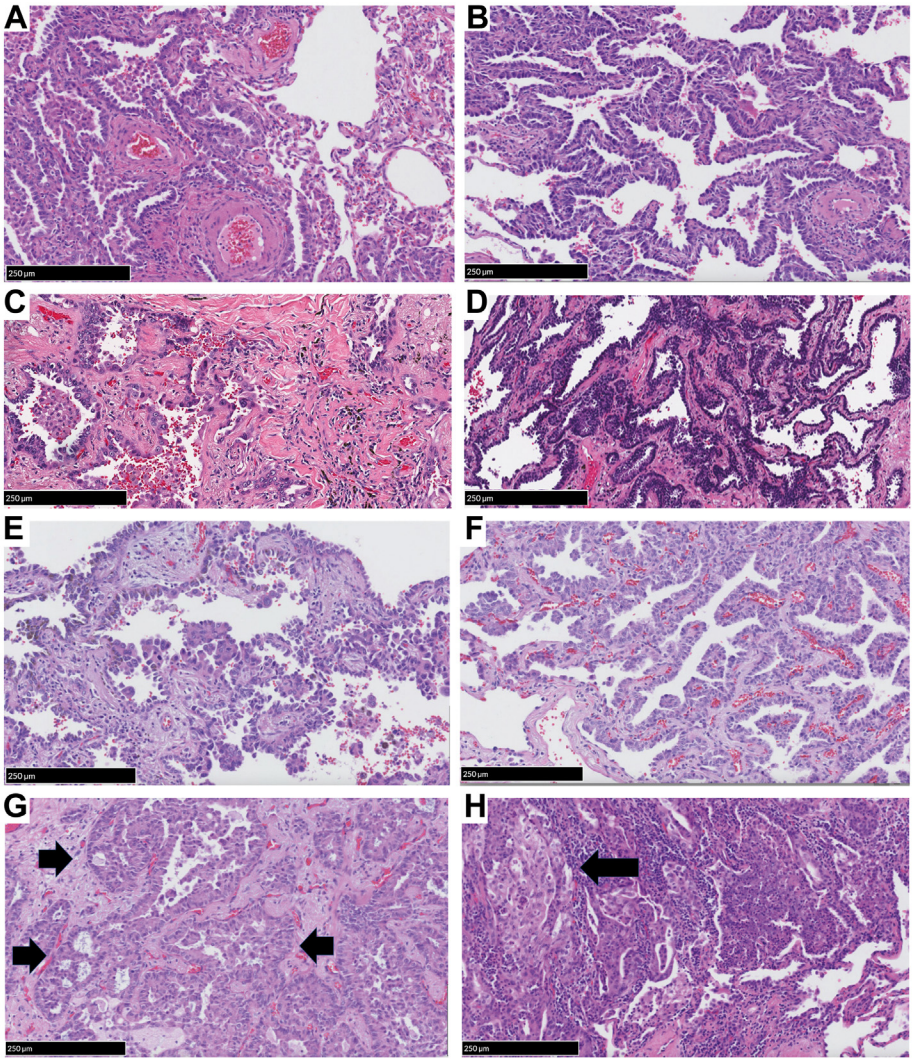

Figure 3. Examples of iatrogenic collapsed lung are found without (*A*, *B*) and with the category of EEP (*C*–*F*) from slight (*C*, *D*) to micropapillary (*E*, *F*), cribriform (*G*, arrows), and solid (*H*, arrow) growth.

thickened by increased stroma with chronic inflammatory cell infiltrate and lined by tumor cells, may lead to collapsed lepidic pattern revealing folding and tufting that mimic papillary, micropapillary, or acinar architectures when cross-sectioned.[9,21] It was recognized that perfusion fixation through the airways and/or transpleural perfusion by needle and syringe may reduce the amount of artifactual collapse and thus may assist in identification of the collapsed lepidic pattern. Nevertheless, in many cases, this process may not fully mitigate this collapse artifact.

Features Favoring Invasion

Cases were categorized as having "definite evidence for invasion" when conventional morphologic criteria for invasion could be identified (Fig. 5*A*–*O*), including effacement of alveolar architecture (Fig. 5*A*) and stromal invasion characterized by desmoplastic stroma infiltrated by single or small nests of tumor cells and/or vascular (Fig. 5*B* and *C*), bronchial or bronchiolar wall (Fig. 5*D* and *E*) or pleural invasion. Desmoplastic stroma defined as collagenous response in relation to invasion[22] (Fig. 5*N*) with morphologically loose fibromyxoid stroma containing fibroblasts (neofibrogenesis or fibroplasia[23]) was frequently, but not always, found in combination with invasive tumor cells in invasive adenocarcinomas (Fig. 5). Fibroelastosis alone was not considered sufficient for invasion. The presence of an occasional subepithelial area of fibromyxoid stroma was interpreted with caution, as this can be found in organizing pneumonia[24] and idiopathic pulmonary fibrosis.[25] Nonetheless, when prevalent within the lesion, desmoplastic stroma is a useful marker of invasive disease.

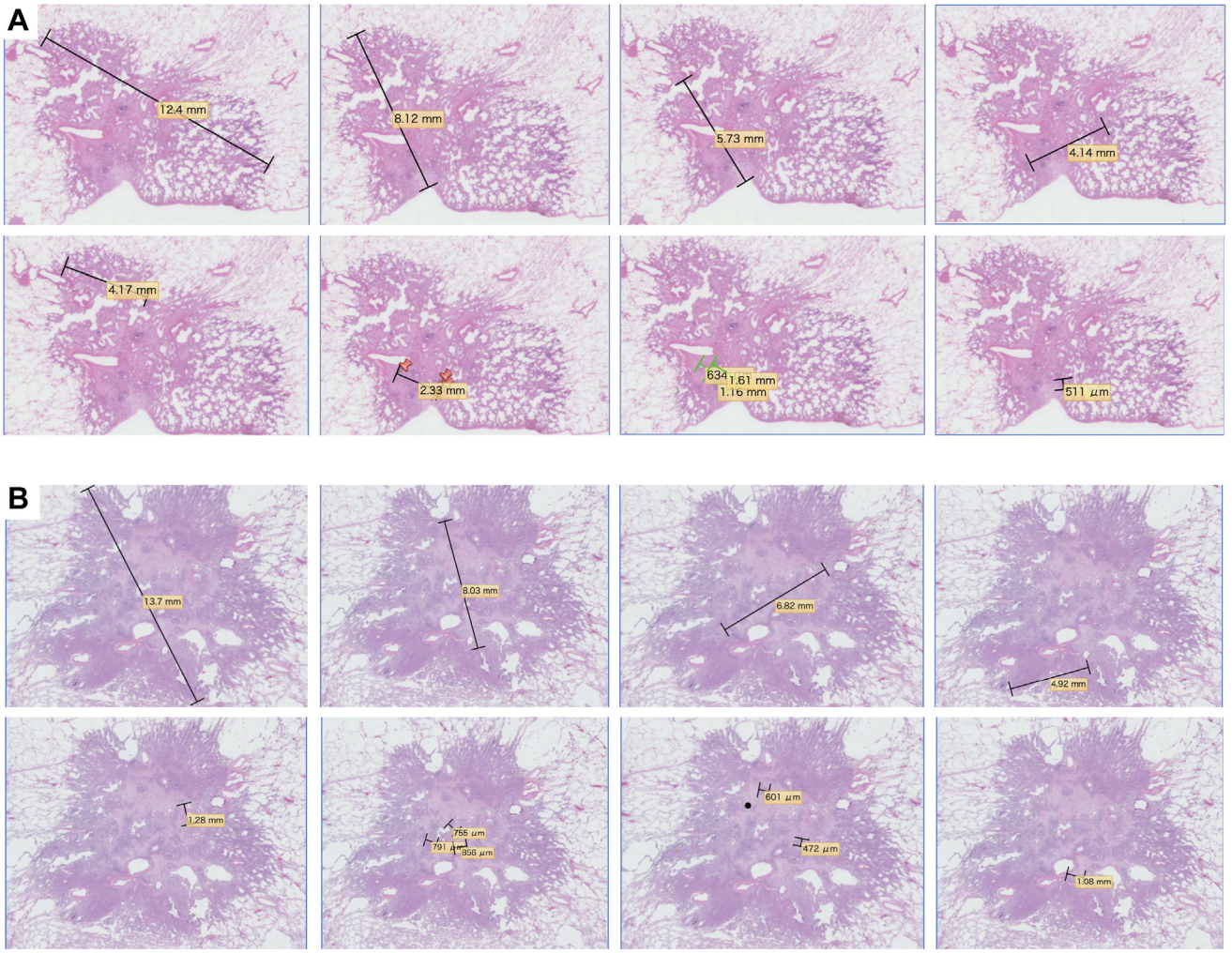

Figure 4. Two examples (*A*, *B*) of adenocarcinoma cases where lines denoting the invasive space as assigned by different observers. In case *A*, 21 of the 27 observers judged this case as invasive and six as noninvasive. In case *B*, nine of the 27 observers judged this as noninvasive. Note the remarkable difference in line size and location of assigned invasive areas and realize that several observers did not interpret these cases as invasive: that is, it did not add a line.

Strikingly, in cases with the above-described features of "definite evidence for invasion" and in some cases lacking conventional invasion criteria, a feature the group descriptively termed "extensive epithelial proliferation" (EEP) was consistently noted (Fig. 5*B–E*). The EEP is characterized by multilayered epithelial cells, usually having high-grade cytologic features (enlarged nuclei, increased nuclear-to-cytoplasmic ratio, nuclear pleomorphism) lining alveolar spaces, which would otherwise be considered as lepidic pattern disease. The EEP is a category of growth that exceeds what could be considered as noninvasive disease. Areas that lack conventional invasion criteria frequently include proliferation that falls short of criteria for micropapillary pattern.

Sometimes, the EEP may involve the whole lesion. As such, the EEP is considered a cellular feature associated with invasion when architectural features of invasion are indeterminate. It is therefore not a "new" adenocarcinoma pattern used for grading but instead one to answer a binary question of lepidic or nonlepidic when definitive architectural features are lacking. Nevertheless, as indeterminate architecture is often found adjacent to established invasive patterns, assignment of such areas to the invasive versus noninvasive lepidic pattern was a major source of interobserver differences. It was acknowledged that the EEP was a subjective assessment, but because it could not be readily explained by tissue compression or cutting artifacts only, it could serve as an independent criterion of invasive transition. To improve consistency, stratification of two or more cells was proposed to define EEP.

Features Favoring Lepidic Growth With Collapse

Noninvasive characteristics were observed in one or more of the cases and could be contrasted with definite evidence of invasion elsewhere in the lesion. These features included the following: (1) presence of iatrogenic

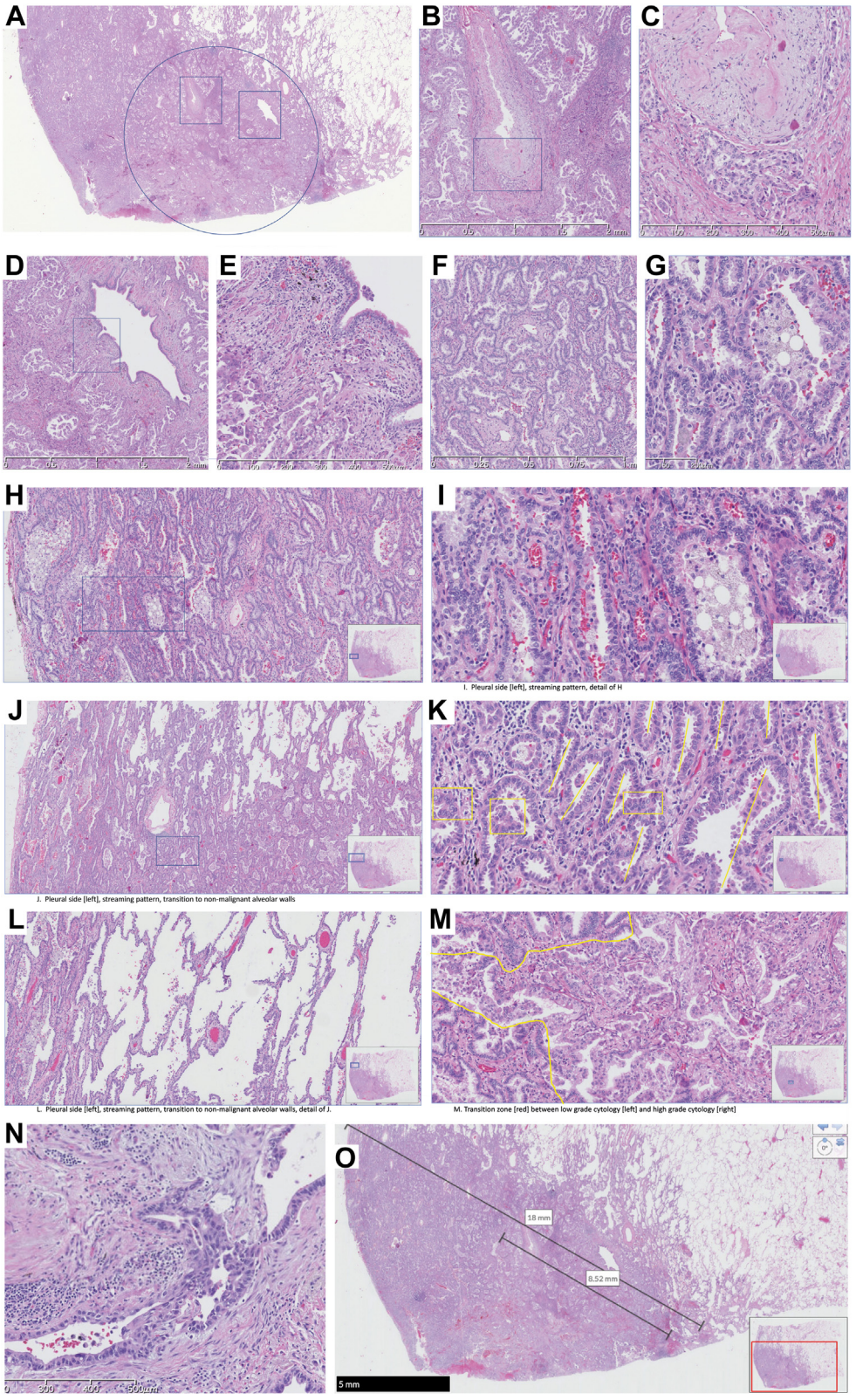

Figure 5. Morphologic appearances observed in an adenocarcinoma. An overview of adenocarcinoma in resection specimen with partly iatrogenic collapse and fixed by bronchial perfusion as example of reduced invasive size compared with total tumor size. (*A*) The tumor with blue circle is revealing the invasive area. (*B*) Higher magnification of left rectangle in (*A*) with luminal EEP category and (*C*) invasive acinar pattern around a pulmonary artery. (*D*) Higher magnification of right rectangle in (*A*) with luminal EEP and invasive growth in bronchial mucosa (*E*). (*F*) Part of iatrogenic collapsed lung with monolayer of tumor cells, which are less atypical than in EEP. (*G*) Luminal smoker's macrophages. (*H*) Iatrogenic collapsed lung with

collapsed (also called compressed) peripheral (alveolar) lung tissue lined predominantly by a single layer of monotonous cells; (2) luminal (alveolar) spaces arranged in a regular manner, often with the long axes of the spaces arranged in parallel (parallel streaming) (Supplementary Figs. 4 and 5 and Fig. 5*F–K*); and (3) an abrupt transition of monolayered tumor cells to type I pneumocytes at the periphery of the lesion, with a continuation of the orientation of compressed or collapsed non-neoplastic alveoli. Recognition of this final pattern helped distinguish lepidic noninvasive from acinar invasive disease, especially when collapse was associated with an increase in fibroelastotic interstitium and shrinkage of alveolar space diameter.[26]

Alveolar macrophages were not infrequently found in collapsed spaces and, when abundant, could influence the shape of the adjacent collapsed alveolar walls (Fig. 5*H–K*). Nevertheless, in some cases with EEP alongside loose luminal epithelial cells, cytokeratin 7–negative morphologic alveolar macrophages could also be discerned. Thus, the presence of alveolar macrophages alone, in what seems to be an airspace, is not an absolute criterion for noninvasive pattern of disease, assuming acceptance of EEP as a surrogate marker for invasion.

"Uninformative" Features

Histologic features that were considered not informative to distinguish between invasive and noninvasive areas (possible pitfalls) are listed in Table 2 and found in Supplementary Figure 4.

Angulated Glands. In contrast to many other organs where angulated spaces or glands lined by tumor cells (glands) may be frequently observed in invasive adenocarcinomas, in pulmonary adenocarcinomas angulated spaces are frequently present in both invasive and collapsed, noninvasive lepidic proliferations (Fig. 5*K*). An area with fibroelastotic or mature fibrotic scar, as distinct from neofibroplasia or desmoplastic reaction, is not considered as evidence to support invasion (Supplementary Fig. 6). Therefore, angulated or round gland-like structures without evidence of desmoplasia are not informative for the distinction between invasive and noninvasive areas.

Similarly, in some cases, the preexisting lepidic growth close to the scar may become either angulated and/or much reduced in size while maintaining a rounded shape. Ultimately, these may be reduced to a tiny focus comprising less than 10 cells, a pitfall which may be compounded by tangential sectioning. The context of such a finding (lack of neofibroplasia and presence of larger and more obvious lepidic areas) helps avoid an erroneous diagnosis of invasion (acinar pattern).

Alveolar Septal Thickening. Alveolar septal thickening is frequently found in association with neoplastic epithelial proliferations without other evidence of invasion and is not of itself considered a morphologic criterion for invasion (Supplementary Fig. 7). The thickening may be due to (1) infiltration with inflammatory cells; (2) fibrosis; or (3) increase in elastin (e.g., as in Noguchi type B[27]). In lepidic proliferations with less than or equal to two cell layers (without other invasive patterns) and the presence of cancer-associated fibroblasts, 100% 5-year survival rate has been reported.[28]

Cytomorphology

Variation in cytomorphology (tumor cell atypia) can be difficult to interpret. At one extreme, it is usual, in the lepidic pattern or adenocarcinoma in situ, for the cytologic features of the tumor cell population to be relatively of low grade and sometimes of hobnail cytology. Nevertheless, this is not absolute, as invasive disease may be cytologically of low grade, whereas lepidic pattern disease can have a range of cytologic grade, some low grade, or some high grade. In tumors where both lepidic and nonlepidic components are present, a transition of cytologic features may be helpful in making a distinction between the two components of the lesion (Fig. 5*M*). This may also help in borderline cases of EEP.

visceral pleura on the left and (*I*) higher magnification. (*J*) Transition of collapsed lung to nonmalignant alveolar walls. Visceral pleura on the left side. (*K*) and (*L*) illustrate details of (*J*). The yellow boxes in (*K*) illustrate focal areas with tangential cutting leading to seemingly multilayering, which are not interpreted as EEP. The yellow lines in (*K*) intend to accentuate the more or less parallel orientation of the collapsed lumina (streaming pattern). Note (1) the streaming pattern in (*H*) and (*J*) and (2) that the luminal collapse is less prominent when filled with, for example, alveolar macrophages. (*M*) Area with transition between low-grade (upper and lower left of yellow lines) and (on the right of the yellow lines) high-grade cytology. All images of HE stain. (*N*) Other adenocarcinoma case with desmoplastic stroma around acinar carcinoma. In (*O*), a line of 8 mm denoting the invasive part (compatible with the oval in *A*) in the section while total tumor size measures 18 mm. Thought process: areas such as in *F* to *L* do (1) not have conventional signs of invasion; (2) not have EEP, desmoplastic stroma, or interstitial growth; (3) not have high-grade cytology but fit in collapsed AIS with partial streaming, monolayer of low-grade tumor cells, and alveolar macrophages. Angulation may be part of the collapse of alveolar tissue and when covered with epithelial tumor cells may still be considered lepidic disease (see, for example, *K*). Likewise, in collapsed lepidic disease, structures may seem round and lack a prominent streaming pattern. These areas are not considered to be invasive and should not be part of the measurement for invasive size. AIS, adenocarcinoma in situ; EEP, extensive epithelial proliferation; HE, hematoxylin and eosin.

Table 3. Features That May Confound the Interpretation of Invasion

Preanalytic	Description
Poor fixation	Delay in fixation may also lead to insufficient or poor fixation recognized by detached cells mimicking micropapillary clusters[42] as is frequently found in autopsy specimen.
Deflation	Prominent deflation leads to iatrogenic collapse of alveolar tissue (Fig. 2) which may lead to inappropriate classification of invasive patterns.
Analytic	
Microscopic examination	The unavoidable need to obtain a histologic section from a nonuniform three-dimensional framework requires crosscutting and may lead to:
Equivocal architecture	Formation of acinar-like or papillary-like structures that mimic invasion, especially when the interstitium is fibrotic. Parallel streaming may be present to aid in distinction between true invasion and tangential cutting, but areas of uncertainty may remain.
	Loss of a regular pattern of tumor-lined spaces (not regular or parallel enough for reliable designation of lepidic disease), but also insufficient for invasion.
Luminal cells	The appearances of both single cells lying freely within the alveoli and multilayering (>1 cell thick) of the epithelium. Interpretation is necessarily subjective though features related to tangential cutting tend to be focal within the acini rather than circumferential.
Stratification	Nuclear or cellular stratification of epithelial tumor cells involving only part of the alveolar space circumference, where the remainder of the "space" is lined by single layers of cells. This may be a biological phenomenon, but also because of tangential cutting.

For example, in a predominantly lepidic pattern lesion, with low-grade hobnail cytologic features, "acini" lined by the same population are likely to be collapsed lepidic foci, whereas spaces lined by larger, more pleomorphic cells with larger nuclei and more eosinophilic cytoplasm probably represent invasive disease, even in the absence of associated neofibrogenesis. The group, however, also recognized that in some cases, lepidic pattern disease may have mixed cytomorphology, some areas of low-grade (presumed preexisting in situ) disease, whereas other areas are of high grade, similar to the invasive components.[29] As such, cytologic change alone without EEP was not considered sufficient criteria for invasion.

Inconclusive Issues

Although the above-described morphologic criteria were helpful in the distinction between invasive and noninvasive areas, some cases had areas with inconclusive findings and are summarized in Table 3. Furthermore, in the whole or part of the resection specimen, delayed or inadequate fixation may lead to sloughing off epithelial cells from the basement membrane. Care has to be taken not to interpret this as sign of micropapillary pattern of invasion.

Diagnostic Algorithm

In daily practice, a pathologist will frequently form a first impression about a possible diagnosis, which is an initial subjective impression (also called gut feeling or gestalt impression). Nevertheless, an analysis based on reproducible criteria may supersede this impression and reduce interobserver variability. A flowchart has been constructed, found in Figure 2, to aid in the consistent identification of invasion in pulmonary adenocarcinoma. To determine the utility of the flowchart elements described previously, a set of 43 images (Supplementary Fig. 8) was scored by the 10 members of invasion working group to assess interobserver concordance. After excluding one member's uninterpretable responses, the remaining nine members had an average concordance of initial subjective impression of 79%. The criteria-based scores for the major criteria (EEP, desmoplasia, and altered alveolar architecture) improved concordance to 84%. Accepting a score of more than or equal to seven of nine as a concordant case, the number of concordant cases increased from 25 (58%) for initial subjective impression to 34 (79%) using the three major criteria (Table 2 and Supplementary Fig. 9; McNemar test: 0.049). The inclusion of minor criteria did not change the agreement rate.

Discussion

For pathologic staging of nonmucinous adenocarcinoma, the measurement of invasive size is required. The large variation in these measurements, especially in adenocarcinomas that at least partly grow along the alveolar walls, prompted a search for more detailed histologic criteria to guide the identification of invasion. A table and flowchart (Table 1 and Fig. 2) evolved with a practical suggestion for day-to-day practice, with the aim to make the decision of invasion versus no invasion more

consistent. It is, however, recognized that these remain proposals focused on improved reproducibility which require wider validation.

The moderate reproducibility of invasive pattern recognition in adenocarcinomas has been found by the pathology committee publication of the IASLC in 2012.[9] In that study, one high magnification static image per case was used for classification, where some pathologists were more inclined toward invasion than others, with a kappa score of 0.55 ± 0.06 and 0.08 ± 0.02, for easy and difficult cases, respectively. The current study was performed on digitized slides, reflecting a closer approximation of daily practice of small adenocarcinomas by allowing a broader assessment of the tumor, and yet again, it reveals that there is major room for improvement. The appearance of differences around interpretation of invasion between four studies on small adenocarcinomas[30–33] is also found in Supplementary Table 3. Moreover, another study used, instead of the histologic patterns for assessment of invasion, an alternative approach defined as a lung adenocarcinoma without nodal involvement, vascular invasion, or lymphatic invasion,[34] implying that the WHO classification was not followed in this respect. These examples of variation in interpretation and use of the actual managerial classification highlight the need for improvement in the assessment of invasion in pulmonary adenocarcinoma.

In the literature, a previous interobserver study on measurement of invasion revealed the following (quotes): "good agreement between (two) observers when classifying tumors as AIS, MIA, and invasive adenocarcinoma" and "significant differences in overall survival between the 3 groups for both observers, and interobserver variability was evident."[35] This contrasts with our study and is explained by several factors. First, the composition of the cases in that study comprised 296 nodules, of which 59% were, in hindsight, agreed invasive adenocarcinomas (including 11% stages III and IV), whereas our study has a focus on pT1 adenocarcinomas. Second, recalculating their data for the remaining 41% of the cases reveals 52 of 123 (42%) discordant cases, of which the largest part is a difference between MIA and invasive adenocarcinoma, similar to our study. Third, their study was performed by two pathologists from one institute, whereas ours involved up to 27 pathologists from different parts of the world, probably reflecting a more global performance and disclosing a marked variability. It is clear from our study that, in applying the WHO classification, there are marked differences in assigned areas of invasion (54% coefficient of variation), as graphically displayed in Figure 4. Nevertheless, if the object is clear (limits of tumor edge), the measurement can be quite precise (7% coefficient of variation for total tumor size).

This raises the following two major questions: Why does so much interobserver variability exist and how can it be improved? The canonical classifications in oncopathology are hybrids of managerial classification grids superimposed on histogenetic classification.[36] Tumor classification and diagnosis is a community activity, and the expert plays an essential regulatory role in that community. In assigning different categories to tumor classification, the reproducibility of the categories or phenomena used for distinction in categories should be high enough to expect generalizability (external validity) throughout the world. The issue of invasion in pulmonary adenocarcinoma seems to be associated with too low kappa score to rely on in a managerial classification. The minimum acceptable value of a kappa score, or alternative ways to express reproducibility,[37] is, however, not known.

Finding solutions to such a problematic issue is difficult. As the issue evolved while applying the approach formulated in the WHO classification of lung cancer, a possible solution required an "out of the box thinking" approach. We used a Delphi procedure to the extent that we did not reach the phase of a recommendation for implementation[38] but developed a concept for further study by the IASLC pathology community. A first step in the evaluation of our cases revealed that classical morphologic criteria of invasion, such as pleural, bronchial, and vascular invasion and desmoplastic stroma with individual cells or small clusters, are not always recognizable in cases with lymphogenic and/or hematogenic metastases. Nevertheless, in cases with established invasive behavior, the presence of EEP, defined as two or more tumor cell layers growing on the luminal side of the alveolar basement membranes, could frequently be discerned even in a background of potentially retained alveolar architecture. In strict morphologic and biological terms, the EEP may not reflect an invasive focus in and of itself, but rather may represent a surrogate for invasive potential and is frequently observed in association with other characteristics of true invasion. The Delphi process at a minimum revealed it as an ambiguous pattern leading to discordance in the assessment of invasive size. In an effort to improve invasive size reproducibility, the expert panel agreed to arbitrarily include the EEP within the invasive size measurement; however, this designation requires further independent validation.

This is a recognition that the EEP should only be used for separation of invasive versus noninvasive areas and not for assigning into one of the currently recognized patterns. When these areas were adjacent to what was agreed on as lepidic, and often in a zone between lepidic and classic invasive patterns, nuclear grade in the EEP was often higher than the adjacent definitive lepidic

component, more closely resembling that in the unambiguous invasive portion. According to the concept of EEP (Fig. 3), the lower end of the category does not meet criteria for papillary, micropapillary, acinar or solid growth, whereas the higher end approaches these conventional patterns of invasion, raising the possibility that the EEP represents partial involvement of an alveolar space by a tumor clone with invasive properties. The EEP is defined as a luminal epithelial proliferation with stratification of two or more cells. Further investigation is needed about this threshold. Furthermore, the extent of stratification needs to be analyzed to avoid inclusion of folds and tangential cutting. During the writing of this document, Yotsukura et al.[28] published a study with emphasis on cancer-associated fibroblasts and revealed that a lepidic pattern with two or less cell layers was associated with a 100% 5-year survival. Whether this is the optimal threshold as opposed to two or three (or more) cell layers requires further study.

The review of images by the panel by initial subjective impression and then scoring of criteria were informative. Major criteria, when found in combination, modestly improve the agreement of observers in invasive versus noninvasive designation, thus supporting their use in assessing invasive size for staging. The use of the minor criteria was not directly supported; however, these remain in the flowchart as their utility may be greater in whole slides than in static images, as they pertain to areas of transition. We recognize that this tool for determination of invasion in pulmonary adenocarcinoma may not be explicit in every single case. The minor criteria may be of value when the major criteria are more ambiguous.

In case of doubt whether an area should be designated as invasive or noninvasive, most of the group decided in favor of "upgrading": that is, after taking into account all the morphologic factors described previously, in cases where there remained doubt as to whether or not invasion was present, we favor concluding that the doubtful area be considered invasive disease. Part of the motivation for this decision is that the overall measurement of the tumor grossly and histologically is reproducible, and in the absence of definitive reason, it should not be superseded. We are aware that this contradicts the recommendations in the TNM staging system, where cases are downstaged, when there is doubt. The conclusion of most of the invasion working group is an attempt to be pragmatic and reflects prevailing diagnostic practice.

The switch from in situ to invasive tumor growth represents a crucial stage in the evolution of lung adenocarcinoma. Nevertheless, the biological understanding of this shift is limited. In some cases, it represents evolution of an invasive subclone, but sometimes, as found by Moore et al.,[29] the in situ component represents peripheral outgrowth of invasion-competent disease rather than a preexisting low-grade precursor. The classic appearance of invasiveness arising in a low-grade lesion was characterized by the following: (1) clear nuclear-grade difference between the in situ and invasive components; (2) architectural asymmetry reflecting the stochastic appearance of the invasive component; and (3) the absence of an in situ "penumbra," with penumbra referring to an in situ component of uniform width at the edge of the lesion. The cytonuclear change (transition) is also presented in Table 1.

In the cases where it is difficult to judge the presence of invasion, the pathologist should err on the side of invasion. This may also diminish the fear of possible underdiagnosis and understaging.

Several issues may modify the morphology of pulmonary adenocarcinoma. First, the recognition of iatrogenic collapse is important. Although the fifth edition of the WHO classification mentions "parenchymal collapse" on page 68,[1] a difference between the pathologic (irreversible) collapse and iatrogenic (at least partly reversible) collapse is not made.[20] A sense of the magnitude of the diagnostic difference is obtained from a small proof-of-principle study, evaluating the effect of iatrogenic collapse on adenocarcinoma classification: in approximately 20% of the cases, the diagnosis was downgraded to AIS.[39] Although perfusion fixation through the airways and/or transpleural perfusion by needle and syringe may reduce the amount of artifactual collapse in many cases, this mitigation effort may not be fully realized: (1) wedge and larger resections may be sectioned fresh for frozen section analysis; (2) despite perfusion fixation, collapse can still be an issue: the lobe volume reached, after perfusion fixation, on average 50% of the lobe volume calculated with computed tomography imaging[40]; (3) in some countries, such as Japan,[41] and some laboratories in the United Kingdom, The Netherlands,[42] Switzerland, and USA, perfusion fixation is part of the routine handling, whereas in many other countries, this is not the case. The group agreed that perfusion fixation is recommended for lung resections, when possible, recognizing that this may be difficult in situations requiring fresh tumor tissue procurement for clinical trials and correlative research.

A second issue may be the effect of preanalytical handling. The routine formaldehyde fixation is usually adequate in small biopsies, but especially in larger surgical specimens, it may be delayed because of low diffusion rate of formalin, especially if fixation depends on immersion in, rather than inflation with, the fixative.[20] Forcing samples into too small containers with insufficient formalin amounts also induces parenchymal compression and inadequate fixation artifacts. This is a

frequent occurrence for those laboratories receiving samples from external hospitals and already fixed in formalin. In addition, although reduction of ischemic time has been a focus for breast specimens, leading to more rapid delivery to pathology, this is not routine in lung specimens. The detachment of the respiratory epithelial cells from the basement membrane is frequently found in autopsy specimens. Likewise, tumor cells undergo the same delay in fixation and may become detached.[42] Recognition of poorly fixed areas is important, as these areas with the misleading appearance should not be considered during diagnostic assessment, if possible.

The use of ancillary stains such as the elastin[20] and cytokeratin 7 stain for recognition of the preexisting alveolar framework and underlying lobular architecture was discussed (Supplementary Table 4 and Supplementary Fig. 10). Most of the group felt that more studies are needed.

A major limitation of this study is the lack of validation in a large independent cohort. The addition of a new feature to a classification may also add further confusion.[36] In this article, we have tried to understand the factors influencing the subjective judgments made by a large group of experienced pulmonary pathologists when assessing early stage adenocarcinomas.

Although the IASLC Staging and Prognostic Factor Committee encouraged further research on what is the best method of measuring size of invasive versus lepidic components,[7] we used a ruler available in the software to measure invasive size and register invasive area but did not attempt to determine what the best method is for establishing invasive area. A one-dimensional ruler is a validated approach for measuring the maximum axis or diameter of an area.[43] Nevertheless, all measurements in this study were conducted with the same method, providing validity for the poor reproducibility on measurements of invasion by pulmonary pathologists from all over the world.

We have identified a number of features, including consistently increased cellularity in a setting of retained alveolar architecture, which we have called EEP, and which seems to lead to a more consistent and accurate discrimination between the binary question of invasive versus noninvasive growth of lung adenocarcinoma. This work and the proposals within require validation and study in other large cohorts; we hope that the wider lung pathology community will take up this challenge.

CRediT Authorship Contribution Statement

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Mary Beth Beasley, Alain Borczuk, Sanja Dacic, Keith M. Kerr, Yuko Minami, Andrew G. Nicholson, Masayuki Noguchi, Lynette Sholl, Ming-Sound Tsao, Erik Thunnissen: Validation.

Birgit Lissenberg-Witte, Alain Borczuk, Erik Thunnissen: Data curation.

Birgit Lissenberg-Witte, Alain Borczuk, Erik Thunnissen: Formal analysis.

Alain Borczuk, Erik Thunnissen: Visualization.

Mary Beth Beasley, Alain Borczuk, Sanja Dacic, Keith M. Kerr, Yuko Minami, Andrew G. Nicholson, Masayuki Noguchi, Lynette Sholl, Ming-Sound Tsao, Erik Thunnissen: Roles: writing—original draft.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at https://doi.org/10.1016/j.jtho.2022.11.026.

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