

Review Article

Lung cancer cytology: potential pitfalls and mimics - a review

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Abstract: Cytology is increasingly being used in the evaluation of lung lesions. There are several potential pitfalls and mimics encountered in the evaluation of respiratory cytology specimens, making interpretation of respiratory cytology challenging. Familiarity with the mimics and the pitfalls is essential in avoiding a misdiagnosis because a false positive or false negative diagnosis may have significant management implications. This article focuses on the main classification of primary lung carcinoma – small cell carcinoma, adenocarcinoma and squamous cell carcinoma - with potential mimics discussed under each tumor category. We have attempted to separate pitfalls from common potential mimics and have suggested general rules when such pitfalls are encountered.

Keywords: Pitfalls, mimics, respiratory cytology, clinical history, pulmonary carcinoma, lung cancer, adenocarcinoma, squamous cell carcinoma, non-small cell carcinoma

Introduction

Lung cancer is the second most common cancer in incidence but the leading cause of cancer deaths in men and women in the United States. According to the American Cancer Society, in 2009 there were estimated 219,440 new cases, which represent approximately 15% of all new cancer cases, excluding basal and squamous cell carcinoma and in situ carcinoma except bladder cancer. The total estimated lung cancer deaths, however, was estimated to be 159,390 representing 30% and 26% of all cancer deaths in males and females respectively [1]. This dismal mortality makes early diagnosis and treatment of utmost importance. Cytology plays an important role in the initial evaluation and diagnosis of patients with lung cancer. Various sampling techniques are currently available to procure specimens for cytologic evaluation of lung tumors. These include exfoliative (induced sputum, thoracentesis), abrasive cytology (bronchioalveolar lavage, bronchial brushing, bronchial washing) and fine needle aspiration [FNA] cytology (endobronchial ultrasound

guided, transesophageal, CT-guided percutaneous / transthoracic). The cytomorphologic diagnosis of lung malignancies is fraught with numerous mimics and pitfalls that may lead to false positive or false negative diagnoses. A zero false positive rate may be unattainable as a false positive rate of approximately 1% was observed even with experienced cytopathologists in a study by Thivolet-Bejui [2]. It is critical to be familiar with the potential pitfalls and mimics in respiratory cytopathology since false positive diagnoses may result in a significant morbidity or even mortality; while false negative diagnosis may result in delayed diagnosis and treatment. This review will discuss common pitfalls and mimics that may be encountered and also highlight an algorithm useful in the evaluation of respiratory cytology.

Adenocarcinoma

Pulmonary adenocarcinoma usually arises in a peripheral location and may thus be sampled by CT-guided percutaneous / transthoracic FNA. There are several variants of adenocarcinoma,

Table 1. Cytomorphologic features and potential mimics of pulmonary adenocarcinoma (ADCA)

Cytologic features	Pulmonary ADCA	Clear cell tumor	Hamartoma	Granular cell Tumor	Atypical Type II Pneumocytes	Reactive Bronchial cells	Bronchial cell hyperplasia	Goblet cell Hyperplasia	Therapy chemoradiation
Cellularity	Usually hypercellular	May be hypercellular	Usually hypocellular	May be Hypercellular	Usually hypocellular	Usually hypocellular	May be hypercellular	Usually hypocellular	Usually hypocellular
Background	Necrotic	Non necrotic Foamy Vacuolated	Non necrotic	Non necrotic Granular	Non necrotic May be inflammatory	Non necrotic	Non necrotic May be inflammatory	Non necrotic	+/- necrosis inflammatory
Pattern	Clusters / acinar Single cells 3 - dimensional	Clusters Single cells	Sheets of bronchial cells Mesenchymal cells	Loose sheets	Cluster with "knobby" border	Cohesive clusters 3- dimensional	Papillary clusters	Small clusters 3-dimensional	Cohesive cluster Individual cells
Cell type	Cuboidal / columnar Polygonal	Polygonal Spindle	Epithelial and Mesenchymal	Polygonal Fusiform	Cuboidal	Cuboidal Columnar	Columnar	Columnar	Variable
Cytoplasm	Scant to moderate Fine to coarse vacuolization	Moderate to abundant Vacuoles	Scant to moderate	Abundant Ill defined Granular	Moderate Vacuolated	Moderate to abundant	Moderate Variable mucus producing cells	Single large mucin-filled cytoplasmic vacuoles	Abundant Cytoplasmic vacuolization: "two toning" of the cytoplasm Enlarged, with hyperchromasia and occasional degeneration
Nuclear	Variably sized; round to oval; High N/C ratio. May be eccentric	Round to oval; Low N/C ratio May have inclusions	Round to oval Uniform	Round to oval. Uniform (occasional atypia)	Round to oval; there may be high N/C ratio	Variably sized; nuclear features may be degenerated	Small and uniform. Round or oval	Small, uniform	Enlarged, with hyperchromasia and occasional degeneration
Nuclear membrane	Irregular	Smooth	Smooth	Smooth	Usually smooth but may be irregular	Usually smooth/regular	Smooth	Smooth	Smooth. May be irregular
Chromatin/ Nucleoli	Finely granular / Prominent (may be multiple) Mitosis +	Finely granular / Small Mitosis +/-	Finely granular / Inconspicuous Mitosis +/-	Finely granular / Indistinct Mitosis -	Variable, +/- coarse / Variable Mitosis +/-	Variable, fine to coarse / Single or multiple Mitosis +/-	Finely granular / Inconspicuous Mitosis +/-	Fine chromatin / Inconspicuous Mitoses +/-	Variable - may be smudgy / Macronucleoli +/- Mitosis +/-
Misc.	There may be intranuclear pseudo-inclusions; no cilia	-Positive for HMB-45. -Related to the PEComa family group -Glycogen in cytoplasm	Cilia are often present, but may be absent. Mesenchymal component helps	Positive for S100	Normal Type II Pneumocytes are morphologically similar to pulmonary macrophages	Cilia and terminal bars are often present (not always. Atypia can be quite significant)	Also known as Creola body. Cilia and terminal bars are often seen.	The normal ratio of goblet: bronchial cells is 1:6.	N/C ratio is maintained (or mildly increased, in spite of the cytomegaly.

including the more common acinar, papillary, mixed acinar-papillary and solid. Bronchioloalveolar carcinoma (BAC), defined by convention as lacking stromal, vascular or capsular invasion, is often included in this category. Typical cytomorphological features of adenocarcinoma include cellular clusters with depth of focus; however, there may also be individual cells or acinar (glandular) arrangements. Depth of focus is one of the major cytological features, because adenocarcinoma is often present as three-dimensional clusters of large vacuolated cells. These cells are columnar, cuboidal or polygonal with variable cell size and variable quantity of fine vacuolated cytoplasm. The nucleus is variably sized round to oval nuclei, often eccentric with high nuclear/cytoplasm (N/C) ratio, finely granular chromatin. Prominent central cherry red nucleoli are variably identified. On a cautionary note, BAC may have a large number of bland neoplastic cells [which may resemble alveolar macrophages/bronchial cells] that may suggest a reactive or reparative process. The presence of papillary fronds with fibrovascular septa and/or psammoma bodies should suggest the diagnosis of BAC.

Mimics and pitfalls for lung adenocarcinoma are summarized in **Table 1**, **Figures 1**, **4** and **5**.

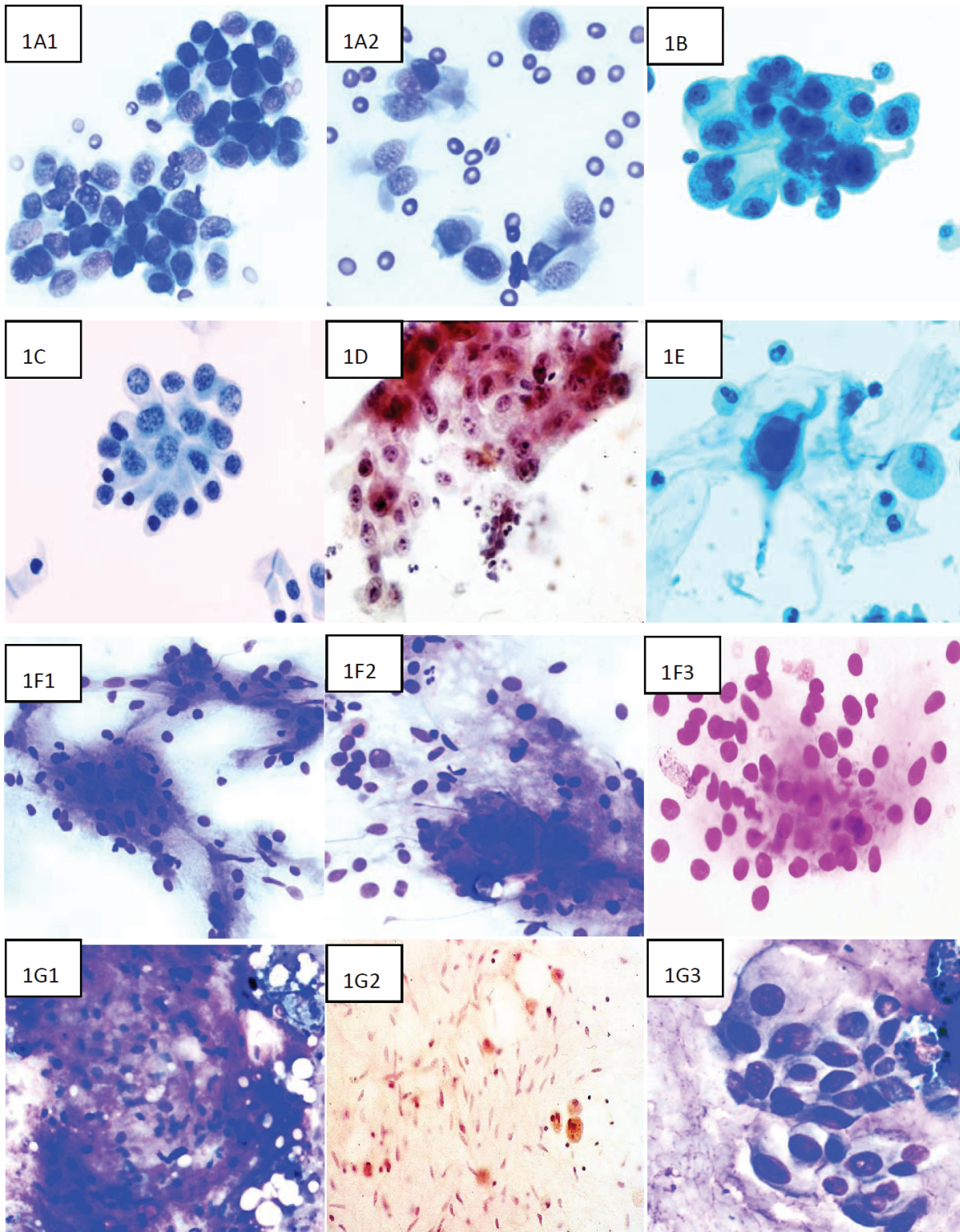
Reactive type II pneumocytes

Reactive type II pneumocytes are often seen in bronchoalveolar lavage (BAL), bronchial washing and sometimes in FNA specimens. The epithelial surface area of the alveoli consists predominantly of thin or flat type I alveolar pneumocytes for efficient gaseous exchange. Type II pneumocytes comprise less than 5% of the alveolar surface area and are important for the synthesis and secretion of surfactant, and regeneration of alveolar epithelium following injury [3]. In view of its functions, significant regenerative atypia may sometimes be in Type II pneumocytes. Over-interpretation of these reactive changes may lead to a false positive diagnosis. Typically, Type II pneumocytes are relatively small, present as individual or loose clusters of cells with finely vacuolated cytoplasm, low N/C ratio and central to eccentric nuclei with inconspicuous nucleoli. Type II Pneumocytes are often difficult to distinguish from macrophages.

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Atypical pneumocytes may be seen in conditions that cause injury to the alveolar epithelium, resulting in regeneration of Type II pneumocytes. Such conditions include organizing pneumonia, infarcts, adult respiratory distress

syndrome, acquired immunodeficiency syndrome and oxygen therapy. Cytomorphologically, the Type II pneumocytes may demonstrate cohesive clusters with high nuclear:cytoplasmic (N/C) ratios, hyperchromatic nuclei with nuclear



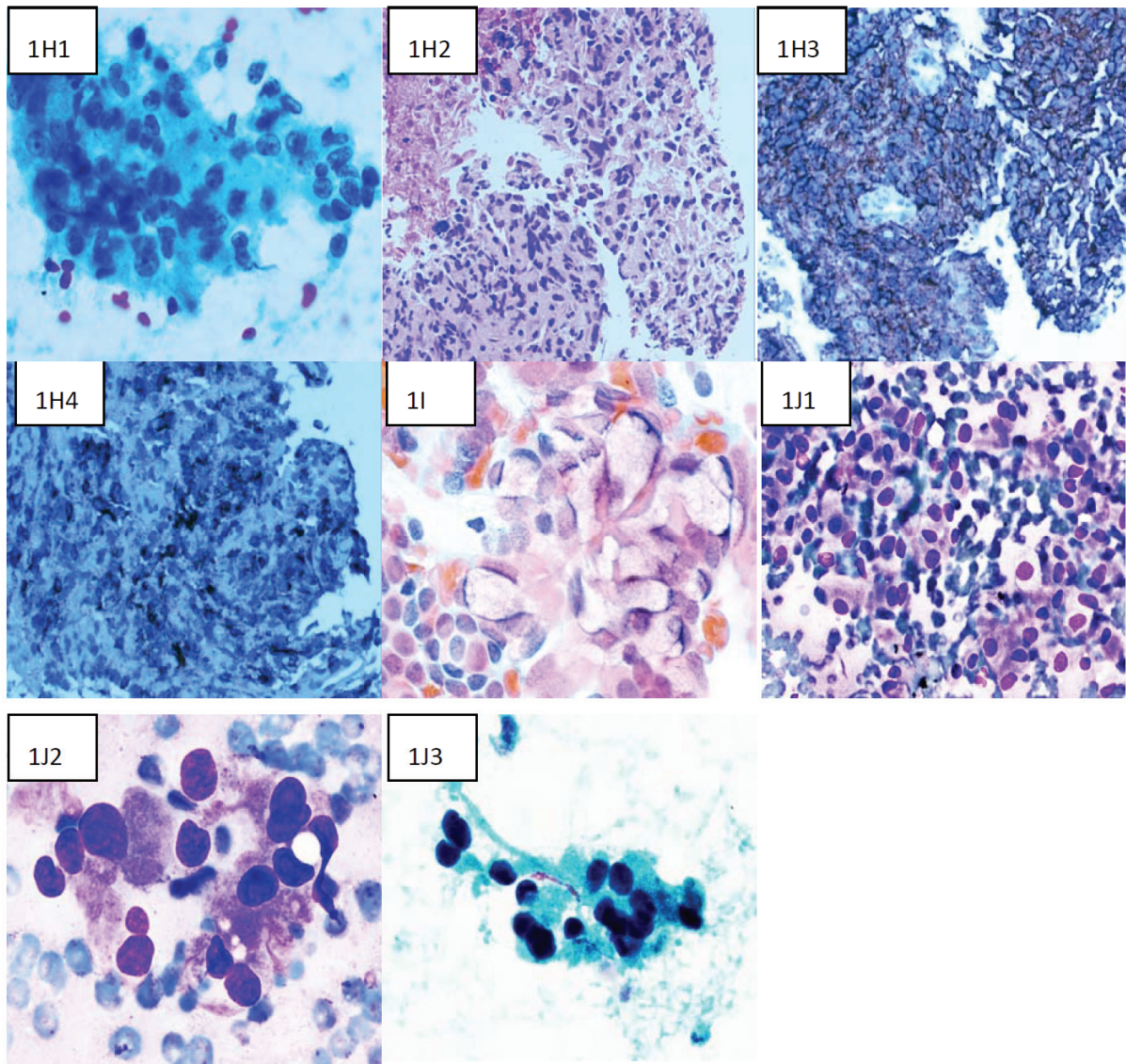


Figure 1. (1A1,1A2). Adenocarcinoma. There are clusters of tumor cells (1A1) and single cells (1A2), with high N/C ratio, irregular nuclear membrane, granular chromatin and nucleoli. Occasional reactive bronchial cells with cilia mixed with the single tumor cells (Diff Quik, 1A1 X200; 1A2 X400); **(1B).** Diffuse alveolar damage. Clusters and individual cells of reactive Type II pneumocytes. Significant atypia may be encountered in cytologic specimens from patients with diffuse alveolar damage. (Papanicolaou stain, X400); **(1C).** Reactive bronchial cells. Cluster of reactive bronchial cells with moderate cytoplasm, enlarged nuclei, with smooth nuclear membrane, granular chromatin and inconspicuous nucleoli. While there is no cilia on identified in the cluster, there are individual reactive bronchial cells with cilia in the background with somewhat similar cytologic features (Papanicolaou stain, X200); **(1D).** Congestive heart failure. Loose clusters of severely atypical bronchial cells with moderate cytoplasm, increased N/C ratio, relatively smooth nuclear membrane, granular chromatin and prominent nucleoli. There are some acute inflammatory cells present (Papanicolaou stain, X200); **(1E).** Atypical pneumocytes. Individual atypical Type II pneumocyte with high N/C ratio and smudged nuclear features is present. The specimen is from a patient status post transplant for myelodysplastic syndrome. There is no evidence of a mass lesion in this patient (Papanicolaou stain, X400); **(1F1-1F3).** Clear cell tumor. Note the loose cluster with delicate transgressing vessel, single cells with stripped cytoplasm and foamy vacuolated background. The nuclei are round to oval, uniform with smooth nuclear membrane, evenly dispersed chromatin and inconspicuous nucleoli. HMB-45 may be useful in differentiating clear cell tumor from renal cell carcinoma (Diff Quik, 1F1 X100, 1F2 X200; Papanicolaou stain 1F3 X200); **(1G1-1G3).** Pulmonary hamartoma. Note the mesenchymal and epithelial components. The chondromyxoid stroma is best appreciated on Diff-Quik stain (1G1 X200) but is also appreciable on Papanicolaou stain (1G2 X200). The epithelial component in this case does not have cilia (1G3, Diff Quik X400). While cilia are often seen in the epithelial component, the absence of cilia does not preclude a diagnosis of hamartoma; *(to be continued to next page)*.

(1H1-1H4). Post transplant lymphoproliferative disorder (PTLD). Loose cluster of atypical cells with moderate amount of cytoplasm, anisonucleosis and prominent nucleoli, features suggestive of an epithelial neoplasm are present. The corresponding H&E shows cohesive pleomorphic tumor cells with moderate amount of cytoplasm and adjacent necrosis. Immunohistochemical stains with CD20 and EBV LMP are positive, cytokeratin stains are negative (not shown). The patient is status post liver transplant for autoimmune hepatitis, presenting with multiple lung nodules and mediastinal adenopathy. The apparent cohesion of the cells may lead to a diagnosis of adenocarcinoma. The clinical history should raise the possibility of a PTLD, with appropriate work-up. (Papanicolaou stain, 1H1 X200; Hematoxylin and eosin stain, 1H2 X100; CD20 immunohistochemistry 1H3 X100; EBV LMP immunohistochemistry 1H4 X100); (1I). Goblet cell hyperplasia. Small clusters of columnar, mucin filled cytoplasmic vacuoles suggestive of signet ring cell. The nuclei are small and uniform with smooth nuclear membrane, fine chromatin and inconspicuous nucleoli. There are reactive bronchial cells in the background (Diff Quik X200); (1J1-1J3). Granular cell tumor. Loose sheets of polygonal cells with abundant ill defined granular cytoplasm. Some of the cells are stripped of their cytoplasm creating a granular background. The nuclei are round to oval with smooth nuclear membrane, fine granular chromatin and inconspicuous nucleoli (Diff Quik, 1J1 X200x, 1J2 X400; Papanicolaou stain 1J3 X200).

membrane irregularity, coarse chromatin and prominent nucleoli, features which mimic adenocarcinoma [4-11]. Awareness of these atypical features and clinical and radiologic information [e.g. absence of a mass or acutely ill patient] are critical in avoiding a false positive diagnosis.

Goblet cell hyperplasia

Goblet cell hyperplasia which consists of an increased area and number of goblet cells in the lower airways may be seen in asthma or chronic obstructive airway disease [12]. Given the location of the goblet cells, goblet cell hyperplasia in cytology specimen is more often encountered in bronchoscopy specimens or endobronchial ultrasound guided FNA, rather than percutaneous FNA. Such hyperplasia may mimic mucin producing adenocarcinoma, like mucinous BAC or metastatic signet ring cell carcinoma. Features suggestive of a benign process include a clean background with single cells containing large distended cytoplasmic vacuoles which indent uniform basal nuclei, as well as smooth nuclear membranes, fine chromatin and inconspicuous nucleoli. Furthermore, the clinical history of chronic obstructive airway disease and/or the absence of discrete mass on imaging may help in correctly diagnosing this process as benign rather than malignant.

Bronchial cell hyperplasia

Bronchial cell hyperplasia may be seen in the context of asthma, tuberculosis, viral infection or instrumentation. Cytologically, it consists of tight papillary clusters with occasional molding and variable numbers of mucus producing cells

– often called Creola bodies. The cells have uniform small, round to oval nuclei with bland finely granular chromatin and inconspicuous nucleoli. Typically, cilia and/or terminal bars are identifiable. Depending on the etiology, the background may be clean or inflammatory - eosinophils may occasionally be seen. There may be increased cellularity and the clusters may sometimes contain atypical cells mimicking adenocarcinoma. Clues to the benign nature of these clusters include the presence of cilia, the bland nuclei, finely granular chromatin and the tightness of the cluster. Furthermore, the clinical history and imaging are critical in avoiding this pitfall.

Reactive/atypical bronchial cells

Reactive bronchial cells may be encountered in the setting of pneumonia, diffuse alveolar damage (including the proliferative/organizing phase), pulmonary infarct, asthma, chronic obstructive airway disease, environmental toxins, radiation/chemotherapy treatment effects and instrumentation [7, 13-17]. Cytologic atypia in these settings can be quite significant, creating a major source of false positive diagnoses. Reactive bronchial cells may form three-dimensional clusters but often have uniform cohesive sheets of cells with a streaming pattern. There is a high N/C ratio but the cytoplasm is still abundant. The nuclei may be hyperchromatic but if so will appear degenerative with loss of nuclear details. The nuclear membranes are regular with fine and evenly distributed chromatin. Nuclei may have coarse chromatin with single or multiple macronucleoli and infrequent mitoses. Cilia and terminal bars are usually present but may be lost due to degeneration

and may not be identified in the specimen [15-21]. Clues to the benign and reactive process include finding identical atypical nuclear features in other cells with cilia and terminal plates, the presence of a few single atypical isolated cells within an obviously benign group, and a spectrum of changes from normal to atypical instead of the distinct two cell population of normal and malignant cells [17]. Nucleomegaly (up to 6 times normal size) may be seen in reactive atypical bronchial cells, larger nuclear size and nuclear pleomorphism should raise the suspicion of malignancy [19]. Multinucleated cells may sometimes be seen, but their nuclei are identical to those of single reactive cells [7]. While it is prudent to identify cilia on atypical cluster of cells and to use this as a sign of benignity, a word of caution is in order, because a hasty dismissal of a cluster with cilia as benign without consideration to all the cytologic features may lead to a missed diagnosis of cancer [17]. It has been suggested that repeat cytology may be useful in demonstrating that the atypia decreases or disappears in cases of reactive bronchial cells [18].

Therapeutic effects

Radiotherapy or chemotherapy induced cellular changes may induce significant cellular atypia, leading to a false positive diagnosis for the urinary [16, 17, 22-24]. Changes related to therapy effects may be seen within a few weeks of the beginning of therapy and may resolve by a month after the cessation of the therapy [17, 22]. However, some radiation induced changes may persist throughout life and seen in cytologic specimens as dispersed individual atypical cells. Responses to radiation or chemotherapy may be patchy or diffuse and often bilateral, and such atypical cells may be obtained by bronchial washings or lavage. Rarely, there may be pulmonary consolidation secondary to these processes, in which case the specimen may be obtained by FNA. Atypical reactive bronchial cells secondary to chemoradiation may occur as cohesive groups or scattered individual cells with cytomegaly (nuclear and cytoplasmic enlargement with mildly increased to normal N/C ratio), nuclear hyperchromasia (and in some cases smudgy nuclei), macronucleoli, and irregular nuclear membranes [16, 17, 22-24]. Like bronchial cells, macrophages and alveolar pneumocytes may show similar atypia. In extreme cases of radiation induced changes, it

may be difficult to distinguish the resulting bizarre cells from carcinoma. This atypia may mimic adenocarcinoma and *squamous cell carcinoma*. Cytologic features favoring radiation/chemotherapy induced changes rather than malignancy include repair-like arrangement with nuclear and cytoplasmic vacuolization, degenerative nuclei and occasional two-toning of the cytoplasm. Non-vacuolated reactive atypical cells can be differentiated from carcinoma by degenerative, smudged nuclei, with no distinct features of malignancy. Clinical history is of utmost importance in avoiding overdiagnosis of these changes as a malignancy [16, 17, 22-24].

Clear cell (sugar) tumor

Cytologic diagnosis of clear cell (sugar) tumor may be challenging because this is a rare benign tumor of the lung [25] infrequently encountered as a cytology specimen. Clear cell (sugar) tumors of the lung were originally described by Liebow and Castleman [26, 27]. Cytomorphologic findings include a dual population of cohesive clusters of polygonal cells and spindle cells with delicate transgressing vessels. Mild anisonucleosis, single spindle-shaped and polygonal cells may also be seen. The cytoplasm is fragile and may be stripped, creating a vacuolated, granular or foamy background and naked nuclei. Nuclei are round to oval with smooth nuclear membranes. There may be intranuclear inclusions, however, no overt cytologic atypia, necrosis or mitoses are present [28, 29]. This tumor is characteristically positive for HMB45, similar to other perivascular epithelioid cell-like tumors (the so called PEComas family). This tumor may mimic clear cell variant of lung adenocarcinoma or acinic cell carcinoma as well as metastatic carcinoma [including renal cell carcinoma, adrenal cortical carcinoma, melanoma and clear cell carcinoma of the female genital tract]. For the clear cell variant of lung adenocarcinoma, other features suggesting of carcinoma, like overt malignant cells with glandular or squamous differentiation will be identifiable. Acinic cell carcinoma consists of cellular smears thick-layered or monolayered clusters and dissociated large polygonal cells with low N/C ratio, uniform round eccentrically or centrally situated nuclei with finely granular cytoplasm and inconspicuous nucleoli [30]. It is also prudent to be cautious in making a diagnosis of metastatic carcinoma when there is a clear cell morphology unless there is supporting clinical and radiologic

information, ancillary tests of the cytology specimen (e.g. immunohistochemistry) and review of the previous pathology material if available [31].

Granular cell tumor

Granular cell tumor is generally a benign tumor of Schwann-cell origin which is usually endobronchial, but may be located in the lung parenchyma [32]. Given the predominant location, it is often sampled by FNA, but may be obtained by bronchial brushing. Cytologically, the tumor consists of variably large, thick syncytial clusters or sheets, with ill-defined, coarsely granular cytoplasm, oval nuclei with smooth nuclear membranes, finely granular chromatin, inconspicuous nucleoli and no mitosis [33]. The cytoplasm is fragile and often stripped off, creating a vacuolated and granular background. This tumor is positive for S100. Careful attention to the cytologic features, the granular cytoplasm and absence of overt malignant features should help in avoiding the pitfall of misdiagnosis [33] of this entity as adenocarcinoma.

Pulmonary hamartoma

Pulmonary hamartoma (PH) is the most common benign neoplasm of the lung consisting of cartilage, adipose tissue, smooth muscle and respiratory epithelium [34]. PH is thought to arise from embryologic rests that are present in fetal life, but generally do not become visible until adulthood [35]. They are often asymptomatic, discovered incidentally on chest radiograph as a coin lesion with "popcorn" calcifications and are usually less than 4 cm. Cytologically, the aspirates consist of variable proportion of both epithelial and mesenchymal elements (such as fat, smooth muscle, cartilage, fibrous tissue and occasionally bone). The epithelial cell component consists of bronchial cells that appear as sheets of small uniform cuboidal or columnar cells with *or without cilia*. The cells have scant to moderate cytoplasm with round to oval cells nuclei, smooth nuclear membranes, finely granular chromatin and inconspicuous nucleoli. The aspirates may be paucicellular (depending on the proportion of the epithelial or mesenchymal elements in the tumor), but the background is clean [34-38]. The cytodiagnosis of PH relies heavily on sampling its mesenchymal component (like cartilage often with chondrocytes in lacunae or fibromyxomatous fragments) and their definite dis-

tingtion from other tissue or cellular elements that may mimic them [36]. The mesenchymal component may be easier to identify on Diff-Quik-stained preparations compared to Papanicolaou-stained preparations. The presence of adipose tissue admixed with the other components may be useful; however, adipose tissue in of itself may be of limited cytologic value because it may be obtained from chest wall adipose tissue. Accurate diagnosis of PH can be challenging as highlighted in a study using the data from College of American Pathologists (CAP) inter-laboratory comparison program, where there was a 22% false positive rate with PH. Commonly selected false positive diagnoses include carcinoid tumor, adenocarcinoma and small cell carcinoma [35]. While 78% of the respondents made a correct classification of benign lesion, the overall accuracy of making a correct specific diagnosis of PH was 26% [35]. Possible explanation for the high false positive rate and the low overall correct specific diagnosis include the failure of the participants to recognize the mesenchymal component which was present but often subtle on Papanicolaou preparations, as well as the failure to recognize the benign nature of the often very cellular epithelial component [35]. The epithelial component may show reactive changes such as large variably sized nuclei (no distinct two cell population of benign and malignant cells), intranuclear invaginations and multinucleated cells, with or without cilia. In some cases reserve cells may be prominent which may lead to a misdiagnosis as neuroendocrine tumor. It is critical to be aware of these pitfalls and pay particular attention the radiographic findings and to the fine cellular details which often will show the dual epithelial and mesenchymal components [35].

Reactive mesothelial cells

Mesothelial cells may be encountered in specimens obtained by CT-guided percutaneous or transthoracic FNA or in pleural effusion. The cytomorphology of mesothelial cells consists of sheets or individual cells with "windows" between the cells, well defined amphophilic cytoplasm, round to oval nuclei with occasional grooves, but smooth nuclear membrane contour and small nucleoli. Occasionally, reactive mesothelial cells show single cells and a few clusters of atypical cells with high N/C ratio, hyperchromatic nuclei and intracytoplasmic vacuoles -

resulting in misinterpretation as adenocarcinoma. Of note, the cytoplasmic vacuoles in reactive mesothelial cells tend to be paranuclear, without indentation of the nuclear membrane unlike in adenocarcinoma. In some cases, it may be necessary to use immunohistochemistry in the determination [21, 24]. GLUT-1, E-cadherin and desmin among others have been reported to be useful in differentiating reactive mesothelial cells from adenocarcinoma with E-cadherin positive in adenocarcinoma and mesothelioma but negative in reactive mesothelial cells; desmin positive in reactive mesothelial and negative in carcinoma; while GLUT-1 is positive in carcinoma and negative in reactive mesothelial cells [39-41].

Contaminants

FNA specimens from the right lower lobe may be contaminated by inadvertent aspiration of the liver. This contamination may lead to the diagnosis of malignancy [16] by the unwary. This possibility needs to be kept in mind to avoid the pitfall of false positive.

Pitfalls in subclassifications of non-small cell lung carcinoma

Although, the classification of primary lung carcinoma into small cell and non small cell carcinoma was adequate for management in the past, the advancement in the understanding of molecular mechanisms of lung cancer and the recent identification of mutation / gene copy number gain of epidermal growth factor receptor (EGFR) in a subset of patients diagnosed with adenocarcinoma which predict response to therapeutic intervention by anti-EGFR tyrosine kinase inhibitors (TKI) [42-56] makes it important to subclassify non small cell carcinoma into adenocarcinoma and squamous cell carcinoma. While this subclassification of NSCLC can be readily made in well differentiated tumors when the characteristic cytomorphologic features are present, it may be a real challenge in poorly differentiated tumors. In difficult cases ancillary studies like immunohistochemistry may be necessary for the subclassification. Preferably cell blocks, but smears and cytopins as well, may be used for ancillary tests, if necessary. In fact, cytology specimens can also be used for the EGFR testing [57-62]. The use of cytology specimens for the subclassification of NSCLC and

EGFR testing may be challenged by some, especially, if the patient is a surgical candidate. On the other hand there is really no point in making a less specific diagnosis, if a more specific diagnosis is possible despite the size of the specimen. Furthermore, it is a good practice to subclassify with cytologic specimens, because this will obviate the need for a more invasive procedure to obtain additional tissue specimens for subclassification.

Primary versus metastatic adenocarcinoma

For management and staging purposes, it is important to distinguish between primary lung cancer and pulmonary metastases. The clinical history of a known extrapulmonary primary and the radiologic findings of multiple nodules in the lung are useful in arriving at the right diagnosis. However, the clinical history may not be available or the pulmonary metastasis may rarely be the first manifestation of an extrapulmonary tumor. Furthermore, pulmonary metastasis may present as a single nodule, while bronchioloalveolar carcinoma is known to present as multiple nodules. In fact primary lung carcinoma may have intrapulmonary metastasis presenting as multiple nodules [63-65]. Some tumors may have sufficient characteristic cytomorphologic features which raise the possibility of metastatic disease. For example, the presence of large clusters of loosely cohesive to discohesive cells with fairly uniform, elongated and hyperchromatic nuclei basally oriented in a "picket fence array" with background dirty necrosis should raise the possibility of colorectal cancer. Similarly, deceptively bland clusters of cells with foamy, multivacuolated cytoplasm [resembling pneumocytes and histiocytes, but with no carbon pigments] with low N/C ratio, round nuclei, fine chromatin and variable nucleoli may raise the possibility of renal cell carcinoma. Malignant melanoma may mimic a variety of tumors. However, it commonly appears as large discohesive, pleomorphic cells. Identification of intranuclear inclusion, double mirror image nuclei and melanin pigment suggests the diagnosis. In the more challenging cases distinctive features are not present to suggest a primary site. Even in these instances, when the potential for a metastasis exists, ancillary immunohistochemistry tests will be necessary to assist in narrowing down the primary site, especially, when there is no previous pathology material (slides or images) avail-

Table 2. Cytomorphologic features and potential mimics of squamous cell carcinoma (SCCA)

Cytologic features	Squamous cell carcinoma (SCCA)	Squamous metaplasia	Pulmonary infarction	Meningioma	Vegetable matter
Cellularity	Usually hypercellular	Usually hypocellular	Usually hypocellular	Variable. May be cellular	Usually hypocellular
Background	Variably necrotic	Non necrotic	Variable Hemosiderin laden macrophages	Non necrotic	Non necrotic
Pattern	Cohesive sheets Single cells Keratin pearl	Single cells Sheets of cells	Sheets and clusters Single cells May be 3-D	Cohesive whorls Syncytia No keratinization	Single cells Loose cluster
Cell type Characteristic cell type	Strap cells Fiber cells Tadpole cells Polygonal cells	Squamous, mature and immature	Cuboidal to columnar Squamous cell	Fibroblastic Fusiform	Rectangular cells Thick refractile cell wall
Cytoplasm	Variable amount Dense	Dense, basophilic	Variable amount	Scant	Abundant. Basophilic
Nuclear	Anisonucleosis High N/C ratio	Variable, round central. Variable N/C ratio	Round to oval, degenerative	Fusiform Intranuclear inclusion	Small amorphous
Nuclear membrane	Irregular	Regular/smooth	May be irregular	Nuclear grooves Smooth	Regular
Chromatin/ Nucleoli	Variable chromatin Variable nucleoli – may be prominent	Variable chromatin Occasional nucleoli	Coarse chromatin Macronucleoli	Fine	Degenerative
Misc.	Poorly differentiated (PD) SCCA difficult to distinguish from PD ADCA	Necrosis possible, if due to mycetoma	Often wedge shaped lesion on imaging	Absence of keratin and bland features may help History helpful	More commonly seen in sputum specimen

able for comparison. Unfortunately in a few cases, it is almost impossible to distinguish primary lung cancer from metastatic carcinoma without the use of ancillary tests.

Squamous cell carcinoma

Squamous cell carcinoma is usually a centrally located tumor. Cytology specimens may be obtained by EBUS, bronchial brush, bronchial wash among others. Cytomorphologically, well differentiated SCCA typically appears as individual cells or cohesive sheets of tumor cells [depending on specimen procurement method] with well defined cell borders and dense cytoplasm. Some of the cells may be tadpole, fiber or strap-shaped. There is variable N/C ratio and there may be keratin and background necrosis or ghost cells. Poorly differentiated SCCA may be indistinguishable from poorly differentiated adenocarcinoma with high cellularity, discohesive or small groups of tumor cells with high N/C ratio, nuclear pleomorphism with variable

chromatin and occasional nucleoli. Without ancillary tests like immunohistochemical stains, it may be impossible to accurately subclassify these poorly differentiated carcinoma into adenocarcinoma or squamous cell carcinoma.

The mimics and pitfalls for squamous cell carcinoma are summarized in **Table 2**, **Figures 2**, **4** and **5**.

Pulmonary infarction

Pulmonary infarction, presenting as a discrete lung lesion with atypical cytology can lead to a false positive diagnosis of either *adenocarcinoma* or squamous cell carcinoma, even for experienced cytopathologists [7, 14, 24]. On imaging, it may but not always present as a wedge-shaped lesion. The atypical cells may present as a three dimensional sheets/clusters of bronchial cells with variable cytoplasm, small to slightly high N/C ratio, enlarged nuclei, coarse chromatin and irregular macronucleoli.

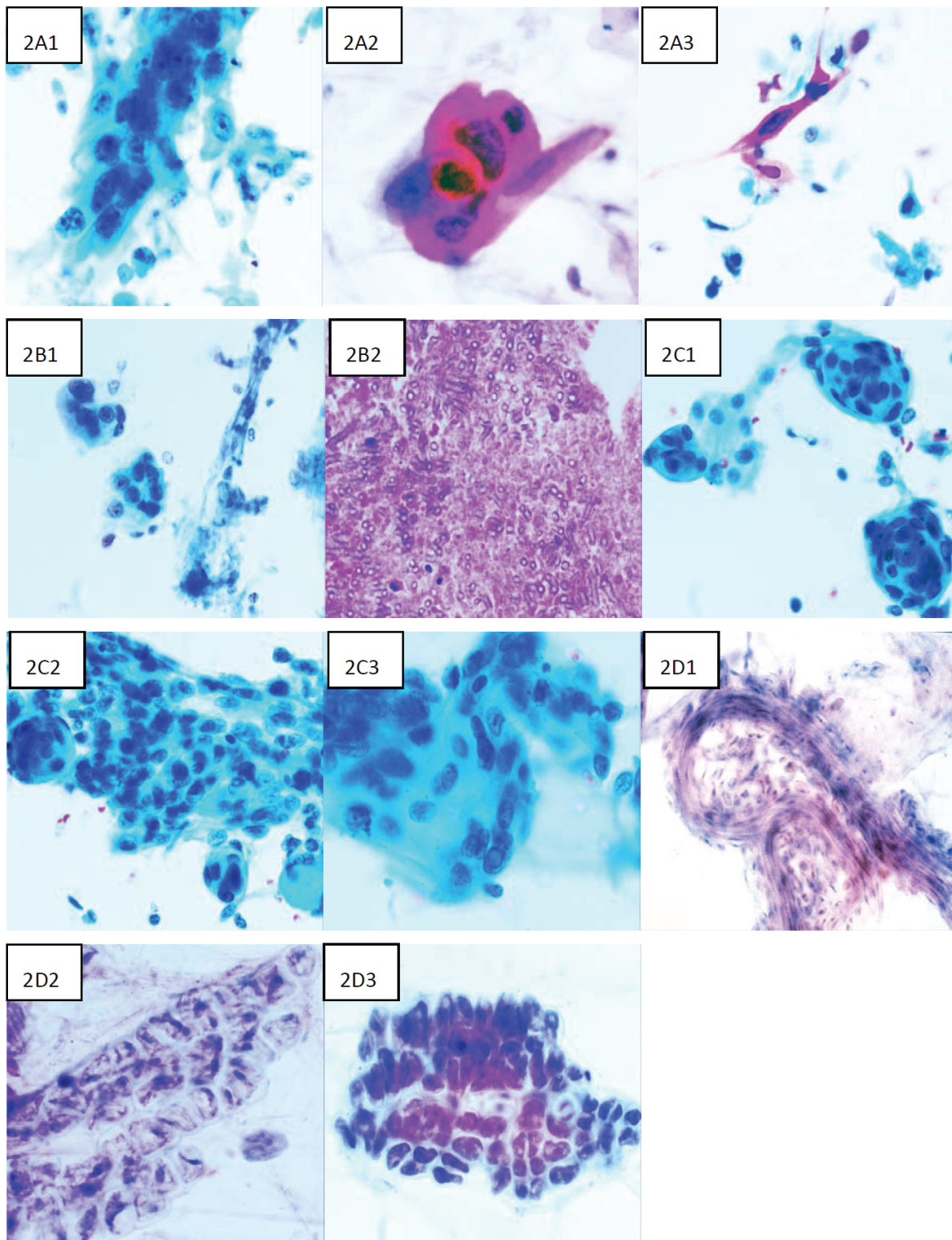


Figure 2. (2A1-3). Squamous cell carcinoma. Loose cohesive sheet of tumor cells and single tumor “strap cells” cells with keratinization are present. Prominent nucleoli may be seen in squamous cell carcinoma (Papanicolaou stain, 2A1 X400, 2A2 X400, 2A3 X200); *(to be continued next page)*

(2B1-2B2). Mycetoma. Loose cluster of atypical cells with adjacent necrosis. The cells have moderate amount of cytoplasm, mild increase in N/C ratio, finely dispersed chromatin and nucleoli. The patient presented with a cavitary lung mass. Corresponding H&E shows that the cavitary mass is due to *Aspergillus* spp. Epithelial atypia around mycetomas may be quite significant (Papanicolaou stain, 2B1 X200; hematoxylin and eosin stain, 2B2 X200); **(2C1-2C3).** Metastatic meningioma. Cohesive whorls and syncytial of cells with no keratinization. The cells have moderate to scant cytoplasm, fusiform nuclei, occasional intranuclear inclusion and nuclear grooves. There is finely dispersed chromatin and inconspicuous nucleoli (Papanicolaou stain, 2C1 X200, 2C2 X200, 2C3 X400); **(2D1-2D3).** Vegetable contaminant. Loose clusters of rectangular cells with basophilic cytoplasm (simulating nuclei), thick clear, sometimes refractile cell wall. The nuclei are inconspicuous. Misinterpretation of basophilic cytoplasm as nuclei may lead to an erroneous diagnosis of squamous cell carcinoma (Papanicolaou stain 2D1 X100, 2D3 X200, 2D2 X200).

Occasionally, there may single keratinized squamous cells with hyperchromatic degenerative and smudged nuclei. These features are sufficiently worrisome to raise the possibility of malignancy. However, careful attention to details will reveal that in the keratinized squamous cells, the nuclei are degenerative. The smears are not cellular and there are numerous hemosiderin laden macrophages, which is suggestive of a reactive process. In view of the worrisome features, caution is warranted and it has been suggested that pulmonary infarction should be considered in the differential diagnosis when the features described above are seen and to repeat the test in equivocal cases [20, 66, 67].

Squamous metaplasia

Continuous irritation of the respiratory epithelium, commonly from cigarette smoking, but also from the presence of a tracheostomy, and instrumentation may lead to squamous metaplasia. In some cases, squamous metaplastic cells from cavitary lesions like mycetoma or tuberculosis [7, 24] may be identified in respiratory specimens. Benign or atypical squamous cells may also exfoliate in patients with *oral lesions or poorly fitted dentures*. Squamous metaplasia is a common finding in sputum and other upper respiratory cytology specimens. Cytologically, metaplastic cells have variable amounts of basophilic cytoplasm with well defined borders, variable N/C ratios, round centrally placed nuclei with smooth nuclear membranes and occasional nucleoli [7]. Metaplastic squamous cells may occasionally be significantly atypical and be mistaken for malignancy. These atypical metaplastic cells may be arranged in clusters or singly with dense eosinophilic cytoplasm, high N/C ratio, hyperchromatic nuclei with irregular nuclear membrane. The concurrent identification of granulomas or fungal elements should

make one hesitate to diagnose a malignancy. Clinical history of instrumentation or tracheostomy may also help avoid this pitfall [7, 16, 17, 24].

Vegetable matter

Vegetable contaminants in respiratory cytology specimens, especially sputum or exfoliative cytology specimens may be mistaken for squamous cell carcinoma, especially when well preserved. Cytologically, vegetable contaminant may appear as single cells or loose clusters of cells with basophilic cytoplasm, thick refractile rectangular cell wall and small amorphous nuclei. The dark staining cytoplasm may be mistaken for nuclei and the nuclei for nucleoli leading to misinterpretation as malignancy. The recognition of a cell wall which is typically refractile is the key to avoid this pitfall. Although, the most significant misinterpretation is the misdiagnosis as squamous cell carcinoma, occasionally, some vegetable cells like asparagus may have a linear arrangement and may be mistaken for metastatic lobular carcinoma [7, 17, 24, 68]

Meningioma/meningothelial nodules

Meningothelial nodules are generally detected incidentally in surgical lung specimen and are found more often in patients with malignant pulmonary tumors than those with benign disease [69]. Intracranial meningiomas rarely metastasize to the lung [70, 71]. Meningioma or meningothelial nodules may rarely be found in cytology specimen and may mimic squamous cell carcinoma. Cytologically, meningioma consists of cohesive whorls and syncytia of bland fibroblastic-type cells with fusiform nuclei. Nuclear grooves and intranuclear inclusions may be seen [72]. The whorls may resemble squamous pearls but there will be no keratiniza-

tion. Of note, meningioma can be atypical and have a different cytologic features from those described above. For example, rhabdoid meningioma will look clearly malignant, mimicking adenocarcinoma, melanoma and other tumors with rhabdoid features [73], however, it is unlikely that a lung metastasis will be the first presentation of rhabdoid meningioma.

Small cell lung carcinoma (SCLC)

Small cell lung carcinoma (SCLC) is a high grade neuroendocrine tumor with poor prognosis. It is usually located centrally and associated with smoking. Using the Surveillance, Epidemiologic and End Results (SEER) data-base, Govinda et al., observed that the proportion of SCLC among all lung cancer decreased from 17.62% in 1986 to 12.95% in 2002, while the proportion of women with SCLC increased from 28% in 1973 to 50% in 2002 [74]. Limited stage disease patients who receive appropriate therapy are reported to have a median survival of 18-20 months and 5-year survival rates of 15-25%, whereas extensive stage disease still has a dismal prognosis [75]. Accurate and early diagnosis of SCLC ensures that patients with the disease receive appropriate treatment with potentially improved survival. Cytologic specimens are frequently used in the diagnosis of small cell carcinoma. Typically, the cytomorphologic features of SCLC consists of biphasic population of viable and degenerating small to intermediate cells present in single or loose clusters with molding and DNA artifact [nuclear encrustation of vessel wall may be seen], scattered individual cell necrosis and tumor diathesis. The viable tumor cells have scant cytoplasm with thin cyanophilic rim, round to oval nuclei with high N/C ratio, smooth nuclear membranes, finely granular evenly dispersed chromatin and inconspicuous or absent nucleoli. Mitoses are present and may be brisk. The nuclei may also be quite hyperchromatic or pyknotic. Occasionally, SCLC may have intermediate sized cells that may have small yet distinct nucleoli and lack definitive neuroendocrine features cytologically leading to a misclassification as NSCLC with significant treatment implications [76]. In addition, there are entities like carcinoid tumors [both typical and atypical], reserve cell hyperplasia and atypical lymphoproliferative process, that may be overdiagnosed as SCLC [77-79].

The mimics and pitfalls for small cell lung carcinoma

are summarized in **Table 3, Figures 3, 4 and 5.**

Reserve/basal cell hyperplasia

Reserve cells are immature cells underneath the surface columnar epithelium that serve to buffer the basal layer when the columnar cells are exfoliated. These immature cells gradually mature into squamous metaplasia. With chronic irritation, reserve cells may be exposed and when sampled mimic SCLC. Cytomorphologically, reserve cell hyperplasia appears as tight cohesive, uniform but hyperchromatic cells that are about the size of lymphocytes [7], somewhat smaller than SCLC. The cells have scant indistinct cytoplasm. The nuclei are round to oval with high N/C ratio, smooth nuclear membrane, finely granular evenly distributed chromatin and inconspicuous or absent nucleoli [80]. There may be nuclear molding, but the clusters show uniformity, however there may be nuclear degeneration, raising possibility of individual cell necrosis [7]. The tight clusters, the uniformity of the cells, smooth nuclear membranes, absence of single cells, absence of tumor necrosis / apoptosis / tumor diathesis and mitosis, should aid in avoiding this pitfall [7, 81, 82].

Typical/atypical carcinoid tumor

Carcinoid tumors are low to intermediate grade neuroendocrine tumors may be misinterpreted as SCLC [77, 78]. Cytomorphologic features of typical carcinoid tumor consist of single cells or loose clusters of cells, occasionally with trabeculae or three dimensional patterns. The cytoplasm may be stripped from the nucleus, but when present is scant to moderate, homogeneous to finely granular. There is moderate to high N/C ratio. The nuclei are small, round to oval and tend to be central or slightly eccentric with smooth nuclear membranes. With the spindle cell variant, the nuclei are elongated, somewhat hyperchromatic with limited cytoplasm. The nuclear features characteristic of this tumor include finely stippled to granular chromatin [“salt and pepper”] with small distinct nucleolus. Nuclear molding and necrosis are not present [83, 84]. There may be scant mitotic activity and prominent vascularity [85]. MIB-1 proliferation index is usually low and this may be useful to avoid misdiagnosis as small cell carcinoma [77, 78].

Table 3. Cytologic features and potential mimics of small cell lung carcinoma (SCLC)

Cytologic features	SCLC	Carcinoid tumor	Atypical carcinoid	Reserve cell hyperplasia	Lymphoma
Cellularity	Hypercellular	Variable cellularity	Variable cellularity	Variable cellularity	Hypercellular
Background	Necrotic	Non necrotic	Focal necrosis	Non necrotic Clean	Non necrotic Lymphoglandular bodies
Pattern	Individual cells Loose clusters Molding Biphasic viable and dead cells.	Trabeculae Glandular 3D clusters No molding No necrosis	Small clusters Acinar groups Necrosis possible	Cohesive clusters No single cells	Discohesive Uniform Usually no molding
Cell type	Neuroendocrine	Neuroendocrine	Neuroendocrine	Cuboidal. Size of lymphocytes	Lymphoid
Cytoplasm	Scant Easily stripped	Moderate Often stripped	Scant to moderate	Scant, indistinct	Scant basophilic rim
Nuclear	Small, Oval Elongated Hyperchromatic High N/C ratio Brisk mitosis	Round to oval; spindle Moderate-high N/C ratio Mitosis rare	Round to oval Moderate-high N/C ratio Occasional mitosis	Round High N/C ratio	Round
Nuclear membrane	Smooth	Smooth	Smooth	Smooth	Smooth
Chromatin/ Nucleoli	Granular evenly distributed	Granular stippled "salt and pepper" Distinct nucleoli	Granular stippled to evenly distributed Distinct nucleoli	Finely granular, evenly distributed	Coarse Nucleoli variable
Misc.	Nuclei may be up to 3 times the size of a lymphocyte	Low Ki-67	Low Ki-67	There may be molding, but there is uniformity of the cells	Lymphoglandular bodies may rarely be seen in SCLC

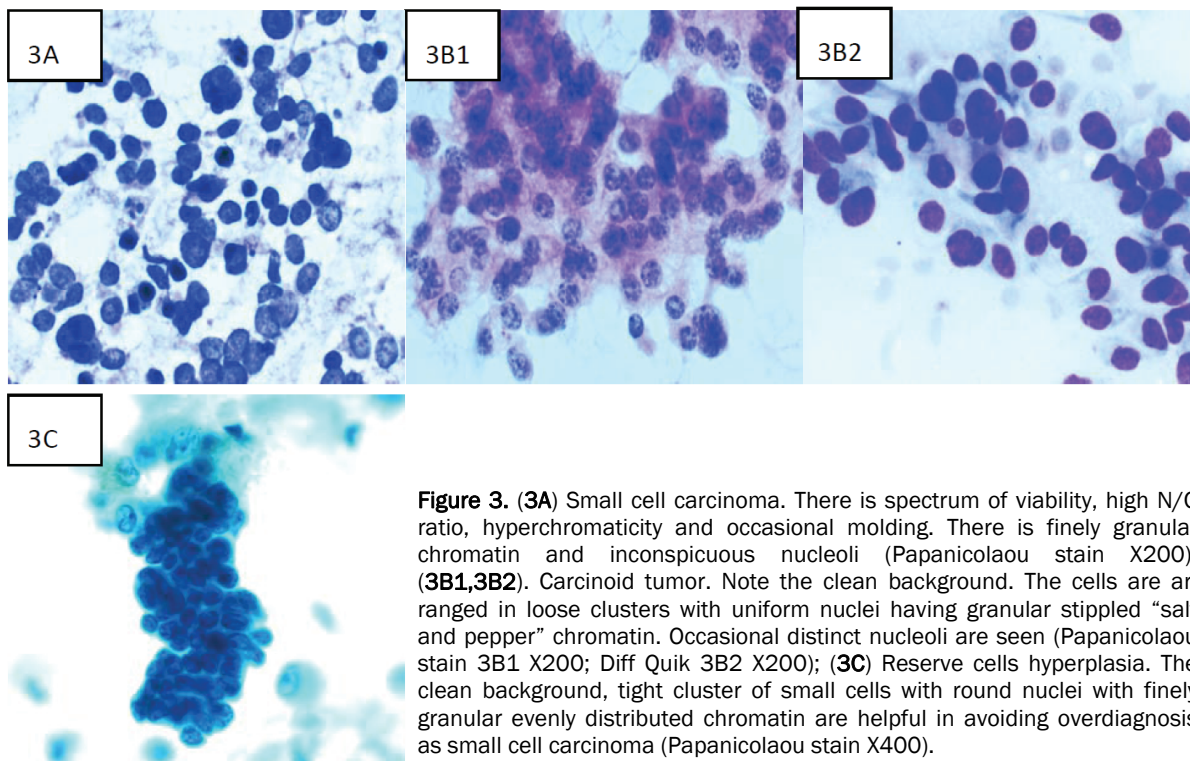


Figure 3. (3A) Small cell carcinoma. There is spectrum of viability, high N/C ratio, hyperchromaticity and occasional molding. There is finely granular chromatin and inconspicuous nucleoli (Papanicolaou stain X200); (3B1,3B2). Carcinoid tumor. Note the clean background. The cells are arranged in loose clusters with uniform nuclei having granular stippled "salt and pepper" chromatin. Occasional distinct nucleoli are seen (Papanicolaou stain 3B1 X200; Diff Quik 3B2 X200); (3C) Reserve cells hyperplasia. The clean background, tight cluster of small cells with round nuclei with finely granular evenly distributed chromatin are helpful in avoiding overdiagnosis as small cell carcinoma (Papanicolaou stain X400).

Lung cancer cytology: pitfalls and mimics

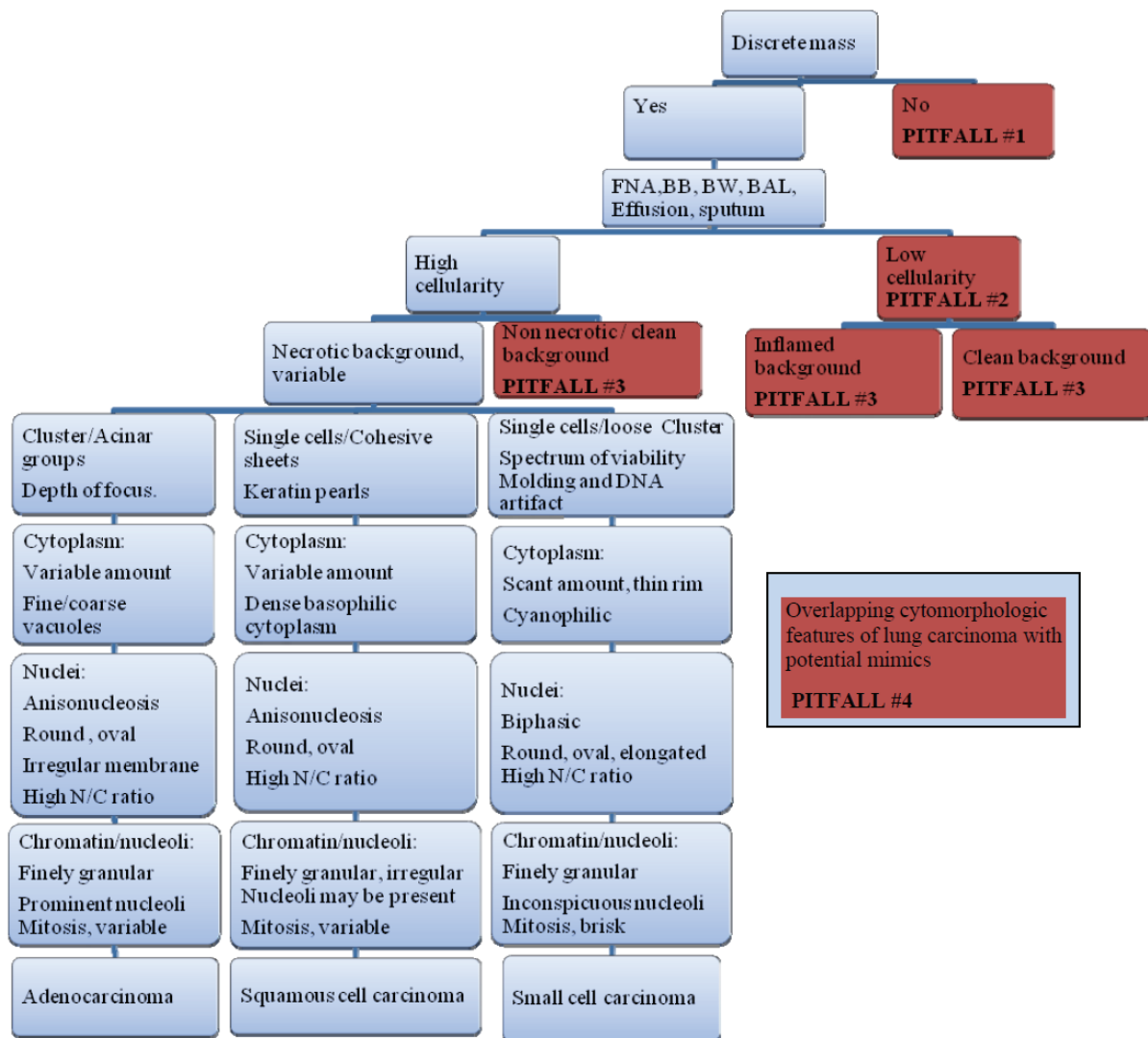


Figure 4. Algorithm for evaluating respiratory cytology and potential pitfalls.

Lymphoma/lymphoproliferative process

Lymphoproliferative processes, like lymphoma or interstitial pneumonitis with lymphoid hyperplasia may also be encountered in pulmonary cytology specimens. Typically, the cells are discohesive and monomorphic with scant basophilic cytoplasm. There are lymphoglandular bodies. The nuclei are usually round and chromatin smooth, with small indistinct nucleoli. It must be pointed out that pseudolymphoglandular bodies may rarely be seen in small cell carcinoma [86]. In well preserved samples, the distinction should be relatively easy. However, in

poor preparations, there may be crushed cells in the smears which mimic DNA artifact seen in SCLC. Furthermore there may be artifactual clustering of cells, which may simulate molding. Awareness of this pitfall is crucial in avoiding a misdiagnosis.

Summary

Respiratory cytology is fraught with numerous potential pitfalls and mimics. Familiarity with these mimics and pitfalls is essential in avoiding a misdiagnosis. There should be hesitation to make a diagnosis of malignancy in an acutely ill

Lung cancer cytology: pitfalls and mimics

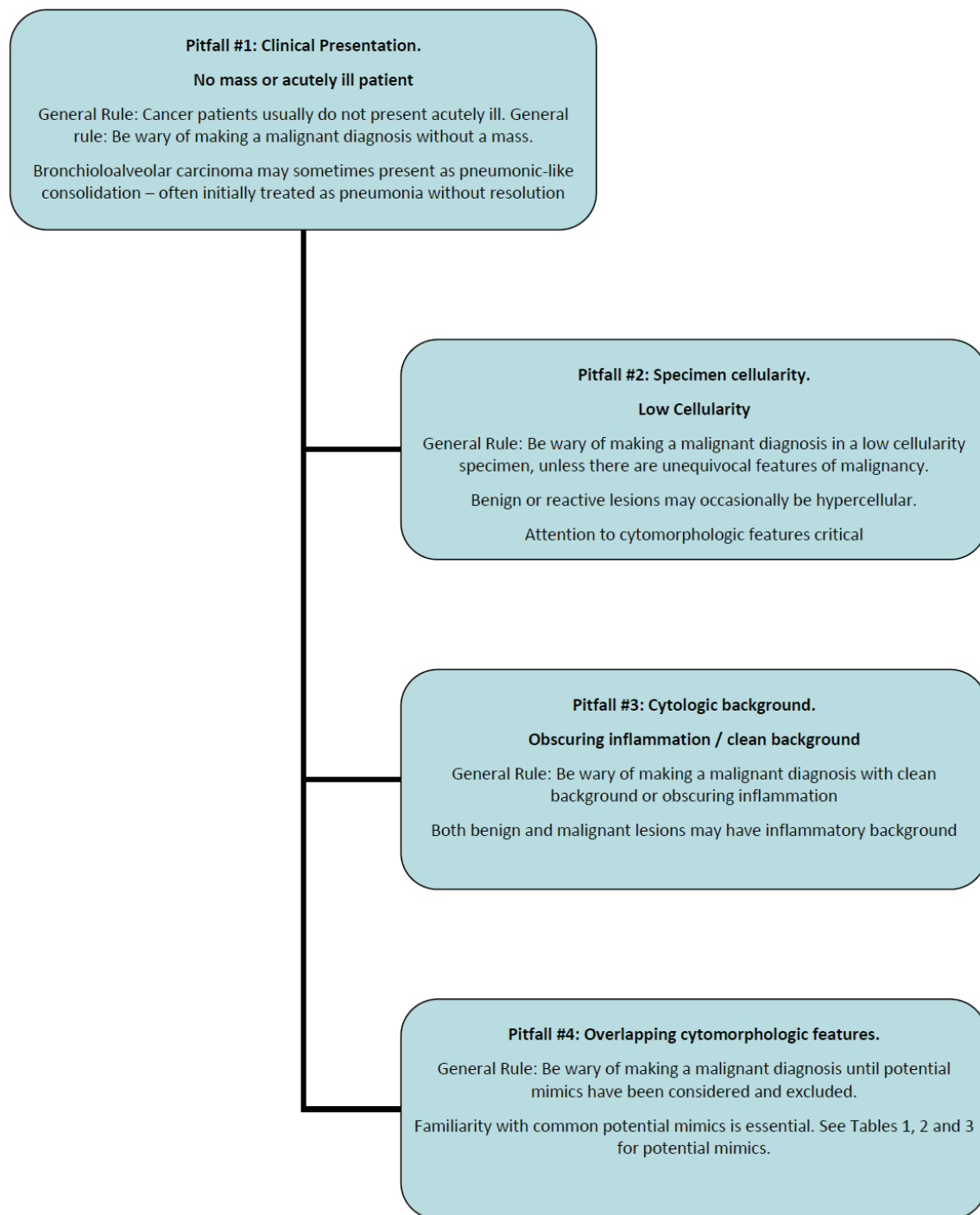


Figure 5. Pitfalls in the evaluation of respiratory cytology specimens

patient, patients without a mass and who have undergone therapy or prior procedures. The potential pitfalls and mimics discussed herein

are not all inclusive. As always, common sense and correlation of the cytomorphologic features with the clinical presentation of the patient

(including radiologic imaging) are absolutely critical in the accurate interpretations of respiratory cytology specimens.

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