Diagnosis of Infectious Diseases in the Lower Respiratory Tract

A Cytopathologist’s Perspective

Rebecca J. Baldassarri, MD; Deepika Kumar, MD; Stephen Baldassarri, MD; Guoping Cai, MD

Context.—Respiratory cytology continues to play an important role in the diagnosis of lower respiratory tract infections. Prompt, accurate diagnosis of causative organisms is of paramount importance, particularly in immunosuppressed patients. In addition, a rapidly expanding arsenal of ancillary testing is now available, aiding tremendously in organism identification.

Objective.—To provide an updated review on the cytomorphologic features of common organisms in lower respiratory tract infection. Relevant ancillary tests, differential diagnoses, and potential pitfalls of organism identification will also be discussed.

Data Sources.—Data for this review were gathered from PubMed searches of infectious diseases of the lower respiratory tract, especially related to the diagnoses.

Conclusions.—The lower respiratory tract is subject to infection by a wide variety of infectious agents. Pathologists should be familiar with common organisms, including their general clinical characteristics, cytomorphologic features, differential diagnoses, and ancillary methods of detection. Above all, correlation with microbiologic and clinical information is necessary to make a confident diagnosis of infection.

Pneumonia is a significant cause of morbidity and mortality in the US population, often affecting adults ages 50 years and older. Despite the ready availability of multiple laboratory diagnostic methods, a causative organism remains unidentified for approximately 40% of patients with community-acquired pneumonia (CAP) requiring hospitalization.1 For this reason, treatment of pneumonia is often empiric. According to the Infectious Diseases Society of America/American Thoracic Society guidelines for CAP, diagnostic tests should be ordered only when the test is likely to have the highest diagnostic yield, and only if results could prompt a significant change from empiric therapy. Although recommendations for diagnostic testing in outpatient CAP are controversial, testing is generally accepted for critically ill patients with pneumonia.2 As a result, the bulk of diagnostic testing is conducted in immunocompromised patients, patients with nosocomial pneumonia, or CAP that is severe or nonresponsive to empiric therapy. The unifying link between these patient populations is a higher likelihood of infection with antibiotic-resistant bacteria or atypical/opportunistic organisms that would lead to a change in antimicrobial therapy. In patients with severe CAP or nosocomial pneumonia, identification of a causative organism has 2-fold significance: tailoring a patient’s treatment and for epidemiologic purposes, such as tracking of infectious organisms and their susceptibility data.2

Cytologic samples for diagnosis of respiratory tract infections can be obtained by 3 methods: exfoliation, abrasion, and fine-needle aspiration (FNA).3 A combination of invasive and noninvasive techniques are often used to obtain material for diagnostic testing. Common sample types include sputum (expectorated and induced), bronchial washings, lung fine-needle aspirate, and pleural fluid. Flexible fiberoptic bronchoscopy is a relatively safe and minimally invasive procedure to obtain cytologic specimens.4 Protected specimen brushes can be used to minimize the amount of upper aerodigestive contamination present in bronchoscopic specimens, rendering these specimens more suitable for microbiologic studies.5

The best diagnostic modality depends upon the location, type, and the radiologic findings of the infection. Sputum analysis and bronchial brushings/washings may yield diagnostic material in case of centrally located lesions and those that involve the major airways. For more peripherally located lesions, BAL remains the more useful modality. Fine-needle aspiration allows sampling of infections that manifest as localized mass lesions, mimicking malignancy. Transbronchial or ultrasound-guided FNA allows sampling of endobronchial lesions or hilar lymph nodes, whereas...
computed tomography–guided FNA can be used for subpleural lesions.

The use of cytologic specimens in diagnosis of lower respiratory tract infection offers many advantages, including minimally invasive approaches to obtain specimens, rapid turnaround time, and cost effectiveness. While respiratory cytology has traditionally been used in the diagnosis of suspected malignancy, its role in the diagnosis of lower respiratory tract infection has expanded considerably, especially with increasing numbers of immunocompromised patients. Though definitive identification of organisms is performed by microbiology laboratory, cytomorphologic analysis plays a critical supporting, and sometimes diagnostic role, largely by increasing specificity when combined with other microbiologic techniques. This article will provide a focused review of common bacterial, viral, and fungal pneumonias, with a focus on immunocompromised patients. Cytomorphologic features of selected organisms and ancillary testing for definitive organism identification will be discussed.

**BACTERIAL INFECTIONS**

The diagnosis of bacterial pneumonia is generally established by correlating clinical and microbiologic results. Cytomorphologic examination of specimens plays a supportive role, predominantly in triaging specimens for further diagnostic testing. Although bacterial pneumonia can have varied radiographic appearances, occasionally a masslike lesion persists after serial imaging such that malignancy cannot be excluded. In these cases, invasive procedures, including transbronchial biopsy, BAL, or lung FNA can be performed for definitive diagnosis.

Sputum is the most commonly obtained cytologic specimen in the initial workup of bacterial pneumonia. Sputum specimens are routinely sent for Gram staining and culture in the microbiology laboratory. Despite the widespread collection of sputum samples, their utility is controversial, owing largely to preanalytic factors, such as specimen collection, processing, and low yield. As a result, sensitivity and specificity of sputum cytology are frequently limited. The presence of neutrophils (>25 cells) and few squamous cells (<10) at low-power examination suggests an adequate specimen, while the opposite suggests a predominance of oral contamination, rendering the specimen suboptimal for interpretation. Identification of a predominant bacterial form by the microbiology laboratory allows for tailoring of antibiotic therapy. The sensitivity of the result depends on the patient’s pretest probability of bacterial pneumonia and whether antibiotics have been previously administered.

The diagnosis of nosocomial pneumonia can be particularly challenging, owing to the often nonspecific clinical and imaging findings. As a result, invasive diagnostic methods using bronchoscopy are frequently performed in an effort to make a definitive diagnosis of pneumonia. Ventilator-associated pneumonia comprises the bulk of nosocomial pneumonia and substantially increases the risk of mortality in critically ill patients. Endotracheal aspirate samples suffer from the same pitfalls as those of nonintubated patients, with wide variations in sensitivity and specificity. The utility of quantitative cultures obtained by invasive methods is controversial, with the American College of Chest Physicians deeming there is insufficient evidence to improve clinical outcomes. Others, however, have reported improved outcomes, with lower morbidity, mortality, and antibiotic use. Morphologic examination of BAL specimens is not helpful, as increased neutrophils can be seen in conditions other than pneumonia, such as idiopathic pulmonary fibrosis and acute respiratory distress syndrome.

Newer molecular techniques have allowed for rapid diagnosis of organisms in BAL specimens. A rapid polymerase chain reaction (PCR) test for methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) on BAL specimens in patients with suspected ventilator-associated pneumonia has an excellent negative predictive value, albeit in a test population with a low prevalence of MRSA. Immuno-chromatographic assays are also available for detection of *Streptococcus pneumoniae* antigen in BAL samples. In particular, antigen detection techniques sidestep the decreased Gram staining and culture sensitivity, given that many patients have already begun antibiotic treatment at the time of testing.

Identification of atypical organisms such as *Mycoplasma, Chlamydia*, and *Legionella*, relies largely on nucleic acid amplification or rapid antigen tests, sometimes in combination with culture. Serology can be nonspecific, and the rise in convalescent antibody titer needed to confirm the diagnosis takes too long to be clinically meaningful. For this reason, most of the atypical organisms are empirically treated. Diagnosis of *Legionella* pneumonia rests primarily on culture of lower respiratory tract specimens (BAL or sputum) on selective buffered charcoal yeast extract agar coupled with urinary *Legionella* antigen detection. Legionella organisms are seldom identified in cytologic specimens. The organisms can be highlighted by Diff-Quik (DQ), Giemsa, silver, and Gram stains, but they stain poorly with Papanicolaou (Pap) and hematoxylin-eosin stains. When present, they are motile short rods, often located within the cytoplasm of neutrophils and macrophages. Of note, certain species of *Legionella* are acid-fast positive and must be differentiated from mycobacterial organisms.

Occasionally, FNA (transbronchial or computed tomography guided) is performed for evaluation of parenchymal lung lesions when the differential diagnosis includes neoplasm versus infection. In these cases, the identification of bacterial organisms is much more meaningful than in sputum or bronchial washing and brushing samples, which are readily contaminated with bacterial from the upper aerodigestive tract. In a study group composed largely of immunocompromised patients, percutaneous lung FNA was uniquely able to identify infectious organisms in patients with abnormal pulmonary imaging findings.

Pulmonary actinomycosis is a rare chronic infection and may cause cavitary lung lesions, lung nodules, or a pleural effusion. It is rare in the developed world and predominantly occurs in adults between the ages of 30 and 60 years. Risk factors include alcoholism, bronchiectasis, and emphysema. Because the imaging findings are nonspecific and may be suggestive of other chronic suppurrative pulmonary infections such as tuberculosis or a malignant process, invasive approaches and a high degree of clinical suspicion are needed to establish the diagnosis. The average time to diagnosis is 6 months, and even when clinical suspicion is high, microbiologic confirmation can still be elusive. Rapid diagnosis can result in prompt treatment with an excellent prognosis, sidestepping avoidance of diagnostic surgery. Fine-needle aspiration of the lesion reveals colonies of *Actinomyces*.
branching filamentous bacteria with a suppurative background (Figures 1 and 2). Sulfur granules may be numerous; however, the presence of sulfur granules is neither sensitive nor specific for actinomycosis, also being seen in nocardiosis, chromomycosis, and botryomycosis. Of note, *Actinomyces* colonization can coexist with necrotic lung cancer. *Actinomyces* organisms are difficult to culture from sputum or bronchoscopy aspirations because these specimens are usually not cultured anaerobically. The overall failure rate of isolation is greater than 50% in microbiologic samples, and as a result, many times the diagnosis is made in a resection specimen for suspected malignancy. Although commercial biochemical tests are available for *Actinomyces* species, reports of sensitivity have been low (<60%). In contrast, broad-range bacterial PCR coupled with mass spectrometry has been reported to identify *Actinomyces* organisms with a high degree of certainty in otherwise culture-negative samples. In addition, PCR amplification of 16S rRNA followed by gene sequencing can also be used to identify *Actinomyces* organisms in cases where cultures are otherwise unrevealing.

In contrast to actinomycosis, pulmonary nocardiosis preferentially affects immunosuppressed patients. *Nocardia* infection occasionally presents as a mass lesion, and FNA reveals a nonspecific suppurative background and slender filaments that branch at right angles, in contrast to the acute angle branching of *Actinomyces* organisms. Both organisms can be identified on routine cytologic Pap and Romanowsky stains. *Nocardia* organisms are also positive by Fite stain (Figure 3). Culture of *Nocardia* organisms can take 5 to 21 days, with species identification determined by biochemical tests. Newer tests such as high-performance liquid chromatography and 16S and 32S ribosomal DNA amplification and sequencing now allow for more rapid and precise identification of organisms.

**Mycobacteria**

Tuberculous and nontuberculous mycobacterial infections of the lower respiratory tract are most commonly identified in immunocompromised patients. Although the organisms are not stained by routine cytologic stains such as the Pap stain and DQ stains, they can be visualized on DQ stain as negative images, often filling the cytoplasm of macrophages. The delicate rod-shaped organisms are highlighted by acid-fast bacillus (AFB) stain (Figure 4). Furthermore, the background cellular composition and diathesis can be highly suggestive of mycobacterial infection, particularly when background necrosis, epithelioid granulomas, and a mixed inflammatory infiltrate are present. These features should always prompt a diligent search for organisms; however, it is important to note that in the absence of definitive organisms, the background diathesis is nonspecific, and malignancy (particularly squamous cell carcinoma) and amoebic abscesses should be included in the differential diagnosis. Differences in the host’s immune status may give rise to varying degrees of organized granulomas, with...
organisms being more readily identified in hosts with compromised versus robust immunity. In an immunosuppressed patient, only loose histiocytic granulomas may be seen, and the differential diagnosis includes both mycobacterial and fungal organisms. In patients with a normal immune system, the presence of well-formed epithelioid granulomas without significant inflammation should raise the possibility of tuberculosis, sarcoidosis, and pneumococcosis.\textsuperscript{16} Infection with \textit{Mycobacterium avium} complex is common, especially in acquired immunodeficiency syndrome (AIDS) patients, and can be highlighted with a Ziehl-Neelsen stain. In mycobacterial infections, cytologic examination is not limited strictly to respiratory specimens and frequently includes rapid assessment of lymph node aspirates. Confirmatory AFB staining can be performed on a variety of preparations, including smears, cytospins, and cell blocks.\textsuperscript{3}

Sputum AFB staining is very specific (90\%-100\%) for diagnosis of mycobacterial infection, but sensitivity is limited. Culture of mycobacteria is time-consuming but is necessary for nontuberculous mycobacterial species’ identification and susceptibility testing. Nucleic acid probes specific to \textit{M tuberculosis} complex, \textit{M avium} complex, \textit{M intracellulare}, \textit{M kansasii}, and \textit{M gordae} are very sensitive and specific (near 100\%), provided there are greater than 100 000 organisms present. With fewer numbers of organisms, nucleic acid amplification is necessary for accurate detection of organisms. Both nucleic acid amplification and probe methods also offer rapid turnaround time, within a matter of hours. Within the \textit{M tuberculosis} complex, further testing (eg, high performance liquid chromatography and restriction fragment length polymorphism) is necessary for species-specific identification.\textsuperscript{24}

\section*{VIRAL INFECTIONS}

Viral infection of the lower respiratory tract is common, accounting for approximately 20\% to 40\% of CAP cases with up to a third of patients with severe CAP requiring intensive care.\textsuperscript{25} Viral infections tend to be self-limited in immunocompetent hosts; however, in certain patients, such as those at the extremes of age, those who are immunocompromised, those who are undergoing mechanical ventilation, and those with chronic respiratory illnesses, viral infections constitute a significant cause of morbidity and mortality. Traditional methods used to isolate viral organisms, such as cell culture, are slow and limited in sensitivity, and have now been largely supplanted by molecular techniques, such as PCR.\textsuperscript{26,27} Despite the ready availability of nucleic acid amplification techniques, cytomorphologic changes observed in respiratory specimens can be useful in suggesting viral infection and suggesting an etiologic agent. Because viruses cannot be detected with routine light microscopy, their presence is inferred through viral cytopathic effect. Cytopathic effect is the mainstay of viral identification through culture; however, viral effects can also be seen on routine cytology specimens, such as sputum, BAL, and bronchial washings and brushings. Upper respiratory tract samples, such as nasal or nasopharyngeal swabs, comprise the bulk of specimens for viral testing. Lower respiratory tract specimens, such as BAL specimens, are generally collected from patients who are immunocompromised or severely ill with respiratory failure. Of note, certain respiratory viruses may cause severe lower respiratory tract infection while being undetectable in upper airway specimens.\textsuperscript{28}

Common morphologic features of viral infection include cytomegaly, syncytia formation, and intracytoplasmic and/or intranuclear inclusions. Ciliocytomphoria is a less specific change consisting of detached fragments of superficial bronchial cell cytoplasm with cilia, suggestive of viral infection, but can also be associated with bronchial carcinoma. Viral changes can also be associated with significant regenerative/reactive atypia; in these cases, care must be taken to distinguish these changes from neoplastic disease.

Most viral inclusions can be detected with ordinary cytology stains, such as the Pap stain or DQ stain. The respiratory tract is susceptible to a wide variety of viruses, with the more common viruses being adenovirus, respiratory syncytial virus, influenza, parainfluenza, and measles viruses.\textsuperscript{3} These diverse viruses may cause overlapping cytomorphologic changes, so clinical history and ancillary studies are necessary for definitive diagnosis.

\section*{Cytomegalovirus}

Cytomegalovirus (CMV) infects most adults. While immunocompetent patients are asymptomatic or experience mild flulike symptoms with primary infection, immunocompromised patients can experience significant disease through primary infection and reactivation of latent disease. One of the most significant manifestations of CMV infection is CMV pneumonitis, which carries a high mortality rate up to 30\% to 50\%, highest in hematopoietic stem cell transplant recipients. Although histologic detection of characteristic intranuclear inclusions or immunohistochemistry has traditionally been the gold standard for diagnosis of CMV pneumonitis, many patients cannot tolerate the invasive procedures required to obtain tissue. As a result, minimally invasive methods such as bronchoscopy with BAL are frequently used. Tan et al\textsuperscript{30} have proposed a set of criteria in making the diagnosis of CMV pneumonitis, based on clinical and radiographic features, as well as identification of CMV in BAL fluid. Polymerase chain reaction was shown to have a higher sensitivity for CMV (compared to viral culture), but a lower specificity, although all specificities remained above 94\%. In a study by Paradis et al\textsuperscript{30} morphologic detection of CMV-infected cells in BAL specimens was only about 20\% sensitive for CMV pneumonitis. Viral culture was 100\% sensitive, but only 70\% specific for CMV pneumonitis.\textsuperscript{30} The varying test characteristics of different diagnostic methods underscore the need for comprehensive criteria to make the diagnosis of CMV pneumonitis. Characteristic CMV-infected cells have been reported in transbronchial FNA in a solid organ transplant patient.\textsuperscript{31}

Seok et al\textsuperscript{32} studied the morphologic features of CMV-infected cells in bronchial washings, comparing liquid-based preparations (LBPs) to conventional preparations. Features include a crisp nuclear contour, a large intranuclear inclusion with a prominent halo, cytomegaly (1.4 and 2.9 times size of histiocytes on conventional preparations and LBPs, respectively), and sometimes intracytoplasmic inclusions (Figure 5). Although infected cells in LBPs were overall larger than those in conventional smears, there were overall fewer infected cells in LBPs than in conventional smears, which also featured higher nuclear to cytoplasmic ratios. Previous studies have shown that addition of CMV immunostain to BAL fluid can increase the sensitivity of BAL in diagnosis of CMV pneumonitis.\textsuperscript{33}
Herpes Simplex Virus

Herpes simplex virus (HSV) is a relatively uncommon cause of viral pneumonia, predominantly affecting immunocompromised patients and patients undergoing mechanical ventilation. HSV I and II are double-stranded DNA viruses that establish latency after primary infection. An estimated 40% to 98% of the population has antibodies to HSV-1. Despite evidence of prior immune exposure, HSV can reactivate in response to certain stimuli, such as hormonal changes, ultraviolet light, trauma, or impaired immunity. It is important to note that primary and reactivation disease can both be asymptomatic; this decreases the specificity of HSV DNA isolation for true HSV infection. Several mechanisms have been proposed to explain HSV infection of the lower respiratory tract, including direct extension from upper to lower respiratory tract, reactivation through vagal ganglia, and hematogenous dissemination.

The pathogenicity of HSV DNA in lower respiratory tract specimens of critically ill patients undergoing mechanical ventilation is still subject to debate. In isolation, clinical, radiographic, and laboratory findings lack sensitivity and specificity for HSV infection. The presence of infected cells (which characteristically show multinucleation, chromatin margination, and nuclear molding) within cytologic specimens is highly specific for HSV infection, although sensitivity of cytology specimens is lower than that of viral isolation (Figure 6). Therefore, in combination, HSV viral isolation with concomitant positive cytologic findings offers excellent sensitivity and specificity. It should also be noted that the background in HSV bronchopneumonia can include many inflammatory cells, necrosis, and marked reactive atypia of squamous/bronchial cells, which should not be mistaken for neoplastic disease.

Herpes simplex virus bronchopneumonia can also manifest in immunocompetent patients undergoing prolonged mechanical ventilation. Definitive diagnosis of bronchopneumonia required fulfillment of 3 criteria: clinical deterioration, detection of HSV by culture and/or PCR, and detection of characteristic viral inclusions on BAL, biopsy, and/or endotracheal aspirate. Risk factors included the presence of oral/labial lesions, HSV isolated in the throat, and visible bronchial lesions during bronchoscopy. Detection of HSV in the lower respiratory tract by PCR was not entirely specific for HSV bronchopneumonitis, while the presence of HSV-specific inclusions in BAL/biopsy was 100% specific.

Varicella-Zoster Virus

Varicella-zoster virus (VZV) is a member of the herpesvirus family of DNA viruses. While many are affected as children (with a low rate of lung involvement), primary infection in adults can potentially produce serious respiratory complications, most commonly pneumonia. While the exact incidence of varicella pneumonia is not known, radiographic incidence of VZV pneumonia was 16% in young men in the military. However, most patients were asymptomatic, suggesting that lung involvement is usually mild and self-limited in otherwise healthy adults.

The consequences of varicella pneumonia confer a much higher rate of morbidity and mortality in immunocompromised patients and those with underlying lung disease. In a study of critically ill patients with VZV pneumonia, most patients were immunocompromised and/or carried comorbidities such as chronic respiratory disease, diabetes, heart disease, among others. Overall mortality rates from adult varicella pneumonia in immunocompromised patients and pregnant women is 30% to 50%, largely attributed to fulminant disease, delayed diagnosis and treatment initiation. In immunocompromised patients, varicella pneumonia can present without the typical cutaneous manifestations.

Varicella-zoster virus cytopathic effect is identical to that of herpes simplex virus. Tzanck smears and viral culture are insensitive compared to PCR. Of note, PCR may not be entirely specific for VZV pneumonia, as contamination from pharyngeal lesions is possible during bronchoscopy. While rare, disseminated VZV infection has been reported to involve pleural fluid in immunocompromised patients. Characteristic viral inclusions were identified in the pleural fluid of patients with a history of malignant lymphoma/leukemia status post chemotherapy and/or radiation. Inclusions are virtually identical to those produced by herpes simplex virus. Infected mesothelial cells demonstrated glassy chromatin with margination and nuclear molding, along with occasionally prominent intranuclear inclusions. A background inflammatory infiltrate of lymphocytes was also noted. Of note, identical viral inclusions can be seen in other herpesvirus infections (namely, HSV and CMV); as such, ancillary testing is necessary for confirmation.

Adenovirus

Adenoviruses are nonenveloped, double-stranded DNA viruses that are spread through respiratory, fecal-oral, and conjunctival routes. The lack of an envelope renders the viruses highly resistant to physical and chemical destruction, and the virus can remain infectious for up to 3 weeks at room temperature. Most commonly, adenoviruses are implicated in self-limited upper respiratory tract infections in young children, with most individuals showing serologic evidence of infection by age 10 years. Adenoviruses can infect a wide variety of organs including the eye, gastrointestinal tract, respiratory tract, bladder, liver, and central nervous system. Following primary infection, certain serotypes of the virus are thought to establish latency in mucosal-related lymphoid tissues, such as the tonsils. In immunocompromised patients, adenoviruses can cause significant morbidity and mortality, through both primary infection and endogenous reactivation.

Viral effect has been characterized in histopathologic specimens. Characteristically, infected cells show nuclear enlargement with smudgy basophilic inclusions, referred to as “smudge” cells (Figure 7). Ancillary testing, such as in situ hybridization, immunohistochemistry, or PCR, is necessary for confirmation of viral infection in formalin-fixed tissue. Descriptions of adenovirus cytopathic effect in cytologic specimens are limited in the literature. An increase in ciliocytophthoria in sputum specimens, a nonspecific finding, has been described in adenoviral infections. Cytopathic changes in pediatric endotracheal aspirates included “rosette” cells, which contained nuclear inclusions with radiated strands, large “smudge” cells, and nuclei with a honeycomb appearance. A case report of adenoviral pneumonia in an immunocompetent patient, complicated by acute respiratory distress syndrome, showed only multinucleated giant cells and necrotic debris on BAL, with no smudge cells identified.
Similar to other viruses, adenovirus can be detected in clinical samples by a variety of methods, including conventional methods (culture, serology, direct antigen detection) and newer molecular techniques. Culture and serology have largely been supplanted by nucleic acid amplification techniques such as PCR. In respiratory specimens, PCR and direct antigen detection have showed reasonably high sensitivity as compared to culture (40%–90%).

Measles Virus

Measles virus is an RNA paramyxovirus spread by droplets. Following the development of the measles vaccine, infection rates decreased dramatically. However, there has been recent resurgence of infection. Typically, infection is self-limited, except in neonates, infants, the elderly, and immunocompromised patients. Immunocompromised individuals tend to have atypical presentations, often without the characteristic skin rash, and higher mortality rates secondary to severe pneumonia or encephalitis. In a small case series of oncologic and human immunodeficiency virus (HIV)–infected patients, mortality rates from measles pneumonia in oncologic and HIV-infected patients was 55% and 33% to 45%, respectively. Because the clinical presentation in immunocompromised patients can be atypical, a high degree of clinical suspicion is necessary to initiate treatment quickly.

Measles cytopathic effect has been described in sputum. In sputum, large multinucleated cells can be identified, thought to be due to fusion of type 2 pneumocytes. In contrast to foreign body giant cells, which have regularly spaced, evenly sized nuclei, the nuclei of measles-infected cells overlap and vary in shape and size. In addition, eosinophilic cytoplasmic inclusions with minute halos are present within the giant cells. While nuclei can overlap, molding is not observed, as in HSV cytopathic effect. Cells with measles cytopathic effect have also been described in nasal secretions and swabbings of the upper respiratory tract. Of note, the multinucleated giant cells of measles must be differentiated from other viruses (such as respiratory syncytial virus, which contains multinucleated cells with basophilic cytoplasmic inclusions, and other herpesviruses), giant cells due to pneumoconiosis, and nonspecific multinucleated reactive bronchial cells, and multinucleated giant cells of granulomatous diseases. A case report of fatal measles pneumonia in an HIV-infected pediatric patient documents the presence of multinucleated giant cells with intranuclear inclusions in a bronchial wash specimen. Characteristic multinucleated giant cells have also been reported in a BAL specimen from an immunocompromised adult patient who died from measles pneumonia. The background of the BAL specimen showed neutrophils and lymphocytes, reactive bronchial cells, and syncytial giant cells with prominent eosinophilic nuclear and cytoplasmic inclusions. The diagnosis of measles pneumonia can be

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difficult, as 21% of patients can present with a normal chest radiograph finding on admission. As a result, laboratory testing is necessary for confirmation of acute measles infection. Measles virus has been isolated by reverse transcriptase–PCR (RT-PCR) in BAL specimens and has also been isolated in blood, urine, and throat swabs. RT-PCR offers a rapid turnaround time, which facilitates prompt treatment of the disease.54–56

**Fungal Infections**

The incidence and severity of opportunistic pulmonary fungal infections has burgeoned over the past several decades. This increase can be attributed to several factors, including the rise of the global AIDS epidemic particularly in the developing world, and the expansion of immunosuppressive treatments used in treatment of transplant patients, autoimmune disorders, and cancer. Early diagnosis and treatment of fungal infections is critical to reduce mortality and prevent overtreatment with broad-spectrum antimicrobials.

Fungal lung infections in immunocompromised individuals are caused predominantly by *Pneumocystis*, Cryptococcus, Aspergillus, Blastomyces, Coccidioides, Histoplasma, *Mucor*, and rarely, Candida organisms. Clinical history and chest imaging can provide some clues to the etiology of the pulmonary process; however, isolation of organism(s) is required to make specific diagnosis for treatment. Minimally invasive procedures, such as bronchoscopy, or traditional invasive techniques are almost always required to obtain a diagnostic specimen. With the increasing use of minimally invasive diagnostic procedures, cytology specimens are commonly examined for the possibility of fungal infection. Ancillary techniques, including special stains, immunocytochemistry, and molecular studies, can be applied to cytology samples, thereby increasing the specificity and sensitivity of diagnosis.

With rare exceptions, most fungal organisms are diagnosed in cytology specimens by their morphologic rather than staining characteristics. This approach, combined with clinical history, is quite accurate for most fungal organisms. The presence of hyphal and/or yeast structures begins a mental algorithm that uses size, shape, budding, and branching characteristics to narrow the differential diagnosis. While fungal organisms can also be visualized on routine Pap and Giemsa stains, GMS and periodic acid–Schiff (PAS) stains can be performed when fungal etiology is suspected in order to highlight fungal cell walls. It is important to note that histologic and cytologic examination can misidentify fungus in up to 20% of cases, compared to the culture.57 In addition, newer urine and serum antigen detection tests can also be prone to cross-reactivity. These potential diagnostic pitfalls underscore the importance of integrating morphology with ancillary laboratory testing in making the correct diagnosis of fungal infection.

**Pneumocystis jirovecii**

*Pneumocystis jirovecii* (formerly *P carinii*) is a leading etiologic agent of pneumonia among immunocompromised patients. Transmission of infection is by airborne route. The highest risk populations for *Pneumocystis* pneumonia are those with HIV infection with low CD4 counts (fewer than 200 cells per cubic milliliter); those receiving glucocorticoids, chemotherapeutic agents, and other immunosuppressive medications; those with hematologic malignancies, solid tumors, inflammatory diseases, and vasculitis.59 Most patients present with low-grade fever, dry cough, and dyspnea.59 Non–HIV-associated patients tend to present with fulminant respiratory failure and hypoxemia at rest or with exertion. The characteristic imaging findings are diffuse, bilateral, interstitial infiltrates and diffuse ground glass opacities.

Diagnosis of *Pneumocystis* pneumonia can be reliably made on examination of BAL, sputum, or tracheal aspirates. The diagnostic utility of BAL in fungal infections is best observed in *Pneumocystis* pneumonia in AIDS patients, with noted sensitivity of up to 98%.60 Ill-defined, pale-green amorphous foamy casts, composed of numerous superimposed cysts, are visualized on Pap stain (Figure 8).61,62 Trophic forms can be seen with Gram-Weigert, Wright-Giemsa, or modified Pap stains, whereas the cell wall of the cysts can be visualized with GMS or toluidine blue stains (Figure 9). Cysts usually range in size from 5 to 8 μm, are crescentic or cup shaped, and contain 6 to 8 small (1- to 2-μm) ovoid trophozoites (Figure 10).

Other rapid stains and techniques, such as immunoperoxidase, have been developed for the detection of *P. jirovecii* in respiratory specimens and may be used if conventional methods yield negative results. Direct fluorescent antibody staining using a fluorescein-conjugated monoclonal antibody is the most commonly used diagnostic modality for BAL and sputum specimens, increasing the detection rates. Both trophic forms and cysts can be visualized by this method. PCR-based assays of respiratory specimens are available and are especially useful in confirmation of clinically suspect cases with negative sputum or BAL smears.63

**Aspergillus**

Aspergillosis encompasses a group of illnesses, ranging from allergic reaction, airway or lung invasion, cutaneous infection, or extrapulmonary dissemination, caused by *Aspergillus* spp, most commonly *A. fumigatus*, *A. flavus*, and *A. terreus*. Invasive pulmonary aspergillosis is usually seen in immunocompromised patients, and is especially common in those with hematologic malignancies or who have undergone hematopoietic stem cell or solid organ transplant.64 *Aspergillus* fungi are narrow (2–4 μm) septate hyaline hyphae demonstrating acute angle branching (Figures 11 and 12).65 Respiratory tract specimens may sometimes show sheaves or rosettes of needle-like, birefringent crystals, representing calcium oxalate.66

Positive culture, along with evidence of tissue invasion on histopathology, or culture from a normally sterile site, is most definitive of invasive aspergillosis.67 However, both microscopic examination and cultures have low sensitivity.68 Serum β-D-glucan and galactomannan antigen are biomarkers for invasive fungal infections, especially aspergillosis.69 Investigational DNA detection assays such as PCR have shown mixed results, with sensitivity and specificity of PCR of BAL fluid estimated to be 67% to 100% and 95% to 95%, respectively.70

**Mucor**

The zygomycetes (phycomycetes) are found in dead, decaying vegetation and soil and include the genera *Mucor, Absidia*, and *Rhizopus* spp. These are opportunistic pathogens with a predilection for causing infection of the head and neck region (orbit, paranasal sinuses, and palate), lungs,
and skin. The close proximity of these infections to the central nervous system and the vasoinvasive nature of the fungi often lead to a rapid, fatal course, making an immediate diagnosis extremely critical. Diabetes mellitus, hematologic malignancies, and solid organ or hematopoietic cell transplants are the most commonly associated underlying conditions.71

*Mucor* is the most commonly identified organism amongst the 3 genera and is often associated with acute inflammation and necrosis. The organisms are best visualized on Pap or hematoxylin-eosin stains and GMS is generally used for confirmation. The characteristic hyphae are broad (5–20 μm), ribbon-like, thin walled, nonpigmented, pauciseptate, and with right-angle irregular branching (Figure 13). Thick-walled spherical structures can be seen at the end of hyphae.

Diagnosis of pulmonary mucormycosis is difficult owing to the nonspecific clinical and radiologic presentation, as well as ubiquitous nature and colonization of airways by the organisms.72 In addition, diagnostic yield from direct examination or culture of BAL or tissues is low. Investigational studies have shown promise in providing rapid and increasingly sensitive results using advanced molecular techniques like PCR and in situ hybridization.73–75

**Histoplasma**

*Histoplasma capsulatum* is a small dimorphic fungus found predominantly intracellularly within histiocytes. Histoplasmosis is the most common endemic mycosis in the United States, and is particularly seen along Ohio and Mississippi River valleys.76 Histoplasmosis is usually an asymptomatic infection that can present as pulmonary disease and occasionally as a severe, systemic illness, particularly in immunocompromised patients.

Respiratory secretions or lung biopsy show the characteristic 2- to 4-μm small ovoid intracellular yeast forms with narrow-based budding. Organisms are better visualized by using GMS or PAS stains (Figure 14). Sensitivity for detection of yeasts, using fungal stains, was noted to be 86% in respiratory secretions and 77% in lung biopsy in a
Numerous endospores ranging from 2 to 5 μm (20–200 μm) can occur as small as Cryptococcus neoformans and serum antigen tests are available, with sensitivity in BAL and urine specimens, and serology are recommended in acute disease, whereas serology and cultures are diagnostic in subacute and chronic pulmonary histoplasmosis.79

**Blastomyces**

*Blastomyces dermatitidis* is a dimorphic fungus that may cause a systemic pyogranulomatous inflammation following inhalation of conidia in both immunocompetent and immunocompromised patients. Blastomycosis is endemic in North America in regions bordering the Great Lakes and the St Lawrence, Ohio, and Mississippi rivers. The clinical manifestations of blastomycosis may range from asymptomatic infection, acute or chronic pneumonia, to extrapulmonary disease.80 Lung is the most common site of infection, followed by the skin, bones, and genitourinary system, secondary to hematogenous spread.

Definitive diagnosis requires isolation of the organism from a biological specimen.81 Unlike Aspergillus and Candida, colonization or contamination with *B dermatitidis* organisms is not known to occur. Therefore, identification of the organism or a positive culture confirms the diagnosis of blastomycosis. Blastomyces organisms are best highlighted by staining the cell wall with silver stains such as GMS or PAS stain. The characteristic appearance of Blastomyces organisms is large, round, yeast form (8–15 μm in diameter), with refractile cell walls, and single, broad-based budding (Figure 15). *Paracoccidioides brasiliensis* can be distinguished from *B dermatitidis* by the presence of multiple, narrow-based buds arranged around the central mother cell giving a “pilot wheel” appearance. (*B dermatitidis* may sometimes be as small as Cryptococcus neoformans, although the capsule and narrow-based budding of the latter aids in differentiation.)

DNA probes and PCR amplification assays for molecular diagnosis of *B dermatitidis* are available. In addition, urine and serum antigen tests are available, with sensitivity reported to be above 90%.82 Serologic testing is not useful for the diagnosis of Blastomyces infection owing to cross-reactivity with other fungal agents.83

**Coccidioides**

*Coccidioides* spp (C immitis and C posadasii) are large, dimorphic fungi endemic in the southwestern United States and Central and South America, capable of causing acute and chronic respiratory infections. The most common form of *C immitis* seen in biological specimens is variably sized (20–200 μm), thick-walled mature spherules containing numerous endospores ranging from 2 to 5 μm (Figure 16).84–86 Chronic respiratory infections with Coccidioides organisms can result in the formation of lung cavities or nodules and the development of mycelial forms with barrel-shaped arthroconidia seen in BAL specimens.87 The lack of visibility of endospores within immature spherules, along with the thick wall, often results in an erroneous identification as *B dermatitidis*.87 Isolated endospores may resemble *H capsulatum* or *Toxoplasma gondii*, and a careful search for older, folded, and fractured spherules is essential for diagnosis.88 Older spherules without intact, mature spherules, may be misdiagnosed as inorganic contaminants.89

*Coccidioides* spp can be grown on any laboratory media, with growth usually noticed within 1 week, but the isolated organisms need confirmation by either “Coccidioides–specific” antigen testing90 or by the preferred rapid detection of *C immitis*–specific DNA sequence.91 Serologic tests for Coccidioides organisms can be used for both screening and confirmation of infection. Urine and serum antigen testing are not useful owing to low sensitivity; however, they can be useful in severely immunocompromised patients with negative serologic testing or those with extensive disease.91

**Cryptococcus**

Cryptococcus organism is an encapsulated ubiquitous yeast that causes cryptococcosis, an invasive fungal infection usually seen in immunocompromised states, like HIV infection and from chronic corticosteroid use. *C neoformans* is responsible for most cases in immunocompromised patients, whereas *C gattii* has been more commonly associated with disease in immunocompetent individuals.92 The infection is usually subacute or chronic and is usually associated with pulmonary and/or central nervous system involvement, although other organs can be rarely affected.93 The clinical and radiographic presentations are usually nonspecific and variable. Pulmonary cryptococcosis is usually underdiagnosed, often leading to severe disseminated disease in immunosuppressed patients.92

Diagnosis of *C neoformans* is primarily based on cytomorphology with confirmation by special stains, culture, or serum cryptococcal antigen when needed.3 Cryptococcus organism, one of the smallest fungi, presents as encapsulated yeast forms, singly or in clusters, ranging from 4 to 10 μm, with clear capsular halos and single, narrow-based buds.81 Organisms can be seen in BAL or pleural fluid specimens. Tear-drop shaped, narrow-based budding is a helpful cytologic feature.3 Special stains may be performed to confirm the cytologic impression. Silver stains are useful for staining the organism itself, while mucicarmine, PAS, and Alcian blue stain the thick mucopolysaccharide capsule (Figures 17 through 20).94 Cryptococcal yeast forms can potentially be mistaken for *Histoplasma* organisms when they are either nonencapsulated or engulfed by histiocytes.

Cryptococcal antigen can be detected in serum and cerebrospinal fluid through latex agglutination or enzyme-linked immunosorbent assay, and is the preferred method for diagnosis.91 The lateral flow assay is an alternative inexpensive method for detection of cryptococcal antigen and can be used on urine, blood, serum, cerebrospinal fluid, or plasma samples. Culture can assist in determining the species of Cryptococcus.

Diagnosis of fungal infection is challenging owing to tremendous overlap in their clinical, radiologic, and cytomorphic presentations. However, there are usually enough clues that allow the cytopathologist to accurately distinguish between (or offer a differential diagnosis) the 4 most common of these fungi: *Histoplasma capsulatum*, Cryptococcus neoformans, Blastomyces dermatitidis, and *Coccidioides immitis*. As with all lower respiratory tract pathogens, integration of clinical, pathologic, radiographic, and individual patient risk factors is critical to establish a definitive diagnosis of disease.

**CONCLUSIONS**

The lower respiratory tract can be affected by a vast array of infectious agents. The goal of this review is to give the...
reader a broad overview of commonly encountered infectious organisms in respiratory cytology specimens, their classic cytomorphologic features, and useful ancillary tests. With the increasing use of minimally invasive methods to obtain diagnostic samples, cytology plays an outsized role in the diagnosis of respiratory infections, particularly in immunocompromised patient populations. Pathologists have in their arsenal cytomorphologic features and ancillary tests such as immunohistochemistry, special stains, and molecular tests to suggest infectious causes. However, numerous additional conditions must be met in order to confidently make a diagnosis of lower respiratory tract infection, including strict adherence to sample collection requirements, appropriate triage of respiratory cytology samples between microbiology and cytology laboratories, and communication between treating clinicians and pathologists with complete integration of pathologic, microbiologic, and clinical information. These factors in combination cannot be overemphasized in making an accurate and confident diagnosis of lower respiratory tract infection.

References


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