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The World Health Organization Reporting System for Lung Cytopathology

Fernando C. Schmitt[a, b, c] Lukas Bubendorf[d] Sule Canberk[c, e, f] Ashish Chandra[g] Ian A. Cree[h] Marianne Engels[i] Kenzo Hiroshima[j] Deepali Jain[k] Ivana Kholová[l] Lester Layfield[m] Ravi Mehrotra[n] Claire W. Michael[o] Robert Osamura[p] Martha B. Pitman[q] Sinchita Roy-Chowdhuri[r] Yukitoshi Satoh[s] Paul VanderLaan[t] Maureen F. Zakowski[u] Andrew S. Field[v]

[a]Department of Pathology, Faculty of Medicine of University of Porto, Porto, Portugal; [b]CINTESIS@RISE, Health Research Network, Porto, Portugal; [c]IPATIMUP, Institute of Molecular Pathology and Immunology of University of Porto, Porto, Portugal; [d]Institute of Medical Genetics and Pathology, University Hospital Basel, Basel, Switzerland; [e]Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Porto, Portugal; [f]Abel Salazar Biomedical Sciences Institute (ICBAS), University of Porto, Porto, Portugal; [g]Department of Cellular Pathology, Guy's & St Thomas' NHS Foundation Trust, London, UK; [h]International Agency for Research on Cancer (IARC), World Health Organization, Lyon, France; [i]Institute of Pathology, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany; [j]Department of Biochemistry and Genetics, Chiba University Graduate School of Medicine, Chiba, Japan; [k]Department of Pathology, All India Institute of Medical Sciences, New Delhi, India; [l]Department of Pathology, Fimlab Laboratories and Tampere University, Faculty of Medicine and Health Technology, Tampere, Finland; [m]Pathology and Anatomic Science Department, University of Missouri, Columbia, MO, USA; [n]Indian Cancer Genomic Atlas, Centre for Health, Innovation and Policy Foundation, Noida, India; [o]Department of Pathology, University Hospitals Cleveland Medical Center/Case Western Reserve University, Cleveland, OH, USA; [p]Department of Diagnostic Pathology, Nippon Koukan Hospital, Kawasaki, Japan; [q]Department of Pathology, Massachusetts General Hospital Harvard Medical School, Boston, MA, USA; [r]Department of Pathology, Molecular Diagnostics Laboratory, Division of Pathology and Laboratory Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [s]Department of Thoracic Surgery, Kitasato University School of Medicine, Tokyo, Japan; [t]Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA; [u]Mount Sinai Medical Center, New York, NY, USA; [v]Department of Anatomical Pathology, St Vincent's Hospital, Sydney, and University of New South Wales and University of Notre Dame, Sydney, NSW, Australia

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Abstract
The International Academy of Cytology has joined with the International Agency for Research on Cancer (IARC) to bring together a group of experts in lung cytopathology to develop a WHO Reporting System for Lung Cytopathology (WHO System). This WHO System defines five categories for reporting lung cytopathology, that is, "Insufficient"/"Inadequate"/"Non-diagnostic," "Benign," "Atypical," "Suspicious for malignancy," and "Malignant," each with a clear descriptive term for the category, a definition, a risk of malignancy and a suggested management algorithm. The key diagnostic cytopathology features of each of the lesions within each category have been established by consensus and will be presented more fully in a subsequent IARC e-book and pub-

Karger@karger.com
www.karger.com/acy

Correspondence to:
Fernando C. Schmitt, fernando.schmitt@ipatimup.pt

lished hard cover book.The WHO System provides the best practice application of ancillary testing, including immunocytochemistry and molecular pathology, and provides a review to guide sampling and processing techniques to optimize the handling and preparation of the cytopathology sample emphasizing the cytomorphological differential diagnosis to aid low-resourced settings. The authors recognize that local medical and pathology resources will vary, particularly in low- and middle-income countries, and have developed the WHO System to make it applicable worldwide based on cytomorphology with options for further diagnostic management of the patient.The online WHO System provides a direct link to the WHO Tumour Classification for Thoracic Tumours 5th Edition. It will raise the profile and use of cytopathology by increasing awareness of its current role and its potential role in the era of personalized medicine based on molecular pathology utilizing "small biopsies." Ultimately, the System will improve patient care and outcomes.This System aims to improve and standardize the reporting of cytopathology, facilitate communication between cytopathologists and clinicians and improve patient care. The System is based on the current role of lung cytopathology and synthesizes the existing evidence while highlighting areas requiring further research and the future potential role of lung cytopathology.

Introduction

The International Academy of Cytology (IAC) has joined with the International Agency for Research on Cancer (IARC) in developing and publishing four international systems for reporting lung, pancreaticobiliary, lymph node, and soft tissue cytopathology. The aims of this project are to improve and standardize the reporting of cytopathology, facilitate communication between cytopathologists and clinicians, improve patient care, synthesize existing evidence, and highlight areas requiring further research. The process of selection of authors, writing and review of the literature, were similar to those used for the WHO Classification of Tumours (the "Blue Books"). In fact, one of the goals of this system is to provide cytopathological correlates with the entities described in the WHO Classification of Tumours, thereby presenting an international approach for reporting cytopathology to mirror the WHO Classification of Tumours, with links particularly on the website between the two series. In addition, information obtained by an international survey promoted by the IAC was taken into account in the final review of the system. This article is a summary of the proposed system for lung cytopathology.

The importance of cytopathology in the investigation of respiratory conditions has been recognized since the earliest days of clinical cytopathology, and respiratory tract cytopathology specimens are established as a vital diagnostic procedure in the evaluation of patients with suspected lung inflammatory/infectious or neoplastic diseases. Lung cancer is the second most common malignancy and the leading cause of cancer-related mortality [1]. In patients with suspected lung cancer, a timely and precise diagnosis is essential to ensure adequate treatment. The study of sputum, bronchial washings (BW), bronchial brushings (BB), bronchoalveolar lavage (BAL) specimens, and fine needle aspirate biopsies (FNAB) provides the cytomorphological basis of the diagnosis. As in other cytopathological fields, the lack of a consistent and standardized system of reporting has hampered communication between disciplines managing these patients.

In 2016, the Papanicolaou Society of Cytopathology (PSC) proposed guidelines for pulmonary cytopathology based on a multidisciplinary approach. This is a 6-tiered reporting system incorporating "Non-diagnostic," "negative for malignancy," "Atypical," "neoplastic, benign neoplasm, low-grade carcinoma," "Suspicious for malignancy" and "Malignant" categories [2, 3]. The most contentious categories of the guideline are "neoplastic, benign neoplasm, low-grade carcinoma," and "Non-diagnostic" [3]. In 2020, the Japan Lung Cancer Society and Japanese Society of Clinical Cytology proposed a new four-tiered cytopathology reporting system for lung carcinoma with the following categories: "negative for malignancy," "Atypical cells," "Suspicious for malignancy," and "malignancy" [4]. In this system, inadequate cases are not categorized because they are excluded in an initial step and only adequate cases are categorized. Although these two proposals had merit in that they attempted to systematize the nomenclature and link the categories with management, neither is used globally or was developed internationally and adapted for all conditions of cytopathology practice.

The aims of the WHO Reporting System for Lung Cytopathology (WHO System) include establishing for the first time a system that can be used internationally in all medical infrastructure settings and provides options for diagnostic management that recognize the variation in availability of ancillary diagnostic and prognostic testing modalities in low- and middle-income countries. The WHO System emphasizes the importance of the cell preparation techniques required to optimize quality and enhance cytopathological diagnosis. Management of the different cytopathological

Table 1. The World Health Organization International System for Reporting Lung Cytopathology on FNAB: implied risk of malignancy and clinical management options by diagnostic category

Diagnostic category	Estimated risk of malignancy , %	Clinical management options
Insufficient/Inadequate/Non-diagnostic	43–53	Correlate with CLIN-IMG-MICRO, ideally discuss at a MDT meeting, and perform repeat FNAB with or without CNB
Benign/negative for malignancy	19–64	Correlate with CLIN-IMG-MICRO, and if these confirm benign diagnosis, then routine follow up at 3–6 months. If no correlation, then perform repeat FNAB with or without CNB
Atypical	46–55	Correlate with CLIN-IMG-MICRO, and ideally discuss at a MDT meeting. If all show a benign diagnosis, then routine follow up at 3–6 months. If no correlation, then perform repeat FNAB with ROSE with or without CNB
Suspicious for malignancy	75–88	Correlate with CLIN-IMG-MICRO, and ideally discuss at a MDT meeting. If all support a diagnosis of malignancy, consider definitive treatment. If no correlation that lesion is Malignant, perform repeat FNAB with ROSE with or without CNB
Malignant	87–100	Correlate with CLIN-IMG-MICRO, and ideally discuss at a MDT meeting. If all support a diagnosis of malignancy, provide definitive treatment. If no correlation that lesion is Malignant, consider repeat FNAB with ROSE with or without CNB

FNAB, fine needle aspiration biopsy; CLIN, clinic; IMG, imaging; MICRO, microbiology; CNB, core needle biopsy; MDT, multidisciplinary team; ROSE, rapid on-site evaluation.

specimens and use of immunocytochemistry (ICC), in situ hybridization and molecular techniques are discussed, since these are extremely important in the rapidly developing era of targeted therapy in lung cancer [5].

The WHO System has five categories that can be stratified by their risk of malignancy (ROM):

- Insufficient/Inadequate/Non-diagnostic
- Benign
- Atypical
- Suspicious for malignancy
- Malignant

The standardized structured report should state one of these five descriptive terms as a heading. A laboratory and its cytopathologists should select one of the terms, "Insufficient/Inadequate/Non-diagnostic," and use this term consistently. The structured report headed by a category term can then include a brief cytopathological description noting where possible the presence or absence of key diagnostic features, which will be detailed in the forthcoming book. In cases with a specific diagnosis, such as "lung adenocarcinoma," some cytopathologists may simply provide the diagnosis with minimal description. This is followed by a conclusion or summary in which the cytopathologist should give as specific a diagnosis of the lesion as possible, such as squamous cell carcinoma, or, if the diagnosis is uncertain, provide the most likely differential diagnoses. A working group consisting of members of the IAC and ICCR is establishing a minimum data set of core and noncore components to be included in the reports of lung malignancies diagnosed by small biopsies and cytopathology specimens.

There are few published papers showing the ROM for each of the WHO System categories and most of them have use previous nomenclature system [2, 3, 6]. Therefore, the ROM provided in this first edition need to be refined by future research by cytopathologists interested in lung cytopathology. The categories are linked with recommendations as to the further workup and diagnostic management, which are dependent of the availability of local practices and medical resources (Table 1).

An international web-based survey was developed by the IAC in consultation with the Lung Expert Editorial Board to establish a snapshot of current lung cytopathology usage from the international community of cytopathology. The survey was based on an earlier survey developed and utilized to assist the authors of the IAC Yokohama System for Reporting Breast FNAB Cytopathology {33369266}. It also included questions related to the pro-

posed lung reporting system. Participants included pathologists and cytotechnologists. The survey demonstrated a diverse practice among various laboratories and countries using a wide spectrum of techniques for specimen processing and handling and various reporting systems. The information obtained assisted the editors and authors to develop the system.

Category: Insufficient/Inadequate/Non-Diagnostic

Definition

A specimen categorized as "Insufficient/Inadequate/Non-diagnostic" lacks sufficient material in quantity or quality for reliable diagnosis.

Discussion

In respiratory cytopathology, specimen adequacy plays a pivotal role in the interpretation and rendering of a diagnosis, the application of ancillary studies and in clinical decision-making. Specimen adequacy is defined in relation to specimen type, medical history, clinical symptoms and imaging findings providing a "triple test" approach [2, 7]. Terms such as Insufficient, Inadequate, and Non-diagnostic have been used interchangeably to describe this category but, until recently, sputum was the only specimen type with relatively well-defined adequacy criteria [8, 9]. The PSC guidelines [2] and the recently revised Cytology Reporting System for Lung Cancer from the Japan Lung Cancer Society and Japanese Society of Clinical Cytology [4] published definitions of an inadequate sample. These reflect the different modalities available for cytopathological diagnosis of pulmonary lesions, each of which strictly requires a different definition of what is "satisfactory" or "adequate." The PSC guidelines include the category "Non-diagnostic" for a "specimen which provides no useful diagnostic information about the pulmonary nodule, cyst, or mass lesion identified by imaging findings." The Japanese system utilizes a stepwise approach with an initial assessment of "Adequate," which is defined as "adequate for cytological evaluation" and clarified as the presence of abundant cellular material, or "Inadequate" where there is no abundant cellular material or obscuring artefact is present, but both of these systems can have limitations in interpretation [10].

In the WHO System the "Insufficient/Inadequate/Non-diagnostic" category is used for cases where there is insufficient material due to low cellularity, poor preparation, fixation or staining, and obscuring by blood, inflammatory or other material. The term "Non-diagnostic" has been used by some cytopathologists for this category to include not only these cases of insufficient material due to technical causes, but also to include cases where there is considerable benign material on the slides, but the material appears to not be representative of a mass lesion or lung nodule seen on imaging. In this situation, an alternative approach is to categorize what is seen on the slides as "Benign" and add a caveat to the report, "that the material may not represent the lesion seen on imaging." Either approach is acceptable, and an institution or cytopathology service should select one term, "Insufficient," "Inadequate," or "Non-diagnostic" and apply it routinely. In order to avoid confusion, it is not recommended to use "Insufficient" or "Inadequate" for cases with technically insufficient material, and "Non-diagnostic" for cases with abundant benign material but a mass lesion on imaging.

The reasons for an "Insufficient/Inadequate/Non-diagnostic" specimen should be documented in the report. If there are any Atypical cells however scant, even if the slides are otherwise insufficient, the case is immediately regarded as "Atypical" and not "Insufficient/Inadequate/Non-diagnostic."

In order to recognize an "Insufficient/Inadequate/Non-diagnostic" cytopathological specimen, the features of an adequate specimen should be established. Specific features to determine adequacy in each of the following specimen types are listed, and it is noted that none require a specified number of cells or cell types. FNAB of lung should include some alveolar, that is, pulmonary, macrophages which generally contain pigment that can be carbon and/or hemosiderin, and may include tissue fragments of collapsed alveolar septa. Transbronchial ultrasound-guided needle aspiration (EBUS-TBNA), when a lymph node has been targeted, should contain moderate to abundant lymphocytes or a large number of anthracotic pigment-laden macrophages. More specifically more than 40 lymphocytes in a high-power field in the area of highest cellularity have been proposed to define an adequate sample, and these should be seen generally in greater numbers than in a CT-guided transthoracic approach [11, 12]. Sputum samples should include at least a few alveolar macrophages and ciliated columnar cells on smears or in a LBC preparation [8, 9]. BB should include abundant bronchial epithelial cells and macrophages may be present. BW and BAL should include readily identified alveolar macrophages and a BAL should have more than 10 alveolar macrophages per high-power field [13]. Bronchial epithelial cells may be present but should not exceed the number of alveolar macrophages.

"Insufficient/Inadequate/Non-diagnostic" rates and the diagnostic yield of lung cytopathology depend on the sampling method and in particular during FNAB on the availability of rapid on-site evaluation (ROSE) [14]. There are considerable variations in the literature concerning "Insufficient/Inadequate/Non-diagnostic" rates. One recent study based on the PSC system categorizing different lung specimen types showed an incidence of 16% [3]. The "Insufficient/Inadequate/Non-diagnostic" rate can be reduced by better initial training of the operator, greater caseload to build the experience of both the operator and the cytopathologist, use of image guidance and immediate feedback on the adequacy and quality of the specimen through ROSE.

Management

Overall, the "Insufficient/Inadequate/Non-diagnostic" category harbours a ROM of approximately 40–60% [2, 3], depending on the mode of sampling and imaging characteristics of a lung mass [15]. Reporting the reasons for inadequacy in a specimen can contribute to improving the diagnostic yield of a repeat examination [15], for example, to perform an adequate "deep cough" to produce sputum after an initial inadequate predominantly buccal sample. In the case of a BB of an endobronchial mass that yields only normal bronchial cells due to its submucosal localization or the fibrotic nature of the tumour, the approach can be changed and an EBUS-TBNA can be performed. After unsuccessful noninvasive or minimally invasive procedures, such as sputum, BW and BB, FNAB, or transthoracic CT-guided FNAB can be utilized. Mediastinoscopy, if available, may be appropriate. In principle, a lung mass more than 30 mm in diameter is considered Malignant [15], and if an "Insufficient/Inadequate/Non-diagnostic" result is obtained after several attempts, then clinical, imaging, and pathological correlation is required to establish further diagnostic management.

Category: Benign

Definition

A specimen categorized as "Benign" demonstrates unequivocal cytopathological features which may or may not be diagnostic of a specific process or benign neoplasm.

Discussion

The "Benign" category includes inflammatory processes and benign neoplasms found in all types of samples of respiratory cytopathology. The category includes those cases where the material is diagnostic of a specific process, such as suppurative or granulomatous inflammation (Fig. 1), or a specific tumour such as a pulmonary hamartoma, as well as, those cases where the normal components of lung tissue, including bronchial cells, alveolar macrophages, alveolar septa and Type 2 pneumocytes are found. Correlation with imaging is required wherever available. If the cytopathological findings do not correlate with the imaging, which may be indeterminate or suspicious, then this should be clearly stated as a caveat in the report and particularly in its conclusion, "that the cytopathological material may not represent the lesion seen on imaging." Recommendations for further diagnostic workup should be given. Tumours that may have an uncertain Malignant potential, but whose cytopathological features are included in the differential diagnosis of benign tumours and whose specific diagnosis can be made with ancillary testing, are included in the benign chapter with a clear discussion of their possible aggressive course, for example, solitary fibrous tumour.

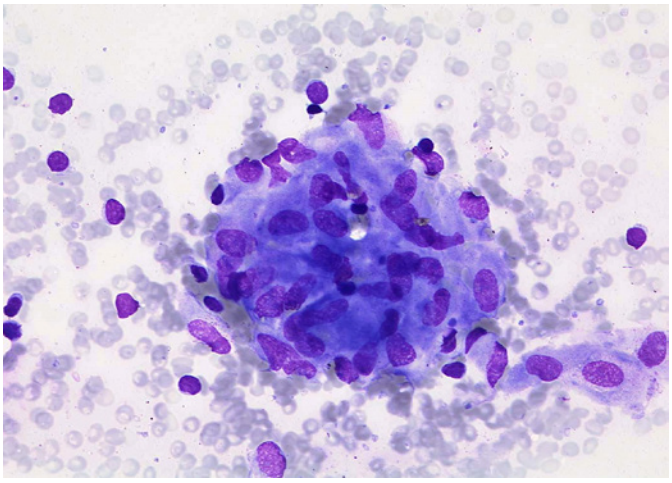

Fig. 1. Benign. Observe granulomatous structure without necrosis. Diagnosis: Granulomatous inflammatory reaction, consistent with sarcoidosis. The diagnosis was based in clinic-radiological correlation (May Grunwald Giemsa stain).

Rates of benign diagnoses vary between different practices and cytopathology specimen types. In one recent series, approximately 50% of the cases were placed in this category [3]. The ROM is reported as in the range from 20 to 40% [2, 3, 6].

Management

Whenever possible, the categorization as "Benign" should be qualified by as specific a diagnosis as possible.

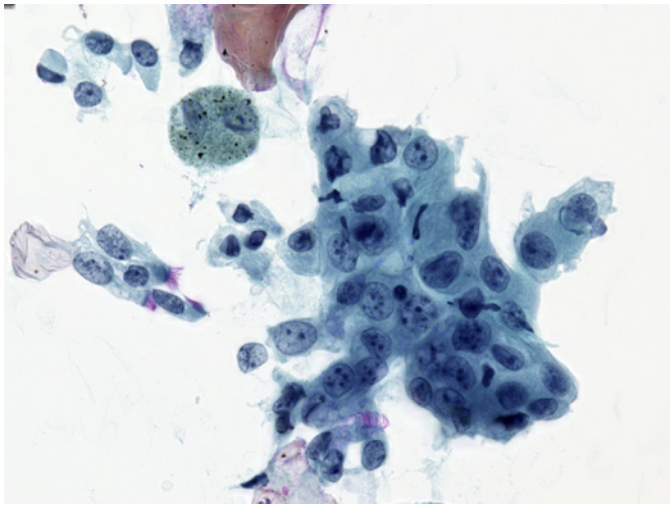

Fig. 2. Atypical. Recently resected Adenocarcinoma of the right lower lobe (pN2). Two months later two infiltrates in the left lower lobe. TBNA shows a group of irregularly arranged Atypical cells. FISH (four probes mix, formerly sold as LAVysion) was normal. Histology showed organizing pneumonia with reactive epithelial changes (repair). Initial category: Atypical. Final category: Benign. Note the reference cells (ciliated) and the pigmented macrophage (Papanicolaou stain).

The final diagnosis should be established in the context of the "Triple Test" with correlation of the cytopathology findings with the clinical and imaging presentation [16]. This is especially important in the benign neoplasms such as hamartoma where the material may resemble normal lung elements. If a specific diagnosis is not established, further evaluation such as repeat core needle biopsy (CNB) or in some cases, if clinically appropriate, limited resection should be performed.

Category: Atypical

Definition

A specimen categorized as "Atypical" demonstrates features predominantly seen in benign lesions and minimal features that may raise the possibility of a Malignant lesion, but with insufficient features either in number or quality to diagnose a benign or Malignant process or lesion.

Discussion

The main causes of an "Atypical" categorization include intrinsic characteristics of the targeted lesion, the expertise of the operator, technical issues related to obtaining and preparing the material, and the experience of the pathologist who is interpreting the specimen [17, 18]. All cellular elements of the respiratory tract including squamous cells lining the buccal mucosa and oropharynx, metaplastic squamous cells, respiratory columnar cells, terminal bronchiolar, or alveolar lining cells and pulmonary macrophages can demonstrate architectural and cellular atypia [19, 20]. Squamous carcinoma precursor lesions of the airways including low, moderate, and high-grade dysplasia and carcinoma in situ are recognized in the WHO Thoracic Tumours 5th Edition, and although not specifically diagnosable on cytopathology, contribute to cases categorizable as "Atypical." Reactive changes, such as metaplasia and hyperplasia, infections particularly viral and post-therapy changes, are commonly associated with an "Atypical" categorization (Fig. 2). It is recommended clinical and imaging findings are reviewed before categorizing a specimen as "Atypical" [5, 18]. Imaging studies can help differentiate between a localized and a diffuse and/or bilateral disease process.

In acute respiratory distress syndrome, a chest radiograph will show bilateral alveolar infiltrates and no evidence of cardiomegaly [21]. Lung malignancy, especially adenocarcinoma, can show a spectrum of changes on CT. These include ground glass that is nonsolid, and part solid nodules, solid pulmonary nodules or masses, cystic lucencies, and multifocal nodules. Lesions may demonstrate calcifications and cavitation [22]. Typically, small cell carcinoma and lymphoma present as extensive mediastinal lymphadenopathy without a dominant pulmonary lesion [22]. Rates of the "Atypical" category vary between different practices and cytopathology specimen types. In the published series, approximately 3–5% of the cases were placed in this category [3, 23]. The ROM is reported as in the range of 50–60%, which is regarded as far from ideal and too high, but there are few published studies [2, 3, 6].

Management

Correlation with imaging and clinical findings is required. Particularly if the imaging features are Atypical or concerning for malignancy, further investigation, is warranted by an additional study usually using a different modality: an "Atypical" sputum could be repeated or bronchoscopy with BW/BB could be conducted; an "Atypical" BW/BB could be followed by an EBUS-TBNA with or without a CNB; and an "Atypical" FNAB could be repeated with a core needle biopsy. However, if the imaging and clinical features account for the "Atypia" described, a confirmatory repeat study may be obtained or the patient may be clinically observed for a period of time.

Category: Suspicious for Malignancy

Definition

A specimen categorized as "Suspicious for malignancy"' demonstrates some cytopathological features suggestive of malignancy but with insufficient features either in number or quality to make an unequivocal diagnosis of malignancy. The category "Suspicious for malignancy" is generally used in respiratory cytopathology to indicate a degree of uncertainty regarding the diagnosis of malignancy and offers a risk stratification for a Malignant diagnosis [2, 4, 18]. This category is often used when there is insufficient quality or quantity of cellular material in an adequate specimen for a definite diagnosis of malignancy. The category remains subjective with a high rate of interobserver disagreement [24], as the threshold for the diagnosis of malignancy depends on the pathologist's experience, the type of cytopathological preparation and the degree of cellular atypia (Fig. 3). The category is used particularly to avoid a false-positive diagnosis of malignancy, which can lead to unnecessary intervention. The diagnosis of "Suspicious for malignancy" should not be used as the sole basis for therapy [2, 4, 25].

When a case is categorized as "Suspicious for malignancy," the report should include a statement as to which malignancy or malignancies in a differential diagnosis are suspected, including nonsmall cell carcinoma, neuroendocrine tumours, small and large cell neuroendocrine carcinomas, lymphoma, sarcoma, and metastatic carcinomas. The category can be used when reporting any respiratory cytopathological specimen. Although there are no defined cytopathological criteria for the category of "Suspicious for malignancy," significant cytopathological atypia is present including nuclear enlargement, anisonucleosis, nuclear crowding, varying chromatin, variability in cell size and shape, and other features associated with malignancy.

ROSE has been shown to reduce the rate of the "Suspicious for malignancy" category [14]. However, the use of the diagnostic category may persist despite utilization of ROSE due to limiting factors related to the intrinsic characteristics of the tumour, the clinical circumstances, and technical aspects. Necrosis, dense inflammatory background, association with a granulomatous reaction, cytopathological features that overlap with benign lesions and tumours with extensive fibrosis are examples of intrinsic tumour characteristics that may produce highly Atypical samples or scant Atypical cellularity independent of the number of passes on ROSE.

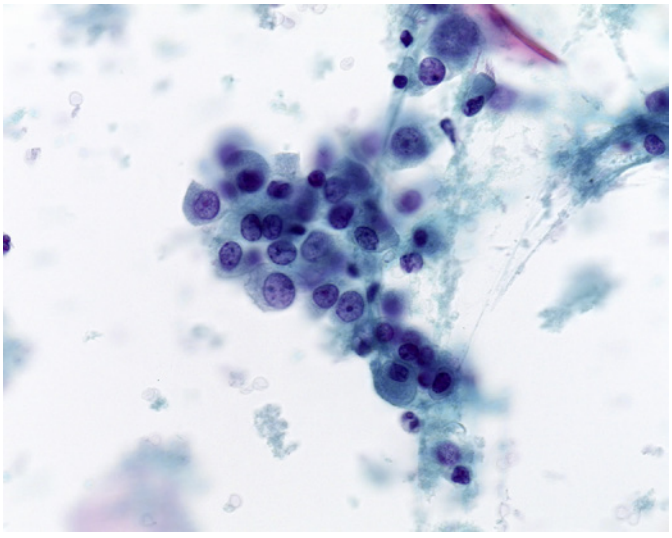

Fig. 3. Suspicious for malignancy. Peripheral lung lesion in a patient with a history of sarcoidosis. TBNA shows epithelial cells suspicious of adenocarcinoma (TTF1 negative). Multitarget FISH with Assay with probes for the EGFR gene (7p12, SpectrumRed), the MYC gene (8q24, SpectrumGold), chromosome 5 (5p12, SpectrumGreen), and chromosome 6 (centromere, SpectrumAqua) showed increased copy numbers for all four probes (3–5 signals instead of 2 signals, each), in favour of malignancy. Initial category: Suspicious for malignancy. Final category: Malignant (Papanicolaou stain).

In contrast, well-differentiated adenocarcinoma of lung with a lepidic pattern can result in sheets of cells with low nuclear atypia that may be categorized as "Suspicious for malignancy" due to the lack of overtly Malignant characteristics. A prior history of radiotherapy and or chemotherapy may result in a "Suspicious for malignancy" categorization because their reactive atypia may mimic carcinoma or there are a small number of highly Atypical cells. Technical aspects including the tumour size particularly if less than 20 mm, location of the targeted lesion and the expertise of the interventionist performing the biopsy can result in specimens with low cellularity [26–29].

The use of ancillary techniques such as ICC may assist in changing a "Suspicious for malignancy" to a "Malignant" categorization, such as when metastatic tumours and neuroendocrine tumours are suspected, but generally, the quality and quantity of the suspicious cells may still prevent definitive classification. For example, the finding of crushed cells on FNAB smears and even in a cell block preparation may lead to a suspicion of a differential diagnosis of a neuroendocrine carcinoma or lymphoma, but insufficient tumour cells may prevent an ICC

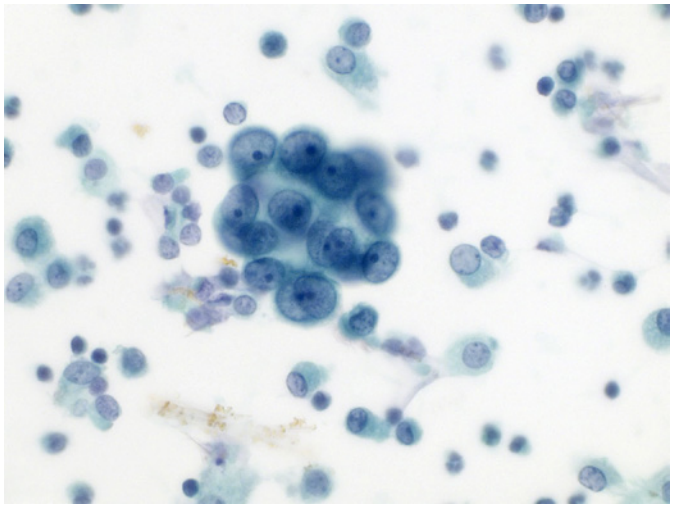

Fig. 4. Malignant. Adenocarcinoma. Clinical suspicion of recurrent adenocarcinoma (lobectomy 3 years earlier). BAL: Malignant. Typical cytological features of ADC. Confirmed by concurrent transbronchial biopsy (lepidic component and KRAS G12C mutation) (Papanicolaou stain).

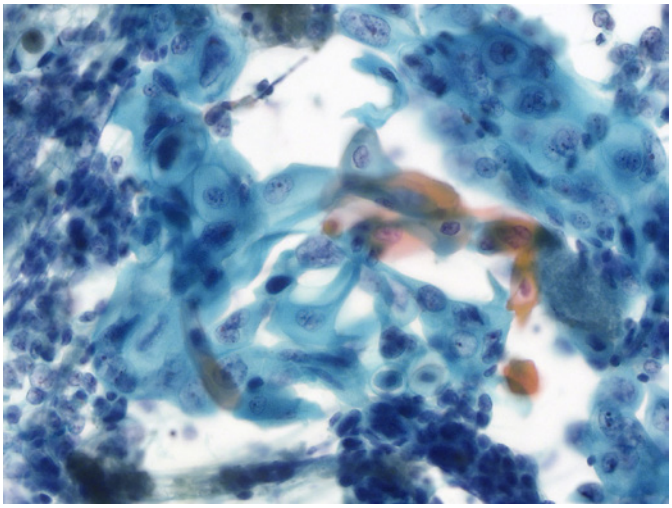

Fig. 5. Malignant. Squamous cell carcinoma. Lung (EBUS-TBNA): keratinizing SCC (Papanicolaou stain).

diagnosis [30, 31]. Similarly, when the features are suspicious for a primary pulmonary "nonsmall cell carcinoma," the thyroid transcription factor-1 (TTF1), a marker of adenocarcinoma, is also expressed in reactive alveolar epithelial cells or in basal cells of the peripheral bronchial tree and does not separate benign from Malignant.

Rates for the category "Suspicious for malignancy" vary between different practices and cytopathology specimen types. In a recent publication, approximately 5% of the cases were placed in this category [3]. The ROM of the "Suspicious for malignancy" category is approximately 82% with a range of 54.5–90% [2, 3, 6], in comparison to the "Atypical" category that carries an estimated ROM of 50–60% [2, 3, 6], demonstrating that the use of the two categories enables risk stratification.

Management

The categorization of a case as "Suspicious for malignancy" is not equivalent to a categorization or final diagnosis of malignancy. It is imperative to have good communication with the clinicians with review of all relevant clinical and imaging information to determine a management plan, in order to avoid unnecessary additional risks and costs. In a minority of cases, surgical management can proceed without a final diagnosis of malignancy if clinically indicated, and in this situation, intraoperative pathological confirmation such as frozen section of the diagnosis is recommended. In most cases, however, if systemic therapy is to be considered, a repeat of the diagnostic cytopathological procedure or more commonly a change to another diagnostic modality such as FNAB or CNB is recommended. Use of ancillary studies has limited value in further classification of these cases. The categories of "Suspicious for malignancy" and "Malignant" are within a spectrum with a high rate of inter-observer variability, and therefore, consultation with a more experienced cytopathologist may be helpful to reach the threshold of malignancy in an otherwise limited specimen.

Category: Malignant

Definition

A specimen classified as "Malignant" demonstrates unequivocal cytopathological features of malignancy.

Discussion

The "Malignant" category should only be used when there is a full constellation of cytopathological findings and no discrepant features. Wherever possible the neoplasm should be subclassified based on the key diagnostic cytopathological features and ICC, if needed. Malignant neoplasms involving the lungs include both primary and secondary tumours. It is important to be aware of the limitations of cytopathology samples, which can be due to qualitative and quantitative reasons [2, 3, 32]. Therefore, a "Malignant" categorization and specific diagnosis

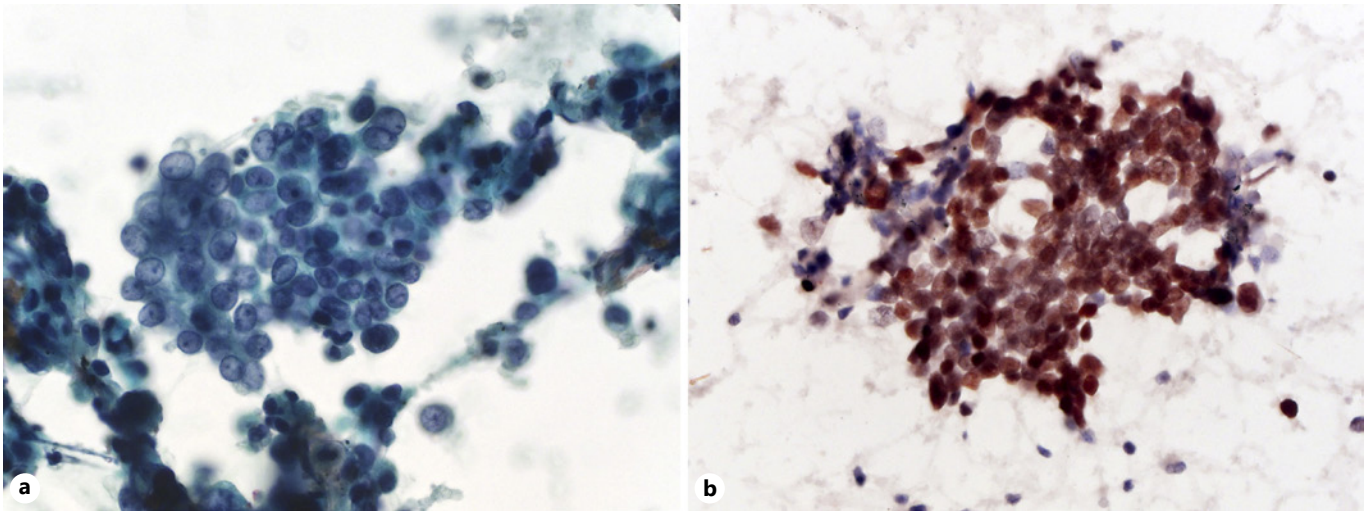

Fig. 6. Malignant. Non-small cell carcinoma. Middle lobe, TBNA: Malignant. First Diagnosis: (**a**) NSCC, NOS (Papanicolaou stain). **b** ICC: p40 positive (immunocytochemistry). Final diagnosis: NSCC, consistent with SCC.

should be rendered only in specimens with adequate cellularity meeting definitive criteria for malignancy [32]. Based on cytomorphology, good accuracy, that is, greater than 70%, can be achieved in differentiating between nonsmall cell carcinoma, including adenocarcinoma and squamous cell carcinoma (Fig. 4, 5), and small cell neuroendocrine carcinoma. However, with the advent of targeted therapies, exact classification of the cancer as adenocarcinoma or squamous cell carcinoma is essential and the standard of care in the current practice of pulmonary cytopathology [33, 34]. A major limitation of pulmonary cytopathology is the higher rate of NSCC-NOS resulting from an inability to subtype as compared to small biopsies and resections [35]. Misclassifications and inability to classify, that is, a final report of NSCC-NOS, on cytopathology are mainly seen in exfoliative cytopathology [36], touch preparations [37], Giemsa-stained smears mainly in cases of SCC [38], samples with low cellularity [36, 39], necrosis [36, 39, 40], poorly differentiated histopathology [36, 39, 41], and in rare instances of large cell carcinoma, sarcomatoid carcinoma or adenosquamous carcinomas [36, 40, 42].

In the case of primary epithelial tumours, differentiation between the commonly encountered adenocarcinoma and squamous cell carcinoma can be achieved using a limited ICC panel consisting of TTF1 and p40 (Fig. 6a, b) [32–34, 40, 43]. Dual stains such as Napsin A/p40 are especially helpful in preserving tissue in very scant samples [44]. Therefore, within the interpretation category "Malignant," it is important that the final diagnosis is as specific as possible. It is also important to be aware of the diagnostic pitfalls associated with ICC stains, especially in cases of secondary malignancies of the lung, for example, TTF1 positivity in metastatic thyroid carcinoma [33, 40, 45].

The Malignant category also includes low-grade neuroendocrine tumours, previously known as carcinoid and Atypical carcinoid, and neuroendocrine carcinomas of predominantly small or large cell types (Fig. 7, 8), which can be diagnosed by cytopathology and confirmed by use of ICC markers such as INSM1, chromogranin, and synaptophysin. The other Malignant neoplasms included in this category are salivary gland-type carcinomas, mesenchymal tumours, and secondary malignancies (Fig. 9). Rates of malignancy diagnosis can vary between different institutions and countries. In a recent series, approximately 20% of the cases were placed in this category [3]. The reported ROM for cytopathology specimens categorized as "Malignant" is greater than 90% and in most cases approaches 100% [2, 3, 6, 46].

Management

A cytopathology categorization as "Malignant" should be correlated with the clinical and imaging findings and if it is concordant, surgical management if appropriate can proceed, while if systemic treatment is planned in the more common situation of advanced disease presentation, definitive therapy can be commenced if material is available for prognostic and predictive biomarkers. Cytopathology of Malignant lesions is often highly diagnostic,

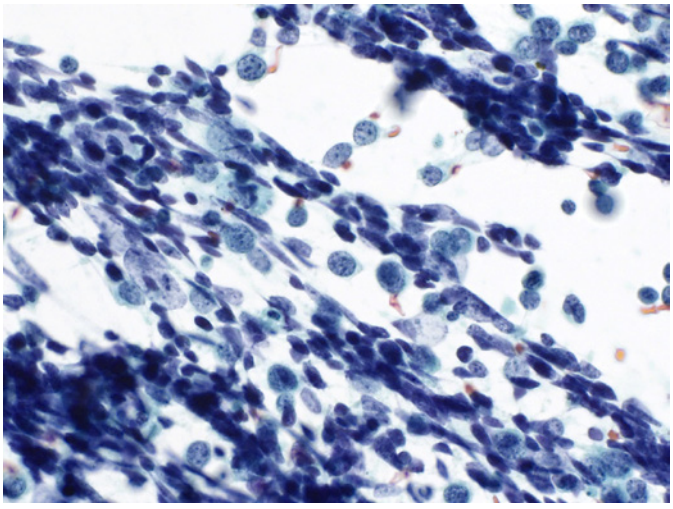

Fig. 7. Malignant. Small cell carcinoma. EBUS-TBNA: typical cytological features of SCC (Papanicolaou stain).

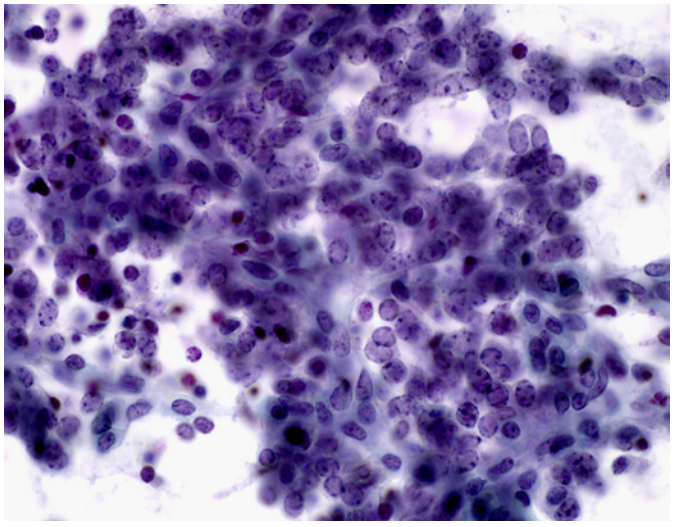

Fig. 8. Malignant Carcinoid. Transthoracic FNA. Typical cytological features. Note the capillaries between the tumour cell clusters. Carcinoids are well capillaries and do easily bleed during biopsies (that's why cytology is often preferred) (Papanicolaou stain).

and in the majority of cases, a large number of cells are present with a high percentage of tumour cells. This enables not only the diagnosis but also ancillary studies including predictive comprehensive molecular profiling using cell blocks and smears. However, if the material is mainly necrotic, all specimens including smears, cell blocks and CNB need to be reviewed for morphologically viable tumour cells to allow for accurate diagnosis and subtyping. Otherwise, a repeat diagnostic procedure is required.

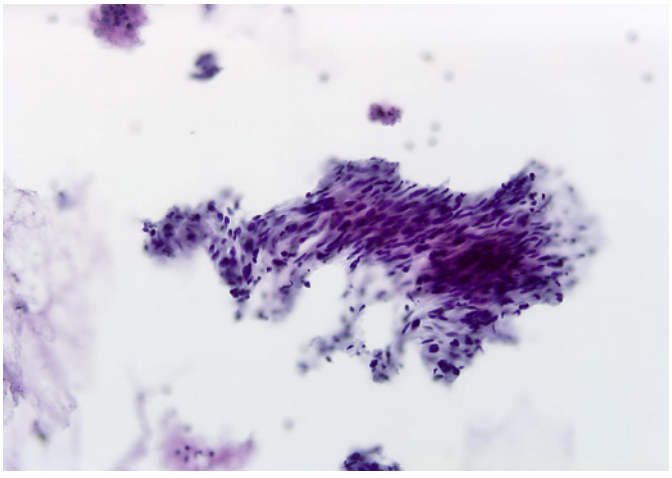

Fig. 9. Malignant. Metastatic leiomyosarcoma. EBUS-TBNA. Note the typical spindle cells (Papanicolaou stain).

Conclusion

As with all reporting systems involving categorization of cytopathology specimens, the new WHO System is designed to improve communication between clinicians and cytopathologists. Each specimen type and its category have a specific ROM and this will directly influence clinical diagnostic management algorithms. The authors recognize that the performance indicators for each category of the system are derived from recent reviews of the literature and hope that the WHO System will encourage research into these indicators to test the current system and its management recommendations and provide ever more precise ROM.

The WHO System also defines through the first international consensus the key diagnostic cytopathological criteria for each lesion or tumour, which is essential to improve the quality of diagnostic assessment and reporting of lung cytopathology. It also provides a differential diagnosis based on these cytopathological criteria of the different entities empowering cytopathologists throughout the world to use the System. Further, the WHO System also provides the current best practice application of ancillary testing, including ICC and molecular pathology, and, importantly, provides detailed descriptions of sam-

pling and processing techniques to optimize the handling and preparation of the cytopathology sample. The integration of ancillary techniques for detecting therapeutic targets in lung cancer has further enhanced the utility of lung cytopathology, and the emergence of prognostic markers and targeted therapies has required more specific classification of the carcinoma and a multidisciplinary approach to patient management.

The authors of the WHO System recognize that local medical and pathology resources and infrastructure will vary, particularly in low- and middle-income countries. To make the WHO System applicable worldwide, the system is based on cytomorphology and provides options for further diagnostic management of the patient.

The WHO System will provide a direct and dynamic link to the WHO Classification for Thoracic Tumours 5th Edition and will raise the profile and use of cytopathology by increasing awareness of its current role and its potential role in the era of personalized medicine based on molecular pathology utilizing "small biopsies." Ultimately, the System will improve patient care and outcomes.

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Conflict of Interest Statement

The content of this article represents the personal views of the authors and does not represent the views of the authors' employers and associated institutions. Where authors are identified as personnel of the IARC/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy, or views of the IARC/World Health Organization."

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Author Contributions

Conceptualization: Fernando C. Schmitt, Lukas Bubendorf, Sule Canberk, Ashish Chandra, Ian A Cree, Marianne Engels, Kenzo Hiroshima, Deepali Jain, Ivana Kholová, Lester Layfield, Ravi Mehrotra, Claire W. Michael, Robert Osamura, Martha B. Pitman, Sinchita Roy-Chowdhuri, Yukitoshi Satoh, Paul VanderLaan, Maureen F. Zakowski, and Andrew S. Field. Writing original draft: Fernando C. Schmitt with collaboration of all authors. Final review: Andrew S. Field. All authors read and approved the final manuscript.

Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

References

1 Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin. 2019;69(1):7–34.
2 Layfield LJ, Baloch Z, Elsheikh T, Litzky L, Rektman N, Travis WD, et al. Standardized terminology and nomenclature for respiratory cytology: the Papanicolaou Society of Cytopathology guidelines. Diagn Cytopathol. 2016;44(5):399–409.
3 Canberk S, Montezuma D, Aydın O, Demirhas MP, Denizci B, Akbas M, et al. The new guidelines of Papanicolaou Society of Cytopathology for respiratory specimens: assessment of risk of malignancy and diagnostic yield in different cytological modalities. Diagn Cytopathol. 2018 Sep;46(9):725–9.
4 Hiroshima K, Yoshizawa A, Takenaka A, Haba R, Kawahara K, Minami Y, et al. Cytology reporting system for lung cancer from the Japan Lung Cancer Society and Japanese Society Of Clinical Cytology: an interobserver reproducibility study and risk of malignancy evaluation on cytology specimens. Acta Cytol. 2020;64(5):452–62.
5 Canberk S, Engels M. Cytology samples and molecular biomarker testing in lung cancer-advantages and challenges. Virchows Arch. 2021;478(1):45–57.
6 Yoshizawa A, Hiroshima K, Takenaka A, Haba R, Kawahara K, Minami Y, et al. Cytology reporting system for lung cancer from the Japan Lung Cancer Society and the Japanese Society of Clinical Cytology: an extensive study containing more benign lesions. Acta Cytol. 2022;66(2):124–33.
7 Tattevin P, Casalino E, Fleury L, Egmann G, Ruel M, Bouvet E. The validity of medical history, classic symptoms, and chest radiographs in predicting pulmonary tuberculosis: derivation of a pulmonary tuberculosis prediction model. Chest. 1999 May;115(5):1248–53.
8 Greenberg SD. Recent advances in pulmonary cytopathology. Hum Pathol. 1983 Oct;14(10):901–12.
9 Choi YD, Han CW, Kim JH, Oh IJ, Lee JS, Nam JH, et al. Effectiveness of sputum cytology using ThinPrep method for evaluation of lung cancer. Diagn Cytopathol. 2008 Mar;36(3):167–71.
10 Canberk S, Tischler V, Engels M. Current topics and practical considerations of cytology practice in lung cancer: reflexions from the lung symposium at the 42nd European Congress Of Cytology, Malmö. Acta Cytol. 2020;64(5):463–70.
11 Cameron SEH, Andrade RS, Pambuccian SE. Endobronchial ultrasound-guided transbronchial needle aspiration cytology: a state of the art review. Cytopathology. 2010 Feb;21(1):6–26.
12 Alsharif M, Andrade RS, Groth SS, Stelow EB, Pambuccian SE. Endobronchial ultrasound-guided transbronchial fine-needle aspiration: the University of Minnesota experience, with emphasis on usefulness, adequacy assessment, and diagnostic difficulties. Am J Clin Pathol. 2008 Sep;130(3):434–43.

13 Chamberlain DW, Braude AC, Rebuck AS. A critical evaluation of bronchoalveolar lavage. Criteria for identifying unsatisfactory specimens. Acta Cytol. 1987 Sep–Oct;31(5):599–605.
14 Jain D, Allen TC, Aisner DL, Beasley MB, Cagle PT, Capelozzi VL, et al. Rapid on-site evaluation of endobronchial ultrasound-guided transbronchial needle aspirations for the diagnosis of lung cancer: a perspective from members of the Pulmonary Pathology Society. Arch Pathol Lab Med. 2018 Feb;142(2):253–62.
15 MacMahon H, Naidich DP, Goo JM, Lee KS, Leung ANC, Mayo JR, et al. Guidelines for management of incidental pulmonary nodules detected on CT images: from the fleischner society 2017. Radiology. 2017 Jul;284(1):228–43.
16 Borczuk AC. Neoplastic and nonneoplastic benign mass lesions of the lung. Arch Pathol Lab Med. 2012 Oct;136(10):1227–33.
17 Idowu MO, Powers CN. Lung cancer cytology: potential pitfalls and mimics – a review. Int J Clin Exp Pathol. 2010 Mar;3(4):367–85.
18 Layfield LJ, Baloch Z. Atypia in pulmonary cytology: morphologic spectrum and causes. Diagn Cytopathol. 2022 Apr;50(4):164–71.
19 Naryshkin S, Young NA. Respiratory cytology: a review of non-neoplastic mimics of malignancy. Diagn Cytopathol. 1993;9(1):89–97.
20 Glatz K, Savic S, Glatz D, Francz G, Barascud A, Grilli B, et al. An online quiz uncovers limitations of morphology in equivocal lung cytology. Cancer. 2006 Dec 25;108(6):480–7.
21 Papazian L, Calfee CS, Chiumello D, Luyt CE, Meyer NJ, Sekiguchi H, et al. Diagnostic workup for ARDS patients. Intensive Care Med. 2016 May;42(5):674–85.
22 Pascoe HM, Knipe HC, Pascoe D, Heinze SB. The many faces of lung adenocarcinoma: a pictorial essay. J Med Imaging Radiat Oncol. 2018 Oct;62(5):654–61.
23 Savic S, Glatz K, Schoenegg R, Spieler P, Feichter G, Tamm M, et al. Multitarget fluorescence in situ hybridization elucidates equivocal lung cytology. Chest. 2006 Jun;129(6):1629–35.
24 Layfield LJ, Esebua M, Dodd L, Giorgadze T, Schmidt RL. The Papanicolaou Society of Cytopathology guidelines for respiratory cytology: reproducibility of categories among observers. Cytojournal. 2018;15:22.
25 Layfield LJ, Esebua M, Schmidt RL, Witt BL. Malignancy risk associated with the EBUS-FNA diagnostic categories nondiagnostic, benign, atypical, suspicious for malignancy, and malignant for mediastinal lymph node aspirate specimens. J Am Soc Cytopathol. 2015 Sep–Oct;4(5):276–81.
26 Ost DE, Ernst A, Lei X, Kovitz KL, Benzaquen S, Diaz-Mendoza J, et al. Diagnostic yield and complications of bronchoscopy for peripheral lung lesions. Results of the AQuIRE registry. Am J Respir Crit Care Med. 2016 Jan;193(1):68–77.
27 Schreiber G, McCrory DC. Performance characteristics of different modalities for diagnosis of suspected lung cancer: summary of published evidence. Chest. 2003 Jan;123(1 Suppl):115S–28S.
28 Zhang X, Kuang Y, Zhang Y, He K, Lechtzin N, Zeng M, et al. Shifted focus of bronchoalveolar lavage in patients with suspected thoracic malignancy: an analysis of 224 patients. J Thorac Dis. 2016 Nov;8(11):3245–54.
29 Rivera MP, Mehta AC; American College of Chest Physicians. Initial diagnosis of lung cancer: ACCP evidence-based clinical practice guidelines (2nd edition). Chest. 2007 Sep;132(3 Suppl):31S–148S.
30 Yatabe Y, Dacic S, Borczuk AC, Warth A, Russell PA, Lantuejoul S, et al. Best practices recommendations for diagnostic immunohistochemistry in lung cancer. J Thorac Oncol. 2019 Mar;14(3):377–407.
31 Thunnissen E, Borczuk AC, Flieder DB, Witte B, Beasley MB, Chung JH, et al. The use of immunohistochemistry improves the diagnosis of small cell lung cancer and its differential diagnosis. An international reproducibility study in a demanding set of cases. J Thorac Oncol. 2017 Feb;12(2):334–46.
32 Patel TS, Shah MG, Gandhi JS, Patel P. Accuracy of cytology in sub typing non small cell lung carcinomas. Diagn Cytopathol. 2017 Jul;45(7):598–603.
33 Layfield LJ, Pearson L, Walker BS, White S, Schmidt R. Diagnostic accuracy of fine-needle aspiration cytology for discrimination of squamous cell carcinoma from adenocarcinoma in non-small cell lung cancer: a systematic review and meta-analysis. Acta Cytol. 2018;62(5–6):318–26.
34 Jain D, Nambirajan A, Chen G, Geisinger K, Hiroshima K, Layfield L, et al. NSCLC subtyping in conventional cytology: results of the International Association for the Study of Lung Cancer Cytology working group survey to determine specific cytomorphologic criteria for adenocarcinoma and squamous cell carcinoma. J Thorac Oncol. 2022;17(6):793–805.
35 Righi L, Graziano P, Fornari A, Rossi G, Barbareschi M, Cavazza A, et al. Immunohistochemical subtyping of nonsmall cell lung cancer not otherwise specified in fine-needle aspiration cytology: a retrospective study of 103 cases with surgical correlation. Cancer. 2011 Aug 1;117(15):3416–23.
36 Rekhtman N, Brandt SM, Sigel CS, Friedlander MA, Riely GJ, Travis WD, et al. Suitability of thoracic cytology for new therapeutic paradigms in non-small cell lung carcinoma: high accuracy of tumor subtyping and feasibility of EGFR and KRAS molecular testing. J Thorac Oncol. 2011 Mar;6(3):451–8.
37 Zakowski MF, Rekhtman N, Auger M, Booth CN, Crothers B, Ghofrani M, et al. Morphologic accuracy in differentiating primary lung adenocarcinoma from squamous cell carcinoma in cytology specimens. Arch Pathol Lab Med. 2016 Oct;140(10):1116–20.
38 Yildiz-Aktas IZ, Sturgis CD, Barkan GA, Souers RJ, Fraig MM, Laucirica R, et al. Primary pulmonary non-small cell carcinomas: the College of American Pathologists Interlaboratory Comparison Program confirms a significant trend toward subcategorization based upon fine-needle aspiration cytomorphology alone. Arch Pathol Lab Med. 2014 Jan;138(1):65–70.
39 Sigel CS, Moreira AL, Travis WD, Zakowski MF, Thornton RH, Riely GJ, et al. Subtyping of non-small cell lung carcinoma: a comparison of small biopsy and cytology specimens. J Thorac Oncol. 2011 Nov;6(11):1849–56.
40 Nizzoli R, Tiseo M, Gelsomino F, Bartolotti M, Majori M, Ferrari L, et al. Accuracy of fine needle aspiration cytology in the pathological typing of non-small cell lung cancer. J Thorac Oncol. 2011 Mar;6(3):489–93.
41 da Cunha Santos G, Lai SW, Saieg MA, Geddie WR, Pintilie M, Tsao MS, et al. Cytohistologic agreement in pathologic subtyping of non small cell lung carcinoma: review of 602 fine needle aspirates with follow-up surgical specimens over a nine year period and analysis of factors underlying failure to subtype. Lung Cancer. 2012 Sep;77(3):501–6.
42 Manucha V, Wang C, Huang Y. Non-small-cell lung carcinoma subtyping on cytology without the use of immunohistochemistry: can we meet the challenge? Acta Cytol. 2012;56(4):413–8.
43 Yao X, Gomes MM, Tsao MS, Allen C, Geddie W, Sekhon H. Fine-needle aspiration biopsy versus core-needle biopsy in diagnosing lung cancer: a systematic review. Curr Oncol. 2012 Feb;19(1):e16–27.
44 Nishino M, Hoang MP, Della Pelle P, Morales-Oyarvide V, Huynh TG, Mark EJ, et al. Napsin A/p40 antibody cocktail for subtyping non-small cell lung carcinoma on cytology and small biopsy specimens. Cancer Cytopathol. 2016 Jul;124(7):472–84.
45 Chen L, Jing H, Gong Y, Tam AL, Stewart J, Staerkel G, et al. Diagnostic efficacy and molecular testing by combined fine-needle aspiration and core needle biopsy in patients with a lung nodule. Cancer Cytopathol. 2020 Mar;128(3):201–6.
46 Boler AK, Banu N, Bose K, Roy S, Bandyopadhyay A. Reproducibility of 'the Papanicolaou Society of Cytopathology system for reporting respiratory cytology': a retrospective analysis of 101 cases of CT-guided FNAC. Diagn Cytopathol. 2020;48(8):701–5.